HSD17B13 VARIANTS AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of US Application No. 62/449,335, filed January 23, 2017, US Application No. 62/472,972, filed March 17, 2017, and US Application No.: 62/581,918, filed November 6, 2017, each of which is herein incorporated by reference in its entirety for all purposes.

REFERENCE TO A SEQUENCE LISTING SUBMITTED AS A TEXT FILE VIA EFS WEB

[0002] The Sequence Listing written in file 507242SEQLIST.txt is 507 kilobytes, was created on January 19, 2018, and is hereby incorporated by reference.

BACKGROUND

[0003] Chronic liver disease and cirrhosis are leading causes of morbidity and mortality in the United States, accounting for 38,170 deaths (1.5% of total deaths) in 2014 (Kochanek et al. (2016) *Natl Vital Stat Rep* 65:1-122, herein incorporated by reference in its entirety for all purposes). The most common etiologies of cirrhosis in the U.S. are alcoholic liver disease, chronic hepatitis C, and nonalcoholic fatty liver disease (NAFLD), together accounting for ~80% of patients awaiting liver transplant between 2004 and 2013 (Wong et al. (2015) *Gastroenterology* 148:547-555, herein incorporated by reference in its entirety for all purposes). The estimated prevalence of NAFLD in the U.S. is between 19 and 46 percent (Browning et al. (2004) *Hepatology* 40:1387-1395; Lazo et al. (2013) *Am J Epidemiol* 178:38-45; and Williams et al. (2011) *Gastroenterology* 140:124-131, each of which is herein incorporated by reference in its entirety for all purposes) and is rising over time (Younossi et al. (2011) *Clin Gastroenterol Hepatol* 9:524-530 e1; quiz e60 (2011), herein incorporated by reference in its entirety for all purposes), likely in conjunction with increased rates of obesity, its primary risk factor (Cohen et al. (2011) *Science* 332:1519-1523, herein incorporated by reference in its entirety for all purposes). While significant advances have been made in the treatment of hepatitis C (Morgan et al. (2013) *Ann Intern Med* 158:329-337 and van der Meer et al. (2012) *JAMA* 308:2584-2593, each of which is herein incorporated by reference in its entirety for all purposes), there are

currently no evidence-based treatments for alcoholic or nonalcoholic liver disease and cirrhosis.

[0004] Previous genome wide association studies (GWAS) have identified a limited number of genes and variants associated with chronic liver disease. The most robustly validated genetic association to date is to a common missense variant in the patatin-like phospholipase domain containing 3 gene (*PNPLA3* p.Ile148Met, rs738409), initially found to be associated with increased risk of nonalcoholic fatty liver disease (NAFLD) (Romeo et al. (2008) *Nat. Genet.* 40:1461-1465 and Speliotes et al. (2011) *PLoS Genet.* 7:e1001324, each of which is herein incorporated by reference in its entirety for all purposes), and subsequently found to be associated with disease severity (Rotman et al. (2010) *Hepatology* 52:894-903 and Sookoian et al. (2009) *J. Lipid Res.* 50:2111-2116, each of which is herein incorporated by reference in its entirety for all purposes) and progression (Trepo et al. (2016) *J. Hepatol.* doi:10.1016/j.jhep.2016.03.011, herein incorporated by reference in its entirety for all purposes). Variation in the transmembrane 6 superfamily member 2 (*TM6SF2*) gene has also been shown to confer increased risk for NAFLD (Kozlitina et al. (2014) *Nat. Genet.* 46:352-356; Liu et al. (2014) *Nat. Commun.* 5:4309; and Sookoian et al. (2015) *Hepatology* 61:515-525, each of which is herein incorporated by reference in its entirety for all purposes). The normal functions of these two proteins are not well understood, though both have been proposed to be involved in hepatocyte lipid metabolism. How variants in *PNPLA3* and *TM6SF2* contribute to increased risk of liver disease has yet to be elucidated. GWAS have also identified several genetic factors to be associated with serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (Chambers et al. (2011) *Nat. Genet.* 43:131-1138 and Yuan et al. (2008) *Am. J. Hum. Genet.* 83:520-528, each of which is herein incorporated by reference in its entirety for all purposes), quantitative markers of hepatocyte injury and liver fat accumulation that are frequently measured clinically. To date, there are no described protective genetic variants for chronic liver disease. The discovery of protective genetic variants in other settings, such as loss-of-function variants in PCSK9 that reduce the risk of cardiovascular disease, has been the catalyst for development of new classes of therapeutics.

[0005] Knowledge of genetic factors underlying the development and progression of chronic liver disease could improve risk stratification and provide the foundation for novel therapeutic strategies. A better understanding of underlying genetic factors is needed to improve risk stratification and generate novel therapies for liver disease.

SUMMARY

[0006] Methods and compositions are provided related to the *HSD17B13* rs72613567 variant gene, variant *HSD17B13* transcripts, and variant HSD17B13 protein isoforms.

[0007] In one aspect, provided are isolated nucleic acids comprising the mutant residue from the *HSD17B13* rs72613567 variant gene. Such isolated nucleic acids can comprise at least 15 contiguous nucleotides of an *HSD17B13* gene and have a thymine inserted between nucleotides corresponding to positions 12665 and 12666 of SEQ ID NO: 1 when optimally aligned with SEQ ID NO: 1. Optionally, the contiguous nucleotides are at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a corresponding sequence in SEQ ID NO: 2 including position 12666 of SEQ ID NO: 2 when optimally aligned with SEQ ID NO: 2. Optionally, the *HSD17B13* gene is a human *HSD17B13* gene. Optionally, the isolated nucleic acid comprises at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 200, at least 300, at least 400, at least 500, at least 600, at least 700, at least 800, at least 900, at least 1000, at least 2000, at least 3000, at least 4000, at least 5000, at least 6000, at least 7000, at least 8000, at least 9000, at least 10000, at least 11000, at least 12000, at least 13000, at least 14000, at least 15000, at least 16000, at least 17000, at least 18000, or at least 19000 contiguous nucleotides of SEQ ID NO: 2.

[0008] Some such isolated nucleic acids comprise an *HSD17B13* minigene in which one or more nonessential segments of the gene have been deleted with respect to a corresponding wild type *HSD17B13* gene. Optionally, the deleted segments comprise one or more intronic sequences. Optionally, the isolated nucleic acid further comprises an intron corresponding to intron 6 of SEQ ID NO: 2 when optimally aligned with SEQ ID NO: 2. Optionally, the intron is intron 6 of SEQ ID NO: 2.

[0009] In another aspect, provided are isolated nucleic acids corresponding with different *HSD17B13* mRNA transcripts or cDNAs. Some such isolated nucleic acids comprise at least 15 contiguous nucleotides encoding all or part of an HSD17B13 protein, wherein the contiguous nucleic acids comprise a segment that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a segment present in SEQ ID NO: 7 (*HSD17B13* Transcript D), SEQ ID NO: 10 (*HSD17B13* Transcript G), and SEQ ID NO: 11 (*HSD17B13*

Transcript H) that is not present in SEQ ID NO: 4 (*HSD17B13* Transcript A). Optionally, the contiguous nucleotides further comprise a segment that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a segment present in SEQ ID NO: 7 (*HSD17B13* Transcript D) that is not present SEQ ID NO: 11 (*HSD17B13* Transcript H), and wherein the contiguous nucleotides further comprise a segment that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a segment present in SEQ ID NO: 7 (*HSD17B13* Transcript D) that is not present in SEQ ID NO: 10 (*HSD17B13* Transcript G). Optionally, the contiguous nucleotides further comprise a segment that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a segment present in SEQ ID NO: 11 (*HSD17B13* Transcript H) that is not present in SEQ ID NO: 7 (*HSD17B13* Transcript D). Optionally, the contiguous nucleotides further comprise a segment that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a segment present in SEQ ID NO: 10 (*HSD17B13* Transcript G) that is not present in SEQ ID NO: 7 (*HSD17B13* Transcript D).

[0010] Some such isolated nucleic acids comprise at least 15 contiguous nucleotides encoding all or part of an HSD17B13 protein, wherein the contiguous nucleotides comprise a segment that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a segment present in SEQ ID NO: 8 (*HSD17B13* Transcript E) that is not present in SEQ ID NO: 4 (*HSD17B13* Transcript A). Optionally, the contiguous nucleotides further comprise a segment that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a segment present in SEQ ID NO: 8 (*HSD17B13* Transcript E) that is not present in SEQ ID NO: 11 (*HSD17B13* Transcript H)

[0011] Some such isolated nucleic acids comprise at least 15 contiguous nucleotides encoding all or part of an HSD17B13 protein, wherein the contiguous nucleotides comprise a segment that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a segment present in SEQ ID NO: 9 (*HSD17B13* Transcript F) that is not present in SEQ ID NO: 4 (*HSD17B13* Transcript A).

[0012] Some such isolated nucleic acids comprise at least 15 contiguous nucleotides encoding all or part of an HSD17B13 protein, wherein the contiguous nucleotides comprise a segment that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a segment present in SEQ ID NO: 6 (*HSD17B13* Transcript C) that is not

present in SEQ ID NO: 4 (*HSD17B13* Transcript A).

[0013] Optionally, the HSD17B13 protein is a human HSD17B13 protein. Optionally, the isolated nucleic acid comprises at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 200, at least 300, at least 400, at least 500, at least 600, at least 700, at least 800, at least 900, at least 1000, or at least 2000 contiguous nucleotides encoding all or part of an HSD17B13 protein.

[0014] Some such isolated nucleic acids comprise a sequence at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the sequence set forth in SEQ ID NO: 6, 7, 8, 9, 10, or 11 (*HSD17B13* Transcript C, D, E, F, G, or H) and encoding an HSD17B13 protein comprising the sequence set forth in SEQ ID NO: 14, 15, 16, 17, 18, or 19 (HSD17B13 Isoform C, D, E, F, G, or H), respectively.

[0015] In any of the above nucleic acids, the contiguous nucleotides can optionally comprise sequence from at least two different exons of an *HSD17B13* gene without an intervening intron.

[0016] In another aspect, provided are proteins encoded by any of the above isolated nucleic acids.

[0017] In another aspect, provided are isolated nucleic acids that hybridize to or near the mutant residue from the *HSD17B13* rs72613567 variant gene. Such isolated nucleic acids can comprise at least 15 contiguous nucleotides that hybridize to an *HSD17B13* gene at a segment that includes or is within 1000, 500, 400, 300, 200, 100, 50, 45, 40, 35, 30, 25, 20, 15, 10, or 5 nucleotides of a position corresponding to position 12666 in SEQ ID NO: 2 when optimally aligned with SEQ ID NO: 2. Optionally, the segment is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a corresponding sequence in SEQ ID NO: 2 when optimally aligned with SEQ ID NO: 2. Optionally, the segment comprises at least 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, or 2000 contiguous nucleotides of SEQ ID NO: 2. Optionally, the segment includes position 12666 in SEQ ID NO: 2 or a position corresponding to position 12666 in SEQ ID NO: 2 when optimally aligned with SEQ ID NO: 2. Optionally, the *HSD17B13* gene is a human *HSD17B13* gene. Optionally, the isolated nucleic acid is up to about 30, 40, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1000 nucleotides in length. Optionally, the isolated nucleic acid is linked to a heterologous nucleic acid or comprises a heterologous label. Optionally, the heterologous label is a fluorescent label.

[0018] In another aspect, provided are isolated nucleic acids that hybridize to different *HSD17B13* mRNA transcripts or cDNAs. Some such isolated nucleic acids hybridize to at least 15 contiguous nucleotides of a nucleic acid encoding an HSD17B13 protein, wherein the contiguous nucleotides comprise a segment that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a segment present in SEQ ID NO: 7 (*HSD17B13* Transcript D), SEQ ID NO: 10 (*HSD17B13* Transcript G), and SEQ ID NO: 11 (*HSD17B13* Transcript H) that is not present within SEQ ID NO: 4 (*HSD17B13* Transcript A).

[0019] Some such isolated nucleic acids hybridize to at least 15 contiguous nucleotides of a nucleic acid encoding an HSD17B13 protein, wherein the contiguous nucleotides comprise a segment that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a segment present in SEQ ID NO: 8 (*HSD17B13* Transcript E) and SEQ ID NO: 11 (*HSD17B13* Transcript H) that is not present in SEQ ID NO: 4 (*HSD17B13* Transcript A).

[0020] Some such isolated nucleic acids hybridize to at least 15 contiguous nucleotides of a nucleic acid encoding an HSD17B13 protein, wherein the contiguous nucleotides comprise a segment that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a segment in SEQ ID NO: 9 (*HSD17B13* Transcript F) that is not present in SEQ ID NO: 4 (*HSD17B13* Transcript A).

[0021] Some such isolated nucleic acids hybridize to at least 15 contiguous nucleotides of a nucleic acid encoding an HSD17B13 protein, wherein the contiguous nucleotides comprise a segment that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a segment present in SEQ ID NO: 6 (*HSD17B13* Transcript C) that is not present in SEQ ID NO: 4 (*HSD17B13* Transcript A).

[0022] Optionally, the HSD17B13 protein is a human HSD17B13 protein. Optionally, the isolated nucleic acid is up to about 30, 40, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1000 nucleotides in length. Optionally, the isolated nucleic acid is linked to a heterologous nucleic acid or comprises a heterologous label. Optionally, the heterologous label is a fluorescent label.

[0023] Optionally, any of the above isolated nucleic acids comprise DNA. Optionally, any of the above isolated nucleic acids comprise RNA. Optionally, any of the above isolated nucleic acids are an antisense RNA, a short hairpin RNA, or a small-interfering RNA. Optionally, any of the above isolated nucleic acids can include a non-natural nucleotide.

[0024] In another aspect, provided are vectors and exogenous donor sequences comprising any of the above isolated nucleic acids and a heterologous nucleic acid sequence.

[0025] In another aspect, provided is the use of any of the above isolated nucleic acids, vectors, or exogenous donor sequences in a method of detecting an *HSD17B13* rs72613567 variant in a subject, a method of detecting the presence of *HSD17B13* Transcript C, D, E, F, G, or H in a subject, a method of determining a subject's susceptibility to developing a chronic liver disease, method of diagnosing a subject with fatty liver disease, or a method of modifying an *HSD17B13* gene in a cell, a method for altering expression of an *HSD17B13* gene in a cell.

[0026] In another aspect, provided are guide RNAs that target the *HSD17B13* gene. Such guide RNAs can be effective to direct a Cas enzyme to bind to or cleave an *HSD17B13* gene, wherein the guide RNA comprises a DNA-targeting segment that hybridizes to a guide RNA recognition sequence within the *HSD17B13* gene. That is, such guide RNAs can be effective to direct a Cas enzyme to bind to or cleave an *HSD17B13* gene, wherein the guide RNA comprises a DNA-targeting segment that targets a guide RNA target sequence within the *HSD17B13* gene. Such guide RNAs can be effective to direct a Cas enzyme to bind to or cleave an *HSD17B13* gene, wherein the guide RNA comprises a DNA-targeting segment that targets a guide RNA target sequence within the *HSD17B13* gene that includes or is proximate to a position corresponding to position 12666 of SEQ ID NO: 2 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 2. Optionally, the guide RNA target sequence comprises, consists essentially of, or consists of any one of SEQ ID NOS: 226-239 and 264-268. Optionally, the DNA-targeting segment comprises, consists essentially of, or consists of any one of SEQ ID NOS: 1629-1642 and 1648-1652. Optionally, the guide RNA comprises, consists essentially of, or consists of any one of SEQ ID NOS: 706-719; 936-949; 1166-1179, 1396-1409, 725-729, 955-959, 1185-1189, and 1415-1419. Optionally, the guide RNA target sequence is selected from SEQ ID NOS: 226-239 or SEQ ID NOS: 230 and 231. Optionally, the guide RNA target sequence is selected from SEQ ID NOS: 226-230 and 264-268. Optionally, the guide RNA target sequence is within a region corresponding to exon 6 and/or intron 6 of SEQ ID NO: 2 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 2. Optionally, the guide RNA target sequence is within a region corresponding to exon 6 and/or intron 6 and/or exon 7 of SEQ ID NO: 2 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 2. Optionally, the guide RNA target sequence is within about 1000, 500, 400, 300, 200, 100, 50, 45, 40, 35, 30, 25,

20, 15, 10, or 5 nucleotides of the position corresponding to position 12666 of SEQ ID NO: 2 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 2. Optionally, the guide RNA target sequence includes the position corresponding to position 12666 of SEQ ID NO: 2 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 2.

[0027] Such guide RNAs can be effective to direct a Cas enzyme to bind to or cleave an *HSD17B13* gene, wherein the guide RNA comprises a DNA-targeting segment that targets a guide RNA target sequence within the *HSD17B13* gene that includes or is proximate to the start codon of the *HSD17B13* gene. Optionally, the guide RNA target sequence comprises, consists essentially of, or consists of any one of SEQ ID NOS: 20-81 and 259-263. Optionally, the DNA-targeting segment comprises, consists essentially of, or consists of any one of SEQ ID NOS: 1423-1484 and 1643-1647. Optionally, the guide RNA comprises, consists essentially of, or consists of any one of SEQ ID NOS: 500-561, 730-791, 960-1021, 1190-1251, 720-724, 950-954, 1180-1184, and 1410-1414. Optionally, the guide RNA target sequence is selected from SEQ ID NOS: 20-81 and 259-263. Optionally, the guide RNA target sequence is selected from SEQ ID NOS: 21-23, 33, and 35. Optionally, the guide RNA target sequence is selected from SEQ ID NOS: 33 and 35. Optionally, the guide RNA target sequence is within a region corresponding to exon 1 of SEQ ID NO: 2 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 2. Optionally, the guide RNA target sequence is within about 1000, 500, 400, 300, 200, 100, 50, 45, 40, 35, 30, 25, 20, 15, 10, or 5 nucleotides of the start codon.

[0028] Such guide RNAs can be effective to direct a Cas enzyme to bind to or cleave an *HSD17B13* gene, wherein the guide RNA comprises a DNA-targeting segment that targets a guide RNA target sequence within the *HSD17B13* gene that includes or is proximate to the stop codon of the *HSD17B13* gene. Optionally, the guide RNA target sequence comprises, consists essentially of, or consists of any one of SEQ ID NOS: 82-225. Optionally, the DNA-targeting segment comprises, consists essentially of, or consists of any one of SEQ ID NOS: 1485-1628. Optionally, the guide RNA comprises, consists essentially of, or consists of any one of SEQ ID NOS: 562-705, 792-935, 1022-1165, and 1252-1395. Optionally, the guide RNA target sequence is selected from SEQ ID NOS: 82-225. Optionally, the guide RNA target sequence is within a region corresponding to exon 7 of SEQ ID NO: 2 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 2. Optionally, the guide RNA target sequence is within about 1000, 500, 400, 300, 200, 100, 50, 45, 40, 35, 30, 25, 20, 15, 10, or 5 nucleotides of the

stop codon.

[0029] Optionally, the *HSD17B13* gene is a human *HSD17B13* gene. Optionally, the *HSD17B13* gene comprises SEQ ID NO: 2.

[0030] Some such guide RNAs comprise a Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) RNA (crRNA) comprising the DNA-targeting segment and a trans-activating CRISPR RNA (tracrRNA). Optionally, the guide RNA is a modular guide RNA in which the crRNA and the tracrRNA are separate molecules that hybridize to each other. Optionally, the crRNA comprises, consists essentially of, or consists of the sequence set forth in SEQ ID NO: 1421 and the tracrRNA comprises, consists essentially of, or consists of the sequence set forth in SEQ ID NO: 1422. Optionally, the guide RNA is a single-guide RNA in which the crRNA is fused to the tracrRNA via a linker. Optionally, the single-guide RNA comprises, consists essentially of, or consists of the sequence set forth in any one of SEQ ID NOS: 1420 and 256-258.

[0031] In another aspect, provided are antisense RNAs, siRNAs, or shRNAs that hybridize to a sequence within an *HSD17B13* transcript disclosed herein. Some such antisense RNAs, siRNAs, or shRNAs hybridize to a sequence within SEQ ID NO: 4 (*HSD17B13* Transcript A). Optionally, the antisense RNA, siRNA, or shRNA can decrease expression of *HSD17B13* Transcript A in a cell. Optionally, the antisense RNA, siRNA, or shRNA hybridizes to a sequence present in SEQ ID NO: 4 (*HSD17B13* Transcript A) that is not present in SEQ ID NO: 7 (*HSD17B13* Transcript D). Optionally, the antisense RNA, siRNA, or shRNA hybridizes to a sequence within exon 7 or a sequence spanning the exon 6-exon 7 boundary of SEQ ID NO: 4 (*HSD17B13* Transcript A). Some such antisense RNAs, siRNAs, or shRNAs hybridize to a sequence within SEQ ID NO: 7 (*HSD17B13* Transcript D). Optionally, the antisense RNA, siRNA, or shRNA can decrease expression of *HSD17B13* Transcript D in a cell. Optionally, the antisense RNA, siRNA, or shRNA hybridizes to a sequence present in SEQ ID NO: 7 (*HSD17B13* Transcript D) that is not present in SEQ ID NO: 4 (*HSD17B13* Transcript A). Optionally, the antisense RNA, siRNA, or shRNA hybridizes to a sequence within exon 7 or a sequence spanning the exon 6-exon 7 boundary of SEQ ID NO: 7 (*HSD17B13* Transcript D).

[0032] In another aspect, provided are DNAs encoding any of the above guide RNAs, antisense RNAs, siRNAs, or shRNAs. In another aspect, provided are vectors comprising a DNA encoding any of the above guide RNAs, antisense RNAs, siRNAs, or shRNAs and a

heterologous nucleic acid. In another aspect, provided is the use of any of the above guide RNAs, antisense RNAs, siRNAs, or shRNAs DNAs encoding guide RNAs, antisense RNAs, siRNAs, or shRNAs or vectors comprising DNAs encoding guide RNAs, antisense RNAs, siRNAs, or shRNAs in a method of modifying an *HSD17B13* gene in a cell or a method for altering expression of an *HSD17B13* gene in a cell.

[0033] In another aspect, provided are compositions comprising any of the above isolated nucleic acids, any of the above guide RNAs, any of the above isolated polypeptides, any of the above antisense RNAs, siRNAs, or shRNAs, any of the above vectors, or any of the above exogenous donor sequences. Optionally, the composition comprises any of the above guide RNAs and a Cas protein, such as a Cas9 protein. Optionally, such compositions comprise a carrier increasing the stability of the isolated polypeptide, the guide RNA, the antisense RNA, the siRNA, the shRNA, the isolated nucleic acid, the vector, or the exogenous donor sequence. Optionally, the carrier comprises a poly(lactic acid) (PLA) microsphere, a poly(D,L-lactic-coglycolic-acid) (PLGA) microsphere, a liposome, a micelle, an inverse micelle, a lipid cochleate, or a lipid microtubule.

[0034] Also provided are cells comprising any of the above isolated nucleic acids, any of the above guide RNAs, any of the above antisense RNAs, siRNAs, or shRNAs, any of the above isolated polypeptides, or any of the above vectors. Optionally, the cell is a human cell, a rodent cell, a mouse cell, or a rat cell. Optionally, any of the above cells are liver cells or pluripotent cells.

[0035] Also provided are uses of any of the above guide RNAs in a method of modifying an *HSD17B13* gene in a cell or a method for altering expression of an *HSD17B13* gene in a cell. Also provided are uses of any of the above antisense RNAs, siRNAs, or shRNAs in a method for altering expression of an *HSD17B13* gene in a cell.

[0036] Also provided are methods of modifying a cell, modifying an *HSD17B13* gene, or altering expression of an *HSD17B13* gene. Some such methods are for modifying an *HSD17B13* gene in a cell, comprising contacting the genome of the cell with: (a) a Cas protein; and (b) a guide RNA that forms a complex with the Cas protein and targets a guide RNA target sequence within the *HSD17B13* gene, wherein the guide RNA target sequence includes or is proximate to a position corresponding to position 12666 of SEQ ID NO: 2 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 2, wherein the Cas protein cleaves the *HSD17B13* gene.

Optionally, the Cas protein is a Cas9 protein. Optionally, the guide RNA target sequence comprises, consists essentially of, or consists of any one of SEQ ID NOS: 226-239 and 264-268. Optionally, the DNA-targeting segment comprises, consists essentially of, or consists of any one of SEQ ID NOS: 1629-1642 and 1648-1652. Optionally, the guide RNA comprises, consists essentially of, or consists of any one of SEQ ID NOS: 706-719; 936-949; 1166-1179, 1396-1409, 725-729, 955-959, 1185-1189, and 1415-1419. Optionally, the guide RNA target sequence is selected from SEQ ID NOS: 226-239, or wherein the guide RNA target sequence is selected from SEQ ID NOS: 230 and 231. Optionally, the guide RNA target sequence is selected from SEQ ID NOS: 226-239 and 264-268 or is selected from SEQ ID NOS: 264-268. Optionally, the guide RNA target sequence is within a region corresponding to exon 6 and/or intron 6 of SEQ ID NO: 2 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 2. Optionally, the guide RNA target sequence is within a region corresponding to exon 6 and/or intron 6 and/or exon 7 of SEQ ID NO: 2 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 2. Optionally, the guide RNA target sequence is within about 1000, 500, 400, 300, 200, 100, 50, 45, 40, 35, 30, 25, 20, 15, 10, or 5 nucleotides of the position corresponding to position 12666 of SEQ ID NO: 2 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 2. Optionally, the guide RNA target sequence includes the position corresponding to position 12666 of SEQ ID NO: 2 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 2.

[0037] Some such methods further comprise contacting the genome with an exogenous donor sequence comprising a 5' homology arm that hybridizes to a target sequence 5' of the position corresponding to position 12666 of SEQ ID NO: 2 and a 3' homology arm that hybridizes to a target sequence 3' of the position corresponding to position 12666 of SEQ ID NO: 2, wherein the exogenous donor sequence recombines with the *HSD17B13* gene. Optionally, the exogenous donor sequence further comprises a nucleic acid insert flanked by the 5' homology arm and the 3' homology arm. Optionally, the nucleic acid insert comprises a thymine, and wherein upon recombination of the exogenous donor sequence with the *HSD17B13* gene, the thymine is inserted between nucleotides corresponding to positions 12665 and 12666 of SEQ ID NO: 1 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 1. Optionally, the exogenous donor sequence is between about 50 nucleotides to about 1 kb in length or between about 80 nucleotides to about 200 nucleotides in length. Optionally, the exogenous donor sequence is a single-stranded oligodeoxynucleotide.

[0038] Some such methods are for modifying an *HSD17B13* gene in a cell, comprising contacting the genome of the cell with: (a) a Cas protein; and (b) a first guide RNA that forms a complex with the Cas protein and targets a first guide RNA target sequence within the *HSD17B13* gene, wherein the first guide RNA target sequence comprises the start codon for the *HSD17B13* gene or is within about 10, 20, 30, 40, 50, 100, 200, 300, 400, 500, or 1,000 nucleotides of the start codon or is selected from SEQ ID NOS: 20-81 or is selected from SEQ ID NOS: 20-81 and 259-263, wherein the Cas protein cleaves or alters expression of the *HSD17B13* gene. Optionally, the first guide RNA target sequence comprises, consists essentially of, or consists of any one of SEQ ID NOS: 20-81 and 259-263. Optionally, the first guide RNA target sequence comprises, consists essentially of, or consists of any one of SEQ ID NOS: 20-41, any one of SEQ ID NOS: 21-23, 33, and 35, or any one of SEQ ID NOS: 33 and 35. Optionally, the first guide RNA comprises, consists essentially of, or consists of a DNA-targeting segment that comprises any one of SEQ ID NOS: 1423-1484 and 1643-1647. Optionally, the first guide RNA comprises, consists essentially of, or consists of a DNA-targeting segment that comprises any one of SEQ ID NOS: 1447-1468, any one of SEQ ID NOS: 1448-1450, 1460, and 1462; or any one of SEQ ID NOS: 1460 and 1462. Optionally, the first guide RNA comprises, consists essentially of, or consists of any one of SEQ ID NOS: 500-561, 730-791, 960-1021, 1190-1251, 720-724, 950-954, 1180-1184, and 1410-1414. Optionally, the first guide RNA comprises, consists essentially of, or consists of any one of SEQ ID NOS: 524-545, 754-775, 984-1005, and 1214-1235, or any one of SEQ ID NOS: 295-297, 525-527, 755-757, 985-987, 1215-1217, 307, 309, 537, 539, 767, 769, 997, 999, 1227, and 1229, or any one of SEQ ID NOS: 307, 309, 537, 539, 767, 769, 997, 999, 1227, and 1229. Optionally, the first guide RNA target sequence is selected from SEQ ID NOS: 20-41, is selected from SEQ ID NOS: 21-23, 33, and 35, or is selected from SEQ ID NOS: 33 and 35. Optionally, the Cas protein is a Cas9 protein. Optionally, the Cas protein is a nuclease-active Cas protein. Optionally, the Cas protein is a nuclease-inactive Cas protein fused to a transcriptional activator domain or a nuclease-inactive Cas protein fused to a transcriptional repressor domain.

[0039] Some such methods further comprise contacting the genome of the cell with a second guide RNA that forms a complex with the Cas protein and targets a second guide RNA target sequence within the *HSD17B13* gene, wherein the second guide RNA target sequence comprises the stop codon for the *HSD17B13* gene or is within about 10, 20, 30, 40, 50, 100, 200, 300, 400,

500, or 1,000 nucleotides of the stop codon or is selected from SEQ ID NOS: 82-225, wherein the cell is modified to comprise a deletion between the first guide RNA target sequence and the second guide RNA target sequence. Optionally, the second guide RNA target sequence comprises, consists essentially of, or consists of any one of SEQ ID NOS: 82-225. Optionally, the second guide RNA comprises, consists essentially of, or consists of a DNA-targeting segment that comprises any one of SEQ ID NOS: 1485-1628. Optionally, the second guide RNA comprises, consists essentially of, or consists of any one of SEQ ID NOS: 562-705, 792-935, 1022-1165, and 1252-1395.

[0040] Some such methods are for decreasing expression of an *HSD17B13* gene in a cell or decreasing expression of a particular *HSD17B13* transcript (e.g., Transcript A or Transcript D) in a cell. Some such methods are for decreasing expression of an *HSD17B13* gene in a cell, comprising: contacting the genome of the cell with an antisense RNA, an siRNA, or an shRNA that hybridizes to a sequence within exon 7 of SEQ ID NO: 4 (*HSD17B13* Transcript A) and decreases expression of *HSD17B13* Transcript A. Some such methods are for decreasing expression of an *HSD17B13* gene in a cell, comprising: contacting the genome of the cell with an antisense RNAs, an siRNA, or an shRNA that hybridizes to a sequence within an *HSD17B13* transcript disclosed herein. In some such methods, the antisense RNA, siRNA, or shRNA hybridizes to a sequence within SEQ ID NO: 4 (*HSD17B13* Transcript A). Optionally, the antisense RNA, siRNA, or shRNA can decrease expression of *HSD17B13* Transcript A in a cell. Optionally, the antisense RNA, siRNA, or shRNA hybridizes to a sequence present in SEQ ID NO: 4 (*HSD17B13* Transcript A) that is not present in SEQ ID NO: 7 (*HSD17B13* Transcript D). Optionally, the antisense RNA, siRNA, or shRNA hybridizes to a sequence within exon 7 or a sequence spanning the exon 6-exon 7 boundary of SEQ ID NO: 4 (*HSD17B13* Transcript A). In some such methods, the antisense RNA, siRNA, or shRNA hybridizes to a sequence within SEQ ID NO: 7 (*HSD17B13* Transcript D). Optionally, the antisense RNA, siRNA, or shRNA can decrease expression of *HSD17B13* Transcript D in a cell. Optionally, the antisense RNA, siRNA, or shRNA hybridizes to a sequence present in SEQ ID NO: 7 (*HSD17B13* Transcript D) that is not present in SEQ ID NO: 4 (*HSD17B13* Transcript A). Optionally, the antisense RNA, siRNA, or shRNA hybridizes to a sequence within exon 7 or a sequence spanning the exon 6-exon 7 boundary of SEQ ID NO: 7 (*HSD17B13* Transcript D).

[0041] In any of the above methods for of modifying an *HSD17B13* gene or altering

expression of an *HSD17B13* gene, the method can further comprise introducing an expression vector into the cell, wherein the expression vector comprises a recombinant *HSD17B13* gene comprising a thymine inserted between nucleotides corresponding to positions 12665 and 12666 of SEQ ID NO: 1 when the recombinant *HSD17B13* gene is optimally aligned with SEQ ID NO: 1. Optionally, the recombinant *HSD17B13* gene is a human gene. Optionally, the recombinant *HSD17B13* gene is an *HSD17B13* minigene in which one or more nonessential segments of the gene have been deleted with respect to a corresponding wild type *HSD17B13* gene. Optionally, the deleted segments comprise one or more intronic sequences. Optionally, the *HSD17B13* minigene comprises an intron corresponding to intron 6 of SEQ ID NO: 2 when optimally aligned with SEQ ID NO: 2.

[0042] In any of the above methods for of modifying an *HSD17B13* gene or altering expression of an *HSD17B13* gene, the method can further comprise introducing an expression vector into the cell, wherein the expression vector comprises a nucleic acid encoding an HSD17B13 protein that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 15 (HSD17B13 Isoform D). Optionally, the nucleic acid encoding the HSD17B13 protein is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 7 (*HSD17B13* Transcript D) when optimally aligned with SEQ ID NO: 7.

[0043] In any of the above methods for of modifying an *HSD17B13* gene or altering expression of an *HSD17B13* gene, the method can further comprise introducing an HSD17B13 protein or fragment thereof into the cell. Optionally, the HSD17B13 protein or fragment thereof is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 15 (HSD17B13 Isoform D).

[0044] Some such methods are for modifying a cell, comprising introducing an expression vector into the cell, wherein the expression vector comprises a recombinant *HSD17B13* gene comprising a thymine inserted between nucleotides corresponding to positions 12665 and 12666 of SEQ ID NO: 1 when the recombinant *HSD17B13* gene is optimally aligned with SEQ ID NO: 1. Optionally, the recombinant *HSD17B13* gene is a human gene. Optionally, the recombinant *HSD17B13* gene is an *HSD17B13* minigene in which one or more nonessential segments of the gene have been deleted with respect to a corresponding wild type *HSD17B13* gene. Optionally, the deleted segments comprise one or more intronic sequences. Optionally, the *HSD17B13*

minigene comprises an intron corresponding to intron 6 of SEQ ID NO: 2 when optimally aligned with SEQ ID NO: 2.

[0045] Some such methods are for modifying a cell, comprising introducing an expression vector into the cell, wherein the expression vector comprises a nucleic acid encoding an HSD17B13 protein that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 15 (HSD17B13 Isoform D). Optionally, the nucleic acid encoding the HSD17B13 protein is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 7 (*HSD17B13* Transcript D) when optimally aligned with SEQ ID NO: 7.

[0046] Some such methods are for modifying a cell, comprising introducing an HSD17B13 protein or fragment thereof into the cell. Optionally, the HSD17B13 protein or fragment thereof is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 15 (HSD17B13 Isoform D).

[0047] In any of the above methods of modifying a cell, modifying an *HSD17B13* gene, or altering expression of an *HSD17B13* gene, the cell can be a human cell, a rodent cell, a mouse cell, or a rat cell. Any of the cells can be pluripotent cells or differentiated cells. Any of the cells can be liver cells. In any of the above methods of modifying a cell, modifying an *HSD17B13* gene, or altering expression of an *HSD17B13* gene, the method or cell can be *ex vivo* or *in vivo*. The guide RNAs used in any of the above methods can be modular guide RNAs comprising separate crRNA and tracrRNA molecules that hybridize to each other or a single-guide RNA in which the crRNA portion is fused to the tracrRNA portion (e.g., by a linker).

[0048] In another aspect, provided are methods of treating a subject who has or is susceptible to developing a chronic liver disease. In another aspect, provided are methods of treating a subject who has or is susceptible to developing an alcoholic or nonalcoholic liver disease. Such subjects can be, for example, a subject who is not a carrier of the *HSD17B13* rs72613567 variant or subject who is not a homozygous carrier of the *HSD17B13* rs72613567 variant. Some such methods comprise a method of treating a subject who is not a carrier of the *HSD17B13* rs72613567 variant and has or is susceptible to developing a chronic liver disease, comprising introducing into the subject: (a) a Cas protein or a nucleic acid encoding the Cas protein; (b) a guide RNA or a nucleic acid encoding the guide RNA, wherein the guide RNA forms a complex with the Cas protein and targets a guide RNA target sequence within an *HSD17B13* gene,

wherein the guide RNA target sequence includes or is proximate to a position corresponding to position 12666 of SEQ ID NO: 2 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 2; and (c) an exogenous donor sequence comprising a 5' homology arm that hybridizes to a target sequence 5' of the position corresponding to position 12666 of SEQ ID NO: 2, a 3' homology arm that hybridizes to a target sequence 3' of the position corresponding to position 12666 of SEQ ID NO: 2, and a nucleic acid insert comprising a thymine flanked by the 5' homology arm and the 3' homology arm, wherein the Cas protein cleaves the *HSD17B13* gene in a liver cell in the subject and the exogenous donor sequence recombines with the *HSD17B13* gene in the liver cell, wherein upon recombination of the exogenous donor sequence with the *HSD17B13* gene, the thymine is inserted between nucleotides corresponding to positions 12665 and 12666 of SEQ ID NO: 1 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 1.

[0049] Optionally, the guide RNA target sequence is selected from SEQ ID NOS: 226-239, or wherein the guide RNA target sequence is selected from SEQ ID NOS: 230 and 231. Optionally, the guide RNA target sequence is selected from SEQ ID NOS: 226-239 and 264-268. Optionally, the guide RNA target sequence is within a region corresponding to exon 6 and/or intron 6 of SEQ ID NO: 2 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 2. Optionally, the guide RNA target sequence is within a region corresponding to exon 6 and/or intron 6 and/or exon 7 of SEQ ID NO: 2 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 2. Optionally, the guide RNA target sequence is within about 1000, 500, 400, 300, 200, 100, 50, 45, 40, 35, 30, 25, 20, 15, 10, or 5 nucleotides of the position corresponding to position 12666 of SEQ ID NO: 2 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 2. Optionally, the guide RNA target sequence includes the position corresponding to position 12666 of SEQ ID NO: 2 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 2.

[0050] Optionally, the exogenous donor sequence is between about 50 nucleotides to about 1 kb in length. Optionally, the exogenous donor sequence is between about 80 nucleotides to about 200 nucleotides in length. Optionally, the exogenous donor sequence is a single-stranded oligodeoxynucleotide.

[0051] Some such methods comprise a method of treating a subject who is not a carrier of the *HSD17B13* rs72613567 variant and has or is susceptible to developing a chronic liver disease, comprising introducing into the subject: (a) a Cas protein or a nucleic acid encoding the

Cas protein; (b) a first guide RNA or a nucleic acid encoding the first guide RNA, wherein the first guide RNA forms a complex with the Cas protein and targets a first guide RNA target sequence within an *HSD17B13* gene, wherein the first guide RNA target sequence comprises the start codon for the *HSD17B13* gene or is within about 10, 20, 30, 40, 50, 100, 200, 300, 400, 500, or 1,000 nucleotides of the start codon or is selected from SEQ ID NOS: 20-81 or is selected from SEQ ID NOS: 20-81 and 259-263; and (c) an expression vector comprising a recombinant *HSD17B13* gene comprising a thymine inserted between nucleotides corresponding to positions 12665 and 12666 of SEQ ID NO: 1 when the recombinant *HSD17B13* gene is optimally aligned with SEQ ID NO: 1, wherein the Cas protein cleaves or alters expression of the *HSD17B13* gene in a liver cell in the subject and the expression vector expresses the recombinant *HSD17B13* gene in the liver cell in the subject. Some such methods comprise a method of treating a subject who is not a carrier of the *HSD17B13* rs72613567 variant and has or is susceptible to developing a chronic liver disease, comprising introducing into the subject: (a) a Cas protein or a nucleic acid encoding the Cas protein; (b) a first guide RNA or a nucleic acid encoding the first guide RNA, wherein the first guide RNA forms a complex with the Cas protein and targets a first guide RNA target sequence within an *HSD17B13* gene, wherein the first guide RNA target sequence comprises the start codon for the *HSD17B13* gene or is within about 10, 20, 30, 40, 50, 100, 200, 300, 400, 500, or 1,000 nucleotides of the start codon or is selected from SEQ ID NOS: 20-81 or is selected from SEQ ID NOS: 20-81 and 259-263; and optionally (c) an expression vector comprising a recombinant *HSD17B13* gene comprising a thymine inserted between nucleotides corresponding to positions 12665 and 12666 of SEQ ID NO: 1 when the recombinant *HSD17B13* gene is optimally aligned with SEQ ID NO: 1, wherein the Cas protein cleaves or alters expression of the *HSD17B13* gene in a liver cell in the subject and the expression vector expresses the recombinant *HSD17B13* gene in the liver cell in the subject.

[0052] Optionally, the first guide RNA target sequence is selected from SEQ ID NOS: 20-41, is selected from SEQ ID NOS: 21-23, 33, and 35, or is selected from SEQ ID NOS: 33 and 35. Optionally, the Cas protein is a nuclease-active Cas protein. Optionally, the Cas protein is a nuclease-inactive Cas protein fused to a transcriptional repressor domain.

[0053] Such methods can further comprise introducing into the subject a second guide RNA, wherein the second guide RNA forms a complex with the Cas protein and targets a second guide

RNA target sequence within the *HSD17B13* gene, wherein the second guide RNA target sequence comprises the stop codon for the *HSD17B13* gene or is within about 10, 20, 30, 40, 50, 100, 200, 300, 400, 500, or 1,000 nucleotides of the stop codon or is selected from SEQ ID NOS: 82-225, wherein the Cas protein cleaves the *HSD17B13* gene in the liver cell within both the first guide RNA target sequence and the second guide RNA target sequence, wherein the liver cell is modified to comprise a deletion between the first guide RNA target sequence and the second guide RNA target sequence.

[0054] Optionally, the recombinant *HSD17B13* gene is an *HSD17B13* minigene in which one or more nonessential segments of the gene have been deleted with respect to a corresponding wild type *HSD17B13* gene. Optionally, the deleted segments comprise one or more intronic sequences. Optionally, the *HSD17B13* minigene comprises an intron corresponding to intron 6 of SEQ ID NO: 2 when optimally aligned with SEQ ID NO: 2.

[0055] In any of the above therapeutic or prophylactic methods, the Cas protein can be a Cas9 proteins. In any of the above therapeutic or prophylactic methods, the subject can be a human. In any of the above therapeutic or prophylactic methods, the chronic liver disease can be a fatty liver disease, a nonalcoholic fatty liver disease (NAFLD), an alcoholic liver fatty liver disease, a cirrhosis, or a hepatocellular carcinoma. Likewise, in any of the above methods, the therapeutic or prophylactic method can be for a liver disease that is an alcoholic liver disease or a nonalcoholic liver disease.

[0056] Some such methods comprise a method of treating a subject who is not a carrier of the *HSD17B13* rs72613567 variant and has or is susceptible to developing a chronic liver disease, comprising introducing into the subject: an antisense RNA, an siRNA, or an shRNA that hybridizes to a sequence within exon 7 or a sequence spanning the exon 6-exon 7 boundary of SEQ ID NO: 4 (*HSD17B13* Transcript A) and decreases expression of *HSD17B13* Transcript A in a liver cell in the subject. Some such methods comprise a method of treating a subject who is not a carrier of the *HSD17B13* rs72613567 variant and has or is susceptible to developing a chronic liver disease, comprising introducing into the subject: an antisense RNAs, an siRNA, or an shRNA that hybridizes to a sequence within an *HSD17B13* transcript disclosed herein. Optionally, the antisense RNA, siRNA, or shRNA hybridizes to a sequence within SEQ ID NO: 4 (*HSD17B13* Transcript A). Optionally, the antisense RNA, siRNA, or shRNA can decrease expression of *HSD17B13* Transcript A in a cell. Optionally, the antisense RNA, siRNA, or

shRNA hybridizes to a sequence present in SEQ ID NO: 4 (*HSD17B13* Transcript A) that is not present in SEQ ID NO: 7 (*HSD17B13* Transcript D). Optionally, the antisense RNA, siRNA, or shRNA hybridizes to a sequence within exon 7 or a sequence spanning the exon 6-exon 7 boundary of SEQ ID NO: 4 (*HSD17B13* Transcript A).

[0057] Optionally, such methods further comprise introducing an expression vector into the subject, wherein the expression vector comprises a recombinant *HSD17B13* gene comprising a thymine inserted between nucleotides corresponding to positions 12665 and 12666 of SEQ ID NO: 1 when the recombinant *HSD17B13* gene is optimally aligned with SEQ ID NO: 1, wherein the expression vector expresses the recombinant *HSD17B13* gene in the liver cell in the subject.

[0058] Optionally, such methods further comprise introducing an expression vector into the subject, wherein the expression vector comprises a nucleic acid encoding an HSD17B13 protein that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 15 (HSD17B13 Isoform D), wherein the expression vector expresses the nucleic acid encoding the HSD17B13 protein in the liver cell in the subject. Optionally, the nucleic acid encoding the HSD17B13 protein is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 7 (*HSD17B13* Transcript D) when optimally aligned with SEQ ID NO: 7.

[0059] Optionally, such methods further comprise introducing a messenger RNA into the subject, wherein the messenger RNA encodes an HSD17B13 protein that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 15 (HSD17B13 Isoform D), wherein the mRNA expresses the HSD17B13 protein in the liver cell in the subject. Optionally, a complementary DNA reverse transcribed from the messenger RNA is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 7 (*HSD17B13* Transcript D) when optimally aligned with SEQ ID NO: 7.

[0060] Optionally, such methods further comprise introducing an HSD17B13 protein or fragment thereof into the subject. Optionally, the HSD17B13 protein or fragment thereof is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 15 (HSD17B13 Isoform D).

[0061] Some such methods comprise a method of treating a subject who is not a carrier of the *HSD17B13* rs72613567 variant and has or is susceptible to developing a chronic liver disease

comprising introducing an expression vector into the subject, wherein the expression vector comprises a recombinant *HSD17B13* gene comprising a thymine inserted between nucleotides corresponding to positions 12665 and 12666 of SEQ ID NO: 1 when the recombinant *HSD17B13* gene is optimally aligned with SEQ ID NO: 1, wherein the expression vector expresses the recombinant *HSD17B13* gene in a liver cell in the subject.

[0062] In any of the above methods, the recombinant *HSD17B13* gene can be a human gene. In any of the above methods, the recombinant *HSD17B13* gene can be at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 2 when optimally aligned with SEQ ID NO: 2. In any of the above methods, the recombinant *HSD17B13* gene can be an *HSD17B13* minigene in which one or more nonessential segments of the gene have been deleted with respect to a corresponding wild type *HSD17B13* gene. Optionally, the deleted segments comprise one or more intronic sequences. Optionally, the *HSD17B13* minigene comprises an intron corresponding to intron 6 of SEQ ID NO: 2 when optimally aligned with SEQ ID NO: 2.

[0063] Some such methods comprise a method of treating a subject who is not a carrier of the *HSD17B13* rs72613567 variant and has or is susceptible to developing a chronic liver disease comprising introducing an expression vector into the subject, wherein the expression vector comprises a nucleic acid encoding an HSD17B13 protein that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 15 (HSD17B13 Isoform D), wherein the expression vector expresses the nucleic acid encoding the HSD17B13 protein in a liver cell in the subject. Optionally, the nucleic acid encoding the HSD17B13 protein is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 7 (*HSD17B13* Transcript D) when optimally aligned with SEQ ID NO: 7.

[0064] Some such methods comprise a method of treating a subject who is not a carrier of the *HSD17B13* rs72613567 variant and has or is susceptible to developing a chronic liver disease comprising introducing a messenger RNA into the subject, wherein the messenger RNA encodes an HSD17B13 protein that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 15 (HSD17B13 Isoform D), wherein the mRNA expresses the HSD17B13 protein in the liver cell in the subject. Optionally, a complementary DNA reverse transcribed from the messenger RNA is at least 90%, at least 95%, at least 96%, at

least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 7 (*HSD17B13* Transcript D) when optimally aligned with SEQ ID NO: 7.

[0065] Some such methods comprise a method of treating a subject who is not a carrier of the *HSD17B13* rs72613567 variant and has or is susceptible to developing a chronic liver disease comprising introducing an HSD17B13 protein or fragment thereof into the liver of the subject. Optionally, the HSD17B13 protein or fragment thereof is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 15 (HSD17B13 Isoform D).

[0066] In any of the above methods, the subject can be a human. In any of the above methods, the chronic liver disease can be nonalcoholic fatty liver disease (NAFLD), alcoholic liver fatty liver disease, cirrhosis, or hepatocellular carcinoma. Likewise, in any of the above methods, the therapeutic or prophylactic method can be for a liver disease that is an alcoholic liver disease or a nonalcoholic liver disease. In any of the above methods, the introducing into the subject can comprise hydrodynamic delivery, virus-mediated delivery, lipid-nanoparticle-mediated delivery, or intravenous infusion.

BRIEF DESCRIPTION OF THE FIGURES

[0067] **Figures 1A** and **1B** show Manhattan plots (left) and quantile-quantile plots (right) of single nucleotide variant associations with median alanine aminotransferase (ALT; **Figure 1A**) and aspartate aminotransferase (AST; **Figure 1B**) levels in the GHS discovery cohort. **Figure 1A** shows that there were 31 variants in 16 genes significantly associated with ALT levels (N=41,414) at $P < 1.0 \times 10^{-7}$. **Figure 1B** shows that there were 12 variants in 10 genes significantly associated with AST levels (N=40,753) at $P < 1.0 \times 10^{-7}$. All significant associations are shown in **Table 2**. There were thirteen variants in nine genes (indicated here by their gene name), including *HSD17B13*, that remained significantly associated with ALT or AST in a replication meta-analysis of three separate European-ancestry cohorts (**Table 3**). The association tests were well calibrated, as shown by exome-wide quantile-quantile plots and genomic control lambda values (**Figure 1A** and **Figure 1B**).

[0068] **Figures 2A** and **2B** show that *HSD17B13* rs72613567:TA is associated with reduced risk of alcoholic and nonalcoholic liver disease phenotypes in the discovery cohort (**Figure 2A**), and with reduced risk of progression from simple steatosis to steatohepatitis and fibrosis in the

bariatric surgery cohort (**Figure 2B**). Odds ratios were calculated using logistic regression, with adjustment for age, age^2, sex, BMI, and principal components of ancestry. Genotypic odds ratios for heterozygous (Het OR) and homozygous (Hom OR) carriers are also shown. In the GHS discovery cohort in **Figure 2A**, variant *HSD17B13* was associated with significantly reduced risk of nonalcoholic and alcoholic liver disease, cirrhosis, and hepatocellular carcinoma in an allele dosage-dependent manner. In the GHS bariatric surgery cohort in **Figure 2B**, *HSD17B13* rs72613567 was associated with 13% and 52% lower odds of nonalcoholic steatohepatitis (NASH), and 13% and 61% lower odds of fibrosis, in heterozygous and homozygous TA carriers, respectively.

[0069] **Figures 3A-3D** show expression of four *HSD17B13* Transcripts (A-D) in homozygous reference (T/T), heterozygous (T/TA), and homozygous alternate (TA/TA) carriers of the *HSD17B13* rs72613567 splice variant. Each transcript is illustrated with a corresponding gene model. Coding regions in gene models are indicated in the striped boxes and untranslated regions in the black boxes. **Figure 3A** shows a representation of Transcript A and expression data for Transcript A. **Figure 3B** shows a representation of Transcript B and expression data for Transcript B. In Transcript B, exon 2 is skipped. **Figure 3C** shows a representation of Transcript C and expression data for Transcript C. In Transcript C, exon 6 is skipped. **Figure 3D** shows a representation of Transcript D and expression data for Transcript D. The asterisk in Transcript D illustrates insertion of G from rs72613567 at the 3' end of exon 6, which leads to premature truncation of the protein. Transcript D becomes the dominant transcript in homozygous carriers of the *HSD17B13* splice variant. Gene expression is displayed in FPKM units (Fragments Per Kilobase of transcript per Million mapped reads). Insets in **Figure 3B** and **Figure 3C** show a zoomed-in view.

[0070] **Figure 4** shows that RNA-Seq studies of human liver reveal eight *HSD17B13* transcripts, including six novel *HSD17B13* transcripts (Transcripts C-H). Expression of the transcripts is displayed in FPKM units (fragments per kilobase of transcript per million mapped reads). Structures of the transcripts are provided on the right side of the figure.

[0071] **Figures 5A** and **5B** show locus-zoom plots of *HSD17B13* (regional association plots in the region around *HSD17B13*) in the GHS discovery cohort for ALT and AST, respectively. No significant recombination across the region was observed. Diamonds indicate the splice variant rs72613567. Each circle indicates a single nucleotide variant with the color of the circle

indicating the linkage disequilibrium (r^2 calculated in the DiscovEHR cohort) between that variant and rs72613567. Lines indicate estimated recombination rates in HapMap. The bottom panels show the relative position and the transcribed strand of each gene in the locus. There were no significant associations between ALT or AST and coding or splice region variants in the neighboring gene *HSD17B11* (most significant P-values $1.4x10^{-1}$ and $4.3x10^{-2}$ for ALT and AST, respectively).

[0072] **Figures 6A-6D** show mRNA expression of four additional novel *HSD17B13* transcripts (E-H) in homozygous reference (T/T), heterozygous (T/TA), and homozygous alternate (TA/TA) carriers of the *HSD17B13* splice variant. Each transcript is illustrated with a corresponding gene model. Coding regions in gene models are indicated in striped boxes and untranslated regions in black boxes. **Figures 6A** and **6D** show that Transcripts E and H contain an additional exon between exons 3 and 4. **Figure 6B** shows that Transcript F involves read-through from exon 6 to intron 6. **Figure 6C** shows that in Transcript G, exon 2 is skipped. The asterisk in Transcripts G and H (**Figures 6C** and **6D**, respectively) illustrates insertion of G from rs72613567 at the 3' end of exon 6, which leads to premature truncation of the protein. The transcripts are differentially expressed according to *HSD17B13* genotype, as shown in the box plots. mRNA expression is displayed in FPKM units (Fragments Per Kilobase of transcript per Million mapped reads).

[0073] **Figures 7A-7B** show a protein sequence alignment of HSD17B13 protein isoforms A-H.

[0074] **Figure 8** shows that *HSD17B13* rs72613567:TA is associated with reduced risk of alcoholic and nonalcoholic liver disease phenotypes. Specifically, **Figure 8** shows in the Dallas Liver Study, *HSD17B13* rs72613567 was associated with lower odds of any liver disease in an allele dosage-dependent manner. Similar allele dosage-dependent effects were observed across liver disease subtypes. Odds ratios were calculated using logistic regression, with adjustment for age, age^2, gender, BMI, and self-reported ethnicity.

[0075] **Figure 9** shows *HSD17B13* rs72613567 is associated with reduced risk of progression from simple steatosis to steatohepatitis and fibrosis. Specifically, it shows that prevalence of histopathologically-characterized liver disease according to *HSD17B13* rs72613567 genotype in 2,391 individuals with liver biopsies from the GHS bariatric surgery cohort. The prevalence of normal liver did not appear to differ by genotype (P = 0.5 by Chi-

squared test for trend in proportions), but the prevalence of NASH decreased ($P = 1.6x10^{-4}$) and that of simple steatosis increased ($P = 1.1x10^{-3}$) with each TA allele.

[0076] **Figures 10A-10E** show expression, subcellular localization, and enzymatic activity of a novel *HSD17B13* transcript. **Figure 10A** shows a Western blot from HepG2 cells overexpressing *HSD17B13* Transcripts A and D and shows that *HSD17B13* Transcript D was translated to a truncated protein with lower molecular weight compared to *HSD17B13* Transcript A. **Figure 10B** shows HSD17B13 western blots from fresh frozen human liver and HEK293 cell samples. Human liver samples are from homozygous reference (T/T), heterozygous (T/TA), and homozygous alternate (TA/TA) carriers of the *HSD17B13* rs72613567 splice variant. Cell samples are from HEK293 cells overexpressing non-tagged *HSD17B13* Transcripts A and D. *HSD17B13* Transcript D was translated to a truncated protein IsoD with lower molecular weight than HSD17B13 IsoA. **Figure 10C** shows HSD17B13 IsoD protein levels were lower than IsoA protein levels from both human liver (left) and cell (right) samples. Protein level normalized to actin is shown in the bar columns; ** $P<0.001$, *$P<0.05$. **Figure 10D** shows enzymatic activity of HSD17B13 isoforms A and D to 17-beta estradiol (estradiol), leukotriene B4 (LTB4), and 13-Hydroxyoctadecadienoic acid (13(S)-HODE). HSD17B13 Isoform D shows <10% enzymatic activity of the corresponding values for Isoform A. **Figure 10E** shows HSD17B13 Isoform D when overexpressed in HEK293 cells did not show much conversion of estradiol (substrate) to estrone (product) when measured in the culture media, while overexpressed HSD17B13 Isoform A showed robust conversion.

[0077] **Figures 11A-11C** show that HSD17B13 Isoform D protein has lower molecular weight and is unstable when overexpressed in HEK 293 cells. **Figure 11A** shows RT-PCR of *HSD17B13* from HEK 293 cells overexpressing *HSD17B13* Transcripts A (IsoA) and D (IsoD), indicating that *HSD17B13* IsoD RNA level was higher than IsoA RNA level. **Figure 11B** shows a western blot from the same cell lines indicating that *HSD17B13* Transcript D was translated to a truncated protein with lower molecular weight compared to *HSD17B13* Transcript A. **Figure 11C** shows that HSD17B13 IsoD protein levels were lower than IsoA protein levels although the RNA level was higher. HSD17B13 protein level was normalized to actin; *$P<0.05$.

[0078] **Figure 12** shows similar localization patterns of HSD17B13 isoform A and isoform D to isolated lipid droplets (LD) derived from HepG2 stable cell lines. ADRP and TIP47 were used as lipid droplet markers. LAMP1, calreticulin, and COX IV were used as markers for the

lysosomal, endoplasmic reticulum, and mitochondrial compartments, respectively. GAPDH was included as a cytosolic marker, and actin was used as a cytoskeletal marker. This experiment was repeated twice in HepG2 cells, with the above being representative of both runs. PNS=Post-nuclear fraction; TM=total membrane.

[0079] **Figures 13A-13D** show oleic acid increased triglyceride content in HepG2 cells overexpressing *HSD17B13* Transcript A or D. **Figure 13A** shows treatment with increasing concentrations of oleic acid increased triglyceride (TG) content to a similar extent in control (GFP overexpressing cells) and *HSD17B13* Transcript A and D cell lines. **Figure 13B** shows *HSD17B13* Transcripts A and D RNA levels were similar in the cell lines. RNA levels are shown reads per kilobase of transcript per million mapped reads (RPKM). **Figure 13C** shows a western blot from HepG2 cells overexpressing *HSD17B13* Transcripts A and D. *HSD17B13* Transcript D was translated to a truncated protein with lower molecular weight compared to *HSD17B13* Transcript A. **Figure 13D** shows HSD17B13 IsoD protein levels were lower than IsoA protein levels. Protein level normalized to actin; **P<0.01.

[0080] **Figure 14** shows K_m and V_{max} values for estradiol using purified recombinant HSD17B13 protein. For K_m and V_{max} determinations, assays were performed with a dose range of 17β-estradiol between 0.2 μM to 200 μM and time points from 5 minutes to 180 minutes, with 500 μM NAD^+ and 228 nM HSD17B13. V_{max} and K_m were then determined using the Michaelis-Menten model and Prism software (GraphPad Software, USA).

[0081] **Figure 15** shows percent genome editing (total number of insertions or deletions observed within a window 20 base pairs on either side of the Cas9-induced DNA break over the total number of sequences read in the PCR reaction from a pool of lysed cells) at the mouse *Hsd17b13* locus as determined by next-generation sequencing (NGS) in primary hepatocytes isolated from hybrid wild type mice (75% C57BL/6NTac 25% 129S6/SvEvTac). The samples tested included hepatocytes treated with ribonucleoprotein complexes containing Cas9 and guide RNAs designed to target the mouse *Hsd17b13* locus.

[0082] **Figure 16** shows percent genome editing (total number of insertions or deletions observed over the total number of sequences read in the PCR reaction from a pool of lysed cells) at the mouse *Hsd17b13* locus as determined by next-generation sequencing (NGS) in samples isolated from mouse livers three weeks post-injection of AAV8 containing sgRNA expression cassettes designed to target mouse *Hsd17b13* into Cas9-ready mice. Wild type mice not

expressing any Cas9 were injected with AAV8 containing all of the sgRNA expression cassettes were used as a negative control.

[0083] **Figures 17A** and **17B** show relative mRNA expression for mouse *Hsd17b13* and a non-target HSD family member, respectively, as determined by RT-qPCR in liver samples from Cas9-ready mice treated with AAV8 carrying guide RNA expression cassettes designed to target mouse *Hsd17b13*. Wild type mice not expressing any Cas9 were injected with AAV8 carrying guide RNA expression cassettes for all of the guide RNAs were used as a negative control.

DEFINITIONS

[0084] The terms "protein," "polypeptide," and "peptide," used interchangeably herein, include polymeric forms of amino acids of any length, including coded and non-coded amino acids and chemically or biochemically modified or derivatized amino acids. The terms also include polymers that have been modified, such as polypeptides having modified peptide backbones.

[0085] Proteins are said to have an "N-terminus" and a "C-terminus." The term "N-terminus" relates to the start of a protein or polypeptide, terminated by an amino acid with a free amine group (-NH2). The term "C-terminus" relates to the end of an amino acid chain (protein or polypeptide), terminated by a free carboxyl group (-COOH).

[0086] The terms "nucleic acid" and "polynucleotide," used interchangeably herein, include polymeric forms of nucleotides of any length, including ribonucleotides, deoxyribonucleotides, or analogs or modified versions thereof. They include single-, double-, and multi-stranded DNA or RNA, genomic DNA, cDNA, DNA-RNA hybrids, and polymers comprising purine bases, pyrimidine bases, or other natural, chemically modified, biochemically modified, non-natural, or derivatized nucleotide bases.

[0087] Nucleic acids are said to have "5' ends" and "3' ends" because mononucleotides are reacted to make oligonucleotides in a manner such that the 5' phosphate of one mononucleotide pentose ring is attached to the 3' oxygen of its neighbor in one direction via a phosphodiester linkage. An end of an oligonucleotide is referred to as the "5' end" if its 5' phosphate is not linked to the 3' oxygen of a mononucleotide pentose ring. An end of an oligonucleotide is referred to as the "3' end" if its 3' oxygen is not linked to a 5' phosphate of another mononucleotide pentose ring. A nucleic acid sequence, even if internal to a larger

oligonucleotide, also may be said to have 5' and 3' ends. In either a linear or circular DNA molecule, discrete elements are referred to as being "upstream" or 5' of the "downstream" or 3' elements.

[0088] The term "wild type" includes entities having a structure and/or activity as found in a normal (as contrasted with mutant, diseased, altered, or so forth) state or context. Wild type genes and polypeptides often exist in multiple different forms (e.g., alleles).

[0089] The term "isolated" with respect to proteins and nucleic acid includes proteins and nucleic acids that are relatively purified with respect to other bacterial, viral, or cellular components that may normally be present *in situ*, up to and including a substantially pure preparation of the protein and the polynucleotide. The term "isolated" also includes proteins and nucleic acids that have no naturally occurring counterpart, have been chemically synthesized and are thus substantially uncontaminated by other proteins or nucleic acids, or has been separated or purified from most other cellular components with which they are naturally accompanied (e.g., other cellular proteins, polynucleotides, or cellular components).

[0090] "Exogenous" molecules or sequences include molecules or sequences that are not normally present in a cell in that form. Normal presence includes presence with respect to the particular developmental stage and environmental conditions of the cell. An exogenous molecule or sequence, for example, can include a mutated version of a corresponding endogenous sequence within the cell or can include a sequence corresponding to an endogenous sequence within the cell but in a different form (i.e., not within a chromosome). In contrast, endogenous molecules or sequences include molecules or sequences that are normally present in that form in a particular cell at a particular developmental stage under particular environmental conditions.

[0091] The term "heterologous" when used in the context of a nucleic acid or a protein indicates that the nucleic acid or protein comprises at least two portions that do not naturally occur together. Likewise, the term "heterologous" when used in the context of a promoter operably linked to a nucleic acid encoding a protein indicates that the promoter and the nucleic acid encoding the protein do not naturally occur together (i.e., are not naturally operably linked). For example, the term "heterologous," when used with reference to portions of a nucleic acid or portions of a protein, indicates that the nucleic acid or protein comprises two or more sub-sequences that are not found in the same relationship to each other (e.g., joined together) in

nature. As one example, a "heterologous" region of a nucleic acid vector is a segment of nucleic acid within or attached to another nucleic acid molecule that is not found in association with the other molecule in nature. For example, a heterologous region of a nucleic acid vector could include a coding sequence flanked by sequences not found in association with the coding sequence in nature. Likewise, a "heterologous" region of a protein is a segment of amino acids within or attached to another peptide molecule that is not found in association with the other peptide molecule in nature (e.g., a fusion protein, or a protein with a tag). Similarly, a nucleic acid or protein can comprise a heterologous label or a heterologous secretion or localization sequence.

[0092] The term "label" refers to a chemical moiety or protein that is directly or indirectly detectable (e.g., due to its spectral properties, conformation, or activity) when attached to a target compound. The label can be directly detectable (fluorophore) or indirectly detectable (hapten, enzyme, or fluorophore quencher). Such labels can be detectable by spectroscopic, photochemical, biochemical, immunochemical, or chemical means. Such labels include, for example, radiolabels that can be measured with radiation-counting devices; pigments, dyes or other chromogens that can be visually observed or measured with a spectrophotometer; spin labels that can be measured with a spin label analyzer; and fluorescent labels (fluorophores), where the output signal is generated by the excitation of a suitable molecular adduct and that can be visualized by excitation with light that is absorbed by the dye or can be measured with standard fluorometers or imaging systems. The label can also be, for example, a chemiluminescent substance, where the output signal is generated by chemical modification of the signal compound; a metal-containing substance; or an enzyme, where there occurs an enzyme-dependent secondary generation of signal, such as the formation of a colored product from a colorless substrate. The term "label" can also refer to a "tag" or hapten that can bind selectively to a conjugated molecule such that the conjugated molecule, when added subsequently along with a substrate, is used to generate a detectable signal. For example, one can use biotin as a tag and then use an avidin or streptavidin conjugate of horseradish peroxidate (HRP) to bind to the tag, and then use a calorimetric substrate (e.g., tetramethylbenzidine (TMB)) or a fluorogenic substrate to detect the presence of HRP. The term "label" can also refer to a tag that can be used, for example, to facilitate purification. Non-limiting examples of such tags include myc, HA, FLAG or 3XFLAG, 6XHis or polyhistidine, glutathione-S-transferase

(GST), maltose binding protein, an epitope tag, or the Fc portion of immunoglobulin. Numerous labels are known and include, for example, particles, fluorophores, haptens, enzymes and their calorimetric, fluorogenic and chemiluminescent substrates and other labels.

[0093] "Codon optimization" takes advantage of the degeneracy of codons, as exhibited by the multiplicity of three-base pair codon combinations that specify an amino acid, and generally includes a process of modifying a nucleic acid sequence for enhanced expression in particular host cells by replacing at least one codon of the native sequence with a codon that is more frequently or most frequently used in the genes of the host cell while maintaining the native amino acid sequence. For example, a polynucleotide encoding a Cas9 protein can be modified to substitute codons having a higher frequency of usage in a given prokaryotic or eukaryotic cell, including a bacterial cell, a yeast cell, a human cell, a non-human cell, a mammalian cell, a rodent cell, a mouse cell, a rat cell, a hamster cell, or any other host cell, as compared to the naturally occurring nucleic acid sequence. Codon usage tables are readily available, for example, at the "Codon Usage Database." These tables can be adapted in a number of ways. *See* Nakamura et al. (2000) *Nucleic Acids Research* 28:292, herein incorporated by reference in its entirety for all purposes. Computer algorithms for codon optimization of a particular sequence for expression in a particular host are also available (*see, e.g.*, Gene Forge).

[0094] The term "locus" refers to a specific location of a gene (or significant sequence), DNA sequence, polypeptide-encoding sequence, or position on a chromosome of the genome of an organism. For example, an "*HSD17B13* locus" may refer to the specific location of an *HSD17B13* gene, HSD17B13 DNA sequence, HSD17B13-encoding sequence, or HSD17B13 position on a chromosome of the genome of an organism that has been identified as to where such a sequence resides. An "*HSD17B13* locus" may comprise a regulatory element of an *HSD17B13* gene, including, for example, an enhancer, a promoter, 5' and/or 3' UTR, or a combination thereof.

[0095] The term "gene" refers to a DNA sequence in a chromosome that codes for a product (e.g., an RNA product and/or a polypeptide product) and includes the coding region interrupted with one or more non-coding introns and sequence located adjacent to the coding region on both the 5' and 3' ends such that the gene corresponds to the full-length mRNA (including the 5' and 3' untranslated sequences). The term "gene" also includes other non-coding sequences including regulatory sequences (e.g., promoters, enhancers, and transcription factor binding sites),

polyadenylation signals, internal ribosome entry sites, silencers, insulating sequence, and matrix attachment regions. These sequences may be close to the coding region of the gene (e.g., within 10 kb) or at distant sites, and they influence the level or rate of transcription and translation of the gene. The term "gene" also encompasses "minigenes."

[0096] The term "minigene" refers to a gene in which one or more nonessential segments of the gene have been deleted with respect to a corresponding naturally occurring germline gene but in which at least one intron remains. Deleted segments can be intronic sequences. For example, deleted segments can be intronic sequences of at least about 500 base pairs to several kilobases. Typically, intronic sequences that do not encompass essential regulatory elements may be deleted. The gene segments comprising a minigene will typically be arranged in the same linear order as is present in the germline gene, but this will not always be the case. Some desired regulatory elements (e.g., enhancers, silencers) may be relatively position-insensitive so that the regulatory element will function correctly even if positioned differently in a minigene than in the corresponding germline gene. For example, an enhancer may be located at a different distance from a promoter, in a different orientation, and/or in a different linear order. For example, an enhancer that is located 3' to a promoter in germline configuration might be located 5' to the promoter in a minigene. Similarly, some genes may have exons which are alternatively spliced at the RNA level. Thus, a minigene may have fewer exons and/or exons in a different linear order than the corresponding germline gene and still encode a functional gene product. A cDNA encoding a gene product may also be used to construct a minigene (e.g., a hybrid cDNA-genomic fusion).

[0097] The term "allele" refers to a variant form of a gene. Some genes have a variety of different forms, which are located at the same position, or genetic locus, on a chromosome. A diploid organism has two alleles at each genetic locus. Each pair of alleles represents the genotype of a specific genetic locus. Genotypes are described as homozygous if there are two identical alleles at a particular locus and as heterozygous if the two alleles differ.

[0098] The term "variant" or "genetic variant" refers to a nucleotide sequence differing from the sequence most prevalent in a population (e.g., by one nucleotide). For example, some variations or substitutions in a nucleotide sequence alter a codon so that a different amino acid is encoded resulting in a genetic variant polypeptide. The term "variant" can also refer to a gene differing in sequence from the sequence most prevalent in a population at a position that does not

change the amino acid sequence of the encoded polypeptide (i.e., a conserved change). Genetic variants can be associated with risk, associated with protection, or can be neutral.

