A RODENT MODEL OF MOOD DISORDERS

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefits of priority from U.S. Provisional Applications 62/793,523 filed January 17, 2019 and 62/899,849 filed September 13, 2019, the contents of both of which are incorporated herein by reference.

FIELD OF THE DISCLOSURE

[0002] This disclosure relates to an animal model of human disease. More specifically, this disclosure relates to a rodent model of mood disorders such as unipolar depression and an anxiety disorder. Disclosed herein are genetically modified rodent animals that carry a humanized G protein-coupled receptor 156 (*GRP156*) gene that encodes a mutant human GPR156 protein comprising Asp at an amino acid position corresponding to position 533 in a full length wild type human GPR156 protein.

INCORPORATION BY REFERENCE OF SEQUENCE LISTING

[0003] The sequence listing in the ASCII text file, named as 36506PCT_10269WO01_SequenceListing.txt of 1299 KB, created on January 8, 2020, and submitted to the United States Patent and Trademark Office via EFS-Web, is incorporated herein by reference.

BACKGROUND ART

[0004] Various references, including patents, patent applications, accession numbers, technical articles, and scholarly articles are cited throughout the specification. Each reference is incorporated by reference herein, in its entirety and for all purposes.

[0005] G protein-coupled receptors (GPCRs) are a large superfamily of cell surface receptors that are characterized by 7 helical transmembrane domains, together with N-terminal

extracellular and C-terminal intracellular domains (Cherezov V, et al., Science 318: 1258-1265 (2007)). GPCRs are expressed in a variety of cell types, and participate in transducing extracellular signals across the cellular membrane and into the cellular interior (Kobilka R, Biochim. Biophys. Acta 1768: 794-807 (2007)). In 2012, the Nobel Prize in Chemistry was awarded to two scientists who identified how GPCRs function (Clark R, Proc. Natl. Acad. Sci. USA 110: 5274-5275 (2013)).

[0006] *GPR156* (G protein-coupled receptor 156) is a human gene which encodes a GPCR belonging to metabotropic glutamate receptor subfamily (Calver A, et al., Brain Res. Mol. Brain Res. 110: 305-307 (2003)). Other names that have been used to reference GPR156 in the literature are GABABL (GABAB-like or $GABA_{BL}$) and PGR28 (Vassilatis D, Proc. Natl. Acad. Sci. USA 100: 4903-4908 (2003)). In mice, GPR156 is referred to as Gpr156 or Gababl.

SUMMARY OF THE DISCLOSURE

[0007] Disclosed herein are genetically modified rodents suitable for use as an animal model of mood disorders such as unipolar depression and anxiety disorders. More specifically, disclosed herein are genetically modified rodent animals (e.g., mouse or rat) that carry a humanized *Gpr156* gene at an endogenous rodent *Gpr156* locus, wherein the humanized *Gpr156* gene encodes a wild type human GPR156 protein or a mutant human GPR156 protein comprising Asp at an amino acid position corresponding to position 533 in a full length wild type human GPR156 protein (also referred to herein as an "E533D variation"). Also disclosed herein are methods and targeting vectors for making a genetically modified rodent, methods of breeding, and use of a genetically modified rodent as an animal model of mood disorders for screening and testing therapeutic agents.

[0008] In one embodiment is a genetically modified rodent whose genome comprises a humanized *Gpr156* gene at an endogenous rodent *Gpr156* locus, wherein the humanized *Gpr156* gene comprises a rodent *Gpr156* nucleic acid portion and a human *GPR156* nucleic acid portion, and wherein expression of the humanized *Gpr156* gene is under control of the rodent *Gpr156* promoter at the endogenous rodent *Gpr156* locus. The rodent *Gpr156* nucleic

acid portion and the human *GPR156* nucleic acid portion each can be a genomic DNA or a cDNA.

[0008a] Disclosed herein is a genetically modified rodent, whose genome comprises a humanized G protein-coupled receptor 156 (GPR156) gene at an endogenous rodent Gpr156 locus, wherein the humanized Gpr156 gene comprises a rodent Gpr156 nucleic acid portion and a human GPR156 nucleic acid portion, wherein the humanized Gpr156 gene encodes a wild type human GPR156 protein or a mutant human GPR156 protein comprising Asp at an amino acid position corresponding to position 533 in a full length wild type human GPR156 protein (E533D variation), wherein expression of the humanized Gpr156 gene is under control of the rodent Gpr156 promoter at the endogenous rodent Gpr156 locus, and wherein the rodent is a mouse or a rat.