[0099] A "promoter" is a regulatory region of DNA usually comprising a TATA box capable of directing RNA polymerase II to initiate RNA synthesis at the appropriate transcription initiation site for a particular polynucleotide sequence. A promoter may additionally comprise other regions which influence the transcription initiation rate. The promoter sequences disclosed herein modulate transcription of an operably linked polynucleotide. A promoter can be active in one or more of the cell types disclosed herein (e.g., a eukaryotic cell, a non-human mammalian cell, a human cell, a rodent cell, a pluripotent cell, a differentiated cell, or a combination thereof). A promoter can be, for example, a constitutively active promoter, a conditional promoter, an inducible promoter, a temporally restricted promoter (e.g., a developmentally regulated promoter), or a spatially restricted promoter (e.g., a cell-specific or tissue-specific promoter). Examples of promoters can be found, for example, in WO 2013/176772, herein incorporated by reference in its entirety for all purposes.

[00100] Examples of inducible promoters include, for example, chemically regulated promoters and physically-regulated promoters. Chemically regulated promoters include, for example, alcohol-regulated promoters (e.g., an alcohol dehydrogenase (alcA) gene promoter), tetracycline-regulated promoters (e.g., a tetracycline-responsive promoter, a tetracycline operator sequence (tetO), a tet-On promoter, or a tet-Off promoter), steroid regulated promoters (e.g., a rat glucocorticoid receptor, a promoter of an estrogen receptor, or a promoter of an ecdysone receptor), or metal-regulated promoters (e.g., a metalloprotein promoter). Physically regulated promoters include, for example temperature-regulated promoters (e.g., a heat shock promoter) and light-regulated promoters (e.g., a light-inducible promoter or a light-repressible promoter).

[00101] Tissue-specific promoters can be, for example, neuron-specific promoters, glia-specific promoters, muscle cell-specific promoters, heart cell-specific promoters, kidney cell-specific promoters, bone cell-specific promoters, endothelial cell-specific promoters, or immune cell-specific promoters (e.g., a B cell promoter or a T cell promoter).

[00102] Developmentally regulated promoters include, for example, promoters active only during an embryonic stage of development, or only in an adult cell.

[00103] "Operable linkage" or being "operably linked" includes juxtaposition of two or more components (e.g., a promoter and another sequence element) such that both components function

normally and allow the possibility that at least one of the components can mediate a function that is exerted upon at least one of the other components. For example, a promoter can be operably linked to a coding sequence if the promoter controls the level of transcription of the coding sequence in response to the presence or absence of one or more transcriptional regulatory factors. Operable linkage can include such sequences being contiguous with each other or acting in trans (e.g., a regulatory sequence can act at a distance to control transcription of the coding sequence).

[00104] The term "primer" refers to an oligonucleotide capable of acting as a point of initiation of polynucleotide synthesis along a complementary strand when placed under conditions in which synthesis of a primer extension product complementary to a polynucleotide is catalyzed. Such conditions include the presence of four different nucleotide triphosphates or nucleoside analogs and one or more agents for polymerization, such as DNA polymerase and/or reverse transcriptase, in an appropriate buffer (including substituents which are cofactors, or which affect pH, ionic strength, and so forth), and at a suitable temperature. Extension of the primer in a sequence specific manner can include, for example, methods of PCR, DNA sequencing, DNA extension, DNA polymerization, RNA transcription, or reverse transcription. A primer must be sufficiently long to prime the synthesis of extension products in the presence of an agent for polymerase. A typical primer is at least about 5 nucleotides in length of a sequence substantially complementary to the target sequence, but longer primers are preferred. Typically, primers are about 15-30 nucleotides in length, but longer primers may also be employed. A primer sequence need not be exactly complementary to a template or target sequence but must be sufficiently complementary to hybridize with a template or target sequence. The term "primer pair" means a set of primers including a 5' upstream primer, which hybridizes to the 5' end of the DNA sequence to be amplified and a 3' downstream primer, which hybridizes to the complement of the 3' end of the sequence to be amplified. Primer pairs can be used for amplification of a target polynucleotide (e.g., by polymerase chain reaction (PCR) or other conventional nucleic-acid amplification methods). "PCR" or "polymerase chain reaction" is a technique used for the amplification of specific DNA segments (*see* US Pat. Nos. 4,683,195 and 4,800,159, each of which is herein incorporated by reference in its entirety for all purposes).

[00105] The term "probe" refers to a molecule which can detectably distinguish between target molecules differing in structure. Detection can be accomplished in a variety of different

ways depending on the type of probe used and the type of target molecule. Thus, for example, detection may be based on discrimination of activity levels of the target molecule, but preferably is based on detection of specific binding. Examples of such specific binding include antibody binding and nucleic acid probe hybridization. Thus, probes can include, for example, enzyme substrates, antibodies and antibody fragments, and nucleic acid hybridization probes. For example, a probe can be an isolated polynucleotide attached to a conventional detectable label or reporter molecule, such as a radioactive isotope, ligand, chemiluminescent agent, enzyme, or the like. Such a probe is complementary to a strand of a target polynucleotide, such as a polynucleotide comprising the *HSD17B13* rs72613567 variant or specific HSD17B13 mRNA transcripts. Deoxyribonucleic acid probes may include those generated by PCR using *HSD17B13*-mRNA/cDNA-specific primers or *HSD17B13*-rs72613567-specific primers, oligonucleotide probes synthesized *in vitro*, or DNA obtained from bacterial artificial chromosome, fosmid, or cosmid libraries. Probes include not only deoxyribonucleic or ribonucleic acids but also polyamides and other probe materials that can specifically detect the presence of a target DNA sequence. For nucleic acid probes, detection reagents can include, for example, radiolabeled probes, enzymatic labeled probes (e.g., horse radish peroxidase and alkaline phosphatase), affinity labeled probes (e.g., biotin, avidin, and streptavidin), and fluorescent labeled probes (e.g., 6-FAM, VIC, TAMRA, MGB, fluorescein, rhodamine, and texas red). The nucleic acid probes described herein can readily be incorporated into one of the established kit formats which are well known.

[00106] The term "antisense RNA" refers to a single-stranded RNA that is complementary to a messenger RNA strand transcribed in a cell.

[00107] The term "small interfering RNA (siRNA)" refers to a typically double-stranded RNA molecule that induces the RNA interference (RNAi) pathway. These molecules can vary in length (generally between 18-30 base pairs) and contain varying degrees of complementarity to their target mRNA in the antisense strand. Some, but not all, siRNAs have unpaired overhanging bases on the 5' or 3' end of the sense strand and/or the antisense strand. The term "siRNA" includes duplexes of two separate strands, as well as single strands that can form hairpin structures comprising a duplex region. The double-stranded structure can be, for example, less than 20, 25, 30, 35, 40, 45, or 50 nucleotides in length. For example, the double-

stranded structure can be from about 21-23 nucleotides in length, from about 19-25 nucleotides in length, or from about 19-23 nucleotides in length.

[00108] The term "short hairpin RNA (shRNA)" refers to a single strand of RNA bases that self-hybridizes in a hairpin structure and can induce the RNA interference (RNAi) pathway upon processing. These molecules can vary in length (generally about 50-90 nucleotides in length, or in some cases up to greater than 250 nucleotides in length, e.g., for microRNA-adapted shRNA). shRNA molecules are processed within the cell to form siRNAs, which in turn can knock down gene expression. shRNAs can be incorporated into vectors. The term "shRNA" also refers to a DNA molecule from which a short, hairpin RNA molecule may be transcribed.

[00109] "Complementarity" of nucleic acids means that a nucleotide sequence in one strand of nucleic acid, due to orientation of its nucleobase groups, forms hydrogen bonds with another sequence on an opposing nucleic acid strand. The complementary bases in DNA are typically A with T and C with G. In RNA, they are typically C with G and U with A. Complementarity can be perfect or substantial/sufficient. Perfect complementarity between two nucleic acids means that the two nucleic acids can form a duplex in which every base in the duplex is bonded to a complementary base by Watson-Crick pairing. "Substantial" or "sufficient" complementary means that a sequence in one strand is not completely and/or perfectly complementary to a sequence in an opposing strand, but that sufficient bonding occurs between bases on the two strands to form a stable hybrid complex in set of hybridization conditions (e.g., salt concentration and temperature). Such conditions can be predicted by using the sequences and standard mathematical calculations to predict the Tm (melting temperature) of hybridized strands, or by empirical determination of Tm by using routine methods. Tm includes the temperature at which a population of hybridization complexes formed between two nucleic acid strands are 50% denatured (i.e., a population of double-stranded nucleic acid molecules becomes half dissociated into single strands). At a temperature below the Tm, formation of a hybridization complex is favored, whereas at a temperature above the Tm, melting or separation of the strands in the hybridization complex is favored. Tm may be estimated for a nucleic acid having a known G+C content in an aqueous 1 M NaCl solution by using, e.g., Tm=81.5+0.41(% G+C), although other known Tm computations take into account nucleic acid structural characteristics.

[00110] "Hybridization condition" includes the cumulative environment in which one nucleic acid strand bonds to a second nucleic acid strand by complementary strand interactions and

hydrogen bonding to produce a hybridization complex. Such conditions include the chemical components and their concentrations (e.g., salts, chelating agents, formamide) of an aqueous or organic solution containing the nucleic acids, and the temperature of the mixture. Other factors, such as the length of incubation time or reaction chamber dimensions may contribute to the environment. *See, e.g.*, Sambrook et al., Molecular Cloning, A Laboratory Manual, 2.sup.nd ed., pp. 1.90-1.91, 9.47-9.51, 1 1.47-11.57 (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989), herein incorporated by reference in its entirety for all purposes.

[00111] Hybridization requires that the two nucleic acids contain complementary sequences, although mismatches between bases are possible. The conditions appropriate for hybridization between two nucleic acids depend on the length of the nucleic acids and the degree of complementation, variables which are well known. The greater the degree of complementation between two nucleotide sequences, the greater the value of the melting temperature (Tm) for hybrids of nucleic acids having those sequences. For hybridizations between nucleic acids with short stretches of complementarity (e.g. complementarity over 35 or fewer, 30 or fewer, 25 or fewer, 22 or fewer, 20 or fewer, or 18 or fewer nucleotides) the position of mismatches becomes important (*see* Sambrook et al., supra, 11.7-11.8). Typically, the length for a hybridizable nucleic acid is at least about 10 nucleotides. Illustrative minimum lengths for a hybridizable nucleic acid include at least about 15 nucleotides, at least about 20 nucleotides, at least about 22 nucleotides, at least about 25 nucleotides, and at least about 30 nucleotides. Furthermore, the temperature and wash solution salt concentration may be adjusted as necessary according to factors such as length of the region of complementation and the degree of complementation.

[00112] The sequence of polynucleotide need not be 100% complementary to that of its target nucleic acid to be specifically hybridizable. Moreover, a polynucleotide may hybridize over one or more segments such that intervening or adjacent segments are not involved in the hybridization event (e.g., a loop structure or hairpin structure). A polynucleotide (e.g., gRNA) can comprise at least 70%, at least 80%, at least 90%, at least 95%, at least 99%, or 100% sequence complementarity to a target region within the target nucleic acid sequence to which they are targeted. For example, a gRNA in which 18 of 20 nucleotides are complementary to a target region, and would therefore specifically hybridize, would represent 90% complementarity. In this example, the remaining noncomplementary nucleotides may be clustered or interspersed

with complementary nucleotides and need not be contiguous to each other or to complementary nucleotides.

[00113] Percent complementarity between particular stretches of nucleic acid sequences within nucleic acids can be determined routinely using BLAST programs (basic local alignment search tools) and PowerBLAST programs (Altschul et al. (1990) *J. Mol. Biol.* 215:403-410; Zhang and Madden (1997) *Genome Res.* 7:649-656) or by using the Gap program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, Madison Wis.), using default settings, which uses the algorithm of Smith and Waterman (Adv. Appl. Math., 1981, 2, 482-489).

[00114] The methods and compositions provided herein employ a variety of different components. Some components throughout the description can have active variants and fragments. Such components include, for example, Cas9 proteins, CRISPR RNAs, tracrRNAs, and guide RNAs. Biological activity for each of these components is described elsewhere herein.

[00115] "Sequence identity" or "identity" in the context of two polynucleotides or polypeptide sequences makes reference to the residues in the two sequences that are the same when aligned for maximum correspondence over a specified comparison window. When percentage of sequence identity is used in reference to proteins, residue positions which are not identical often differ by conservative amino acid substitutions, where amino acid residues are substituted for other amino acid residues with similar chemical properties (e.g., charge or hydrophobicity) and therefore do not change the functional properties of the molecule. When sequences differ in conservative substitutions, the percent sequence identity may be adjusted upwards to correct for the conservative nature of the substitution. Sequences that differ by such conservative substitutions are said to have "sequence similarity" or "similarity." Means for making this adjustment are well known. Typically, this involves scoring a conservative substitution as a partial rather than a full mismatch, thereby increasing the percentage sequence identity. Thus, for example, where an identical amino acid is given a score of 1 and a non-conservative substitution is given a score of zero, a conservative substitution is given a score between zero and 1. The scoring of conservative substitutions is calculated, e.g., as implemented in the program PC/GENE (Intelligenetics, Mountain View, California).

[00116] "Percentage of sequence identity" includes the value determined by comparing two optimally aligned sequences (greatest number of perfectly matched residues) over a comparison window, wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (i.e., gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison, and multiplying the result by 100 to yield the percentage of sequence identity. Unless otherwise specified (e.g., the shorter sequence includes a linked heterologous sequence), the comparison window is the full length of the shorter of the two sequences being compared.

[00117] Unless otherwise stated, sequence identity/similarity values include the value obtained using GAP Version 10 using the following parameters: % identity and % similarity for a nucleotide sequence using GAP Weight of 50 and Length Weight of 3, and the nwsgapdna.cmp scoring matrix; % identity and % similarity for an amino acid sequence using GAP Weight of 8 and Length Weight of 2, and the BLOSUM62 scoring matrix; or any equivalent program thereof. "Equivalent program" includes any sequence comparison program that, for any two sequences in question, generates an alignment having identical nucleotide or amino acid residue matches and an identical percent sequence identity when compared to the corresponding alignment generated by GAP Version 10.

[00118] The term "conservative amino acid substitution" refers to the substitution of an amino acid that is normally present in the sequence with a different amino acid of similar size, charge, or polarity. Examples of conservative substitutions include the substitution of a non-polar (hydrophobic) residue such as isoleucine, valine, or leucine for another non-polar residue. Likewise, examples of conservative substitutions include the substitution of one polar (hydrophilic) residue for another such as between arginine and lysine, between glutamine and asparagine, or between glycine and serine. Additionally, the substitution of a basic residue such as lysine, arginine, or histidine for another, or the substitution of one acidic residue such as aspartic acid or glutamic acid for another acidic residue are additional examples of conservative substitutions. Examples of non-conservative substitutions include the substitution of a non-polar (hydrophobic) amino acid residue such as isoleucine, valine, leucine, alanine, or methionine for a

polar (hydrophilic) residue such as cysteine, glutamine, glutamic acid or lysine and/or a polar residue for a non-polar residue. Typical amino acid categorizations are summarized below.

Alanine	Ala	A	Nonpolar	Neutral	1.8
Arginine	Arg	R	Polar	Positive	-4.5
Asparagine	Asn	N	Polar	Neutral	-3.5
Aspartic acid	Asp	D	Polar	Negative	-3.5
Cysteine	Cys	C	Nonpolar	Neutral	2.5
Glutamic acid	Glu	E	Polar	Negative	-3.5
Glutamine	Gln	Q	Polar	Neutral	-3.5
Glycine	Gly	G	Nonpolar	Neutral	-0.4
Histidine	His	H	Polar	Positive	-3.2
Isoleucine	Ile	I	Nonpolar	Neutral	4.5
Leucine	Leu	L	Nonpolar	Neutral	3.8
Lysine	Lys	K	Polar	Positive	-3.9
Methionine	Met	M	Nonpolar	Neutral	1.9
Phenylalanine	Phe	F	Nonpolar	Neutral	2.8
Proline	Pro	P	Nonpolar	Neutral	-1.6
Serine	Ser	S	Polar	Neutral	-0.8
Threonine	Thr	T	Polar	Neutral	-0.7
Tryptophan	Trp	W	Nonpolar	Neutral	-0.9
Tyrosine	Tyr	Y	Polar	Neutral	-1.3
Valine	Val	V	Nonpolar	Neutral	4.2

[00119] A subject nucleic acid such as a primer or a guide RNA hybridizes to or targets a position or includes a position proximate to a specified nucleotide position in a reference nucleic acid when it is within about 1000, 500, 400, 300, 200, 100, 50, 45, 40, 35, 30, 25, 20, 15, 10, or 5 nucleotides of the position.

[00120] The term "biological sample" refers to a sample of biological material, within or obtainable from a subject, from which a nucleic acid or protein is recoverable. The term biological sample can also encompass any material derived by processing the sample, such as cells or their progeny. Processing of the biological sample may involve one or more of filtration, distillation, extraction, concentration, fixation, inactivation of interfering components, and the like. In some embodiments, a biological sample comprises a nucleic acid, such as genomic DNA, cDNA, or mRNA. In some embodiments, a biological sample comprises a protein. A subject can be any organism, including, for example, a human, a non-human mammal, a rodent, a mouse, or a rat. The biological sample can be derived from any cell, tissue, or biological fluid

from the subject. The sample may comprise any clinically relevant tissue, such as a bone marrow sample, a tumor biopsy, a fine needle aspirate, or a sample of bodily fluid, such as blood, plasma, serum, lymph, ascitic fluid, cystic fluid, or urine. In some cases, the sample comprises a buccal swab. The sample used in the methods disclosed herein will vary based on the assay format, nature of the detection method, and the tissues, cells, or extracts that are used as the sample.

[00121] The term "control sample" refers to a sample obtained from a subject who does not have the *HSD17B13* rs72613567 variant, and preferably is homozygous for the wild type allele of the *HSD17B13* gene. Such samples can be obtained at the same time as a biological sample or on a different occasion. A biological sample and a control sample can both be obtained from the same tissue or bodily fluid.

[00122] A "homologous" sequence (e.g., nucleic acid sequence) includes a sequence that is either identical or substantially similar to a known reference sequence, such that it is, for example, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the known reference sequence. Homologous sequences can include, for example, orthologous sequence and paralogous sequences. Homologous genes, for example, typically descend from a common ancestral DNA sequence, either through a speciation event (orthologous genes) or a genetic duplication event (paralogous genes). "Orthologous" genes include genes in different species that evolved from a common ancestral gene by speciation. Orthologs typically retain the same function in the course of evolution. "Paralogous" genes include genes related by duplication within a genome. Paralogs can evolve new functions in the course of evolution.

[00123] The term "*in vitro*" includes artificial environments and to processes or reactions that occur within an artificial environment (e.g., a test tube). The term "*in vivo*" includes natural environments (e.g., a cell or organism or body, such as a cell within an organism or body) and to processes or reactions that occur within a natural environment. The term "*ex vivo*" includes cells that have been removed from the body of an individual and to processes or reactions that occur within such cells.

[00124] Compositions or methods "comprising" or "including" one or more recited elements may include other elements not specifically recited. For example, a composition that

"comprises" or "includes" a protein may contain the protein alone or in combination with other ingredients. The transitional phrase "consisting essentially of" means that the scope of a claim is to be interpreted to encompass the specified elements recited in the claim and those that do not materially affect the basic and novel characteristic(s) of the claimed invention. Thus, the term "consisting essentially of" when used in a claim of this invention is not intended to be interpreted to be equivalent to "comprising."

[00125] "Optional" or "optionally" means that the subsequently described event or circumstance may or may not occur and that the description includes instances in which the event or circumstance occurs and instances in which it does not.

[00126] Designation of a range of values includes all integers within or defining the range, and all subranges defined by integers within the range.

[00127] Unless otherwise apparent from the context, the term "about" encompasses values within a standard margin of error of measurement (e.g., SEM) of a stated value.

[00128] The term "and/or" refers to and encompasses any and all possible combinations of one or more of the associated listed items, as well as the lack of combinations when interpreted in the alternative ("or").

[00129] The term "or" refers to any one member of a particular list and also includes any combination of members of that list.

[00130] The singular forms of the articles "a," "an," and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a Cas9 protein" or "at least one Cas9 protein" can include a plurality of Cas9 proteins, including mixtures thereof.

[00131] Statistically significant means $p \leq 0.05$.

DETAILED DESCRIPTION

I. Overview

[00132] Provided herein is an *HSD17B13* variant discovered to be associated with reduced alanine and aspartate transaminase levels; a reduced risk of chronic liver diseases including nonalcoholic and alcoholic liver fatty liver disease, cirrhosis, and hepatocellular carcinoma; and reduced progression from simple steatosis to more clinically advanced stages of chronic liver disease. Also provided herein are previously unidentified transcripts of the *HSD17B13* gene associated with the variant.

[00133] Isolated nucleic acids and proteins related to variants of *HSD17B13*, and cells comprising those nucleic acids and proteins are provided herein. Also provided are methods for modifying a cell through use of any combination of nuclease agents, exogenous donor sequences, transcriptional activators, transcriptional repressors, and expression vectors for expressing a recombinant *HSD17B13* gene or a nucleic acid encoding an HSD17B13 protein. Also provided are therapeutic and prophylactic methods for treating a subject having or at risk of developing chronic liver disease.

II. HSD17B13 Variants

[00134] Provided herein are isolated nucleic acids and proteins related to variants of *HSD17B13* (also known as hydroxysteroid 17-beta dehydrogenase 13, 17-beta-hydroxysteroid dehydrogenase 13, 17β-hydroxysteroid dehydrogenase-13, 17β-HSD13, short-chain dehydrogenase/reductase 9, *SCDR9, HMFN0376, NIIL497*, and *SDR16C3*). The human *HSD17B13* gene is approximately 19 kb in length and includes seven exons and six introns located at 4q22.1 in the genome. Exemplary human HSD17B13 protein sequences are assigned UniProt Accession No. Q7Z5P4 (SEQ ID NOS: 240 and 241; Q7Z5P4-1 and Q7Z5P4-2, respectively) and NCBI Reference Sequence Nos. NP_835236 and NP_001129702 (SEQ ID NOS: 242 and 243, respectively). Exemplary human HSD17B13 mRNAs are assigned NCBI Reference Sequence Nos. NM_178135 and NM_001136230 (SEQ ID NOS: 244 and 245, respectively).

[00135] In particular, provided herein is a splice variant of *HSD17B13* (rs72613567) having an insertion of an adenine adjacent to the donor splice site in intron 6. The adenine is an insertion on the forward (plus) strand of the chromosome, which corresponds to an inserted thymine on the reverse (minus) strand of the chromosome. Because the human *HSD17B13* gene is transcribed in the reverse direction, this nucleotide insertion is reflected as an inserted thymine in the exemplary *HSD17B13* rs72613567 variant sequence provided in SEQ ID NO: 2 relative to the exemplary wild type *HSD17B13* gene sequence provided in SEQ ID NO: 1. The insertion will therefore be referred to herein as a thymine inserted between positions 12665 and 12666 in SEQ ID NO: 1 or at position 12666 in SEQ ID NO: 2.

[00136] Two mRNA transcripts (A and B; SEQ ID NOS: 4 and 5, respectively) were previously identified to be expressed in subjects with the wild type *HSD17B13* gene. Transcript

A includes all seven exons of the *HSD17B13* gene, whereas exon 2 is skipped in Transcript B. Transcript A is the dominant transcript in wild type subjects. Provided herein, however, are six additional, previously unidentified, *HSD17B13* transcripts that are expressed (C-H, SEQ ID NOS: 6 to 11, respectively). These transcripts are shown in Figure 4. In Transcript C, exon 6 is skipped compared to Transcript A. In Transcript D, there is an insertion of a guanine 3' of exon 6, resulting in a frameshift in and premature truncation of exon 7 compared to Transcript A. In Transcript E, there is an additional exon between exons 3 and 4 compared to Transcript A. In Transcript F, which is expressed only in *HSD17B13* rs72613567 variant carriers, there is read-through from exon 6 into intron 6 compared to Transcript A. In Transcript G, exon 2 is skipped, and there is an insertion of a guanine 3' of exon 6, resulting in a frameshift in and premature truncation of exon 7 compared to Transcript A. In Transcript H, there is an additional exon between exons 3 and 4, and there is an insertion of a guanine 3' of exon 6, resulting in a frameshift in and premature truncation of exon 7 compared to Transcript A. Transcripts C, D, F, G, and H are dominant in *HSD17B13* rs72613567 variant carriers, with Transcript D being the most abundant transcript in carriers of the *HSD17B13* rs72613567 variant. Also provided herein is one additional, previously unidentified, *HSD17B13* transcript that is expressed at low levels (F', SEQ ID NO: 246). Like Transcript F, Transcript F' also includes a read-through from exon 6 into intron 6 compared to Transcript A, but, in contrast to Transcript F, the read-through does not include the inserted thymine present in the *HSD17B13* rs72613567 variant gene. The nucleotide positions of the exons within the *HSD17B13* genes for each Transcript are provided below.

[00137] Nucleotide Positions in SEQ ID NO: 1 for Exons of HSD17B13 Transcripts More Prevalent in Subjects Homozygous for Wild Type *HSD17B13* Gene.

	Transcript A	**Transcript B**	**Transcript E**	**Transcript F'**
Exon 1	1-275	1-275	1-275	1-275
Exon 2	4471-4578	skipped	4471-4578	4471-4578
Exon 3	5684-5815	5684-5815	5684-5815	5684-5815
Exon 3'	not present	not present	6210-6281	not present
Exon 4	7308-7414	7308-7414	7308-7414	7308-7414
Exon 5	8947-9084	8947-9084	8947-9084	8947-9084
Exon 6	12548-12664	12548-12664	12548-12664	12548-13501*
Exon 7	17599-19118	17599-19118	17599-19118	skipped

*Includes read-through from exon 6 into intron 6; read-through = positions 12665-13501

[00138] Nucleotide Positions in SEQ ID NO: 2 for Exons of HSD17B13 Transcripts More Prevalent in Subjects Homozygous for rs72613567 *HSD17B13* Variant Gene (Insertion of T at Position 12666).

	Transcript C	Transcript D	Transcript F	Transcript G	Transcript H
Exon 1	1-275	1-275	1-275	1-275	1-275
Exon 2	4471-4578	4471-4578	4471-4578	skipped	4471-4578
Exon 3	5684-5815	5684-5815	5684-5815	5684-5815	5684-5815
Exon 3'	not present	not present	not present	not present	6210-6281
Exon 4	7308-7414	7308-7414	7308-7414	7308-7414	7308-7414
Exon 5	8947-9084	8947-9084	8947-9084	8947-9084	8947-9084
Exon 6	skipped	12548-12665^	12548-13502*	12548-12665^	12548-12665^
Exon 7	17600-19119	17600-19119	skipped	17600-19119	17600-19119

^Includes additional residue 12665 at 3' end compared to Transcript A
*Includes read-through from exon 6 into intron 6; read-through = positions 12665-13502

[00139] As explained in more detail elsewhere herein, the *HSD17B13* rs72613567 variant is associated with reduced alanine and aspartate transaminase levels and a reduced risk of chronic liver diseases including nonalcoholic and alcoholic liver fatty liver disease, cirrhosis, and hepatocellular carcinoma. The *HSD17B13* rs72613567 variant is also associated with reduced progression from simple steatosis to more clinically advanced stages of chronic liver disease.

A. Nucleic Acids

[00140] Disclosed herein are isolated nucleic related to *HSD17B13* variants and variant HSD17B13 transcripts. Also disclosed are isolated nucleic acids that hybridize under stringent or moderate conditions with any of the nucleic acids disclosed herein. Such nucleic acids can be useful, for example, to express *HSD17B13* variant proteins or as primers, probes, exogenous donor sequences, guide RNAs, antisense RNAs, shRNAs, and siRNAs, each of which is described in more detail elsewhere herein.

[00141] Also disclosed are functional nucleic acids that can interact with the disclosed polynucleotides. Functional nucleic acids are nucleic acid molecules that have a specific function, such as binding a target molecule or catalyzing a specific reaction. Examples of functional nucleic acids include antisense molecules, aptamers, ribozymes, triplex forming molecules, and external guide sequences. The functional nucleic acid molecules can act as effectors, inhibitors, modulators, and stimulators of a specific activity possessed by a target molecule, or the functional nucleic acid molecules can possess a *de novo* activity independent of any other molecules.

[00142] Antisense molecules are designed to interact with a target nucleic acid molecule through either canonical or non-canonical base pairing. The interaction of the antisense molecule and the target molecule is designed to promote the destruction of the target molecule through, for example, RNase-H-mediated RNA-DNA hybrid degradation. Alternatively, the antisense molecule is designed to interrupt a processing function that normally would take place on the target molecule, such as transcription or replication. Antisense molecules can be designed based on the sequence of the target molecule. Numerous methods for optimization of antisense efficiency by finding the most accessible regions of the target molecule exist. Exemplary methods would be *in vitro* selection experiments and DNA modification studies using DMS and DEPC. Antisense molecules generally bind the target molecule with a dissociation constant (k_d) less than or equal to 10^{-6}, 10^{-8}, 10^{-10}, or 10^{-12}. A representative sample of methods and techniques which aid in the design and use of antisense molecules can be found in the following non-limiting list of US patents: 5,135,917; 5,294,533; 5,627,158; 5,641,754; 5,691,317; 5,780,607; 5,786,138; 5,849,903; 5,856,103; 5,919,772; 5,955,590; 5,990,088; 5,994,320; 5,998,602; 6,005,095; 6,007,995; 6,013,522; 6,017,898; 6,018,042; 6,025,198; 6,033,910; 6,040,296; 6,046,004; 6,046,319; and 6,057,437, each of which is herein incorporated by reference in its entirety for all purposes. Examples of antisense molecules include antisense RNAs, small interfering RNAs (siRNAs), and short hairpin RNAs (shRNAs), which are described in greater detail elsewhere herein.

[00143] The isolated nucleic acids disclosed herein can comprise RNA, DNA, or both RNA and DNA. The isolated nucleic acids can also be linked or fused to a heterologous nucleic acid sequence, such as in a vector, or a heterologous label. For example, the isolated nucleic acids disclosed herein can be in a vector or exogenous donor sequences comprising the isolated nucleic acid and a heterologous nucleic acid sequence. The isolated nucleic acids can also be linked or fused to a heterologous label, such as a fluorescent label. Other examples of labels are disclosed elsewhere herein.

[00144] The disclosed nucleic acids molecules can be made up of, for example, nucleotides or non-natural or modified nucleotides, such as nucleotide analogs or nucleotide substitutes. Such nucleotides include a nucleotide that contains a modified base, sugar or phosphate group, or that incorporates a non-natural moiety in its structure. Examples of non-natural nucleotides include dideoxynucleotides, biotinylated, aminated, deaminated, alkylated, benzylated and fluorophor-

labeled nucleotides.

[00145] The nucleic acids molecules disclosed herein can comprise one or more nucleotide analogs or substitutions. A nucleotide analog is a nucleotide which contains some type of modification to either the base, sugar, or phosphate moieties. Modifications to the base moiety would include natural and synthetic modifications of A, C, G, and T/U as well as different purine or pyrimidine bases, such as pseudouridine, uracil-5-yl, hypoxanthin-9-yl (I), and 2-aminoadenin-9-yl. Modified bases include, for example, 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl uracil and cytosine, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-deazaadenine and 3-deazaguanine and 3-deazaadenine. Additional base modifications can be found, for example, in US Pat. No. 3,687,808; Englisch et al. (1991) *Angewandte Chemie, International Edition* 30:613; and Sanghvi, Y. S., Chapter 15, Antisense Research and Applications, pages 289-302, Crooke, S. T. and Lebleu, B. ed., CRC Press, 1993, each of which is herein incorporated by reference in its entirety for all purposes. Certain nucleotide analogs, such as 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and O-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil, 5-propynylcytosine, and 5-methylcytosine can increase the stability of duplex formation. Often base modifications can be combined with, for example, a sugar modification, such as 2'-O-methoxyethyl, to achieve unique properties such as increased duplex stability. There are numerous US patents, such as 4,845,205; 5,130,302; 5,134,066; 5,175,273; 5,367,066; 5,432,272; 5,457,187; 5,459,255; 5,484,908; 5,502,177; 5,525,711; 5,552,540; 5,587,469; 5,594,121, 5,596,091; 5,614,617; and 5,681,941, which detail and describe a range of base modifications. Each of these is herein incorporated by reference in its entirety for all purposes.

[00146] Nucleotide analogs can also include modifications of the sugar moiety. Modifications to the sugar moiety can include, for example, natural modifications of the ribose and deoxy ribose as well as synthetic modifications. Sugar modifications include, for example, the

following modifications at the 2' position: OH; F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S- or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl, and alkynyl may be substituted or unsubstituted C1 to C10, alkyl or C2 to C10 alkenyl and alkynyl. Exemplary 2' sugar modifications also include, for example, -O[(CH2)$_n$ O]m CH3, -O(CH2)$_n$OCH3, -O(CH2)$_n$ NH2, -O(CH2)$_n$ CH3, -O(CH2)$_n$ -ONH2, and -O(CH2)$_n$ON[(CH2)$_n$CH3)]2, where n and m are from 1 to about 10.

[00147] Other modifications at the 2' position include, for example, C_1 to C_{10} lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH3, OCN, Cl, Br, CN, CF3, OCF_3, $SOCH_3$, SO_2 CH_3, ONO_2, NO_2, N_3, NH_2, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an oligonucleotide, or a group for improving the pharmacodynamic properties of an oligonucleotide, and other substituents having similar properties. Similar modifications may also be made at other positions on the sugar, particularly the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked oligonucleotides and the 5' position of 5' terminal nucleotide. Modified sugars can also include those that contain modifications at the bridging ring oxygen, such as CH_2 and S. Nucleotide sugar analogs may also have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar. There are numerous US patents that teach the preparation of such modified sugar structures such as 4,981,957; 5,118,800; 5,319,080; 5,359,044; 5,393,878; 5,446,137; 5,466,786; 5,514,785; 5,519,134; 5,567,811; 5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,053; 5,639,873; 5,646,265; 5,658,873; 5,670,633; and 5,700,920, each of which is herein incorporated by reference in its entirety for all purposes.

[00148] Nucleotide analogs can also be modified at the phosphate moiety. Modified phosphate moieties include, for example, those that can be modified so that the linkage between two nucleotides contains a phosphorothioate, chiral phosphorothioate, phosphorodithioate, phosphotriester, aminoalkylphosphotriester, methyl and other alkyl phosphonates including 3'-alkylene phosphonate and chiral phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates. These phosphate or modified phosphate linkage between two nucleotides can be through a 3'-5' linkage or a 2'-5' linkage, and the linkage can contain inverted polarity such as 3'-5' to 5'-3' or 2'-5' to 5'-2'.

Various salts, mixed salts and free acid forms are also included. Numerous US patents teach how to make and use nucleotides containing modified phosphates and include, for example, 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5,177,196; 5,188,897; 5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,939; 5,453,496; 5,455,233; 5,466,677; 5,476,925; 5,519,126; 5,536,821; 5,541,306; 5,550,111; 5,563,253; 5,571,799; 5,587,361; and 5,625,050, each of which is herein incorporated by reference in its entirety for all purposes.

[00149] Nucleotide substitutes include molecules having similar functional properties to nucleotides, but which do not contain a phosphate moiety, such as peptide nucleic acid (PNA). Nucleotide substitutes include molecules that will recognize nucleic acids in a Watson-Crick or Hoogsteen manner, but which are linked together through a moiety other than a phosphate moiety. Nucleotide substitutes are able to conform to a double helix type structure when interacting with the appropriate target nucleic acid.

[00150] Nucleotide substitutes also include nucleotides or nucleotide analogs that have had the phosphate moiety or sugar moieties replaced. Nucleotide substitutes may not contain a standard phosphorus atom. Substitutes for the phosphate can be, for example, short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatom and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. These include those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH_2 component parts. Numerous US patents disclose how to make and use these types of phosphate replacements and include, but are not limited to, 5,034,506; 5,166,315; 5,185,444; 5,214,134; 5,216,141; 5,235,033; 5,264,562; 5,264,564; 5,405,938; 5,434,257; 5,466,677; 5,470,967; 5,489,677; 5,541,307; 5,561,225; 5,596,086; 5,602,240; 5,610,289; 5,602,240; 5,608,046; 5,610,289; 5,618,704; 5,623,070; 5,663,312; 5,633,360; 5,677,437; and 5,677,439, each of which is herein incorporated by reference in its entirety for all purposes.

[00151] It is also understood in a nucleotide substitute that both the sugar and the phosphate moieties of the nucleotide can be replaced, by, for example, an amide type linkage (aminoethylglycine) (PNA). US patents 5,539,082; 5,714,331; and 5,719,262 teach how to make

and use PNA molecules, each of which is herein incorporated by reference in its entirety for all purposes. *See also* Nielsen et al. (1991) *Science* 254:1497-1500, herein incorporated by reference in its entirety for all purposes.

[00152] It is also possible to link other types of molecules (conjugates) to nucleotides or nucleotide analogs to enhance, for example, cellular uptake. Conjugates can be chemically linked to the nucleotide or nucleotide analogs. Such conjugates include, for example, lipid moieties such as a cholesterol moiety (Letsinger et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:6553-6556, herein incorporated by reference in its entirety for all purposes), cholic acid (Manoharan et al. (1994) *Bioorg. Med. Chem. Let.* 4:1053-1060, herein incorporated by reference in its entirety for all purposes), a thioether such as hexyl-S-tritylthiol (Manoharan et al. (1992) *Ann. N.Y. Acad. Sci.* 660:306-309; Manoharan et al. (1993) *Bioorg. Med. Chem. Let.* 3:2765-2770, herein incorporated by reference in its entirety for all purposes), a thiocholesterol (Oberhauser et al. (1992) *Nucl. Acids Res.* 20:533-538, herein incorporated by reference in its entirety for all purposes), an aliphatic chain such as dodecandiol or undecyl residues (Saison-Behmoaras et al. (1991) *EMBO J.* 10:1111-1118; Kabanov et al. (1990) *FEBS Lett.* 259:327-330; Svinarchuk et al. (1993) *Biochimie* 75:49-54, each of which is herein incorporated by reference in its entirety for all purposes), a phospholipid such as di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan et al. (1995) *Tetrahedron Lett.* 36:3651-3654; Shea et al. (1990) *Nucl. Acids Res.* 18:3777-3783, each of which is herein incorporated by reference in its entirety for all purposes), a polyamine or a polyethylene glycol chain (Manoharan et al. (1995) *Nucleosides & Nucleotides* 14:969-973, herein incorporated by reference in its entirety for all purposes), or adamantane acetic acid (Manoharan et al. (1995) *Tetrahedron Lett.* 36:3651-3654, herein incorporated by reference in its entirety for all purposes), a palmityl moiety (Mishra et al. (1995) *Biochim. Biophys. Acta* 1264:229-237), or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety (Crooke et al. (1996) *J. Pharmacol. Exp. Ther.* 277:923-937, herein incorporated by reference in its entirety for all purposes). Numerous US patents teach the preparation of such conjugates and include, for example, US Pat. Nos. 4,828,979; 4,948,882; 5,218,105; 5,525,465; 5,541,313; 5,545,730; 5,552,538; 5,578,717, 5,580,731; 5,580,731; 5,591,584; 5,109,124; 5,118,802; 5,138,045; 5,414,077; 5,486,603; 5,512,439; 5,578,718; 5,608,046; 4,587,044; 4,605,735; 4,667,025; 4,762,779; 4,789,737; 4,824,941; 4,835,263; 4,876,335; 4,904,582; 4,958,013; 5,082,830;

5,112,963; 5,214,136; 5,082,830; 5,112,963; 5,214,136; 5,245,022; 5,254,469; 5,258,506; 5,262,536; 5,272,250; 5,292,873; 5,317,098; 5,371,241, 5,391,723; 5,416,203, 5,451,463; 5,510,475; 5,512,667; 5,514,785; 5,565,552; 5,567,810; 5,574,142; 5,585,481; 5,587,371; 5,595,726; 5,597,696; 5,599,923; 5,599,928 and 5,688,941, each of which is herein incorporated by reference in its entirety for all purposes.

[00153] The isolated nucleic acids disclosed herein can comprise a nucleotide sequence of a naturally occurring *HSD17B13* gene or mRNA transcript, or can comprise a non-naturally occurring sequence. In one example, the non-naturally occurring sequence can differ from the non-naturally occurring sequence due to synonymous mutations or mutations that do not affect the encoded HSD17B13 protein. For example, the sequence can be identical with the exception of synonymous mutations or mutations that do not affect the encoded HSD17B13 protein. A synonymous mutation or substitution is the substitution of one nucleotide for another in an exon of a gene coding for a protein such that the produced amino acid sequence is not modified. This is possible because of the degeneracy of the genetic code, with some amino acids being coded for by more than one three-base pair codon. Synonymous substitutions are used, for example, in the process of codon optimization.

[00154] Also disclosed herein are proteins encoded by the nucleic acids disclosed herein and compositions comprising an isolated nucleic acid or protein disclosed herein and a carrier increasing the stability of the isolated nucleic acid or protein (e.g., prolonging the period under given conditions of storage (e.g., -20°C, 4°C, or ambient temperature) for which degradation products remain below a threshold, such below 0.5% by weight of the starting nucleic acid or protein; or increasing the stability in vivo). Non-limiting examples of such carriers include poly(lactic acid) (PLA) microspheres, poly(D,L-lactic-coglycolic-acid) (PLGA) microspheres, liposomes, micelles, inverse micelles, lipid cochleates, and lipid microtubules.

(1) Nucleic Acids Including Mutant Residue of HSD17B13 *rs72613567 Variant*

[00155] Disclosed herein are isolated nucleic acids comprising at least 15 contiguous nucleotides of an *HSD17B13* gene and having a thymine at a position corresponding to position 12666 (or thymines at positions corresponding to positions 12666 and 12667) of the *HSD17B13* rs72613567 variant (SEQ ID NO: 2) when optimally aligned with the *HSD17B13* rs72613567

variant. That is, disclosed herein are isolated nucleic acids comprising at least 15 contiguous nucleotides of an *HSD17B13* gene and having a thymine inserted between nucleotides corresponding to positions 12665 and 12666 of the wild type *HSD17B13* gene (SEQ ID NO: 1) when optimally aligned with the wild type *HSD17B13* gene. Such isolated nucleic acids can be useful, for example, to express *HSD17B13* variant transcripts and proteins or as exogenous donor sequences. Such isolated nucleic acids can also be useful, for example, as guide RNAs, primers, and probes.

[00156] The *HSD17B13* gene can be an *HSD17B13* gene from any organism. For example, the *HSD17B13* gene can be a human *HSD17B13* gene or an ortholog from another organism, such as a non-human mammal, a rodent, a mouse, or a rat.

[00157] It is understood that gene sequences within a population can vary due to polymorphisms such as single-nucleotide polymorphisms. The examples provided herein are only exemplary sequences. Other sequences are also possible. As one example, the at least 15 contiguous nucleotides can be at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a corresponding sequence in the *HSD17B13* rs72613567 variant (SEQ ID NO: 2) including position 12666 or positions 12666 and 12667 of SEQ ID NO: 2 when optimally aligned with SEQ ID NO: 2. Optionally, the isolated nucleic acid comprises at least 15 contiguous nucleotides of SEQ ID NO: 2 including position 12666 or positions 12666 and 12667 of SEQ ID NO: 2. As another example, the at least 15 contiguous nucleotides can be at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a corresponding sequence in the wild type *HSD17B13* gene (SEQ ID NO: 1) including positions 12665 and 12666 of SEQ ID NO: 1 when optimally aligned with SEQ ID NO: 1, wherein a thymine is present between the positions corresponding to positions 12665 and 12666 of SEQ ID NO: 1. Optionally, the isolated nucleic acid comprises at least 15 contiguous nucleotides of SEQ ID NO: 1 including positions 12665 and 12666 of SEQ ID NO: 1, wherein a thymine is present between the positions corresponding to positions 12665 and 12666 of SEQ ID NO: 1.

[00158] The isolated nucleic acid can comprise, for example, at least 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1000 contiguous nucleotides of an *HSD17B13* gene. Alternatively, the isolated nucleic acid can comprise, for example, at least 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000, 10000, 11000, 12000, 13000, 14000, 15000, 16000, 17000, 18000, or 19000 contiguous nucleotides of an *HSD17B13* gene.

[00159] In some cases, the isolated nucleic acid can comprise an *HSD17B13* minigene in which one or more nonessential segments of the gene have been deleted with respect to a corresponding wild type *HSD17B13* gene. As one example, the deleted segments comprise one or more intronic sequences. Such *HSD17B13* minigenes can comprise, for example, exons corresponding to exons 1-7 from *HSD17B13* Transcript D and an intron corresponding to intron 6 in SEQ ID NO: 2 when optimally aligned with SEQ ID NO: 2. As one example, an *HSD17B13* minigene may comprise exons 1-7 and intron 6 from SEQ ID NO: 2. Minigenes are described in more detail elsewhere herein.

(2) Nucleic Acids Hybridizing to Sequence Adjacent to or Including Mutant Residue of HSD17B13 *rs72613567 Variant*

[00160] Also disclosed herein are isolated nucleic acids comprising at least 15 contiguous nucleotides that hybridize to an *HSD17B13* gene (e.g., an *HSD17B13* minigene) at a segment that includes or is within 1000, 500, 400, 300, 200, 100, 50, 45, 40, 35, 30, 25, 20, 15, 10, or 5 nucleotides of a position corresponding to position 12666 or positions 12666 and 12667 of the *HSD17B13* rs72613567 variant (SEQ ID NO: 2) when optimally aligned with the *HSD17B13* rs72613567 variant. Such isolated nucleic acids can be useful, for example, as guide RNAs, primers, probes, or exogenous donor sequences.

[00161] The *HSD17B13* gene can be an *HSD17B13* gene from any organism. For example, the *HSD17B13* gene can be a human *HSD17B13* gene or an ortholog from another organism, such as a non-human mammal, a mouse, or a rat.

[00162] As one example, the at least 15 contiguous nucleotides can hybridize to a segment of the *HSD17B13* gene or *HSD17B13* minigene that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a corresponding sequence in the *HSD17B13* rs72613567 variant (SEQ ID NO: 2) when optimally aligned with SEQ ID NO: 2. Optionally, the isolated nucleic acid can hybridize to at least 15 contiguous nucleotides of SEQ ID NO: 2. Optionally, the isolated nucleic acid hybridizes to a segment including position 12666 or positions 12666 and 12667 in SEQ ID NO: 2 or a position corresponding to position 12666 or positions 12666 and 12667 in SEQ ID NO: 2 when optimally aligned with SEQ ID NO: 2.

[00163] The segment to which the isolated nucleic acid can hybridize can comprise, for example, at least 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 75, 90, 95, 100, 200, 300, 400,

500, 600, 700, 800, 900, or 1000 contiguous nucleotides of an *HSD17B13* gene. Alternatively, the isolated nucleic acid can comprise, for example, at least 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000, 10000, 11000, 12000, 13000, 14000, 15000, 16000, 17000, 18000, or 19000 contiguous nucleotides of an *HSD17B13* gene. Alternatively, the segment to which the isolated nucleic acid can hybridize can be, for example, up to 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 75, 90, 95, 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1000 contiguous nucleotides of an *HSD17B13* gene. For example, the segment can be about 15 to 100 nucleotides in length, or about 15-35 nucleotides in length.

(3) cDNAs and Variant Transcripts Produced by HSD17B13 *rs72613567 Variant*

[00164] Also provided are nucleic acids corresponding to all or part of an mRNA transcript or a cDNA corresponding to any one of Transcripts A-H (SEQ ID NOS: 4-11, respectively), and particularly Transcripts C-H, when optimally aligned with the any one of Transcripts A-H. It is understood that gene sequences and within a population and mRNA sequences transcribed from such genes can vary due to polymorphisms such as single-nucleotide polymorphisms. The sequences provided herein for each Transcript are only exemplary sequences. Other sequences are also possible. Specific, non-limiting examples are provided below. Such isolated nucleic acids can be useful, for example, to express *HSD17B13* variant transcripts and proteins.

[00165] The isolated nucleic acid can be of any length. For example, the isolated nucleic acid can comprise at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, or 2000 contiguous nucleotides encoding all or part of an HSD17B13 protein. In some cases, the isolated nucleic acids comprises contiguous nucleotides encoding all or part of an HSD17B13 protein, wherein the contiguous nucleotides comprise sequence from at least two different exons of an *HSD17B13* gene (e.g., spanning at least one exon-exon boundary of an *HSD17B13* gene without an intervening intron).

[00166] *HSD17B13* Transcript D (SEQ ID NO: 7), Transcript G (SEQ ID NO: 10), and Transcript H (SEQ ID NO: 11) include an insertion of a guanine at the 3' end of exon 6, resulting in a frameshift in exon 7 and premature truncation of the region of the HSD17B13 protein encoded by exon 7 compared to Transcript A. Accordingly, provided herein are isolated nucleic acids comprising a segment (e.g., at least 15 contiguous nucleotides) present in Transcripts D, G,

and H (or fragments or homologs thereof) that is not present in Transcript A (or a fragment or homolog thereof). Such regions can be readily identified by comparing the sequences of the Transcripts. For example, provided herein are isolated nucleic acids comprising at least 15 contiguous nucleotides (e.g., at least 20 contiguous nucleotides or at least 30 contiguous nucleotides) encoding all or part of an HSD17B13 protein, wherein a segment of the contiguous nucleotides (e.g., at least 5 contiguous nucleotides, at least 10 contiguous nucleotides or at least 15 contiguous nucleotides) is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a region spanning the exon 6-exon 7 boundary in SEQ ID NO: 7 (*HSD17B13* Transcript D), SEQ ID NO: 10 (*HSD17B13* Transcript G), or SEQ ID NO: 11 (*HSD17B13* Transcript H) when optimally aligned with SEQ ID NO: 7, 10, or 11, respectively, and the segment includes a guanine at a residue corresponding to residue 878 at the 3' end of exon 6 in SEQ ID NO: 7 (i.e., an insertion of a guanine at the 3' end of exon 6 relative to Transcript A in addition to the guanine at the start of exon 7), a residue corresponding to residue 770 at the 3' end of exon 6 in SEQ ID NO: 10 (i.e., an insertion of a guanine at the 3' end of exon 6 relative to Transcript B in addition to the guanine at the start of exon 7), or a residue corresponding to residue 950 at the 3' end of exon 6 in SEQ ID NO: 11 (i.e., an insertion of a guanine at the 3' end of exon 6 relative to Transcript E in addition to the guanine at the start of exon 7). It is understood that such a nucleic acid would include a sufficient number of nucleotides in each of exons 6 and 7 to distinguish the inserted guanine from other features in the *HSD17B13* Transcripts (e.g., from the guanine at the start of exon 7, from the read-through into intron 6 in Transcript F, or from the deleted exon 6 in Transcript C).

[00167] As one example, the isolated nucleic acid can comprise at least 15 contiguous nucleotides (e.g., at least 20 contiguous nucleotides or at least 30 contiguous nucleotides) of SEQ ID NO: 7 spanning the exon 6-exon 7 boundary, optionally comprising exons 6 and 7 of SEQ ID NO: 7, and optionally comprising the entire sequence of SEQ ID NO: 7.

[00168] Optionally, the isolated nucleic acid further comprises a segment present in Transcript D (or a fragment or homolog thereof) that is not present in Transcript G (or a fragment or homolog thereof), and the isolated nucleic acid further comprises a segment present in Transcript D (or a fragment or homolog thereof) that is not present in Transcript H (or a fragment or homolog thereof). Such regions can be readily identified by comparing the sequences of the Transcripts. For example, such isolated nucleic acids can comprise a segment of the contiguous

nucleotides (e.g., at least 5 contiguous nucleotides, at least 10 contiguous nucleotides or at least 15 contiguous nucleotides) that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a region spanning the boundary of exons 3 and 4 of SEQ ID NO: 7 (*HSD17B13* Transcript D) when optimally aligned with SEQ ID NO: 7 to distinguish from Transcript H. Likewise, such isolated nucleic acids can comprise a segment of the contiguous nucleotides (e.g., at least 5 contiguous nucleotides, at least 10 contiguous nucleotides or at least 15 contiguous nucleotides) that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a region within exon 2 of SEQ ID NO: 7 (*HSD17B13* Transcript D), a region spanning the exon 1-exon 2 boundary of SEQ ID NO: 7, or a region spanning the exon 2-exon 3 boundary of SEQ ID NO: 7 when optimally aligned with SEQ ID NO: 7 to distinguish from Transcript G. Optionally, the isolated nucleic acid comprises a sequence at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the sequence set forth in SEQ ID NO: 7 (*HSD17B13* Transcript D) and encodes an HSD17B13 protein comprising the sequence set forth in SEQ ID NO: 15 (HSD17B13 Isoform D).Like Transcript D, Transcript H (SEQ ID NO: 11) includes an insertion of a guanine 3' of exon 6 compared to Transcript A. Transcript H further includes an additional exon (exon 3') between exons 3 and 4 compared to Transcript A and Transcript D. Accordingly, provided herein are isolated nucleic acids as described for above comprising a segment present in Transcripts D, G, and H (or fragments or homologs thereof) that is not present in Transcript A (or a fragment or homolog thereof) but further comprising a segment (e.g., at least 15 contiguous nucleotides) of Transcript H (or a fragment or homolog thereof) that is not present in Transcript D (or a fragment or homolog thereof). Such regions can be readily identified by comparing the sequences of the Transcripts. For example, provided herein are isolated nucleic acids as described for Transcript D, wherein a segment of the contiguous nucleotides (e.g., at least 5 contiguous nucleotides, at least 10 contiguous nucleotides or at least 15 contiguous nucleotides) is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a region within exon 3' of SEQ ID NO: 11 (*HSD17B13* Transcript H), a region spanning the exon 3-exon 3' boundary of SEQ ID NO: 11, or a region spanning the exon 3'-exon 4 boundary of SEQ ID NO: 11 when optimally aligned with SEQ ID NO: 11. It is understood that such a nucleic acid would include a sufficient number of nucleotides in each of exons 3 and 3' or each of exons 3' and 4 to distinguish from other features in the *HSD17B13* transcripts (e.g., from the

boundary of exons 3 and 4). For example, the region of exon 3' can comprise the entire exon 3'. Optionally, the isolated nucleic acid comprises a sequence at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the sequence set forth in SEQ ID NO: 11 (*HSD17B13* Transcript H) and encodes an HSD17B13 protein comprising the sequence set forth in SEQ ID NO: 19 (HSD17B13 Isoform H).

[00169] As one example, the isolated nucleic acid can comprise at least 15 contiguous nucleotides (e.g., at least 20 contiguous nucleotides or at least 30 contiguous nucleotides) of SEQ ID NO: 11 including a region within exon 3', a region spanning the exon 3-exon 3' boundary, or a region spanning the exon 3'-exon 4 boundary, optionally comprising the entire exon 3' of SEQ ID NO: 11, and optionally comprising the entire sequence of SEQ ID NO: 11.

[00170] Like Transcript D, Transcript G (SEQ ID NO: 10) includes an insertion of a guanine 3' of exon 6 compared to Transcript A. In addition, however, Transcript G is missing exon 2 compared to Transcript A and Transcript D (i.e., Transcript G includes an exon 1-exon 3 boundary not present in Transcripts A and D). Accordingly, provided herein are isolated nucleic acids as described above comprising a segment present in Transcripts D, G, and H (or fragments or homologs thereof) that is not present in Transcript A (or a fragment or homolog thereof) but further comprising a segment (e.g., at least 15 contiguous nucleotides) from Transcript G (or a fragment or homolog thereof) that is not present in Transcript D (or a fragment or homolog thereof). Such regions can be readily identified by comparing the sequences of the Transcripts. For example, provided herein are isolated nucleic acids as described for Transcript D, wherein a segment of the contiguous nucleotides (e.g., at least 5 contiguous nucleotides, at least 10 contiguous nucleotides or at least 15 contiguous nucleotides) is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a region spanning the exon 1-exon 3 boundary in SEQ ID NO: 10 (*HSD17B13* Transcript G) when optimally aligned with SEQ ID NO: 10. It is understood that such a nucleic acid would include a sufficient number of nucleotides in each of exons 1 and 3 to distinguish from other features in the *HSD17B13* Transcripts (e.g., the boundary of exons 1 and 2 or the boundary of exons 2 and 3). For example, the region can comprise the entirety of exons 1 and 3 in SEQ ID NO: 10. Optionally, the isolated nucleic acid comprises a sequence at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the sequence set forth in SEQ ID NO: 10 (*HSD17B13* Transcript G) and encodes an HSD17B13 protein comprising the sequence set forth in SEQ ID

NO: 18 (HSD17B13 Isoform G).

[00171] As one example, the isolated nucleic acid can comprise at least 15 contiguous nucleotides (e.g., at least 20 contiguous nucleotides or at least 30 contiguous nucleotides) of SEQ ID NO: 10 including a region spanning the exon 1-exon 3 boundary, optionally comprising the exons 1 and 3 of SEQ ID NO: 10, and optionally comprising the entire sequence of SEQ ID NO: 10.

[00172] Also provided herein are isolated nucleic acids comprising a segment (e.g., at least 15 contiguous nucleotides) present in Transcript E (or a fragment or homolog thereof) that is not present in Transcript A (or a fragment or homolog thereof). Such regions can be readily identified by comparing the sequences of the Transcripts. Transcript E (SEQ ID NO: 8) includes an additional exon between exons 3 and 4 compared to Transcript A. Accordingly, provided herein are isolated nucleic acids comprising at least 15 contiguous nucleotides (e.g., at least 20 contiguous nucleotides or at least 30 contiguous nucleotides) encoding all or part of an HSD17B13 protein, wherein a segment of the contiguous nucleotides (e.g., at least 5 contiguous nucleotides, at least 10 contiguous nucleotides or at least 15 contiguous nucleotides) is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a region within exon 3' of SEQ ID NO: 8 (*HSD17B13* Transcript E), a region spanning the exon 3-exon 3' boundary of SEQ ID NO: 8, or a region spanning the exon 3'-exon 4 boundary of SEQ ID NO: 8 when optimally aligned with SEQ ID NO: 8. It is understood that such a nucleic acid would include a sufficient number of nucleotides in each of exons 3 and 3' or each of exons 3' and 4 to distinguish from other features in the *HSD17B13* transcripts (e.g., from the boundary of exons 3 and 4). For example, the region of exon 3' can comprise the entire exon 3'. Optionally, the isolated nucleic acid further comprises a segment (e.g., at least 15 contiguous nucleotides) from Transcript E (or a fragment or homolog thereof) that is not present in Transcript H (or a fragment or homolog thereof). Such regions can be readily identified by comparing the sequences of the Transcripts. For example, provided herein are isolated nucleic acids as described above, wherein a segment of the contiguous nucleotides (e.g., at least 5 contiguous nucleotides, at least 10 contiguous nucleotides or at least 15 contiguous nucleotides) is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a region spanning the exon 6-exon 7 boundary in SEQ ID NO: 8 (*HSD17B13* Transcript E) when optimally aligned with SEQ ID NO: 8. It is understood that such a nucleic acid would include a

sufficient number of nucleotides in each of exons 6 and 7 to distinguish from other features in the *HSD17B13* Transcripts (particularly the additional guanine at the 3' end of exon 6 in Transcript H)). For example, the region can comprise the entirety of exons 6 and 7 in SEQ ID NO: 8. Optionally, the isolated nucleic acid comprises a sequence at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the sequence set forth in SEQ ID NO: 8 (*HSD17B13* Transcript E) and encodes an HSD17B13 protein comprising the sequence set forth in SEQ ID NO: 16 (HSD17B13 Isoform E).

[00173] As one example, the isolated nucleic acid can comprise at least 15 contiguous nucleotides (e.g., at least 20 contiguous nucleotides or at least 30 contiguous nucleotides) of SEQ ID NO: 8 including a region within exon 3', a region spanning the exon 3-exon 3' boundary, or a region spanning the exon 3'-exon 4 boundary, optionally comprising the entire exon 3' of SEQ ID NO: 8, and optionally comprising the entire sequence of SEQ ID NO: 8.

[00174] Also provided herein are isolated nucleic acids comprising a segment (e.g., at least 15 contiguous nucleotides) present in Transcript F (or a fragment or homolog thereof) that is not present in Transcript A (or a fragment or homolog thereof). Such regions can be readily identified by comparing the sequences of the Transcripts. Transcript F (SEQ ID NO: 9) includes a read-through from exon 6 into intron 6 compared to Transcript A, and the read-through includes the inserted thymine present in the *HSD17B13* rs72613567 variant gene. Accordingly, provided herein are isolated nucleic acids comprising at least 15 contiguous nucleotides (e.g., at least 20 contiguous nucleotides or at least 30 contiguous nucleotides) encoding all or part of an HSD17B13 protein, wherein a segment of the contiguous nucleotides (e.g., at least 5 contiguous nucleotides, at least 10 contiguous nucleotides or at least 15 contiguous nucleotides) is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a region within the read-through into intron 6 in SEQ ID NO: 9 (*HSD17B13* Transcript F) or a region spanning the boundary between the read-through into intron 6 and the rest of exon 6 in SEQ ID NO: 9 when optimally aligned with SEQ ID NO: 9. It is understood that such a nucleic acid would a sufficient number of nucleotides in the read-through to distinguish the read-through from other features in the *HSD17B13* Transcripts (e.g., from boundary of exons 6 and 7 in other *HSD17B13* Transcripts). Optionally, the contiguous nucleotides comprise a sequence present in Transcript F (i.e., the inserted thymine) that is not present in Transcript F' (SEQ ID NO: 246). Transcript F' also includes a read-through from exon 6 into intron 6 compared to Transcript A,

but the read-through does not include the inserted thymine present in the *HSD17B13* rs72613567 variant gene. For example, the region can be the entire read-through into intron 6 in SEQ ID NO: 9. Optionally, the isolated nucleic acid comprises a sequence at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the sequence set forth in SEQ ID NO: 9 (*HSD17B13* Transcript F) and encodes an HSD17B13 protein comprising the sequence set forth in SEQ ID NO: 17 (HSD17B13 Isoform F).

[00175] As one example, the isolated nucleic acid can comprise at least 15 contiguous nucleotides (e.g., at least 20 contiguous nucleotides or at least 30 contiguous nucleotides) of SEQ ID NO: 9 including a region within the read-through into intron 6 or a region spanning the boundary between the read-through into intron 6 and the rest of exon 6, optionally comprising the entire read-through into intron 6, and optionally comprising the entire sequence of SEQ ID NO: 9.

[00176] Also provided herein are isolated nucleic acids comprising a segment (e.g., at least 15 contiguous nucleotides) present in Transcript F' (or a fragment or homolog thereof) that is not present in Transcript A (or a fragment or homolog thereof). Such regions can be readily identified by comparing the sequences of the Transcripts. Transcript F' (SEQ ID NO: 246) includes a read-through from exon 6 into intron 6 compared to Transcript A, and the read-through does not include the inserted thymine present in the *HSD17B13* rs72613567 variant gene. Accordingly, provided herein are isolated nucleic acids comprising at least 15 contiguous nucleotides (e.g., at least 20 contiguous nucleotides or at least 30 contiguous nucleotides) encoding all or part of an HSD17B13 protein, wherein a segment of the contiguous nucleotides (e.g., at least 5 contiguous nucleotides, at least 10 contiguous nucleotides or at least 15 contiguous nucleotides) is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a region within the read-through into intron 6 in SEQ ID NO: 246 (*HSD17B13* Transcript F') or a region spanning the boundary between the read-through into intron 6 and the rest of exon 6 in SEQ ID NO: 246 when optimally aligned with SEQ ID NO: 246. It is understood that such a nucleic acid would a sufficient number of nucleotides in the read-through to distinguish the read-through from other features in the *HSD17B13* Transcripts (e.g., from boundary of exons 6 and 7 in other *HSD17B13* Transcripts). Optionally, the contiguous nucleotides comprise a sequence present in Transcript F' that is not present in Transcript F (SEQ ID NO: 9). The read-through in Transcript F includes the inserted thymine

present in the *HSD17B13* rs72613567 variant gene, whereas the read-through in Transcript F' does not. For example, the region can be the entire read-through into intron 6 in SEQ ID NO: 246. Optionally, the isolated nucleic acid comprises a sequence at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the sequence set forth in SEQ ID NO: 246 (*HSD17B13* Transcript F') and encodes an HSD17B13 protein comprising, consisting essentially of, or consisting of the sequence set forth in SEQ ID NO: 247 (HSD17B13 Isoform F').

[00177] As one example, the isolated nucleic acid can comprise at least 15 contiguous nucleotides (e.g., at least 20 contiguous nucleotides or at least 30 contiguous nucleotides) of SEQ ID NO: 246 including a region within the read-through into intron 6 or a region spanning the boundary between the read-through into intron 6 and the rest of exon 6, optionally comprising the entire read-through into intron 6, and optionally comprising the entire sequence of SEQ ID NO: 246.

[00178] Also provided herein are isolated nucleic acids comprising a segment (e.g., at least 15 contiguous nucleotides) present in Transcript C (or a fragment or homolog thereof) that is not present in Transcript A (or a fragment or homolog thereof). Such regions can be readily identified by comparing the sequences of the Transcripts. Transcript C (SEQ ID NO: 6) is missing exon 6 compared to Transcript A (i.e., Transcript C includes an exon 5-exon 7 boundary not present in Transcript A). Accordingly, provided herein are isolated nucleic acids comprising at least 15 contiguous nucleotides (e.g., at least 20 contiguous nucleotides or at least 30 contiguous nucleotides) encoding all or part of an HSD17B13 protein, wherein a segment of the contiguous nucleotides (e.g., at least 5 contiguous nucleotides, at least 10 contiguous nucleotides or at least 15 contiguous nucleotides) is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a region spanning the exon 5-exon 7 boundary in SEQ ID NO: 6 (*HSD17B13* Transcript C) when optimally aligned with SEQ ID NO: 6. It is understood that such a nucleic acid would a sufficient number of nucleotides in each of exons 5 and 7 to distinguish from other features in the *HSD17B13* Transcripts (e.g., from boundary of exons 5 and 6 or of exons 6 and 7 in other *HSD17B13* Transcripts). For example, the region can comprise the entirety of exons 5 and 7 in SEQ ID NO: 6. Optionally, the isolated nucleic acid comprises a sequence at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the sequence set forth in SEQ ID NO: 6 (*HSD17B13* Transcript C) and encodes an

HSD17B13 protein comprising the sequence set forth in SEQ ID NO: 14 (HSD17B13 Isoform C).

[00179] As one example, the isolated nucleic acid can comprise at least 15 contiguous nucleotides (e.g., at least 20 contiguous nucleotides or at least 30 contiguous nucleotides) of SEQ ID NO: 6 including a region spanning the exon 5-exon 7 boundary, optionally comprising the entirety of exons 5 and 7 in SEQ ID NO: 6, and optionally comprising the entire sequence of SEQ ID NO: 6.

(4) Nucleic Acids Hybridizing to cDNAs and Variant HSD17B13 *Transcripts*

[00180] Also provided are nucleic acids hybridizing to segments of an mRNA transcript or a cDNA corresponding to any one of Transcripts A-H (SEQ ID NOS: 4-11, respectively), and particularly Transcripts C-H, when optimally aligned with the any one of Transcripts A-H. Specific, non-limiting examples are provided below. Such isolated nucleic acids can be useful, for example, primers, probes, antisense RNAs, siRNAs, or shRNAs.

[00181] The segment to which the isolated nucleic acid can hybridize can comprise, for example, at least 5, at least 10, or at least 15 contiguous nucleotides of a nucleic acid encoding an HSD17B13 protein. The segment to which the isolated nucleic acid can hybridize can comprise, for example, at least 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 75, 90, 95, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, or 2000 contiguous nucleotides of a nucleic acid encoding an HSD17B13 protein. Alternatively, the segment to which the isolated nucleic acid can hybridize can be, for example, up to 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 75, 90, 95, 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1000 contiguous nucleotides of a nucleic acid encoding an HSD17B13 protein. For example, the segment can be about 15 to 100 nucleotides in length, or about 15-35 nucleotides in length.

[00182] *HSD17B13* Transcript D (SEQ ID NO: 7), Transcript G (SEQ ID NO: 10), and Transcript H (SEQ ID NO: 11) include an insertion of a guanine at the 3' end of exon 6, resulting in a frameshift in and premature truncation of exon 7 compared to Transcript A. Accordingly, provided herein are isolated nucleic acids comprising a region (e.g., at least 15 contiguous nucleotides) that hybridizes to a segment present in Transcripts D, G, and H (or fragments or homologs thereof) that is not present in Transcript A (or a fragment or homolog thereof). Such regions can be readily identified by comparing the sequences of the Transcripts. For example,

provided herein are isolated nucleic acids that hybridize to at least 15 contiguous nucleotides of a nucleic acid encoding an HSD17B13 protein, wherein contiguous nucleotides comprise a segment (e.g., at least 5 contiguous nucleotides, at least 10 contiguous nucleotides or at least 15 contiguous nucleotides) that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a region spanning the exon 6–exon 7 boundary in SEQ ID NO: 7 (*HSD17B13* Transcript D) when optimally aligned with SEQ ID NO: 7, and the segment includes a guanine at a residue corresponding to residue 878 at the 3' end of exon 6 in SEQ ID NO: 7 (i.e., an insertion of a guanine at the 3' end of exon 6 relative to Transcript A in addition to the guanine at the start of exon 7). Alternatively, provided herein are isolated nucleic acids that hybridize to at least 15 contiguous nucleotides of a segment of a nucleic acid encoding an HSD17B13 protein, wherein the contiguous nucleotides comprise a segment (e.g., at least 5 contiguous nucleotides, at least 10 contiguous nucleotides or at least 15 contiguous nucleotides) that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a region spanning the exon 6-exon 7 boundary in SEQ ID NO: 10 (*HSD17B13* Transcript G) when optimally aligned with SEQ ID NO: 10, and the segment includes a guanine at a residue corresponding to residue 770 at the 3' end of exon 6 in SEQ ID NO: 10 (i.e., an insertion of a guanine at the 3' end of exon 6 relative to Transcript B in addition to the guanine at the start of exon 7). Alternatively, provided herein are isolated nucleic acids comprising that hybridize to at least 15 contiguous nucleotides of a nucleic acid encoding an HSD17B13 protein, wherein the contiguous nucleotides comprise a segment (e.g., at least 5 contiguous nucleotides, at least 10 contiguous nucleotides or at least 15 contiguous nucleotides) that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a region spanning the exon 6-exon 7 boundary in SEQ ID NO: 11 (*HSD17B13* Transcript H) when optimally aligned with SEQ ID NO: 11, and the segment includes a guanine at a residue corresponding to residue 950 at the 3' end of exon 6 in SEQ ID NO: 11 (i.e., an insertion of a guanine at the 3' end of exon 6 relative to Transcript E in addition to the guanine at the start of exon 7). It is understood that such nucleic acids would be designed to hybridize to a sufficient number of nucleotides in each of exons 6 and 7 to distinguish the inserted guanine from other features in the *HSD17B13* Transcripts (e.g., from the read-through into intron 6 in Transcript F or from the deleted exon 6 in Transcript C).

[00183] As one example, the segment can comprise a region of SEQ ID NO: 7 spanning the

exon 6-exon 7 boundary (i.e., including the guanine at residue 878 of SEQ ID NO: 7). As another example, the segment can comprise a region of SEQ ID NO: 10 spanning the exon 6-exon 7 boundary (i.e., including the guanine at residue 770 of SEQ ID NO: 10). As another example, the segment can comprise a region of SEQ ID NO: 11 spanning the exon 6-exon 7 boundary (i.e., including the guanine at residue 950 of SEQ ID NO: 11).