[0009] In some embodiments, a humanized *Gpr156* gene in a rodent encodes a mutant human GPR156 protein comprising an E533D variation.

[0010] In some embodiments, the human *GPR156* nucleic acid portion in a humanized *Gpr156* gene comprises a coding sequence of a human *GPR156* gene, wherein the coding sequence comprises the ATG start codon in the first coding exon through the stop codon in the last coding exon of the human *GPR156* gene, and wherein the human *GPR156* gene comprises nucleotides that encode an E533D variation. In some embodiments, the human *GPR156* nucleic acid portion in a humanized *Gpr156* gene further comprises a 5' non-coding exonic sequence or a 3' non-coding exonic sequence of a human *GPR156* gene, or a combination thereof. A 5' non-coding exonic sequence of a human *GPR156* gene can include, for example, any 5' non-coding exon, and/or the 5' non-coding portion of the first coding exon of a human *GPR156* gene. A 3' non-coding exonic sequence of a human *GPR156* gene can include, for example, the 3' UTR of a human *GPR156* gene. In specific embodiments, the human *GPR156* nucleic acid portion in a humanized *Gpr156* gene comprises the ATG start codon in the first coding exon through the last exon (i.e., including the 3' UTR) of a human *GPR156* gene, with the human *GPR156* gene comprising nucleotides that encode an E533D variation.

[0011] In some embodiments, the rodent *Gpr156* nucleic acid portion in a humanized *Gpr156* gene comprises a 5' non-coding exonic sequence or a 3' non-coding exonic sequence of a rodent *Gpr156* gene, or a combination thereof. In embodiments where a rodent *Gpr156* gene is a mouse *Gpr156* gene, a 5' non-coding exonic sequence can include, for example, exon 1, the 5' non-coding portion of exon 2 (the first coding exon), or a combination thereof, of the mouse *Gpr156* gene; and a 3' non-coding exonic sequence can include, for example, the 3' UTR of exon 10 (the 9th and last coding exon) of the mouse *Gpr156* gene. In embodiments where a rodent *Gpr156* gene is a rat *Gpr156* gene, a 5' non-coding exonic sequence can include, for example, the 5' non-coding portion of exon 1 (also being the first coding exon) of the rat *Gpr156* gene; and a 3' non-coding exonic sequence can include, for example, the 3' UTR of exon 9 (also being the last coding exon) of the rat *Gpr156* gene.

[0012] In specific embodiments, a humanized *Gpr156* gene comprises a '5 non-coding exonic sequence of a rodent *Gpr156* gene, and the ATG start codon in the first coding exon through the last exon of a human *GPR156* gene, with the human *GPR156* gene comprising nucleotides that encode an E533D variation; and in certain embodiments, such humanized *Gpr156* gene further comprises a 3' non-coding exonic sequence of a rodent *Gpr156* gene, placed downstream of the last exon of the human *GPR156* gene.

[0013] In some embodiments, the rodent *Gpr156* gene that provides the rodent *Gpr156* nucleic acid portion in a humanized *Gpr156* gene is the endogenous rodent *Gpr156* gene.

[0014] In some embodiments, a humanized *Gpr156* gene in a rodent is formed from a replacement of a rodent genomic fragment of an endogenous rodent *Gpr156* gene at an endogenous rodent *Gpr156* locus with a human *GPR156* nucleic acid encoding a mutant human GPR156 protein comprising an E533D variation. The human *GPR156* nucleic acid can be a genomic DNA or a cDNA.

[0015] In some embodiments, the human *GPR156* nucleic acid encoding the mutant human GPR156 protein comprises a coding sequence (e.g., a genomic or cDNA sequence) comprising the ATG start codon in the first coding exon through the stop codon in the last coding exon of a human *GPR156* gene, wherein the human *GPR156* gene comprises

nucleotides that encode an E533D variation. In some embodiments, the human *GPR156* nucleic acid portion in a humanized *Gpr156* gene further comprises a 5' non-coding exonic sequence or a 3' non-coding exonic sequence of a human *GPR156* gene, or a combination thereof. In some embodiments, the human *GPR156* nucleic acid comprises the 5' non-coding sequence of the first coding exon or the 3' UTR of a human *GPR156* gene, or a combination thereof. In specific embodiments, the human *GPR156* nucleic acid comprises the ATG start codon in the first coding exon through the last exon (i.e., through the 3' UTR) of a human *GPR156* gene, with the human *GPR156* gene comprising nucleotides that encode an E533D variation.

[0016] In some embodiments, the rodent genomic fragment being replaced comprises the ATG start codon (in the first coding exon) through the stop codon (in the last coding exon) of the endogenous rodent *Gpr156* gene. In some embodiments, the rodent genomic fragment being replaced further comprises a 5' non-coding exonic sequence or a 3' non-coding exonic sequence of a rodent *Gpr156* gene, or a combination thereof.

[0017] In some embodiments, a humanized *Gpr156* gene encodes a mutant human GPR156 protein comprising an E533D variation, wherein the mutant protein is a full length GPR156 protein of 814 amino acids. In some embodiments, the mutant human GPR156 protein comprises the amino acid sequence set forth in SEQ ID NO: 5. In some embodiments, the mutant human GPR156 protein comprises the amino acid sequence set forth in SEQ ID NO: 6. In some embodiments, the mutant human GPR156 protein comprises an amino acid sequence that is substantially identical to SEQ ID NO: 5 or SEQ ID NO: 6.

[0018] In some embodiments, a humanized *Gpr156* gene encodes a mutant human GPR156 protein that is a short isoform GPR156 protein of 810 amino acids and comprises an E533D variation. In some embodiments, the mutant human GPR156 protein comprises the amino acid sequence set forth in SEQ ID NO: 7. In some embodiments, the mutant human GPR156 protein comprises the amino acid sequence set forth in SEQ ID NO: 8. In some embodiments, the mutant human GPR156 protein comprises an amino acid sequence that is substantially identical to SEQ ID NO: 7 or SEQ ID NO: 8.

[0019] In further embodiments, a humanized *Gpr156* gene in a rodent encodes a wild type human GPR156 protein.

[0020] In some embodiments, the human *GPR156* nucleic acid portion in a humanized *Gpr156* gene comprises a coding sequence (e.g., a genomic or cDNA sequence) of a human *GPR156* gene, wherein the coding sequence comprises the ATG start codon in the first coding exon through the stop codon in the last coding exon of the human *GPR156* gene. In some embodiments, the human *GPR156* nucleic acid portion in a humanized *Gpr156* gene further comprises a 5' non-coding exonic sequence or a 3' non-coding exonic sequence of a human *GPR156* gene, or a combination thereof. In some embodiments, the human *GPR156* nucleic acid portion in a humanized *Gpr156* gene comprises the 5' non-coding sequence of the first coding exon or the 3' UTR of a human *GPR156* gene, or a combination thereof. In specific embodiments, the human *GPR156* nucleic acid portion in a humanized *Gpr156* gene comprises the ATG start codon in the first coding exon through the last exon (i.e., through the 3' UTR) of a human *GPR156* gene.

[0021] In some embodiments, the rodent *Gpr156* nucleic acid portion in a humanized *Gpr156* gene comprises a 5' non-coding exonic sequence or a 3' non-coding exonic sequence of a rodent *Gpr156* gene, or a combination thereof. In embodiments where the rodent *Gpr156* gene is a mouse *Gpr156* gene, the rodent *Gpr156* nucleic acid portion in a humanized *Gpr156* may comprise exon 1, the 5' non-coding sequence in exon 2, or the 3' UTR of exon 10 of the mouse *Gpr156* gene, or a combination thereof.

[0022] In specific embodiments, a humanized *Gpr156* gene comprises a 5' non-coding exonic sequence of a rodent *Gpr156* gene, and the ATG start codon in the first coding exon through the last exon of a human *GPR156* gene; and in certain embodiments, such humanized *Gpr156* gene further comprises a 3' non-coding exonic sequence of a rodent *Gpr156* gene, placed downstream of the last exon of the human *GPR156* gene. In embodiments where the rodent *Gpr156* gene is a mouse *Gpr156* gene, a humanized *Gpr156* gene may comprise exon 1, the 5' non-coding sequence in exon 2, or the 3' UTR of exon 10, of the mouse *Gpr156* gene, or a

combination thereof, wherein the 3' UTR of exon 10 of the mouse *Gpr156* gene, when present, is placed downstream of the last exon of the human *GPR156* gene.