[00184] Optionally, the isolated nucleic acid further comprises a region (e.g., 15 contiguous nucleotides) that hybridizes to a segment present in Transcript D (or a fragment or homolog thereof) that is not present in Transcript G (or a fragment or homolog thereof), and the isolated nucleic acid further comprises a region that hybridizes to a segment present in Transcript D (or a fragment or homolog thereof) that is not present in Transcript H (or a fragment or homolog thereof). Such segments can be readily identified by comparing the sequences of the Transcripts. For example, the segment (e.g., at least 5 contiguous nucleotides, at least 10 contiguous nucleotides or at least 15 contiguous nucleotides) present in Transcript D (or a fragment or homolog thereof) that is not present in Transcript H (or a fragment or homolog thereof) can be at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a region spanning the boundary of exons 3 and 4 of SEQ ID NO: 7 (*HSD17B13* Transcript D) when optimally aligned with SEQ ID NO: 7 to distinguish from Transcript H. Likewise, the segment (e.g., at least 5 contiguous nucleotides, at least 10 contiguous nucleotides or at least 15 contiguous nucleotides) present in Transcript D (or a fragment or homolog thereof) that is not present in Transcript G (or a fragment or homolog thereof) can be at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a region within exon 2 of SEQ ID NO: 7 (*HSD17B13* Transcript D), a region spanning the exon 1-exon 2 boundary of SEQ ID NO: 7, or a region spanning the exon 2-exon 3 boundary of SEQ ID NO: 7 when optimally aligned with SEQ ID NO: 7 to distinguish from Transcript G.

[00185] Like Transcript D, Transcript H (SEQ ID NO: 11) includes an insertion of a guanine at the 3' end of exon 6 compared to Transcript A. Transcript H further includes an additional exon between exons 3 and 4 compared to Transcript A and Transcript D. Accordingly, provided herein are isolated nucleic acids as described above comprising a region that hybridizes to a segment present in Transcripts D, G, and H (or fragments or homologs thereof) that is not present in Transcript A (or a fragment or homolog thereof) but further comprising a region (e.g., at least 15 contiguous nucleotides) that hybridizes to a segment that is present in Transcript H (or

a fragment or homolog thereof) but not in Transcript D (or a fragment or homolog thereof). Such regions can be readily identified by comparing the sequences of the Transcripts. For example, the segment can be at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a region (e.g., at least 5 contiguous nucleotides, at least 10 contiguous nucleotides or at least 15 contiguous nucleotides) within exon 3' of SEQ ID NO: 11 (*HSD17B13* Transcript H), a region spanning the exon 3-exon 3' boundary of SEQ ID NO: 11, or a region spanning the exon 3'-exon 4 boundary of SEQ ID NO: 11 when optimally aligned with SEQ ID NO: 11. It is understood that such a nucleic acid would be designed to hybridize to a sufficient number of nucleotides in each of exons 3 and 3' or each of exons 3' and 4 to distinguish from other features in the *HSD17B13* transcripts (e.g., from the boundary of exons 3 and 4).

[00186] As one example, the segment can comprise a region of SEQ ID NO: 11 within exon 3', spanning the exon 3-exon 3' boundary, or spanning the exon 3'-exon 4 boundary.

[00187] Like Transcript D, Transcript G (SEQ ID NO: 10) includes an insertion of a guanine at the 3' end of exon 6 compared to Transcript A. In addition, however, Transcript G is missing exon 2 compared to Transcript A and Transcript D (i.e., Transcript G includes an exon 1-exon 3 boundary not present in Transcripts A and D). Accordingly, provided herein are isolated nucleic acids as described above comprising a region that hybridizes to a segment present in Transcripts D, G, and H (or fragments or homologs thereof) that is not present in Transcript A (or a fragment or homolog thereof) but further comprising a region (e.g., at least 15 contiguous nucleotides) that hybridizes to a segment present in Transcript G (or a fragment or homolog thereof) but not in Transcript D (or a fragment or homolog thereof). Such regions can be readily identified by comparing the sequences of the Transcripts. For example, the segment can be at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a region (e.g., at least 5 contiguous nucleotides, at least 10 contiguous nucleotides or at least 15 contiguous nucleotides) spanning the exon 1-exon 3 boundary in SEQ ID NO: 10 (*HSD17B13* Transcript G) when optimally aligned with SEQ ID NO: 10. It is understood that such a nucleic acid would be designed to hybridize to a sufficient number of nucleotides in each of exons 1 and 3 to distinguish from other features in the *HSD17B13* Transcripts (e.g., the boundary of exons 1 and 2 or the boundary of exons 2 and 3).

[00188] As one example, the segment can comprise a region of SEQ ID NO: 10 spanning the

exon 1-exon 3 boundary.

[00189] Also provided are isolated nucleic acids comprising a region (e.g., at least 15 contiguous nucleotides) that hybridizes to a segment of a nucleic acid encoding an HSD17B13 protein that is present in Transcript E (or a fragment or homolog thereof) but not in Transcript A (or a fragment or homolog thereof). Such regions can be readily identified by comparing the sequences of the Transcripts. Transcript E (SEQ ID NO: 8) includes an additional exon between exons 3 and 4 compared to Transcript A. Accordingly, provided herein are isolated nucleic acids that hybridize to at least 15 contiguous nucleotides of a nucleic acid encoding an HSD17B13 protein, wherein the contiguous nucleotides comprise a segment that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a region (e.g., at least 5 contiguous nucleotides, at least 10 contiguous nucleotides or at least 15 contiguous nucleotides) within exon 3' of SEQ ID NO: 8 (*HSD17B13* Transcript E), a region spanning the exon 3-exon 3' boundary of SEQ ID NO: 8, or a region spanning the exon 3'-exon 4 boundary of SEQ ID NO: 8 when optimally aligned with SEQ ID NO: 8. It is understood that such a nucleic acid would be designed to hybridize to a sufficient number of nucleotides in each of exons 3 and 3' or each of exons 3' and 4 to distinguish from other features in the *HSD17B13* transcripts (e.g., from the boundary of exons 3 and 4).

[00190] As one example, the segment can comprise a region of SEQ ID NO: 8 within exon 3', spanning the exon 3-exon 3' boundary of SEQ ID NO: 8, or spanning the exon 3'-exon 4 boundary.

[00191] Optionally, the isolated nucleic acid further comprises a region (e.g., 15 contiguous nucleotides) that hybridizes to a segment present in Transcript E (or a fragment or homolog thereof) that is not present in Transcript H (or a fragment or homolog thereof). Such segments can be readily identified by comparing the sequences of the Transcripts. For example, the segment (e.g., at least 5 contiguous nucleotides, at least 10 contiguous nucleotides or at least 15 contiguous nucleotides) present in Transcript E (or a fragment or homolog thereof) that is not present in Transcript H (or a fragment or homolog thereof) can be at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a region spanning the boundary of exons 6 and 7 of SEQ ID NO: 8 (*HSD17B13* Transcript E) when optimally aligned with SEQ ID NO: 8 to distinguish from Transcript G. It is understood that such a nucleic acid would be designed to hybridize to a sufficient number of nucleotides in each of exons 6 and 7 to

distinguish from other features in the *HSD17B13* Transcripts (particularly the additional guanine at the 3' end of exon 6 in Transcript H)).

[00192] Also provided are isolated nucleic acids comprising a region (e.g., at least 15 contiguous nucleotides) that hybridizes to a segment of a nucleic acid encoding an HSD17B13 protein that is present in Transcript F (or a fragment or homolog thereof) but not in Transcript A (or a fragment or homolog thereof). Such regions can be readily identified by comparing the sequences of the Transcripts. Transcript F (SEQ ID NO: 9) includes a read-through from exon 6 to intron 6 compared to Transcript A. Accordingly, provided herein are isolated nucleic acids that hybridize to at least 15 contiguous nucleotides of a nucleic acid encoding an HSD17B13 protein, wherein the contiguous nucleotides comprise a segment (e.g., at least 5 contiguous nucleotides, at least 10 contiguous nucleotides or at least 15 contiguous nucleotides) that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a region within the read-through into intron 6 in SEQ ID NO: 9 (*HSD17B13* Transcript F) or a region spanning the boundary between the read-through into intron 6 and the rest of exon 6 in SEQ ID NO: 9 when optimally aligned with SEQ ID NO: 9. It is understood that such a nucleic acid would be designed to hybridize to a sufficient number of nucleotides in the read-through to distinguish the read-through from other features in the *HSD17B13* Transcripts (e.g., from boundary of exons 6 and 7 in other *HSD17B13* Transcripts). Optionally, the contiguous nucleotides comprise a sequence present in Transcript F (i.e., the inserted thymine) that is not present in Transcript F' (SEQ ID NO: 246). Transcript F' also includes a read-through from exon 6 into intron 6 compared to Transcript A, but the read-through does not include the inserted thymine present in the *HSD17B13* rs72613567 variant gene.

[00193] As one example, the segment can comprise a region of SEQ ID NO: 9 within the read-through into intron 6 or spanning the boundary between the read-through into intron 6 and the rest of exon 6.

[00194] Also provided are isolated nucleic acids comprising a region (e.g., at least 15 contiguous nucleotides) that hybridizes to a segment of a nucleic acid encoding an HSD17B13 protein that is present in Transcript F' (or a fragment or homolog thereof) but not in Transcript A (or a fragment or homolog thereof). Such regions can be readily identified by comparing the sequences of the Transcripts. Transcript F' (SEQ ID NO: 246) includes a read-through from exon 6 to intron 6 compared to Transcript A. Accordingly, provided herein are isolated nucleic

acids that hybridize to at least 15 contiguous nucleotides of a nucleic acid encoding an HSD17B13 protein, wherein the contiguous nucleotides comprise a segment (e.g., at least 5 contiguous nucleotides, at least 10 contiguous nucleotides or at least 15 contiguous nucleotides) that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a region within the read-through into intron 6 in SEQ ID NO: 246 (*HSD17B13* Transcript F') or a region spanning the boundary between the read-through into intron 6 and the rest of exon 6 in SEQ ID NO: 246 when optimally aligned with SEQ ID NO: 246. It is understood that such a nucleic acid would be designed to hybridize to a sufficient number of nucleotides in the read-through to distinguish the read-through from other features in the *HSD17B13* Transcripts (e.g., from boundary of exons 6 and 7 in other *HSD17B13* Transcripts). Optionally, the contiguous nucleotides comprise a sequence present in Transcript F' that is not present in Transcript F (SEQ ID NO: 9). The read-through in Transcript F includes the inserted thymine present in the *HSD17B13* rs72613567 variant gene, whereas the read-through in Transcript F' does not.

[00195] As one example, the segment can comprise a region of SEQ ID NO: 246 within the read-through into intron 6 or spanning the boundary between the read-through into intron 6 and the rest of exon 6.

[00196] Also provided are isolated nucleic acids comprising a region (e.g., at least 15 contiguous nucleotides) that hybridizes to a segment of a nucleic acid encoding an HSD17B13 protein that is present in Transcript C (or a fragment or homolog thereof) but not in Transcript A (or a fragment or homolog thereof). Such regions can be readily identified by comparing the sequences of the Transcripts. Transcript C (SEQ ID NO: 6) is missing exon 6 compared to Transcript A (i.e., Transcript C includes an exon 5-exon 7 boundary not present in Transcript A). Accordingly, provided herein are isolated nucleic acids that hybridize to at least 15 contiguous nucleotides of a nucleic acid encoding an HSD17B13 protein, wherein the contiguous nucleotides comprise a segment (e.g., at least 5 contiguous nucleotides, at least 10 contiguous nucleotides or at least 15 contiguous nucleotides) that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a region spanning the exon 5-exon 7 boundary in SEQ ID NO: 6 (*HSD17B13* Transcript C) when optimally aligned with SEQ ID NO: 6. It is understood that such a nucleic acid would be designed to hybridize to a sufficient number of nucleotides in exons 5 and 7 to distinguish from other features in the *HSD17B13*

Transcripts (e.g., from boundary of exons 5 and 6 or of exons 6 and 7 in other *HSD17B13* Transcripts).

[00197] As one example, the segment can comprise a region from SEQ ID NO: 6 spanning the exon 5-exon 7 boundary.

[00198] Also provided herein are isolated nucleic acids (e.g., antisense RNAs, siRNAs, or shRNAs) that hybridize to at least 15 contiguous nucleotides of a nucleic acid encoding an HSD17B13 protein, wherein contiguous nucleotides comprise a segment (e.g., at least 5 contiguous nucleotides, at least 10 contiguous nucleotides or at least 15 contiguous nucleotides) that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a region of *HSD17B13* Transcript D (SEQ ID NO: 7). The isolated nucleic acids can comprise a region (e.g., at least 15 contiguous nucleotides) that hybridizes to a segment present in Transcript D (or fragments or homologs thereof) that is not present in Transcript A (or a fragment or homolog thereof). Such regions can be readily identified by comparing the sequences of the Transcripts. *HSD17B13* Transcript D (SEQ ID NO: 7) includes an insertion of a guanine at the 3' end of exon 6, resulting in a frameshift in and premature truncation of exon 7 compared to Transcript A (SEQ ID NO: 4). For example, provided herein are isolated nucleic acids that hybridize to at least 15 contiguous nucleotides of a nucleic acid encoding an HSD17B13 protein, wherein contiguous nucleotides comprise a segment (e.g., at least 5 contiguous nucleotides, at least 10 contiguous nucleotides or at least 15 contiguous nucleotides) that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a region spanning the exon 6–exon 7 boundary in SEQ ID NO: 7 (*HSD17B13* Transcript D) when optimally aligned with SEQ ID NO: 7. The segment can include a guanine at a residue corresponding to residue 878 at the 3' end of exon 6 in SEQ ID NO: 7 (i.e., an insertion of a guanine at the 3' end of exon 6 relative to Transcript A in addition to the guanine at the start of exon 7). It is understood that such nucleic acids would be designed to hybridize to a sufficient number of nucleotides in each of exons 6 and 7 to distinguish the inserted guanine from other features in the *HSD17B13* Transcripts (e.g., from the read-through into intron 6 in Transcript F or from the deleted exon 6 in Transcript C).

[00199] Also provided herein are isolated nucleic acids (e.g., antisense RNAs, siRNAs, or shRNAs) that hybridize to at least 15 contiguous nucleotides of a nucleic acid encoding an HSD17B13 protein, wherein contiguous nucleotides comprise a segment (e.g., at least 5

contiguous nucleotides, at least 10 contiguous nucleotides or at least 15 contiguous nucleotides) that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a region of *HSD17B13* Transcript A (SEQ ID NO: 4). The isolated nucleic acids can comprise a region (e.g., at least 15 contiguous nucleotides) that hybridizes to a segment present in Transcript A (or fragments or homologs thereof) that is not present in Transcript D (or a fragment or homolog thereof). Such regions can be readily identified by comparing the sequences of the Transcripts. *HSD17B13* Transcript D (SEQ ID NO: 7) includes an insertion of a guanine at the 3' end of exon 6, resulting in a frameshift in and premature truncation of exon 7 compared to Transcript A (SEQ ID NO: 4). For example, provided herein are isolated nucleic acids that hybridize to at least 15 contiguous nucleotides of a nucleic acid encoding an HSD17B13 protein, wherein contiguous nucleotides comprise a segment (e.g., at least 5 contiguous nucleotides, at least 10 contiguous nucleotides or at least 15 contiguous nucleotides) that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a region spanning the exon 6–exon 7 boundary in SEQ ID NO: 4 (*HSD17B13* Transcript A) when optimally aligned with SEQ ID NO: 4.

(5) Vectors

[00200] Also provided are vectors comprising any of the nucleic acids disclosed herein and a heterologous nucleic acid. The vectors can be viral or nonviral vectors capable of transporting a nucleic acid. In some cases, a vector can be a plasmid (e.g., a circular double-stranded DNA into which additional DNA segments can be ligated). In some cases, a vector can be a viral vector, wherein additional DNA segments can be ligated into the viral genome. In some cases, a vector can autonomously replicate in a host cell into which it is introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). In other cases, vectors (e.g., non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell and thereby are replicated along with the host genome. Moreover, certain vectors can direct the expression of genes to which they are operatively linked. Such vectors can be referred to as "recombinant expression vectors" or "expression vectors." Such vectors can also be targeting vectors (i.e., exogenous donor sequences) as disclosed elsewhere herein.

[00201] In some cases, the proteins encoded by the disclosed genetic variants are expressed by

inserting nucleic acids encoding the disclosed genetic variants into expression vectors such that the genes are operatively linked to necessary expression control sequences such as transcriptional and translational control sequences. Expression vectors can include, for example, plasmids, retroviruses, adenoviruses, adeno-associated viruses (AAV), plant viruses such as cauliflower mosaic virus, tobacco mosaic virus, cosmids, YACs, EBV derived episomes, and the like. In some instances, nucleic acids comprising the disclosed genetic variants can be ligated into a vector such that transcriptional and translational control sequences within the vector serve their intended function of regulating the transcription and translation of the genetic variant. The expression vector and expression control sequences are chosen to be compatible with the expression host cell used. Nucleic acid sequences comprising the disclosed genetic variants can be inserted into separate vectors or into the same expression vector. A nucleic acid sequence comprising the disclosed genetic variants can be inserted into the expression vector by standard methods (e.g., ligation of complementary restriction sites on the nucleic acid comprising the disclosed genetic variants and vector, or blunt end ligation if no restriction sites are present).

[00202] In addition to a nucleic acid sequence comprising the disclosed genetic variants, the recombinant expression vectors can carry regulatory sequences that control the expression of the genetic variant in a host cell. The design of the expression vector, including the selection of regulatory sequences can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, and so forth. Preferred regulatory sequences for mammalian host cell expression can include, for example, viral elements that direct high levels of protein expression in mammalian cells, such as promoters and/or enhancers derived from retroviral LTRs, cytomegalovirus (CMV) (such as the CMV promoter/enhancer), Simian Virus 40 (SV40) (such as the SV40 promoter/enhancer), adenovirus, (e.g., the adenovirus major late promoter (AdMLP)), polyoma and strong mammalian promoters such as native immunoglobulin and actin promoters. Further description of viral regulatory elements, and sequences thereof is provided in US Pat. Nos. 5,168,062; 4,510,245; and 4,968,615, each of which is herein incorporated by reference in its entirety for all purposes. Methods of expressing polypeptides in bacterial cells or fungal cells (e.g., yeast cells) are also well known.

[00203] In addition to a nucleic acid sequence comprising the disclosed genetic variants and regulatory sequences, the recombinant expression vectors can carry additional sequences, such as sequences that regulate replication of the vector in host cells (e.g., origins of replication) and

selectable marker genes. A selectable marker gene can facilitate selection of host cells into which the vector has been introduced (*see e.g.*, US Pat. Nos. 4,399,216; 4,634,665; and 5,179,017, each of which is herein incorporated by reference in its entirety for all purposes). For example, a selectable marker gene can confer resistance to drugs, such as G418, hygromycin, or methotrexate, on a host cell into which the vector has been introduced. Exemplary selectable marker genes include the dihydrofolate reductase (DHFR) gene (for use in dhfr-host cells with methotrexate selection/amplification), the neo gene (for G418 selection), and the glutamate synthetase (GS) gene.

B. Proteins

[00204] Disclosed herein are isolated *HSD17B13* proteins and fragments thereof, and particularly *HSD17B13* proteins and fragments thereof produced by the *HSD17B13* rs72613567 variant.

[00205] The isolated proteins disclosed herein can comprise an amino acid sequence of a naturally occurring HSD17B13 protein, or can comprise a non-naturally occurring sequence. In one example, the non-naturally occurring sequence can differ from the non-naturally occurring sequence due to conservative amino acid substitutions. For example, the sequence can be identical with the exception of conservative amino acid substitutions.

[00206] The isolated proteins disclosed herein can be linked or fused to heterologous polypeptides or heterologous molecules or labels, numerous examples of which are disclosed elsewhere herein. For example, the proteins can be fused to a heterologous polypeptide providing increased or decreased stability. The fused domain or heterologous polypeptide can be located at the N-terminus, the C-terminus, or internally within the protein. A fusion partner may, for example, assist in providing T helper epitopes (an immunological fusion partner), or may assist in expressing the protein (an expression enhancer) at higher yields than the native recombinant protein. Certain fusion partners are both immunological and expression enhancing fusion partners. Other fusion partners may be selected so as to increase the solubility of the polypeptide or to enable the polypeptide to be targeted to desired intracellular compartments. Still further fusion partners include affinity tags, which facilitate purification of the polypeptide.

[00207] A fusion protein may be directly fused to the heterologous molecule or may be linked to the heterologous molecule via a linker, such as a peptide linker. Suitable peptide linker

sequences may be chosen, for example, based on the following factors: (1) their ability to adopt a flexible extended conformation; (2) their inability to adopt a secondary structure that could interact with functional epitopes on the first and second polypeptides; and (3) the lack of hydrophobic or charged residues that might react with the polypeptide functional epitopes. For example, peptide linker sequences may contain Gly, Asn and Ser residues. Other near neutral amino acids, such as Thr and Ala may also be used in the linker sequence. Amino acid sequences which may be usefully employed as linkers include those disclosed in Maratea et al. (1985) *Gene* 40:39-46; Murphy et al. (1986) *Proc. Natl. Acad. Sci. USA* 83:8258-8262; US Pat. No. 4,935,233; and US Pat. No. 4,751,180, each of which is herein incorporated by reference in its entirety. A linker sequence may generally be, for example, from 1 to about 50 amino acids in length. Linker sequences are generally not required when the first and second polypeptides have non-essential N-terminal amino acid regions that can be used to separate the functional domains and prevent steric interference.

[00208] The proteins can also be operably linked to a cell-penetrating domain. For example, the cell-penetrating domain can be derived from the HIV-1 TAT protein, the TLM cell-penetrating motif from human hepatitis B virus, MPG, Pep-1, VP22, a cell-penetrating peptide from Herpes simplex virus, or a polyarginine peptide sequence. *See, e.g.*, WO 2014/089290, herein incorporated by reference in its entirety for all purposes. The cell-penetrating domain can be located at the N-terminus, the C-terminus, or anywhere within the protein.

[00209] The proteins can also be operably linked to a heterologous polypeptide for ease of tracking or purification, such as a fluorescent protein, a purification tag, or an epitope tag. Examples of fluorescent proteins include green fluorescent proteins (e.g., GFP, GFP-2, tagGFP, turboGFP, eGFP, Emerald, Azami Green, Monomeric Azami Green, CopGFP, AceGFP, ZsGreenl), yellow fluorescent proteins (e.g., YFP, eYFP, Citrine, Venus, YPet, PhiYFP, ZsYellowl), blue fluorescent proteins (e.g., eBFP, eBFP2, Azurite, mKalamal, GFPuv, Sapphire, T-sapphire), cyan fluorescent proteins (e.g., eCFP, Cerulean, CyPet, AmCyanl, Midoriishi-Cyan), red fluorescent proteins (e.g., mKate, mKate2, mPlum, DsRed monomer, mCherry, mRFP1, DsRed-Express, DsRed2, DsRed-Monomer, HcRed-Tandem, HcRedl, AsRed2, eqFP611, mRaspberry, mStrawberry, Jred), orange fluorescent proteins (e.g., mOrange, mKO, Kusabira-Orange, Monomeric Kusabira-Orange, mTangerine, tdTomato), and any other suitable fluorescent protein. Examples of tags include glutathione-S-transferase (GST), chitin binding

protein (CBP), maltose binding protein, thioredoxin (TRX), poly(NANP), tandem affinity purification (TAP) tag, myc, AcV5, AU1 , AU5, E, ECS, E2, FLAG, hemagglutinin (HA), nus, Softag 1, Softag 3, Strep, SBP, Glu-Glu, HSV, KT3, S, S1, T7, V5, VSV-G, histidine (His), biotin carboxyl carrier protein (BCCP), and calmodulin.

[00210] The isolated proteins herein can also comprise non-natural or modified amino acids or peptide analogs. For example, there are numerous D-amino acids or amino acids which have a different functional substituent than the naturally occurring amino acids. The opposite stereo isomers of naturally occurring peptides are disclosed, as well as the stereo isomers of peptide analogs. These amino acids can readily be incorporated into polypeptide chains by charging tRNA molecules with the amino acid of choice and engineering genetic constructs that utilize, for example, amber codons, to insert the analog amino acid into a peptide chain in a site-specific way (Thorson et al. (1991) *Methods Molec. Biol.* 77:43-73; Zoller (1992) *Current Opinion in Biotechnology* 3:348-354; Ibba, (1995) *Biotechnology & Genetic Engineering Reviews* 13:197-216; Cahill et al. (1989) *TIBS* 14(10):400-403; Benner (1993) *TIB Tech* 12:158-163; and Ibba and Hennecke (1994) *Biotechnology* 12:678-682, each of which are herein incorporated by reference in its entirety for all purposes).

[00211] Molecules can be produced that resemble peptides, but that are not connected via a natural peptide linkage. For example, linkages for amino acids or amino acid analogs can include CH2NH--, --CH2S--, --CH2-- --, --CH=CH-- (cis and trans), --COCH2 --, --CH(OH)CH2--, and --CHH2SO-- (*see, e.g.*, Spatola, A.F. in Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins, B. Weinstein, eds., Marcel Dekker, New York, p. 267 (1983); Spatola, A. F., Vega Data (March 1983), Vol. 1, Issue 3, Peptide Backbone Modifications (general review); Morley (1994) *Trends Pharm Sci* 15(12):463-468; Hudson et al. (1979) *Int J Pept Prot Res* 14:177-185; Spatola et al. (1986) *Life Sci* 38:1243-1249; Hann (1982) *Chem. Soc Perkin Trans.* I 307-314; Almquist et al. (1980) *J. Med. Chem.* 23:1392-1398; Jennings-White et al. (1982) *Tetrahedron Lett* 23:2533); Szelke et al. European Appln, EP 45665 CA (1982): 97:39405 (1982); Holladay et al. (1983) *Tetrahedron. Lett* 24:4401-4404; and Hruby (1982) *Life Sci* 31:189-199; each of which is incorporated herein by reference in its entirety for all purposes. Peptide analogs can have more than one atom between the bond atoms, such as b-alanine, gaminobutyric acid, and the like.

[00212] Amino acid analogs and peptide analogs often have enhanced or desirable properties,

such as, more economical production, greater chemical stability, enhanced pharmacological properties (half-life, absorption, potency, efficacy, and so forth), altered specificity (e.g., a broad-spectrum of biological activities), reduced antigenicity, and others desirable properties.

[00213] D-amino acids can be used to generate more stable peptides because D amino acids are not recognized by peptidases and such. Systematic substitution of one or more amino acids of a consensus sequence with a D-amino acid of the same type (e.g., D-lysine in place of L-lysine) can be used to generate more stable peptides. Cysteine residues can be used to cyclize or attach two or more peptides together. This can be beneficial to constrain peptides into particular conformations (*see, e.g.*, Rizo and Gierasch (1992) *Ann. Rev. Biochem.* 61:387, herein by reference in its entirety for all purposes).

[00214] Also disclosed herein are nucleic acids encoding any of the proteins disclosed herein. This includes all degenerate sequences related to a specific polypeptide sequence (i.e., all nucleic acids having a sequence that encodes one particular polypeptide sequence as well as all nucleic acids, including degenerate nucleic acids, encoding the disclosed variants and derivatives of the protein sequences). Thus, while each particular nucleic acid sequence may not be written out herein, each and every sequence is in fact disclosed and described herein through the disclosed polypeptide sequences.

[00215] Also disclosed herein are compositions comprising an isolated polypeptide or protein disclosed herein and a carrier increasing the stability of the isolated polypeptide. Non-limiting examples of such carriers include poly(lactic acid) (PLA) microspheres, poly(D,L-lactic-coglycolic-acid) (PLGA) microspheres, liposomes, micelles, inverse micelles, lipid cochleates, and lipid microtubules.

(1) HSD17B13 Proteins and Fragments

[00216] Disclosed herein are isolated HSD17B13 proteins and fragments thereof, particularly HSD17B13 proteins and fragments thereof produced by the *HSD17B13* rs72613567 variant, or particularly HSD17B13 Isoforms C, D, E, F, F', G, and H. Such proteins can include, for example an isolated polypeptide comprising at least 5, 6, 8, 10, 12, 14, 15, 16, 18, 20, 22, 24, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 150, 200, 250, or 300 contiguous amino acids of HSD17B13 Isoform C, D, E, F, F', G, or H or a fragment thereof. It is understood that gene sequences within a population and proteins encoded by such genes can vary due to

polymorphisms such as single-nucleotide polymorphisms. The sequences provided herein for each HSD17B13 isoform are only exemplary sequences. Other sequences are also possible. For example, the isolated polypeptide comprises an amino acid sequence (e.g., a sequence of contiguous amino acids) at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to HSD17B13 Isoform C, D, E, F, F', G, or H when optimally aligned with Isoform C, D, E, F, F', G, or H, respectively. Optionally, the isolated polypeptide comprises a sequence identical to HSD17B13 Isoform C, D, E, F, F', G, or H.

[00217] As one example, the isolated polypeptide can comprise a segment (e.g., at least 8 contiguous amino acids) that is present in Isoforms D, G, and H (or fragments or homologs thereof) that is not present in Isoform A (or a fragment or homolog thereof). Such regions can be readily identified by comparing the sequences of the Isoforms. The region encoded by exon 7 in Isoforms D, G, and H is frameshifted and truncated compared to the region encoded by exon 7 in Isoform A. Thus, such an isolated polypeptide can comprise at least 5, 6, 8, 10, 12, 14, 15, 16, 18, 20, 22, 24, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 150, or 200 contiguous amino acids of an HSD17B13 protein (e.g., at least 8 contiguous amino acids, at least 10 contiguous amino acids, or at least 15 contiguous amino acids of an HSD17B13 protein), wherein a segment of the contiguous amino acids (e.g., at least 3 contiguous amino acids, at least 5 contiguous amino acids, at least 8 contiguous amino acids, at least 10 contiguous amino acids, or at least 15 contiguous amino acids) is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a segment including at least a portion of the region encoded by exon 7 in SEQ ID NO: 15 (HSD17B13 Isoform D), SEQ ID NO: 18 (HSD17B13 Isoform G), or SEQ ID NO: 19 (HSD17B13 Isoform H) when the isolated polypeptide is optimally aligned with SEQ ID NO: 15, 18, or 19, respectively.

[00218] Such isolated polypeptides can further comprise a segment present in Isoform D (or a fragment or homolog thereof) that is not present in Isoform G (or a fragment or homolog thereof), and can further comprise a segment present in Isoform D (or a fragment or homolog thereof) that is not present in Isoform H (or a fragment or homolog thereof). Such regions can be readily identified by comparing the sequences of the Isoforms. For example, such isolated polypeptides can comprise a segment of the contiguous amino acids (e.g., at least 3 contiguous amino acids, at least 5 contiguous amino acids, at least 8 contiguous amino acids, at least 10 contiguous amino acids, or at least 15 contiguous amino acids) that is at least 90%, at least 95%,

at least 96%, at least 97%, at least 98%, or at least 99% identical to a segment spanning the boundary of the regions encoded by exons 3 and 4 of SEQ ID NO: 15 (HSD17B13 Isoform D) when optimally aligned with SEQ ID NO: 15 to distinguish from Isoform H. Likewise, such isolated polypeptides can comprise a segment of the contiguous amino acids (e.g., at least 3 contiguous amino acids, at least 5 contiguous amino acids, at least 8 contiguous amino acids, at least 10 contiguous amino acids, or at least 15 contiguous amino acids) that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a segment within the region encoded by exon 2 in SEQ ID NO: 15 (HSD17B13 Isoform D), a segment spanning the boundary of the regions encoded by exons 1 and 2 in SEQ ID NO: 15, or a segment spanning the boundary of the regions encoded by exons 2 and 3 in SEQ ID NO: 15 when optimally aligned with SEQ ID NO: 15 to distinguish from Isoform G.

[00219] Like Isoform D, the region encoded by exon 7 in Isoform H (SEQ ID NO: 19) is frameshifted and truncated compared to Isoform A. In addition, however, Isoform H includes a region encoded by an additional exon (exon 3') between exons 3 and 4 compared to Isoforms A and D. Accordingly, such an isolated polypeptide can be as described above comprising a segment that is present in Isoforms D, G, and H (or fragments or homologs thereof) that is not present in Isoform A (or a fragment or homolog thereof) but further comprising a segment (e.g., at least 8 contiguous amino acids) from Isoform H (or a fragment or homolog thereof) that is not present in Isoform D (or a fragment or homolog thereof). Such regions can be readily identified by comparing the sequences of the Isoforms. For example, such an isolated polypeptide can further comprise a segment of the contiguous amino acids (e.g., at least 3 contiguous amino acids, at least 5 contiguous amino acids, at least 8 contiguous amino acids, at least 10 contiguous amino acids, or at least 15 contiguous amino acids) that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a segment including at least a portion of the region encoded by exon 3' in SEQ ID NO: 19 (HSD17B13 Isoform H) when the isolated polypeptide is optimally aligned with SEQ ID NO: 19.

[00220] Like Isoform D, the region encoded by exon 7 in Isoform G (SEQ ID NO: 18) is frameshifted and truncated compared to Isoform A. In addition, however, Isoform G is missing the region encoded by exon 2 compared to Isoforms A and D and thus includes an exon 1-exon 3 boundary not present in Isoforms A and D. Accordingly, such an isolated polypeptide can be as described above comprising a segment that is present in Isoforms D, G, and H (or fragments or

homologs thereof) that is not present in Isoform A (or a fragment or homolog thereof) but further comprising a segment (e.g., at least 8 contiguous amino acids) from Isoform G (or a fragment or homolog thereof) that is not present in Isoform D (or a fragment or homolog thereof). Such regions can be readily identified by comparing the sequences of the Isoforms. For example, such an isolated polypeptide can further comprise a segment of the contiguous amino acids (e.g., at least 3 contiguous amino acids, at least 5 contiguous amino acids, at least 8 contiguous amino acids, at least 10 contiguous amino acids, or at least 15 contiguous amino acids) that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a segment spanning the boundary of the regions encoded by exons 1 and 3 in SEQ ID NO: 18 (HSD17B13 Isoform G) when the isolated polypeptide is optimally aligned with SEQ ID NO: 18.

[00221] Also provided herein are isolated polypeptides comprising a segment (e.g., at least 8 contiguous amino acids) that is present in Isoform E (or a fragment or homolog thereof) that is not present in Isoform A (or a fragment or homolog thereof). Isoform E includes a region encoded by an additional exon (exon 3') between exons 3 and 4 that is not present in Isoform A. Such regions can be readily identified by comparing the sequences of the Isoforms. Accordingly, the isolated polypeptide can comprise at least 5, 6, 8, 10, 12, 14, 15, 16, 18, 20, 22, 24, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 150, or 200 contiguous amino acids of an HSD17B13 protein (e.g., at least 8 contiguous amino acids, at least 10 contiguous amino acids, or at least 15 contiguous amino acids of an HSD17B13 protein), wherein a segment of the contiguous amino acids (e.g., at least 3 contiguous amino acids, at least 5 contiguous amino acids, at least 8 contiguous amino acids, at least 10 contiguous amino acids, or at least 15 contiguous amino acids) is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a segment including at least a portion of the region encoded by exon 3' in SEQ ID NO: 16 (HSD17B13 Isoform E) or SEQ ID NO: 19 (HSD17B13 Isoform H) when the isolated polypeptide is optimally aligned with SEQ ID NO: 16 or 19, respectively. Optionally, such an isolated polypeptide can further comprise a segment (e.g., at least 8 contiguous amino acids) from Isoform E (or a fragment or homolog thereof) that is not present in Isoform H (or a fragment or homolog thereof). Such regions can be readily identified by comparing the sequences of the Isoforms. For example, such an isolated polypeptide can further comprise a segment of the contiguous amino acids (e.g., at least 3 contiguous amino acids, at

least 5 contiguous amino acids, at least 8 contiguous amino acids, at least 10 contiguous amino acids, or at least 15 contiguous amino acids) that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical a segment spanning the boundary of the regions encoded by exons 6 and 7 in SEQ ID NO: 16 (HSD17B13 Isoform E) when the isolated polypeptide is optimally aligned with SEQ ID NO: 16.

[00222] Also provided is an isolated polypeptide comprising a segment (e.g., at least 8 contiguous amino acids) present in Isoform F (or a fragment or homolog thereof) that is not present in Isoform A (or a fragment or homolog thereof). Isoform F includes a region encoded by read-through from exon 6 into intron 6 that is not present in Isoform A. Such regions can be readily identified by comparing the sequences of the Isoforms. Accordingly, the isolated polypeptide can comprise at least 5, 6, 8, 10, 12, 14, 15, 16, 18, 20, 22, 24, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 150, or 200 contiguous amino acids of an HSD17B13 protein (e.g., at least 8 contiguous amino acids, at least 10 contiguous amino acids, or at least 15 contiguous amino acids of an HSD17B13 protein), wherein a segment of the contiguous amino acids (e.g., at least 3 contiguous amino acids, at least 5 contiguous amino acids, at least 8 contiguous amino acids, at least 10 contiguous amino acids, or at least 15 contiguous amino acids) is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a segment including at least a portion of the region encoded by the read-through into intron 6 in SEQ ID NO: 17 (HSD17B13 Isoform F) when the isolated polypeptide is optimally aligned with SEQ ID NO: 17.

[00223] Also provided is an isolated polypeptide comprising a segment (e.g., at least 8 contiguous amino acids) present in Isoform C (or a fragment or homolog thereof) that is not present in Isoform A (or a fragment or homolog thereof). Isoform C is missing the region encoded by exon 6 compared to Isoform A and includes an exon 5-exon 7 boundary not present in Isoform A. Such regions can be readily identified by comparing the sequences of the Isoforms. Accordingly, the isolated polypeptide can comprise at least 5, 6, 8, 10, 12, 14, 15, 16, 18, 20, 22, 24, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 150, or 200 contiguous amino acids of an HSD17B13 protein (e.g., at least 8 contiguous amino acids, at least 10 contiguous amino acids, or at least 15 contiguous amino acids of an HSD17B13 protein), wherein a segment of the contiguous amino acids (e.g., at least 3 contiguous amino acids, at least 5 contiguous amino acids, at least 8 contiguous amino acids, at least 10 contiguous amino acids, or at least 15

contiguous amino acids) is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a segment spanning the boundary of the regions encoded by exons 5 and 7 in SEQ ID NO: 14 (HSD17B13 Isoform C) when the isolated polypeptide is optimally aligned with SEQ ID NO: 14.

[00224] Any of the isolated polypeptides disclosed herein can be linked to a heterologous molecule or heterologous label. Examples of such heterologous molecules or labels are disclosed elsewhere herein. For example, the heterologous molecule can be an immunoglobulin Fc domain, a peptide tag as disclosed elsewhere herein, poly(ethylene glycol), polysialic acid, or glycolic acid.

(2) Methods of Producing HSD17B13 Proteins or Fragments

[00225] Also disclosed are methods of producing any of the HSD17B13 proteins or fragments thereof disclosed herein. Such HSD17B13 proteins or fragments thereof can be produced by any suitable method. For example, HSD17B13 proteins or fragments thereof can be produced from host cells comprising nucleic acids (e.g., recombinant expression vectors) encoding such HSD17B13 proteins or fragments thereof. Such methods can comprise culturing a host cell comprising a nucleic acid (e.g., recombinant expression vector) encoding an HSD17B13 protein or fragment thereof, thereby producing the HSD17B13 protein or fragment thereof. The nucleic acid can be operably linked to a promoter active in the host cell, and the culturing can be under conditions whereby the nucleic acid is expressed. Such methods can further comprise recovering the expressed HSD17B13 protein or fragment thereof. The recovering can further comprise purifying the HSD17B13 protein or fragment thereof.

[00226] Examples of suitable systems for protein expression include bacterial cell expression systems (e.g., *Escherichia coli*, *Lactococcus lactis*), yeast cell expression systems (e.g., *Saccharomyces cerevisiae*, *Pichia pastoris*), insect cell expression systems (e.g., baculovirus-mediated protein expression), and mammalian cell expression systems.

[00227] Examples of nucleic acids encoding HSD17B13 proteins or fragments thereof are disclosed in more detail elsewhere herein. Optionally, such nucleic acids are codon optimized for expression in the host cell. Optionally, such nucleic acids are operably linked to a promoter active in the host cell. The promoter can be a heterologous promoter (i.e., a promoter than is not a naturally occurring *HSD17B13* promoter). Examples of promoters suitable for *Escherichia*

coli include arabinose, *lac*, *tac*, and T7 promoters. Examples of promoters suitable for *Lactococcus lactis* include P170 and nisin promoters. Examples of promoters suitable for *Saccharomyces cerevisiae* include constitutive promoters such as alcohol dehydrogenase (ADHI) or enolase (ENO) promoters or inducible promoters such as PHO, CUP1, GAL1, and G10. Examples of promoters suitable for *Pichia pastoris* include the alcohol oxidase I (AOX I) promoter, the glyceraldehyde 3 phosphate dehydrogenase (GAP) promoter, and the glutathione dependent formaldehyde dehydrogenase (FLDI) promoter. An example of a promoter suitable for a baculovirus-mediated system is the late viral strong polyhedrin promoter.

[00228] Optionally, the nucleic acid further encodes a tag in frame with the HSD17B13 protein or fragment thereof to facilitate protein purification. Examples of tags are disclosed elsewhere herein. Such tags can, for example, bind to a partner ligand (e.g., immobilized on a resin) such that the tagged protein can be isolated from all other proteins (e.g., host cell proteins). Affinity chromatography, high performance liquid chromatography (HPLC), and size exclusion chromatography (SEC) are examples of methods that can be used to improve the purity of the expressed protein.

[00229] Other methods can also be used to produce HSD17B13 proteins or fragments thereof. For example, two or more peptides or polypeptides can be linked together by protein chemistry techniques. For example, peptides or polypeptides can be chemically synthesized using either Fmoc (9-fluorenylmethyloxycarbonyl) or Boc (*tert* -butyloxycarbonoyl) chemistry. Such peptides or polypeptides can be synthesized by standard chemical reactions. For example, a peptide or polypeptide can be synthesized and not cleaved from its synthesis resin, whereas the other fragment of a peptide or protein can be synthesized and subsequently cleaved from the resin, thereby exposing a terminal group which is functionally blocked on the other fragment. By peptide condensation reactions, these two fragments can be covalently joined via a peptide bond at their carboxyl and amino termini, respectively. (Grant GA (1992) Synthetic Peptides: A User Guide. W.H. Freeman and Co., N.Y. (1992); and Bodansky M and Trost B., Ed. (1993) Principles of Peptide Synthesis. Springer-Verlag Inc., NY, each of which is herein incorporated by reference in its entirety for all purposes). Alternatively, the peptide or polypeptide can be independently synthesized *in vivo* as described herein. Once isolated, these independent peptides or polypeptides may be linked to form a peptide or fragment thereof via similar peptide condensation reactions.

[00230] For example, enzymatic ligation of cloned or synthetic peptide segments allow relatively short peptide fragments to be joined to produce larger peptide fragments, polypeptides, or whole protein domains (Abrahmsen L et al. (1991) *Biochemistry* 30:4151, herein incorporated by reference in its entirety for all purposes). Alternatively, native chemical ligation of synthetic peptides can be utilized to synthetically construct large peptides or polypeptides from shorter peptide fragments. This method can consist of a two-step chemical reaction (Dawson et al. (1994) *Science* 266:776-779, herein incorporated by reference in its entirety for all purposes). The first step can be the chemoselective reaction of an unprotected synthetic peptide--thioester with another unprotected peptide segment containing an amino-terminal Cys residue to give a thioester-linked intermediate as the initial covalent product. Without a change in the reaction conditions, this intermediate can undergo spontaneous, rapid intramolecular reaction to form a native peptide bond at the ligation site (Baggiolini et al. (1992) *FEBS Lett* 307:97-101; Clark-Lewis et al. (1994) *J Biol Chem* 269:16075; Clark-Lewis et al. (1991) *Biochemistry* 30:3128; and Rajarathnam et al. (1994) *Biochemistry* 33:6623-6630, each of which is herein incorporated by reference in its entirety for all purposes).

[00231] Alternatively, unprotected peptide segments can be chemically linked where the bond formed between the peptide segments as a result of the chemical ligation is an unnatural (non-peptide) bond (Schnolzer et al. (1992) *Science* 256:221, herein incorporated by reference in its entirety for all purposes). This technique has been used to synthesize analogs of protein domains as well as large amounts of relatively pure proteins with full biological activity (deLisle Milton RC et al., Techniques in Protein Chemistry IV. Academic Press, New York, pp. 257-267 (1992), herein incorporated by reference in its entirety for all purposes).

C. Cells

[00232] Also provided herein are cells (e.g., recombinant host cells) comprising any of the nucleic acids and proteins disclosed herein. The cells can be *in vitro*, *ex vivo*, or *in vivo*. Nucleic acids can be linked to a promoter and other regulatory sequences so they are expressed to produce an encoded protein. Any type of cell is provided.

[00233] The cell can be, for example, a totipotent cell or a pluripotent cell (e.g., an embryonic stem (ES) cell such as a rodent ES cell, a mouse ES cell, or a rat ES cell). Totipotent cells include undifferentiated cells that can give rise to any cell type, and pluripotent cells include

undifferentiated cells that possess the ability to develop into more than one differentiated cell types. Such pluripotent and/or totipotent cells can be, for example, ES cells or ES-like cells, such as an induced pluripotent stem (iPS) cells. ES cells include embryo-derived totipotent or pluripotent cells that are capable of contributing to any tissue of the developing embryo upon introduction into an embryo. ES cells can be derived from the inner cell mass of a blastocyst and are capable of differentiating into cells of any of the three vertebrate germ layers (endoderm, ectoderm, and mesoderm).

[00234] The cell can also be a primary somatic cell, or a cell that is not a primary somatic cell. Somatic cells can include any cell that is not a gamete, germ cell, gametocyte, or undifferentiated stem cell. The cell can also be a primary cell. Primary cells include cells or cultures of cells that have been isolated directly from an organism, organ, or tissue. Primary cells include cells that are neither transformed nor immortal. They include any cell obtained from an organism, organ, or tissue which was not previously passed in tissue culture or has been previously passed in tissue culture but is incapable of being indefinitely passed in tissue culture. Such cells can be isolated by conventional techniques and include, for example, somatic cells, hematopoietic cells, endothelial cells, epithelial cells, fibroblasts, mesenchymal cells, keratinocytes, melanocytes, monocytes, mononuclear cells, adipocytes, preadipocytes, neurons, glial cells, hepatocytes, skeletal myoblasts, and smooth muscle cells. For example, primary cells can be derived from connective tissues, muscle tissues, nervous system tissues, or epithelial tissues.

[00235] Such cells also include would normally not proliferate indefinitely but, due to mutation or alteration, have evaded normal cellular senescence and instead can keep undergoing division. Such mutations or alterations can occur naturally or be intentionally induced. Examples of immortalized cells include Chinese hamster ovary (CHO) cells, human embryonic kidney cells (e.g., HEK 293 cells), and mouse embryonic fibroblast cells (e.g., 3T3 cells). Numerous types of immortalized cells are well known. Immortalized or primary cells include cells that are typically used for culturing or for expressing recombinant genes or proteins.

[00236] The cell can also be a differentiated cell, such as a liver cell (e.g., a human liver cell).

[00237] The cell can be from any source. For example, the cell can be a eukaryotic cell, an animal cell, a plant cell, or a fungal (e.g., yeast) cell. Such cells can be fish cells or bird cells, or such cells can be mammalian cells, such as human cells, non-human mammalian cells, rodent cells, mouse cells or rat cells. Mammals include, for example, humans, non-human primates,

monkeys, apes, cats dogs, horses, bulls, deer, bison, sheep, rodents (e.g., mice, rats, hamsters, guinea pigs), livestock (e.g., bovine species such as cows, steer, etc.; ovine species such as sheep, goats, etc.; and porcine species such as pigs and boars). Birds include, for example, chickens, turkeys, ostrich, geese, ducks, etc. Domesticated animals and agricultural animals are also included. The term "non-human animal" excludes humans.

[00238] For mouse cells, the mouse can be any strain, including, for example, from a 129 strain, a C57BL/6 strain, a BALB/c strain, a Swiss Webster strain, a mix of 129 and C57BL/6, strains, a mix of BALB/c and C57BL/6 strains, a mix of 129 and BALB/c strains, and a mix of BALB/c, C57BL/6, and 129 strains. For example, a mouse can be at least partially from a BALB/c strain (e.g., at least about 25%, at least about 50%, at least about 75% derived from a BALB/c strain, or about 25%, about 50%, about 75%, or about 100% derived from a BALB/c strain). In one example, the mouse is a strain comprising 50% BALB/c, 25% C57BL/6, and 25% 129. Alternatively, the mouse comprises a strain or strain combination that excludes BALB/c.

[00239] Examples of 129 strains include 129P1, 129P2, 129P3, 129X1, 129S1 (e.g., 129S1/SV, 129S1/Svlm), 129S2, 129S4, 129S5, 129S9/SvEvH, 129S6 (129/SvEvTac), 129S7, 129S8, 129T1, and 129T2. *See, e.g.*, Festing et al. (1999) *Mammalian Genome* 10(8):836, herein incorporated by reference in its entirety for all purposes. Examples of C57BL strains include C57BL/A, C57BL/An, C57BL/GrFa, C57BL/Kal_wN, C57BL/6, C57BL/6J, C57BL/6ByJ, C57BL/6NJ, C57BL/10, C57BL/10ScSn, C57BL/10Cr, and C57BL/Ola. Mouse cells also be from a mix of an aforementioned 129 strain and an aforementioned C57BL/6 strain (e.g., 50% 129 and 50% C57BL/6). Likewise, mouse cells can be from a mix of aforementioned 129 strains or a mix of aforementioned BL/6 strains (e.g., the 129S6 (129/SvEvTac) strain).

[00240] For rat cells, the rat can be any rat strain, including, for example, an ACI rat strain, a Dark Agouti (DA) rat strain, a Wistar rat strain, a LEA rat strain, a Sprague Dawley (SD) rat strain, or a Fischer rat strain such as Fisher F344 or Fisher F6. Rats can also be from a strain derived from a mix of two or more strains recited above. For example, the rat can be from a DA strain or an ACI strain. The ACI rat strain is characterized as having black agouti, with white belly and feet and an $RT1^{avl}$ haplotype. Such strains are available from a variety of sources including Harlan Laboratories. The Dark Agouti (DA) rat strain is characterized as having an agouti coat and an $RT1^{avl}$ haplotype. Such rats are available from a variety of sources including Charles River and Harlan Laboratories. In some cases, the rats are from an inbred rat strain.

See, e.g., US 2014/0235933 A1, herein incorporated by reference in its entirety for all purposes.

III. Methods of Modifying or Altering Expression of HSD17B13

[00241] Various methods are provided for modifying a cell through use of any combination of nuclease agents, exogenous donor sequences, transcriptional activators, transcriptional repressors, antisense molecules such as antisense RNA, siRNA, and shRNA, HSD17B13 proteins or fragments thereof, and expression vectors for expressing a recombinant *HSD17B13* gene or a nucleic acid encoding an HSD17B13 protein. The methods can occur *in vitro*, *ex vivo*, or *in vivo*. The nuclease agents, exogenous donor sequences, transcriptional activators, transcriptional repressors, antisense molecules such as antisense RNA, siRNA, and shRNA, HSD17B13 proteins or fragments thereof, and expression vectors can be introduced into the cell in any form and by any means as described elsewhere herein, and all or some can be introduced simultaneously or sequentially in any combination. Some methods involve only altering an endogenous *HSD17B13* gene in a cell. Some methods involve only altering expression of an endogenous *HSD17B13* gene through use of transcriptional activators or repressors or through use of antisense molecules such as antisense RNA, siRNA, and shRNA. Some methods involve only introducing a recombinant *HSD17B13* gene or nucleic acid encoding an HSD17B13 protein or fragment thereof into a cell. Some methods involve only introducing an HSD17B13 protein or fragment thereof into a cell (e.g., any one of or any combination of the HSD17B13 proteins or fragments thereof disclosed herein or any one of or any combination of HSD17B13 Isoforms A-H or fragments thereof disclosed herein). For example, such methods can involve introducing one or more of HSD17B13 Isoforms C, D, F, G, and H (or fragments thereof) into a cell or introducing HSD17B13 Isoform D (or a fragment thereof) into a cell. Alternatively, such methods can involve introducing one or more of HSD17B13 Isoforms A, B, and E or Isoforms A, B, E, and F' (or fragments thereof) into a cell or introducing HSD17B13 Isoform A (or a fragment thereof) into a cell. Other methods can involve both altering an endogenous *HSD17B13* gene in a cell and introducing an HSD17B13 protein or fragment thereof or recombinant *HSD17B13* gene or nucleic acid encoding an HSD17B13 protein or fragment thereof into the cell. Yet other methods can involve both altering expression of an endogenous *HSD17B13* gene in a cell and introducing an HSD17B13 protein or fragment thereof or recombinant *HSD17B13* gene or nucleic acid encoding an HSD17B13 protein or fragment

thereof into the cell.

A. Methods of Modifying *HSD17B13* Nucleic Acids

[00242] Various methods are provided for modifying an *HSD17B13* gene in a genome within a cell (e.g., a pluripotent cell or a differentiated cell such as a liver cell) through use of nuclease agents and/or exogenous donor sequences. The methods can occur *in vitro*, *ex vivo*, or *in vivo*. The nuclease agent can be used alone or in combination with an exogenous donor sequence. Alternatively, the exogenous donor sequence can be used alone or in combination with a nuclease agent.

[00243] Repair in response to double-strand breaks (DSBs) occurs principally through two conserved DNA repair pathways: non-homologous end joining (NHEJ) and homologous recombination (HR). *See* Kasparek & Humphrey (2011) *Seminars in Cell & Dev. Biol.* 22:886-897, herein incorporated by reference in its entirety for all purposes. NHEJ includes the repair of double-strand breaks in a nucleic acid by direct ligation of the break ends to one another or to an exogenous sequence without the need for a homologous template. Ligation of non-contiguous sequences by NHEJ can often result in deletions, insertions, or translocations near the site of the double-strand break.

[00244] Repair of a target nucleic acid (e.g., an *HSD17B13* gene) mediated by an exogenous donor sequence can include any process of exchange of genetic information between the two polynucleotides. For example, NHEJ can also result in the targeted integration of an exogenous donor sequence through direct ligation of the break ends with the ends of the exogenous donor sequence (i.e., NHEJ-based capture). Such NHEJ-mediated targeted integration can be preferred for insertion of an exogenous donor sequence when homology directed repair (HDR) pathways are not readily usable (e.g., in non-dividing cells, primary cells, and cells which perform homology-based DNA repair poorly). In addition, in contrast to homology-directed repair, knowledge concerning large regions of sequence identity flanking the cleavage site (beyond the overhangs created by Cas-mediated cleavage) is not needed, which can be beneficial when attempting targeted insertion into organisms that have genomes for which there is limited knowledge of the genomic sequence. The integration can proceed via ligation of blunt ends between the exogenous donor sequence and the cleaved genomic sequence, or via ligation of sticky ends (i.e., having 5' or 3' overhangs) using an exogenous donor sequence that is flanked

by overhangs that are compatible with those generated by the Cas protein in the cleaved genomic sequence. *See, e.g.*, US 2011/020722, WO 2014/033644, WO 2014/089290, and Maresca *et al.* (2013) *Genome Res.* 23(3):539-546, each of which is herein incorporated by reference in its entirety for all purposes. If blunt ends are ligated, target and/or donor resection may be needed to generation regions of microhomology needed for fragment joining, which may create unwanted alterations in the target sequence.

[00245] Repair can also occur via homology directed repair (HDR) or homologous recombination (HR). HDR or HR includes a form of nucleic acid repair that can require nucleotide sequence homology, uses a "donor" molecule as a template for repair of a "target" molecule (i.e., the one that experienced the double-strand break), and leads to transfer of genetic information from the donor to target. Without wishing to be bound by any particular theory, such transfer can involve mismatch correction of heteroduplex DNA that forms between the broken target and the donor, and/or synthesis-dependent strand annealing, in which the donor is used to resynthesize genetic information that will become part of the target, and/or related processes. In some cases, the donor polynucleotide, a portion of the donor polynucleotide, a copy of the donor polynucleotide, or a portion of a copy of the donor polynucleotide integrates into the target DNA. *See* Wang *et al.* (2013) *Cell* 153:910-918; Mandalos *et al.* (2012) *PLOS ONE* 7:e45768:1-9; and Wang *et al.* (2013) *Nat Biotechnol.* 31:530-532, each of which is herein incorporated by reference in its entirety for all purposes.

[00246] Targeted genetic modifications to an *HSD17B13* gene in a genome can be generated by contacting a cell with an exogenous donor sequence comprising a 5' homology arm that hybridizes to a 5' target sequence at a target genomic locus within the *HSD17B13* gene and a 3' homology arm that hybridizes to a 3' target sequence at the target genomic locus within the *HSD17B13* gene. The exogenous donor sequence can recombine with the target genomic locus to generate the targeted genetic modification to the *HSD17B13* gene. As one example, the 5' homology arm can hybridize to a target sequence 5' of the position corresponding to position 12666 of SEQ ID NO: 2, and the 3' homology arm can hybridize to a target sequence 3' of the position corresponding to position 12666 of SEQ ID NO: 2 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 2. Such methods can result, for example, in an *HSD17B13* gene in which a thymine is inserted between nucleotides corresponding to positions 12665 and 12666 of SEQ ID NO: 1 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 1 (or

an adenine is inserted at the corresponding position on the opposite strand). As another example, the 5' and 3' homology arms can hybridize to 5' and 3' target sequences, respectively, at positions corresponding to those flanking exon 6 in SEQ ID NO: 1 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 1. Such methods can result, for example, in an *HSD17B13* gene in which a sequence corresponding to exon 6 of SEQ ID NO: 1 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 1 has been deleted. As another example, the 5' and 3' homology arms can hybridize to 5' and 3' target sequences, respectively, at positions corresponding to those flanking exon 2 in SEQ ID NO: 1 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 1. Such methods can result, for example, in an *HSD17B13* gene in which a sequence corresponding to exon 2 of SEQ ID NO: 1 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 1 has been deleted. As another example, the 5' and 3' homology arms can hybridize to 5' and 3' target sequences, respectively, at positions corresponding to the exon 6/intron 6 boundary in SEQ ID NO: 1 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 1. As another example, the 5' and 3' homology arms can hybridize to 5' and 3' target sequences, respectively, at positions corresponding to exon 6 and exon 7 in SEQ ID NO: 1 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 1. Such methods can result, for example, in an *HSD17B13* gene in which a thymine is inserted between nucleotides corresponding to positions 12665 and 12666 of SEQ ID NO: 1 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 1 (or an adenine is inserted at the corresponding position on the opposite strand). As another example, the 5' and 3' homology arms can hybridize to 5' and 3' target sequences, respectively, at positions corresponding to those flanking or within the region corresponding to the donor splice site in intron 6 of SEQ ID NO: 1 (i.e., the region at the 5' end of intron 6 in SEQ ID NO: 1). Such methods can result, for example, in an *HSD17B13* gene in which the donor splice site in intron 6 is disrupted. Examples of exogenous donor sequences are disclosed elsewhere herein.

[00247] Targeted genetic modifications to an *HSD17B13* gene in a genome can also be generated by contacting a cell with a nuclease agent that induces one or more nicks or double-strand breaks at a target sequence at a target genomic locus within the *HSD17B13* gene. Such methods can result, for example, in an *HSD17B13* gene in which the region corresponding to the donor splice site in intron 6 of SEQ ID NO: 1 is disrupted (i.e., the region at the 5' end of intron 6 in SEQ ID NO: 1). Examples and variations of nuclease agents that can be used in the methods

are described elsewhere herein.

[00248] For example, targeted genetic modifications to an *HSD17B13* gene in a genome can be generated by contacting a cell or the genome of a cell with a Cas protein and one or more guide RNAs that hybridize to one or more guide RNA recognition sequences within a target genomic locus in the *HSD17B13* gene. That is, targeted genetic modifications to an *HSD17B13* gene in a genome can be generated by contacting a cell or the genome of a cell with a Cas protein and one or more guide RNAs that target one or more guide RNA target sequences within a target genomic locus in the *HSD17B13* gene. For example, such methods can comprise contacting a cell with a Cas protein and a guide RNA that target a guide RNA target sequence within the *HSD17B13* gene. As one example, the guide RNA target sequence is within a region corresponding to exon 6 and/or intron 6 of SEQ ID NO: 2 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 2. As one example, the guide RNA target sequence is within a region corresponding to exon 6 and/or intron 6 and/or exon 7 (e.g., exon 6 and/or intron 6, or exon 6 and/or exon 7), of SEQ ID NO: 2 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 2. As another example, the guide RNA target sequence can includes or is proximate to a position corresponding to position 12666 of SEQ ID NO: 2 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 2. For example, the guide RNA target sequence can be within about 1000, 500, 400, 300, 200, 100, 50, 45, 40, 35, 30, 25, 20, 15, 10, or 5 nucleotides of the position corresponding to position 12666 of SEQ ID NO: 2 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 2. As yet another example, the guide RNA target sequence can include or be proximate to the start codon of an *HSD17B13* gene or the stop codon of an *HSD17B13* gene. For example, the guide RNA target sequence can be within about 10, 20, 30, 40, 50, 100, 200, 300, 400, 500, or 1,000 nucleotides of the start codon or the stop codon. The Cas protein and the guide RNA form a complex, and the Cas protein cleaves the guide RNA target sequence. Cleavage by the Cas protein can create a double-strand break or a single-strand break (e.g., if the Cas protein is a nickase). Such methods can result, for example, in an *HSD17B13* gene in which the region corresponding to the donor splice site in intron 6 of SEQ ID NO: 1 is disrupted (i.e., the region at the 5' end of intron 6 in SEQ ID NO: 1), the start codon is disrupted, the stop codon is disrupted, or the coding sequence is deleted. Examples and variations of Cas (e.g., Cas9) proteins and guide RNAs that can be used in the methods are described elsewhere herein.

[00249] In some methods, two or more nuclease agents can be used. For example, two nuclease agents can be used, each targeting a nuclease target sequence within a region corresponding to exon 6 and/or intron 6, or exon 6 and/or exon 7, of SEQ ID NO: 2 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 2, or including or proximate to a position corresponding to position 12666 of SEQ ID NO: 2 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 2 (e.g., within about 1000, 500, 400, 300, 200, 100, 50, 45, 40, 35, 30, 25, 20, 15, 10, or 5 nucleotides of the position corresponding to position 12666 of SEQ ID NO: 2 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 2). For example, two nuclease agents can be used, each targeting a nuclease target sequence within a region corresponding to exon 6 and/or intron 6 and/or exon 7, of SEQ ID NO: 2 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 2. As another example, two or more nuclease agents can be used, each targeting a nuclease target sequence including or proximate to the start codon. As another example, two nuclease agents can be used, one targeting a nuclease target sequence including or proximate to the start codon, and one targeting a nuclease target sequence including or proximate to the stop codon, wherein cleavage by the nuclease agents can result in deletion of the coding region between the two nuclease target sequences. As yet another example, three or more nuclease agents can be used, with one or more (e.g., two) targeting nuclease target sequences including or proximate to the start codon, and one or more (e.g., two) targeting nuclease target sequences including or proximate to the stop codon, wherein cleavage by the nuclease agents can result in deletion of the coding region between the nuclease target sequences including or proximate to the start codon and the nuclease target sequence including or proximate to the stop codon.

[00250] Optionally, the cell can be further contacted with one or more additional guide RNAs that target additional guide RNA target sequences within the target genomic locus in the *HSD17B13* gene. By contacting the cell with one or more additional guide RNAs (e.g., a second guide RNA that target a second guide RNA target sequence), cleavage by the Cas protein can create two or more double-strand breaks or two or more single-strand breaks (e.g., if the Cas protein is a nickase).

[00251] Optionally, the cell can additionally be contacted with one or more exogenous donor sequences which recombine with the target genomic locus in the *HSD17B13* gene to generate a targeted genetic modification. Examples and variations of exogenous donor sequences that can

be used in the methods are disclosed elsewhere herein.

[00252] The Cas protein, guide RNA(s), and exogenous donor sequence(s) can be introduced into the cell in any form and by any means as described elsewhere herein, and all or some of the Cas protein, guide RNA(s), and exogenous donor sequence(s) can be introduced simultaneously or sequentially in any combination.

[00253] In some such methods, the repair of the target nucleic acid (e.g., the *HSD17B13* gene) by the exogenous donor sequence occurs via homology-directed repair (HDR). Homology-directed repair can occur when the Cas protein cleaves both strands of DNA in the *HSD17B13* gene to create a double-strand break, when the Cas protein is a nickase that cleaves one strand of DNA in the target nucleic acid to create a single-strand break, or when Cas nickases are used to create a double-strand break formed by two offset nicks. In such methods, the exogenous donor sequence comprises 5' and 3' homology arms corresponding to 5' and 3' target sequences. The guide RNA target sequence(s) or cleavage site(s) can be adjacent to the 5' target sequence, adjacent to the 3' target sequence, adjacent to both the 5' target sequence and the 3' target sequence, or adjacent to neither the 5' target sequence nor the 3' target sequence. Optionally, the exogenous donor sequence can further comprise a nucleic acid insert flanked by the 5' and 3' homology arms, and the nucleic acid insert is inserted between the 5' and 3' target sequences. If no nucleic acid insert is present, the exogenous donor sequence can function to delete the genomic sequence between the 5' and 3' target sequences. Examples of exogenous donor sequences are disclosed elsewhere herein.

[00254] Alternatively, the repair of the *HSD17B13* gene mediated by the exogenous donor sequence can occur via non-homologous end joining (NHEJ)-mediated ligation. In such methods, at least one end of the exogenous donor sequence comprises a short single-stranded region that is complementary to at least one overhang created by Cas-mediated cleavage in the *HSD17B13* gene. The complementary end in the exogenous donor sequence can flank a nucleic acid insert. For example, each end of the exogenous donor sequence can comprise a short single-stranded region that is complementary to an overhang created by Cas-mediated cleavage in the *HSD17B13* gene, and these complementary regions in the exogenous donor sequence can flank a nucleic acid insert.

[00255] Overhangs (i.e., staggered ends) can be created by resection of the blunt ends of a double-strand break created by Cas-mediated cleavage. Such resection can generate the regions

of microhomology needed for fragment joining, but this can create unwanted or uncontrollable alterations in the *HSD17B13* gene. Alternatively, such overhangs can be created by using paired Cas nickases. For example, the cell can be contacted with first and second nickases that cleave opposite strands of DNA, whereby the genome is modified through double nicking. This can be accomplished by contacting a cell with a first Cas protein nickase, a first guide RNA that target a first guide RNA target sequence within the target genomic locus in the *HSD17B13* gene, a second Cas protein nickase, and a second guide RNA that targets a second guide RNA target sequence within target genomic locus in the *HSD17B13* gene. The first Cas protein and the first guide RNA form a first complex, and the second Cas protein and the second guide RNA form a second complex. The first Cas protein nickase cleaves a first strand of genomic DNA within the first guide RNA target sequence, the second Cas protein nickase cleaves a second strand of genomic DNA within the second guide RNA target sequence, and optionally the exogenous donor sequence recombines with the target genomic locus in the *HSD17B13* gene to generate the targeted genetic modification.

[00256] The first nickase can cleave a first strand of genomic DNA (i.e., the complementary strand), and the second nickase can cleave a second strand of genomic DNA (i.e., the non-complementary strand). The first and second nickases can be created, for example, by mutating a catalytic residue in the RuvC domain (e.g., the D10A mutation described elsewhere herein) of Cas9 or mutating a catalytic residue in the HNH domain (e.g., the H840A mutation described elsewhere herein) of Cas9. In such methods, the double nicking can be employed to create a double-strand break having staggered ends (i.e., overhangs). The first and second guide RNA target sequences can be positioned to create a cleavage site such that the nicks created by the first and second nickases on the first and second strands of DNA create a double-strand break. Overhangs are created when the nicks within the first and second CRISPR RNA target sequences are offset. The offset window can be, for example, at least about 5 bp, 10 bp, 20 bp, 30 bp, 40 bp, 50 bp, 60 bp, 70 bp, 80 bp, 90 bp, 100 bp or more. *See, e.g.*, Ran *et al.* (2013) *Cell* 154:1380-1389; Mali *et al.* (2013) *Nat. Biotech.*31:833-838; and Shen *et al.* (2014) *Nat. Methods* 11:399-404, each of which is herein incorporated by reference in its entirety for all purposes.

(1) Types of Targeted Genetic Modifications

[00257] Various types of targeted genetic modifications can be introduced using the methods

described herein. Such targeted modifications can include, for example, additions of one or more nucleotides, deletions of one or more nucleotides, substitutions of one or more nucleotides, a point mutation, or a combination thereof. For example, at least 1, 2, 3, 4, 5, 7, 8, 9, 10 or more nucleotides can be changed (e.g., deleted, inserted, or substituted) to form the targeted genomic modification. The deletions, insertions, or substitutions can be of any size, as disclosed elsewhere herein. *See, e.g.*, Wang et al. (2013) *Cell* 153:910-918; Mandalos et al. (2012) *PLOS ONE* 7:e45768:1-9; and Wang et al. (2013) *Nat Biotechnol.* 31:530-532, each of which is herein incorporated by reference in its entirety for all purposes.

[00258] Such targeted genetic modifications can result in disruption of a target genomic locus. Disruption can include alteration of a regulatory element (e.g., promoter or enhancer), a missense mutation, a nonsense mutation, a frame-shift mutation, a truncation mutation, a null mutation, or an insertion or deletion of small number of nucleotides (e.g., causing a frameshift mutation), and it can result in inactivation (i.e., loss of function) or loss of an allele. For example, a targeted modification can comprise disruption of the start codon of an *HSD17B13* gene such that the start codon is no longer functional.

[00259] In a specific example, a targeted modification can comprise a deletion between first and second guide RNA target sequences or Cas cleavage sites. If an exogenous donor sequence (e.g., repair template or targeting vector) is used, the modification can comprise a deletion between first and second guide RNA target sequences or Cas cleavage sites as well as an insertion of a nucleic acid insert between the 5' and 3' target sequences.

[00260] Alternatively, if an exogenous donor sequence is used, alone or in combination with a nuclease agent, the modification can comprise a deletion between the 5' and 3' target sequences as well as an insertion of a nucleic acid insert between the 5' and 3' target sequences in the pair of first and second homologous chromosomes, thereby resulting in a homozygous modified genome. Alternatively, if the exogenous donor sequence comprises 5' and 3' homology arms with no nucleic acid insert, the modification can comprise a deletion between the 5' and 3' target sequences.

[00261] The deletion between the first and second guide RNA target sequences or the deletion between the 5' and 3' target sequences can be a precise deletion wherein the deleted nucleic acid consists of only the nucleic acid sequence between the first and second nuclease cleavage sites or only the nucleic acid sequence between the 5' and 3' target sequences such that there are no

additional deletions or insertions at the modified genomic target locus. The deletion between the first and second guide RNA target sequences can also be an imprecise deletion extending beyond the first and second nuclease cleavage sites, consistent with imprecise repair by non-homologous end joining (NHEJ), resulting in additional deletions and/or insertions at the modified genomic locus. For example, the deletion can extend about 1 bp, about 2 bp, about 3 bp, about 4 bp, about 5 bp, about 10 bp, about 20 bp, about 30 bp, about 40 bp, about 50 bp, about 100 bp, about 200 bp, about 300 bp, about 400 bp, about 500 bp, or more beyond the first and second Cas protein cleavage sites. Likewise, the modified genomic locus can comprise additional insertions consistent with imprecise repair by NHEJ, such as insertions of about 1 bp, about 2 bp, about 3 bp, about 4 bp, about 5 bp, about 10 bp, about 20 bp, about 30 bp, about 40 bp, about 50 bp, about 100 bp, about 200 bp, about 300 bp, about 400 bp, about 500 bp, or more.

[00262] The targeted genetic modification can be, for example, a biallelic modification or a monoallelic modification. Biallelic modifications include events in which the same modification is made to the same locus on corresponding homologous chromosomes (e.g., in a diploid cell), or in which different modifications are made to the same locus on corresponding homologous chromosomes. In some methods, the targeted genetic modification is a monoallelic modification. A monoallelic modification includes events in which a modification is made to only one allele (i.e., a modification to the *HSD17B13* gene in only one of the two homologous chromosomes). Homologous chromosomes include chromosomes that have the same genes at the same loci but possibly different alleles (e.g., chromosomes that are paired during meiosis). The term allele includes any of one or more alternative forms of a genetic sequence. In a diploid cell or organism, the two alleles of a given sequence typically occupy corresponding loci on a pair of homologous chromosomes.

[00263] A monoallelic mutation can result in a cell that is heterozygous for the targeted *HSD17B13* modification. Heterozygosity includes situation in which only one allele of the *HSD17B13* gene (i.e., corresponding alleles on both homologous chromosomes) have the targeted modification.

[00264] A biallelic modification can result in homozygosity for a targeted modification. Homozygosity includes situations in which both alleles of the *HSD17B13* gene (i.e., corresponding alleles on both homologous chromosomes) have the targeted modification. Alternatively, a biallelic modification can result in compound heterozygosity (e.g.,

hemizygosity) for the targeted modification. Compound heterozygosity includes situations in which both alleles of the target locus (i.e., the alleles on both homologous chromosomes) have been modified, but they have been modified in different ways (e.g., a targeted modification in one allele and inactivation or disruption of the other allele). For example, in the allele without the targeted modification, a double-strand break created by the Cas protein may have been repaired by non-homologous end joining (NHEJ)-mediated DNA repair, which generates a mutant allele comprising an insertion or a deletion of a nucleic acid sequence and thereby causes disruption of that genomic locus. For example, a biallelic modification can result in compound heterozygosity if the cell has one allele with the targeted modification and another allele that is not capable of being expressed. Compound heterozygosity includes hemizygosity. Hemizygosity includes situations in which only one allele (i.e., an allele on one of two homologous chromosomes) of the target locus is present. For example, a biallelic modification can result in hemizygosity for a targeted modification if the targeted modification occurs in one allele with a corresponding loss or deletion of the other allele.

(2) Identifying Cells with Targeted Genetic Modifications

[00265] The methods disclosed herein can further comprise identifying a cell having a modified *HSD17B13* gene. Various methods can be used to identify cells having a targeted genetic modification, such as a deletion or an insertion. Such methods can comprise identifying one cell having the targeted genetic modification in the *HSD17B13* gene. Screening can be done to identify such cells with modified genomic loci.

[00266] The screening step can comprise a quantitative assay for assessing modification of allele (MOA) (e.g., loss-of-allele (LOA) and/or gain-of-allele (GOA) assays) of a parental chromosome. For example, the quantitative assay can be carried out via a quantitative PCR, such as a real-time PCR (qPCR). The real-time PCR can utilize a first primer set that recognizes the target genomic locus and a second primer set that recognizes a non-targeted reference locus. The primer set can comprise a fluorescent probe that recognizes the amplified sequence. The loss-of-allele (LOA) assay inverts the conventional screening logic and quantifies the number of copies of the native locus to which the mutation was directed. In a correctly targeted cell clone, the LOA assay detects one of the two native alleles (for genes not on the X or Y chromosome), the other allele being disrupted by the targeted modification. The same principle can be applied

in reverse as a gain-of-allele (GOA) assay to quantify the copy number of the inserted targeting vector. For example, the combined use of GOA and LOA assays will reveal a correctly targeted heterozygous clone as having lost one copy of the native target gene and gained one copy of the drug resistance gene or other inserted marker.

[00267] As an example, quantitative polymerase chain reaction (qPCR) can be used as the method of allele quantification, but any method that can reliably distinguish the difference between zero, one, and two copies of the target gene or between zero, one, and two copies of the nucleic acid insert can be used to develop a MOA assay. For example, TAQMAN® can be used to quantify the number of copies of a DNA template in a genomic DNA sample, especially by comparison to a reference gene (*see, e.g.*, US 6,596,541, herein incorporated by reference in its entirety for all purposes). The reference gene is quantitated in the same genomic DNA as the target gene(s) or locus(loci). Therefore, two TAQMAN® amplifications (each with its respective probe) are performed. One TAQMAN® probe determines the "Ct" (Threshold Cycle) of the reference gene, while the other probe determines the Ct of the region of the targeted gene(s) or locus(loci) which is replaced by successful targeting (i.e., a LOA assay). The Ct is a quantity that reflects the amount of starting DNA for each of the TAQMAN® probes, i.e. a less abundant sequence requires more cycles of PCR to reach the threshold cycle. Decreasing by half the number of copies of the template sequence for a TAQMAN® reaction will result in an increase of about one Ct unit. TAQMAN® reactions in cells where one allele of the target gene(s) or locus(loci) has been replaced by homologous recombination will result in an increase of one Ct for the target TAQMAN® reaction without an increase in the Ct for the reference gene when compared to DNA from non-targeted cells. For a GOA assay, another TAQMAN® probe can be used to determine the Ct of the nucleic acid insert that is replacing the targeted gene(s) or locus(loci) by successful targeting.

[00268] Other examples of suitable quantitative assays include fluorescence-mediated in situ hybridization (FISH), comparative genomic hybridization, isothermic DNA amplification, quantitative hybridization to an immobilized probe(s), INVADER® Probes, TAQMAN® Molecular Beacon probes, or ECLIPSE™ probe technology (*see, e.g.*, US 2005/0144655, herein incorporated by reference in its entirety for all purposes). Conventional assays for screening for targeted modifications, such as long-range PCR, Southern blotting, or Sanger sequencing, can also be used. Such assays typically are used to obtain evidence for a linkage between the

inserted targeting vector and the targeted genomic locus. For example, for a long-range PCR assay, one primer can recognize a sequence within the inserted DNA while the other recognizes a target genomic locus sequence beyond the ends of the targeting vector's homology arms.

[00269] Next generation sequencing (NGS) can also be used for screening. Next-generation sequencing can also be referred to as "NGS" or "massively parallel sequencing" or "high throughput sequencing." In the methods disclosed herein, it is not necessary to screen for targeted cells using selection markers. For example, the MOA and NGS assays described herein can be relied on without using selection cassettes.

B. Methods of Altering Expression of *HSD17B13* Nucleic Acids

[00270] Various methods are provided for altering expression of nucleic acids encoding HSD17B13 proteins. In some methods, expression is altered through cleavage with a nuclease agent to cause disruption of the nucleic acid encoding the HSD17B13 protein, as described in further detail elsewhere herein. In some methods, expression is altered through use of a DNA-binding protein fused or linked to a transcription activation domain or a transcription repression domain. In some methods, expression is altered through use of RNA interference compositions, such as antisense RNA, shRNA, or siRNA.

[00271] In one example, expression of an *HSD17B13* gene or a nucleic acid encoding an HSD17B13 protein can be modified by contacting a cell or the genome within a cell with a nuclease agent that induces one or more nicks or double-strand breaks at a target sequence at a target genomic locus within the *HSD17B13* gene or nucleic acid encoding an HSD17B13 protein. Such cleavage can result in disruption of expression of the *HSD17B13* gene or nucleic acid encoding an HSD17B13 protein. For example, the nuclease target sequence can include or be proximate to the start codon of an *HSD17B13* gene. For example, the target sequence can be within about 10, 20, 30, 40, 50, 100, 200, 300, 400, 500, or 1,000 nucleotides of the start codon, and cleavage by the nuclease agent can disrupt the start codon. As another example, two or more nuclease agents can be used, each targeting a nuclease target sequence including or proximate to the start codon. As another example, two nuclease agents can be used, one targeting a nuclease target sequence including or proximate to the start codon, and one targeting a nuclease target sequence including or proximate to the stop codon, wherein cleavage by the nuclease agents can result in deletion of the coding region between the two nuclease target sequences. As yet another

example, three or more nuclease agents can be used, with one or more (e.g., two) targeting nuclease target sequences including or proximate to the start codon, and one or more (e.g., two) targeting nuclease target sequences including or proximate to the stop codon, wherein cleavage by the nuclease agents can result in deletion of the coding region between the nuclease target sequences including or proximate to the start codon and the nuclease target sequence including or proximate to the stop codon. Other examples of modifying an *HSD17B13* gene or a nucleic acid encoding an HSD17B13 protein are disclosed elsewhere herein.

[00272] In another example, expression of an *HSD17B13* gene or a nucleic acid encoding an HSD17B13 protein can be modified by contacting a cell or the genome within a cell with a DNA-binding protein that binds to a target genomic locus within the *HSD17B13* gene. The DNA-binding protein can be, for example, a nuclease-inactive Cas protein fused to a transcriptional activator domain or a transcriptional repressor domain. Other examples of DNA-binding proteins include zinc finger proteins fused to a transcriptional activator domain or a transcriptional repressor domain, or Transcription Activator-Like Effector (TALE) proteins fused to a transcriptional activator domain or a transcriptional repressor domain. Examples of such proteins are disclosed elsewhere herein. For example, in some methods, a transcriptional repressor can be used to decrease expression of a wild type *HSD17B13* gene or an *HSD17B13* gene that is not the rs72613567 variant (e.g., to decrease expression of *HSD17B13* Transcript or Isoform A). Likewise, in some methods, a transcriptional activator can be used to increase expression of an *HSD17B13* gene rs72613567 variant gene (e.g., to increase expression of *HSD17B13* Transcript or Isoform D).

[00273] The target sequence (e.g., guide RNA target sequence) for the DNA-binding protein can be anywhere within the *HSD17B13* gene or a nucleic acid encoding an HSD17B13 protein suitable for altering expression. As one example, the target sequence can be within a regulatory element, such as an enhancer or promoter, or can be in proximity to a regulatory element. For example, the target sequence can include or be proximate to the start codon of an *HSD17B13* gene. For example, the target sequence can be within about 10, 20, 30, 40, 50, 100, 200, 300, 400, 500, or 1,000 nucleotides of the start codon.

[00274] In another example, antisense molecules can be used to alter expression of an *HSD17B13* gene or a nucleic acid encoding an HSD17B13 protein. Examples of antisense molecules include antisense RNAs, small interfering RNAs (siRNAs), and short hairpin RNAs

(shRNAs). Such antisense RNAs, siRNAs, or shRNAs can be designed to target any region of an mRNA. For example, the antisense RNAs, siRNAs, or shRNAs can be designed to target a region unique to one or more of the HSD17B13 transcripts disclosed herein, or a region common to one or more of the HSD17B13 transcripts disclosed herein. Examples of nucleic acids hybridizing to cDNAs and variant *HSD17B13* transcripts are disclosed in more detail elsewhere herein. For example, the antisense RNA, siRNA, or shRNA can hybridize to a sequence within SEQ ID NO: 4 (*HSD17B13* Transcript A). Optionally, the antisense RNA, siRNA, or shRNA can decrease expression of *HSD17B13* Transcript A in a cell. Optionally, the antisense RNA, siRNA, or shRNA hybridizes to a sequence present in SEQ ID NO: 4 (*HSD17B13* Transcript A) that is not present in SEQ ID NO: 7 (*HSD17B13* Transcript D). Optionally, the antisense RNA, siRNA, or shRNA hybridizes to a sequence within exon 7 or a sequence spanning the exon 6-exon 7 boundary of SEQ ID NO: 4 (*HSD17B13* Transcript A).

[00275] As another example, the antisense RNA, siRNA, or shRNA can hybridize to a sequence within SEQ ID NO: 7 (*HSD17B13* Transcript D). Optionally, the antisense RNA, siRNA, or shRNA can decrease expression of *HSD17B13* Transcript D in a cell. Optionally, the antisense RNA, siRNA, or shRNA hybridizes to a sequence present in SEQ ID NO: 7 (*HSD17B13* Transcript D) that is not present in SEQ ID NO: 4 (*HSD17B13* Transcript A). Optionally, the antisense RNA, siRNA, or shRNA hybridizes to a sequence within exon 7 or a sequence spanning the exon 6-exon 7 boundary of SEQ ID NO: 7 (*HSD17B13* Transcript D).

C. Introducing Nucleic Acids and Proteins into Cells

[00276] The nucleic acids and proteins disclosed herein can be introduced into a cell by any means. "Introducing" includes presenting to the cell the nucleic acid or protein in such a manner that the sequence gains access to the interior of the cell. The introducing can be accomplished by any means, and one or more of the components (e.g., two of the components, or all of the components) can be introduced into the cell simultaneously or sequentially in any combination. For example, an exogenous donor sequence can be introduced prior to the introduction of a nuclease agent, or it can be introduced following introduction of nuclease agent (e.g., the exogenous donor sequence can be administered about 1, 2, 3, 4, 8, 12, 24, 36, 48, or 72 hours before or after introduction of the nuclease agent). *See, e.g.,* US 2015/0240263 and US 2015/0110762, each of which is herein incorporated by reference in its entirety for all purposes.

Contacting the genome of a cell with a nuclease agent or exogenous donor sequence can comprise introducing one or more nuclease agents or nucleic acids encoding nuclease agents (e.g., one or more Cas proteins or nucleic acids encoding one or more Cas proteins, and one or more guide RNAs or nucleic acids encoding one or more guide RNAs (i.e., one or more CRISPR RNAs and one or more tracrRNAs)) and/or one or more exogenous donor sequences into the cell. Contacting the genome of cell (i.e., contacting a cell) can comprise introducing only one of the above components, one or more of the components, or all of the components into the cell.

[00277] A nuclease agent can be introduced into the cell in the form of a protein or in the form of a nucleic acid encoding the nuclease agent, such as an RNA (e.g., messenger RNA (mRNA)) or DNA. When introduced in the form of a DNA, the DNA can be operably linked to a promoter active in the cell. Such DNAs can be in one or more expression constructs.

[00278] For example, a Cas protein can be introduced into the cell in the form of a protein, such as a Cas protein complexed with a gRNA, or in the form of a nucleic acid encoding the Cas protein, such as an RNA (e.g., messenger RNA (mRNA)) or DNA. A guide RNA can be introduced into the cell in the form of an RNA or in the form of a DNA encoding the guide RNA. When introduced in the form of a DNA, the DNA encoding the Cas protein and/or the guide RNA can be operably linked to a promoter active in the cell. Such DNAs can be in one or more expression constructs. For example, such expression constructs can be components of a single nucleic acid molecule. Alternatively, they can be separated in any combination among two or more nucleic acid molecules (i.e., DNAs encoding one or more CRISPR RNAs, DNAs encoding one or more tracrRNAs, and DNA encoding a Cas protein can be components of separate nucleic acid molecules).

[00279] In some methods, DNA encoding a nuclease agent (e.g., a Cas protein and a guide RNA) and/or DNA encoding an exogenous donor sequence can be introduced into a cell via DNA minicircles. *See, e.g.*, WO 2014/182700, herein incorporated by reference in its entirety for all purposes. DNA minicircles are supercoiled DNA molecules that can be used for non-viral gene transfer that have neither an origin of replication nor an antibiotic selection marker. Thus, DNA minicircles are typically smaller in size than plasmid vector. These DNAs are devoid of bacterial DNA, and thus lack the unmethylated CpG motifs found in bacterial DNA.

[00280] The methods provided herein do not depend on a particular method for introducing a nucleic acid or protein into the cell, only that the nucleic acid or protein gains access to the

interior of a least one cell. Methods for introducing nucleic acids and proteins into various cell types are known and include, for example, stable transfection methods, transient transfection methods, and virus-mediated methods.

[00281] Transfection protocols as well as protocols for introducing nucleic acids or proteins into cells may vary. Non-limiting transfection methods include chemical-based transfection methods using liposomes; nanoparticles; calcium phosphate (Graham et al. (1973) *Virology* 52 (2): 456–67, Bacchetti et al. (1977) *Proc. Natl. Acad. Sci. USA* 74 (4): 1590–4, and Kriegler, M (1991). Transfer and Expression: A Laboratory Manual. New York: W. H. Freeman and Company. pp. 96–97); dendrimers; or cationic polymers such as DEAE-dextran or polyethylenimine. Non-chemical methods include electroporation, Sono-poration, and optical transfection. Particle-based transfection includes the use of a gene gun, or magnet-assisted transfection (Bertram (2006) *Current Pharmaceutical Biotechnology* 7, 277–28). Viral methods can also be used for transfection.

[00282] Introduction of nucleic acids or proteins into a cell can also be mediated by electroporation, by intracytoplasmic injection, by viral infection, by adenovirus, by adeno-associated virus, by lentivirus, by retrovirus, by transfection, by lipid-mediated transfection, or by nucleofection. Nucleofection is an improved electroporation technology that enables nucleic acid substrates to be delivered not only to the cytoplasm but also through the nuclear membrane and into the nucleus. In addition, use of nucleofection in the methods disclosed herein typically requires much fewer cells than regular electroporation (e.g., only about 2 million compared with 7 million by regular electroporation). In one example, nucleofection is performed using the LONZA® NUCLEOFECTOR™ system.

[00283] Introduction of nucleic acids or proteins into a cell can also be accomplished by microinjection. Microinjection of an mRNA is preferably into the cytoplasm (e.g., to deliver mRNA directly to the translation machinery), while microinjection of a protein or a DNA encoding a DNA encoding a Cas protein is preferably into the nucleus. Alternatively, microinjection can be carried out by injection into both the nucleus and the cytoplasm: a needle can first be introduced into the nucleus and a first amount can be injected, and while removing the needle from the cell a second amount can be injected into the cytoplasm. If a nuclease agent protein is injected into the cytoplasm, the protein preferably comprises a nuclear localization signal to ensure delivery to the nucleus/pronucleus. Methods for carrying out microinjection are

well known. *See, e.g.*, Nagy et al. (Nagy A, Gertsenstein M, Vintersten K, Behringer R., 2003, Manipulating the Mouse Embryo. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press); Meyer et al. (2010) *Proc. Natl. Acad. Sci. USA* 107:15022-15026 and Meyer et al. (2012) *Proc. Natl. Acad. Sci. USA* 109:9354-9359.

[00284] Other methods for introducing nucleic acid or proteins into a cell can include, for example, vector delivery, particle-mediated delivery, exosome-mediated delivery, lipid-nanoparticle-mediated delivery, cell-penetrating-peptide-mediated delivery, or implantable-device-mediated delivery. Methods of administering nucleic acids or proteins to a subject to modify cells in vivo are disclosed elsewhere herein.

[00285] Introduction of nucleic acids and proteins into cells can also be accomplished by hydrodynamic delivery (HDD). Hydrodynamic delivery has emerged as a near-perfect method for intracellular DNA delivery *in vivo*. For gene delivery to parenchymal cells, only essential DNA sequences need to be injected via a selected blood vessel, eliminating safety concerns associated with current viral and synthetic vectors. When injected into the bloodstream, DNA is capable of reaching cells in the different tissues accessible to the blood. Hydrodynamic delivery employs the force generated by the rapid injection of a large volume of solution into the incompressible blood in the circulation to overcome the physical barriers of endothelium and cell membranes that prevent large and membrane-impermeable compounds from entering parenchymal cells. In addition to the delivery of DNA, this method is useful for the efficient intracellular delivery of RNA, proteins, and other small compounds *in vivo*. *See, e.g.*, Bonamassa et al. (2011) *Pharm. Res.* 28(4):694-701, herein incorporated by reference in its entirety for all purposes.

[00286] Other methods for introducing nucleic acid or proteins into a cell can include, for example, vector delivery, particle-mediated delivery, exosome-mediated delivery, lipid-nanoparticle-mediated delivery, cell-penetrating-peptide-mediated delivery, or implantable-device-mediated delivery. As specific examples, a nucleic acid or protein can be introduced into a cell in a carrier such as a poly(lactic acid) (PLA) microsphere, a poly(D,L-lactic-coglycolic-acid) (PLGA) microsphere, a liposome, a micelle, an inverse micelle, a lipid cochleate, or a lipid microtubule.

[00287] The introduction of nucleic acids or proteins into the cell can be performed one time or multiple times over a period of time. For example, the introduction can be performed at least

two times over a period of time, at least three times over a period of time, at least four times over a period of time, at least five times over a period of time, at least six times over a period of time, at least seven times over a period of time, at least eight times over a period of time, at least nine times over a period of times, at least ten times over a period of time, at least eleven times, at least twelve times over a period of time, at least thirteen times over a period of time, at least fourteen times over a period of time, at least fifteen times over a period of time, at least sixteen times over a period of time, at least seventeen times over a period of time, at least eighteen times over a period of time, at least nineteen times over a period of time, or at least twenty times over a period of time.

[00288] In some cases, the cells employed in the methods and compositions have a DNA construct stably incorporated into their genome. In such cases, the contacting can comprise providing a cell with the construct already stably incorporated into its genome. For example, a cell employed in the methods disclosed herein may have a preexisting Cas-encoding gene stably incorporated into its genome (i.e., a Cas-ready cell). "Stably incorporated" or "stably introduced" or "stably integrated" includes the introduction of a polynucleotide into the cell such that the nucleotide sequence integrates into the genome of the cell and is capable of being inherited by progeny thereof. Any protocol may be used for the stable incorporation of the DNA constructs or the various components of the targeted genomic integration system.

D. Nuclease Agents and DNA-Binding Proteins

[00289] Any nuclease agent that induces a nick or double-strand break into a desired target sequence or any DNA-binding protein that binds to a desired target sequence can be used in the methods and compositions disclosed herein. A naturally occurring or native nuclease agent can be employed so long as the nuclease agent induces a nick or double-strand break in a desired target sequence. Likewise, a naturally occurring or native DNA-binding protein can be employed so long as the DNA-binding protein binds to the desired target sequence. Alternatively, a modified or engineered nuclease agent or DNA-binding protein can be employed. An "engineered nuclease agent or DNA-binding protein" includes a nuclease agent or DNA-binding protein that is engineered (modified or derived) from its native form to specifically recognize a desired target sequence. Thus, an engineered nuclease agent or DNA-binding protein can be derived from a native, naturally occurring nuclease agent or DNA-binding protein

or it can be artificially created or synthesized. The engineered nuclease agent or DNA-binding protein can recognize a target sequence, for example, wherein the target sequence is not a sequence that would have been recognized by a native (non-engineered or non-modified) nuclease agent or DNA-binding protein. The modification of the nuclease agent or DNA-binding protein can be as little as one amino acid in a protein cleavage agent or one nucleotide in a nucleic acid cleavage agent. Producing a nick or double-strand break in a target sequence or other DNA can be referred to herein as "cutting" or "cleaving" the target sequence or other DNA.

[00290] Active variants and fragments of nuclease agents or DNA-binding proteins (i.e., an engineered nuclease agent or DNA-binding protein) are also provided. Such active variants can comprise at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to the native nuclease agent or DNA-binding protein, wherein the active variants retain the ability to cut at a desired target sequence and hence retain nick or double-strand-break-inducing activity or retain the ability to bind a desired target sequence. For example, any of the nuclease agents described herein can be modified from a native endonuclease sequence and designed to recognize and induce a nick or double-strand break at a target sequence that was not recognized by the native nuclease agent. Thus, some engineered nucleases have a specificity to induce a nick or double-strand break at a target sequence that is different from the corresponding native nuclease agent target sequence. Assays for nick or double-strand-break-inducing activity are known and generally measure the overall activity and specificity of the endonuclease on DNA substrates containing the target sequence.

[00291] The term "target sequence for a nuclease agent" includes a DNA sequence at which a nick or double-strand break is induced by a nuclease agent. Likewise, the term "target sequence for a DNA-binding protein" includes a DNA sequence to which a DNA-binding protein will bind. The target sequence can be endogenous (or native) to the cell or the target sequence can be exogenous to the cell. A target sequence that is exogenous to the cell is not naturally occurring in the genome of the cell. The target sequence can also exogenous to the polynucleotides of interest that one desires to be positioned at the target locus. In some cases, the target sequence is present only once in the genome of the host cell.

[00292] Active variants and fragments of the exemplified target sequences are also provided. Such active variants can comprise at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%,

94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to the given target sequence, wherein the active variants retain biological activity and hence are capable of being recognized and cleaved by a nuclease agent in a sequence-specific manner. Assays to measure the double-strand break of a target sequence by a nuclease agent are known (e.g., TAQMAN® qPCR assay, Frendewey et al. (2010) *Methods in Enzymology* 476:295-307, herein incorporated by reference in its entirety for all purposes).

[00293] The length of the target sequence can vary, and includes, for example, target sequences that are about 30-36 bp for a zinc finger protein or zinc finger nuclease (ZFN) pair (i.e., about 15-18 bp for each ZFN), about 36 bp for a Transcription Activator-Like Effector (TALE) protein or Transcription Activator-Like Effector Nuclease (TALEN), or about 20 bp for a CRISPR/Cas9 guide RNA.

[00294] The target sequence of the DNA-binding protein or nuclease agent can be positioned anywhere in or near the target genomic locus. The target sequence can be located within a coding region of a gene (e.g., the *HSD17B13* gene), or within regulatory regions that influence the expression of the gene. A target sequence of the DNA-binding protein or nuclease agent can be located in an intron, an exon, a promoter, an enhancer, a regulatory region, or any non-protein coding region.

[00295] One type of DNA-binding protein that can be employed in the various methods and compositions disclosed herein is a Transcription Activator-Like Effector (TALE). A TALE can be fused or linked to, for example, an epigenetic modification domain, a transcriptional activation domain, or a transcriptional repressor domain. Examples of such domains are described with respect to Cas proteins, below, and can also be found, for example, in WO 2011/145121, herein incorporated by reference in its entirety for all purposes. Correspondingly, one type of nuclease agent that can be employed in the various methods and compositions disclosed herein is a Transcription Activator-Like Effector Nuclease (TALEN). TAL effector nucleases are a class of sequence-specific nucleases that can be used to make double-strand breaks at specific target sequences in the genome of a prokaryotic or eukaryotic organism. TAL effector nucleases are created by fusing a native or engineered transcription activator-like (TAL) effector, or functional part thereof, to the catalytic domain of an endonuclease such as *FokI*. The unique, modular TAL effector DNA binding domain allows for the design of proteins with potentially any given DNA recognition specificity. Thus, the DNA binding domains of the TAL

effector nucleases can be engineered to recognize specific DNA target sites and thus, used to make double-strand breaks at desired target sequences. *See* WO 2010/079430; Morbitzer et al. (2010) *Proc. Natl. Acad. Sci. U.S.A.* 107(50:21617-21622; Scholze & Boch (2010) *Virulence* 1:428-432; Christian et al. (2010) *Genetics* 186:757-761; Li et al. (2011) *Nucleic Acids Res.* 39(1):359-372; and Miller et al. (2011) *Nature Biotechnology* 29:143–148, each of which is herein incorporated by reference in its entirety for all purposes.

[00296] Examples of suitable TAL nucleases, and methods for preparing suitable TAL nucleases, are disclosed, e.g., in US 2011/0239315 A1, US 2011/0269234 A1, US 2011/0145940 A1, US 2003/0232410 A1, US 2005/0208489 A1, US 2005/0026157 A1, US 2005/0064474 A1, US 2006/0188987 A1, and US 2006/0063231 A1, each of which is herein incorporated by reference in its entirety for all purposes. In various embodiments, TAL effector nucleases are engineered that cut in or near a target nucleic acid sequence in, for example, a genomic locus of interest, wherein the target nucleic acid sequence is at or near a sequence to be modified by a an exogenous donor sequence. The TAL nucleases suitable for use with the various methods and compositions provided herein include those that are specifically designed to bind at or near target nucleic acid sequences to be modified by exogenous donor sequences as described elsewhere herein.

[00297] In some TALENs, each monomer of the TALEN comprises 33-35 TAL repeats that recognize a single base pair via two hypervariable residues. In some TALENs, the nuclease agent is a chimeric protein comprising a TAL-repeat-based DNA binding domain operably linked to an independent nuclease such as a FokI endonuclease. For example, the nuclease agent can comprise a first TAL-repeat-based DNA binding domain and a second TAL-repeat-based DNA binding domain, wherein each of the first and the second TAL-repeat-based DNA binding domains is operably linked to a FokI nuclease, wherein the first and the second TAL-repeat-based DNA binding domain recognize two contiguous target DNA sequences in each strand of the target DNA sequence separated by a spacer sequence of varying length (12-20 bp), and wherein the FokI nuclease subunits dimerize to create an active nuclease that makes a double strand break at a target sequence.

[00298] Another example of a DNA-binding protein is a zinc finger protein. Such zinc finger proteins can be linked or fused to, for example, an epigenetic modification domain, a transcriptional activation domain, or a transcriptional repressor domain. Examples of such

domains are described with respect to Cas proteins, below, and can also be found, for example, in WO 2011/145121, herein incorporated by reference in its entirety for all purposes. Correspondingly, another example of a nuclease agent that can be employed in the various methods and compositions disclosed herein is a zinc-finger nuclease (ZFN). In some ZFNs, each monomer of the ZFN comprises three or more zinc finger-based DNA binding domains, wherein each zinc finger-based DNA binding domain binds to a 3 bp subsite. In other ZFNs, the ZFN is a chimeric protein comprising a zinc finger-based DNA binding domain operably linked to an independent nuclease such as a FokI endonuclease. For example, the nuclease agent can comprise a first ZFN and a second ZFN, wherein each of the first ZFN and the second ZFN is operably linked to a FokI nuclease subunit, wherein the first and the second ZFN recognize two contiguous target DNA sequences in each strand of the target DNA sequence separated by about 5-7 bp spacer, and wherein the FokI nuclease subunits dimerize to create an active nuclease that makes a double strand break. *See, e.g.*, US 2006/0246567; US 2008/0182332; US 2002/0081614; US 2003/0021776; WO 2002/057308 A2; US 2013/0123484; US 2010/0291048; WO 2011/017293 A2; and Gaj et al. (2013) *Trends in Biotechnology* 31(7):397-405, each of which is herein incorporated by reference in its entirety for all purposes.

[00299] Other suitable DNA-binding proteins and nuclease agents for use in the methods and compositions described herein include CRISPR-Cas systems, which are described elsewhere herein.

[00300] The DNA-binding protein or nuclease agent may be introduced into the cell by any known means. A polypeptide encoding the DNA-binding protein or nuclease agent may be directly introduced into the cell. Alternatively, a polynucleotide encoding the DNA-binding protein or nuclease agent can be introduced into the cell. When a polynucleotide encoding the DNA-binding protein or nuclease agent is introduced into the cell, the DNA-binding protein or nuclease agent can be transiently, conditionally, or constitutively expressed within the cell. For example, the polynucleotide encoding the DNA-binding protein or nuclease agent can be contained in an expression cassette and be operably linked to a conditional promoter, an inducible promoter, a constitutive promoter, or a tissue-specific promoter. Such promoters are discussed in further detail elsewhere herein. Alternatively, the DNA-binding protein or nuclease agent can be introduced into the cell as an mRNA encoding a DNA-binding protein or a nuclease agent.

[00301] A polynucleotide encoding a DNA-binding protein or nuclease agent can be stably integrated in the genome of the cell and operably linked to a promoter active in the cell. Alternatively, a polynucleotide encoding a DNA-binding protein or nuclease agent can be in a targeting vector or in a vector or a plasmid that is separate from the targeting vector comprising the insert polynucleotide.

[00302] When the DNA-binding protein or nuclease agent is provided to the cell through the introduction of a polynucleotide encoding the DNA-binding protein or nuclease agent, such a polynucleotide encoding a DNA-binding protein or nuclease agent can be modified to substitute codons having a higher frequency of usage in the cell of interest, as compared to the naturally occurring polynucleotide sequence encoding the DNA-binding protein or nuclease agent. For example, the polynucleotide encoding the DNA-binding protein or nuclease agent can be modified to substitute codons having a higher frequency of usage in a given prokaryotic or eukaryotic cell of interest, including a bacterial cell, a yeast cell, a human cell, a non-human cell, a mammalian cell, a rodent cell, a mouse cell, a rat cell or any other host cell of interest, as compared to the naturally occurring polynucleotide sequence.

E. CRISPR-Cas Systems

[00303] The methods disclosed herein can utilize Clustered Regularly Interspersed Short Palindromic Repeats (CRISPR)/CRISPR-associated (Cas) systems or components of such systems to modify a genome within a cell. CRISPR-Cas systems include transcripts and other elements involved in the expression of, or directing the activity of, Cas genes. A CRISPR-Cas system can be a type I, a type II, or a type III system. Alternatively a CRISPR/Cas system can be, for example, a type V system (e.g., subtype V-A or subtype V-B). The methods and compositions disclosed herein can employ CRISPR-Cas systems by utilizing CRISPR complexes (comprising a guide RNA (gRNA) complexed with a Cas protein) for site-directed cleavage of nucleic acids.

[00304] The CRISPR-Cas systems used in the methods disclosed herein can be non-naturally occurring. A "non-naturally occurring" system includes anything indicating the involvement of the hand of man, such as one or more components of the system being altered or mutated from their naturally occurring state, being at least substantially free from at least one other component with which they are naturally associated in nature, or being associated with at least one other

component with which they are not naturally associated. For example, non-naturally occurring CRISPR/Cas systems can employ CRISPR complexes comprising a gRNA and a Cas protein that do not naturally occur together, a Cas protein that does not occur naturally, or a gRNA that does not occur naturally.

(1) Cas Proteins and Polynucleotides Encoding Cas Proteins

[00305] Cas proteins generally comprise at least one RNA recognition or binding domain that can interact with guide RNAs (gRNAs, described in more detail below). Cas proteins can also comprise nuclease domains (e.g., DNase or RNase domains), DNA binding domains, helicase domains, protein-protein interaction domains, dimerization domains, and other domains. A nuclease domain possesses catalytic activity for nucleic acid cleavage, which includes the breakage of the covalent bonds of a nucleic acid molecule. Cleavage can produce blunt ends or staggered ends, and it can be single-stranded or double-stranded. For example, a wild type Cas9 protein will typically create a blunt cleavage product. Alternatively, a wild type Cpf1 protein (e.g., FnCpf1) can result in a cleavage product with a 5-nucleotide 5' overhang, with the cleavage occurring after the 18th base pair from the PAM sequence on the non-targeted strand and after the 23rd base on the targeted strand. A Cas protein can have full cleavage activity to create a double-strand break in the *HSD17B13* gene (e.g., a double-strand break with blunt ends), or it can be a nickase that creates a single-strand break in the *HSD17B13* gene.

[00306] Examples of Cas proteins include Cas1, Cas1B, Cas2, Cas3, Cas4, Cas5, Cas5e (CasD), Cas6, Cas6e, Cas6f, Cas7, Cas8a1, Cas8a2, Cas8b, Cas8c, Cas9 (Csn1 or Csx12), Cas10, Cas10d, CasF, CasG, CasH, Csy1, Csy2, Csy3, Cse1 (CasA), Cse2 (CasB), Cse3 (CasE), Cse4 (CasC), Csc1, Csc2, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1 , Cmr3, Cmr4, Cmr5, Cmr6, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csx1, Csx15, Csf1, Csf2, Csf3, Csf4, and Cu1966, and homologs or modified versions thereof.

[00307] An exemplary Cas protein is a Cas9 protein or a protein derived from a Cas9 protein from a type II CRISPR/Cas system. Cas9 proteins are from a type II CRISPR/Cas system and typically share four key motifs with a conserved architecture. Motifs 1, 2, and 4 are RuvC-like motifs, and motif 3 is an HNH motif. Exemplary Cas9 proteins are from *Streptococcus pyogenes*, *Streptococcus thermophilus*, *Streptococcus sp.*, *Staphylococcus aureus*, *Nocardiopsis dassonvillei*, *Streptomyces pristinaespiralis*, *Streptomyces viridochromogenes*, *Streptomyces*

viridochromogenes, *Streptosporangium roseum*, *Streptosporangium roseum*, *Alicyclobacillus acidocaldarius*, *Bacillus pseudomycoides*, *Bacillus selenitireducens*, *Exiguobacterium sibiricum*, *Lactobacillus delbrueckii*, *Lactobacillus salivarius*, *Microscilla marina*, *Burkholderiales bacterium*, *Polaromonas naphthalenivorans*, *Polaromonas sp.*, *Crocosphaera watsonii*, *Cyanothece sp.*, *Microcystis aeruginosa*, *Synechococcus sp.*, *Acetohalobium arabaticum*, *Ammonifex degensii*, *Caldicelulosiruptor becscii*, *Candidatus Desulforudis*, *Clostridium botulinum*, *Clostridium difficile*, *Finegoldia magna*, *Natranaerobius thermophilus*, *Pelotomaculum thermopropionicum*, *Acidithiobacillus caldus*, *Acidithiobacillus ferrooxidans*, *Allochromatium vinosum*, *Marinobacter sp.*, *Nitrosococcus halophilus*, *Nitrosococcus watsoni*, *Pseudoalteromonas haloplanktis*, *Ktedonobacter racemifer*, *Methanohalobium evestigatum*, *Anabaena variabilis*, *Nodularia spumigena*, *Nostoc sp.*, *Arthrospira maxima*, *Arthrospira platensis*, *Arthrospira sp.*, *Lyngbya sp.*, *Microcoleus chthonoplastes*, *Oscillatoria sp.*, *Petrotoga mobilis*, *Thermosipho africanus*, or *Acaryochloris marina*. Additional examples of the Cas9 family members are described in WO 2014/131833, herein incorporated by reference in its entirety for all purposes. Cas9 from *S. pyogenes* (SpCas9) (assigned SwissProt accession number Q99ZW2) is an exemplary Cas9 protein. Cas9 from *S. aureus* (SaCas9) (assigned UniProt accession number J7RUA5) is another exemplary Cas9 protein.

[00308] Another example of a Cas protein is a Cpf1 (CRISPR from *Prevotella* and *Francisella* 1) protein. Cpf1 is a large protein (about 1300 amino acids) that contains a RuvC-like nuclease domain homologous to the corresponding domain of Cas9 along with a counterpart to the characteristic arginine-rich cluster of Cas9. However, Cpf1 lacks the HNH nuclease domain that is present in Cas9 proteins, and the RuvC-like domain is contiguous in the Cpf1 sequence, in contrast to Cas9 where it contains long inserts including the HNH domain. *See, e.g.*, Zetsche et al. (2015) *Cell* 163(3):759-771, herein incorporated by reference in its entirety for all purposes. Exemplary Cpf1 proteins are from *Francisella tularensis 1*, *Francisella tularensis subsp. novicida*, *Prevotella albensis*, *Lachnospiraceae bacterium MC2017 1*, *Butyrivibrio proteoclasticus*, *Peregrinibacteria bacterium GW2011_GWA2_33_10*, *Parcubacteria bacterium GW2011_GWC2_44_17*, *Smithella sp. SCADC*, *Acidaminococcus sp. BV3L6*, *Lachnospiraceae bacterium MA2020*, *Candidatus Methanoplasma termitum*, *Eubacterium eligens*, *Moraxella bovoculi 237*, *Leptospira inadai*, *Lachnospiraceae bacterium ND2006*, *Porphyromonas crevioricanis 3*, *Prevotella disiens*, and *Porphyromonas macacae*.

Cpf1 from *Francisella novicida* U112 (FnCpf1; assigned UniProt accession number A0Q7Q2) is an exemplary Cpf1 protein.

[00309] Cas proteins can be wild type proteins (i.e., those that occur in nature), modified Cas proteins (i.e., Cas protein variants), or fragments of wild type or modified Cas proteins. Cas proteins can also be active variants or fragments with respect to catalytic activity of wild type or modified Cas proteins. Active variants or fragments with respect to catalytic activity can comprise at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to the wild type or modified Cas protein or a portion thereof, wherein the active variants retain the ability to cut at a desired cleavage site and hence retain nick-inducing or double-strand-break-inducing activity. Assays for nick-inducing or double-strand-break-inducing activity are known and generally measure the overall activity and specificity of the Cas protein on DNA substrates containing the cleavage site.

[00310] Cas proteins can be modified to increase or decrease one or more of nucleic acid binding affinity, nucleic acid binding specificity, and enzymatic activity. Cas proteins can also be modified to change any other activity or property of the protein, such as stability. For example, one or more nuclease domains of the Cas protein can be modified, deleted, or inactivated, or a Cas protein can be truncated to remove domains that are not essential for the function of the protein or to optimize (e.g., enhance or reduce) the activity of the Cas protein.

[00311] Cas proteins can comprise at least one nuclease domain, such as a DNase domain. For example, a wild type Cpf1 protein generally comprises a RuvC-like domain that cleaves both strands of target DNA, perhaps in a dimeric configuration. Cas proteins can also comprise at least two nuclease domains, such as DNase domains. For example, a wild type Cas9 protein generally comprises a RuvC-like nuclease domain and an HNH-like nuclease domain. The RuvC and HNH domains can each cut a different strand of double-stranded DNA to make a double-stranded break in the DNA. *See, e.g.*, Jinek et al. (2012) *Science* 337:816-821, herein incorporated by reference in its entirety for all purposes.

[00312] One or more of the nuclease domains can be deleted or mutated so that they are no longer functional or have reduced nuclease activity. For example, if one of the nuclease domains is deleted or mutated in a Cas9 protein, the resulting Cas9 protein can be referred to as a nickase and can generate a single-strand break at a guide RNA target sequence within a double-stranded DNA but not a double-strand break (i.e., it can cleave the complementary strand or the non-

complementary strand, but not both). If both of the nuclease domains are deleted or mutated, the resulting Cas protein (e.g., Cas9) will have a reduced ability to cleave both strands of a double-stranded DNA (e.g., a nuclease-null or nuclease-inactive Cas protein, or a catalytically dead Cas protein (dCas)). An example of a mutation that converts Cas9 into a nickase is a D10A (aspartate to alanine at position 10 of Cas9) mutation in the RuvC domain of Cas9 from *S. pyogenes*. Likewise, H939A (histidine to alanine at amino acid position 839) or H840A (histidine to alanine at amino acid position 840) in the HNH domain of Cas9 from *S. pyogenes* can convert the Cas9 into a nickase. Other examples of mutations that convert Cas9 into a nickase include the corresponding mutations to Cas9 from *S. thermophilus*. *See, e.g.*, Sapranauskas et al. (2011) *Nucleic Acids Research* 39:9275-9282 and WO 2013/141680, each of which is herein incorporated by reference in its entirety for all purposes. Such mutations can be generated using methods such as site-directed mutagenesis, PCR-mediated mutagenesis, or total gene synthesis. Examples of other mutations creating nickases can be found, for example, in WO 2013/176772 and WO 2013/142578, each of which is herein incorporated by reference in its entirety for all purposes.

[00313] Cas proteins (e.g., nuclease-active Cas proteins or nuclease-inactive Cas proteins) can also be operably linked to heterologous polypeptides as fusion proteins. For example, a Cas protein can be fused to a cleavage domain, an epigenetic modification domain, a transcriptional activation domain, or a transcriptional repressor domain. *See* WO 2014/089290, herein incorporated by reference in its entirety for all purposes. Examples of transcriptional activation domains include a herpes simplex virus VP16 activation domain, VP64 (which is a tetrameric derivative of VP16), a NFκB p65 activation domain, p53 activation domains 1 and 2, a CREB (cAMP response element binding protein) activation domain, an E2A activation domain, and an NFAT (nuclear factor of activated T-cells) activation domain. Other examples include activation domains from Oct1, Oct-2A, SP1, AP-2, CTF1, P300, CBP, PCAF, SRC1, PvALF, ERF-2, OsGAI, HALF-1, C1, AP1, ARF-5, ARF-6, ARF-7, ARF-8, CPRF1, CPRF4, MYC-RP/GP, TRAB1PC4, and HSF1. *See, e.g.*, US 2016/0237456, EP3045537, and WO 2011/145121, each of which is incorporated by reference in its entirety for all purposes. In some cases, a transcriptional activation system can be used comprising a dCas9-VP64 fusion protein paired with MS2-p65-HSF1. Guide RNAs in such systems can be designed with aptamer sequences appended to sgRNA tetraloop and stem-loop 2 designed to bind dimerized MS2 bacteriophage

coat proteins. *See, e.g.*, Konermann et al. (2015) *Nature* 517(7536):583-588, herein incorporated by reference in its entirety for all purposes. Examples of transcriptional repressor domains include inducible cAMP early repressor (ICER) domains, Kruppel-associated box A (KRAB-A) repressor domains, YY1 glycine rich repressor domains, Sp1 -like repressors, E(spl) repressors, IκB repressor, and MeCP2. Other examples include transcriptional repressor domains from A/B, KOX, TGF-beta-inducible early gene (TIEG), v-erbA, SID, SID4X, MBD2, MBD3, DNMT1, DNMG3A, DNMT3B, Rb, ROM2, *See, e.g.*, EP3045537 and WO 2011/145121, each of which is incorporated by reference in its entirety for all purposes. Cas proteins can also be fused to a heterologous polypeptide providing increased or decreased stability. The fused domain or heterologous polypeptide can be located at the N-terminus, the C-terminus, or internally within the Cas protein.

[00314] As one example, a Cas protein can be fused to a heterologous polypeptide that provides for subcellular localization. Such heterologous polypeptides can include, for example, one or more nuclear localization signals (NLS) such as the SV40 NLS for targeting to the nucleus, a mitochondrial localization signal for targeting to the mitochondria, an ER retention signal, and the like. *See, e.g.*, Lange et al. (2007) *J. Biol. Chem.* 282:5101-5105, herein incorporated by reference in its entirety for all purposes. Such subcellular localization signals can be located at the N-terminus, the C-terminus, or anywhere within the Cas protein. An NLS can comprise a stretch of basic amino acids, and can be a monopartite sequence or a bipartite sequence.

[00315] Cas proteins can also be operably linked to a cell-penetrating domain. For example, the cell-penetrating domain can be derived from the HIV-1 TAT protein, the TLM cell-penetrating motif from human hepatitis B virus, MPG, Pep-1, VP22, a cell penetrating peptide from Herpes simplex virus, or a polyarginine peptide sequence. *See, e.g.*, WO 2014/089290, herein incorporated by reference in its entirety for all purposes. The cell-penetrating domain can be located at the N-terminus, the C-terminus, or anywhere within the Cas protein.

[00316] Cas proteins can also be operably linked to a heterologous polypeptide for ease of tracking or purification, such as a fluorescent protein, a purification tag, or an epitope tag. Examples of fluorescent proteins include green fluorescent proteins (e.g., GFP, GFP-2, tagGFP, turboGFP, eGFP, Emerald, Azami Green, Monomeric Azami Green, CopGFP, AceGFP, ZsGreenl), yellow fluorescent proteins (e.g., YFP, eYFP, Citrine, Venus, YPet, PhiYFP,

ZsYellowl), blue fluorescent proteins (e.g., eBFP, eBFP2, Azurite, mKalamal, GFPuv, Sapphire, T-sapphire), cyan fluorescent proteins (e.g., eCFP, Cerulean, CyPet, AmCyanl, Midoriishi-Cyan), red fluorescent proteins (e.g., mKate, mKate2, mPlum, DsRed monomer, mCherry, mRFP1, DsRed-Express, DsRed2, DsRed-Monomer, HcRed-Tandem, HcRedl, AsRed2, eqFP611, mRaspberry, mStrawberry, Jred), orange fluorescent proteins (e.g., mOrange, mKO, Kusabira-Orange, Monomeric Kusabira-Orange, mTangerine, tdTomato), and any other suitable fluorescent protein. Examples of tags include glutathione-S-transferase (GST), chitin binding protein (CBP), maltose binding protein, thioredoxin (TRX), poly(NANP), tandem affinity purification (TAP) tag, myc, AcV5, AU1 , AU5, E, ECS, E2, FLAG, hemagglutinin (HA), nus, Softag 1, Softag 3, Strep, SBP, Glu-Glu, HSV, KT3, S, S1, T7, V5, VSV-G, histidine (His), biotin carboxyl carrier protein (BCCP), and calmodulin.

[00317] Cas proteins can also be tethered to exogenous donor sequences or labeled nucleic acids. Such tethering (i.e., physical linking) can be achieved through covalent interactions or noncovalent interactions, and the tethering can be direct (e.g., through direct fusion or chemical conjugation, which can be achieved by modification of cysteine or lysine residues on the protein or intein modification), or can be achieved through one or more intervening linkers or adapter molecules such as streptavidin or aptamers. *See, e.g.*, Pierce et al. (2005) *Mini Rev. Med. Chem.* 5(1):41-55; Duckworth et al. (2007) *Angew. Chem. Int. Ed. Engl.* 46(46):8819-8822; Schaeffer and Dixon (2009) *Australian J. Chem.* 62(10):1328-1332; Goodman et al. (2009) *Chembiochem.* 10(9):1551-1557; and Khatwani et al. (2012) *Bioorg. Med. Chem.* 20(14):4532-4539, each of which is herein incorporated by reference in its entirety for all purposes. Noncovalent strategies for synthesizing protein-nucleic acid conjugates include biotin-streptavidin and nickel-histidine methods. Covalent protein-nucleic acid conjugates can be synthesized by connecting appropriately functionalized nucleic acids and proteins using a wide variety of chemistries. Some of these chemistries involve direct attachment of the oligonucleotide to an amino acid residue on the protein surface (e.g., a lysine amine or a cysteine thiol), while other more complex schemes require post-translational modification of the protein or the involvement of a catalytic or reactive protein domain. Methods for covalent attachment of proteins to nucleic acids can include, for example, chemical cross-linking of oligonucleotides to protein lysine or cysteine residues, expressed protein-ligation, chemoenzymatic methods, and the use of photoaptamers. The exogenous donor sequence or labeled nucleic acid can be tethered to the C-terminus, the N-

terminus, or to an internal region within the Cas protein. Preferably, the exogenous donor sequence or labeled nucleic acid is tethered to the C-terminus or the N-terminus of the Cas protein. Likewise, the Cas protein can be tethered to the 5' end, the 3' end, or to an internal region within the exogenous donor sequence or labeled nucleic acid. That is, the exogenous donor sequence or labeled nucleic acid can be tethered in any orientation and polarity. Preferably, the Cas protein is tethered to the 5' end or the 3' end of the exogenous donor sequence or labeled nucleic acid.

[00318] Cas proteins can be provided in any form. For example, a Cas protein can be provided in the form of a protein, such as a Cas protein complexed with a gRNA. Alternatively, a Cas protein can be provided in the form of a nucleic acid encoding the Cas protein, such as an RNA (e.g., messenger RNA (mRNA)) or DNA. Optionally, the nucleic acid encoding the Cas protein can be codon optimized for efficient translation into protein in a particular cell or organism. For example, the nucleic acid encoding the Cas protein can be modified to substitute codons having a higher frequency of usage in a bacterial cell, a yeast cell, a human cell, a non-human cell, a mammalian cell, a rodent cell, a mouse cell, a rat cell, or any other host cell of interest, as compared to the naturally occurring polynucleotide sequence. When a nucleic acid encoding the Cas protein is introduced into the cell, the Cas protein can be transiently, conditionally, or constitutively expressed in the cell.

[00319] Nucleic acids encoding Cas proteins can be stably integrated in the genome of the cell and operably linked to a promoter active in the cell. Alternatively, nucleic acids encoding Cas proteins can be operably linked to a promoter in an expression construct. Expression constructs include any nucleic acid constructs capable of directing expression of a gene or other nucleic acid sequence of interest (e.g., a Cas gene) and which can transfer such a nucleic acid sequence of interest to a target cell. For example, the nucleic acid encoding the Cas protein can be in a targeting vector comprising a nucleic acid insert and/or a vector comprising a DNA encoding a gRNA. Alternatively, it can be in a vector or plasmid that is separate from the targeting vector comprising the nucleic acid insert and/or separate from the vector comprising the DNA encoding the gRNA. Promoters that can be used in an expression construct include promoters active, for example, in one or more of a eukaryotic cell, a human cell, a non-human cell, a mammalian cell, a non-human mammalian cell, a rodent cell, a mouse cell, a rat cell, a hamster cell, a rabbit cell, a pluripotent cell, an embryonic stem (ES) cell, or a zygote. Such promoters can be, for

example, conditional promoters, inducible promoters, constitutive promoters, or tissue-specific promoters. Optionally, the promoter can be a bidirectional promoter driving expression of both a Cas protein in one direction and a guide RNA in the other direction. Such bidirectional promoters can consist of (1) a complete, conventional, unidirectional Pol III promoter that contains 3 external control elements: a distal sequence element (DSE), a proximal sequence element (PSE), and a TATA box; and (2) a second basic Pol III promoter that includes a PSE and a TATA box fused to the 5′ terminus of the DSE in reverse orientation. For example, in the H1 promoter, the DSE is adjacent to the PSE and the TATA box, and the promoter can be rendered bidirectional by creating a hybrid promoter in which transcription in the reverse direction is controlled by appending a PSE and TATA box derived from the U6 promoter. *See, e.g.*, US 2016/0074535, herein incorporated by references in its entirety for all purposes. Use of a bidirectional promoter to express genes encoding a Cas protein and a guide RNA simultaneously allow for the generation of compact expression cassettes to facilitate delivery.

(2) Guide RNAs

[00320] A "guide RNA" or "gRNA" is an RNA molecule that binds to a Cas protein (e.g., Cas9 protein) and targets the Cas protein to a specific location within a target DNA (e.g., the *HSD17B13* gene). In particular, disclosed herein are guide RNAs effective to direct a Cas enzyme to bind to or cleave an *HSD17B13* locus or *HSD17B13* gene. One exemplary guide RNA is a guide RNA effective to direct a Cas enzyme to bind to or cleave an *HSD17B13* gene, wherein the guide RNA comprises a DNA-targeting segment that hybridizes to a guide RNA recognition sequence (i.e., targets a guide RNA target sequence) within the *HSD17B13* gene that includes or is proximate to a position corresponding to position 12666 of SEQ ID NO: 2 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 2. By target a guide RNA target sequence is meant hybridize to the complementary strand sequence that is the reverse complement of the guide RNA target sequence on the non-complementary strand. For example, the guide RNA target sequence can be within about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 100, 200, 300, 400, 500, or 1,000 nucleotides of a position corresponding to position 12666 of SEQ ID NO: 2 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 2. Other exemplary guide RNAs comprise a DNA-targeting segment that targets a guide RNA target sequence within the *HSD17B13* gene that is within a region corresponding to exon 6 and/or intron 6 of SEQ ID

NO: 2 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 2. Other exemplary guide RNAs comprise a DNA-targeting segment that targets a guide RNA target sequence within the *HSD17B13* gene that is within a region corresponding to exon 6 and/or intron 6 and/or exon 7 of SEQ ID NO: 2 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 2. Other exemplary guide RNAs comprise a DNA-targeting segment that hybridizes to a guide RNA recognition sequence (i.e., targets a guide RNA target sequence) within the *HSD17B13* gene that includes or is proximate to the start codon of the *HSD17B13* gene or includes or is proximate to the stop codon of the *HSD17B13* gene. For example, the guide RNA target sequence can be within about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 100, 200, 300, 400, 500, or 1,000 nucleotides of the start codon or within about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 100, 200, 300, 400, 500, or 1,000 nucleotides of the stop codon. For example, the guide RNA target sequence can be within a region corresponding to exon 1 of SEQ ID NO: 1 or 2 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 1 or 2. Likewise, the guide RNA target sequence can be within a region corresponding to exon 7 of SEQ ID NO: 1 or 2 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 1 or 2. The *HSD17B13* gene can be an *HSD17B13* gene from any organism. For example, the *HSD17B13* gene can be a human *HSD17B13* gene or an ortholog from another organism, such as a non-human mammal, a rodent, a mouse, or a rat.

[00321] Examples of guide RNA target sequences at the 5' end of the human *HSD17B13* gene comprise, consist essentially of, or consist of the sequences set forth in SEQ ID NOS: 20-81 and are set forth in the table below. Examples of guide RNA DNA-targeting segments corresponding to SEQ ID NOS: 20-81 are set forth in in the table below and are identical to SEQ ID NOS: 20-81 except with uracils instead of thymines. A guide RNA DNA-targeting segment can comprise, consist essentially of, or consist of any the DNA-targeting segment sequences set forth in the table below. Examples of guide RNA target sequences adjacent to the transcription start site (TSS) of the human *HSD17B13* gene comprise, consist essentially of, or consist of the sequences set forth in SEQ ID NOS: 20-41 and are set forth in the table below. Exemplary guide RNA target sequences adjacent to the TSS include SEQ ID NOS: 21-23, 33, and 35. SEQ ID NOS: 33 and 35 are closest to the TSS. Exemplary crRNAs and sgRNAs (comprising scaffold version 1, 2, 3, or 4) corresponding to the guide RNA target sequences at the 5' end of the human *HSD17B13* gene comprise, consist essentially of, or consist of any of the sequences set forth in the table below.

[00322] Guide RNA Target Sequences at 5' End of the Human *HSD17B13* Gene

Strand	PAM	Guide RNA Target Sequence	SEQ ID NO						
			gRNA Target Seq	DNA-Targeting Segment	crRNA	sgRNA			
						v1	v2	v3	v4
-	GGG	TGTCAGGTTAGTTAGATGAA	42	1423	270	500	730	960	1190
-	AGG	GTGTCAGGTTAGTTAGATGA	43	1424	271	501	731	961	1191
+	AGG	CCTGACACATATACAGACTA	44	1425	272	502	732	962	1192
+	GGG	CTGACACATATACAGACTAA	45	1426	273	503	733	963	1193
-	AGG	CCTTAGTCTGTATATGTGTC	46	1427	274	504	734	964	1194
+	AGG	CATATACAGACTAAGGGACC	47	1428	275	505	735	965	1195
+	GGG	ATATACAGACTAAGGGACCA	48	1429	276	506	736	966	1196
-	TGG	TCAAAGTTTGATAAATTCCC	49	1430	277	507	737	967	1197
+	TGG	AAAATACAAAGATAAGTAGA	50	1431	278	508	738	968	1198
+	TGG	ACTCTGTGACTTTAAAAAGT	51	1432	279	509	739	969	1199
-	AGG	GGTTCTGTGGGATATTAATA	52	1433	280	510	740	970	1200
-	GGG	ACAGAGCATATTGGTTCTGT	53	1434	281	511	741	971	1201
-	TGG	GACAGAGCATATTGGTTCTG	54	1435	282	512	742	972	1202
-	TGG	TGCAAAACGACAGAGCATAT	55	1436	283	513	743	973	1203
-	AGG	GAGCTGGGCATGGAATAGGC	56	1437	284	514	744	974	1204
-	AGG	ACTGGAGCTGGGCATGGAAT	57	1438	285	515	745	975	1205
-	TGG	CTCATTACTGGAGCTGGGCA	58	1439	286	516	746	976	1206
-	GGG	TTGTTCTCATTACTGGAGCT	59	1440	287	517	747	977	1207
-	TGG	ATTGTTCTCATTACTGGAGC	60	1441	288	518	748	978	1208
-	TGG	GGGGAGATTGTTCTCATTAC	61	1442	289	519	749	979	1209
-	GGG	GAGGAGAAAATCTGTGGCTG	62	1443	290	520	750	980	1210
-	GGG	AGAGGAGAAAATCTGTGGCT	63	1444	291	521	751	981	1211
-	TGG	CAGAGGAGAAAATCTGTGGC	64	1445	292	522	752	982	1212
-	TGG	TCCTCAGAGGAGAAAATCTG	65	1446	293	523	753	983	1213
-	AGG	TGAAGTTTTTCATTCCTCAG	20	1447	294	524	754	984	1214
+	AGG	CTTCACCAACGACTCCAAGT	21	1448	295	525	755	985	1215
-	TGG	CTACTCCTACTTGGAGTCGT	22	1449	296	526	756	986	1216
+	TGG	CTCCAAGTAGGAGTAGATGA	23	1450	297	527	757	987	1217
-	TGG	CACCATCATCTACTCCTACT	24	1451	298	528	758	988	1218
+	AGG	TGATGGTGATCAGAAGCAGA	25	1452	299	529	759	989	1219
+	AGG	TCAGAAGCAGAAGGATTTCT	26	1453	300	530	760	990	1220
+	TGG	GATTTCTAGGATGATGTTCA	27	1454	301	531	761	991	1221
+	TGG	TTGCTCTGTCCTCTTCCTTC	28	1455	302	532	762	992	1222
-	AGG	AGGACTGAACCAGAAGGAAG	29	1456	303	533	763	993	1223
-	AGG	TACACAAGGACTGAACCAGA	30	1457	304	534	764	994	1224
+	AGG	TTCAGTCCTTGTGTAGTCCT	31	1458	305	535	765	995	1225
+	GGG	TCAGTCCTTGTGTAGTCCTA	32	1459	306	536	766	996	1226
+	AGG	GTCCTTGTGTAGTCCTAGGG	33	1460	307	537	767	997	1227
+	AGG	CTTGTGTAGTCCTAGGGAGG	34	1461	308	538	768	998	1228
-	AGG	CTCCTCCCTAGGACTACACA	35	1462	309	539	769	999	1229
-	AGG	GTAGACAGTACCTCCTCCCT	36	1463	310	540	770	1000	1230
+	AGG	TACTGTCTACACAGAGCTCT	37	1464	311	541	771	1001	1231
+	GGG	ACTGTCTACACAGAGCTCTA	38	1465	312	542	772	1002	1232
+	AGG	TCTACACAGAGCTCTAGGGA	39	1466	313	543	773	1003	1233
+	GGG	CTACACAGAGCTCTAGGGAA	40	1467	314	544	774	1004	1234
+	GGG	TACACAGAGCTCTAGGGAAG	41	1468	315	545	775	1005	1235
+	TGG	GGGGTGTGCCCAGTTGTTAA	66	1469	316	546	776	1006	1236
+	GGG	GGGTGTGCCCAGTTGTTAAT	67	1470	317	547	777	1007	1237
-	GGG	TGGTAGTCCCATTAACAACT	68	1471	318	548	778	1008	1238
-	TGG	CTGGTAGTCCCATTAACAAC	69	1472	319	549	779	1009	1239
+	TGG	TTGTTAATGGGACTACCAGA	70	1473	320	550	780	1010	1240
+	TGG	TACCAGATGGAAGCCAGCTT	71	1474	321	551	781	1011	1241
-	TGG	TTCCAAAGCTGGCTTCCATC	72	1475	322	552	782	1012	1242

Strand	PAM	Guide RNA Target Sequence	SEQ ID NO						
			gRNA Target Seq	DNA-Targeting Segment	crRNA	sgRNA			
						v1	v2	v3	v4
+	AGG	TGGAAGCCAGCTTTGGAAGC	73	1476	323	553	783	1013	1243
-	TGG	ACAAGGCCTGCTTCCAAAGC	74	1477	324	554	784	1014	1244
+	TGG	GCCTTGTTCACGTGTTCTAA	75	1478	325	555	785	1015	1245
+	GGG	CCTTGTTCACGTGTTCTAAT	76	1479	326	556	786	1016	1246
-	AGG	CCCATTAGAACACGTGAACA	77	1480	327	557	787	1017	1247
-	AGG	TTGGCATCACTTCATATTTG	78	1481	328	558	788	1018	1248
-	TGG	CTTGTGCTCTTGGCATCACT	79	1482	329	559	789	1019	1249
-	TGG	AGCACACTCTCTTGTGCTCT	80	1483	330	560	790	1020	1250
+	TGG	GCACAAGAGAGTGTGCTCTC	81	1484	331	561	791	1021	1251

[00323] Examples of guide RNA target sequences at the 3' end of the human *HSD17B13* gene comprise, consist essentially of, or consist of the sequences set forth in SEQ ID NOS: 82-225 and are set forth in the table below. Examples of guide RNA DNA-targeting segments corresponding to SEQ ID NOS: 82-225 are set forth in SEQ ID NOS: 1485-1628, respectively, which are identical to SEQ ID NOS: 82-225 except with uracils instead of thymines. A guide RNA DNA-targeting segment can comprise, consist essentially of, or consist of any the sequences set forth in SEQ ID NOS: 1485-1628. Exemplary crRNAs and sgRNAs (comprising scaffold version 1, 2, 3, or 4) corresponding to the guide RNA target sequences at the 3' end of the human *HSD17B13* gene comprise, consist essentially of, or consist of any of the sequences set forth in the table below.

[00324] Guide RNA Target Sequences at 3' End of the Human *HSD17B13* Gene

Strand	PAM	Guide RNA Target Sequence	SEQ ID NO					
			gRNA Target Seq	crRNA	sgRNA			
					v1	v2	v3	v4
+	AGG	GCTTAATCTCACACATAGAA	82	332	562	792	1022	1252
+	GGG	CTTAATCTCACACATAGAAA	83	333	563	793	1023	1253
+	GGG	TTAATCTCACACATAGAAAG	84	334	564	794	1024	1254
-	TGG	AGGAGTGCTGGTTTATCAAC	85	335	565	795	1025	1255
-	TGG	TTCTTTGACAGCAGGAGTGC	86	336	566	796	1026	1256
-	AGG	ACTCTGGTTTCTTTGACAGC	87	337	567	797	1027	1257
+	TGG	ACCAGAGTTGAGAAAACCCC	88	338	568	798	1028	1258
-	TGG	TCCAGGGGTTTTCTCAACTC	89	339	569	799	1029	1259
-	GGG	CAGTTATTAAATGAATCCAG	90	340	570	800	1030	1260
-	GGG	GCAGTTATTAAATGAATCCA	91	341	571	801	1031	1261
-	AGG	GGCAGTTATTAAATGAATCC	92	342	572	802	1032	1262
-	TGG	TGGATGGTAACAGCTACATC	93	343	573	803	1033	1263
+	TGG	GCTGTTACCATCCACATCCT	94	344	574	804	1034	1264
-	TGG	TCAAGAACCAAGGATGTGGA	95	345	575	805	1035	1265
-	TGG	TCCTTCAAGAACCAAGGATG	96	346	576	806	1036	1266
-	AGG	TGAGTGTCCTTCAAGAACCA	97	347	577	807	1037	1267
+	AGG	TTTTATTTTATAACTACAAG	98	348	578	808	1038	1268
+	AGG	TTGTTTTTAATAAAAACAAG	99	349	579	809	1039	1269
-	TGG	TATTATAGAATGCTTTTGCA	100	350	580	810	1040	1270

Strand	PAM	Guide RNA Target Sequence	SEQ ID NO					
			gRNA Target Seq	crRNA	sgRNA			
					v1	v2	v3	v4
+	TGG	CAAGATTAGTCTTGATGTAG	101	351	581	811	1041	1271
+	GGG	AAGATTAGTCTTGATGTAGT	102	352	582	812	1042	1272
+	CGG	AGTCTTGATGTAGTGGGAGT	103	353	583	813	1043	1273
+	AGG	TTTTTCTATTAAAAAAAAAA	104	354	584	814	1044	1274
+	TGG	TCTATTAAAAAAAAAAAGGC	105	355	585	815	1045	1275
+	GGG	CTATTAAAAAAAAAAAGGCT	106	356	586	816	1046	1276
+	CGG	AAAAAAAAAAAGGCTGGGCA	107	357	587	817	1047	1277
+	TGG	AAAAAAAAGGCTGGGCACGG	108	358	588	818	1048	1278
+	TGG	CACCCGTAATCCCAGCACTT	109	359	589	819	1049	1279
+	GGG	ACCCGTAATCCCAGCACTTT	110	360	590	820	1050	1280
+	AGG	CGTAATCCCAGCACTTTGGG	111	361	591	821	1051	1281
-	GGG	TCCCAAAGTGCTGGGATTAC	112	362	592	822	1052	1282
-	CGG	CTCCCAAAGTGCTGGGATTA	113	363	593	823	1053	1283
+	AGG	CCCAGCACTTTGGGAGGCCG	114	364	594	824	1054	1284
-	GGG	CCTCGGCCTCCCAAAGTGCT	115	365	595	825	1055	1285
+	AGG	GCACTTTGGGAGGCCGAGGC	116	366	596	826	1056	1286
+	TGG	CTTTGGGAGGCCGAGGCAGG	117	367	597	827	1057	1287
+	AGG	GCCGAGGCAGGTGGATCACG	118	368	598	828	1058	1288
-	CGG	ACCTCGTGATCCACCTGCCT	119	369	599	829	1059	1289
+	AGG	GGCAGGTGGATCACGAGGTC	120	370	600	830	1060	1290
+	TGG	TCAGGAGATCGAGACCATCT	121	371	601	831	1061	1291
+	TGG	CGAGACCATCTTGGCTAACA	122	372	602	832	1062	1292
-	TGG	TTTCACCATGTTAGCCAAGA	123	373	603	833	1063	1293
-	GGG	TTGTATTTTTTGTAGAGACG	124	374	604	834	1064	1294
-	GGG	TTTGTATTTTTTGTAGAGAC	125	375	605	835	1065	1295
-	CGG	TTTTGTATTTTTTGTAGAGA	126	376	606	836	1066	1296
+	CGG	AAAAAATACAAAAAATTAGC	127	377	607	837	1067	1297
+	GGG	AAAAATACAAAAAATTAGCC	128	378	608	838	1068	1298
+	TGG	TACAAAAAATTAGCCGGGTG	129	379	609	839	1069	1299
+	TGG	AAAAAATTAGCCGGGTGTGG	130	380	610	840	1070	1300
+	CGG	AAATTAGCCGGGTGTGGTGG	131	381	611	841	1071	1301
+	GGG	AATTAGCCGGGTGTGGTGGC	132	382	612	842	1072	1302
-	CGG	CAGGCGCCCGCCACCACACC	133	383	613	843	1073	1303
+	AGG	GCCTGTAGTCCCAGCTACTC	134	384	614	844	1074	1304
+	AGG	TGTAGTCCCAGCTACTCAGG	135	385	615	845	1075	1305
-	AGG	TCCTGAGTAGCTGGGACTAC	136	386	616	846	1076	1306
+	AGG	CCCAGCTACTCAGGAGGCTG	137	387	617	847	1077	1307
-	GGG	CCTCAGCCTCCTGAGTAGCT	138	388	618	848	1078	1308
-	TGG	GCCTCAGCCTCCTGAGTAGC	139	389	619	849	1079	1309
+	TGG	AGGAGGCTGAGGCAGGAGAA	140	390	620	850	1080	1310
+	CGG	GCAGGAGAATGGCGTGAACC	141	391	621	851	1081	1311
+	GGG	CAGGAGAATGGCGTGAACCC	142	392	622	852	1082	1312
+	AGG	GAGAATGGCGTGAACCCGGG	143	393	623	853	1083	1313
+	TGG	AATGGCGTGAACCCGGGAGG	144	394	624	854	1084	1314
-	GGG	CACTGCAAGCTCCACCTCCC	145	395	625	855	1085	1315
-	CGG	TCACTGCAAGCTCCACCTCC	146	396	626	856	1086	1316
+	TGG	CATACCACTGCACTCCAGCC	147	397	627	857	1087	1317
+	GGG	ATACCACTGCACTCCAGCCT	148	398	628	858	1088	1318
-	TGG	TCGCCCAGGCTGGAGTGCAG	149	399	629	859	1089	1319
-	TGG	TCTCACTCTTTCGCCCAGGC	150	400	630	860	1090	1320
-	AGG	GGAGTCTCACTCTTTCGCCC	151	401	631	861	1091	1321
-	TGG	TGTTTTTTGTTTTTTTGAGA	152	402	632	862	1092	1322
-	TGG	AGGAAGAAAGAAAGGTTTTT	153	403	633	863	1093	1323
-	AGG	AGAAGAAAAGGAAGAAAGAA	154	404	634	864	1094	1324
+	TGG	CTTTCTTCCTTTTCTTCTCT	155	405	635	865	1095	1325

Strand	PAM	Guide RNA Target Sequence	SEQ ID NO					
			gRNA Target Seq	crRNA	sgRNA			
					v1	v2	v3	v4
+	GGG	TTTCTTCCTTTTCTTCTCTT	156	406	636	866	1096	1326
-	AGG	AATGGACCCAAGAGAAGAAA	157	407	637	867	1097	1327
-	TGG	GGCTATTACATAAGAAACAA	158	408	638	868	1098	1328
-	TGG	CACAGGAAAAGGAACTGTAC	159	409	639	869	1099	1329
-	AGG	ATTAAAGCTAACACAGGAAA	160	410	640	870	1100	1330
-	AGG	TCAAAAATTAAAGCTAACAC	161	411	641	871	1101	1331
+	TGG	TAAAATTGTCTAAACATCTC	162	412	642	872	1102	1332
-	AGG	AGAGATGTTTAGACAATTTT	163	413	643	873	1103	1333
+	AGG	TCTAAACATCTCTGGGACCA	164	414	644	874	1104	1334
-	TGG	TTTATGCTTTCATATATCCT	165	415	645	875	1105	1335
+	AGG	AGCATAAATTACAAAGAAAA	166	416	646	876	1106	1336
+	TGG	TACAAAGAAAAAGGTTATCA	167	417	647	877	1107	1337
+	GGG	ACAAAGAAAAAGGTTATCAT	168	418	648	878	1108	1338
+	GGG	CAAAGAAAAAGGTTATCATG	169	419	649	879	1109	1339
+	CGG	TCTGAGATTTAAAATAGAGT	170	420	650	880	1110	1340
-	AGG	CTTATAAGATACATTATGAA	171	421	651	881	1111	1341
+	AGG	TATCTTATAAGACTATAAAA	172	422	652	882	1112	1342
+	GGG	ATCTTATAAGACTATAAAAA	173	423	653	883	1113	1343
+	AGG	TTATAAGACTATAAAAAGGG	174	424	654	884	1114	1344
+	AGG	TAAAAAGGGAGGAAATATAG	175	425	655	885	1115	1345
+	GGG	AAAAAGGGAGGAAATATAGA	176	426	656	886	1116	1346
+	TGG	AAATATAGAGGGTCCACTTT	177	427	657	887	1117	1347
+	TGG	TATAGAGGGTCCACTTTTGG	178	428	658	888	1118	1348
-	TGG	ACTCTGAAGTCCACCAAAAG	179	429	659	889	1119	1349
+	TGG	AGAATAGAGTTGCACCGTTT	180	430	660	890	1120	1350
-	TGG	AAAACGGTGCAACTCTATTC	181	431	661	891	1121	1351
+	AGG	CCGTTTTGGGCTAATGAAAA	182	432	662	892	1122	1352
-	CGG	CCTTTTTCATTAGCCCAAAA	183	433	663	893	1123	1353
+	AGG	TGGGCTAATGAAAAAGGAAG	184	434	664	894	1124	1354
+	AGG	TAATGAAAAAGGAAGAGGCT	185	435	665	895	1125	1355
+	GGG	AATGAAAAAGGAAGAGGCTA	186	436	666	896	1126	1356
+	AGG	CTGAATCTTAAAATATGTCC	187	437	667	897	1127	1357
-	TGG	CAGGCAGCTTTATCTCAACC	188	438	668	898	1128	1358
-	AGG	CTAAGAGATCAAGTTTCAGC	189	439	669	899	1129	1359
+	TGG	GTGTTCTTGTTGATATTCTG	190	440	670	900	1130	1360
+	TGG	CTTGTTGATATTCTGTGGCA	191	441	671	901	1131	1361
+	TGG	TCTGTGGCATGGCTACAGAT	192	442	672	902	1132	1362
-	AGG	AGAACTTATTTACACAGGGA	193	443	673	903	1133	1363
-	GGG	AAAGAGAACTTATTTACACA	194	444	674	904	1134	1364
-	AGG	CAAAGAGAACTTATTTACAC	195	445	675	905	1135	1365
+	AGG	TTCTCTTTGTATTTACTTTT	196	446	676	906	1136	1366
+	GGG	TCTCTTTGTATTTACTTTTA	197	447	677	907	1137	1367
+	AGG	CTTTGTATTTACTTTTAGGG	198	448	678	908	1138	1368
+	TGG	AGCTTTTGTCCACCTTTAAA	199	449	679	909	1139	1369
-	TGG	TTTATTTTTCCATTTAAAGG	200	450	680	910	1140	1370
-	AGG	TATTTTATTTTTCCATTTAA	201	451	681	911	1141	1371
-	AGG	CTTACATAAACATACTTAAA	202	452	682	912	1142	1372
+	AGG	TAAGCACAGAAGTTTTTAAG	203	453	683	913	1143	1373
+	AGG	AAGTTTTTAAGAGGCATGAA	204	454	684	914	1144	1374
-	AGG	ATATTTACGTAGTTTTTCAT	205	455	685	915	1145	1375
+	AGG	CGTAAATATTCTTGAGAAAC	206	456	686	916	1146	1376
+	AGG	TTCTTGAGAAACAGGAAGAC	207	457	687	917	1147	1377
-	TGG	TAATATTAAAAACATTGGTT	208	458	688	918	1148	1378
+	AGG	CCAATGTTTTTAATATTATC	209	459	689	919	1149	1379
-	TGG	CCTGATAATATTAAAAACAT	210	460	690	920	1150	1380

Strand	PAM	Guide RNA Target Sequence	SEQ ID NO					
			gRNA Target Seq	crRNA	sgRNA			
					v1	v2	v3	v4
+	TGG	CATTATCATGCATACATCTC	211	461	691	921	1151	1381
+	TGG	ATCATGCATACATCTCTGGC	212	462	692	922	1152	1382
+	TGG	TTCATTTCATTTTGATTTTG	213	463	693	923	1153	1383
-	TGG	ATTCAATTTGAAGCAGTGGT	214	464	694	924	1154	1384
-	TGG	GAATATTCAATTTGAAGCAG	215	465	695	925	1155	1385
+	AGG	CATACGATTTAAAATCGCTG	216	466	696	926	1156	1386
+	AGG	AAAATCGCTGAGGCGCGTTC	217	467	697	927	1157	1387
-	AGG	TTTTTTTTTCTTTTTTGTAC	218	468	698	928	1158	1388
-	TGG	CTGTTGTCAAAGATTTTAAA	219	469	699	929	1159	1389
+	TGG	TGACAACAGAGTTCTGTTTT	220	470	700	930	1160	1390
+	TGG	AGAATACGCTGAGAGTTATC	221	471	701	931	1161	1391
-	AGG	GCAAGAGAAGAAAAGAACGG	222	472	702	932	1162	1392
-	CGG	GTTGCAAGAGAAGAAAAGAA	223	473	703	933	1163	1393
-	TGG	ATGCACACGTAAAAGAGAGG	224	474	704	934	1164	1394
-	AGG	AAGATGCACACGTAAAAGAG	225	475	705	935	1165	1395

[00325] Examples of guide RNA target sequences proximate to a position corresponding to position 12666 of SEQ ID NO: 2 comprise, consist essentially of, or consist of the sequences set forth in SEQ ID NOS: 226-239 and are set forth in the table below. Examples of guide RNA DNA-targeting segments corresponding to SEQ ID NOS: 226-239 are set forth in SEQ ID NOS: 1629-1642, respectively, which are identical to SEQ ID NOS: 226-239 except with uracils instead of thymines. A guide RNA DNA-targeting segment can comprise, consist essentially of, or consist of any the sequences set forth in SEQ ID NOS: 1629-1642. Exemplary guide RNA target sequences proximate to a position corresponding to position 12666 of SEQ ID NO: 2 include SEQ ID NOS: 230 and 231. Exemplary crRNAs and sgRNAs (comprising scaffold version 1, 2, 3, or 4) corresponding to the guide RNA target sequences proximate to a position corresponding to position 12666 of SEQ ID NO: 2 comprise, consist essentially of, or consist of any of the sequences set forth in the table below.