[0023] In some embodiments, the rodent *Gpr156* gene that provides the rodent *Gpr156* nucleic acid portion in a humanized *Gpr156* gene is an endogenous rodent *Gpr156* gene.

[0024] In some embodiments, a humanized *Gpr156* gene is formed from a replacement of a rodent genomic fragment of an endogenous rodent *Gpr156* gene at an endogenous rodent *Gpr156* locus with a human *GPR156* nucleic acid encoding a wild type human GPR156 protein. The human *GPR156* nucleic acid can be a genomic DNA or a cDNA.

[0025] In some embodiments, the human *GPR156* nucleic acid encoding a wild type human GPR156 protein comprises a coding sequence (e.g., a genomic or cDNA sequence) comprising the ATG start codon in the first coding exon through the stop codon in the last coding exon of a human *GPR156* gene. In some embodiments, the human *GPR156* nucleic acid further comprises a 5' and/or a 3' non-coding exonic sequence of a human *GPR156* gene. In some embodiments, the human *GPR156* nucleic acid comprises the 5' non-coding sequence in the first coding exon or the 3' UTR of a human *GPR156* gene, or a combination thereof. In specific embodiments, the human *GPR156* nucleic acid comprises the ATG start codon in the first coding exon through the last exon (i.e., through the 3' UTR) of a human *GPR156* gene.

[0026] In some embodiments, the rodent genomic fragment being replaced comprises the ATG start codon in the first coding exon through the stop codon in the last coding exon of the endogenous rodent *Gpr156* gene. In some embodiments, the rodent genomic fragment being replaced further comprises a 5' and/or a 3' non-coding exonic sequence of the endogenous rodent *Gpr156* gene. In embodiments where the rodent is a mouse, the mouse genomic fragment being replaced may comprise exon 1, the 5' non-coding sequence in exon 2, or the 3' UTR of exon 10 of the endogenous mouse *Gpr156* gene, or a combination thereof.

[0027] In some embodiments, a humanized *Gpr156* gene encodes a wild type human GPR156 protein, wherein the wild type protein is a full length wild type human GPR156 protein of 814 amino acids. In some embodiments, the full length wild type human GPR156 protein

comprises the amino acid sequence set forth in SEQ ID NO: 1. In some embodiments, the full length wild type human GPR156 protein comprises the amino acid sequence set forth in SEQ ID NO: 2. In some embodiments, the full length wild type human GPR156 protein comprises an amino acid sequence that is substantially identical to SEQ ID NO: 1 or SEQ ID NO: 2, and comprises Glu at position 533.

[0028] In some embodiments, a humanized *Gpr156* gene encodes a wild type human GPR156 protein that is a short isoform wild type human GPR156 protein of 810 amino acids. In some embodiments, the wild type human GPR156 protein comprises the amino acid sequence set forth in SEQ ID NO: 3. In some embodiments, the wild type human GPR156 protein comprises the amino acid sequence set forth in SEQ ID NO: 4. In some embodiments, the wild type human GPR156 protein comprises an amino acid sequence of 810 amino acids that is substantially identical to SEQ ID NO: 3 or SEQ ID NO: 4 and that comprises Glu at position 529.

[0029] In some embodiments, a rodent disclosed herein is heterozygous for a humanized *Gpr156* gene. In some embodiments, a rodent disclosed herein is homozygous for a humanized *Gpr156* gene.

[0030] In some embodiments, a rodent disclosed herein is incapable of expressing an endogenous rodent Gpr156 protein.

[0031] In some embodiments, a rodent disclosed herein is a mouse or a rat.

[0032] In another embodiment is an isolated rodent cell or tissue, whose genome comprises a humanized *Gpr156* gene at an endogenous rodent *Gpr156* locus, wherein the humanized *Gpr156* gene comprises a rodent *Gpr156* nucleic acid portion and a human *GPR156* nucleic acid portion, and wherein expression of the humanized *Gpr156* gene is under control of the rodent *Gpr156* promoter at the endogenous rodent *Gpr156* locus. The rodent *Gpr156* nucleic acid portion and the human *GPR156* nucleic acid portion each can be a genomic DNA or a cDNA.