[00326] Guide RNA Target Sequences Near rs72613567 Variation

Strand	PAM	Guide RNA Target Sequence	Distance to Variation (bp)	SEQ ID NO					
				gRNA Target Seq	crRNA	sgRNA			
						v1	v2	v3	v4
+	TGG	ATCATGCATACATCTCTGGC	107	226	476	706	936	1166	1396
+	TGG	TTCATTTCATTTTGATTTTG	74	227	477	707	937	1167	1397
-	TGG	ATTCAATTTGAAGCAGTGGT	62	228	478	708	938	1168	1398
-	TGG	GAATATTCAATTTGAAGCAG	58	229	479	709	939	1169	1399
+	AGG	CATACGATTTAAAATCGCTG	22	230	480	710	940	1170	1400
+	AGG	AAAATCGCTGAGGCGCGTTC	12	231	481	711	941	1171	1401
-	AGG	TTTTTTTTTCTTTTTTGTAC	22	232	482	712	942	1172	1402
-	TGG	CTGTTGTCAAAGATTTTAAA	40	233	483	713	943	1173	1403
+	TGG	TGACAACAGAGTTCTGTTTT	65	234	484	714	944	1174	1404
+	TGG	AGAATACGCTGAGAGTTATC	94	235	485	715	945	1175	1405
-	AGG	GCAAGAGAAGAAAAGAACGG	121	236	486	716	946	1176	1406
-	CGG	GTTGCAAGAGAAGAAAAGAA	124	237	487	717	947	1177	1407
-	TGG	ATGCACACGTAAAAGAGAGG	146	238	488	718	948	1178	1408
-	AGG	AAGATGCACACGTAAAAGAG	149	239	489	719	949	1179	1409

[00327] Examples of guide RNA target sequences in the mouse *Hsd17b13* gene proximate to a position corresponding to position 12666 of SEQ ID NO: 2 when the mouse *Hsd17b13* gene is optimally aligned with SEQ ID NO: 2 comprise, consist essentially of, or consist of the sequences set forth in Table 12 in Example 4. Examples of guide RNA target sequences at the 5' end of the mouse *Hsd17b13* gene comprise, consist essentially of, or consist of the sequences set forth Table 12 in Example 4. Examples of guide RNA DNA-targeting segments corresponding to those guide RNA target sequences are also set forth in Table 12 in Example 4. A guide RNA DNA-targeting segment can comprise, consist essentially of, or consist of any those sequences. Exemplary crRNAs and sgRNAs (comprising scaffold version 1, 2, 3, or 4) corresponding to the guide RNA target sequences in Table 12 in Example 4 can comprise, consist essentially of, or consist of any of the crRNA or sgRNA sequences set forth in Table 12 in Example 4.

[00328] Guide RNAs can comprise two segments: a "DNA-targeting segment" and a "protein-binding segment." "Segment" includes a section or region of a molecule, such as a contiguous stretch of nucleotides in an RNA. Some gRNAs, such as those for Cas9, can comprise two separate RNA molecules: an "activator-RNA" (e.g., tracrRNA) and a "targeter-RNA" (e.g., CRISPR RNA or crRNA). Other gRNAs are a single RNA molecule (single RNA polynucleotide), which can also be called a "single-molecule gRNA," a "single-guide RNA," or an "sgRNA." *See, e.g.*, WO 2013/176772, WO 2014/065596, WO 2014/089290, WO

2014/093622, WO 2014/099750, WO 2013/142578, and WO 2014/131833, each of which is herein incorporated by reference in its entirety for all purposes. For Cas9, for example, a single-guide RNA can comprise a crRNA fused to a tracrRNA (e.g., via a linker). For Cpf1, for example, only a crRNA is needed to achieve binding to and/or cleavage of a target sequence. The terms "guide RNA" and "gRNA" include both double-molecule (i.e., modular) gRNAs and single-molecule gRNAs.

[00329] An exemplary two-molecule gRNA comprises a crRNA-like ("CRISPR RNA" or "targeter-RNA" or "crRNA" or "crRNA repeat") molecule and a corresponding tracrRNA-like ("trans-acting CRISPR RNA" or "activator-RNA" or "tracrRNA") molecule. A crRNA comprises both the DNA-targeting segment (single-stranded) of the gRNA and a stretch of nucleotides (i.e., the crRNA tail) that forms one half of the dsRNA duplex of the protein-binding segment of the gRNA. An example of a crRNA tail, located downstream (3') of the DNA-targeting segment, comprises, consists essentially of, or consists of GUUUUAGAGCUAUGCU (SEQ ID NO: 1421). Any of the DNA-targeting segments disclosed herein can be joined to the 5' end of SEQ ID NO: 1421 to form a crRNA.

[00330] A corresponding tracrRNA (activator-RNA) comprises a stretch of nucleotides that forms the other half of the dsRNA duplex of the protein-binding segment of the gRNA. A stretch of nucleotides of a crRNA are complementary to and hybridize with a stretch of nucleotides of a tracrRNA to form the dsRNA duplex of the protein-binding domain of the gRNA. As such, each crRNA can be said to have a corresponding tracrRNA. An example of a tracrRNA sequence comprises, consists essentially of, or consists of AGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACC GAGUCGGUGCUUU (SEQ ID NO: 1422).

[00331] In systems in which both a crRNA and a tracrRNA are needed, the crRNA and the corresponding tracrRNA hybridize to form a gRNA. In systems in which only a crRNA is needed, the crRNA can be the gRNA. The crRNA additionally provides the single-stranded DNA-targeting segment that targets a guide RNA target sequence by hybridizing to the opposite strand (i.e., the complementary strand). If used for modification within a cell, the exact sequence of a given crRNA or tracrRNA molecule can be designed to be specific to the species in which the RNA molecules will be used. *See, e.g.*, Mali et al. (2013) *Science* 339:823-826; Jinek et al. (2012) *Science* 337:816-821; Hwang et al. (2013) *Nat. Biotechnol.* 31:227-229; Jiang et al.

(2013) *Nat. Biotechnol.* 31:233-239; and Cong et al. (2013) *Science* 339:819-823, each of which is herein incorporated by reference in its entirety for all purposes.

[00332] The DNA-targeting segment (crRNA) of a given gRNA comprises a nucleotide sequence that is complementary to a sequence (i.e., the complementary strand of the guide RNA recognition sequence on the strand opposite of the guide RNA target sequence) in a target DNA. The DNA-targeting segment of a gRNA interacts with a target DNA (e.g., the *HSD17B13* gene) in a sequence-specific manner via hybridization (i.e., base pairing). As such, the nucleotide sequence of the DNA-targeting segment may vary and determines the location within the target DNA with which the gRNA and the target DNA will interact. The DNA-targeting segment of a subject gRNA can be modified to hybridize to any desired sequence within a target DNA. Naturally occurring crRNAs differ depending on the CRISPR/Cas system and organism but often contain a targeting segment of between 21 to 72 nucleotides length, flanked by two direct repeats (DR) of a length of between 21 to 46 nucleotides (*see, e.g.*, WO 2014/131833, herein incorporated by reference in its entirety for all purposes). In the case of *S. pyogenes*, the DRs are 36 nucleotides long and the targeting segment is 30 nucleotides long. The 3' located DR is complementary to and hybridizes with the corresponding tracrRNA, which in turn binds to the Cas protein.

[00333] The DNA-targeting segment can have a length of at least about 12 nucleotides, at least about 15 nucleotides, at least about 17 nucleotides, at least about 18 nucleotides, at least about 19 nucleotides, at least about 20 nucleotides, at least about 25 nucleotides, at least about 30 nucleotides, at least about 35 nucleotides, or at least about 40 nucleotides. Such DNA-targeting segments can have a length from about 12 nucleotides to about 100 nucleotides, from about 12 nucleotides to about 80 nucleotides, from about 12 nucleotides to about 50 nucleotides, from about 12 nucleotides to about 40 nucleotides, from about 12 nucleotides to about 30 nucleotides, from about 12 nucleotides to about 25 nucleotides, or from about 12 nucleotides to about 20 nucleotides. For example, the DNA targeting segment can be from about 15 nucleotides to about 25 nucleotides (e.g., from about 17 nucleotides to about 20 nucleotides, or about 17 nucleotides, about 18 nucleotides, about 19 nucleotides, or about 20 nucleotides). *See, e.g.*, US 2016/0024523, herein incorporated by reference in its entirety for all purposes. For Cas9 from *S. pyogenes*, a typical DNA-targeting segment is between 16 and 20 nucleotides in length or between 17 and 20 nucleotides in length. For Cas9 from *S. aureus*, a typical DNA-targeting

segment is between 21 and 23 nucleotides in length. For Cpf1, a typical DNA-targeting segment is at least 16 nucleotides in length or at least 18 nucleotides in length.

[00334] TracrRNAs can be in any form (e.g., full-length tracrRNAs or active partial tracrRNAs) and of varying lengths. They can include primary transcripts or processed forms. For example, tracrRNAs (as part of a single-guide RNA or as a separate molecule as part of a two-molecule gRNA) may comprise or consist of all or a portion of a wild type tracrRNA sequence (e.g., about or more than about 20, 26, 32, 45, 48, 54, 63, 67, 85, or more nucleotides of a wild type tracrRNA sequence). Examples of wild type tracrRNA sequences from *S. pyogenes* include 171-nucleotide, 89-nucleotide, 75-nucleotide, and 65-nucleotide versions. *See, e.g.*, Deltcheva et al. (2011) *Nature* 471:602-607; WO 2014/093661, each of which is herein incorporated by reference in its entirety for all purposes. Examples of tracrRNAs within single-guide RNAs (sgRNAs) include the tracrRNA segments found within +48, +54, +67, and +85 versions of sgRNAs, where "+n" indicates that up to the +n nucleotide of wild type tracrRNA is included in the sgRNA. *See* US 8,697,359, herein incorporated by reference in its entirety for all purposes.

[00335] The percent complementarity between the DNA-targeting sequence and the complementary strand of the guide RNA recognition sequence within the target DNA can be at least 60% (e.g., at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, at least 99%, or 100%). The percent complementarity between the DNA-targeting sequence and the complementary strand of the guide RNA recognition sequence within the target DNA can be at least 60% over about 20 contiguous nucleotides. As an example, the percent complementarity between the DNA-targeting sequence and the complementary strand of the guide RNA recognition sequence within the target DNA is 100% over the 14 contiguous nucleotides at the 5' end of the complementary strand of the guide RNA recognition sequence within the complementary strand of the target DNA and as low as 0% over the remainder. In such a case, the DNA-targeting sequence can be considered to be 14 nucleotides in length. As another example, the percent complementarity between the DNA-targeting sequence and the complementary strand of the guide RNA recognition sequence within the target DNA is 100% over the seven contiguous nucleotides at the 5' end of the complementary strand of the guide RNA recognition sequence within the complementary strand of the target DNA and as low as 0% over the remainder. In such a case, the DNA-targeting

sequence can be considered to be 7 nucleotides in length. In some guide RNAs, at least 17 nucleotides within the DNA-targeting sequence are complementary to the target DNA. For example, the DNA-targeting sequence can be 20 nucleotides in length and can comprise 1, 2, or 3 mismatches with the complementary strand of the guide RNA recognition sequence. Preferably, the mismatches are not adjacent to a protospacer adjacent motif (PAM) sequence (e.g., the mismatches are in the 5' end of the DNA-targeting sequence, or the mismatches are at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, or 19 base pairs away from the PAM sequence).

[00336] The protein-binding segment of a gRNA can comprise two stretches of nucleotides that are complementary to one another. The complementary nucleotides of the protein-binding segment hybridize to form a double-stranded RNA duplex (dsRNA). The protein-binding segment of a subject gRNA interacts with a Cas protein, and the gRNA directs the bound Cas protein to a specific nucleotide sequence within target DNA via the DNA-targeting segment.

[00337] Single-guide RNAs have the DNA-targeting segment and a scaffold sequence (i.e., the protein-binding or Cas-binding sequence of the guide RNA). For example, such guide RNAs have a 5' DNA-targeting segment and a 3' scaffold sequence. Exemplary scaffold sequences comprise, consist essentially of, or consist of:
GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGA AAAAGUGGCACCGAGUCGGUGCU (version 1; SEQ ID NO: 1420);
GUUGGAACCAUUCAAAACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCA ACUUGAAAAAGUGGCACCGAGUCGGUGC (version 2; SEQ ID NO: 256);
GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGA AAAAGUGGCACCGAGUCGGUGC (version 3; SEQ ID NO: 257); and
GUUUAAGAGCUAUGCUGGAAACAGCAUAGCAAGUUUAAAUAAGGCUAGUCCGUU AUCAACUUGAAAAAGUGGCACCGAGUCGGUGC (version 4; SEQ ID NO: 258). Guide RNAs targeting any of the guide RNA target sequences disclosed herein (e.g., SEQ ID NOS: 20-239 and 259-268) can include, for example, a DNA-targeting segment on the 5' end of the guide RNA fused to any of the exemplary guide RNA scaffold sequences on the 3' end of the guide RNA. That is, any of the DNA-targeting segments disclosed herein can be joined to the 5' end of any one of SEQ ID NOS: 1420, 256, 257, or 258 to form a single guide RNA (chimeric guide

RNA). Guide RNA versions 1, 2, 3, and 4 as disclosed elsewhere herein refer to DNA-targeting segments joined with scaffold versions 1, 2, 3, and 4, respectively.

[00338] Guide RNAs can include modifications or sequences that provide for additional desirable features (e.g., modified or regulated stability; subcellular targeting; tracking with a fluorescent label; a binding site for a protein or protein complex; and the like). Examples of such modifications include, for example, a 5' cap (e.g., a 7-methylguanylate cap (m7G)); a 3' polyadenylated tail (i.e., a 3' poly(A) tail); a riboswitch sequence (e.g., to allow for regulated stability and/or regulated accessibility by proteins and/or protein complexes); a stability control sequence; a sequence that forms a dsRNA duplex (i.e., a hairpin); a modification or sequence that targets the RNA to a subcellular location (e.g., nucleus, mitochondria, chloroplasts, and the like); a modification or sequence that provides for tracking (e.g., direct conjugation to a fluorescent molecule, conjugation to a moiety that facilitates fluorescent detection, a sequence that allows for fluorescent detection, and so forth); a modification or sequence that provides a binding site for proteins (e.g., proteins that act on DNA, including transcriptional activators, transcriptional repressors, DNA methyltransferases, DNA demethylases, histone acetyltransferases, histone deacetylases, and the like); and combinations thereof. Other examples of modifications include engineered stem loop duplex structures, engineered bulge regions, engineered hairpins 3' of the stem loop duplex structure, or any combination thereof. *See, e.g.*, US 2015/0376586, herein incorporated by reference in its entirety for all purposes. A bulge can be an unpaired region of nucleotides within the duplex made up of the crRNA-like region and the minimum tracrRNA-like region. A bulge can comprise, on one side of the duplex, an unpaired 5′-XXXY-3′ where X is any purine and Y can be a nucleotide that can form a wobble pair with a nucleotide on the opposite strand, and an unpaired nucleotide region on the other side of the duplex.

[00339] In some cases, a transcriptional activation system can be used comprising a dCas9-VP64 fusion protein paired with MS2-p65-HSF1. Guide RNAs in such systems can be designed with aptamer sequences appended to sgRNA tetraloop and stem-loop 2 designed to bind dimerized MS2 bacteriophage coat proteins. *See, e.g.*, Konermann et al. (2015) *Nature* 517(7536):583-588, herein incorporated by reference in its entirety for all purposes.

[00340] Guide RNAs can be provided in any form. For example, the gRNA can be provided in the form of RNA, either as two molecules (separate crRNA and tracrRNA) or as one molecule

(sgRNA), and optionally in the form of a complex with a Cas protein. For example, gRNAs can be prepared by *in vitro* transcription using, for example, T7 RNA polymerase (*see, e.g.*, WO 2014/089290 and WO 2014/065596, each of which is herein incorporated by reference in its entirety for all purposes). Guide RNAs can also be prepared by chemical synthesis.

[00341] The gRNA can also be provided in the form of DNA encoding the gRNA. The DNA encoding the gRNA can encode a single RNA molecule (sgRNA) or separate RNA molecules (e.g., separate crRNA and tracrRNA). In the latter case, the DNA encoding the gRNA can be provided as one DNA molecule or as separate DNA molecules encoding the crRNA and tracrRNA, respectively.

[00342] When a gRNA is provided in the form of DNA, the gRNA can be transiently, conditionally, or constitutively expressed in the cell. DNAs encoding gRNAs can be stably integrated into the genome of the cell and operably linked to a promoter active in the cell. Alternatively, DNAs encoding gRNAs can be operably linked to a promoter in an expression construct. For example, the DNA encoding the gRNA can be in a vector comprising a heterologous nucleic acid. The vector can further comprise an exogenous donor sequence and/or the vector can further comprise a nucleic acid encoding a Cas protein. Alternatively, the DNA encoding the gRNA can be in a vector or a plasmid that is separate from the vector comprising an exogenous donor sequence and/or the vector comprising the nucleic acid encoding the Cas protein. Promoters that can be used in such expression constructs include promoters active, for example, in one or more of a eukaryotic cell, a human cell, a non-human cell, a mammalian cell, a non-human mammalian cell, a rodent cell, a mouse cell, a rat cell, a hamster cell, a rabbit cell, a pluripotent cell, an embryonic stem (ES) cell, an adult stem cell, a developmentally restricted progenitor cell, an induced pluripotent stem (iPS) cell, or a one-cell stage embryo. Such promoters can be, for example, conditional promoters, inducible promoters, constitutive promoters, or tissue-specific promoters. Such promoters can also be, for example, bidirectional promoters. Specific examples of suitable promoters include an RNA polymerase III promoter, such as a human U6 promoter, a rat U6 polymerase III promoter, or a mouse U6 polymerase III promoter.

[00343] Also disclosed herein are compositions comprising one or more guide RNAs (e.g., 1, 2, 3, 4, or more guide RNAs) disclosed herein and a carrier increasing the stability of the isolated nucleic acid or protein (e.g., prolonging the period under given conditions of storage (e.g., -

,20°C, 4°C, or ambient temperature) for which degradation products remain below a threshold, such below 0.5% by weight of the starting nucleic acid or protein; or increasing the stability in vivo). Non-limiting examples of such carriers include poly(lactic acid) (PLA) microspheres, poly(D,L-lactic-coglycolic-acid) (PLGA) microspheres, liposomes, micelles, inverse micelles, lipid cochleates, and lipid microtubules. Such compositions can further comprise a Cas protein, such as a Cas9 protein, or a nucleic acid encoding a Cas protein. Such compositions can further comprise one or more (e.g., 1, 2, 3, 4, or more) exogenous donor sequences and/or one or more (e.g., 1, 2, 3, 4, or more) targeting vectors and/or one or more (e.g., 1, 2, 3, 4, or more) expression vectors as disclosed elsewhere herein.

(3) Guide RNA Recognition Sequences and Guide RNA Target Sequences

[00344] The term "guide RNA recognition sequence" includes nucleic acid sequences present in a target DNA (e.g., the *HSD17B13* gene) to which a DNA-targeting segment of a gRNA will bind, provided sufficient conditions for binding exist. The term guide RNA recognition sequence as used herein encompasses both strands of the target double-stranded DNA (i.e., the sequence on the complementary strand to which the guide RNA hybridizes and the corresponding sequence on the non-complementary strand adjacent to the protospacer adjacent motif (PAM)). The term "guide RNA target sequence" as used herein refers specifically to the sequence on the non-complementary strand adjacent to the PAM (i.e., upstream or 5' of the PAM). That is, the guide RNA target sequence refers to the sequence on the non-complementary strand corresponding to the sequence to which the guide RNA hybridizes on the complementary strand. A guide RNA target sequence is equivalent to the DNA-targeting segment of a guide RNA, but with thymines instead of uracils. As one example, a guide RNA target sequence for a Cas9 enzyme would refer to the sequence on the non-complementary strand adjacent to the 5'-NGG-3' PAM. Guide RNA recognition sequences include sequences to which a guide RNA is designed to have complementarity, where hybridization between the complementary strand of a guide RNA recognition sequence and a DNA targeting sequence of a guide RNA promotes the formation of a CRISPR complex. Full complementarity is not necessarily required, provided that there is sufficient complementarity to cause hybridization and promote formation of a CRISPR complex. Guide RNA recognition sequences or guide RNA target sequences also include cleavage sites for Cas proteins, described in more detail below. A guide RNA

recognition sequence or guide RNA target sequence can comprise any polynucleotide, which can be located, for example, in the nucleus or cytoplasm of a cell or within an organelle of a cell, such as a mitochondrion or chloroplast.

[00345] The guide RNA recognition sequence within a target DNA can be targeted by (i.e., be bound by, or hybridize with, or be complementary to) a Cas protein or a gRNA. Suitable DNA/RNA binding conditions include physiological conditions normally present in a cell. Other suitable DNA/RNA binding conditions (e.g., conditions in a cell-free system) are known (*see, e.g.*, Molecular Cloning: A Laboratory Manual, 3rd Ed. (Sambrook et al., Harbor Laboratory Press 2001), herein incorporated by reference in its entirety for all purposes). The strand of the target DNA that is complementary to and hybridizes with the Cas protein or gRNA can be called the "complementary strand," and the strand of the target DNA that is complementary to the "complementary strand" (and is therefore not complementary to the Cas protein or gRNA) can be called "non-complementary strand" or "template strand."

[00346] The Cas protein can cleave the nucleic acid at a site within or outside of the nucleic acid sequence present in the target DNA to which the DNA-targeting segment of a gRNA will bind. The "cleavage site" includes the position of a nucleic acid at which a Cas protein produces a single-strand break or a double-strand break. For example, formation of a CRISPR complex (comprising a gRNA hybridized to the complementary strand of a guide RNA recognition sequence and complexed with a Cas protein) can result in cleavage of one or both strands in or near (e.g., within 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 50, or more base pairs from) the nucleic acid sequence present in a target DNA to which a DNA-targeting segment of a gRNA will bind. If the cleavage site is outside of the nucleic acid sequence to which the DNA-targeting segment of the gRNA will bind, the cleavage site is still considered to be within the "guide RNA recognition sequence" or guide RNA target sequence. The cleavage site can be on only one strand or on both strands of a nucleic acid. Cleavage sites can be at the same position on both strands of the nucleic acid (producing blunt ends) or can be at different sites on each strand (producing staggered ends (i.e., overhangs)). Staggered ends can be produced, for example, by using two Cas proteins, each of which produces a single-strand break at a different cleavage site on a different strand, thereby producing a double-strand break. For example, a first nickase can create a single-strand break on the first strand of double-stranded DNA (dsDNA), and a second nickase can create a single-strand break on the second strand of dsDNA such that overhanging sequences

are created. In some cases, the guide RNA recognition sequence or guide RNA target sequence of the nickase on the first strand is separated from the guide RNA recognition sequence or guide RNA target sequence of the nickase on the second strand by at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 75, 100, 250, 500, or 1,000 base pairs.

[00347] Site-specific binding and/or cleavage of target DNA by Cas proteins can occur at locations determined by both (i) base-pairing complementarity between the gRNA and the target DNA and (ii) a short motif, called the protospacer adjacent motif (PAM), in the target DNA. The PAM can flank the guide RNA target sequence on the non-complementary strand opposite of the strand to which the guide RNA hybridizes. Optionally, the guide RNA target sequence can be flanked on the 3' end by the PAM. Alternatively, the guide RNA target sequence can be flanked on the 5' end by the PAM. For example, the cleavage site of Cas proteins can be about 1 to about 10 or about 2 to about 5 base pairs (e.g., 3 base pairs) upstream or downstream of the PAM sequence. In some cases (e.g., when Cas9 from *S. pyogenes* or a closely related Cas9 is used), the PAM sequence of the non-complementary strand can be 5'-N_1GG-3', where $N_1$is any DNA nucleotide and is immediately 3' of the guide RNA recognition sequence of the non-complementary strand of the target DNA (i.e., immediately 3' of the guide RNA target sequence). As such, the PAM sequence of the complementary strand would be 5'-CCN_2-3', where N_2 is any DNA nucleotide and is immediately 5' of the guide RNA recognition sequence of the complementary strand of the target DNA. In some such cases, N_1 and N_2 can be complementary and the N_1- N_2 base pair can be any base pair (e.g., N_1=C and N_2=G; N_1=G and N_2=C; N_1=A and N_2=T; or N_1=T, and N_2=A). In the case of Cas9 from *S. aureus*, the PAM can be NNGRRT or NNGRR, where N can be A, G, C, or T, and R can be G or A. In some cases (e.g., for FnCpf1), the PAM sequence can be upstream of the 5' end and have the sequence 5'-TTN-3'.

[00348] Examples of guide RNA target sequences or guide RNA target sequences in addition to a PAM sequence are provided below. For example, the guide RNA target sequence can be a 20-nucleotide DNA sequence immediately preceding an NGG motif recognized by a Cas9 protein. Examples of such guide RNA target sequence plus a PAM sequence are GN_{19}NGG (SEQ ID NO: 248) or N_{20}NGG (SEQ ID NO: 249). *See, e.g.*, WO 2014/165825, herein incorporated by reference in its entirety for all purposes. The guanine at the 5' end can facilitate transcription by RNA polymerase in cells. Other examples of guide RNA target sequences plus

a PAM sequence can include two guanine nucleotides at the 5' end (e.g., $GGN_{20}NGG$; SEQ ID NO: 250) to facilitate efficient transcription by T7 polymerase *in vitro*. *See, e.g.*, WO 2014/065596, herein incorporated by reference in its entirety for all purposes. Other guide RNA target sequences plus a PAM sequence can have between 4-22 nucleotides in length of SEQ ID NOS: 248-250, including the 5' G or GG and the 3' GG or NGG. Yet other guide RNA target sequences can have between 14 and 20 nucleotides in length of SEQ ID NOS: 248-250.

[00349] The guide RNA recognition sequence or guide RNA target sequence can be any nucleic acid sequence endogenous or exogenous to a cell. The guide RNA recognition sequence or guide RNA target sequence can be a sequence coding a gene product (e.g., a protein) or a non-coding sequence (e.g., a regulatory sequence) or can include both.

[00350] As one example, the guide RNA recognition sequence or guide RNA target sequence can be within a region corresponding to exon 6 and/or intron 6, exon 6 and/or exon 7, or exon 6 and/or intron 6 and/or exon 7 of SEQ ID NO: 2 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 2 . As another example, the guide RNA recognition sequence or guide RNA target sequence can include or is proximate to a position corresponding to position 12666 of SEQ ID NO: 2 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 2. For example, the guide RNA recognition sequence or guide RNA target sequence can be within about 1000, 500, 400, 300, 200, 100, 50, 45, 40, 35, 30, 25, 20, 15, 10, or 5 nucleotides of the position corresponding to position 12666 of SEQ ID NO: 2 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 2. As yet another example, the guide RNA recognition sequence or guide RNA target sequence can include or be proximate to the start codon of an *HSD17B13* gene or the stop codon of an *HSD17B13* gene. For example, the guide RNA recognition sequence or guide RNA target sequence can be within about 10, 20, 30, 40, 50, 100, 200, 300, 400, 500, or 1,000 nucleotides of the start codon or the stop codon. Examples of such guide RNA target sequences and of guide RNAs targeting such guide RNA target sequences are disclosed elsewhere herein.

F. Exogenous Donor Sequences or Targeting Vectors

[00351] The methods and compositions disclosed herein can utilize exogenous donor sequences (e.g., targeting vectors or repair templates) to modify an *HSD17B13* gene, either without cleavage of the *HSD17B13* gene or following cleavage of the *HSD17B13* gene with a

nuclease agent. An exogenous donor sequence refers to any nucleic acid or vector that includes the elements that are required to enable site-specific recombination with a target sequence. Using exogenous donor sequences in combination with nuclease agents may result in more precise modifications within the *HSD17B13* gene by promoting homology-directed repair.

[00352] In such methods, the nuclease agent cleaves the *HSD17B13* gene to create a single-strand break (nick) or double-strand break, and the exogenous donor sequence recombines the *HSD17B13* gene via non-homologous end joining (NHEJ)-mediated ligation or through a homology-directed repair event. Optionally, repair with the exogenous donor sequence removes or disrupts the nuclease cleavage site so that alleles that have been targeted cannot be re-targeted by the nuclease agent.

[00353] Exogenous donor sequences can comprise deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), they can be single-stranded or double-stranded, and they can be in linear or circular form. For example, an exogenous donor sequence can be a single-stranded oligodeoxynucleotide (ssODN). *See, e.g.*, Yoshimi et al. (2016) *Nat. Commun.* 7:10431, herein incorporated by reference in its entirety for all purposes. An exemplary exogenous donor sequence is between about 50 nucleotides to about 5 kb in length, is between about 50 nucleotides to about 3 kb in length, or is between about 50 to about 1,000 nucleotides in length. Other exemplary exogenous donor sequences are between about 40 to about 200 nucleotides in length. For example, an exogenous donor sequence can be between about 50 to about 60, about 60 to about 70, about 70 to about 80, about 80 to about 90, about 90 to about 100, about 100 to about 110, about 110 to about 120, about 120 to about 130, about 130 to about 140, about 140 to about 150, about 150 to about 160, about 160 to about 170, about 170 to about 180, about 180 to about 190, or about 190 to about 200 nucleotides in length. Alternatively, an exogenous donor sequence can be between about 50 to about 100, about 100 to about 200, about 200 to about 300, about 300 to about 400, about 400 to about 500, about 500 to about 600, about 600 to about 700, about 700 to about 800, about 800 to about 900, or about 900 to about 1,000 nucleotides in length. Alternatively, an exogenous donor sequence can be between about 1 kb to about 1.5 kb, about 1.5 kb to about 2 kb, about 2 kb to about 2.5 kb, about 2.5 kb to about 3 kb, about 3 kb to about 3.5 kb, about 3.5 kb to about 4 kb, about 4 kb to about 4.5 kb, or about 4.5 kb to about 5 kb in length. Alternatively, an exogenous donor sequence can be, for example, no more than 5 kb, 4.5 kb, 4 kb, 3.5 kb, 3 kb, 2.5 kb, 2 kb, 1.5 kb, 1 kb, 900 nucleotides, 800 nucleotides, 700

nucleotides, 600 nucleotides, 500 nucleotides, 400 nucleotides, 300 nucleotides, 200 nucleotides, 100 nucleotides, or 50 nucleotides in length.

[00354] In one example, an exogenous donor sequence is an ssODN that is between about 80 nucleotides and about 200 nucleotides in length (e.g., about 120 nucleotides in length). In another example, an exogenous donor sequences is an ssODN that is between about 80 nucleotides and about 3 kb in length. Such an ssODN can have homology arms, for example, that are each between about 40 nucleotides and about 60 nucleotides in length. Such an ssODN can also have homology arms, for example, that are each between about 30 nucleotides and 100 nucleotides in length. The homology arms can be symmetrical (e.g., each 40 nucleotides or each 60 nucleotides in length), or they can be asymmetrical (e.g., one homology arm that is 36 nucleotides in length, and one homology arm that is 91 nucleotides in length).

[00355] Exogenous donor sequences can include modifications or sequences that provide for additional desirable features (e.g., modified or regulated stability; tracking or detecting with a fluorescent label; a binding site for a protein or protein complex; and so forth). Exogenous donor sequences can comprise one or more fluorescent labels, purification tags, epitope tags, or a combination thereof. For example, an exogenous donor sequence can comprise one or more fluorescent labels (e.g., fluorescent proteins or other fluorophores or dyes), such as at least 1, at least 2, at least 3, at least 4, or at least 5 fluorescent labels. Exemplary fluorescent labels include fluorophores such as fluorescein (e.g., 6-carboxyfluorescein (6-FAM)), Texas Red, HEX, Cy3, Cy5, Cy5.5, Pacific Blue, 5-(and-6)-carboxytetramethylrhodamine (TAMRA), and Cy7. A wide range of fluorescent dyes are available commercially for labeling oligonucleotides (e.g., from Integrated DNA Technologies). Such fluorescent labels (e.g., internal fluorescent labels) can be used, for example, to detect an exogenous donor sequence that has been directly integrated into a cleaved *HSD17B13* gene having protruding ends compatible with the ends of the exogenous donor sequence. The label or tag can be at the 5' end, the 3' end, or internally within the exogenous donor sequence. For example, an exogenous donor sequence can be conjugated at 5' end with the IR700 fluorophore from Integrated DNA Technologies (5'IRDYE®700).

[00356] Exogenous donor sequences can also comprise nucleic acid inserts including segments of DNA to be integrated in the *HSD17B13* gene. Integration of a nucleic acid insert in the *HSD17B13* gene can result in addition of a nucleic acid sequence of interest in the *HSD17B13* gene, deletion of a nucleic acid sequence of interest in the *HSD17B13* gene, or

replacement of a nucleic acid sequence of interest in the *HSD17B13* gene (i.e., deletion and insertion). Some exogenous donor sequences are designed for insertion of a nucleic acid insert in the *HSD17B13* gene without any corresponding deletion in the *HSD17B13* gene. Other exogenous donor sequences are designed to delete a nucleic acid sequence of interest in the *HSD17B13* gene without any corresponding insertion of a nucleic acid insert. Yet other exogenous donor sequences are designed to delete a nucleic acid sequence of interest in the *HSD17B13* gene and replace it with a nucleic acid insert.

[00357] The nucleic acid insert or the corresponding nucleic acid in the *HSD17B13* gene being deleted and/or replaced can be various lengths. An exemplary nucleic acid insert or corresponding nucleic acid in the *HSD17B13* gene being deleted and/or replaced is between about 1 nucleotide to about 5 kb in length or is between about 1 nucleotide to about 1,000 nucleotides in length. For example, a nucleic acid insert or a corresponding nucleic acid in the *HSD17B13* gene being deleted and/or replaced can be between about 1 to about 10, about 10 to about 20, about 20 to about 30, about 30 to about 40, about 40 to about 50, about 50 to about 60, about 60 to about 70, about 70 to about 80, about 80 to about 90, about 90 to about 100, about 100 to about 110, about 110 to about 120, about 120 to about 130, about 130 to about 140, about 140 to about 150, about 150 to about 160, about 160 to about 170, about 170 to about 180, about 180 to about 190, or about 190 to about 200 nucleotides in length. Likewise, a nucleic acid insert or a corresponding nucleic acid in the *HSD17B13* gene being deleted and/or replaced can be between about 1 to about 100, about 100 to about 200, about 200 to about 300, about 300 to about 400, about 400 to about 500, about 500 to about 600, about 600 to about 700, about 700 to about 800, about 800 to about 900, or about 900 to about 1,000 nucleotides in length. Likewise, a nucleic acid insert or a corresponding nucleic acid in the *HSD17B13* gene being deleted and/or replaced can be between about 1 kb to about 1.5 kb, about 1.5 kb to about 2 kb, about 2 kb to about 2.5 kb, about 2.5 kb to about 3 kb, about 3 kb to about 3.5 kb, about 3.5 kb to about 4 kb, about 4 kb to about 4.5 kb, or about 4.5 kb to about 5 kb in length.

[00358] The nucleic acid insert can comprise genomic DNA or any other type of DNA. For example, the nucleic acid insert can comprise cDNA.

[00359] The nucleic acid insert can comprise a sequence that is homologous to all or part of the *HSD17B13* gene (e.g., a portion of the gene encoding a particular motif or region of a HSD17B13 protein). For example, the nucleic acid insert can comprise a sequence that

comprises one or more point mutations (e.g., 1, 2, 3, 4, 5, or more) or one or more nucleotide insertions or deletions compared with a sequence targeted for replacement in the *HSD17B13* gene.

[00360] The nucleic acid insert or the corresponding nucleic acid in the *HSD17B13* gene being deleted and/or replaced can be a coding region such as an exon; a non-coding region such as an intron, an untranslated region, or a regulatory region (e.g., a promoter, an enhancer, or a transcriptional repressor-binding element); or any combination thereof.

[00361] The nucleic acid insert can also comprise a conditional allele. The conditional allele can be a multifunctional allele, as described in US 2011/0104799, herein incorporated by reference in its entirety for all purposes. For example, the conditional allele can comprise: (a) an actuating sequence in sense orientation with respect to transcription of a target gene; (b) a drug selection cassette (DSC) in sense or antisense orientation; (c) a nucleotide sequence of interest (NSI) in antisense orientation; and (d) a conditional by inversion module (COIN, which utilizes an exon-splitting intron and an invertible gene-trap-like module) in reverse orientation. *See, e.g.*, US 2011/0104799. The conditional allele can further comprise recombinable units that recombine upon exposure to a first recombinase to form a conditional allele that (i) lacks the actuating sequence and the DSC; and (ii) contains the NSI in sense orientation and the COIN in antisense orientation. *See, e.g.*, US 2011/0104799.

[00362] Nucleic acid inserts can also comprise a polynucleotide encoding a selection marker. Alternatively, the nucleic acid inserts can lack a polynucleotide encoding a selection marker. The selection marker can be contained in a selection cassette. Optionally, the selection cassette can be a self-deleting cassette. *See, e.g.,* US 8,697,851 and US 2013/0312129, each of which is herein incorporated by reference in its entirety for all purposes. As an example, the self-deleting cassette can comprise a Crei gene (comprises two exons encoding a Cre recombinase, which are separated by an intron) operably linked to a mouse *Prm1* promoter and a neomycin resistance gene operably linked to a human ubiquitin promoter. Exemplary selection markers include neomycin phosphotransferase (neo^r), hygromycin B phosphotransferase (hyg^r), puromycin-N-acetyltransferase ($puro^r$), blasticidin S deaminase (bsr^r), xanthine/guanine phosphoribosyl transferase (gpt), or herpes simplex virus thymidine kinase (HSV-k), or a combination thereof. The polynucleotide encoding the selection marker can be operably linked to a promoter active in a cell being targeted. Examples of promoters are described elsewhere herein.

[00363] The nucleic acid insert can also comprise a reporter gene. Exemplary reporter genes include those encoding luciferase, β-galactosidase, green fluorescent protein (GFP), enhanced green fluorescent protein (eGFP), cyan fluorescent protein (CFP), yellow fluorescent protein (YFP), enhanced yellow fluorescent protein (eYFP), blue fluorescent protein (BFP), enhanced blue fluorescent protein (eBFP), DsRed, ZsGreen, MmGFP, mPlum, mCherry, tdTomato, mStrawberry, J-Red, mOrange, mKO, mCitrine, Venus, YPet, Emerald, CyPet, Cerulean, T-Sapphire, and alkaline phosphatase. Such reporter genes can be operably linked to a promoter active in a cell being targeted. Examples of promoters are described elsewhere herein.

[00364] The nucleic acid insert can also comprise one or more expression cassettes or deletion cassettes. A given cassette can comprise one or more of a nucleotide sequence of interest, a polynucleotide encoding a selection marker, and a reporter gene, along with various regulatory components that influence expression. Examples of selectable markers and reporter genes that can be included are discussed in detail elsewhere herein.

[00365] The nucleic acid insert can comprise a nucleic acid flanked with site-specific recombination target sequences. Alternatively, the nucleic acid insert can comprise one or more site-specific recombination target sequences. Although the entire nucleic acid insert can be flanked by such site-specific recombination target sequences, any region or individual polynucleotide of interest within the nucleic acid insert can also be flanked by such sites. Site-specific recombination target sequences, which can flank the nucleic acid insert or any polynucleotide of interest in the nucleic acid insert can include, for example, loxP, lox511, lox2272, lox66, lox71, loxM2, lox5171, FRT, FRT11, FRT71, attp, att, FRT, rox, or a combination thereof. In one example, the site-specific recombination sites flank a polynucleotide encoding a selection marker and/or a reporter gene contained within the nucleic acid insert. Following integration of the nucleic acid insert in the *HSD17B13* gene, the sequences between the site-specific recombination sites can be removed. Optionally, two exogenous donor sequences can be used, each with a nucleic acid insert comprising a site-specific recombination site. The exogenous donor sequences can be targeted to 5' and 3' regions flanking a nucleic acid of interest. Following integration of the two nucleic acid inserts into the target genomic locus, the nucleic acid of interest between the two inserted site-specific recombination sites can be removed.

[00366] Nucleic acid inserts can also comprise one or more restriction sites for restriction endonucleases (i.e., restriction enzymes), which include Type I, Type II, Type III, and Type IV endonucleases. Type I and Type III restriction endonucleases recognize specific recognition sequences, but typically cleave at a variable position from the nuclease binding site, which can be hundreds of base pairs away from the cleavage site (recognition sequence). In Type II systems the restriction activity is independent of any methylase activity, and cleavage typically occurs at specific sites within or near to the binding site. Most Type II enzymes cut palindromic sequences, however Type IIa enzymes recognize non-palindromic recognition sequences and cleave outside of the recognition sequence, Type IIb enzymes cut sequences twice with both sites outside of the recognition sequence, and Type IIs enzymes recognize an asymmetric recognition sequence and cleave on one side and at a defined distance of about 1-20 nucleotides from the recognition sequence. Type IV restriction enzymes target methylated DNA. Restriction enzymes are further described and classified, for example in the REBASE database (webpage at rebase.neb.com; Roberts et al., (2003) *Nucleic Acids Res.* 31:418-420; Roberts et al., (2003) *Nucleic Acids Res.* 31:1805-1812; and Belfort et al. (2002) in Mobile DNA II, pp. 761-783, Eds. Craigie et al., (ASM Press, Washington, DC)).

(1) Donor Sequences for Non-Homologous-End-Joining-Mediated Insertion

[00367] Some exogenous donor sequences have short single-stranded regions at the 5' end and/or the 3' end that are complementary to one or more overhangs created by nuclease-mediated or Cas-protein-mediated cleavage at the target genomic locus (e.g., in the *HSD17B13* gene). These overhangs can also be referred to as 5' and 3' homology arms. For example, some exogenous donor sequences have short single-stranded regions at the 5' end and/or the 3' end that are complementary to one or more overhangs created by Cas-protein-mediated cleavage at 5' and/or 3' target sequences at the target genomic locus. Some such exogenous donor sequences have a complementary region only at the 5' end or only at the 3' end. For example, some such exogenous donor sequences have a complementary region only at the 5' end complementary to an overhang created at a 5' target sequence at the target genomic locus or only at the 3' end complementary to an overhang created at a 3' target sequence at the target genomic locus. Other such exogenous donor sequences have complementary regions at both the 5' and 3' ends. For example, other such exogenous donor sequences have complementary regions at both

the 5' and 3' ends e.g., complementary to first and second overhangs, respectively, generated by Cas-mediated cleavage at the target genomic locus. For example, if the exogenous donor sequence is double-stranded, the single-stranded complementary regions can extend from the 5' end of the top strand of the donor sequence and the 5' end of the bottom strand of the donor sequence, creating 5' overhangs on each end. Alternatively, the single-stranded complementary region can extend from the 3' end of the top strand of the donor sequence and from the 3' end of the bottom strand of the template, creating 3' overhangs.

[00368] The complementary regions can be of any length sufficient to promote ligation between the exogenous donor sequence and the *HSD17B13* gene. Exemplary complementary regions are between about 1 to about 5 nucleotides in length, between about 1 to about 25 nucleotides in length, or between about 5 to about 150 nucleotides in length. For example, a complementary region can be at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleotides in length. Alternatively, the complementary region can be about 5 to about 10, about 10 to about 20, about 20 to about 30, about 30 to about 40, about 40 to about 50, about 50 to about 60, about 60 to about 70, about 70 to about 80, about 80 to about 90, about 90 to about 100, about 100 to about 110, about 110 to about 120, about 120 to about 130, about 130 to about 140, about 140 to about 150 nucleotides in length, or longer.

[00369] Such complementary regions can be complementary to overhangs created by two pairs of nickases. Two double-strand breaks with staggered ends can be created by using first and second nickases that cleave opposite strands of DNA to create a first double-strand break, and third and fourth nickases that cleave opposite strands of DNA to create a second double-strand break. For example, a Cas protein can be used to nick first, second, third, and fourth guide RNA target sequences corresponding with first, second, third, and fourth guide RNAs. The first and second guide RNA target sequences can be positioned to create a first cleavage site such that the nicks created by the first and second nickases on the first and second strands of DNA create a double-strand break (i.e., the first cleavage site comprises the nicks within the first and second guide RNA target sequences). Likewise, the third and fourth guide RNA target sequences can be positioned to create a second cleavage site such that the nicks created by the third and fourth nickases on the first and second strands of DNA create a double-strand break (i.e., the second cleavage site comprises the nicks within the third and fourth guide RNA target sequences). Preferably, the nicks within the first and second guide RNA target sequences and/or

the third and fourth guide RNA target sequences can be off-set nicks that create overhangs. The offset window can be, for example, at least about 5 bp, 10 bp, 20 bp, 30 bp, 40 bp, 50 bp, 60 bp, 70 bp, 80 bp, 90 bp, 100 bp or more. *See* Ran et al. (2013) *Cell* 154:1380-1389; Mali et al. (2013) *Nat. Biotech.*31:833-838; and Shen et al. (2014) *Nat. Methods* 11:399-404, each of which is herein incorporated by reference in its entirety for all purposes. In such cases, a double-stranded exogenous donor sequence can be designed with single-stranded complementary regions that are complementary to the overhangs created by the nicks within the first and second guide RNA target sequences and by the nicks within the third and fourth guide RNA target sequences. Such an exogenous donor sequence can then be inserted by non-homologous-end-joining-mediated ligation.

(2) Donor Sequences for Insertion by Homology-Directed Repair

[00370] Some exogenous donor sequences (i.e., targeting vectors) comprise homology arms. If the exogenous donor sequence also comprises a nucleic acid insert, the homology arms can flank the nucleic acid insert. For ease of reference, the homology arms are referred to herein as 5' and 3' (i.e., upstream and downstream) homology arms. This terminology relates to the relative position of the homology arms to the nucleic acid insert within the exogenous donor sequence. The 5' and 3' homology arms correspond to regions within the *HSD17B13* gene, which are referred to herein as "5' target sequence" and "3' target sequence," respectively.

[00371] A homology arm and a target sequence "correspond" or are "corresponding" to one another when the two regions share a sufficient level of sequence identity to one another to act as substrates for a homologous recombination reaction. The term "homology" includes DNA sequences that are either identical or share sequence identity to a corresponding sequence. The sequence identity between a given target sequence and the corresponding homology arm found in the exogenous donor sequence can be any degree of sequence identity that allows for homologous recombination to occur. For example, the amount of sequence identity shared by the homology arm of the exogenous donor sequence (or a fragment thereof) and the target sequence (or a fragment thereof) can be at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity, such that the sequences undergo homologous recombination. Moreover, a corresponding region of homology between the homology arm and

the corresponding target sequence can be of any length that is sufficient to promote homologous recombination. Exemplary homology arms are between about 25 nucleotides to about 2.5 kb in length, are between about 25 nucleotides to about 1.5 kb in length, or are between about 25 to about 500 nucleotides in length. For example, a given homology arm (or each of the homology arms) and/or corresponding target sequence can comprise corresponding regions of homology that are between about 25 to about 30, about 30 to about 40, about 40 to about 50, about 50 to about 60, about 60 to about 70, about 70 to about 80, about 80 to about 90, about 90 to about 100, about 100 to about 150, about 150 to about 200, about 200 to about 250, about 250 to about 300, about 300 to about 350, about 350 to about 400, about 400 to about 450, or about 450 to about 500 nucleotides in length, such that the homology arms have sufficient homology to undergo homologous recombination with the corresponding target sequences within the *HSD17B13* gene. Alternatively, a given homology arm (or each homology arm) and/or corresponding target sequence can comprise corresponding regions of homology that are between about 0.5 kb to about 1 kb, about 1 kb to about 1.5 kb, about 1.5 kb to about 2 kb, or about 2 kb to about 2.5 kb in length. For example, the homology arms can each be about 750 nucleotides in length. The homology arms can be symmetrical (each about the same size in length), or they can be asymmetrical (one longer than the other).

[00372] The homology arms can correspond to a locus that is native to a cell (e.g., the targeted locus). Alternatively, for example, they can correspond to a region of a heterologous or exogenous segment of DNA that was integrated into the genome of the cell, including, for example, transgenes, expression cassettes, or heterologous or exogenous regions of DNA. Alternatively, the homology arms of the targeting vector can correspond to a region of a yeast artificial chromosome (YAC), a bacterial artificial chromosome (BAC), a human artificial chromosome, or any other engineered region contained in an appropriate host cell. Still further, the homology arms of the targeting vector can correspond to or be derived from a region of a BAC library, a cosmid library, or a P1 phage library, or can be derived from synthetic DNA.

[00373] When a nuclease agent is used in combination with an exogenous donor sequence, the 5' and 3' target sequences are preferably located in sufficient proximity to the nuclease cleavage site so as to promote the occurrence of a homologous recombination event between the target sequences and the homology arms upon a single-strand break (nick) or double-strand break at the nuclease cleavage site. The term "nuclease cleavage site" includes a DNA sequence at which a

nick or double-strand break is created by a nuclease agent (e.g., a Cas9 protein complexed with a guide RNA). The target sequences within the *HSD17B13* gene that correspond to the 5' and 3' homology arms of the exogenous donor sequence are "located in sufficient proximity" to a nuclease cleavage site if the distance is such as to promote the occurrence of a homologous recombination event between the 5' and 3' target sequences and the homology arms upon a single-strand break or double-strand break at the nuclease cleavage site. Thus, the target sequences corresponding to the 5' and/or 3' homology arms of the exogenous donor sequence can be, for example, within at least 1 nucleotide of a given nuclease cleavage site or within at least 10 nucleotides to about 1,000 nucleotides of a given nuclease cleavage site. As an example, the nuclease cleavage site can be immediately adjacent to at least one or both of the target sequences.

[00374] The spatial relationship of the target sequences that correspond to the homology arms of the exogenous donor sequence and the nuclease cleavage site can vary. For example, target sequences can be located 5' to the nuclease cleavage site, target sequences can be located 3' to the nuclease cleavage site, or the target sequences can flank the nuclease cleavage site.

IV. Therapeutic and Prophylactic Applications

[00375] Also provided are therapeutic methods and methods of treatment or prophylaxis of a chronic liver disease in a subject having or at risk for the disease using the methods disclosed herein for modifying or altering expression of an endogenous *HSD17B13* gene. Also provided are therapeutic methods and methods of treatment or prophylaxis of a liver disease such as an alcoholic liver disease or a nonalcoholic liver disease in a subject having or at risk for the disease using the methods disclosed herein for modifying or altering expression of an endogenous *HSD17B13* gene. Also provided are therapeutic methods and methods of treatment or prophylaxis of a chronic liver disease in a subject having or at risk for the disease using methods for decreasing expression of HSD17B13 mRNA transcripts or using methods for providing recombinant nucleic acids encoding HSD17B13 proteins, providing mRNAs encoding HSD17B13 proteins, or providing HSD17B13 proteins to the subject. Also provided are therapeutic methods and methods of treatment or prophylaxis of a liver disease such as an alcoholic liver disease or a nonalcoholic liver disease in a subject having or at risk for the disease using methods for decreasing expression of HSD17B13 mRNA transcripts or using methods for

providing recombinant nucleic acids encoding HSD17B13 proteins, providing mRNAs encoding HSD17B13 proteins, or providing HSD17B13 proteins to the subject. The methods can comprise introducing one or more nucleic acids or proteins into the subject, into the liver of the subject, or into a cell (e.g., liver cell) of the subject (e.g., *in vivo* or *ex vivo*).

[00376] Chronic liver diseases include diseases of the liver which last over a period of six months and can include, for example, diseases of the liver involving progressive destruction and regeneration of the liver parenchyma that can lead to fibrosis and cirrhosis. Chronic liver diseases can be alcoholic liver diseases or nonalcoholic liver diseases. Liver pathologies encompassed by chronic liver diseases can include, for example, inflammation (e.g., chronic hepatitis), liver cirrhosis, and hepatocellular carcinoma. Types of chronic liver disease are disclosed elsewhere herein and include, for example, fatty liver disease, nonalcoholic fatty liver disease, alcoholic fatty liver disease, cirrhosis, and hepatocellular carcinoma. Symptoms and signs of chronic liver diseases are known and can include, for example, enlarged liver, fatigue, pain in the upper right abdomen, abdominal swelling (ascites), enlarged blood vessels just beneath the skin's surface, enlarged breasts in men, enlarged spleen, red palms, and yellowing of the skin and eyes (jaundice). Testing for chronic liver diseases can involve blood tests, imaging of the liver, and biopsy of the liver. An individual is at increased risk of a chronic liver disease if the subject has at least one known risk-factor (e.g., genetic factor such as a disease-causing mutation) placing individuals with that risk factor at a statistically significant greater risk of developing the disease than individuals without the risk factor. Risk factors for chronic liver diseases are also well known and can include, for example, excessive alcohol use, obesity, high cholesterol, high levels of triglycerides in the blood, polycystic ovary syndrome, sleep apnea, type 2 diabetes, underactive thyroid (hypothyroidism), underactive pituitary gland (hypopituitarism), and metabolic syndromes including raised blood lipids.

[00377] The term "subject" includes human and other mammalian subjects (e.g., feline, canine, rodent, mouse, or rat) or non-mammalian subjects (e.g., poultry) that receive either prophylactic or therapeutic treatment. Such subjects can be, for example, a subject (e.g., a human) who is not a carrier of the *HSD17B13* rs72613567 variant (or is only a heterozygous carrier of the *HSD17B13* rs72613567 variant) and has or is susceptible to developing a chronic liver disease. Various methods are possible for detecting the presence of the *HSD17B13* rs72613567 variant in a biological sample comprising genomic DNA, for detecting the presence

or levels of any one of or a combination of *HSD17B13* Transcripts C, D, E, F, F', G, and H, and particularly D, in a biological sample comprising mRNA or cDNA, or for detecting the presence or levels of any one of or a combination of HSD17B13 protein Isoforms C, D, E, F, F', G, or H, and particularly D, in a biological sample comprising protein. Methods for detecting the presence of a sequence in genomic DNA and for detecting the presence of a particular mRNA transcript or protein isoform are well-known. It is understood that gene sequences within a population and mRNAs and proteins encoded by such genes can vary due to polymorphisms such as single-nucleotide polymorphisms. The sequences provided herein for the *HSD17B13* gene and for each *HSD17B13* Transcript and HSD17B13 Isoform are only exemplary sequences for the *HSD17B13* gene and for each *HSD17B13* Transcript and HSD17B13 isoform. Other sequences for the *HSD17B13* gene and for each *HSD17B13* Transcript and HSD17B13 Isoform are also possible.

[00378] For example, a method for detecting an *HSD17B13* rs72613567 variant in a cell or in a subject such as a human subject can comprise, for example, obtaining a biological sample from the subject comprising an *HSD17B13* gene, and performing an assay on the biological sample that determines that a position of the *HSD17B13* gene corresponding to position 12666 of SEQ ID NO: 2 when the *HSD17B13* gene and SEQ ID NO: 2 are optimally aligned is occupied by a thymine or that a thymine is inserted between positions corresponding to positions 12665 and 12666 when the *HSD17B13* gene and SEQ ID NO: 1 are optimally aligned. It is understood that determining that a position of the *HSD17B13* gene corresponding to position 12666 of SEQ ID NO: 2 when the *HSD17B13* gene and SEQ ID NO: 2 are optimally aligned is occupied by a thymine means that the identity of a sufficient number of nucleotides is determined in the positions flanking the positions corresponding to positions 12665 and 12666 of SEQ ID NO: 1 that it can be determined that a thymine is inserted between the positions corresponding to positions 12665 and 12666 of SEQ ID NO: 1. Such assays can comprise, for example determining the identity of positions of the *HSD17B13* gene corresponding to position 12666 of SEQ ID NO: 2 (or positions 12665 and 12666 of SEQ ID NO: 1) and one or more surrounding positions (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 positions flanking one side or each side of position 12666 of SEQ ID NO: 2 or positions 12665 and 12666 of SEQ ID NO: 1) when the *HSD17B13* gene and SEQ ID NO: 2 (or SEQ ID NO: 1) are optimally aligned. The assay in such a method can comprise, for example, sequencing a portion of the *HSD17B13* gene including

a position corresponding to position 12666 or positions 12666 and 12667 of SEQ ID NO: 2 when the *HSD17B13* gene and SEQ ID NO: 2 are optimally aligned. Likewise, the assay can comprise sequencing a portion of the *HSD17B13* gene including positions corresponding to positions 12665 and 12666 of SEQ ID NO: 1 when the *HSD17B13* gene and SEQ ID NO: 1 are optimally aligned. Alternatively, the assay in such a method can comprise contacting the biological sample with a primer or probe that specifically hybridizes to the *HSD17B13* rs72613567 variant and not the corresponding wild type *HSD17B13* sequence (e.g., under stringent conditions), and determining whether hybridization has occurred.

[00379] Such methods can comprise genome editing or gene therapy. For example, an endogenous *HSD17B13* gene that is not the *HSD17B13* rs72613567 variant can be modified to comprise the variation associated with the *HSD17B13* rs72613567 variant (i.e., an insertion of a thymine between nucleotides corresponding to positions 12665 and 12666 of SEQ ID NO: 1 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 1, or an insertion of an adenine at the corresponding position on the opposite strand). As another example, an endogenous *HSD17B13* gene that is not the *HSD17B13* rs72613567 variant can be knocked out or inactivated. Likewise, an endogenous *HSD17B13* gene that is not the *HSD17B13* rs72613567 variant can be knocked out or inactivated, and an *HSD17B13* gene comprising the modification associated with the *HSD17B13* rs72613567 variant (e.g., the full *HSD17B13* rs72613567 variant or a minigene comprising the modification) can be introduced and expressed. Similarly, an endogenous *HSD17B13* gene that is not the *HSD17B13* rs72613567 variant can be knocked out or inactivated, and a recombinant DNA encoding any one of or any combination of HSD17B13 Isoforms C, D, F, G, and H (or fragments thereof) can be introduced and expressed, an mRNA encoding any one of or any combination of HSD17B13 Isoforms C, D, F, G, and H (or fragments thereof) can be introduced and expressed (e.g., intracellular protein replacement therapy), or any one of or any combination of HSD17B13 Isoforms C, D, F, G, and H (or fragments thereof) can be introduced (e.g., protein replacement therapy). In particular embodiments, the combination of HSD17B13 Isoforms (or DNA or mRNA encoding) is a combination comprising HSD17B13 Isoform D (e.g., D, DC, DF, DG, DH, DCF, DCG, DCH, DFG, DFH, DGH, DCFG, DCFH, DCGH, DFGH, or DCFGH).

[00380] Other such methods can comprise introducing and expressing a recombinant *HSD17B13* gene comprising the modification associated with the *HSD17B13* rs72613567 variant

(e.g., the full *HSD17B13* rs72613567 variant or a minigene comprising the modification), introducing and expressing recombinant nucleic acids (e.g., DNA) encoding any one of or any combination of HSD17B13 Isoforms C, D, F, G, and H or fragments thereof, introducing and expressing one or more mRNAs encoding any one of or any combination of HSD17B13 Isoforms C, D, F, G, and H or fragments thereof (e.g., intracellular protein replacement therapy), or introducing any one of or any combination of HSD17B13 Isoforms C, D, F, G, and H or fragments thereof (e.g., protein replacement therapy) without knocking out or inactivating an endogenous *HSD17B13* gene that is not the *HSD17B13* rs72613567 variant. In particular embodiments, the combination of HSD17B13 isoforms (or DNA or mRNA encoding) is a combination comprising HSD17B13 Isoform D (e.g., D, DC, DF, DG, DH, DCF, DCG, DCH, DFG, DFH, DGH, DCFG, DCFH, DCGH, DFGH, or DCFGH). Optionally, such methods can also be done in combination with methods in which an *HSD17B13* transcript whose expression decreases in carriers of the *HSD17B13* rs72613567 variant (e.g., Transcripts A, B, E, and F') is targeted for reduced expression, such as through use of antisense RNA, siRNA, or shRNA. In particular embodiments, the *HSD17B13* transcripts targeted for reduced expression are a combination comprising Transcript A (e.g., A, AB, AE, AF', ABE, ABF', AEF', or ABEF').

[00381] An *HSD17B13* gene or minigene or a DNA encoding any one of or any combination of HSD17B13 Isoforms C, D, F, G, and H or fragments thereof can be introduced and expressed in the form of an expression vector that does not modify the genome, it can be introduced in the form of a targeting vector such that it genomically integrates into an *HSD17B13* locus, or it can be introduced such that it genomically integrates into a locus other than the *HSD17B13* locus, such as a safe harbor locus. The genomically integrated *HSD17B13* gene can be operably linked to an *HSD17B13* promoter or to another promoter, such as an endogenous promoter at the site of integration. Safe harbor loci are chromosomal sites where transgenes can be stably and reliably expressed in all tissues of interest without adversely affecting gene structure or expression. Safe harbor loci can have, for example, one or more or all of the following characteristics: (1) distance of greater than 50 kb from the 5' end of any gene; distance of greater than 300 kb from any cancer-related gene; distance of greater than 300 kb from any microRNA; outside a gene transcription unit, and outside of ultra-conserved regions. Examples of suitable safe harbor loci include adeno-associated virus site 1 (AAVS1), the chemokine (CC motif) receptor 5 (CCR5) gene locus, and the human orthologue of mouse ROSA26 locus.

[00382] Combinations of HSD17B13 protein isoforms or nucleic acids encoding HSD17B13 protein isoforms that can be introduced and expressed include, for example, C, D, F, G, H, CD, CF, CG, CH, DF, DG, DH, FG, FH, GH, CDF, CDG, CDH, CFG, CFH, CGH, DFG, DFH, DGH, FGH, CDFG, CDFH, CFGH, DFGH, and CDFGH. In particular methods, HSD17B13 Isoform D or a nucleic acid encoding Isoform D (alone or in combination with other isoforms) is introduced or expressed. Exemplary sequences for each of these isoforms and transcripts are provided elsewhere herein. It is understood, however, that gene sequences and within a population, mRNA sequences transcribed from such genes, and proteins translated from such mRNAs can vary due to polymorphisms such as single-nucleotide polymorphisms. The sequences provided herein for each transcript and isoform are only exemplary sequences. Other sequences are also possible.

[00383] Combinations of HSD17B13 Transcripts whose expression can be targeted for reduction through antisense RNA, shRNA, or siRNA include, for example, A, B, E, F', AB, AE, AF', BE, BF', ABE, ABF', AEF', BEF', and ABEF'. In particular methods, HSD17B13 Transcript A (alone or in combination with other transcripts) is targeted. For example, the antisense RNA, siRNA, or shRNA can hybridize to a sequence within SEQ ID NO: 4 (*HSD17B13* Transcript A). Optionally, the antisense RNA, siRNA, or shRNA hybridizes to a sequence present in SEQ ID NO: 4 (*HSD17B13* Transcript A) that is not present in SEQ ID NO: 7 (*HSD17B13* Transcript D). Optionally, the antisense RNA, siRNA, or shRNA hybridizes to a sequence within exon 7 or a sequence spanning the exon 6-exon 7 boundary of SEQ ID NO: 4 (*HSD17B13* Transcript A).

[00384] For example, some such methods comprise a method of treating a subject who is not a carrier of the *HSD17B13* rs72613567 variant (or is only a heterozygous carrier of the *HSD17B13* rs72613567 variant) and has or is susceptible to developing a chronic liver disease, comprising introducing into the subject or introducing into a liver cell in the subject: (a) a nuclease agent (or nucleic acid encoding) that binds to a nuclease target sequence within an *HSD17B13* gene, wherein the nuclease target sequence includes or is proximate to a position corresponding to position 12666 of SEQ ID NO: 2 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 2; and (b) an exogenous donor sequence comprising a 5' homology arm that hybridizes to a target sequence 5' of the position corresponding to position 12666 of SEQ ID NO: 2, a 3' homology arm that hybridizes to a target sequence 3' of the position corresponding to position

12666 of SEQ ID NO: 2, and a nucleic acid insert comprising a thymine flanked by the 5' homology arm and the 3' homology arm. The nuclease agent can cleave the *HSD17B13* gene in a liver cell in the subject, and the exogenous donor sequence can recombine with the *HSD17B13* gene in the liver cell, wherein upon recombination of the exogenous donor sequence with the *HSD17B13* gene, the thymine is inserted between nucleotides corresponding to positions 12665 and 12666 of SEQ ID NO: 1 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 1. Examples of nuclease agents (e.g., a Cas9 protein and a guide RNA) that can be used in such methods are disclosed elsewhere herein. Examples of suitable guide RNAs and guide RNA target sequences are disclosed elsewhere herein. Examples of exogenous donor sequences that can be used in such methods are disclosed elsewhere herein.

[00385] As another example, some such methods comprise a method of treating a subject who is not a carrier of the *HSD17B13* rs72613567 variant (or is only a heterozygous carrier of the *HSD17B13* rs72613567 variant) and has or is susceptible to developing a chronic liver disease, comprising introducing into the subject or introducing into a liver cell in the subject an exogenous donor sequence comprising a 5' homology arm that hybridizes to a target sequence 5' of the position corresponding to position 12666 of SEQ ID NO: 2, a 3' homology arm that hybridizes to a target sequence 3' of the position corresponding to position 12666 of SEQ ID NO: 2, and a nucleic acid insert comprising a thymine flanked by the 5' homology arm and the 3' homology arm. The exogenous donor sequence can recombine with the *HSD17B13* gene in the liver cell, wherein upon recombination of the exogenous donor sequence with the *HSD17B13* gene, the thymine is inserted between nucleotides corresponding to positions 12665 and 12666 of SEQ ID NO: 1 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 1. Examples of exogenous donor sequences that can be used in such methods are disclosed elsewhere herein.

[00386] Some such methods comprise a method of treating a subject who is not a carrier of the *HSD17B13* rs72613567 variant (or is only a heterozygous carrier of the *HSD17B13* rs72613567 variant) and has or is susceptible to developing a chronic liver disease, comprising introducing into the subject or introducing into a liver cell in the subject: (a) a nuclease agent (or nucleic acid encoding) that binds to a nuclease target sequence within an *HSD17B13* gene, wherein the nuclease target sequence comprises the start codon for the *HSD17B13* gene or is within about 10, 20, 30, 40, 50, 100, 200, 300, 400, 500, or 1,000 nucleotides of the start codon or is selected from SEQ ID NOS: 20-81. The nuclease agent can cleave and disrupt expression

of the *HSD17B13* gene in a liver cell in the subject. Some such methods comprise a method of treating a subject who is not a carrier of the *HSD17B13* rs72613567 variant (or is only a heterozygous carrier of the *HSD17B13* rs72613567 variant) and has or is susceptible to developing a chronic liver disease, comprising introducing into the subject or introducing into a liver cell in the subject: (a) a nuclease agent (or nucleic acid encoding) that binds to a nuclease target sequence within an *HSD17B13* gene, wherein the nuclease target sequence comprises the start codon for the *HSD17B13* gene or is within about 10, 20, 30, 40, 50, 100, 200, 300, 400, 500, or 1,000 nucleotides of the start codon or is selected from SEQ ID NOS: 20-81; and (b) an expression vector comprising a recombinant *HSD17B13* gene comprising a thymine inserted between nucleotides corresponding to positions 12665 and 12666 of SEQ ID NO: 1 when the recombinant *HSD17B13* gene is optimally aligned with SEQ ID NO: 1. The expression vector can be one that does not genomically integrate. Alternatively, a targeting vector (i.e., exogenous donor sequence) can be introduced comprising a recombinant *HSD17B13* gene comprising a thymine inserted between nucleotides corresponding to positions 12665 and 12666 of SEQ ID NO: 1 when the recombinant *HSD17B13* gene is optimally aligned with SEQ ID NO: 1. The nuclease agent can cleave and disrupt expression of the *HSD17B13* gene in a liver cell in the subject, and the expression vector can express the recombinant *HSD17B13* gene in the liver cell in the subject. Alternatively, the genomically integrated, recombinant *HSD17B13* gene can express in the liver cell in the subject. Examples of nuclease agents (e.g., a nuclease-active Cas9 protein and guide RNA) that can be used in such methods are disclosed elsewhere herein. Examples of suitable guide RNAs and guide RNA target sequences are disclosed elsewhere herein. Step (b) can alternatively comprise introducing an expression vector or targeting vector comprising a nucleic acid (e.g., DNA) encoding an HSD17B13 protein that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to HSD17B13 Isoform C, D, F, G, or H or a fragment thereof and/or comprising a sequence that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to *HSD17B13* Transcript C, D, F, G, or H or a fragment thereof. Likewise, step (b) can alternatively comprise introducing an mRNA encoding an HSD17B13 protein that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to HSD17B13 Isoform C, D, F, G, or H or a fragment thereof and/or having a complementary DNA (or a portion thereof) that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at

least 99%, or 100% identical to *HSD17B13* Transcript C, D, F, G, or H or a fragment thereof. Likewise, step (b) can alternatively comprise introducing a protein comprising a sequence that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to HSD17B13 Isoform C, D, F, G, or H or a fragment thereof. In specific methods, the transcript can be *HSD17B13* Transcript D (e.g., SEQ ID NO: 7), or the isoform can be HSD17B13 Isoform D (e.g., SEQ ID NO: 15). In other specific methods, a combination of HSD17B13 Isoforms, or expression vectors or targeting vectors encoding a combination of HSD17B13 Isoforms, or mRNAs encoding a combination of HSD17B13 Isoforms can be introduced (e.g., D, DC, DF, DG, DH, DCF, DCG, DCH, DFG, DFH, DGH, DCFG, DCFH, DCGH, DFGH, or DCFGH).

[00387] In some such methods, a second nuclease agent is also introduced into the subject or into the liver cell in the subject, wherein the second nuclease agent binds to a second nuclease target sequence within the *HSD17B13* gene, wherein the second nuclease target sequence comprises the stop codon for the *HSD17B13* gene or is within about 10, 20, 30, 40, 50, 100, 200, 300, 400, 500, or 1,000 nucleotides of the stop codon or is selected from SEQ ID NOS: 82-225, wherein the nuclease agent cleaves the *HSD17B13* gene in the liver cell within both the first nuclease target sequence and the second nuclease target sequence, wherein the liver cell is modified to comprise a deletion between the first nuclease target sequence and the second nuclease target sequence. For example, the second nuclease agent can be a Cas9 protein and a guide RNA. Suitable guide RNAs and guide RNA target sequences in proximity to the stop codon are disclosed elsewhere herein.

[00388] Such methods can also comprise a method of treating a subject who is not a carrier of the *HSD17B13* rs72613567 variant (or is only a heterozygous carrier of the *HSD17B13* rs72613567 variant) and has or is susceptible to developing a chronic liver disease, comprising introducing into the subject or introducing into a liver cell in the subject: (a) a DNA-binding protein (or nucleic acid encoding) that binds to a DNA-binding protein target sequence within an *HSD17B13* gene, wherein the DNA-binding protein target sequence comprises the start codon for the *HSD17B13* gene or is within about 10, 20, 30, 40, 50, 100, 200, 300, 400, 500, or 1,000 nucleotides of the start codon or is selected from SEQ ID NOS: 20-81. The DNA-binding protein can alter (e.g., reduce) expression of the *HSD17B13* gene in a liver cell in the subject. Such methods can also comprise a method of treating a subject who is not a carrier of the

HSD17B13 rs72613567 variant (or is only a heterozygous carrier of the *HSD17B13* rs72613567 variant) and has or is susceptible to developing a chronic liver disease, comprising introducing into the subject or introducing into a liver cell in the subject: (a) a DNA-binding protein (or nucleic acid encoding) that binds to a DNA-binding protein target sequence within an *HSD17B13* gene, wherein the DNA-binding protein target sequence comprises the start codon for the *HSD17B13* gene or is within about 10, 20, 30, 40, 50, 100, 200, 300, 400, 500, or 1,000 nucleotides of the start codon or is selected from SEQ ID NOS: 20-81; and (b) an expression vector comprising a recombinant *HSD17B13* gene comprising a thymine inserted between nucleotides corresponding to positions 12665 and 12666 of SEQ ID NO: 1 when the recombinant *HSD17B13* gene is optimally aligned with SEQ ID NO: 1. The expression vector can be one that does not genomically integrate. Alternatively, a targeting vector (i.e., exogenous donor sequence) can be introduced comprising a recombinant *HSD17B13* gene comprising a thymine inserted between nucleotides corresponding to positions 12665 and 12666 of SEQ ID NO: 1 when the recombinant *HSD17B13* gene is optimally aligned with SEQ ID NO: 1. The DNA-binding protein can alter (e.g., reduce) expression of the *HSD17B13* gene in a liver cell in the subject, and the expression vector can express the recombinant *HSD17B13* gene in the liver cell in the subject. Alternatively, the genomically integrated, recombinant *HSD17B13* gene can express in the liver cell in the subject. Examples of DNA-binding proteins suitable for use in such methods are disclosed elsewhere herein. Such DNA-binding proteins (e.g., Cas9 protein and guide RNA) can be fused or operably linked to a transcriptional repressor domain. For example, the DNA-binding protein can be a catalytically inactive Cas9 protein fused to a transcriptional repressor domain. Such a DNA-binding protein fused to a transcriptional repressor domain can be used, for example, to decrease expression of a wild type *HSD17B13* gene or an *HSD17B13* gene that is not the rs72613567 variant (e.g., to decrease expression of *HSD17B13* Transcript or Isoform A). Examples of suitable guide RNAs and guide RNA target sequences are disclosed elsewhere herein. Step (b) can alternatively comprise introducing an expression vector or targeting vector comprising a nucleic acid (e.g., DNA) encoding an HSD17B13 protein that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to HSD17B13 Isoform C, D, F, G, or H or a fragment thereof and/or comprising a sequence that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to *HSD17B13* Transcript C, D, F, G, or H or a

fragment thereof. Likewise, step (b) can alternatively comprise introducing an mRNA encoding an HSD17B13 protein that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to HSD17B13 Isoform C, D, F, G, or H or a fragment thereof and/or having a complementary DNA (or a portion thereof) that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to *HSD17B13* Transcript C, D, F, G, or H or a fragment thereof. Likewise, step (b) can alternatively comprise introducing a protein comprising a sequence that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to HSD17B13 Isoform C, D, F, G, or H or a fragment thereof. In specific methods, the transcript can be *HSD17B13* Transcript D (e.g., SEQ ID NO: 7), or the isoform can be HSD17B13 Isoform D (e.g., SEQ ID NO: 15). In other specific methods, a combination of HSD17B13 Isoforms, or expression vectors or targeting vectors encoding a combination of HSD17B13 Isoforms, or mRNAs encoding a combination of HSD17B13 Isoforms can be introduced (e.g., D, DC, DF, DG, DH, DCF, DCG, DCH, DFG, DFH, DGH, DCFG, DCFH, DCGH, DFGH, or DCFGH).

[00389] Such methods can also comprise a method of treating a subject who is not a carrier of the *HSD17B13* rs72613567 variant (or is only a heterozygous carrier of the *HSD17B13* rs72613567 variant) and has or is susceptible to developing a chronic liver disease, comprising introducing into the subject or introducing into a liver cell in the subject: an antisense RNA, an siRNA, or an shRNA that hybridizes to a sequence within a region of one or more of *HSD17B13* Transcripts A, B, E, and F' (and particularly A) that optionally is not present in one or more *HSD17B13* Transcripts C, D, F, G, and H (and particularly D). Optionally, the antisense RNA, siRNA, or shRNA hybridizes to a sequence within SEQ ID NO: 4 (*HSD17B13* Transcript A), and the antisense RNA, siRNA, or shRNA can decrease expression of *HSD17B13* Transcript A in a cell. Optionally, the antisense RNA, siRNA, or shRNA hybridizes to a sequence present in SEQ ID NO: 4 (*HSD17B13* Transcript A) that is not present in SEQ ID NO: 7 (*HSD17B13* Transcript D). Optionally, the antisense RNA, siRNA, or shRNA hybridizes to a sequence within exon 7 or a sequence spanning the exon 6-exon 7 boundary of SEQ ID NO: 4 (*HSD17B13* Transcript A). For example, the antisense RNA, siRNA, or shRNA can hybridize to sequence within a region in exon 7 or a region spanning the exon 6-exon 7 boundary of SEQ ID NO: 4 (*HSD17B13* Transcript A) and decrease expression of *HSD17B13* Transcript A in a liver cell in the subject. Optionally, such methods can further comprise introducing into the subject an

expression vector comprising a recombinant *HSD17B13* gene comprising a thymine inserted between nucleotides corresponding to positions 12665 and 12666 of SEQ ID NO: 1 when the recombinant *HSD17B13* gene is optimally aligned with SEQ ID NO: 1. The expression vector can be one that does not genomically integrate. Alternatively, a targeting vector (i.e., exogenous donor sequence) can be introduced comprising a recombinant *HSD17B13* gene comprising a thymine inserted between nucleotides corresponding to positions 12665 and 12666 of SEQ ID NO: 1 when the recombinant *HSD17B13* gene is optimally aligned with SEQ ID NO: 1. In methods in which an expression vector is used, the expression vector can express the recombinant *HSD17B13* gene in the liver cell in the subject. Alternatively, in methods in which a recombinant *HSD17B13* gene is genomically integrated, the recombinant *HSD17B13* gene can express in the liver cell in the subject. Such methods can alternatively comprise introducing an expression vector or targeting vector comprising a nucleic acid (e.g., DNA) encoding an HSD17B13 protein that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to HSD17B13 Isoform C, D, F, G, or H or a fragment thereof and/or comprising a sequence that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to *HSD17B13* Transcript C, D, F, G, or H or a fragment thereof. Likewise, such methods can alternatively comprise introducing an mRNA encoding an HSD17B13 protein that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to HSD17B13 Isoform C, D, F, G, or H or a fragment thereof and/or having a complementary DNA (or a portion thereof) that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to *HSD17B13* Transcript C, D, F, G, or H or a fragment thereof. Likewise, such methods can alternatively comprise introducing a protein comprising a sequence that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to HSD17B13 Isoform C, D, F, G, or H or a fragment thereof. In specific methods, the transcript can be *HSD17B13* Transcript D (e.g., SEQ ID NO: 7), or the isoform can be HSD17B13 Isoform D (e.g., SEQ ID NO: 15). In other specific methods, a combination of HSD17B13 Isoforms, or expression vectors or targeting vectors encoding a combination of HSD17B13 Isoforms, or mRNAs encoding a combination of HSD17B13 Isoforms can be introduced (e.g., D, DC, DF, DG, DH, DCF, DCG, DCH, DFG, DFH, DGH, DCFG, DCFH, DCGH, DFGH, or DCFGH).

[00390] Other such methods can comprise method of treating a subject who is not a carrier of

the *HSD17B13* rs72613567 variant (or is only a heterozygous carrier of the *HSD17B13* rs72613567 variant) and has or is susceptible to developing a chronic liver disease, comprising introducing into the subject or introducing into a liver cell in the subject an expression vector, wherein the expression vector comprises a recombinant *HSD17B13* gene comprising a thymine inserted between nucleotides corresponding to positions 12665 and 12666 of SEQ ID NO: 1 when the recombinant *HSD17B13* gene is optimally aligned with SEQ ID NO: 1, wherein the expression vector expresses the recombinant *HSD17B13* gene in a liver cell in the subject. The expression vector can be one that does not genomically integrate. Alternatively, a targeting vector (i.e., exogenous donor sequence) can be introduced comprising a recombinant *HSD17B13* gene comprising a thymine inserted between nucleotides corresponding to positions 12665 and 12666 of SEQ ID NO: 1 when the recombinant *HSD17B13* gene is optimally aligned with SEQ ID NO: 1. In methods in which an expression vector is used, the expression vector can express the recombinant *HSD17B13* gene in the liver cell in the subject. Alternatively, in methods in which a recombinant *HSD17B13* gene is genomically integrated, the recombinant *HSD17B13* gene can express in the liver cell in the subject. Such methods can alternatively comprise introducing an expression vector or targeting vector comprising a nucleic acid (e.g., DNA) encoding an HSD17B13 protein that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to HSD17B13 Isoform C, D, F, G, or H or a fragment thereof and/or comprising a sequence that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to *HSD17B13* Transcript C, D, F, G, or H or a fragment thereof. Likewise, such methods can alternatively comprise introducing an mRNA encoding an HSD17B13 protein that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to HSD17B13 Isoform C, D, F, G, or H or a fragment thereof and/or having a complementary DNA (or a portion thereof) that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to *HSD17B13* Transcript C, D, F, G, or H or a fragment thereof. Likewise, such methods can alternatively comprise introducing a protein comprising a sequence that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to HSD17B13 Isoform C, D, F, G, or H or a fragment thereof. In specific methods, the transcript can be *HSD17B13* Transcript D (e.g., SEQ ID NO: 7), or the isoform can be HSD17B13 Isoform D (e.g., SEQ ID NO: 15). In other specific methods, a combination of HSD17B13 Isoforms, or expression vectors or targeting

vectors encoding a combination of HSD17B13 Isoforms, or mRNAs encoding a combination of HSD17B13 Isoforms can be introduced (e.g., D, DC, DF, DG, DH, DCF, DCG, DCH, DFG, DFH, DGH, DCFG, DCFH, DCGH, DFGH, or DCFGH).

[00391] Suitable expression vectors and recombinant *HSD17B13* genes for use in any of the above methods are disclosed elsewhere herein. For example, the recombinant *HSD17B13* gene can be the full rs72613567 variant gene or can be an *HSD17B13* minigene in which one or more nonessential segments of the gene have been deleted with respect to a corresponding wild type *HSD17B13* gene. As an example, the deleted segments can comprise one or more intronic sequences, and the minigene can comprise an intron corresponding to intron 6 of SEQ ID NO: 2 when optimally aligned with SEQ ID NO: 2. An example of a full rs72613567 variant gene is one that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 2 when optimally aligned with SEQ ID NO: 2.

[00392] Some such methods comprise a method of modifying a cell (e.g., a liver cell) in a subject having or susceptible to developing a chronic liver disease. In such methods, the nuclease agents and/or exogenous donor sequences and/or recombinant expression vectors can be introduced into the cell via administration in an effective regime meaning a dosage, route of administration and frequency of administration that delays the onset, reduces the severity, inhibits further deterioration, and/or ameliorates at least one sign or symptom of a chronic liver disease being treated. The term "symptom" refers to a subjective evidence of a disease as perceived by the subject, and a "sign" refers to objective evidence of a disease as observed by a physician. If a subject is already suffering from a disease, the regime can be referred to as a therapeutically effective regime. If the subject is at elevated risk of the disease relative to the general population but is not yet experiencing symptoms, the regime can be referred to as a prophylactically effective regime. In some instances, therapeutic or prophylactic efficacy can be observed in an individual patient relative to historical controls or past experience in the same subject. In other instances, therapeutic or prophylactic efficacy can be demonstrated in a preclinical or clinical trial in a population of treated subjects relative to a control population of untreated subjects.

[00393] Delivery can be any suitable method, as disclosed elsewhere herein. For example, the nuclease agents or exogenous donor sequences or recombinant expression vectors can be delivered by vector delivery, viral delivery, particle-mediated delivery, nanoparticle-mediated

delivery, liposome-mediated delivery, exosome-mediated delivery, lipid-mediated delivery, lipid-nanoparticle-mediated delivery, cell-penetrating-peptide-mediated delivery, or implantable-device-mediated delivery. Some specific examples include hydrodynamic delivery, virus-mediated delivery, and lipid-nanoparticle-mediated delivery.

[00394] Administration can be by any suitable route including, for example, parenteral, intravenous, oral, subcutaneous, intra-arterial, intracranial, intrathecal, intraperitoneal, topical, intranasal, or intramuscular. A specific example which is often used, for example, for protein replacement therapies is intravenous infusion. The frequency of administration and the number of dosages can be depend on the half-life of the nuclease agents or exogenous donor sequences or recombinant expression vectors, the condition of the subject, and the route of administration among other factors. Pharmaceutical compositions for administration are preferably sterile and substantially isotonic and manufactured under GMP conditions. Pharmaceutical compositions can be provided in unit dosage form (i.e., the dosage for a single administration). Pharmaceutical compositions can be formulated using one or more physiologically and pharmaceutically acceptable carriers, diluents, excipients or auxiliaries. The formulation depends on the route of administration chosen. The term "pharmaceutically acceptable" means that the carrier, diluent, excipient, or auxiliary is compatible with the other ingredients of the formulation and not substantially deleterious to the recipient thereof.

[00395] Other such methods comprise an *ex vivo* method in a cell from a subject having or susceptible to developing a chronic liver disease. The cell with the targeted genetic modification can then be transplanted back into the subject.

[00396] Any of the therapeutic or prophylactic methods disclosed herein can further comprise administering a therapeutic tailored to prevent or alleviate one or more symptoms associated with progression to more clinically advanced stages of chronic liver disease (e.g., progression from simple steatosis to more clinically advanced stages of chronic liver disease, or progression from simple steatosis to one or more of steatohepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma). For examples, such treatments could be focused on preventing or reducing inflammation or preventing or reducing fibrosis. Examples of such therapeutics in development are provided below.

Drug (Company)	Stage	Type	Gene Target	Notes
OCA – Obeticholic acid (Intercept)	Phase III	Agonist	NR1H4 (FXR)	Improved NAS, reversed fibrosis in Phase Iib
GS-9674 (Gilead)	Phase I			
Simtuzumab (Gilead)	Phase II	Inhibitor	LOXL2	Potential to reverse fibrosis (NASH/PSC)
GS-4997 (Gilead)	Phase II	Inhibitor	MAP3K5	Reduces oxidative stress
NDI-010976 (Gilead)	Phase I	Inhibitor	ACACA	Prevent lipogenesis
			ACACB	
GFT505 / Elafibranor (Genfit)	Phase III	Agonist	PPARA	Break down fatty acids, block fat & glucose production, dec inflammation
			PPARD	
Aramchol (Galmed)	Phase II	Inhibitor	SCD	Fatty acid-bile acid conjugate; boosts liver fat metabolism
			(ABCA1)	
Cenicriviroc (Tobira)	Phase IIb	Inhibitor	CCR2	Chemokine receptors are involved in inflammation and fibrosis
			CCR5	
GR-MD-02 (Galectin Therapeutics)	Phase II	Inhibitor	LGALS3	Galectin-3 is upregulated in fibrosis
TD139 (Galecto Biotech)	Phase I			
SHP626 (Shire)	Phase I	Inhibitor	SLC10A2	Interferes with bile acid recycling
PXS4728A – (Boehringer Ingelheim)	Phase I	Inhibitor	AOC3	Anti-inflammatory
RP103 – Cysteamine bitartrate (Raptor)	Phase II	Depleting agent	CTNS	Cysteine-depleting; potential anti-oxidant

[00397] All patent filings, websites, other publications, accession numbers and the like cited above or below are incorporated by reference in their entirety for all purposes to the same extent as if each individual item were specifically and individually indicated to be so incorporated by reference. If different versions of a sequence are associated with an accession number at different times, the version associated with the accession number at the effective filing date of this application is meant. The effective filing date means the earlier of the actual filing date or filing date of a priority application referring to the accession number if applicable. Likewise, if different versions of a publication, website or the like are published at different times, the version most recently published at the effective filing date of the application is meant unless otherwise indicated. Any feature, step, element, embodiment, or aspect of the invention can be used in combination with any other unless specifically indicated otherwise. Although the present

invention has been described in some detail by way of illustration and example for purposes of clarity and understanding, it will be apparent that certain changes and modifications may be practiced within the scope of the appended claims.

BRIEF DESCRIPTION OF THE SEQUENCES

[00398] The nucleotide and amino acid sequences listed in the accompanying sequence listing are shown using standard letter abbreviations for nucleotide bases, and three-letter code for amino acids. The nucleotide sequences follow the standard convention of beginning at the 5' end of the sequence and proceeding forward (i.e., from left to right in each line) to the 3' end. Only one strand of each nucleotide sequence is shown, but the complementary strand is understood to be included by any reference to the displayed strand. The amino acid sequences follow the standard convention of beginning at the amino terminus of the sequence and proceeding forward (i.e., from left to right in each line) to the carboxy terminus.

SEQ ID NO	Type	Description
1	DNA	HSD17B13 Wild Type Genomic Sequence (Human Genome Assembly GRCh38) ***Transcripts More Prevalent in Subjects with Wild Type HSD17B13 Gene:*** **Transcript A** – Exon 1 = 1-275 – Exon 2 = 4471-4578 – Exon 3 = 5684-5815 – Exon 4 = 7308-7414 – Exon 5 = 8947-9084 – Exon 6v1 = 12548-12664 – Exon 7 = 17599-19118 **Transcript B** – Exon 1 = 1-275 – Exon 2 = skipped – Exon 3 = 5684-5815 – Exon 4 = 7308-7414 – Exon 5 = 8947-9084 – Exon 6v1 = 12548-12664 – Exon 7 = 17599-19118 **Transcript E** – Exon 1 = 1-275 – Exon 2 = 4471-4578 – Exon 3 = 5684-5815 – Exon 3' = 6210-6281 – Exon 4 = 7308-7414 – Exon 5 = 8947-9084 – Exon 6v1 = 12548-12664 – Exon 7 = 17599-19118 **Transcript F'** – Exon 1 = 1-275 – Exon 2 = 4471-4578 – Exon 3 = 5684-5815 – Exon 4 = 7308-7414 – Exon 5 = 8947-9084 – Exon 6v3 = 12548-13501 (Read-through from exon 6 into intron 6 = 12665-13501) – Exon 7 = skipped

SEQ ID NO	Type	Description
2	DNA	HSD17B13 Genomic Sequence Variant (Human Genome Assembly GRCh38; rs72613567—insertion of T at chr4: 87310241-87310240): Insertion of T at position 12666 ***Transcripts More Prevalent in Subjects with rs72613567 HSD17B13 Gene Variant:*** **Transcript C** – Exon 1 = 1-275 – Exon 2 = 4471-4578 – Exon 3 = 5684-5815 – Exon 4 = 7308-7414 – Exon 5 = 8947-9084 – Exon 6 = skipped – Exon 7 = 17600-19119 **Transcript D** – Exon 1 = 1-275 – Exon 2 = 4471-4578 – Exon 3 = 5684-5815 – Exon 4 = 7308-7414 – Exon 5 = 8947-9084 – Exon 6v2 = 12548-12665 (Includes additional residue 12665 at 3' end) – Exon 7 = 17600-19119 **Transcript F** – Exon 1 = 1-275 – Exon 2 = 4471-4578 – Exon 3 = 5684-5815 – Exon 4 = 7308-7414 – Exon 5 = 8947-9084 – Exon 6v3 = 12548-13502 (Read-through from exon 6 into intron 6 = 12665-13502) – Exon 7 = skipped **Transcript G** – Exon 1 = 1-275 – Exon 2 = skipped – Exon 3 = 5684-5815 – Exon 4 = 7308-7414 – Exon 5 = 8947-9084 – Exon 6v2 = 12548-12665 (Includes additional residue 12665 at 3' end) – Exon 7 = 17600-19119 **Transcript H** – Exon 1 = 1-275 – Exon 2 = 4471-4578 – Exon 3 = 5684-5815 – Exon 3' = 6210-6281 – Exon 4 = 7308-7414 – Exon 5 = 8947-9084 – Exon 6v2 = 12548-12665 (Includes additional residue 12665 at 3' end) – Exon 7 = 17600-19119
3	DNA	Endogenous HSD17B13 Promoter (-499 to 100 relative to transcription start site (TSS))
4	DNA	HSD17B13 Transcript A cDNA
5	DNA	HSD17B13 Transcript B cDNA
6	DNA	HSD17B13 Transcript C cDNA
7	DNA	HSD17B13 Transcript D cDNA
8	DNA	HSD17B13 Transcript E cDNA

SEQ ID NO	Type	Description
9	DNA	HSD17B13 Transcript F cDNA
10	DNA	HSD17B13 Transcript G cDNA
11	DNA	HSD17B13 Transcript H cDNA
12	Protein	HSD17B13 Protein Isoform A
13	Protein	HSD17B13 Protein Isoform B
14	Protein	HSD17B13 Protein Isoform C
15	Protein	HSD17B13 Protein Isoform D
16	Protein	HSD17B13 Protein Isoform E
17	Protein	HSD17B13 Protein Isoform F
18	Protein	HSD17B13 Protein Isoform G
19	Protein	HSD17B13 Protein Isoform H
20-41	DNA	Human *HSD17B13* TSS Guide RNA Target Sequences
42-81	DNA	Other Human *HSD17B13* 5' Guide RNA Target Sequences
82-225	DNA	Human *HSD17B13* 3' Guide RNA Target Sequences
226-239	DNA	Human *HSD17B13* Guide RNA Target Sequences Near rs72613567 Variation
240	Protein	Human HSD17B13 Protein Q7Z5P4-1
241	Protein	Human HSD17B13 Protein Q7Z5P4-2
242	Protein	Human HSD17B13 Protein NP_835236.2
243	Protein	Human HSD17B13 Protein NP_001129702.1
244	DNA	Human HSD17B13 cDNA NM_178135.4
245	DNA	Human HSD17B13 cDNA NM_001136230.2
246	DNA	HSD17B13 Transcript F'
247	Protein	HSD17B13 Protein Isoform F'
248-250	DNA	Guide RNA Target Sequences Plus PAM
251	DNA	PST516 Primer
252	DNA	PST517 Primer
253	DNA	DE002 Primer
254	DNA	HSD17B13 Primer 1
255	DNA	HSD17B13 Primer 2
256-258	RNA	Guide RNA Scaffolds v2-v4
259-263	DNA	Mouse 5' Guide RNA Target Sequences
264-268	DNA	Mouse Exon 6/7 Guide RNA Target Sequences
269	DNA	Mouse *Hsd17b13* Locus
270-489	RNA	Human *HSD17B13* crRNAs
490-499	RNA	Mouse *Hsd17b13* crRNAs
500-719	RNA	Human *HSD17B13* sgRNAs v1
720-729	RNA	Mouse *Hsd17b13* sgRNAs v1
730-949	RNA	Human *HSD17B13* sgRNAs v2
950-959	RNA	Mouse *Hsd17b13* sgRNAs v2
960-1179	RNA	Human *HSD17B13* sgRNAs v3
1180-1189	RNA	Mouse *Hsd17b13* sgRNAs v3
1190-1409	RNA	Human *HSD17B13* sgRNAs v4
1410-1419	RNA	Mouse *Hsd17b13* sgRNAs v4
1420	RNA	Guide RNA Scaffold v1
1421	RNA	crRNA tail
1422	RNA	tracrRNA
1423-1642	RNA	Human *HSD17B13* Guide RNA DNA-Targeting Segments
1643-1652	RNA	Mouse *Hsd17b13* Guide RNA DNA-Targeting Segments

EXAMPLES

Example 1. Variant 17beta-hydroxysteroid dehydrogenase 13 protects against chronic liver disease.

[00399] Chronic liver disease and cirrhosis are leading causes of morbidity and mortality in the U.S. (Kochanek et al. (2016) *Natl Vital Stat Rep* 65:1-122, herein incorporated by reference in its entirety for all purposes). The most common etiologies of cirrhosis are alcoholic liver disease, chronic hepatitis C, and nonalcoholic fatty liver disease (NAFLD), together accounting for ~80% of patients awaiting liver transplant (Wong et al. (2015) *Gastroenterology* 148:547-555, herein incorporated by reference in its entirety for all purposes). Notably, the estimated prevalence of NAFLD in the U.S. is between 19 and 46 percent (Browning et al. (2004) *Hepatology* 40:1387-1395; Lazo et al. (2013) *Am J Epidemiol* 178:38-45; and Williams et al. (2011) *Gastroenterology* 140:124-131, each of which is herein incorporated by reference in its entirety for all purposes) and is rising over time (Younossi et al. (2011) *Clin Gastroenterol Hepatol* 9:524-530 e1; quiz e60 (2011), herein incorporated by reference in its entirety for all purposes), likely in conjunction with increased rates of obesity. To date, there remains much uncertainty about the inter-individual variation in NAFLD progression and outcomes; knowledge of underlying genetic factors could improve risk stratification and provide the foundation for novel therapeutic strategies. Here, we show that carriers of a splice variant in *HSD17B13* (encoding hydroxysteroid-17-beta dehydrogenase 13) have reduced risk of alcoholic and nonalcoholic liver disease, and reduced risk of NAFLD progression. Association studies of whole exome sequence data linked to electronic health records from 46,544 European ancestry participants in the DiscovEHR study led to the identification of a splice variant in *HSD17B13* (rs72613567) associated with reduced alanine transaminase and aspartate transaminase levels; these findings were replicated in three separate cohorts comprising 12,528 individuals. In the discovery cohort, variant *HSD17B13* was associated with reduced risk of alcoholic and nonalcoholic liver disease, cirrhosis, and hepatocellular carcinoma. In a bariatric surgery cohort, the variant was associated with reduced risk of histopathological steatohepatitis in individuals with steatosis. RNA sequencing of human liver samples from the bariatric surgery cohort revealed that homozygous carriers of the splice variant predominantly express a novel transcript coding for a truncated *HSD17B13* isoform. These findings shed new light on the role of *HSD17B13* in promoting liver disease progression, and its potential as a therapeutic target for

steatohepatitis and cirrhosis.

[00400] Previous genome wide association studies (GWAS) have identified a limited number of genes and variants associated with chronic liver disease. The most robustly validated genetic association to date is to a common missense variant in the patatin-like phospholipase domain containing 3 gene (*PNPLA3* p.Ile148Met, rs738409), initially found to be associated with increased risk of nonalcoholic fatty liver disease (NAFLD) (Romeo et al. (2008) *Nat Genet* 40:1461-1465 and Speliotes et al. (2011) *PLoS Genet* 7:e1001324, each of which is herein incorporated by reference in its entirety for all purposes), and subsequently found to be associated with disease severity (Rotman et al. (2010) *Hepatology* 52:894-903 and Sookoian et al. (2009) *J Lipid Res* 50:2111-2116, each of which is herein incorporated by reference in its entirety for all purposes) and progression (Trepo et al. (2016) *J Hepatol* doi:10.1016/j.jhep.2016.03.011, herein incorporated by reference in its entirety for all purposes). Variation in the transmembrane 6 superfamily member 2 (*TM6SF2*) gene has also been shown to confer increased risk for NAFLD (Kozlitina et al. (2014) *Nat Genet* 46:352-356, Liu et al. (2014) *Nat Commun* 5:4309, and Sookoian et al. (2015) *Hepatology* 61:515-525, each of which is herein incorporated by reference in its entirety for all purposes). The normal functions of these two proteins are not well understood, though both have been proposed to be involved in hepatocyte lipid metabolism. How variants in *PNPLA3* and *TM6SF2* contribute to increased risk of liver disease has yet to be elucidated. GWAS have also identified several genetic factors to be associated with serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (Chambers et al. (2011) *Nat Genet* 43:1131-1138 and Yuan et al. (2008) *Am J Hum Genet* 83:520-528, each of which is herein incorporated by reference in its entirety for all purposes), quantitative markers of hepatocyte injury and liver fat accumulation that are frequently measured clinically. To date, there are no described protective genetic variants for chronic liver disease. The discovery of protective genetic variants in other settings, such as loss-of-function variants in *PCSK9* that reduce the risk of cardiovascular disease, has been the catalyst for development of new classes of therapeutics.

[00401] The DiscovEHR collaboration between the Regeneron Genetics Center and the Geisinger Health System (GHS) couples exome sequencing to de-identified electronic health record (EHR) data to enable genetic discoveries and precision medicine (Dewey et al. (2016) *Science* 354(6319) doi:10.1126/science.aaf6814, herein incorporated by reference in its entirety

for all purposes). The DiscovEHR cohort is comprised of patients recruited from primary and specialty medical care cohorts across the GHS integrated healthcare system, including bariatric surgery patients with liver biopsy specimens (Gorden et al. (2013) *Hum Hered* 75:34-43, herein incorporated by reference in its entirety for all purposes). In this study, we undertook a comprehensive functional genomics approach to assess the contribution of exome sequence variation to quantitative traits, disease diagnoses, and histopathologic phenotypes relevant to chronic liver disease and cirrhosis in 49,188 individuals of European descent from the DiscovEHR cohort, with follow-up studies using whole exome sequencing of 9,883 individuals of European ancestry.

[00402] Using whole exome sequence data linked to EHR-derived phenotypes, we first carried out an association study of serum ALT and AST measures in 46,544 individuals of European descent from the DiscovEHR cohort ("GHS discovery cohort"). Clinical characteristics of the cohort are described in **Table 1A**. There were 41,908 individuals with EHR-documented transaminase measures (including 40,561 individuals with both ALT and AST measures). We used a linear mixed model (Yang et al. (2011) *Am J Hum Genet* 88:76-82, herein incorporated by reference in its entirety for all purposes) to detect associations between $\log_{10}$-transformed median ALT and AST levels (adjusted for sex, age, age^2, body mass index (BMI) and the first four principle components of ancestry) and 502,219 biallelic single variants with minor allele frequency greater than 0.1%. Using an exome-wide significance threshold of $P < 1.0 \times 10^{-7}$, we identified 35 variants in 19 genes significantly associated with ALT or AST, including eight variants in seven genes that were associated with both ALT and AST (**Fig. 1** and **Table 2**).

[00403] **Table 1A**. Demographics and clinical characteristics of sequenced European-ancestry individuals from the discovery and replication cohorts.

Characteristic	**Discovery Cohort (N = 46,544)**	**Bariatric Surgery Cohort (N = 2,644)**	**Dallas Heart Study (N = 1,357)**	**Penn Medicine Biobank (N = 8,526)**
Age (years) – median (IQR)	62.9 (49.6 - 73.8)	52.9 (44.1 - 61.2)	46.0 (38.0 - 54.0)	68.0 (60.0 - 76.0)
Female sex – number (%)	26,875 (57.7)	2,119 (80.1)	724 (53.4)	3,242 (38.0)
Body mass index – median (IQR)	29.9 (35.4 - 44.8)	47.4 (42.0 - 53.7)	28 (25-32)	30 (25-32)
Transaminase level (U/L) – median (IQR)				
Alanine aminotransferase (ALT)	22.0 (17.0 - 29.0)	23.0 (17.5 - 29.5)	20.0 (15.0 - 27.0)	22.0 (17.0 - 30.0)
Aspartate aminotransferase (AST)	23.0 (20.0 - 27.5)	23.0 (20.0 - 27.0)	21.0 (18.0 - 25.0)	24.0 (20.0 - 30.5)
Presence of liver disease (by ICD-9 code) – N (%)				
Alcoholic liver disease	197 (0.4)	7 (0.3)	-	-
Alcoholic cirrhosis	130 (0.3)	3 (0.1)	-	-
Nonalcoholic, non-viral liver disease	1,938 (4.2)	1,543 (58.4)	-	-
Nonalcoholic cirrhosis	382 (0.8)	24 (0.9)	-	-
Hepatocellular carcinoma	76 (0.2)	1 (0.04)	-	-
No liver disease	30,628 (65.8)	1 (0.04)	-	-

[00404] **Table 1B**. Demographics and clinical characteristics of genotyped multi-ethnic cases and controls from the Dallas Liver and Pediatric Liver Studies.

Characteristic	**Dallas Liver Study Cases (N = 517)**	**Dallas Liver Study Controls (N = 4,279)**	**Dallas Pediatric Liver Study Cases (N = 203)**	**Dallas Pediatric Liver Study Controls (N = 244)**
Age (years) – median (IQR)	55 (48 - 60)	44 (36 - 53)	12 (10 - 15)	12 (11 - 14)
Female sex – number (%)	277 (54)	2,494 (58)	65 (32)	126 (52)
Body mass index – median (IQR)	30 (27 - 35)	30 (26 - 35)	30 (27 - 34)	31 (28 - 35)
Self-reported ethnicity				
African American	33 (6)	2,291 (54)	-	-
European American	158 (31)	1,266 (30)	-	-
Hispanic American	326 (63)	722 (17)	203 (100)	244 (100)
Presence of liver disease (by ICD-9 code) – N (%)				
Alcoholic liver disease	223 (43)	-	-	-
Alcoholic cirrhosis	215 (42)	-	-	-
Nonalcoholic, non-viral liver disease	212 (20)	-	-	-
Nonalcoholic cirrhosis	100 (19)	-	-	-
Hepatocellular carcinoma	44 (9)	-	-	-
No liver disease	-	4,279 (100)	-	-244 (100)

[00405] **Table 2.** Single nucleotide variants associated with serum transaminase levels at $P < 1.0x10^{-7}$ in the discovery cohort.

													N			Mean AST or ALT level (U/L)		
Trait	CHR	BP	REF	ALT	rsID	Gene	Annotation	AA Substitution	Beta (SE)	P	AAF	N	REF/ REF	REF/ ALT	ALT/ ALT	REF/ REF	REF/ ALT	ALT/ ALT
ALT	1	220970028	A	G	rs2642438	*MARC1*	missense	p.Thr165Ala	0.008 (0.001)	4.67E-08	0.7067	41,414	3,515	17,262	20,637	23.88	24.52	24.92
	4	88231392	T	TA	***rs72613567**	*HSD17B13*	splice donor		-0.009 (0.001)	4.16E-12	0.2634	41,414	22,441	16,130	2,843	25.02	24.26	24.1
	8	144997604	C	T	rs371119003	*PLEC*	missense	p.Ala2302Thr	-0.160 (0.026)	1.30E-09	0.0005	41,413	41,373	40	0	24.67	18.1	NA
	8	145008502	G	A		*PLEC*	missense	p.Arg522Cys	-0.268 (0.032)	3.26E-17	0.0003	41,414	41,387	27	0	24.67	13.8	NA
	8	145692918	G	A	rs35968570	*KIFC2*	missense	p.Glu174Lys	-0.033 (0.005)	1.40E-11	0.0139	41,414	40,271	1,133	10	24.67	12.07	NA
	8	145730072	G	A	rs143408057	*GPT*	missense	p.Arg83His	-0.314 (0.036)	3.28E-18	0.0003	41,414	41,393	21	0	24.67	12.07	NA
	8	145730161	C	T	rs201815297	*GPT*	missense	p.Ala87Val	-0.224 (0.014)	6.28E-59	0.0018	41,414	41,270	144	0	24.7	14.68	NA
	8	145730221	G	A	rs112574791	*GPT*	missense	p.Arg107Lys	-0.033 (0.005)	4.25E-11	0.0136	41,414	40,293	1,111	10	24.71	23.09	18.35
	8	145731636	T	G	rs145155876	*GPT*	stop gained	p.Tyr326*	-0.235 (0.031)	1.76E-14	0.0004	41,394	41,364	30	0	24.67	14.07	NA
	8	145732114	G	C	rs141505249	*GPT*	missense	p.Glu430Gln	-0.224 (0.013)	8.84E-64	0.0019	41,375	41,223	150	2	24.7	14.48	13.75
	8	145732151	G	A	rs143462595	*GPT*	missense	p.Arg442His	-0.077 (0.013)	1.18E-09	0.0021	41,406	41,232	174	0	24.68	20.87	NA
	8	145732180	G	C	rs147998249	*GPT*	missense	p.Val452Leu	-0.225 (0.013)	8.19E-65	0.0019	41,413	41,254	159	0	24.7	14.74	NA
	8	145732305	G	GC		*GPT*	frameshift	p.Glu475fs	-0.271 (0.031)	1.00E-18	0.0004	41,414	41,385	29	0	24.67	14.24	NA
	8	145748532	A	G	rs567402720	*LRRC24*	missense	p.Leu290Ser	-0.185 (0.028)	3.42E-11	0.0004	41,393	41,358	35	0	24.67	17.71	NA
	9	117122202	C	T	rs3748177	*AKNA*	synonymous	p.Glu755Glu	-0.007 (0.001)	9.51E-09	0.5232	41,414	9,414	20,645	11,355	25.12	24.72	24.18
	9	117124731	G	A	rs3748176	*AKNA*	missense	p.Pro624Leu	-0.007 (0.001)	4.31E-09	0.5230	41,412	9,427	20,634	11,351	25.12	24.73	24.17
	10	101595996	T	A	rs17222723	*ABCC2*	missense	p.Val1188Glu	-0.015 (0.003)	2.97E-08	0.0608	41,414	36,543	4,704	167	24.77	23.97	22.12
	10	101606861	G	T	rs1137968	*ABCC2*	synonymous	p.Val1430Val	-0.015 (0.003)	2.71E-08	0.0608	41,414	36,543	4,704	167	24.77	23.97	22.04
	10	101610533	C	T	rs8187707	*ABCC2*	synonymous	p.His1496His	-0.015 (0.003)	2.77E-08	0.0608	41,414	36,542	4,706	166	24.77	23.97	22.03
	10	101611294	G	A	rs8187710	*ABCC2*	missense	p.Cys1515Tyr	-0.015 (0.003)	2.15E-08	0.0611	41,414	36,519	4,726	169	24.77	23.97	21.99
	10	101912064	T	C	***rs2862954**	*ERLIN1*	missense	p.Ile291Val	-0.012 (0.001)	2.43E-21	0.4755	41,414	11,318	20,819	9,277	25.32	24.71	23.77
	10	101977883	C	T	rs2230804	*CHUK*	missense	p.Val268Ile	-0.009 (0.001)	1.93E-13	0.5072	41,414	10,048	20,733	10,633	25.18	24.75	24.01
	10	113917085	T	A	rs2254537	*GPAM*	synonymous	p.Pro681Pro	-0.008 (0.001)	4.61E-10	0.7073	41,414	3,627	16,984	20,803	25	24.97	24.36
	10	113940329	T	C	rs2792751	*GPAM*	missense	p.Ile43Val	-0.008 (0.001)	2.54E-10	0.7097	41,412	3,567	16,910	20,935	25	24.98	24.35
	14	94844947	C	T	***rs28929474**	*SERPINA1*	missense	p.Glu366Lys	0.042 (0.005)	9.28E-21	0.0171	41,414	40,006	1,399	9	24.58	26.91	43.89
	19	19379549	C	T	***rs58542926**	*TM6SF2*	missense	p.Glu167Lys	0.014 (0.002)	4.76E-09	0.0759	41,413	35,388	5,780	245	24.52	25.46	26.84
	22	44324727	C	G	***rs738409**	*PNPLA3*	missense	p.Ile148Met	0.023 (0.002)	1.34E-50	0.2351	41,414	24,257	14,837	2,320	24.06	24.99	28.91
	22	44324730	C	T	***rs738408**	*PNPLA3*	synonymous	p.Pro149Pro	0.023 (0.002)	1.11E-50	0.2349	41,414	24,273	14,824	2,317	24.06	24.98	28.92
	22	44342116	A	G	rs2294918	*PNPLA3*	missense	p.Lys434Glu	0.007 (0.001)	8.26E-08	0.5986	41,412	6,691	19,833	14,888	24.15	24.47	25.15
	22	44368122	A	G	***rs3761472**	*SAMM50*	missense	p.Asp110Gly	0.019 (0.002)	8.85E-30	0.1682	41,413	28,626	11,618	1,169	24.23	25.36	28.45
	22	44395451	T	C	***rs1007863**	*PARVB*	missense	p.Trp37Arg	0.011 (0.001)	7.98E-16	0.3963	41,414	15,036	19,920	6,458	24.15	24.6	26.09
AST	4	88231392	T	TA	***rs72613567**	*HSD17B13*	splice donor		-0.005 (0.001)	6.24E-10	0.2638	40,753	22,068	15,870	2,815	24.47	24.1	23.96
	10	18242311	A	G	rs10764176	*SLC39A12*	missense	p.Ser36Gly	-0.006 (0.001)	1.09E-10	0.2881	40,753	20,645	16,738	3,370	24.47	24.15	23.85
	10	101157378	CGTT	C		*GOT1*	inframe indel	p.Asn389del	-0.221 (0.024)	1.96E-20	0.0002	40,753	40,733	20	0	24.29	14.7	NA
	10	101165533	G	C	rs374966349	*GOT1*	missense	p.Gln208Glu	0.271 (0.027)	2.43E-24	0.0002	40,753	40,736	17	0	24.28	44.5	NA
	10	101912064	T	C	***rs2862954**	*ERLIN1*	missense	p.Ile291Val	-0.005 (0.001)	4.82E-09	0.4754	40,753	11,138	20,486	9,129	24.59	24.26	23.99
	11	22271870	A	T	rs7481951	*ANO5*	missense	p.Leu322Phe	0.004 (0.001)	9.61E-08	0.5833	40,722	7,123	19,686	13,913	24.03	24.22	24.53
	14	94844947	C	T	***rs28929474**	*SERPINA1*	missense	p.Glu366Lys	0.027 (0.003)	2.44E-20	0.0172	40,753	39,361	1,384	8	24.24	25.76	34.5
	19	19379549	C	T	***rs58542926**	*TM6SF2*	missense	p.Glu167Lys	0.008 (0.002)	6.54E-08	0.0760	40,752	34,811	5,698	243	24.21	24.74	25.43
	22	44324727	C	G	***rs738409**	*PNPLA3*	missense	p.Ile148Met	0.014 (0.001)	8.31E-46	0.2343	40,753	23,889	14,622	2,242	23.96	24.48	26.62
	22	44324730	C	T	***rs738408**	*PNPLA3*	synonymous	p.Pro149Pro	0.014 (0.001)	8.93E-46	0.2341	40,753	23,905	14,609	2,239	23.96	24.47	26.63
	22	44368122	A	G	***rs3761472**	*SAMM50*	missense	p.Asp110Gly	0.011 (0.001)	1.22E-22	0.1680	40,752	28,170	11,450	1,132	24.07	24.64	26.24
	22	44395451	T	C	***rs1007863**	*PARVB*	missense	p.Trp37Arg	0.006 (0.001)	1.31E-13	0.3961	40,753	14,761	19,678	6,314	24.02	24.23	25.1

* Indicates variants having exome-wide significant associations with both ALT and AST.

Abbreviations: AAF, alternate allele frequency; Alt, alternate allele; ALT, alanine aminotransferase; AST, aspartate aminotransferase; Ref, reference allele; SE, standard error.

[00406] To replicate these associations, we analyzed the 35 AST- or ALT-associated variants ascertained via whole exome sequencing in three separate European-ancestry cohorts: 2,644 bariatric surgery patients from DiscovEHR ("GHS bariatric surgery cohort"), 1,357 individuals from the Dallas Heart Study, and 8,526 individuals from the Penn Medicine Biobank (**Table 1A**). In meta-analysis of the replication cohorts, thirteen variants in nine genes were significantly associated (Bonferroni significance threshold of $P<1.43x10^{-3}$) with ALT or AST (**Table 3**). These included previously reported liver disease-associated genes and variants, such as *PNPLA3* p.Ile148Met (Romeo et al. (2008) *Nat Genet* 40:1461-1465, herein incorporated by reference in its entirety for all purposes), *TM6SF2* p.Glu167Lys (Kozlitina et al. (2014) *Nat Genet* 46:352-356, herein incorporated by reference in its entirety for all purposes), and *SERPINA1* p.Glu366Lys (Z allele associated with alpha-1-anti-trypsin deficiency) (Brantly et al. (1988) *Am J Med* 84:13-31, herein incorporated by reference in its entirety for all purposes), *SAMM50*, and *ERLIN1*. *SERPINA1* encodes alpha-1-antitrypsin, whose functional deficiency is known to cause hereditary liver disease; the association with *SAMM50* may be mediated via linkage disequilibrium with variation in *PNPLA3*, and *ERLIN1* has been implicated in liver fat deposition. Several variants in *GPT* and *GOT1*, the genes encoding ALT and AST, respectively, were significantly associated with either ALT or AST levels but have not been previously reported to be associated with liver disease. *SLC39A12* has not previously been linked to transaminases or liver disease. Meta-analysis also replicated novel associations in our discovery cohort between decreased levels of ALT (beta (SE) -0.009 (0.001); $P=4.16x10^{-12}$) and AST (beta (SE) -0.005 (0.001); $P=6.24x10^{-10}$) and a splice variant in *HSD17B13,* the gene encoding hydroxysteroid 17-beta dehydrogenase 13, an uncharacterized member of the 17-beta hydroxysteroid dehydrogenase family. This variant, rs72613567, corresponds to the insertion of an A nucleotide adjacent to the donor splice site (TA allele). Replication meta-analysis P-values for these associations were $3.85x10^{-5}$ and $9.38x10^{-5}$, and joint meta-analysis P-values were $1.17x10^{-15}$ and $6.82x10^{-13}$ for ALT and AST, respectively (**Table 3**). A prior GWAS identified a nearby locus at 4q22 (rs6834314) as being associated with ALT levels (Chambers et al. (2011) *Nat Genet* 43:1131-1138, herein incorporated by reference in its entirety for all purposes); to our knowledge, there are no previous studies describing any association with rs72613567.

[00407] **Table 3**. Replication and joint meta-analysis of 35 exome-wide significant single nucleotide variants from the discovery cohort in three separate European-ancestry cohorts.

									GHS Discovery Cohort			Replication Cohorts									**Replication Meta-Analysis (N=3)		***Joint Meta-Analysis (N = 4)	
												GHS Bariatric Surgery Cohort			Dallas Heart Study			U. Penn						
Trait	Chr	BP	Ref	Alt	RSID	Gene	Ann	AA Substitution	Beta (SE)	P	N	Beta (SE)	P	N	Beta (SE)	P	N	Beta (SE)	P	N	Beta (SE)	P	Beta (SE)	P
ALT	1	220970028	A	G	rs2642438	*MARC1*	mis	p.Thr165Ala	0.008 (0.001)	4.67 E-08	41,414	0.005 (0.005)	3.10 E-01	2475	0.011 (0.008)	1.76 E-01	1357	0.007 (0.004)	1.02 E-01	6158	0.007 (0.003)	2.31E-02	0.008 (0.001)	3.38E-09
	4	88231392	T	TA	rs72613567	*HSD17B13*	spl		-0.009 (0.001)	4.16 E-12	41,414	-0.010 (0.005)	5.57 E-02	2475	-0.016 (0.008)	6.60 E-02	1357	-0.013 (0.004)	1.33 E-03	6158	-0.013 (0.003)	***3.85E-05**	-0.010 (0.001)	1.17E-15
	8	144997604	C	T	rs371119003	*PLEC*	mis	p.Ala2302Thr	-0.160 (0.026)	1.30 E-09	41,413	-0.492 (0.165)	2.84 E-03	2475	NA (NA)	NA	NA	-0.051 (0.072)	4.79 E-01	6158	-0.121 (0.066)	6.56E-02	-0.155 (0.025)	2.68E-10
	8	145008502	G	A		*PLEC*	mis	p.Arg522Cys	-0.268 (0.032)	3.26 E-17	41,414	-0.161 (0.165)	3.29 E-01	2475	NA (NA)	NA	NA	-0.247 (0.143)	8.48 E-02	6158	-0.210 (0.108)	5.23E-02	-0.264 (0.031)	5.54E-18
	8	145692918	G	A	rs35968570	*KIFC2*	mis	p.Glu174Lys	-0.033 (0.005)	1.40 E-11	41,414	-0.009 (0.020)	6.48 E-01	2475	0.032 (0.036)	3.76 E-01	1356	-0.053 (0.018)	3.72 E-03	6158	-0.025 (0.013)	4.69E-02	-0.032 (0.005)	2.25E-12
	8	145730072	G	A	rs143408057	*GPT*	mis	p.Arg83His	-0.314 (0.036)	3.28 E-18	41,414	-0.189 (0.165)	2.50 E-01	2475	NA (NA)	NA	NA	-0.298(0.101)	3.26 E-03	6158	-0.268 (0.086)	1.88E-03	-0.308 (0.033)	2.79E-20
	8	145730161	C	T	rs201815297	*GPT*	mis	p.Ala87Val	-0.224 (0.014)	6.28 E-59	41,414	-0.341 (0.074)	3.64 E-06	2475	NA (NA)	NA	NA	-0.143 (0.054)	8.50 E-03	6158	-0.213 (0.044)	***1.14E-06**	-0.223 (0.013)	4.49E-64
	8	145730221	G	A	rs112574791	*GPT*	mis	p.Arg107Lys	-0.033 (0.005)	4.25 E-11	41,414	-0.009 (0.020)	6.45 E-01	2475	0.028 (0.036)	4.37 E-01	1357	-0.060 (0.018)	5.60 E-04	6158	-0.031 (0.013)	1.36E-02	-0.033 (0.005)	1.92E-12
	8	145731636	T	G	rs145155876	*GPT*	stop	p.Tyr326*	-0.235 (0.031)	1.76 E-14	41,394	-0.314 (0.165)	5.71 E-02	2475	-0.317 (0.140)	2.35 E-02	1356	-0.148 (0.143)	3.04 E-01	6157	-0.256 (0.086)	2.79E-03	-0.237 (0.029)	1.94E-16
	8	145732114	G	C	rs141505249	*GPT*	mis	p.Glu430Gln	-0.224 (0.013)	8.84 E-64	41,375	-0.273 (0.048)	9.83 E-09	2474	-0.240 (0.075)	1.36 E-03	1357	-0.197 (0.041)	1.31 E-06	6157	-0.231 (0.029)	***7.24E-16**	-0.225 (0.012)	6.06E-78
	8	145732151	G	A	rs143462595	*GPT*	mis	p.Arg442His	-0.077 (0.013)	1.18 E-09	41,406	-0.115 (0.058)	4.82 E-02	2475	-0.106 (0.099)	2.86 E-01	1356	-0.049 (0.041)	2.27 E-01	6157	-0.074 (0.032)	1.88E-02	-0.076 (0.012)	7.03E-11

						GHS Discovery Cohort		Replication Cohorts							**Replication Meta-Analysis (N=3)		***Joint Meta-Analysis (N = 4)	
								GHS Bariatric Surgery Cohort		Dallas Heart Study		U. Penn						
Trait	Chr	BP	Ref	Alt	RSID	Gene	Ann	AA Substitution	Beta (SE)	P	N	Beta (SE)	P	N	Beta (SE)	P	Beta (SE)	P
	8	145732180	G	C	rs147998249	*GPT*	mis	p.Val452Leu	-0.225 (0.013)	8.19 E-65	41,413	-0.273 (0.050)	4.26 E-08	2475	-0.191 (0.070)	6.58 E-03	-0.197 (0.041)	1.31 E-06	6158	-0.221 (0.029)	**1.41E-14**	-0.224 (0.012)	1.04E-77
	8	145732305	G	GC		*GPT*	fs	p.Glu475fs	-0.271 (0.031)	1.00 E-18	41,414	-0.161 (0.165)	3.29 E-01	2475	NA (NA)	NA	-0.509 (0.203)	1.21 E-02	6158	-0.299 (0.128)	1.93E-02	-0.273 (0.030)	6.44E-20
	8	145748532	A	G	rs567402720	*LRRC24*	mis	p.Leu290Ser	-0.185 (0.028)	3.42 E-11	41,393	-0.161 (0.165)	3.29 E-01	2475	NA (NA)	NA	-0.307 (0.143)	3.21 E-02	6158	-0.244 (0.108)	2.40E-02	-0.189 (0.027)	2.93E-12
	9	117122202	C	T	rs3748177	*AKNA*	syn	p.Glu755Glu	-0.007 (0.001)	9.51 E-09	41,414	-0.004 (0.005)	4.09 E-01	2475	0.004 (0.008)	6.18 E-01	-0.007 (0.004)	5.29 E-02	6158	-0.005 (0.003)	8.42E-02	-0.007 (0.001)	3.08E-09
	9	117124731	G	A	rs3748176	*AKNA*	mis	p.Pro624Leu	-0.007 (0.001)	4.31 E-09	41,412	-0.004 (0.005)	3.90 E-01	2475	0.003 (0.008)	7.33 E-01	-0.007 (0.004)	4.24 E-02	6158	-0.005 (0.003)	6.15E-02	-0.007 (0.001)	1.00E-09
	10	101595996	T	A	rs172222723	*ABCC2*	mis	p.Val1188Glu	-0.015 (0.003)	2.97 E-08	41,414	-0.002 (0.010)	8.01 E-01	2475	-0.007 (0.017)	6.88 E-01	-0.017 (0.007)	1.55 E-02	6158	-0.012 (0.005)	3.43E-02	-0.014 (0.002)	3.44E-09
	10	101606861	G	T	rs1137968	*ABCC2*	syn	p.Val1430Val	-0.015 (0.003)	2.71 E-08	41,414	-0.003 (0.010)	7.74 E-01	2475	-0.008 (0.017)	6.28 E-01	-0.017 (0.007)	1.70 E-02	6158	-0.012 (0.005)	3.25E-02	-0.014 (0.002)	2.99E-09
	10	101610533	C	T	rs8187707	*ABCC2*	syn	p.His1496His	-0.015 (0.003)	2.77 E-08	41,414	-0.003 (0.010)	7.93 E-01	2475	-0.008 (0.017)	6.28 E-01	-0.017 (0.007)	1.76 E-02	6158	-0.012 (0.005)	3.43E-02	-0.014 (0.002)	3.23E-09
	10	101611294	G	A	rs8187710	*ABCC2*	mis	p.Cys1515Tyr	-0.015 (0.003)	2.15 E-08	41,414	-0.001 (0.010)	9.11 E-01	2475	-0.010 (0.017)	5.40 E-01	-0.016 (0.007)	2.77 E-02	6158	-0.011 (0.005)	5.21E-02	-0.014 (0.002)	4.09E-09
	10	101912064	T	C	rs2862954	*ERLIN1*	mis	p.Ile291Val	-0.012 (0.001)	2.43 E-21	40,834	-0.010 (0.005)	2.91 E-02	2475	-0.006 (0.007)	4.02 E-01	-0.009 (0.004)	2.06 E-02	6158	-0.009 (0.003)	**1.14E-03**	-0.011 (0.001)	1.76E-23
	10	101977883	C	T	rs2230804	*CHUK*	mis	p.Val268Ile	-0.009 (0.001)	1.93 E-13	41,414	-0.006 (0.005)	2.05 E-01	2475	0.0001 (0.008)	9.94 E-01	-0.011 (0.004)	3.91 E-03	6158	-0.008 (0.003)	4.33E-03	-0.009 (0.001)	3.59E-15
	10	113917085	T	A	rs2254537	*GPAM*	syn	p.Pro681Pro	-0.008 (0.001)	4.61 E-10	41,414	-0.003 (0.005)	5.80 E-01	2475	-0.013 (0.008)	1.15 E-01	-0.008 (0.004)	5.12 E-02	6158	-0.007 (0.003)	2.07E-02	-0.008 (0.001)	3.28E-11
	10	113940329	T	C	rs2792751	*GPAM*	mis	p.Ile643Val	-0.008 (0.001)	2.54 E-10	41,412	-0.003 (0.005)	5.61 E-01	2475	-0.013 (0.008)	1.33 E-01	-0.008 (0.004)	4.77 E-02	6158	-0.007 (0.003)	2.00E-02	-0.008 (0.001)	1.77E-11

						GHS Discovery Cohort		Replication Cohorts						**Replication Meta-Analysis (N=3)		***Joint Meta-Analysis (N = 4)	
								GHS Bariatric Surgery Cohort		Dallas Heart Study		U. Penn					
Trait	Chr	BP	Ref	Alt	RSID	Gene	Ann	AA Substitution	Beta (SE)	P	N	Beta (SE)	P	N	Beta (SE)	P	Beta (SE)	P
AST	14	94844947	C	T	rs28929474	*SERPINA1*	mis	p.Glu366Lys	0.042 (0.005)	9.28 E-21	41,414	0.035 (0.020)	7.97 E-02	2473	0.034 (0.032)	2.92 E-01	1357	0.054 (0.013)	1.63 E-05	6158	0.047 (0.010)	**2.82E-06**	0.043 (0.004)	1.59E-25
	19	19379549	C	T	rs58542926	*TM6SF2*	mis	p.Glu167Lys	0.014 (0.002)	4.76 E-09	41,413	0.040 (0.010)	2.40 E-05	2473	0.024 (0.014)	9.50 E-02	1357	0.013 (0.008)	7.51 E-02	6158	0.024 (0.006)	**1.37E-05**	0.016 (0.002)	1.15E-12
	22	44324727	C	G	rs738409	*PNPLA3*	mis	p.Ile148Met	0.023 (0.002)	1.34 E-50	41,414	0.019 (0.006)	5.54 E-04	2473	0.006 (0.009)	5.43 E-01	1357	0.016 (0.004)	2.05 E-04	6158	0.016 (0.003)	**7.45E-07**	0.021 (0.001)	3.55E-55
	22	44324730	C	T	rs738408	*PNPLA3*	syn	p.Pro149Pro	0.023 (0.002)	1.11 E-50	41,414	0.019 (0.006)	5.51 E-04	2473	0.006 (0.009)	5.43 E-01	1357	0.016 (0.004)	2.14 E-04	6158	0.016 (0.003)	**7.73E-07**	0.021 (0.001)	3.10E-55
	22	44342116	A	G	rs2294918	*PNPLA3*	mis	p.Lys434Glu	0.007 (0.001)	8.26 E-08	41,412	0.001 (0.005)	7.77 E-01	2473	0.005 (0.008)	5.18 E-01	1357	0.005 (0.004)	2.16 E-01	6158	0.004 (0.003)	1.91E-01	0.006 (0.001)	6.24E-08
	22	44368122	A	G	rs3761472	*SAMM50*	mis	p.Asp110Gly	0.019 (0.002)	8.85 E-30	41,413	0.009 (0.006)	1.66 E-01	2473	-0.001 (0.01)	9.37 E-01	1357	0.018 (0.005)	4.02 E-04	6158	0.012 (0.004)	**7.69E-04**	0.018 (0.002)	1.08E-31
	22	44395451	T	C	rs1007863	*PARVB*	mis	p.Trp37Arg	0.011 (0.001)	7.98 E-16	41,414	0.003 (0.005)	5.22 E-01	2473	0.008 (0.008)	3.13 E-01	1357	0.009 (0.004)	2.50 E-02	6158	0.007 (0.003)	1.78E-02	0.010 (0.001)	1.16E-16
	4	88231392	T	TA	rs72613567	*HSD17B13*	spl		-0.005 (0.001)	6.24 E-10	40,753	-0.010 (0.003)	3.12 E-03	2469	-0.012 (0.006)	5.32 E-02	1357	-0.007 (0.004)	5.56 E-02	6166	-0.009 (0.002)	**8.38E-05**	-0.006 (0.001)	6.82E-13
	10	18242311	A	G	rs10764176	*SLC39A12*	mis	p.Ser36Gly	-0.006 (0.001)	1.09 E-10	40,753	-0.010 (0.003)	2.91 E-03	2469	-0.003 (0.006)	5.80 E-01	1357	-0.009 (0.004)	1.03 E-02	6166	-0.009 (0.002)	**1.16E-04**	-0.006 (0.001)	1.10E-13
	10	101157378	CGTT	C		*GOT1*	inf	p.Asn389del	-0.221 (0.024)	1.96 E-20	40,753	-0.205 (0.062)	8.57 E-04	2469	NA (NA)	NA	NA	-0.243 (0.088)	5.97 E-03	6165	-0.218 (0.051)	**1.66E-05**	-0.220 (0.022)	1.68E-24
	10	101165533	G	C	rs374966349	*GOT1*	mis	p.Gln208Glu	0.271 (0.027)	2.43 E-24	40,753	NA (NA)	NA	NA	NA (NA)	NA	NA	0.339 (0.079)	1.85 E-05	6166	0.339 (0.079)	**1.85E-05**	0.278 (0.025)	3.25E-28
	10	101912064	T	C	rs2862954	*ERLIN1*	mis	p.Ile291Val	-0.005 (0.001)	4.82 E-09	40,753	-0.004 (0.003)	1.54 E-01	2469	-0.007 (0.006)	2.21 E-01	1357	-0.004 (0.003)	1.94 E-01	6166	-0.005 (0.002)	2.51E-02	-0.005 (0.001)	3.68E-10
	11	22271870	A	T	rs7481951	*ANO5*	mis	p.Leu322Phe	0.004 (0.001)	9.61 E-08	40,722	-0.001 (0.003)	7.85 E-01	2466	0.006 (0.006)	2.85 E-01	1357	-0.002 (0.003)	5.46 E-01	6165	0.000 (0.002)	8.43E-01	0.004 (0.001)	1.13E-06

										GHS Discovery Cohort			Replication Cohorts									**Replication Meta-Analysis (N=3)		***Joint Meta-Analysis (N = 4)	
													GHS Bariatric Surgery Cohort			Dallas Heart Study			U. Penn						
Trait	Chr	BP	Ref	Alt	RSID	Gene	Ann	AA Substitution		Beta (SE)	P	N	Beta (SE)	P	N	Beta (SE)	P	N	Beta (SE)	P	N	Beta (SE)	P	Beta (SE)	P
	14	94844947	C	T	rs28929474	*SERPINA1*	mis	p.Glu366Lys		0.027 (0.003)	2.44 E-20	40,753	0.023 (0.013)	7.79 E-02	2469	0.044 (0.024)	6.98 E-02	1357	0.055 (0.011)	4.01 E-07	6166	0.042 (0.008)	***9.54E-08**	0.029 (0.003)	6.71E-26
	19	19379549	C	T	rs58542926	*TM6SF2*	mis	p.Glu167Lys		0.008 (0.002)	6.54 E-08	40,192	0.023 (0.006)	1.99 E-04	2469	0.010 (0.011)	3.42 E-01	1356	0.004 (0.007)	5.94 E-01	6166	0.014 (0.004)	***1.20E-03**	0.009 (0.002)	5.92E-10
	22	44324727	C	G	rs738409	*PNPLA3*	mis	p.Ile148Met		0.014 (0.001)	8.31 E-46	40,753	0.014 (0.004)	1.27 E-04	2469	0.004 (0.007)	5.44 E-01	1357	0.015 (0.004)	4.87 E-05	6166	0.013 (0.002)	***5.51E-08**	0.014 (0.001)	3.14E-52
	22	44324730	C	T	rs738408	*PNPLA3*	syn	p.Pro149Pro		0.014 (0.001)	8.93 E-46	40,753	0.014 (0.004)	1.32 E-04	2469	0.004 (0.007)	5.44 E-01	1357	0.015 (0.004)	4.96 E-05	6166	0.013 (0.002)	***5.81E-08**	0.014 (0.001)	3.55E-52
	22	44368122	A	G	rs3761472	*SAMM50*	mis	p.Asp110Gly		0.011 (0.001)	1.22 E-22	40,752	0.008 (0.004)	6.03 E-02	2469	-0.001 (0.008)	9.45 E-01	1357	0.016 (0.004)	2.64 E-04	6166	0.010 (0.003)	***3.40E-04**	0.011 (0.001)	1.91E-25
	22	44395451	T	C	rs1007863	*PARVB*	mis	p.Trp37Arg		0.006 (0.001)	1.31 E-13	40,753	0.003 (0.003)	4.12 E-01	2469	0.006 (0.006)	2.95 E-01	1357	0.009 (0.003)	6.17 E-03	6166	0.006 (0.002)	7.34E-03	0.006 (0.001)	3.62E-15

* Indicates P-values meeting the Bonferroni significance threshold of $P < 1.43 \times 10^{-3}$.

** Replication meta-analysis includes the three replication cohorts: GHS Bariatric Surgery Cohort, Dallas Heart Study, and Penn Medicine Biobank.

*** Joint meta-analysis includes the discovery cohort and the three replication cohorts: GHS Discovery Cohort, GHS Bariatric Surgery Cohort, Dallas Heart Study, and Penn Medicine Biobank.

Abbreviations: AAF, alternate allele frequency; Alt, alternate allele; ALT, alanine aminotransferase; AST, aspartate aminotransferase; Ref, reference allele; SE, standard error; ann, annotation; mis, missense; syn, synonymous; spl, splice donor; stop, stop gained; fs, frameshift; inf, inframe indel.

[00408] *HSD17B13* is 30 kb upstream of *HSD17B11*, a member of the same gene family, and both genes lie within a single large haplotype block in Europeans. We did not observe any association between coding or splice variants in *HSD17B11* and transaminase levels in the discovery cohort (**Fig. 5A** and **5B**; most significant discovery P-values $1.36x10^{-1}$ for ALT and $4.32x10^{-2}$ for AST) or in the joint meta-analysis of the discovery cohort and three replication cohorts (most significant P-values $6.25x10^{-3}$ and $1.17x10^{-5}$ for ALT and AST, respectively). Furthermore, linkage disequilibrium of rs72613567 with variants in *HSD17B11* was modest across all ancestry groups, including in European Americans that largely comprise our discovery group, and also in Hispanic and African Americans represented in the Dallas Heart Study ($r^2 <$ 0.4 with all ascertained variants in *HSD17B11* in all ancestry groups; data not shown). Collectively, these findings suggest *HSD17B13* as the gene in the genomic region that is most likely to be functionally related to transaminase levels.