[0032a] Disclosed herein is an isolated rodent cell or tissue, whose genome comprises a humanized Gpr156 gene at an endogenous rodent Gpr156 locus, wherein the humanized Gpr156 gene comprises a rodent Gpr156 nucleic acid portion and a human GPR156 nucleic acid portion, wherein the humanized Gpr156 gene encodes a wild type human GPR156 protein or a mutant human GPR156 protein comprising an E533D variation, wherein expression of the humanized Gpr156 gene is under control of the rodent Gpr156 promoter at the endogenous rodent Gpr156 locus, and wherein the rodent cell or tissue is a mouse cell or tissue, or a rat cell or tissue.

[0033] In some embodiments, a humanized *Gpr156* gene in an isolated rodent cell or tissue encodes a mutant human GPR156 protein comprising an E533D variation.

[0034] In some embodiments, the human *GPR156* nucleic acid portion in a humanized *Gpr156* gene in an isolated rodent cell or tissue comprises a coding sequence of a human *GPR156* gene, wherein the coding sequence comprises the ATG start codon in the first coding exon through the stop codon in the last coding exon of the human *GPR156* gene, and wherein the human *GPR156* gene comprises nucleotides (in the last coding exon) that encode an E533D variation. In some embodiments, the human *GPR156* nucleic acid portion in a humanized *Gpr156* gene further comprises a 5' and/or a 3' non-coding exonic sequence of a human *GPR156* gene. In some embodiments, the human *GPR156* nucleic acid portion in a humanized *Gpr156* gene comprises the 5' non-coding sequence of the first coding exon or the 3' UTR of a human *GPR156* gene, or a combination thereof. In specific embodiments, the human *GPR156* nucleic acid portion in a humanized *Gpr156* gene comprises the ATG start codon in the first coding exon through the last exon (i.e., through the 3' UTR) of a human *GPR156* gene, with the human *GPR156* gene comprising nucleotides that encode an E533D variation.

[0035] In some embodiments, the rodent *Gpr156* nucleic acid portion in a humanized *Gpr156* gene in an isolated rodent cell or tissue comprises a 5' non-coding exonic sequence or a 3' non-coding exonic sequence of a rodent *Gpr156* gene, or a combination thereof. In embodiments where the rodent *Gpr156* gene is a mouse *Gpr156* gene, the rodent *Gpr156*

nucleic acid portion in a humanized *Gpr156* gene in an isolated rodent cell or tissue may comprise exon 1, the 5' non-coding sequence in exon 2, or the 3' UTR of exon 10 of the mouse *Gpr156* gene, or a combination thereof.

[0036] In specific embodiments, a humanized *Gpr156* gene in an isolated rodent cell or tissue comprises a 5' non-coding exonic sequence of a rodent *Gpr156* gene, and the ATG start codon in the first coding exon through the last exon of a human *GPR156* gene, with the human *GPR156* gene comprising nucleotides that encode an E533D variation; and in certain embodiments, such humanized *Gpr156* gene further comprises a 3' non-coding exonic sequence of a rodent *Gpr156* gene, placed downstream of the last exon of the human *GPR156* gene.

[0037] In some embodiments, the rodent *Gpr156* gene that provides the rodent *Gpr156* nucleic acid portion in a humanized *Gpr156* gene in an isolated rodent cell or tissue is the endogenous rodent *Gpr156* gene.

[0038] In some embodiments, a humanized *Gpr156* gene in an isolated rodent cell or tissue is formed from a replacement of a rodent genomic fragment of an endogenous rodent *Gpr156* gene at an endogenous rodent *Gpr156* locus with a human *GPR156* nucleic acid encoding a mutant human GPR156 protein comprising an E533D variation. The human *GPR156* nucleic acid portion can be a genomic DNA or a cDNA.

[0039] In some embodiments, the human *GPR156* nucleic acid encoding the mutant human GPR156 protein comprises a coding sequence (e.g., a genomic or cDNA sequence) comprising the ATG start codon in the first coding exon through the stop codon in the last coding exon of a human *GPR156* gene, wherein the human *GPR156* gene comprises nucleotides that encode an E533D variation. In some embodiments, the human *GPR156* nucleic acid further comprises a 5' and/or a 3' non-coding exonic sequence of a human *GPR156* gene. In some embodiments, the human *GPR156* nucleic acid comprises the 5' non-coding portion of the first coding exon or the 3' UTR of a human *GPR156* gene, or a combination thereof. In specific embodiments, the human *GPR156* nucleic acid comprises the ATG start codon in the first coding exon through the last exon (i.e., including the 3' UTR)

of a human *GPR156* gene, with the human *GPR156* gene comprising nucleotides that encode an E533D variation.