[00409] Next, we sought to establish whether variants associated with ALT or AST levels were also associated with chronic liver disease. In the discovery cohort, we used EHR diagnosis codes to broadly define cases of alcoholic and nonalcoholic (non-viral) liver disease, as well as the following disease sequelae: alcoholic cirrhosis, nonalcoholic cirrhosis, and hepatocellular carcinoma (HCC). A common control group ("no liver disease") was defined as individuals with no diagnosis codes for any type of liver disease (**Table 1**). We tested the twelve transaminase-associated variants from the discovery and replication cohorts for association with chronic liver disease, using a Bonferroni significance threshold of $P<0.05/24$ ($P<2.08x10^{-3}$) to account for the thirteen variants and two broad chronic liver disease categories (alcoholic and nonalcoholic) tested (**Table 4**). Overall, we found significant associations between six variants in five genes (*HSD17B13*, *SERPINA1*, *TM6SF2*, *PNPLA3*, and *SAMM50*) and chronic liver disease phenotypes. The *SERPINA1*, *TM6SF2*, *PNPLA3*, and *SAMM50* associations confirm previously reported associations. Variants in *GPT*, *GOT1*, *ERLIN1*, and *SLC39A12* were not significantly associated with any liver disease phenotype. The *HSD17B13* association with liver disease reported here is novel and the first potentially protective genetic variant described.

[00410] **Table 4.** Association of twelve exome-wide significant and replicating single nucleotide variants with liver disease phenotypes in the discovery cohort.

CHR:BP:Ref:Alt	Gene	rsID	Alcoholic liver disease		Alcoholic cirrhosis		Nonalcoholic liver disease		Nonalcoholic cirrhosis		Hepatocellular carcinoma	
			OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value
4:88231392:T:TA	*HSD17B13*	rs72613567	0.62 (0.48-0.81)	***1.82E-04**	0.56 (0.41-0.78)	***3.35E-04**	0.84 (0.78-0.91)	***1.31E-05**	0.74 (0.62-0.88)	***4.48E-04**	0.67 (0.45-1.00)	4.66E-02
8:145730161:C:T	*GPT*	rs201815297	3.83 (1.05-13.94)	8.88E-02	6.33 (1.71-23.43)	2.88E-02	0.23 (0.04-1.14)	1.86E-02	1.25 (0.24-6.38)	7.98E-01	3.66 (0.70-19.01)	2.01E-01
8:145732114:G:C	*GPT*	rs141505249	0.77 (0.06-10.73)	8.43E-01	1.13 (0.08-15.39)	9.30E-01	1.02 (0.49-2.11)	9.70E-01	0.36 (0.02-5.37)	3.82E-01	1.84 (0.15-23.25)	6.88E-01
8:145732180:G:C	*GPT*	rs147998249	0.73 (0.05-11.76)	8.17E-01	1.07 (0.07-17.16)	9.60E-01	1.03 (0.49-2.17)	9.30E-01	0.34 (0.02-5.59)	3.67E-01	1.74 (0.11-27.05)	7.21E-01
10:18242311:A:G	*SLC39A12*	rs10764176	0.85 (0.68-1.07)	1.64E-01	0.92 (0.70-1.22)	5.80E-01	0.92 (0.86 (0.99)	3.43E-02	1.03 (0.88-1.21)	7.15E-01	1.29 (0.93-1.79)	1.37E-01
10:101157378:CGTT:C	*GOT1*		4.60 (0.25-86.41)	3.93E-01	7.11 (0.38-133.19)	3.00E-01	2.37 (0.61-9.27)	2.50E-01	8.27 (1.44-47.49)	5.92E-02	9.81 (0.52-183.54)	2.43E-01
10:101165533:G:C	*GOT1*	rs374966349	2.20 (0.13-37.68)	6.24E-01	3.47 (0.20 - 59.04)	4.70E-01	1.63 (0.53-4.96)	4.20E-01	1.17 (0.07-20.09)	9.13E-01	5.37 (0.32-91.12)	3.55E-01
14:94844947:C:T	*SERPINA1*	rs28929474	2.49 (1.49-4.17)	2.30E-03	3.35 (1.93-5.83)	***3.01E-04**	1.50 (1.21-1.87)	***5.29E-04**	2.99 (2.11-4.24)	***9.08E-08**	1.86 (0.74-4.67)	2.40E-01
19:19379549:C:T	*TM6SF2*	rs58542926	1.47 (1.06-2.04)	2.76E-02	1.35 (0.89-2.04)	1.80E-01	1.36 (1.21-1.52)	***2.42E-07**	1.64 (1.31-2.05)	***6.04E-05**	1.93 (1.22-3.04)	1.08E-02
22:44324727:C:G	*PNPLA3*	rs738409	1.76 (1.43-2.18)	***4.98E-07**	2.07 (1.60-2.67)	***1.08E-07**	1.65 (1.54-1.78)	***1.31E-41**	2.05 (1.76-2.38)	***1.70E-19**	2.20 (1.60-3.02)	***5.59E-06**
22:44324730:C:T	*PNPLA3*	rs738408	1.77 (1.43-2.18)	***4.70E-07**	2.07 (1.61-2.67)	***1.03E-07**	1.65 (1.54-1.78)	***1.42E-41**	2.05 (1.77-2.38)	***1.45E-19**	2.20 (1.60-3.03)	***5.41E-06**
22:44368122:A:G	*SAMM50*	rs3761472	1.90 (1.52-2.38)	***1.36E-07**	2.28 (1.75-2.98)	***1.83E-08**	1.52 (1.41-1.65)	***7.33E-24**	1.86 (1.58-2.19)	***1.81E-12**	1.66 (1.16-2.39)	1.05E-02

* Indicates P-values meeting the Bonferroni significance threshold of $P < 2.08 \times 10^{-3}$.

[00411] The alternate (TA) allele of *HSD17B13* rs72613567 was observed at higher frequency in controls compared to participants with any of the chronic liver disease phenotypes evaluated (**Fig. 2A** and **Table 5**). After adjustment for age, age^2, sex, BMI, and ancestry, we observed 38% lower odds of alcoholic liver disease (odds ratio [OR] 0.62; 95% confidence interval [CI] 0.48-0.81, P=1.8x10^{-4}) and 16% lower odds of nonalcoholic (non-viral) liver disease (OR 0.84, 95% CI 0.78-0.91, P=1.3x10^{-5}) per TA allele. When restricting to cases with cirrhosis, the TA allele was associated with 44% lower odds of alcoholic (OR 0.56, 95% CI 0.41-0.78, P=3.4x10^{-4}) and 26% lower odds of nonalcoholic (OR 0.74, 95% CI 0.62-0.88, P=4.5x10^{-4}) cirrhosis. The TA allele was nominally associated with 33% lower odds of HCC per allele (OR 0.67, 95% CI 0.45-1.00, P=4.7x10^{-2}). Unadjusted genotypic ORs suggested a co-dominant effect; for example, for alcoholic cirrhosis, the OR was 0.59 (95% CI 0.40-0.86) for heterozygous T/TA carriers and 0.26 (95% CI 0.08-0.82) for homozygous TA/TA carriers, and for nonalcoholic cirrhosis, the OR was 0.75 (95% CI 0.61-0.93) for heterozygous and 0.55 (95% CI 0.34-0.91) for homozygous carriers.

[00412] Thus , in the discovery cohort, the alternate (TA) allele of *HSD17B13* rs72613567 was associated with lower odds of all EHR-derived chronic liver disease phenotypes evaluated, in a consistent allele dosage-dependent manner (**Fig. 2A**): all categories of alcoholic liver disease, heterozygous odds ratio (OR_{het}) [95% confidence interval] 0.58 [0.42-0.79], homozygous OR (OR_{hom}) 0.46 [0.23-0.94], allelic OR ($OR_{allelic}$) 0.62 [0.48-0.81], P = 1.82x10^{-4}; all categories of nonalcoholic liver disease, OR_{het} 0.84 [0.76-0.92], OR_{hom} 0.73 [0.59-0.89], $OR_{allelic}$ 0.84 [0.78-0.91], P = 1.31x10^{-5}. The TA allele was also associated with lower odds of the most advanced forms of these chronic liver diseases (as defined by EHR-derived diagnostic codes), namely alcoholic and nonalcoholic cirrhosis and HCC. The TA allele was associated with 42% and 73% lower odds of alcoholic cirrhosis for heterozygotes and homozygotes, respectively (OR_{het} 0.59 [0.40-0.86], OR_{hom} 0.26 [0.08-0.82], $OR_{allelic}$ 0.56 [0.41-0.78], P = 3.35x10^{-4}), 26% and 49% lower odds of nonalcoholic cirrhosis for heterozygotes and homozygotes, respectively (OR_{het} 0.75 [0.61-0.93], OR_{hom} 0.55 [0.34-0.91], $OR_{allelic}$ 0.74 [0.62-0.88], P = 4.48x10^{-4}). The TA allele was also nominally associated with lower odds of HCC.

[00413] Next, we sought to confirm and extend these findings in the multi-ethnic Dallas Liver Study (DLS) and the Dallas Pediatric Liver Study (DPLS), including African American, European American, and Hispanic American adults and children (**Table 1B**). In the DLS, the

TA allele was associated with lower odds of any liver disease in an allele-dosage dependent manner (OR_{het} 0.74 [0.57-0.97], OR_{hom} 0.41 [0.21-0.83], $OR_{allelic}$ 0.70 [0.5-0.88], $P = 1.77x10^{-3}$, **Fig. 8**). Similar allele dosage-dependent effects were observed across EHR-derived liver disease subtypes, including protective associations with advanced, cirrhotic forms of alcoholic ($OR_{allelic}$ 0.72 [0.53-0.99], $P = 4.37x10^{-2}$) and nonalcoholic ($OR_{allelic}$ 0.65 [0.40-1.07], $P = 8.96x10^{-2}$) liver disease. In subset analyses of individuals grouped by self-reported ethnicity, the association with liver disease remained significant in Hispanic Americans, in particular, because of the high rate of liver disease in this subpopulation (n = 326 cases and 722 controls, $OR_{allelic}$ 0.51 [0.35-0.74], $P = 3.98x10^{-4}$); similar numerical trends, which did not achieve statistical significance, were also noted in the African American (n = 33 cases and 2,291 controls, $OR_{allelic}$ 0.74 [0.25-2.47], P = 0.67) and European American (n = 158 cases and 1,266 controls, $OR_{allelic}$ 0.87 [0.65-1.15], P = 0.32) subsets of the DLS. In the DPLS, a separate study of Hispanic American pediatric liver disease patients and obese controls (**Table 1B**), the TA allele was also associated with lower odds of liver disease ($OR_{allelic}$ 0.59 [0.36-0.97], $P = 3.6x10^{-2}$). Thus, the *HSD17B13* rs72613567:TA allele was associated with reduced odds of multiple forms of chronic liver disease, including cirrhosis, in adults and children in three independent populations.

[00414] NAFLD describes a spectrum of disease ranging from fatty liver without evidence of significant inflammation (designated as "simple steatosis" upon histopathological examination) to more clinically impactful manifestations (designated as "nonalcoholic steatohepatitis" (NASH), with histopathological evidence of lobular inflammation, hepatocyte ballooning, and/or fibrosis). To understand the relationship between the *HSD17B13* TA allele and histologically defined NAFLD and NASH, we performed tests of association of rs72613567 in 2,391 whole exome sequenced individuals with liver biopsy samples from the GHS bariatric surgery cohort. Among these individuals, there were 555 (23%) with no evidence of steatosis, steatohepatitis, or fibrosis ("normal"), 830 (35%) with simple steatosis, and 1006 (42%) with NASH (i.e. evidence of lobular inflammation, hepatocyte ballooning, or fibrosis). The *HSD17B13* TA allele was not significantly associated with simple steatosis (OR 1.11, 95% CI 0.94-1.32, P=0.21) or NASH (OR 0.86, 95% CI 0.72-1.02, P=0.09) compared to normal liver (**Fig. 2B** and **Table 5**). When comparing prevalence of normal liver, simple steatosis, and NASH by genotype, it was observed that the prevalence of normal liver did not appear to differ by genotype (23%, 24%, and 23% for T/T, T/TA, and TA/TA carriers, respectively, P = 0.5 by Chi-squared test for trend in

proportions), but that the prevalence of NASH decreased (45%, 40%, and 31% for T/T, T/TA, and TA/TA carriers, respectively, $P = 1.6x10^{-4}$) and that of simple steatosis increased (33%, 35%, and 47% for T/T, T/TA, and TA/TA carriers, respectively, $P = 1.1x10^{-3}$) with each TA allele (**Fig. 9**). Among individuals with steatosis, the TA allele was associated with statistically significantly lower odds of NASH, as compared to simple steatosis, in an allele dosage-dependent manner. On the background of simple steatosis, the TA allele was associated with 23% lower odds of NASH (OR 0.77, 95% CI 0.66-0.90, $P=6.5x10^{-4}$), suggesting a role for *HSD17B13* in mediating the progression of NAFLD to more advanced stages of NASH and fibrosis. Genotypic association results were consistent with a co-dominant effect; in the NASH *vs*. simple steatosis comparison, the OR was 0.84 (95% CI 0.69-1.02) for heterozygous T/TA carriers, and 0.48 (95% CI 0.34-0.68) for homozygous TA/TA carriers.

[00415] **Table 5.** *HSD17B13* rs72613567 is associated with reduced risk of alcoholic and nonalcoholic liver disease phenotypes in the discovery cohort, and with reduced risk of progression from nonalcoholic fatty liver disease to nonalcoholic steatohepatitis and fibrosis in the bariatric surgery cohort.

		Cases				Controls									
Cohort	**Definitions**	**N**	**REF/ REF**	**REF/ ALT**	**ALT/ ALT**	**Definitions**	**N**	**REF/ REF**	**REF/ ALT**	**ALT/ ALT**	**AAF**	**Het OR (95% CI)**	**Hom OR (95% CI)**	**Per-allele OR (95% CI)**	**P-value**
Discovery cohort	Alcoholic liver disease	197	133	56	8	No liver disease	30,522	16413	11969	2140	0.266	0.58 (0.42-0.79)	0.46 (0.23-0.94)	0.62 (0.48-0.81)	1.82E-04
	Alcoholic cirrhosis	130	89	38	3						0.266	0.59 (0.40-0.86)	0.26 (0.08-0.82)	0.56 (0.41-0.78)	3.35E-04
	Nonalcoholic liver disease	1930	1131	692	107						0.264	0.84 (0.76-0.92)	0.73 (0.59-0.89)	0.84 (0.78-0.91)	1.31E-05
	Nonalcoholic cirrhosis	381	235	129	17						0.266	0.75 (0.61-0.93)	0.55 (0.34-0.91)	0.74 (0.62-0.88)	4.48E-04
	Hepatocellular carcinoma	76	49	24	3						0.266	0.67 (0.41-1.10)	0.47 (0.15-1.51)	0.67 (0.45-1.00)	4.66E-02
Bariatric surgery cohort	Simple steatosis	830	421	321	88	Normal	555	288	224	43	0.291	0.98 (0.78-1.23)	1.39 (0.94-2.08)	1.11 (0.94-1.32)	2.11E-01
	NASH	1006	578	370	58						0.255	0.82 (0.66-1.02)	0.67 (0.44-1.02)	0.86 (0.72-1.02)	8.53E-02
	NASH	1006	578	370	58	Simple steatosis	830	421	321	88	0.268	0.84 (0.69-1.02)	0.48 (0.34-0.68)	0.77 (0.66-0.90)	6.47E-04

[00416] We next sought to understand how the *HSD17B13* TA allele affects expression of known and novel transcripts of the gene. We used RNA sequencing to assess *HSD17B13* mRNA expression in histologically normal liver samples from 22 homozygous reference (T/T), 30 heterozygous (T/TA), and 17 homozygous alternate (TA/TA) carriers of the *HSD17B13* rs72613567 splice variant (**Fig. 3**). In addition to the two known *HSD17B13* transcripts, A and B, two novel transcripts were identified: transcript C lacking exon 6, and transcript D characterized by the insertion of a G nucleotide at the 3' end of exon 6, leading to premature protein truncation. Novel transcripts were validated by RT-PCR, and the D transcript was additionally validated by long read cDNA sequencing. The expression levels of these transcripts varied according to *HSD17B13* rs72613567 genotype; levels of transcripts A and B decreased, while those of transcripts C and D increased in an allele-dose-dependent manner in T/TA heterozygotes and TA/TA homozygotes (**Fig. 3**). Transcript A, encoding a 300 amino acid protein, was the predominant transcript in T/T in T/T homozygotes (**Fig. 3A**), while transcript D, encoding the prematurely truncated protein, was the predominant transcript in TA/TA homozygotes (**Fig. 3D**). These expression patterns suggest a functional role for *HSD17B13* rs72613567 in determining HSD17B13 isoform expression. Four additional transcripts (E-H) with very low levels of expression were also identified (**Fig. 6A-6D**). Protein sequence alignment of all identified *HSD17B13* isoforms is shown in **Fig. 7A-7B**.

[00417] HSD17B13 has been previously described as a lipid-droplet associated protein in human hepatocytes (Su et al. (2014) *Proc Natl Acad Sci USA* 111:11437-11442, herein incorporated by reference in its entirety for all purposes). We evaluated protein isoform expression and localization in a perpetual human liver cell line (HepG2 hepatoma cells) stably transduced with lentivirus expressing known and novel isoforms A-D of HSD17B13. HSD17B13 isoform A localized to lipid droplets in untreated and oleic acid-treated cells. Isoform A was mainly detected on membranes surrounding BODIPY-labeled lipid droplets, and co-localized with the lipid droplet coat protein perilipin (PLIN). Similar subcellular localization was observed for HSD17B13 isoform D at the lipid droplet surface; however, lipid droplets appeared larger following oleic acid treatment. In contrast, isoforms B and C co-localized with the endoplasmic reticulum marker calnexin.

[00418] In summary, using exome sequence data linked to EHR and liver biopsy data from 49,188 individuals from the DiscovEHR study population, and in follow-up studies of exome

sequence data from 9,883 additional individuals with ALT and AST measurements, we discovered a novel association between a splice variant in *HSD17B13*, transaminase levels, and chronic liver disease phenotypes. In our study, variant *HSD17B13* reduced the risk of nonalcoholic and alcoholic liver disease, and cirrhosis. This, to our knowledge, is the first report of an exonic variant with a protective association with chronic liver disease phenotypes. The *HSD17B13* TA allele was not associated with simple steatosis, but reduced the risk of histopathologic steatohepatitis in individuals with steatosis, suggesting a role for *HSD17B13* in progression to more clinically advanced stages of chronic liver disease. The consistency of protective associations in four independent cohorts (GHS discovery, GHS bariatric, DLS, and DPLS) across several different liver disease categories, characterized using EHR diagnosis codes as well as histopathological definitions of liver disease, together with the striking allele dosage-dependence of the associations, support the notion that the reported *HSD17B13* variant protects from progression to more clinically advanced stages of chronic liver disease. The observed allele dosage-dependence also argues that more profound regulation of HSD17B13 function may result in more profound effects on disease risk and progression.

[00419] Other 17beta-hydroxysteroid dehydrogenase family members are known to be involved in sex steroid and fatty acid metabolism (Moeller and Adamski (2009) *Mol Cell Endocrinol* 301:7-19, herein incorporated by reference in its entirety for all purposes), but little is known about the function of HSD17B13. *HSD17B13* is expressed primarily in the liver (Liu et al. (2007) *Acta Biochim Pol* 54:213-218, herein incorporated by reference in its entirety for all purposes), where it localizes to lipid droplets (Su et al. (2014) *Proc Natl Acad Sci USA* 111:11437-11442, herein incorporated by reference in its entirety for all purposes), consistent with a role for *HSD17B13* in the pathogenesis of fatty liver disease. Our data are consistent with recent findings that *HSD17B13* overexpression increased lipogenesis in mouse liver, and increased the number and size of lipid droplets in cultured hepatocytes (Su et al. (2014) *Proc Natl Acad Sci USA* 111:11437-11442, herein incorporated by reference in its entirety for all purposes). Two previous studies have also shown that hepatic expression of HSD17B13 protein is increased in patients with fatty liver (Su et al. (2014) *Proc Natl Acad Sci USA* 111:11437-11442 and Kampf et al. (2014) *FASEB J* 28:2901-2914, each of which is herein incorporated by reference in its entirety for all purposes). Two genes with variants that have been reported to be associated with increased risk of liver disease – *PNPLA3* and *TM6SF2* – also have physiological

roles in hepatocyte lipid metabolism. The variant in *HSD17B13* that we describe here is the first protective variant for liver disease, and may provide an avenue to new therapeutic strategies targeting chronic liver disease, similar to genetic variants that have guided the way to new therapeutics in other domains.

[00420] Overall, our data support *HSD17B13* as a novel therapeutic target to reduce the risk of chronic liver disease in humans. Importantly, our data indicate that targeting of *HSD17B13* could reduce progression from NAFLD to later stages of NASH, fibrosis, and cirrhosis, which are associated with significant morbidity and mortality, and for which there are currently no effective treatments.

Methods

[00421] **Study Participants.** Human genetics studies were conducted as part of the DiscovEHR collaboration of the Regeneron Genetics Center and the Geisinger Health System (GHS). The study was approved by the GHS Institutional Review Board. The two DiscovEHR study populations (discovery cohort and bariatric surgery cohort) originated from the first 50,726 consented participants ≥ 18 years of age from the MYCODE® Community Health Initiative of GHS (Dewey et al. (2016) *Science* 354(6319) doi:10.1126/science.aaf6814, herein incorporated by reference in its entirety for all purposes). The GHS discovery cohort consisted of 46,544 European individuals recruited from outpatient primary care and specialty clinics between 2007 and 2016, excluding all those recruited to the bariatric surgery cohort. The GHS bariatric surgery cohort consisted of 2,644 European individuals who had been referred for bariatric surgery.

[00422] Replication studies included 1,357 European individuals from the Dallas Heart Study and 8,527 European individuals from the Penn Medicine Biobank. The Dallas Heart Study is a probability-based population cohort study of Dallas County residents aged 30 to 65 years (Victor et al. (2004) *Am J Cardiol* 93:1473-1480, herein incorporated by reference in its entirety for all purposes. The Penn Medicine Biobank includes participants recruited from the University of Pennsylvania Health System and consented for biospecimen storage, access to EHR data, and permission to recontact.

[00423] Replication studies of the associations with chronic liver disease included 517 individuals from the Dallas Liver Study (DLS) and 447 individuals from the Dallas Pediatric Liver Study (DPLS). The DLS is a biobank of patients with liver disease of non-viral etiology.

Recruitment began in January 2015 and is ongoing. Participants were recruited from liver clinics at UT Southwestern and Parkland Health and Hospital System, Dallas. The biobank was approved by the UT Southwestern Institutional Review Board. Participants provided written informed consent. Participants completed a questionnaire on ethnic/racial background, medical history, lifestyle factors, and family history of liver disease and other diseases. Additional clinical information was extracted from medical records by a trained technician. We included all African American, European American, and Hispanic American patients with DNA available at the time of the present study (n=517). The DPLS is a biobank of children recruited from pediatric liver clinics at UT Southwestern and Parkland Health and Hospital System, Dallas, and from an obesity clinic at Children's Medical Center, Dallas. The biobank was approved by the UT Southwestern Institutional Review Board. The legal guardians of the participants provided written informed consent. Clinical information was extracted from medical records by a trained technician. As more than 95% of the patients were Hispanic Americans, we only included Hispanic American patients and controls in the present study (n=203 patients and 244 controls).

[00424] Sample Preparation and Sequencing. Sample preparation and whole exome sequencing were performed at the Regeneron Genetics Center as previously described (Dewey et al. (2016) *Science* 354(6319) doi:10.1126/science.aaf6814, herein incorporated by reference in its entirety for all purposes). In brief, exome capture was performed using NimbleGen probes according to the manufacturer's recommended protocol (Roche NimbleGen). The captured DNA was PCR amplified and quantified by qRT-PCR (Kapa Biosystems). The multiplexed samples were sequenced using 75 bp paired-end sequencing on an Illumina v4 HiSeq 2500 to a coverage depth sufficient to provide greater than 20x haploid read depth of over 85% of targeted bases in 96% of samples (approximately 80x mean haploid read depth of targeted bases). Raw sequence data from each Illumina Hiseq 2500 run were uploaded to the DNAnexus platform (Reid et al. (2014) *BMC Bioinformatics* 15, 30 doi:10.1186/1471-2105-15-30) for sequence read alignment and variant identification. In brief, raw sequence data were converted from BCL files to sample-specific FASTQ-files, which were aligned to the human reference build GRCh37.p13 with BWA-mem (Li and Durbin (2009) *Bioinformatics* 25:1754-1760, herein incorporated by reference in its entirety for all purposes). Single nucleotide variants (SNV) and insertion/deletion (indel) sequence variants were identified using the Genome Analysis Toolkit

(McKenna et al. (2010) *Genome Res* 20:1297-1303, herein incorporated by reference in its entirety for all purposes).

[00425] Targeted Genotyping of rs72613567 in the Dallas Liver and Pediatric Liver Studies. *HSD17B13* rs72613567 was genotyped by TAQMAN® assay in the Dallas Liver Study and Dallas Pediatric Liver Study, and by exome sequencing in the Dallas Heart Study. TAQMAN® calls were verified by Sanger sequencing of 5 individuals with each genotype.

[00426] Clinical Measurements and Chronic Liver Disease Definitions in the Discovery Cohort. Clinical laboratory measurements for ALT and AST were extracted from EHRs of participants from the GHS discovery cohort and bariatric surgery cohort. Median ALT and AST values were calculated for all participants with two or more measurements, and were $\log_{10}$ transformed to normalize the distribution prior to association analyses.

[00427] *International Classification of Diseases, Ninth Revision* (ICD-9) disease codes were extracted from EHRs and collapsed into clinical disease categories for non-viral, nonalcoholic (ICD-9 571.40, 571.41, 571.49, 571.5, 571.8, 571.9) or alcoholic (ICD-9 571.0, 571.1, 571.2, 571.3) liver disease case definitions. Additional case definitions based on single diagnosis codes included: alcoholic cirrhosis (ICD-9 571.2), nonalcoholic cirrhosis (ICD-9 571.5), and HCC (ICD-9 155.0). For these case definitions, a common control group without liver disease was defined as participants with no case criteria or single-encounter or problem-list diagnosis code indicating any type of liver disease.

[00428] Liver Histopathologic Phenotype Definitions in the Bariatric Surgery Cohort. The GHS bariatric surgery cohort consisted of 2,644 individuals of European descent, with intra-operative liver biopsy specimens available from 2,391 of these individuals. Liver biopsy specimens were formalin-fixed and stained with hematoxylin and eosin for routine histology, and Masson's trichrome stain for assessment of fibrosis, as previously described (Gerhard et al. (2011) *Patient Saf Surg* 5, 1, doi:10.1186/1754-9493-5-1, herein incorporated by reference in its entirety for all purposes). Histologic diagnoses were determined by hepatopathologists using previously established criteria (Brunt et al. (1999) *Am J Gastroenterol* 94:2467-2474, herein incorporated by reference in its entirety for all purposes). Histologic diagnoses were used to defined the following phenotypes: 1) Normal: no evidence of steatosis, NASH, or fibrosis; 2) Simple steatosis: Steatosis (regardless of grade) with no evidence of NASH or fibrosis; 3) NASH/fibrosis: Any presence of lobular inflammation or hepatocyte ballooning (regardless of

grade), or any presence of fibrosis (regardless of stage); 4) Fibrosis: Any presence of fibrosis (regardless of stage).

[00429] Exome-Wide Association Analysis of Liver Enzymes. In the GHS discovery cohort, we tested 502,219 biallelic variants with missing data rate < 1%, Hardy-Weinberg equilibrium p-value > $1.0x10^{-6}$, and minor allele frequency > 0.1% for association with transaminase levels. Log_{10}-transformed median ALT and AST were adjusted for age, age^2, sex, BMI, and the first four principal components of ancestry. To account for relatedness among study participants, we also fit a genetic relatedness matrix as a random-effects covariate. Both principal components and the genetic relatedness matrix were constructed from 39,858 non-MHC markers in approximate linkage equilibrium and with minor allele frequency > 0.1%. We used linear mixed models as implemented in the GCTA package (Yang et al. (2011) *Am J Hum Genet* 88:76-82, herein incorporated by references in its entirety for all purposes) to test for association between trait residuals and single nucleotide variants. The tests were well-calibrated, as shown by exome-wide quantile-quantile plots and genomic control lambda values (**Fig. 1**).

[00430] Replication Meta-Analysis of Liver Enzyme Associations. We attempted to replicate associations in the GHS discovery cohort in three separate European-ancestry cohorts: the GHS bariatric surgery cohort, the Dallas Heart Study, and the Penn Medicine Biobank (described above). ALT and AST measures in the GHS bariatric surgery cohort and from Penn Medicine Biobank were log_{10}-transformed and adjusted for age, age^2, sex, BMI, and the first four principal components of ancestry. ALT and AST measures from the Penn Medicine Biobank samples were log_{10}-transformed and adjusted for age, age^2, sex, BMI, and the first four principal components of ancestry. Genetic relatedness matrices were included as random-effects covariates, and analysis was performed using linear mixed models in GCTA. In the Dallas Heart study, log_{10}-transformed ALT and AST measures were adjusted for age, age^2, sex, and the first ten principal components of ancestry, and analysis was performed using linear regression implemented in PLINK. Summary statistics for the three replication cohorts were meta-analyzed using METAL (replication meta-analysis) (Willer et al. (2010) *Bioinformatics* 26:2190-2191, herein incorporated by reference in its entirety for all purposes). Summary statistics for the discovery cohort and the three replication cohorts were meta-analyzed similarly (joint meta-analysis).

[00431] **Association Analysis with Chronic Liver Disease Phenotypes.** We analyzed nine significant and replicated single nucleotide variants from the liver enzyme ExWAS for associations with binary liver disease phenotypes defined from the GHS discovery cohort, as described above. We used a Bonferroni significance threshold of $P<0.05/26$ ($P<1.92x10^{-3}$) to account for the thirteen variants and two broad chronic liver disease categories (alcoholic and nonalcoholic) tested. Variant *HSD17B13* was further tested for association with histopathologically defined liver phenotypes from the GHS bariatric surgery cohort, as described above. Odds ratios were estimated with the use of Firth's penalized likelihood method of logistic regression after adjustment for age, age^2, sex, BMI, and the first four principal components of ancestry. Unadjusted genotypic odds ratios were also estimated for *HSD17B13* rs72613567.

[00432] Odds ratios for liver disease in the DLS were estimated by logistic regression, adjusted for age, age^2, gender, BMI, and self-reported ethnicity. Participants from the Dallas Heart Study with available rs72613567 genotypes were used as normal controls (n=4,279). Odds ratios in the DPLS were estimated by logistic regression.

[00433] **Software.** Genetic association analyses were performed using GCTA software, version 1.25.0 (Yang et al. (2011) *Am J Hum Genet* 88:76-82, herein incorporated by reference in its entirety for all purposes), and PLINK, version 1.9.0. Quantile-quantile and Manhattan plots were generated using R software, version 3.2.1 (R Project for Statistical Computing). Regional association plots were generated using LocusZoom (Pruim et al. (2010) *Bioinformatics* 26:2336-2337, herein incorporated by reference in its entirety for all purposes).

[00434] **RNA Sequencing Studies.** RNA quality and concentration was evaluated by running total RNA on an Agilent RNA Nano Bioanalyzer chip; all samples had an RNA integrity number (RIN) greater than 8. Polyadenlylated RNA transcripts were isolated using two rounds of enrichment with oligo(dT)25 beads (Thermo Fisher Scientific). Samples were purified and concentrated with RNAclean XP beads (Beckman Coulter) and heat-fragmented to approximately 140 base pairs. First-strand synthesis was completed with SuperScript III reverse transcriptase (Thermo Fisher Scientific) using random hexamers; dTTP was replaced with dUTP during second-strand synthesis. Samples were processed according to our standard DNA library preparation method referenced above for exomes with the addition of a uracil DNA-glycosylase step to generate strand-specific sequencing libraries. Samples were pooled and sequenced using 75 bp paired-end sequencing on an Illumina v4 HiSeq 2500.

[00435] Identification of Novel *HSD17B13* Transcripts. Reads were mapped to the Human.B38 using ARRAYSTUDIO® software (OMICSOFT®, Cary, NC) allowing two mismatches. Two approaches were employed to identify novel *HSD17B13* transcripts. Novel exon junctions were discovered based on Gencode v24. *De novo* transcript assembly was run using Trinity (v2.2.0) in default setting. Custom gene models were built to incorporate novel transcripts of *HSD17B13*, and transcript quantification was estimated by read alignment to the custom gene model. Protein sequence alignment of all identified HSD17B13 isoforms is shown in **Fig. 7A** and **7B**.

[00436] RT-PCR Validation of Novel Transcripts. RT-PCR on total RNA from human liver samples was performed using the SUPERSCRIPT™ One-Step RT-PCR System with PlatinumTM Taq DNA Polymerase (Thermofisher). Each 50 uL RT-PCR reaction contained 1X Reaction Mix, 500 nM each forward and reverse primers (PST516: ATGAACATCATCCTAGAAATCCTTC (SEQ ID NO: 251) and PST517: ATCATGCATACATCTCTGGCTGGAG (SEQ ID NO: 252)), 1 μL of RT/Platinum Taq, and 75 ng RNA. Cycling conditions were: one cycle of 45°C for 30 min; one cycle of 94°C for 2 min; 40 cycles of 94°C for 20 s, 53°C for 30 s, and 72°C for 90 s; one cycle of 72°C for 5 min; then a 10°C hold. Products were purified using the QIAquick PCR Purification Kit (Qiagen) and submitted for direct Sanger sequencing using the primer DE002 (ATCAGAACTTCAGGCCTTGG (SEQ ID NO: 253)). To identify the B and C transcripts, the RT-PCR products were run out on a 2% agarose gel stained with SYBR GOLDSYBR® Gold Nucleic Acid Gel Stain (Thermofisher), and bands of the expected molecular weight were excised and purified using the QIAquick Gel Extraction Kit (Qiagen), then subjected to cloning with the TOPO® TA Cloning Kit (Thermofisher). Sequencing of the TOPO clones was performed using, M13F and M13R sequencing primers. Sequence analysis was performed using the Sequencher DNA analysis software (Gene Codes Corporation).

[00437] PacBio Validation of Novel Transcripts. Full-length *HSD17B13* transcripts were amplified directly from 50ng of total RNA with the SuperScript III One-step RT-PCR System with Platinum Taq High Fidelity (Thermo Fisher Scientific) using gene-specific primers in the first (GCAAAGCCATGAACATCATCC (SEQ ID NO: 254) and last exons (TCTTGATGTAGTGGGAGTCGGATT (SEQ ID NO: 255)) to generate an amplicon of ~2.2 kb (maximum predicted size transcript). Amplicons were verified on an Agilent Bioanalyzer.

PacBio-compatible barcoded adapters were ligated to the amplicons and cleaned with PacBio PB beads (Pacific Biosciences). Libraries were pooled in equal amounts and sequenced on one SMRT cell for 180 min on the PacBio RSII platform. The data were demultiplexed using PacBio software smrtanalysis v2.3 tool labelzmw and then analyzed with ConsensusTools AmpliconAnalysis. Resulting amplicons were compared to *HSD17B13* RefSeq genes to determine isoform and genotype status.

[00438] Subcellular Localization of HSD17B13 Isoforms. HepG2 cells were cultured in Eagle's Minimum Essential Medium supplemented with 10% fetal bovine serum. HSD17B13 Transcripts A, B, C, and D were sub-cloned into Myc-DDK backbone lentivirus constructs, and lentivirus were generated. HepG2 cells were infected with lentivirus carrying the various HSD17B13 transcripts. Stable cell lines expressing each HSD17B13 transcript were selected with 1-3 mg/ml Geneticin G-418 sulfate in complete culture medium for two weeks. Selected HepG2 cells were treated with or without 200 μM oleic acid overnight then fixed. HSD17B13 isoforms were labeled with mouse anti-Myc antibody. Lipid droplets were labeled with BODIPY FL dye (Sigma). Lipid coat protein and endoplasmic reticulum were labeled with rabbit anti-PLIN antibody (Sigma) and rabbit anti-calnexin antibody (Cell Signaling Technology), respectively. Secondary antibodies for immunofluorescence were Alexa Fluor 488 donkey anti-rabbit IgG and Alexa Fluor 594 donkey anti-mouse IgG (Jackson ImmunoResearch).

Example 2. Effect of rs72613567:TA on HSD17B13 mRNA and HSD17B13 Protein Expression.

[00439] The effect of the HSD17B13 rs72613567:TA allele on expression of known and novel transcripts of the gene was examined. RNA sequencing was used to assess HSD17B13 mRNA expression in histologically normal liver samples from 22 T/T homozygous, 30 T/TA heterozygous, and 17 TA/TA homozygous carriers of the HSD17B13 rs72613567 splice variant. In addition to the two known HSD17B13 transcripts, A and B, two novel transcripts were identified: Transcript C, which lacked exon 6, and Transcript D which contained an insertion of a guanine nucleotide at the 3' end of exon 6, which would be predicted to result in premature truncation of the protein. The transcripts were validated by RT-PCR and Sanger sequencing (data not shown). The D transcript was also validated using long read cDNA sequencing. The expression levels of these transcripts varied according to HSD17B13 rs72613567 genotype;

levels of transcript A decreased, while the level of transcripts D increased in an allele dosage-dependent manner with each TA allele (*see* **Figures 3A, 3D**, and **10B**). Transcript A, which encodes the full-length 300 amino acid protein, was the predominant transcript in T/T homozygotes, while transcript D, which encodes the prematurely truncated protein, was the predominant transcript in TA/TA homozygotes. In human liver biopsy tissue, the truncated isoform D protein was minimally present in heterozygotes and TA/TA homozygotes, and isoform A protein abundance was reduced in an allele dosage-dependent manner (*see* **Figures 10B** and **10C**). These data are consistent with HSD17B13 rs72613567 altering mRNA splicing, resulting in the synthesis of a truncated form of the protein with substantially reduced expression in human liver.

[00440] Referring to **Figures 10A-10E**, expression, subcellular localization, and enzymatic activity of a novel HSD17B13 transcript is shown. Expression of HSD17B13 transcripts A and D in homozygous reference (T/T), heterozygous (T/TA), and homozygous alternate (TA/TA) carriers of the HSD17B13 rs72613567 splice variant is shown in **Figures 3A** and **3D**. Coding regions in gene models are indicated in the striped boxes and untranslated regions in the black boxes. The asterisk in transcript D indicates the insertion of G from rs72613567 at the 3' end of exon 6, which leads to premature truncation of the protein. mRNA expression is displayed in FPKM units (Fragments Per Kilobase of transcript per Million mapped reads). A Western blot from HepG2 cells overexpressing HSD17B13 transcripts A and D shows that HSD17B13 transcript D was translated to a truncated protein with lower molecular weight compared to HSD17B13 transcript A (*see* **Figure 10A**). Similar results were observed with an HSD17B13 western blot from fresh frozen human liver and HEK293 cell samples (*see* **Figure 10B**). Human liver samples were from homozygous reference (T/T), heterozygous (T/TA), and homozygous alternate (TA/TA) carriers of the HSD17B13 rs72613567 splice variant. Cell samples were from HEK293 cells overexpressing non-tagged HSD17B13 transcripts A and D. HSD17B13 Transcript D was translated to a truncated protein IsoD with lower molecular weight than HSD17B13 IsoA. HSD17B13 IsoD protein levels were lower than IsoA protein levels from both human liver (left) and cell (right) samples (*see* **Figure 10C**). Protein level normalized to actin is shown in the bar columns in **Figure 10C**; ** $P<0.001$, *$P<0.05$. Both HSD17B13 Isoforms A and D were localized on lipid droplet membrane in HepG2 stably overexpressing HSD17B13 transcripts A or D were labelled with BODIPY to show lipid droplets and anti-Myc to show

HSD17B13 localization (data not shown). Enzymatic activity of HSD17B13 isoforms A and D to 17-beta estradiol (estradiol), leukotriene B4 (LTB4), and 13-Hydroxyoctadecadienoic acid (13(S)-HODE) was also assessed (*see* **Figure 10D**). HSD17B13 Isoform D showed <10% enzymatic activity of the corresponding values for Isoform A. HSD17B13 Isoform D when overexpressed in HEK293 cells did not show much conversion of estradiol (substrate) to estrone (product) when measured in the culture media, while overexpressed HSD17B13 Isoform A showed robust conversion (*see* **Figure 10E**).

[00441] HSD17B13 is expressed primarily in the liver (Liu et al., *Acta Biochim. Pol.*, 2007, 54, 213-8, herein incorporated by reference in its entirety for all purposes), where it localizes to lipid droplets (Su et al., *Proc. Natl. Acad. Sci. USA*, 2014, 111, 11437-42, herein incorporated by reference in its entirety for all purposes), consistent with a role in the pathogenesis of fatty liver disease. The expression of HSD171B3 and its localization was evaluated in an immortalized human liver cell line stably transduced with lentivirus expressing HSD17B13 Transcripts A and D. HSD17B13 Isoform A was mainly detected on membranes surrounding BODIPY-labeled lipid droplets (data not shown). Similar subcellular localization was observed for HSD17B13 Isoform D at the lipid droplet surface (*see* **Figure 10D**).

[00442] To understand the functional consequences of premature truncation of HSD17B13 protein due to rs72613567:TA, the enzymatic activity of Isoforms A and D was evaluated *in vitro* using recombinant protein. Greater than 300 putative substrates were examined, of which estradiol, leukotriene B4, and 13-Hydroxyoctadecadienoic acid were enzymatically converted by HSD17B13, resulting in oxidation of a hydroxyl to a ketone group. HSD17B13 Isoform D showed greatly reduced activity towards the three substrates (*see* **Figure 10D**).

[00443] Compared to GFP control, HSD17B13-Transcript-A-overexpressing cells had lower concentration of estradiol as well as higher concentration of estrone in the cell culture medium, suggesting enzyme activity against estradiol (*see* **Figure 10E**). HSD17B13-Transcript-D-overexpressing cells had similar ratio of estrone/estradiol to GFP control cells, suggesting that HSD17B13 Transcript D has significant loss of function. The mass spectrometry analysis revealed rapid conversion of estrone into hydroxyestrone and other products accounting for the low accumulation of estrone compared to consumed estradiol.

[00444] Through large-scale exome sequencing, a novel association was identified between a splice variant in HSD17B13 and decreased serum transaminase levels, as well as reduced risk of

nonalcoholic and alcoholic forms of liver disease, including advanced cirrhotic forms of liver disease and HCC. To our knowledge, this is the first report of a protein-altering variant that has a protective association with liver disease. The HSD17B13 rs72613567:TA allele was not associated with simple steatosis, but reduced the risk of progression to NASH. The consistency of the dosage-dependent protective associations in four independent cohorts (DiscovEHR, an independent bariatric surgery cohort in DiscovEHR, DLS, and DPLS) across several different liver disease categories and ethnicities support the notion that the reported HSD17B13 variant protects from progression to more clinically advanced stages of chronic liver disease. The observed allele dosage-dependence also argues that more profound regulation of HSD17B13 function may result in more profound effects on disease risk and progression.

[00445] The association findings described herein were primarily based on observations in European and Hispanic Americans who have elevated BMI. HSD17B13 is in close proximity with HSD17B11, a member of the same gene family with high sequence similarity to HSD17B13 but broader tissue distribution. Overall, the data presented herein support the position that HSD17B13 is a potential therapeutic target for prevention and treatment of fatty liver disease in humans. The data presented herein indicate that targeting of HSD17B13 could reduce progression of liver disease from steatosis to later stages of NASH, fibrosis, and cirrhosis, which are associated with significant morbidity and mortality, and for which there are currently no effective treatments.

Example 3. Variant 17Beta-Hydroxysteroid Dehydrogenase 13 Protects Against Chronic Liver Disease.

[00446] To identify genetic factors contributing to chronic liver disease, we utilized exome sequence data and electronic health records from 46,544 participants in the DiscovEHR human genetics study. We identified genetic variants associated with established biomarkers of hepatic injury (serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST)) to nominate candidates that might be associated with chronic liver disease. Candidate variants replicating in three additional cohorts (12,527 individuals) were subsequently evaluated for association with clinical diagnoses of chronic liver disease in DiscovEHR and two independent cohorts (total of 37,892 individuals). We also examined the association with histopathological severity of liver disease in an independent bariatric surgery cohort (n=2,391 human liver

samples).

[00447] A splice variant (rs72613567:TA) in HSD17B13, encoding the hepatic lipid droplet protein 17-beta hydroxysteroid dehydrogenase 13, was reproducibly associated with reduced ALT ($P=4.2x10^{-12}$) and AST ($P=6.2x10^{-10}$) levels. In DiscovEHR, this variant was associated with reduced risk of alcoholic and nonalcoholic liver disease (by 38%, 95% confidence interval (CI) 19%-52%; and by 16%, 95% CI 9%-22%, respectively, for each rs72613567:TA allele) and cirrhosis (by 44%, 95% CI 22-59%; and by 26%, 95% CI 12%-38% for alcoholic and nonalcoholic cirrhosis, respectively, for each rs72613567:TA allele) in an allele dosage-dependent manner; associations were confirmed in two independent cohorts. rs72613567:TA was associated with decreased severity of histological features of nonalcoholic steatohepatitis (NASH) (23% reduction, 95% CI 10%-34% for each rs72613567:TA allele among individuals with fatty liver disease). rs72613567:TA results in an unstable and truncated protein with reduced enzymatic activity against steroid substrates.

[00448] A loss-of-function variant in HSD17B13 was associated with reduced risk of alcoholic and nonalcoholic liver disease, and progression from steatosis to NASH.

Study Design and Participants

[00449] Human genetics studies were conducted as part of the DiscovEHR collaboration of the Regeneron Genetics Center and Geisinger Health System (GHS). The two DiscovEHR study populations (discovery cohort and bariatric surgery cohort) originated from the first 50,726 consented participants ≥ 18 years of age from the MyCode® Community Health Initiative of GHS. The GHS discovery cohort consisted of 46,544 European individuals recruited from outpatient primary care and specialty clinics between 2007 and 2016, excluding all those recruited to the bariatric surgery cohort. The GHS bariatric surgery cohort consisted of 2,644 European individuals who had been referred for bariatric surgery.

[00450] Replication studies of associations with liver transaminases included 1,357 European individuals from the Dallas Heart Study and 8,527 European individuals from the Penn Medicine Biobank. The Dallas Heart Study is a probability-based population cohort study of Dallas County residents aged 30 to 65 years (Victor et al., *Am. J. Cardiol.*, 2004; 93, 1473-80, herein incorporated by reference in its entirety for all purposes). The Penn Medicine Biobank includes participants recruited from the University of Pennsylvania Health System and consented for

biospecimen storage, access to EHR data, and permission to recontact.

[00451] Replication studies of the associations with chronic liver disease included 517 individuals from the Dallas Liver Study (DLS) and 447 individuals from the Dallas Pediatric Liver Study (DPLS). The DLS is a biobank of patients with liver disease of non-viral etiology. Recruitment began in January 2015 and is ongoing. Participants were recruited from liver clinics at UT Southwestern and Parkland Health and Hospital System, Dallas. Participants completed a questionnaire on ethnic/racial background, medical history, lifestyle factors, and family history of liver disease and other diseases. Additional clinical information was extracted from medical records by a trained technician. We included all African American, European American, and Hispanic American patients with DNA available at the time of the present study (n=517) with controls from the Dallas Heart Study. The DPLS is a biobank of Hispanic children recruited from pediatric liver clinics at UT Southwestern and Parkland Health and Hospital System, Dallas, and from an obesity clinic at Children's Medical Center, Dallas. Clinical information was extracted from medical records by a trained technician. As more than 95% of the patients were Hispanic Americans, we only included Hispanic American patients and controls in the present study (n=205 patients and 234 controls).

Clinical Measurements and Chronic Liver Disease Definitions in the Discovery Cohort

[00452] Clinical laboratory measurements for ALT and AST were extracted from EHRs of participants from the GHS discovery cohort and bariatric surgery cohort. Median ALT and AST values were calculated for all participants with two or more measurements, and were $\log_{10}$-transformed to normalize the distribution prior to association analyses.

[00453] International Classification of Diseases, Ninth Revision (ICD-9) disease diagnosis codes were extracted from EHRs and collapsed into clinical disease categories for non-viral, nonalcoholic (ICD-9 571.40, 571.41, 571.49, 571.5, 571.8, 571.9) or alcoholic (ICD-9 571.0, 571.1, 571.2, 571.3) liver disease case definitions. Additional case definitions based on single diagnosis codes included: alcoholic cirrhosis (ICD-9 571.2), nonalcoholic cirrhosis (ICD-9 571.5), and HCC (ICD-9 155.0). For these case definitions, a common control group without liver disease ("no liver disease") was defined as participants with no case criteria or single-encounter or problem-list diagnosis code indicating any type of liver disease.

Liver Histopathologic Phenotype Definitions in the Bariatric Surgery Cohort

[00454] The GHS bariatric surgery cohort consisted of 2,644 individuals of European descent. Wedge biopsies of the liver were obtained intraoperatively during bariatric surgery from 2,391 of these individuals. The biopsies were consistently obtained 10 cm to the left of falciform ligament prior to any liver retraction or surgery on the stomach. The biopsy was divided into sections, with the primary section delivered to the clinical pathologists for liver histology (fixed in 10% neutral buffered formalin and stained with hematoxylin and eosin for routine histology and Masson's trichrome for assessment of fibrosis) and remaining sections stored within a research biobank (frozen in RNAlater and/or liquid nitrogen). Liver histology was conducted by an experienced pathologist and subsequently re-reviewed by a second experienced pathologist using the NASH Clinical Research Network scoring system (Kleiner et al., *Hepatology*, 2005, 41, 1313-21, herein incorporated by reference in its entirety for all purposes) as follows: steatosis grade 0 (<5% parenchymal involvement), 1 (5 to <33 %), 2 (34 to <66 %), and 3 (>67 %); lobular inflammation grade 0 (no foci), grade 1 (mild, <2 foci per 200X field), grade 2 (moderate, 2-4 foci per 200X field), grade 3 (severe, >4 foci per 200X field); fibrosis Stage 0 (none), Stage 1 (perisinusoidal or periportal fibrosis), Stage 2 (perisinusoidal and periportal fibrosis), Stage 3 (bridging fibrosis), and Stage 4 (cirrhosis). These histologic diagnoses were used to defined the following phenotypes: 1) Normal: no evidence of steatosis, NASH, or fibrosis; 2) Simple steatosis: Steatosis (regardless of grade) with no evidence of NASH or fibrosis; 3) NASH: Any presence of lobular inflammation or hepatocyte ballooning (regardless of grade), or any presence of fibrosis (regardless of stage); 4) Fibrosis: Any presence of fibrosis (regardless of stage).

Sample Preparation, Sequencing, and Genotyping

[00455] DNA sample preparation and whole exome sequencing for the participants in the DiscovEHR study, the Dallas Heart Study, and the Penn Medicine Biobank were performed at the Regeneron Genetics (Dewey et al., *Science* In Press, 2016, herein incorporated by reference in its entirety for all purposes). HSD17B13 rs72613567 was genotyped by Taqman assay (and verified by Sanger sequencing in 5 individuals of each genotype) in the Dallas Liver Study and Dallas Pediatric Liver Study.

[00456] In particular, exome capture was performed using NimbleGen probes according to the

manufacturer's recommended protocol (Roche NimbleGen). The captured DNA was PCR amplified and quantified by qRT-PCR (Kapa Biosystems). The multiplexed samples were sequenced using 75 bp paired-end sequencing on an Illumina v4 HiSeq 2500 to a coverage depth sufficient to provide greater than 20x haploid read depth of over 85% of targeted bases in 96% of samples (approximately 80x mean haploid read depth of targeted bases). Raw sequence data from each Illumina Hiseq 2500 run were uploaded to the DNAnexus platform (Reid et al., *BMC Bioinformatics*, 2014, 15, 30, herein incorporated by reference in its entirety for all purposes) for sequence read alignment and variant identification. In brief, raw sequence data were converted from BCL files to sample-specific FASTQ-files, which were aligned to the human reference build GRCh37.p13 with BWA-mem (Li et al., *Bioinformatics*, 2009, 25, 1754-60, herein incorporated by reference in its entirety for all purposes). Single nucleotide variants (SNV) and insertion/deletion (indel) sequence variants were identified using the Genome Analysis Toolkit (McKenna et al., *Genome Res.*, 2010, 20, 1297-303, herein incorporated by reference in its entirety for all purposes).

Exome-Wide Association Analysis of Liver Enzymes and Chronic Liver Disease Phenotypes

[00457] We used linear mixed models to test 502,219 biallelic variants that had missing data rate of < 1%, Hardy-Weinberg equilibrium P-value > $1.0x10^{-6}$, and minor allele frequency > 0.1% for association with transaminase levels. For variants with exome wide significant associations with transaminases ($p<1x10^{-7}$) in the GHS discovery cohort, we performed association analyses and meta-analysis, in the European-ancestry replication studies described above. We used a Bonferroni significance threshold determined by the number of variants tested to define replicated associations. Meta-analysis of discovery and replication studies was also performed. All P-values reported in the text correspond to the allelic model.

[00458] We subsequently tested transaminase-associated single nucleotide variants for associations with chronic liver disease phenotypes. We used a Bonferroni significance threshold determined by the number of variants and broad chronic liver disease categories tested to determine significance of associations. We further tested replicated novel variants for association with histopathologically defined liver phenotypes from the GHS bariatric surgery cohort. We also performed a phenome-wide study of associations of replicated novel variants with 405 quantitative clinical measurements and 3,168 clinical diagnoses.

[00459] In particular, we tested 502,219 biallelic variants with missing data rate < 1%, Hardy-Weinberg equilibrium P-value > $1.0x10^{-6}$, and minor allele frequency > 0.1% for association with transaminase levels. Log_{10}-transformed median ALT and AST were adjusted for age, age^2, sex, BMI, and the first four principal components of ancestry. To account for relatedness among study participants, we also fit a genetic relatedness matrix as a random-effects covariate. Both principal components and the genetic relatedness matrix were constructed from 39,858 non-MHC markers in approximate linkage equilibrium and with minor allele frequency > 0.1%. We used linear mixed models as implemented in the GCTA package (Yang et al., *Am. J. Hum. Genet.*, 2011, 88, 76-82, herein incorporated by reference in its entirety for all purposes) to test for association between trait residuals and single nucleotide variants. All P-values reported in the text correspond to the allelic model.

[00460] We attempted to replicate associations in the GHS discovery cohort in three separate European-ancestry cohorts: the GHS bariatric surgery cohort, the Dallas Heart Study, and the Penn Medicine Biobank (described above). ALT and AST measures from the GHS bariatric surgery cohort and from Penn Medicine Biobank were log_{10}-transformed and adjusted for age, age^2, sex, BMI, and the first four principal components of ancestry. Genetic relatedness matrices were included as random-effects covariates, and analysis was performed using linear mixed models in GCTA. In the Dallas Heart study, log_{10}-transformed ALT and AST measures were adjusted for age, age^2, sex, BMI, and the first ten principal components of ancestry, and analysis was performed using linear regression implemented in PLINK. Summary statistics for the three replication cohorts were meta-analyzed using METAL (Willer et al., *Bioinformatics*, 2010, 26, 2190-1, herein incorporated by reference in its entirety for all purposes) (replication meta-analysis). Summary statistics for the discovery cohort and the three replication cohorts were meta-analyzed similarly (joint meta-analysis).

Association Analysis with Chronic Liver Disease Phenotypes

[00461] We analyzed thirteen significant and replicated single nucleotide variants from the liver enzyme ExWAS for associations with chronic liver disease phenotypes defined from the GHS discovery cohort, as described above. We used a Bonferroni significance threshold of $P<0.05/26$ ($P<1.92x10^{-3}$) to account for the thirteen variants and two broad chronic liver disease categories (alcoholic and nonalcoholic) tested. The HSD17B13 rs72613567 variant was further

tested for association with histopathologically defined liver phenotypes from the GHS bariatric surgery cohort, as described above. Odds ratios were estimated with the use of Firth's penalized likelihood method of logistic regression after adjustment for age, age^2, sex, BMI, and the first four principal components of ancestry. Genotypic odds ratios were estimated for HSD17B13 rs72613567 using the same covariates.

[00462] Odds ratios for liver disease in the DLS were estimated by logistic regression, adjusted for age, age^2, sex, body mass index, and self-reported ethnicity. Participants from the Dallas Heart Study with available rs72613567 genotypes were used as normal controls (n=4,279). Odds ratios in the DPLS were estimated by logistic regression.

Phenome-Wide Association Study of HSD17B13 rs72613567

[00463] We performed a phenome-wide study of associations of HSD17B13 rs72613567 with 405 quantitative EHR-derived anthropometric, vital sign, laboratory, electrocardiographic, echocardiographic, and bone densitometry measurements, and also with 3,168 EHR-derived clinical diagnoses. Median laboratory values for individuals with serial outpatient measures were calculated following removal of likely spurious values that were > 3 standard deviations from the intra-individual median value; maximum and minimum values were also calculated. We then calculated trait residuals for all laboratory traits after adjustment for age, age^2, sex, and the first ten principal components of ancestry, and applied appropriate transformations prior to association analysis. ICD-9 based diagnosis codes were collapsed to hierarchical clinical disease groups and corresponding controls using a modified version of the groupings proposed by Denny et al (Denny et al., *Nature Biotechnology*, 2013, 31, 1102-10 and Denny et al., *Bioinformatics*, 2010, 26, 1205-10, each of which is herein incorporated by reference in its entirety for all purposes). ICD-9 based diagnoses required one or more of the following: a problem list entry of the diagnosis code or an encounter diagnosis code entered for two separate clinical encounters on separate calendar days.

[00464] Analyses of association with transformed quantitative clinical measurement residuals were performed using linear regression, and analyses of association with clinical diagnoses were performed using logistic regression adjusted for age, age^2, sex, and the first four principal components. Alleles were coded using both additive (0 for reference allele homozygotes, 1 for heterozygotes, and 2 for alternative allele homozygotes) and recessive (0 for reference allele

homozygotes and heterozygotes, 1 for alternative allele homozygotes) models.

Software

[00465] Genetic association analyses were performed using GCTA software, version 1.25.07 and PLINK, version 1.9.0. Quantile-quantile and Manhattan plots were generated using R software, version 3.2.1 (R Project for Statistical Computing). Regional association plots were generated using LocusZoom (Pruim et al., *Bioinformatics*, 2010, 26, 2336-7, herein incorporated by reference in its entirety for all purposes).

RNA Sequencing Studies

[00466] RNA quality and concentration was evaluated by running total RNA on an Agilent RNA Nano Bioanalyzer chip; all samples had an RNA integrity number (RIN) greater than 8. Polyadenlylated RNA transcripts were isolated using two rounds of enrichment with oligo(dT)25 beads (Thermo Fisher Scientific). Samples were purified and concentrated with RNAclean XP beads (Beckman Coulter) and heat-fragmented to approximately 140 base pairs. First-strand synthesis was completed with SuperScript III reverse transcriptase (Thermo Fisher Scientific) using random hexamers; dTTP was replaced with dUTP during second-strand synthesis. Samples were processed according to our standard DNA library preparation method referenced above for exomes with the addition of a uracil DNA-glycosylase step to generate strand-specific sequencing libraries.

Identification and Validation of Novel HSD17B13 Transcripts

[00467] Reads were mapped to the Human.B38 using ArrayStudio® software (OmicSoft®, Cary, NC) allowing two mismatches. Two approaches were employed to identify novel HSD17B13 transcripts. Novel exon junctions were discovered based on Gencode v24 using ArrayStudio. *De novo* transcript assembly was carried out using Trinity (v2.2.0) in default setting. Custom gene models were built to incorporate novel transcripts of HSD17B13, and transcript quantification was estimated by read alignment to the custom gene model. Protein sequence alignment of all identified HSD17B13 isoforms is shown in **Figures 7A** and **7B**. RT-PCR was performed on total RNA from human liver samples was performed using the SuperScriptTM One-Step RT-PCR System with PlatinumTM Taq DNA Polymerase (Thermo

Fisher). Each 50 µL RT-PCR reaction contained 1X Reaction Mix, 500 nM each forward and reverse primers (PST516: ATGAACATCATCCTAGAAATCCTTC (SEQ ID NO: 251) and PST517: ATCATGCATACATCTCTGGCTGGAG (SEQ ID NO: 252)), 1 µL of RT/Platinum Taq, and 75 ng RNA. Cycling conditions were: one cycle of 45°C for 30 minutes; one cycle of 94°C for 2 minutes; 40 cycles of 94°C for 20 seconds, 53°C for 30 seconds, and 72°C for 90 seconds; one cycle of 72°C for 5 minutes; then a 10°C hold. Products were purified using the QIAquick PCR Purification Kit (Qiagen) and submitted for direct Sanger sequencing using the primer DE002 (ATCAGAACTTCAGGCCTTGG (SEQ ID NO: 253)). To identify the B and C transcripts, the RT-PCR products were run out on a 2% agarose gel stained with SYBR GoldSYBR® Gold Nucleic Acid Gel Stain (ThermoFisher), and bands of the expected molecular weight were excised and purified using the QIAquick Gel Extraction Kit (Qiagen), then subjected to cloning with the TOPO® TA Cloning Kit (ThermoFisher). Sequencing of the TOPO clones was performed using M13F and M13R sequencing primers. Sequence analysis was performed using the Sequencher DNA analysis software (Gene Codes Corporation).
Full-length HSD17B13 transcripts were amplified directly from 50 ng of total RNA with the SuperScript III One-step RT-PCR System with Platinum Taq High Fidelity (ThermoFisher Scientific) using gene-specific primers in the first (GCAAAGCCATGAACATCATCC (SEQ ID NO: 254)) and last exons (TCTTGATGTAGTGGGAGTCGGATT (SEQ ID NO: 255)) to generate an amplicon of about 2.2 kb (maximum predicted size transcript). Amplicons were verified on an Agilent Bioanalyzer. PacBio-compatible barcoded adapters were ligated to the amplicons and cleaned with PacBio PB beads (Pacific Biosciences). Libraries were pooled in equal amounts and sequenced on one SMRT cell for 180 minutes on the PacBio RSII platform. The data was demultiplexed using PacBio software smrtanalysis v2.3 tool labelzmw and then analyzed with ConsensusTools AmpliconAnalysis. Resulting amplicons were compared to HSD17B13 RefSeq genes to determine isoform and genotype status.

Subcellular Localization of HSD17B13 Isoforms

[00468] HepG2 cells were cultured in Eagle's Minimum Essential Medium supplemented with 10% fetal bovine serum. HSD17B13 transcripts A and D were sub-cloned into Myc-DDK backbone lentivirus constructs, and lentivirus were generated. HepG2 cells were infected with lentivirus carrying the HSD17B13 transcripts. Stable cell lines expressing each HSD17B13

transcript were selected with 1-3 mg/ml Geneticin G-418 sulfate in complete culture medium for two weeks. Following fixation, HSD17B13 isoforms were detected with mouse anti-Myc antibody. Lipid droplets were labeled with BODIPY FL dye (Sigma). Secondary antibodies for immunofluorescence were Alexa Fluor 488 donkey anti-rabbit IgG and Alexa Fluor 594 donkey anti-mouse IgG (Jackson ImmunoResearch).

Quantification of HSD171B3 Protein Expression in Human Liver Biopsy Tissue and Stable Cell Lines

[00469] Human liver and cell pellet samples were homogenized in ice-cold 1x RIPA lysis buffer (EMD Millipore) in the presence of protease and phosphatase inhibitor mixtures (ThermoFisher). Supernatant was collected and used for protein concentration using BCA protein assay (ThermoFisher). Human tissue and cell lysates were loaded and separated on SDS/PAGE gels (Bio-Rad) and transferred to PVDF membranes (Bio-Rad). The membranes were blocked for 1 hour with 5% (wt/vol) milk in 1x TBS supplemented with 0.1% Tween20 (Bio-Rad). Membranes were incubated with antibody at 4°C overnight against HSD17B13 (1:200, Thermo-Fisher) and B-Actin (1:500, Cell Signaling Technology). Bound antibody was detected using HRP-conjugated anti-rabbit antibody (1:10,000, Jackson ImmunoResearch) and enhanced using chemi-luminescence reagent (ThermoFisher). Band intensities were quantified using Image J software.

Real-Time Semi-Quantitative PCR

[00470] RNA was extracted from cell using TRIzol® (Invitrogen, Carlsbad, CA). First-strand cDNA was synthesized using Superscript III RT (Invitrogen) and utilized for Semi-Quantitative PCR based on intron-spanning primers. A QuantStudio 6 Flex Real-Time PCR System was used to measure the expression level of transcripts. Primers of HSD17B13 and TBP were ordered from IDT (Integrated DNA Technologies). Relative gene expression was analyzed with the ΔΔCt method, providing a fold-change of expression normalized to the house-keeping gene TBP (ΔCt).

Lipid Droplet Isolation and Characterization by Western Blotting

[00471] Lipid droplets were prepared from HepG2 cells stably expressing HSD17B13

transcript A (IsoA) or transcript D (IsoD) as previously reported (Brasaemle DL, Wolins NE. Isolation of lipid droplets from cells by density gradient centrifugation, Current protocols in cell biology 2006; Chapter 3:Unit 3 15 and Ding et al., *Nature Protocols*, 2013, 8, 43-51, each of which is herein incorporated by reference in its entirety for all purposes). In brief, HepG2 cells stably expressing HSD17B13 IsoA, IsoD, or the parental line were incubated overnight with 1 mM oleic acid. The following lipid loading, cells were scraped and resuspended in hypotonic lysis buffer (20 mM Tris, pH 7.5, 1 mM EDTA) supplemented with 1X HaltTM protease/phosphatase inhibitors (Thermo) and lysed by cavitation at 50 bar for 8 minutes. Lysates were centrifuged at 1000g/4°C for 10 minutes, and the post-nuclear supernatant (PNS) was mixed with sucrose to a final volume of 2 mL and concentration of 20% in ultracentrifuge tubes. Then 1.5 mL of 5% sucrose and another 1.5 mL of hypotonic lysis buffer was layered on top of the lysate. Tubes were centrifuged at 182,000g/4°C for 40 minutes, and the lipid droplet (LD) layers were transferred to new tubes. The remaining volume in the tube was aspirated, and the pelleted (total membrane, TM) was resuspended in 0.5 mL hypotonic lysis buffer. The PNS, LD, and TM fractions were mixed with 1x radioimmunoprecipitation (RIPA) buffer (EMD) + NuPAGETM LDS Sample Buffer (Thermo) and β-mercaptoethanol and sonicated for 3 hours at 37°C. The TM lysate was diluted 2.5-fold to normalize to the PNS. Lysates were run on 4-20% SDS-PAGE gels (Biorad), transferred using the Trans-Blot (Biorad) onto low fluorescence PVDF membranes, and blocked for 1 hour in Odyssey TBS Blocking Buffer. Membranes were incubated overnight with the following antibodies: α-HSD17B13 (Abgent, cat # AP5729a 1:500); LD marker: α-ADRP (Proteintech, 152-94-1-AP, 1:2500); LD marker: α-TIP47 (Proteintech, 10694 1:2000); lysosome marker: α-LAMP1 (Novus, NBP2-25183, 1:1000); cytosolic marker: α-GAPDH (Proteintech, 60004-1-Ig, 1:2000); endoplasmic reticulum marker: α-calreticulin (Abcam, ab92516, 1:1000); mitochondrial marker: α-COX IV (Abcam, ab33985, 1:500); cytoskeleton marker: α-actin (Sigma, A5441, 1:4000). The next day membranes were washed 4 times with Tris-buffered saline + 0.1% Tween, then incubated for 1 hour at room temperature with blocking buffer containing IRDye® α-rabbit (800CW) and α-mouse (680RD) secondary antibodies (Li-Cor) at 1:5,000 and 1:10,000 dilutions, respectively. Gels were washed again with TBST and imaged using the Odyssey.

Quantification of Intracellular Triglyceride Content

[00472] The triglyceride (TG) content from the stable cells was determined using a TG quantification kit (Abcam). In the assay, TG are converted to free fatty acids and glycerol. The glycerol is then oxidized to generate a product which is quantified (spectrophotometry at λ= 570 nm).

Substrate Screening of Steroid and Bioactive Lipid Libraries Against Purified Recombinant HSD17B13

[00473] Reactions were performed in a final volume of 40 µL of assay buffer (0.2 M Tris-HCl, pH 7.5) which contained 500 µM NAD^+, 5 µM bioactive lipid or 50 µM steroid (all in a final concentration of 5% DMSO), and 100 ng recombinant human HSD17B13. Reactions were incubated for 3 hours, at 23°C, after which an equal volume NADH-Glo Detection Reagent (Promega) was added. Following a 1 hour incubation at 23°C, the relative light units (RLUs) were measured on an Envision Plate Reader (Perkin Elmer). Raw RLU values were normalized as percent of control (50 µM estradiol) following subtraction of negative control (5% DMSO) using the following formula: Percent of control (POC) = 100 x (Sample (RLU) – Negative CTRLaverage) / (Positive CTRLaverage – Negative CTRLaverage).

In Vitro and Cellular Characterization of HSD17B13 Enzymatic Activity

[00474] Recombinant human HSD17B13 protein was purified from *E. coli* (Genscript) transformed with plasmid DNA harboring HSD17B13 transcript A or transcript D. The HSD17B13 variants contained a 10xHis tag at the C terminus and were purified from soluble fraction using a $Ni2^+$ affinity purification. Enzymatic activity was determined through measurement of NADH production using the NAD(P)H-Glo Detection System (Promega). Reactions were performed for 3 hours at 25°C in 0.2 M Tris-HCl, pH 7.5, 0.5mM NAD^+, 75 µM of substrate (Sigma) and 500 ng purified enzyme in a final volume of 100 µL. After incubation, 20 µL of the reaction was combined with 20 µL luciferase reagent (Promega), incubated at room temperature for 1 hour and read on an Envision Plate Reader (Perkin Elmer).

[00475] HEK293 cells overexpressing HSD17B13 transcript A, transcript D or green fluorescent protein (GFP, control) were used to investigate the activity of HSD17B13 against estradiol in a cell-based assay. Estradiol (1 µM) was fed to each cell type. After 48 hours, the

media was collected and the concentration of estradiol and its converted product estrone were identified and quantified by LC-MS.

Association of Exonic Variants with Aspartate and Alanine Aminotransferases

[00476] We tested 502,219 biallelic single genetic variants for association with serum ALT or AST levels in 46,544 individuals of European descent from the DiscovEHR study ("GHS discovery cohort"; basic demographics in **Table 6**). A total of 35 variants in 19 genes were found to be associated with ALT or AST at $P<1.0x10^{-7}$ (**Figures 1A** and **1B**, and **Table 7**). We performed replication studies in three cohorts of European-ancestry individuals: 1) bariatric surgery patients (n=2,644) from DiscovEHR ("GHS bariatric surgery cohort"); 2) 1,357 individuals from the Dallas Heart Study; and 3) 8,526 individuals from the Penn Medicine Biobank. In meta-analysis of the replication cohorts, thirteen variants in nine genes were significantly associated with serum levels of ALT or AST (Bonferroni significance threshold of $P<1.43x10^{-3}$ for 35 variants tested, **Table 8**). These included variants that were previously reported to be associated with elevated transaminase levels, such as PNPLA37, TM6SF211, SERPINA122, SAMM5023, and ERLIN124. SERPINA1 encodes alpha-1-antitrypsin, whose functional deficiency causes liver disease; the association with SAMM50 is mediated via linkage disequilibrium with variation in PNPLA3, and ERLIN1 has been implicated in liver fat deposition. We also identified variants that were not previously reported to be associated with liver disease. These included several variants in GPT and GOT1, the genes encoding ALT and AST, respectively, and SLC39A12, which encodes solute carrier family 39 member 12.