[0040] In some embodiments, the rodent genomic fragment being replaced comprises the ATG start codon in the first coding exon through the stop codon in the last coding exon of the endogenous rodent *Gpr156* gene. In some embodiments, the rodent genomic fragment being replaced further comprises a 5' and/or a 3' non-coding exonic sequence of the endogenous rodent *Gpr156* gene. In embodiments where the rodent cell or tissue is a mouse cell or tissue, the mouse genomic fragment being replaced may comprise exon 1, the 5' non-coding sequence in exon 2, or the 3' UTR of exon 10 of the endogenous mouse *Gpr156* gene, or a combination thereof.

[0041] In some embodiments, a humanized *Gpr156* gene in an isolated rodent cell or tissue encodes a mutant human GPR156 protein comprising an E533D variation, wherein the mutant protein is a full length GPR156 protein of 814 amino acids. In some embodiments, the mutant human GPR156 protein comprises the amino acid sequence set forth in SEQ ID NO: 5. In some embodiments, the mutant human GPR156 protein comprises the amino acid sequence set forth in SEQ ID NO: 6. In some embodiments, the mutant human GPR156 protein comprises an amino acid sequence that is substantially identical to SEQ ID NO: 5 or SEQ ID NO: 6.

[0042] In some embodiments, a humanized *Gpr156* gene in an isolated rodent cell or tissue encodes a mutant human GPR156 protein that is a short isoform GPR156 protein of 810 amino acids and comprises an E533D variation. In some embodiments, the mutant human GPR156 protein comprises the amino acid sequence set forth in SEQ ID NO: 7. In some embodiments, the mutant human GPR156 protein comprises the amino acid sequence set forth in SEQ ID NO: 8. In some embodiments, the mutant human GPR156 protein comprises an amino acid sequence that is substantially identical to SEQ ID NO: 7 or SEQ ID NO: 8.

[0043] In further embodiments, a humanized *Gpr156* gene in an isolated rodent cell or tissue encodes a wild type human GPR156 protein.

[0044] In some embodiments, the human *GPR156* nucleic acid portion in a humanized *Gpr156* gene in an isolated rodent cell or tissue comprises a coding sequence (e.g., a genomic or cDNA sequence) of a human *GPR156* gene, wherein the coding sequence comprises the ATG start codon in the first coding exon through the stop codon in the last coding exon of the human *GPR156* gene. In some embodiments, the human *GPR156* nucleic acid portion in a humanized *Gpr156* gene further comprises a 5' and/or a 3' non-coding exonic sequence of a human *GPR156* gene. In some embodiments, the human *GPR156* nucleic acid portion in a humanized *Gpr156* gene comprises the 5' non-coding portion of the first coding exon or the 3' UTR of a human *GPR156* gene, or a combination thereof. In specific embodiments, the human *GPR156* nucleic acid portion in a humanized *Gpr156* gene in an isolated rodent cell or tissue comprises the ATG start codon in the first coding exon through the last exon (i.e., including the 3' UTR) of a human *GPR156* gene.

[0045] In some embodiments, the rodent *Gpr156* nucleic acid portion in a humanized *Gpr156* gene in an isolated rodent cell or tissue comprises a 5' non-coding exonic sequence and/or a 3' non-coding exonic sequence. In embodiments where the rodent cell or tissue is a mouse cell or tissue, the rodent *Gpr156* nucleic acid portion in a humanized *Gpr156* gene in the isolated mouse cell or tissue may comprise exon 1, the 5' non-coding sequence in exon 2, or the 3' UTR of exon 10 of a mouse *Gpr156* gene, or a combination thereof.

[0046] In specific embodiments, a humanized *Gpr156* gene in an isolated rodent cell or tissue comprises a 5' non-coding exonic sequence of a rodent *Gpr156* gene, and the ATG start codon in the first coding exon through the last exon of a human *GPR156* gene; and in certain embodiments, such humanized *Gpr156* gene further comprises a 3' non-coding exonic sequence of a rodent *Gpr156* gene, placed downstream of the last exon of the human *GPR156* gene.

[0047] In some embodiments, the rodent *Gpr156* gene that provides the rodent *Gpr156* nucleic acid portion in a humanized *Gpr156* gene in an isolated rodent cell or tissue is the endogenous rodent *Gpr156* gene.