[00477] We also identified a reproducible association between a variant in HSD17B13, the gene encoding hydroxysteroid 17-beta dehydrogenase 13, an uncharacterized member of the 17-beta hydroxysteroid dehydrogenase family, and decreased levels of ALT (discovery $P=4.2x10^{-12}$, replication $P=1.7x10^{-4}$) and AST (discovery $P=6.2x10^{-10}$, replication $P=1.7x10^{-4}$, **Table 8**). The associated variant, rs72613567, is an insertion of an adenine adjacent to the donor splice site of exon six (TA allele), and had an allele frequency of 26.0% in the GHS discovery cohort. Previously, Chambers et al. identified a nearby locus at 4q22 (rs6834314) associated with ALT levels (Chambers et al., *Nat. Genet.*, 2011, 43, 1131-1138, doi:10.1038/ng.970, herein incorporated by reference in its entirety for all purposes); rs72613567 has not heretofore been reported to be associated with transaminase levels. HSD17B13 is 30 kb upstream of HSD17B11,

another member of the same gene family. We did not observe exome-wide significant associations between coding or splice variants in HSD17B11 and transaminase levels in the discovery cohort (**Figures 5A** and **5B**) or in the joint meta-analysis of the discovery cohort and three replication cohorts. Furthermore, linkage disequilibrium of rs72613567 with variants in HSD17B11 was modest across all ancestry groups (r^2<0.4 with all ascertained variants in HSD17B11 in all ancestry groups). Collectively, these findings suggest HSD17B13 as the gene in the genomic region that is most likely to be functionally related to transaminase levels.

[00478] Table 6. Demographics and clinical characteristics of sequenced European-ancestry individuals from the discovery and replication cohorts.

Characteristic	**Discovery Cohort (N = 46,544)**	**Bariatric Surgery Cohort (N = 2,644)**	**Dallas Heart Study (N = 1,357)**	**Penn Medicine Biobank (N = 8,526)**
Age (years) – median (IQR)	62.9 (49.6 - 73.8)	52.9 (44.1 - 61.2)	46.0 (38.0 - 54.0)	68.0 (60.0 - 76.0)
Female sex – number (%)	26,875 (57.7)	2,119 (80.1)	724 (53.4)	3,242 (38.0)
Body mass index – median (IQR)	29.9 (35.4 - 44.8)	47.4 (42.0 - 53.7)	28 (25-32)	30 (25-32)
Transaminase level (U/L) – median (IQR)				
Alanine aminotransferase (ALT)	22.0 (17.0 - 29.0)	23.0 (17.5 - 29.5)	20.0 (15.0 - 27.0)	22.0 (17.0 - 30.0)
Aspartate aminotransferase (AST)	23.0 (20.0 - 27.5)	23.0 (20.0 - 27.0)	21.0 (18.0 - 25.0)	24.0 (20.0 - 30.5)
Presence of liver disease (by ICD-9 code) – N (%)				
Alcoholic liver disease	197 (0.4)	7 (0.3)	-	-
Alcoholic cirrhosis	130 (0.3)	3 (0.1)	-	-
Nonalcoholic, non-viral liver disease	1,938 (4.2)	1,543 (58.4)	-	-
Nonalcoholic cirrhosis	382 (0.8)	24 (0.9)	-	-
Hepatocellular carcinoma	76 (0.2)	1 (0.04)	-	-
No liver disease	30,628 (65.8)	1 (0.04)	-	-

[00479] Table 7. Single nucleotide variants associated with serum transaminase levels at $P < 1.0x10^{-7}$ in the discovery cohort.

Trait	CHR	BP	REF	ALT	rsID	Gene	Annotation	AA Substitution	Beta (SE)
ALT	1	220970028	A	G	rs2642438	*MARC1*	missense	p.Thr165Ala	0.008 (0.001)
	4	88231392	T	TA	***rs72613567**	*HSD17B13*	splice donor		-0.009 (0.001)
	8	144997604	C	T	rs371119003	*PLEC*	missense	p.Ala2302Thr	-0.160 (0.026)
	8	145008502	G	A		*PLEC*	missense	p.Arg522Cys	-0.268 (0.032)
	8	145692918	G	A	rs35968570	*KIFC2*	missense	p.Glu174Lys	-0.033 (0.005)
	8	145730072	G	A	rs143408057	*GPT*	missense	p.Arg83His	-0.314 (0.036)
	8	145730161	C	T	rs201815297	*GPT*	missense	p.Ala87Val	-0.224 (0.014)
	8	145730221	G	A	rs112574791	*GPT*	missense	p.Arg107Lys	-0.033 (0.005)
	8	145731636	T	G	rs145155876	*GPT*	stop gained	p.Tyr326*	-0.235 (0.031)
	8	145732114	G	C	rs141505249	*GPT*	missense	p.Glu430Gln	-0.224 (0.013)
	8	145732151	G	A	rs143462595	*GPT*	missense	p.Arg442His	-0.077 (0.013)
	8	145732180	G	C	rs147998249	*GPT*	missense	p.Val452Leu	-0.225 (0.013)
	8	145732305	G	GC		*GPT*	frameshift	p.Glu475fs	-0.271 (0.031)
	8	145748532	A	G	rs567402720	*LRRC24*	missense	p.Leu290Ser	-0.185 (0.028)
	9	117122202	C	T	rs3748177	*AKNA*	synonymous	p.Glu755Glu	-0.007 (0.001)
	9	117124731	G	A	rs3748176	*AKNA*	missense	p.Pro624Leu	-0.007 (0.001)
	10	101595996	T	A	rs17222723	*ABCC2*	missense	p.Val1188Glu	-0.015 (0.003)
	10	101606861	G	T	rs1137968	*ABCC2*	synonymous	p.Val1430Val	-0.015 (0.003)
	10	101610533	C	T	rs8187707	*ABCC2*	synonymous	p.His1496His	-0.015 (0.003)
	10	101611294	G	A	rs8187710	*ABCC2*	missense	p.Cys1515Tyr	-0.015 (0.003)
	10	101912064	T	C	***rs2862954**	*ERLIN1*	missense	p.Ile291Val	-0.012 (0.001)
	10	101977883	C	T	rs2230804	*CHUK*	missense	p.Val268Ile	-0.009 (0.001)
	10	113917085	T	A	rs2254537	*GPAM*	synonymous	p.Pro681Pro	-0.008 (0.001)
	10	113940329	T	C	rs2792751	*GPAM*	missense	p.Ile43Val	-0.008 (0.001)
	14	94844947	C	T	***rs28929474**	*SERPINA1*	missense	p.Glu366Lys	0.042 (0.005)
	19	19379549	C	T	***rs58542926**	*TM6SF2*	missense	p.Glu167Lys	0.014 (0.002)
	22	44324727	C	G	***rs738409**	*PNPLA3*	missense	p.Ile148Met	0.023 (0.002)
	22	44324730	C	T	***rs738408**	*PNPLA3*	synonymous	p.Pro149Pro	0.023 (0.002)
	22	44342116	A	G	rs2294918	*PNPLA3*	missense	p.Lys434Glu	0.007 (0.001)
	22	44368122	A	G	***rs3761472**	*SAMM50*	missense	p.Asp110Gly	0.019 (0.002)
	22	44395451	T	C	***rs1007863**	*PARVB*	missense	p.Trp37Arg	0.011 (0.001)
AST	4	88231392	T	TA	***rs72613567**	*HSD17B13*	splice donor		-0.005 (0.001)
	10	18242311	A	G	rs10764176	*SLC39A12*	missense	p.Ser36Gly	-0.006 (0.001)
	10	101157378	CGTT	C		*GOT1*	inframe indel	p.Asn389del	-0.221 (0.024)
	10	101165533	G	C	rs374966349	*GOT1*	missense	p.Gln208Glu	0.271 (0.027)
	10	101912064	T	C	***rs2862954**	*ERLIN1*	missense	p.Ile291Val	-0.005 (0.001)
	11	22271870	A	T	rs7481951	*ANO5*	missense	p.Leu322Phe	0.004 (0.001)
	14	94844947	C	T	***rs28929474**	*SERPINA1*	missense	p.Glu366Lys	0.027 (0.003)
	19	19379549	C	T	***rs58542926**	*TM6SF2*	missense	p.Glu167Lys	0.008 (0.002)
	22	44324727	C	G	***rs738409**	*PNPLA3*	missense	p.Ile148Met	0.014 (0.001)
	22	44324730	C	T	***rs738408**	*PNPLA3*	synonymous	p.Pro149Pro	0.014 (0.001)
	22	44368122	A	G	***rs3761472**	*SAMM50*	missense	p.Asp110Gly	0.011 (0.001)
	22	44395451	T	C	***rs1007863**	*PARVB*	missense	p.Trp37Arg	0.006 (0.001)

[00480] Table 7 (cont.)

								N			Mean AST or ALT level (U/L)		
Trait	CHR	BP	REF	ALT	P	AAF	N	REF/ REF	REF/ ALT	ALT/ ALT	REF/ REF	REF/ ALT	ALT/ ALT
ALT	1	220970028	A	G	4.67E-08	0.7067	41,414	3,515	17,262	20,637	23.88	24.52	24.92
	4	88231392	T	TA	4.16E-12	0.2634	41,414	22,441	16,130	2,843	25.02	24.26	24.1
	8	144997604	C	T	1.30E-09	0.0005	41,413	41,373	40	0	24.67	18.1	NA
	8	145008502	G	A	3.26E-17	0.0003	41,414	41,387	27	0	24.67	13.8	NA
	8	145692918	G	A	1.40E-11	0.0139	41,414	40,271	1,133	10	24.67	12.07	NA
	8	145730072	G	A	3.28E-18	0.0003	41,414	41,393	21	0	24.67	12.07	NA
	8	145730161	C	T	6.28E-59	0.0018	41,414	41,270	144	0	24.7	14.68	NA
	8	145730221	G	A	4.25E-11	0.0136	41,414	40,293	1,111	10	24.71	23.09	18.35
	8	145731636	T	G	1.76E-14	0.0004	41,394	41,364	30	0	24.67	14.07	NA
	8	145732114	G	C	8.84E-64	0.0019	41,375	41,223	150	2	24.7	14.48	13.75
	8	145732151	G	A	1.18E-09	0.0021	41,406	41,232	174	0	24.68	20.87	NA
	8	145732180	G	C	8.19E-65	0.0019	41,413	41,254	159	0	24.7	14.74	NA
	8	145732305	G	GC	1.00E-18	0.0004	41,414	41,385	29	0	24.67	14.24	NA
	8	145748532	A	G	3.42E-11	0.0004	41,393	41,358	35	0	24.67	17.71	NA
	9	117122202	C	T	9.51E-09	0.5232	41,414	9,414	20,645	11,355	25.12	24.72	24.18
	9	117124731	G	A	4.31E-09	0.5230	41,412	9,427	20,634	11,351	25.12	24.73	24.17
	10	101595996	T	A	2.97E-08	0.0608	41,414	36,543	4,704	167	24.77	23.97	22.12
	10	101606861	G	T	2.71E-08	0.0608	41,414	36,543	4,704	167	24.77	23.97	22.04
	10	101610533	C	T	2.77E-08	0.0608	41,414	36,542	4,706	166	24.77	23.97	22.03
	10	101611294	G	A	2.15E-08	0.0611	41,414	36,519	4,726	169	24.77	23.97	21.99
	10	101912064	T	C	2.43E-21	0.4755	41,414	11,318	20,819	9,277	25.32	24.71	23.77
	10	101977883	C	T	1.93E-13	0.5072	41,414	10,048	20,733	10,633	25.18	24.75	24.01
	10	113917085	T	A	4.61E-10	0.7073	41,414	3,627	16,984	20,803	25	24.97	24.36
	10	113940329	T	C	2.54E-10	0.7097	41,412	3,567	16,910	20,935	25	24.98	24.35
	14	94844947	C	T	9.28E-21	0.0171	41,414	40,006	1,399	9	24.58	26.91	43.89
	19	19379549	C	T	4.76E-09	0.0759	41,413	35,388	5,780	245	24.52	25.46	26.84
	22	44324727	C	G	1.34E-50	0.2351	41,414	24,257	14,837	2,320	24.06	24.99	28.91
	22	44324730	C	T	1.11E-50	0.2349	41,414	24,273	14,824	2,317	24.06	24.98	28.92
	22	44342116	A	G	8.26E-08	0.5986	41,412	6,691	19,833	14,888	24.15	24.47	25.15
	22	44368122	A	G	8.85E-30	0.1682	41,413	28,626	11,618	1,169	24.23	25.36	28.45
	22	44395451	T	C	7.98E-16	0.3963	41,414	15,036	19,920	6,458	24.15	24.6	26.09
AST	4	88231392	T	TA	6.24E-10	0.2638	40,753	22,068	15,870	2,815	24.47	24.1	23.96
	10	18242311	A	G	1.09E-10	0.2881	40,753	20,645	16,738	3,370	24.47	24.15	23.85
	10	101157378	CGTT	C	1.96E-20	0.0002	40,753	40,733	20	0	24.29	14.7	NA
	10	101165533	G	C	2.43E-24	0.0002	40,753	40,736	17	0	24.28	44.5	NA
	10	101912064	T	C	4.82E-09	0.4754	40,753	11,138	20,486	9,129	24.59	24.26	23.99
	11	22271870	A	T	9.61E-08	0.5833	40,722	7,123	19,686	13,913	24.03	24.22	24.53
	14	94844947	C	T	2.44E-20	0.0172	40,753	39,361	1,384	8	24.24	25.76	34.5
	19	19379549	C	T	6.54E-08	0.0760	40,752	34,811	5,698	243	24.21	24.74	25.43
	22	44324727	C	G	8.31E-46	0.2343	40,753	23,889	14,622	2,242	23.96	24.48	26.62
	22	44324730	C	T	8.93E-46	0.2341	40,753	23,905	14,609	2,239	23.96	24.47	26.63
	22	44368122	A	G	1.22E-22	0.1680	40,752	28,170	11,450	1,132	24.07	24.64	26.24
	22	44395451	T	C	1.31E-13	0.3961	40,753	14,761	19,678	6,314	24.02	24.23	25.1

* Indicates variants having exome-wide significant associations with both ALT and AST.

Abbreviations: AAF, alternate allele frequency; Alt, alternate allele; ALT, alanine aminotransferase; AST, aspartate aminotransferase; Ref, reference allele; SE, standard error.

[00481] Table 8. Replication and joint meta-analysis of 35 exome-wide significant single nucleotide variants from the discovery cohort in three separate European-ancestry cohorts.

									GHS Discovery Cohort		
Trait	Chr	BP	Ref	Alt	RSID	Gene	Ann	AA Substitution	Beta (SE)	P	N
ALT	1	220970028	A	G	rs2642438	*MARC1*	mis	p.Thr165Ala	0.008 (0.001)	4.67E-08	41,414
	4	88231392	T	TA	rs72613567	*HSD17B13*	spl		-0.009 (0.001)	4.16E-12	41,414
	8	144997604	C	T	rs371119003	*PLEC*	mis	p.Ala2302Thr	-0.160 (0.026)	1.30E-09	41,413
	8	145008502	G	A		*PLEC*	mis	p.Arg522Cys	-0.268 (0.032)	3.26E-17	41,414
	8	145692918	G	A	rs35968570	*KIFC2*	mis	p.Glu174Lys	-0.033 (0.005)	1.40E-11	41,414
	8	145730072	G	A	rs143408057	*GPT*	mis	p.Arg83His	-0.314 (0.036)	3.28E-18	41,414
	8	145730161	C	T	rs201815297	*GPT*	mis	p.Ala87Val	-0.224 (0.014)	6.28E-59	41,414
	8	145730221	G	A	rs112574791	*GPT*	mis	p.Arg107Lys	-0.033 (0.005)	4.25E-11	41,414
	8	145731636	T	G	rs145155876	*GPT*	stop	p.Tyr326*	-0.235 (0.031)	1.76E-14	41,394
	8	145732114	G	C	rs141505249	*GPT*	mis	p.Glu430Gln	-0.224 (0.013)	8.84E-64	41,375
	8	145732151	G	A	rs143462595	*GPT*	mis	p.Arg442His	-0.077 (0.013)	1.18E-09	41,406
	8	145732180	G	C	rs147998249	*GPT*	mis	p.Val452Leu	-0.225 (0.013)	8.19E-65	41,413
	8	145732305	G	GC		*GPT*	fs	p.Glu475fs	-0.271 (0.031)	1.00E-18	41,414
	8	145748532	A	G	rs567402720	*LRRC24*	mis	p.Leu290Ser	-0.185 (0.028)	3.42E-11	41,393
	9	117122202	C	T	rs3748177	*AKNA*	syn	p.Glu755Glu	-0.007 (0.001)	9.51E-09	41,414
	9	117124731	G	A	rs3748176	*AKNA*	mis	p.Pro624Leu	-0.007 (0.001)	4.31E-09	41,412
	10	101595996	T	A	rs17222723	*ABCC2*	mis	p.Val1188Glu	-0.015 (0.003)	2.97E-08	41,414
	10	101606861	G	T	rs1137968	*ABCC2*	syn	p.Val1430Val	-0.015 (0.003)	2.71E-08	41,414
	10	101610533	C	T	rs8187707	*ABCC2*	syn	p.His1496His	-0.015 (0.003)	2.77E-08	41,414
	10	101611294	G	A	rs8187710	*ABCC2*	mis	p.Cys1515Tyr	-0.015 (0.003)	2.15E-08	41,414
	10	101912064	T	C	rs2862954	*ERLIN1*	mis	p.Ile291Val	-0.012 (0.001)	2.43E-21	40,834
	10	101977883	C	T	rs2230804	*CHUK*	mis	p.Val268Ile	-0.009 (0.001)	1.93E-13	41,414
	10	113917085	T	A	rs2254537	*GPAM*	syn	p.Pro681Pro	-0.008 (0.001)	4.61E-10	41,414
	10	113940329	T	C	rs2792751	*GPAM*	mis	p.Ile43Val	-0.008 (0.001)	2.54E-10	41,412

									GHS Discovery Cohort		
Trait	Chr	BP	Ref	Alt	RSID	Gene	Ann	AA Substitution	Beta (SE)	P	N
	14	94844947	C	T	rs28929474	*SERPINA1*	mis	p.Glu366Lys	0.042 (0.005)	9.28E-21	41,414
	19	19379549	C	T	rs58542926	*TM6SF2*	mis	p.Glu167Lys	0.014 (0.002)	4.76E-09	41,413
	22	44324727	C	G	rs738409	*PNPLA3*	mis	p.Ile148Met	0.023 (0.002)	1.34E-50	41,414
	22	44324730	C	T	rs738408	*PNPLA3*	syn	p.Pro149Pro	0.023 (0.002)	1.11E-50	41,414
	22	44342116	A	G	rs2294918	*PNPLA3*	mis	p.Lys434Glu	0.007 (0.001)	8.26E-08	41,412
	22	44368122	A	G	rs3761472	*SAMM50*	mis	p.Asp110Gly	0.019 (0.002)	8.85E-30	41,413
	22	44395451	T	C	rs1007863	*PARVB*	mis	p.Trp37Arg	0.011 (0.001)	7.98E-16	41,414
AST	4	88231392	T	TA	rs72613567	*HSD17B13*	spl		-0.005 (0.001)	6.24E-10	40,753
	10	18242311	A	G	rs10764176	*SLC39A12*	mis	p.Ser36Gly	-0.006 (0.001)	1.09E-10	40,753
	10	101157378	CGTT	C		*GOT1*	inf	p.Asn389del	-0.221 (0.024)	1.96E-20	40,753
	10	101165533	G	C	rs374966349	*GOT1*	mis	p.Gln208Glu	0.271 (0.027)	2.43E-24	40,753
	10	101912064	T	C	rs2862954	*ERLIN1*	mis	p.Ile291Val	-0.005 (0.001)	4.82E-09	40,753
	11	22271870	A	T	rs7481951	*ANO5*	mis	p.Leu322Phe	0.004 (0.001)	9.61E-08	40,722
	14	94844947	C	T	rs28929474	*SERPINA1*	mis	p.Glu366Lys	0.027 (0.003)	2.44E-20	40,753
	19	19379549	C	T	rs58542926	*TM6SF2*	mis	p.Glu167Lys	0.008 (0.002)	6.54E-08	40,192
	22	44324727	C	G	rs738409	*PNPLA3*	mis	p.Ile148Met	0.014 (0.001)	8.31E-46	40,753
	22	44324730	C	T	rs738408	*PNPLA3*	syn	p.Pro149Pro	0.014 (0.001)	8.93E-46	40,753
	22	44368122	A	G	rs3761472	*SAMM50*	mis	p.Asp110Gly	0.011 (0.001)	1.22E-22	40,752
	22	44395451	T	C	rs1007863	*PARVB*	Mis	p.Trp37Arg	0.006 (0.001)	1.31E-13	40,753

[00482] Table 8 (cont.)

			GHS Bariatric Surgery Cohort			Dallas Heart Study			U. Penn		
Trait	Chr	BP	Beta (SE)	P	N	Beta (SE)	P	N	Beta (SE)	P	N
ALT	1	220970028	0.005 (0.005)	3.10E-01	2475	0.011 (0.008)	1.76E-01	1357	0.007 (0.004)	1.02E-01	6158
	4	88231392	-0.010 (0.005)	5.57E-02	2475	-0.016 (0.008)	6.60E-02	1357	-0.013 (0.004)	1.33E-03	6158
	8	144997604	-0.492 (0.165)	2.84E-03	2475	NA (NA)	NA	NA	-0.051 (0.072)	4.79E-01	6158
	8	145008502	-0.161 (0.165)	3.29E-01	2475	NA (NA)	NA	NA	-0.247 (0.143)	8.48E-02	6158
	8	145692918	-0.009 (0.020)	6.48E-01	2475	0.032 (0.036)	3.76E-01	1356	-0.053 (0.018)	3.72E-03	6158
	8	145730072	-0.189 (0.165)	2.50E-01	2475	NA (NA)	NA	NA	-0.298(0.101)	3.26E-03	6158
	8	145730161	-0.341 (0.074)	3.64E-06	2475	NA (NA)	NA	NA	-0.143 (0.054)	8.50E-03	6158

			GHS Bariatric Surgery Cohort			Dallas Heart Study			U. Penn		
Trait	Chr	BP	Beta (SE)	P	N	Beta (SE)	P	N	Beta (SE)	P	N
	8	145730221	-0.009 (0.020)	6.45E-01	2475	0.028 (0.036)	4.37E-01	1357	-0.060 (0.018)	5.60E-04	6158
	8	145731636	-0.314 (0.165)	5.71E-02	2475	-0.317 (0.140)	2.35E-02	1356	-0.148 (0.143)	3.04E-01	6157
	8	145732114	-0.273 (0.048)	9.83E-09	2474	-0.240 (0.075)	1.36E-03	1357	-0.197 (0.041)	1.31E-06	6157
	8	145732151	-0.115 (0.058)	4.82E-02	2475	-0.106 (0.099)	2.86E-01	1356	-0.049 (0.041)	2.27E-01	6157
	8	145732180	-0.273 (0.050)	4.26E-08	2475	-0.191 (0.070)	6.58E-03	1357	-0.197 (0.041)	1.31E-06	6158
	8	145732305	-0.161 (0.165)	3.29E-01	2475	NA (NA)	NA	NA	-0.509 (0.203)	1.21E-02	6158
	8	145748532	-0.161 (0.165)	3.29E-01	2475	NA (NA)	NA	NA	-0.307 (0.143)	3.21E-02	6158
	9	117122202	-0.004 (0.005)	4.09E-01	2475	0.004 (0.008)	6.18E-01	1357	-0.007 (0.004)	5.29E-02	6158
	9	117124731	-0.004 (0.005)	3.90E-01	2475	0.003 (0.008)	7.33E-01	1356	-0.007 (0.004)	4.24E-02	6158
	10	101595996	-0.002 (0.010)	8.01E-01	2475	-0.007 (0.017)	6.88E-01	1357	-0.017 (0.007)	1.55E-02	6158
	10	101606861	-0.003 (0.010)	7.74E-01	2475	-0.008 (0.017)	6.28E-01	1357	-0.017 (0.007)	1.70E-02	6158
	10	101610533	-0.003 (0.010)	7.93E-01	2475	-0.008 (0.017)	6.28E-01	1357	-0.017 (0.007)	1.76E-02	6158
	10	101611294	-0.001 (0.010)	9.11E-01	2475	-0.010 (0.017)	5.40E-01	1357	-0.016 (0.007)	2.77E-02	6158
	10	101912064	-0.010 (0.005)	2.91E-02	2475	-0.006 (0.007)	4.02E-01	1356	-0.009 (0.004)	2.06E-02	6158
	10	101977883	-0.006 (0.005)	2.05E-01	2475	0.0001 (0.008)	9.94E-01	1357	-0.011 (0.004)	3.91E-03	6158
	10	113917085	-0.003 (0.005)	5.80E-01	2475	-0.013 (0.008)	1.15E-01	1357	-0.008 (0.004)	5.12E-02	6158
	10	113940329	-0.003 (0.005)	5.61E-01	2475	-0.013 (0.008)	1.33E-01	1357	-0.008 (0.004)	4.77E-02	6158
	14	94844947	0.035 (0.020)	7.97E-02	2475	0.034 (0.032)	2.92E-01	1357	0.054 (0.013)	1.63E-05	6158
	19	19379549	0.040 (0.010)	2.40E-05	2475	0.024 (0.014)	9.50E-02	1357	0.013 (0.008)	7.51E-02	6158
	22	44324727	0.019 (0.006)	5.54E-04	2475	0.006 (0.009)	5.43E-01	1357	0.016 (0.004)	2.05E-04	6158
	22	44324730	0.019 (0.006)	5.51E-04	2475	0.006 (0.009)	5.43E-01	1357	0.016 (0.004)	2.14E-04	6158
	22	44342116	0.001 (0.005)	7.77E-01	2475	0.005 (0.008)	5.18E-01	1357	0.005 (0.004)	2.16E-01	6158
	22	44368122	0.009 (0.006)	1.66E-01	2475	-0.001 (0.01)	9.37E-01	1357	0.018 (0.005)	4.02E-04	6158
	22	44395451	0.003 (0.005)	5.22E-01	2475	0.008 (0.008)	3.13E-01	1357	0.009 (0.004)	2.50E-02	6158
AST	4	88231392	-0.010 (0.003)	3.12E-03	2469	-0.012 (0.006)	5.32E-02	1357	-0.007 (0.004)	5.56E-02	6166
	10	18242311	-0.010 (0.003)	2.91E-03	2469	-0.003 (0.006)	5.80E-01	1357	-0.009 (0.004)	1.03E-02	6166
	10	101157378	-0.205 (0.062)	8.57E-04	2469	NA (NA)	NA	NA	-0.243 (0.088)	5.97E-03	6165
	10	101165533	NA (NA)	NA	NA	NA (NA)	NA	NA	0.339 (0.079)	1.85E-05	6166
	10	101912064	-0.004 (0.003)	1.54E-01	2469	-0.007 (0.006)	2.21E-01	1357	-0.004 (0.003)	1.94E-01	6166
	11	22271870	-0.001 (0.003)	7.85E-01	2466	0.006 (0.006)	2.85E-01	1357	-0.002 (0.003)	5.46E-01	6165
	14	94844947	0.023 (0.013)	7.79E-02	2469	0.044 (0.024)	6.98E-02	1357	0.055 (0.011)	4.01E-07	6166
	19	19379549	0.023 (0.006)	1.99E-04	2469	0.010 (0.011)	3.42E-01	1356	0.004 (0.007)	5.94E-01	6166
	22	44324727	0.014 (0.004)	1.27E-04	2469	0.004 (0.007)	5.44E-01	1357	0.015 (0.004)	4.87E-05	6166
	22	44324730	0.014 (0.004)	1.32E-04	2469	0.004 (0.007)	5.44E-01	1357	0.015 (0.004)	4.96E-05	6166
	22	44368122	0.008 (0.004)	6.03E-02	2469	-0.001 (0.008)	9.45E-01	1357	0.016 (0.004)	2.64E-04	6166
	22	44395451	0.003 (0.003)	4.12E-01	2469	0.006 (0.006)	2.95E-01	1357	0.009 (0.003)	6.17E-03	6166

[00483] Table 8 (cont.)

			**Replication Meta-Analysis (N=3)		***Joint Meta-Analysis (N = 4)	
Trait	Chr	BP	Beta (SE)	P	Beta (SE)	P
ALT	1	220970028	0.007 (0.003)	2.31E-02	0.008 (0.001)	3.38E-09
	4	88231392	-0.013 (0.003)	***3.85E-05**	-0.010 (0.001)	1.17E-15
	8	144997604	-0.121 (0.066)	6.56E-02	-0.155 (0.025)	2.68E-10
	8	145008502	-0.210 (0.108)	5.23E-02	-0.264 (0.031)	5.54E-18
	8	145692918	-0.025 (0.013)	4.69E-02	-0.032 (0.005)	2.25E-12

			**Replication Meta-Analysis (N=3)		***Joint Meta-Analysis (N = 4)	
Trait	**Chr**	**BP**	**Beta (SE)**	**P**	**Beta (SE)**	**P**
	8	145730072	-0.268 (0.086)	1.88E-03	-0.308 (0.033)	2.79E-20
	8	145730161	-0.213 (0.044)	***1.14E-06**	-0.223 (0.013)	4.49E-64
	8	145730221	-0.031 (0.013)	1.36E-02	-0.033 (0.005)	1.92E-12
	8	145731636	-0.256 (0.086)	2.79E-03	-0.237 (0.029)	1.94E-16
	8	145732114	-0.231 (0.029)	***7.24E-16**	-0.225 (0.012)	6.06E-78
	8	145732151	-0.074 (0.032)	1.88E-02	-0.076 (0.012)	7.03E-11
	8	145732180	-0.221 (0.029)	***1.41E-14**	-0.224 (0.012)	1.04E-77
	8	145732305	-0.299 (0.128)	1.93E-02	-0.273 (0.030)	6.44E-20
	8	145748532	-0.244 (0.108)	2.40E-02	-0.189 (0.027)	2.93E-12
	9	117122202	-0.005 (0.003)	8.42E-02	-0.007 (0.001)	3.08E-09
	9	117124731	-0.005 (0.003)	6.15E-02	-0.007 (0.001)	1.00E-09
	10	101595996	-0.012 (0.005)	3.43E-02	-0.014 (0.002)	3.44E-09
	10	101606861	-0.012 (0.005)	3.25E-02	-0.014 (0.002)	2.99E-09
	10	101610533	-0.012 (0.005)	3.43E-02	-0.014 (0.002)	3.23E-09
	10	101611294	-0.011 (0.005)	5.21E-02	-0.014 (0.002)	4.09E-09
	10	101912064	-0.009 (0.003)	***1.14E-03**	-0.011 (0.001)	1.76E-23
	10	101977883	-0.008 (0.003)	4.33E-03	-0.009 (0.001)	3.59E-15
	10	113917085	-0.007 (0.003)	2.07E-02	-0.008 (0.001)	3.28E-11
	10	113940329	-0.007 (0.003)	2.00E-02	-0.008 (0.001)	1.77E-11
	14	94844947	0.047 (0.010)	***2.82E-06**	0.043 (0.004)	1.59E-25
	19	19379549	0.024 (0.006)	***1.37E-05**	0.016 (0.002)	1.15E-12
	22	44324727	0.016 (0.003)	***7.45E-07**	0.021 (0.001)	3.55E-55
	22	44324730	0.016 (0.003)	***7.73E-07**	0.021 (0.001)	3.10E-55
	22	44342116	0.004 (0.003)	1.91E-01	0.006 (0.001)	6.24E-08

			**Replication Meta-Analysis (N=3)		***Joint Meta-Analysis (N = 4)	
Trait	**Chr**	**BP**	**Beta (SE)**	**P**	**Beta (SE)**	**P**
	22	44368122	0.012 (0.004)	***7.69E-04**	0.018 (0.002)	1.08E-31
	22	44395451	0.007 (0.003)	1.78E-02	0.010 (0.001)	1.16E-16
AST	4	88231392	-0.009 (0.002)	***8.38E-05**	-0.006 (0.001)	6.82E-13
	10	18242311	-0.009 (0.002)	***1.16E-04**	-0.006 (0.001)	1.10E-13
	10	101157378	-0.218 (0.051)	***1.66E-05**	-0.220 (0.022)	1.68E-24
	10	101165533	0.339 (0.079)	***1.85E-05**	0.278 (0.025)	3.25E-28
	10	101912064	-0.005 (0.002)	2.51E-02	-0.005 (0.001)	3.68E-10
	11	22271870	0.000 (0.002)	8.43E-01	0.004 (0.001)	1.13E-06
	14	94844947	0.042 (0.008)	***9.54E-08**	0.029 (0.003)	6.71E-26
	19	19379549	0.014 (0.004)	***1.20E-03**	0.009 (0.002)	5.92E-10
	22	44324727	0.013 (0.002)	***5.51E-08**	0.014 (0.001)	3.14E-52
	22	44324730	0.013 (0.002)	***5.81E-08**	0.014 (0.001)	3.55E-52
	22	44368122	0.010 (0.003)	***3.40E-04**	0.011 (0.001)	1.91E-25
	22	44395451	0.006 (0.002)	7.34E-03	0.006 (0.001)	3.62E-15

* Indicates P-values meeting the Bonferroni significance threshold of $P < 1.43 \times 10^{-3}$.

** Replication meta-analysis includes the three replication cohorts: GHS Bariatric Surgery Cohort, Dallas Heart Study, and Penn Medicine Biobank.

*** Joint meta-analysis includes the discovery cohort and the three replication cohorts: GHS Discovery Cohort, GHS Bariatric Surgery Cohort, Dallas Heart Study, and Penn Medicine Biobank.

Abbreviations: AAF, alternate allele frequency; Alt, alternate allele; ALT, alanine aminotransferase; AST, aspartate aminotransferase; Ref, reference allele; SE, standard error; ann, annotation; mis, missense; syn, synonymous; spl, splice donor; stop, stop gained; fs, frameshift; inf, inframe indel.

Association of Exonic Variants with Clinical Diagnoses of Chronic Liver Disease

[00484] Next, we analyzed the relationship between the thirteen transaminase-associated variants in the nine genes found in the discovery and replication cohorts and chronic liver disease, including alcoholic and nonalcoholic (non-viral) liver disease, as well as the most advanced forms of chronic liver disease: alcoholic cirrhosis, nonalcoholic cirrhosis, and

hepatocellular carcinoma (HCC). Using a Bonferroni significance threshold of $P<1.92x10^{-3}$ for the thirteen variants tested, we found significant associations between six variants in five genes (HSD17B13, SERPINA1, TM6SF2, PNPLA3, and SAMM50) and chronic liver disease phenotypes (**Table 9**). The SERPINA1, TM6SF2, PNPLA3, and SAMM50 associations confirm previously reported associations. In the discovery cohort, HSD17B13 rs72613567:TA was associated with lower odds of all EHR-derived categories of both alcoholic and nonalcoholic liver disease in an allele dosage-dependent manner (**Figure 2A**): all categories of alcoholic liver disease, heterozygous odds ratio (OR_{het}) (95% confidence interval) 0.58 (0.42-0.80), homozygous OR (OR_{hom}) 0.47 (0.23-0.97), allelic OR ($OR_{allelic}$) 0.62 (0.48-0.81), $P=1.8x10^{-4}$; all categories of nonalcoholic liver disease, OR_{het} 0.83 (0.75-0.92), OR_{hom} 0.70 (0.57-0.87), $OR_{allelic}$ 0.84 (0.78-0.91), $P=1.3x10^{-5}$. HSD17B13 rs72613567:TA was also associated with lower odds of alcoholic and nonalcoholic cirrhosis, with 42% and 73% lower odds of alcoholic cirrhosis for heterozygotes and homozygotes, respectively, (OR_{het} 0.58 (0.39-0.86), OR_{hom} 0.27 (0.09-0.85), $OR_{allelic}$ 0.56 (0.41-0.78), $P=3.4x10^{-4}$) and 26% and 49% lower odds of nonalcoholic cirrhosis for heterozygotes and homozygotes, respectively (OR_{het} 0.74 (0.60-0.93), OR_{hom} 0.51 (0.31-0.85), $OR_{allelic}$ 0.74 (0.62-0.88), $P=4.5x10^{-4}$). HSD17B13 rs72613567:TA was also nominally associated with lower odds of HCC.

[00485] We sought to confirm and extend these findings in the multi-ethnic Dallas Liver Study (DLS) and the Dallas Pediatric Liver Study (DPLS, **Table 10**). In the DLS, the TA allele was associated with lower odds of any liver disease in an allele-dosage dependent manner (OR_{het} 0.74 (0.57-0.97), OR_{hom} 0.41 (0.21-0.83), $OR_{allelic}$ 0.70 (0.5-0.88), $P=1.8x10^{-3}$, **Figure 8**). Similar effects were observed across EHR-derived liver disease subtypes, including protective associations with advanced, cirrhotic forms of alcoholic ($OR_{allelic}$ 0.72 (0.53-0.99), $P=4.4x10^{-2}$) and nonalcoholic ($OR_{allelic}$ 0.65 (0.40-1.07), $P=9.0x10^{-2}$) liver disease. In subset analyses of individuals grouped by self-reported ethnicity, the association with liver disease was significant in Hispanic Americans (n=326 cases and 722 controls, $OR_{allelic}$ 0.51 (0.35-0.74), $P=4.0x10^{-4}$); similar numerical trends, which did not achieve statistical significance, were also noted in the African American (n=33 cases and 2,291 controls, $OR_{allelic}$ 0.74 (0.25-2.47), P=0.67) and European American (n=158 cases and 1,266 controls, $OR_{allelic}$ 0.87 (0.65-1.15), P=0.32) subsets of the DLS. In the DPLS, a separate study of Hispanic American pediatric liver disease patients and obese controls, the TA allele was also associated with lower odds of liver disease ($OR_{allelic}$

0.61 (0.37-0.99), P=4.6x10^{-2}). Thus, HSD17B13 rs72613567:TA was associated with reduced odds of multiple forms of chronic liver disease, including cirrhosis, in adults and children in three independent populations.

[00486] Table 9. Association of twelve exome-wide significant and replicating single nucleotide variants with liver disease phenotypes in the discovery cohort.

CHR:BP:Ref:Alt	Gene	rsID	Alcoholic liver disease		Alcoholic cirrhosis	
			OR (95% CI)	P-value	OR (95% CI)	P-value
4:88231392:T:TA	*HSD17B13*	rs72613567	0.62 (0.48-0.81)	***1.82E-04**	0.56 (0.41-0.78)	***3.35E-04**
8:145730161:C:T	*GPT*	rs201815297	3.83 (1.05-13.94)	8.88E-02	6.33 (1.71-23.43)	2.88E-02
8:145732114:G:C	*GPT*	rs141505249	0.77 (0.06-10.73)	8.43E-01	1.13 (0.08-15.39)	9.30E-01
8:145732180:G:C	*GPT*	rs147998249	0.73 (0.05-11.76)	8.17E-01	1.07 (0.07-17.16)	9.60E-01
10:18242311:A:G	*SLC39A12*	rs10764176	0.85 (0.68-1.07)	1.64E-01	0.92 (0.70-1.22)	5.80E-01
10:101157378:CGTT:C	*GOT1*		4.60 (0.25-86.41)	3.93E-01	7.11 (0.38-133.19)	3.00E-01
10:101165533:G:C	*GOT1*	rs374966349	2.20 (0.13-37.68)	6.24E-01	3.47 (0.20 - 59.04)	4.70E-01
14:94844947:C:T	*SERPINA1*	rs28929474	2.49 (1.49-4.17)	2.30E-03	3.35 (1.93-5.83)	***3.01E-04**
19:19379549:C:T	*TM6SF2*	rs58542926	1.47 (1.06-2.04)	2.76E-02	1.35 (0.89-2.04)	1.80E-01
22:44324727:C:G	*PNPLA3*	rs738409	1.76 (1.43-2.18)	***4.98E-07**	2.07 (1.60-2.67)	***1.08E-07**
22:44324730:C:T	*PNPLA3*	rs738408	1.77 (1.43-2.18)	***4.70E-07**	2.07 (1.61-2.67)	***1.03E-07**
22:44368122:A:G	*SAMM50*	rs3761472	1.90 (1.52-2.38)	***1.36E-07**	2.28 (1.75-2.98)	***1.83E-08**

* Indicates P-values meeting the Bonferroni significance threshold of P < 2.08x10^{-3}.

[00487] Table 9 (cont.)

CHR:BP:Ref:Alt	Gene	rsID	Nonalcoholic liver disease		Nonalcoholic cirrhosis		Hepatocellular carcinoma	
			OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value
4:88231392:T:TA	*HSD17B13*	rs72613567	0.84 (0.78-0.91)	***1.31E-05**	0.74 (0.62-0.88)	***4.48E-04**	0.67 (0.45-1.00)	4.66E-02
8:145730161:C:T	*GPT*	rs201815297	0.23 (0.04-1.14)	1.86E-02	1.25 (0.24-6.38)	7.98E-01	3.66 (0.70-19.01)	2.01E-01
8:145732114:G:C	*GPT*	rs141505249	1.02 (0.49-2.11)	9.70E-01	0.36 (0.02-5.37)	3.82E-01	1.84 (0.15-23.25)	6.88E-01
8:145732180:G:C	*GPT*	rs147998249	1.03 (0.49-2.17)	9.30E-01	0.34 (0.02-5.59)	3.67E-01	1.74 (0.11-27.05)	7.21E-01
10:18242311:A:G	*SLC39A12*	rs10764176	0.92 (0.86 (0.99)	3.43E-02	1.03 (0.88-1.21)	7.15E-01	1.29 (0.93-1.79)	1.37E-01
10:101157378:CGTT:C	*GOT1*		2.37 (0.61-9.27)	2.50E-01	8.27 (1.44-47.49)	5.92E-02	9.81 (0.52-183.54)	2.43E-01
10:101165533:G:C	*GOT1*	rs374966349	1.63 (0.53-4.96)	4.20E-01	1.17 (0.07-20.09)	9.13E-01	5.37 (0.32-91.12)	3.55E-01
14:94844947:C:T	*SERPINA1*	rs28929474	1.50 (1.21-1.87)	***5.29E-04**	2.99 (2.11-4.24)	***9.08E-08**	1.86 (0.74-4.67)	2.40E-01
19:19379549:C:T	*TM6SF2*	rs58542926	1.36 (1.21-1.52)	***2.42E-07**	1.64 (1.31-2.05)	***6.04E-05**	1.93 (1.22-3.04)	1.08E-02
22:44324727:C:G	*PNPLA3*	rs738409	1.65 (1.54-1.78)	***1.31E-41**	2.05 (1.76-2.38)	***1.70E-19**	2.20 (1.60-3.02)	***5.59E-06**
22:44324730:C:T	*PNPLA3*	rs738408	1.65 (1.54-1.78)	***1.42E-41**	2.05 (1.77-2.38)	***1.45E-19**	2.20 (1.60-3.03)	***5.41E-06**
22:44368122:A:G	*SAMM50*	rs3761472	1.52 (1.41-1.65)	***7.33E-24**	1.86 (1.58-2.19)	***1.81E-12**	1.66 (1.16-2.39)	1.05E-02

[00488] Table 10. Demographics and clinical characteristics of genotyped multi-ethnic cases and controls from the Dallas Liver and Pediatric Liver Studies.

Characteristic	Dallas Liver Study Cases (N = 517)	Dallas Liver Study Controls (N = 4,279)	Dallas Pediatric Liver Study Cases (N = 203)	Dallas Pediatric Liver Study Controls (N = 244)
Age (years) – median (IQR)	55 (48 - 60)	44 (36 - 53)	12 (10 - 15)	12 (11 - 14)
Female sex – number (%)	277 (54)	2,494 (58)	65 (32)	126 (52)
Body mass index – median (IQR)	30 (27 - 35)	30 (26 - 35)	30 (27 - 34)	31 (28 - 35)
Self-reported ethnicity				
African American	33 (6)	2,291 (54)	-	-
European American	158 (31)	1,266 (30)	-	-
Hispanic American	326 (63)	722 (17)	203 (100)	244 (100)
Presence of liver disease (by ICD-9 code) – N (%)				
Alcoholic liver disease	223 (43)	-	-	-
Alcoholic cirrhosis	215 (42)	-	-	-
Nonalcoholic, non-viral liver disease	212 (20)	-	-	-
Nonalcoholic cirrhosis	100 (19)	-	-	-
Hepatocellular carcinoma	44 (9)	-	-	-
No liver disease	-	4,279 (100)	-	-244 (100)

Association of HSD17B13 rs72613567:TA with Liver Pathology

[00489] NAFLD describes a disease spectrum ranging from liver fat accumulation without evidence of significant inflammation (simple steatosis), to more clinically impactful NASH. To confirm the association between the HSD17B13 rs72613567:TA and EHR-derived liver disease diagnoses codes, and to further understand its association with histopathological progression of steatosis to NASH, we performed tests of association in the GHS bariatric surgery cohort. In this cohort of 2,391 of the whole exome sequenced individuals assessed by liver biopsy at the time of bariatric surgery, a total of 555 (23%) individuals had no evidence of steatosis, steatohepatitis, or fibrosis ("normal"), 830 (35%) had simple steatosis, and 1006 (42%) had NASH. When comparing prevalence of normal liver, simple steatosis, and NASH by genotype, it was observed that the prevalence of normal liver did not appear to differ by genotype (23%, 24%, and 23% for T/T, T/TA, and TA/TA carriers, respectively, P = 0.5 by Chi-squared test for trend in proportions), but that the prevalence of NASH decreased (45%, 40%, and 31% for T/T, T/TA, and TA/TA carriers, respectively, $P = 1.6x10^{-4}$) and that of simple steatosis increased (33%, 35%, and 47% for T/T, T/TA, and TA/TA carriers, respectively, $P = 1.1x10^{-3}$) with each TA allele (**Figure 9**). Among individuals with steatosis, the TA allele was associated with

statistically significantly lower odds of both NASH and fibrosis, as compared to simple steatosis ($OR_{allelic}$ 0.77 (0.66-0.90), P=6.5x10^{-4} for NASH; $OR_{allelic}$ 0.74 (0.62-0.88), P=4.15x10^{-4} for fibrosis; **Figure 2B**), in an allele dosage-dependent manner. Altogether, these data suggest a role for HSD17B13 in mediating NAFLD progression from simple steatosis to more advanced stages of NASH and fibrosis.

Association of HSD17B13 rs72613567:TA with Clinical Quantitative Traits and Diagnoses

[00490] To more comprehensively examine the clinical consequences of the HSD17B13 splice variant, we performed a phenome-wide study of associations of HSD17B13 rs72613567:TA with 405 quantitative EHR-derived anthropometric, vital sign, laboratory, electrocardiographic, echocardiographic, and bone densitometry measurements, and also with 3,168 EHR-derived clinical diagnoses. Using Bonferroni significance thresholds of 1.23x10^{-4} and 1.58x10^{-5} for associations with quantitative clinical measurements and clinical diagnoses, respectively, we identified statistically significant associations of the HSD17B13 rs72613567:TA allele with higher platelet counts, in addition to the associations with hepatic transaminases (**Table 11**). There were no statistically significant associations with clinical diagnoses other than chronic liver disease (OR (95% CI) =0.88 (0.84-0.93); P = 9.14x10^{-6}; AAF = 0.263; N Cases total = 4031, T/T = 2331, T/TA = 1449, TA/TA = 251; N Controls Total = 35701, T/T = 19238, T/TA = 13984, TA/TA = 2479).

[00491] Table 11. Phenome-Wide Study of Associations of HSD17B13 rs72613567:TA with Quantitative Clinical Measurements.

Phenotype	Effect	SE	P	AAF	N			
					Total	T/T	T/TA	TA/TA
Alanine Aminotransferase median:Adjusted(Residual Log)	-0.009	0.001	***1.74E-12***	0.264	44038	23868	17115	3055
Aspartate Aminotransferase median:Adjusted(Residual Log)	-0.006	0.001	***2.75E-11***	0.264	43370	23493	16851	3026
Alanine Aminotransferase max:Adjusted(Residual Log)	-0.013	0.002	***1.39E-09***	0.264	43905	23797	17065	3043
Aspartate Aminotransferase max:Adjusted(Residual Log)	-0.010	0.002	***8.73E-09***	0.264	42733	23145	16609	2979
Platelets median:Adjusted(Residual Log)	0.004	0.001	***1.44E-08***	0.264	46182	25020	17944	3218
Alanine Aminotransferase min:Adjusted(Residual Log)	-0.008	0.002	***2.47E-07***	0.264	44029	23864	17111	3054
Platelets min:Adjusted(Residual)	1.919	0.443	***1.47E-05***	0.264	46181	25020	17943	3218
Platelets max:Adjusted(Residual Log)	0.004	0.001	***3.03E-05***	0.264	46165	25014	17936	3215
Aspartate Aminotransferase min:Adjusted(Residual Log)	-0.004	0.001	***5.00E-05***	0.264	43327	23471	16831	3025
Bolding and italicization indicates P-values meeting the Bonferroni significance threshold of P < 1.23x10^{-4}.								
Abbreviations: AAF, alternate allele frequency; SE, standard error.								

Effect of HSD17B13 rs72613567:TA on HSD17B13 mRNA and HSD17B13 Protein Expression

[00492] We next examined the effect of the HSD17B13 rs72613567:TA allele on expression of known and novel transcripts of the gene. We used RNA sequencing to assess HSD17B13 mRNA expression in histologically normal liver samples from 22 T/T homozygous, 30 T/TA heterozygous, and 17 TA/TA homozygous carriers of the HSD17B13 rs72613567 splice variant. In addition to the two known HSD17B13 transcripts, A and B, two novel transcripts were identified: transcript C, which lacked exon 6, and transcript D which contained an insertion of a guanine nucleotide at the 3' end of exon 6, which would be predicted to result in premature truncation of the protein. Four additional transcripts (E-H) were expressed at very low levels (**Figures 3A-3D and 6A-6D**). The transcripts were validated by RT-PCR and Sanger sequencing. The D transcript was also validated using long read cDNA sequencing. Protein sequence alignment of all identified HSD17B13 isoforms (A-H) is shown in **Figures 7A** and **7B**. The expression levels of these transcripts varied according to HSD17B13 rs72613567 genotype; levels of transcripts A and B decreased, while those of transcripts C and D increased in an allele dosage-dependent manner with each TA allele (**Figures 3A-3D**). Transcript A, which encodes the full-length 300 amino acid protein, was the predominant transcript in T/T homozygotes, while transcript D, which encodes the prematurely truncated protein, was the predominant transcript in TA/TA homozygotes. In human liver biopsy tissue, the truncated isoform D protein was minimally present in heterozygotes and TA/TA homozygotes, and isoform A protein abundance was reduced in an allele dosage-dependent manner (**Figures 10B** and **10C**). Heterologous expression of isoforms A and D in HEK 293 cells indicated reduced abundance of isoform D relative to mRNA expression, suggesting instability of the D isoform when compared to isoform A (**Figures 11A-11C**). These data are consistent with HSD17B13 rs72613567 altering mRNA splicing, resulting in the synthesis of a truncated form of the protein with substantially reduced expression in human liver.

Expression of HSD17B13 in Human Liver Cells

[00493] HSD17B13 is expressed primarily in the liver (Liu et al., *Acta Biochim. Pol.* 2007, 54, 213-218, herein incorporated by reference in its entirety for all purposes), where it localizes to lipid droplets (Su et al., *Proc. Natl. Acad. Sci. USA*, 2014, 111, 11437-11442,

doi:10.1073/pnas.1410741111, herein incorporated by reference in its entirety for all purposes), consistent with a role in the pathogenesis of fatty liver disease. We evaluated the expression of HSD17B13 and its localization in an immortalized human liver cell line stably transduced with lentivirus expressing HSD17B13 transcript A or D. HSD17B13 isoform A was mainly detected on membranes surrounding BODIPY-labeled lipid droplets (data not shown). Similar subcellular localization was observed for HSD17B13 isoform D at the lipid droplet surface (data not shown and **Figure 12**). No differences in intracellular triglyceride content were observed with oleic acid treatment of cell lines overexpressing GFP control or HSD17B13 isoforms A or D (**Figures 13A-13D**).

Effect of rs72613567:TA on HSD17B13 Activity in vitro and in Cellular Models

[00494] To understand the functional consequences of premature truncation of the HSD17B13 protein due to rs72613567:TA, we evaluated the enzymatic activity of isoforms A and D *in vitro* using recombinant protein and nicotinamide adenosine dinucleotide as cofactor. We tested 265 unique putative substrates, and identified steroid substrates and bioactive lipids (e.g. leukotriene B4) as enzymatic substrates of HS17B13. We focused subsequent characterization of HSD17B13 enzymatic activity on enzymatic conversion of estradiol (V_{max} and K_m values in **Figure 14**), which resulted in oxidation of a hydroxyl to a ketone group. HSD17B13 isoform D showed greatly reduced activity towards estradiol *in vitro* (**Figure 10D**) and in cell-based enzymatic conversion assays (**Figure 10E**) when compared to HSD17B13 isoform A.

[00495] By linking large-scale exome sequencing to EHR-derived clinical phenotypes, we identified a novel association between a splice variant in HSD17B13 and decreased serum transaminase levels, as well as reduced risk of nonalcoholic and alcoholic forms of liver disease. These associations were observed consistently in four independent cohorts, and across several different liver disease categories, including advanced cirrhotic forms of liver disease and HCC. The HSD17B13 rs72613567:TA allele was not associated with simple steatosis, but was associated with reduced risk of NASH and fibrosis, suggesting that this variant allele protects from progression to more clinically advanced stages of chronic liver disease. In a phenome-wide association study, HSD17B13 rs72613567:TA was not significantly associated with clinical diagnoses or measurements other than chronic liver disease and associated clinical measurements (hepatic transaminases and platelet counts), suggesting that the clinical effects of the variant

allele may be specific to chronic liver disease.

[00496] Other hydroxysteroid 17-beta dehydrogenase family members are involved in sex steroid and fatty acid metabolism (Moeller, *Mol. Cell. Endocrinol.*, 2009, 301, 7-19, doi:10.1016/j.mce.2008.10.040, herein incorporated by reference in its entirety for all purposes), but little is known about the function of HSD17B13. HSD17B13 overexpression was shown previously to increase lipogenesis in mouse liver, and to increase the number and size of lipid droplets in cultured hepatocytes (Su et al., *Proc. Natl. Acad. Sci. USA*, 2014, 111, 11437-11442, doi:10.1073/pnas.1410741111, herein incorporated by reference in its entirety for all purposes). Two previous studies also showed that hepatic expression of HSD17B13 protein is increased in patients with fatty liver (Su et al., *Proc. Natl. Acad. Sci. USA*, 2014, 111, 11437-11442, doi:10.1073/pnas.1410741111 and Kampf et al., *FASEB J.*, 2014, 28, 2901-2914, doi:10.1096/fj.14-250555, each of which is herein incorporated by reference in its entirety for all purposes). Our data suggest that both HSD17B13 isoforms are expressed on the lipid droplet membrane, but do not appear to modulate intracellular neutral fat content, a finding that mirrors the lack of an association between the HSD17B13 rs72613567:TA and simple steatosis in humans. Although the physiological substrates of HSD17B13 are not known, enzymatic studies demonstrate that the HSD17B13 isoform encoded by the HSD17B13 rs72613567:TA allele is catalytically defective against estradiol. While at this time it is not clear if any of the substrates tested are critical for liver disease, it is intriguing that HSD17B13 has enzymatic activity against several bioactive lipid species (e.g. leukotriene B4) that have previously been implicated in lipid-mediated inflammation (Li et al., *Nature Medicine*, 2015, 21, 239-247, doi:10.1038/nm.3800, herein incorporated by reference in its entirety for all purposes).

[00497] This HSD17B13 variant may provide an avenue to new therapeutic strategies targeting chronic liver disease, similar to genetic variants that have guided the way to new therapeutics in other domains. Our data indicate that HSD17B13 modulates progression of liver disease from steatosis to later stages of NASH, fibrosis, and cirrhosis, which are associated with significant morbidity and mortality, and for which there are currently no effective treatments.

Example 4. Modification of Mouse *Hsd17b13* Locus Using CRISPR/Cas9 *Ex Vivo* and *In Vivo*.

[00498] As a proof of concept for targeting *Hsd17b13* using the CRISPR/Cas9 system, mouse *Hsd17b13* guide RNAs targeting either the exon 1 region or the exon 6/7 region of the mouse

Hsd17b13 locus were tested. The guide RNA target sequences are provided in **Table 12**. The guide RNA DNA-targeting segments corresponding to SEQ ID NOS: 259-268 are set forth in SEQ ID NOS: 1643-1652, respectively, which are identical to SEQ ID NOS: 259-268 except with uracils instead of thymines. The NCBI Gene ID for mouse *Hsd17b13* (hydroxysteroid (17-beta) dehydrogenase 13 is 243168 (SEQ ID NO: 269). The mouse genomic locus is on chromosome 5, NC_000071.6 (103955442..103977388, complement).

[00499] Table 12. Guide RNA Target Sequences for Mouse

Region of *Hsd17b13*	#	Guide RNA Target Sequence	SEQ ID NO					
			gRNA Target Seq	crRNA	sgRNA v1	sgRNA v2	sgRNA v3	sgRNA v4
Exon 1	1	GGCAGACCGTTCTCATCACG	259	490	720	950	1180	1410
	2	CTTTACCAGTGACTCCAGGT	260	491	721	951	1181	1411
	3	GTCACAGATTTCCTTCTCCG	261	492	722	952	1182	1412
	4	AGATGATGACGCCCACCAGA	262	493	723	953	1183	1413
	5	GGAGAAGGAAATCTGTGACC	263	494	724	954	1184	1414
Exons 6/7	1	TGCGAGGAACTTACTTTTCC	264	495	725	955	1185	1415
	2	AGAGAAATATTGATATAGGA	265	496	726	956	1186	1416
	3	TATCAATATTTCTCTGATCC	266	497	727	957	1187	1417
	4	ATCGCTTTTAAGGCACGCTC	267	498	728	958	1188	1418
	5	TATACGACTGATCGCTTTTA	268	499	729	959	1189	1419

[00500] The guide RNAs were first tested *ex vivo* in primary mouse hepatocytes isolated from hybrid wild type mice (75% C57BL/6NTac 25% 129S6/SvEvTac). Livers from mice were perfused with 50 mL liver perfusion medium containing 1X PenStrep, followed by 50 mL liver digestion medium (HBSS, 100 mM $CaCl_2$, 500 mM HEPES, collagenase). Once livers appeared digested, they were placed into wash medium containing 1X PenStrep and L-glutamine. The livers were torn to release the hepatocytes from the liver through gentle shaking. Once cells were released, they were put through a 70 μm mesh filter and spun at 50 g for 4 minutes at 4°C. The pellets were washed 2X with wash buffer. The pellets were then re-suspended in 20 mL of 38-40% Percoll and spun at 200g x 10 min at 4°C. The pellet was washed 2X and re-suspended in plating medium (Williams E Media, 1X Penstrep, 1X L-glutamine, 5% FBS). Cells were plated at 300,000 cells per well in 24-well collagen-coated tissue culture plates. After the cells were allowed to attach for 6-18 hrs, the plating medium was replaced with medium without FBS. Reagents used are shown in **Table 13.**

[00501] Table 13. Reagents for Isolation of Primary Hepatocytes.

Material	Catalog Number
Liver Perfusion Media	Gibco [17701-038]
HBSS (1x)	Gibco [14175-079]
Hepatocyte Wash Media	Gibco [17704-024]
Williams E media	Gibco [A12176-01]
Penstrep (100x)	Gibco [15140163]
L-glutamine (200mM)	Gibco [25030081]
FBS supplement	Gibco [A13450]
HEPES	Gibco [15630080]
Collagen	Gibco [A1048301]
Acetic acid	Sigma [A6283]
Liberase TM	Roche [TM05401119001]
Primary Hepatocyte Thawing and Plating Supplements	Gibco [CM3000]
Primary Hepatocyte Maintenance Supplements	Gibco [CM4000]
Percoll	GE [17-0891-01]

[00502] Ribonucleoprotein complexes (RNPs) containing Cas9 and a mouse *Hsd17b13* gRNA were added to the freshly isolated primary mouse hepatocytes. For *ex vivo* experiments in primary mouse hepatocytes, modular guide RNAs having a separate crRNA and tracrRNA were used. The crRNA SEQ ID NOs are set forth in Table 12, and the tracrRNA sequence is set forth in SEQ ID NO: 1422. Each Cas9/gRNA RNP complex was transfected at a final concentration of 2 nM using CRISPRMAX™. After 48 hrs, DNA lysates were prepared from the cells, and next-generation sequencing was performed for each guide RNA tested to determine insertion/deletion (indel) frequency over the predicted cut sites.

[00503] **Figure 15** shows editing levels (% reads with indels) in the mouse *Hsd17b13* gene with each of the guide RNAs in primary mouse hepatocytes, including each of the five guide RNAs targeting the exon 1 region and each of the five guide RNAs targeting the exon 6/7 region. Editing efficiency refers to the total number of insertions or deletions observed over the total number of sequences read in the PCR reaction from a pool of lysed cells as determined by next generation sequencing. Nearly all of the guide RNAs showed an editing efficiency of at least 20%.

[00504] Next, the five mouse *Hsd17b13* guide RNAs were tested *in vivo* in mice with a genomically integrated Cas9 gene (Cas9-ready mice). For *in vivo* experiments in mice, chimeric single guide RNAs were used. The DNA-targeting sequence for each guide RNA is equivalent to the guide RNA target sequence set forth in Table 12, with uracils replacing the thymines. Each single guide RNA included the DNA-target sequence upstream (5') of the gRNA scaffold

set forth in SEQ ID NO: 1420. The sgRNA SEQ ID NOs are set forth in Table 12 (column for sgRNA v1). Other sgRNA variations using different guide RNA scaffold are included in Table 12 but were not tested. For each guide RNA, three Cas9-ready male mice were dosed per group. Guide RNAs were introduced via adeno-associated virus (AAV8) carrying an sgRNA expression cassette by tail vein injection (1E11 per mouse in 100 μL PBS). Wild type mice that do not express any Cas9 were dosed with all five guide RNAs as a negative control. Three weeks post-injection, the animals were euthanized, and blood serum was harvested along with liver and other tissues. The tissues were processed into DNA lysates that were then analyzed by NGS sequencing.

[00505] As shown in **Figure 16**, NGS sequencing showed significant editing in liver for all five guide RNAs (percent editing of at least 20% for each). Editing efficiency refers to the total number of insertions or deletions observed over the total number of sequences read in the PCR reaction from a pool of lysed cells. Minimal or no statistically significant levels of gene editing were observed in other tissues (data not shown).

[00506] Serum chemistry analysis for the liver enzymes ALT, AST, triglycerides, total cholesterol, HDL, LDL, non-esterified fatty acids (NEFA), and albumin showed little difference between various treatment groups (data not shown).

[00507] *Hsd17b13* expression was evaluated by assessing equal mass amounts of RNA from liver by RT-qPCR. The genomic DNA was degraded so that it would not count towards the qPCR reaction. The RNA was reverse transcribed and then an assay specific to Cas9 was used to detect Cas9 transcripts. Each individual *Hsd17b13* guide RNA showed at least 50% ablation of *Hsd17b13* mRNA expression. *See* **Figure 17A**. In contrast, no significant decreases were observed in expression of a non-target HSD family member. *See* **Figure 17B**.

CLAIMS

1. A composition comprising a Cas protein or a nucleic acid encoding the Cas protein and a guide RNA or a DNA encoding the guide RNA for use in a method of modifying an *HSD17B13* gene in a cell,

wherein the guide RNA comprises a CRISPR RNA (crRNA) portion and a trans-activating CRISPR RNA (tracrRNA) portion and targets a guide RNA target sequence within the *HSD17B13* gene, wherein the guide RNA target sequence includes or is proximate to a position corresponding to position 12666 of SEQ ID NO: 2 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 2, and

wherein the method comprises introducing the Cas protein or the nucleic acid encoding the Cas protein and the guide RNA or the DNA encoding the guide RNA into the cell, wherein the guide RNA forms a complex with the Cas protein and targets the guide RNA target sequence, and wherein the Cas protein cleaves the guide RNA target sequence to generate a targeted genetic modification in the *HSD17B13* gene.

2. The composition for use according to claim 1, wherein the *HSD17B13* gene does not have a thymine inserted between nucleotides corresponding to positions 12665 and 12666 of SEQ ID NO: 1 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 1.

3. The composition for use according to claim 1 or 2, wherein:

(a) the guide RNA target sequence comprises any one of SEQ ID NOS: 226-239 and 264-268; and/or

(b) the guide RNA comprises a DNA-targeting segment comprising any one of SEQ ID NOS: 1629-1642 and 1648-1652; and/or

(c) the guide RNA comprises any one of SEQ ID NOS: 706-719; 936-949; 1166-1179, 1396-1409, 725-729, 955-959, 1185-1189, and 1415-1419.

4. The composition for use according to any one of claims 1-3, wherein the guide RNA target sequence is within a region corresponding to exon 6 and/or intron 6 of SEQ ID NO: 2 or is within a region corresponding to intron 6 and/or exon 7 of SEQ ID NO: 2 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 2.

5. The composition for use according to any one of claims 1-4, wherein the guide RNA target sequence is within about 1000, 500, 400, 300, 200, 100, 50, 45, 40, 35, 30, 25, 20, 15, 10, or 5 nucleotides of the position corresponding to position 12666 of SEQ ID NO: 2 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 2.

6. The composition for use according to any one of claims 1-5, wherein the guide RNA target sequence includes the position corresponding to position 12666 of SEQ ID NO: 2 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 2.

7. The composition for use according to any one of claims 1-6, further comprising one or more additional guide RNAs or one or more DNAs encoding the one or more additional guide RNAs, wherein the one or more additional guide RNAs target one or more additional guide RNA target sequences within the *HSD17B13* gene, and

wherein the method further comprises introducing the one or more additional guide RNAs or the one or more DNAs encoding the one or more additional guide RNAs into the cell, wherein the one or more additional guide RNAs form one or more complexes with the Cas protein and target the one or more additional guide RNA target sequences, and

wherein the Cas protein cleaves the one or more additional guide RNA target sequences.

8. The composition for use according to any one of claims 1-7, wherein the method results in disruption of a donor splice site in intron 6 of the *HSD17B13* gene.

9. The composition for use according to any one of claims 1-8, wherein the method results in loss of function of the *HSD17B13* gene.

10. The composition for use according to any one of claims 1-9, wherein the targeted genetic modification is generated by repair of the cleaved guide RNA target sequence by non-homologous end-joining.

11. The composition for use according to any one of claims 1-10, further comprising an exogenous donor sequence,

wherein the method further comprises introducing into the cell the exogenous donor sequence, wherein the exogenous donor sequence recombines with a target genomic locus in the *HSD17B13* gene to generate the targeted genetic modification.

12. The composition for use according to claim 11, wherein repair of the *HSD17B13* gene by the exogenous donor sequence occurs via non-homologous-end-joining-mediated insertion.

13. The composition for use according to claim 11, wherein repair of the *HSD17B13* gene by the exogenous donor sequence occurs via homology-directed repair.

14. The composition for use according to claim 13, wherein the exogenous donor sequence comprises a 5' homology arm that hybridizes to a target sequence 5' of the position corresponding to position 12666 of SEQ ID NO: 2 and a 3' homology arm that hybridizes to a target sequence 3' of the position corresponding to position 12666 of SEQ ID NO: 2, wherein the exogenous donor sequence recombines with the *HSD17B13* gene.

15. The composition for use according to claim 14, wherein the exogenous donor sequence further comprises a nucleic acid insert flanked by the 5' homology arm and the 3' homology arm.

16. The composition for use according to claim 15, wherein the nucleic acid insert comprises a thymine, and wherein upon recombination of the exogenous donor sequence with the *HSD17B13* gene, the thymine is inserted between nucleotides corresponding to positions 12665 and 12666 of SEQ ID NO: 1 when the *HSD17B13* gene is optimally aligned with SEQ ID NO: 1.

17. The composition for use according to any one of claims 11-16, wherein the exogenous donor sequence is between about 50 nucleotides to about 1 kb in length.

18. The composition for use according to claim 17, wherein the exogenous donor sequence is between about 80 nucleotides to about 200 nucleotides in length.

19. The composition for use according to any one of claims 11-18, wherein the exogenous donor sequence is a single-stranded oligodeoxynucleotide.

20. The composition for use according to any one of claims 1-19, wherein the Cas protein is a Cas9 protein.

21. The composition for use according to any one of claims 1-20, wherein the composition comprises the nucleic acid encoding the Cas protein.

22. The composition for use according to claim 21, wherein the nucleic acid encoding the Cas protein comprises DNA.

23. The composition for use according to claim 21, wherein the nucleic acid encoding the Cas protein comprises RNA.

24. The composition for use according to any one of claims 1-23, wherein the composition comprises the guide RNA in the form of RNA.

25. The composition for use according to any one of claims 1-23, wherein the composition comprises the DNA encoding the guide RNA.

26. The composition for use according to any one of claims 1-25, wherein the Cas protein or the nucleic acid encoding the Cas protein and/or the guide RNA or the DNA encoding the guide RNA are in a lipid-nanoparticle.

27. The composition for use according to any one of claims 1-25, wherein the nucleic acid encoding the Cas protein and/or the DNA encoding the guide RNA are in an adeno-associated virus vector.

28. The composition for use according to any one of claims 1-27, wherein the guide RNA is a single-molecule guide RNA in which the crRNA portion is linked to the tracrRNA portion.

29. The composition for use according to claim 28, wherein the guide RNA comprises the sequence set forth in SEQ ID NO: 1420, 256, 257, or 258.

30. The composition for use according to any one of claims 1-27, wherein the crRNA portion and the tracrRNA portion are separate RNA molecules.

31. The composition for use according to claim 30, wherein the crRNA portion comprises the sequence set forth in SEQ ID NO: 1421 and/or the tracrRNA portion comprises the sequence set forth in SEQ ID NO: 1422.

32. The composition for use according to any one of claims 1-31, wherein the guide RNA comprises a modification providing for modified or regulated stability.

33. The composition for use according to any one of claims 1-32, wherein the cell is *ex vivo* or *in vivo.*

34. The composition for use according to any one of claims 1-33, wherein the cell is a mouse cell, a rat cell or a human cell.

35. The composition for use according to any one of claims 1-34, wherein the cell is a human liver cell, a mouse liver cell, a mouse pluripotent cell, or a rat pluripotent cell.

36. The composition for use according to claim 35, wherein the cell is a human liver cell.

37. The composition for use according to claim 36, wherein the cell is *in vivo*.

38. The composition for use according to claim 37, wherein the Cas protein or a nucleic acid encoding the Cas protein and the guide RNA or a DNA encoding the guide RNA are introduced into a liver *in vivo*.

39. The composition for use according to any one of claims 1-38, wherein the cell is a human cell, and wherein:

(a) the guide RNA target sequence comprises any one of SEQ ID NOS: 226-239; and/or

(b) the DNA-targeting segment comprises any one of SEQ ID NOS: 1629-1642; and/or

(c) the guide RNA comprises any one of SEQ ID NOS: 706-719; 936-949; 1166-1179, and 1396-1409.

40. The composition for use according to any one of claims 1-35, wherein the cell is a mouse cell, and wherein:

(a) the guide RNA target sequence comprises any one of SEQ ID NOS: 264-268; and/or

(b) the DNA-targeting segment comprises any one of SEQ ID NOS: 1648-1652; and/or

(c) the guide RNA comprises any one of SEQ ID NOS: 725-729, 955-959, 1185-1189, and 1415-1419.