[0048] In some embodiments, a humanized *Gpr156* gene is formed from a replacement of a rodent genomic fragment of an endogenous rodent *Gpr156* gene at an endogenous rodent *Gpr156* locus with a human *GPR156* nucleic acid encoding a wild type human GPR156 protein. The human *GPR156* nucleic acid can be a genomic DNA or a cDNA.

[0049] In some embodiments, the human *GPR156* nucleic acid encoding a wild type human GPR156 protein comprises a coding sequence (e.g., a genomic or cDNA sequence) comprising the ATG start codon in the first coding exon through the stop codon in the last coding exon of a human *GPR156* gene. In some embodiments, the human *GPR156* nucleic acid further comprises a 5' and/or a 3' non-coding exonic sequence of a human *GPR156* gene. In some embodiments, the human *GPR156* nucleic acid comprises the 5' non-coding portion of the first coding exon or the 3' UTR of a human *GPR156* gene, or a combination thereof. In specific embodiments, the human *GPR156* nucleic acid comprises the ATG start codon in the first coding exon through the last exon (i.e., including the 3' UTR) of a human *GPR156* gene.

[0050] In some embodiments, the rodent genomic fragment being replaced comprises the ATG start codon in the first coding exon through the stop codon in the last coding exon of the endogenous rodent *Gpr156* gene.

[0051] In some embodiments, a humanized *Gpr156* gene in an isolated rodent cell or tissue encodes a wild type human GPR156 protein, wherein the wild type protein is a full length wild type human GPR156 protein of 814 amino acids. In some embodiments, the wild type human GPR156 protein comprises the amino acid sequence set forth in SEQ ID NO: 1. In some embodiments, the full length wild type human GPR156 protein comprises the amino acid sequence set forth in SEQ ID NO: 2. In some embodiments, the full length wild type human GPR156 protein comprises an amino acid sequence that is substantially identical to SEQ ID NO: 1 or SEQ ID NO: 2, and comprises Glu at position 533.

[0052] In some embodiments, a humanized *Gpr156* gene in an isolated rodent cell or tissue encodes a wild type human GPR156 protein that is a short isoform wild type human GPR156 protein of 810 amino acids. In some embodiments, the wild type human GPR156 protein

comprises the amino acid sequence set forth in SEQ ID NO: 3. In some embodiments, the wild type human GPR156 protein comprises the amino acid sequence set forth in SEQ ID NO: 4. In some embodiments, the wild type human GPR156 protein comprises an amino acid sequence of 810 amino acids that is substantially identical to SEQ ID NO: 3 or SEQ ID NO: 4 and that comprises Glu at position 529.

[0053] In some embodiments, an isolated rodent cell or tissue disclosed herein is heterozygous for a humanized *Gpr156* gene. In some embodiments, an isolated rodent cell or tissue disclosed herein is homozygous for a humanized *Gpr156* gene.

[0054] In some embodiments, an isolated rodent cell or tissue disclosed herein is incapable of expressing an endogenous rodent Gpr156 protein.

[0055] In some embodiments, an isolated rodent cell is a rodent embryonic stem (ES) cell.

[0056] In some embodiments, an isolated rodent cell or tissue is a mouse cell or tissue, or a rat cell or tissue.

[0057] In a further embodiment is a method of making a genetically modified rodent, the method comprising modifying a rodent genome so that the modified genome comprises a humanized *Gpr156* gene at an endogenous rodent *Gpr156* locus, wherein the humanized *Gpr156* gene comprises a rodent *Gpr156* nucleic acid portion and a human *GPR156* nucleic acid portion, and wherein expression of the humanized *Gpr156* gene is under control of the rodent *Gpr156* promoter at the endogenous rodent *Gpr156* locus; and obtaining a rodent comprising the modified genome. The rodent *Gpr156* nucleic acid portion and the human *GPR156* nucleic acid portion each can be a genomic DNA or a cDNA.

[0057a] Disclosed herein is a method of making a genetically modified rodent, comprising: modifying a rodent genome so that the modified genome comprises a humanized Gpr156 gene at an endogenous rodent Gpr156 locus, wherein the humanized Gpr156 gene comprises a rodent Gpr156 nucleic acid portion and a human GPR156 nucleic acid portion, wherein the humanized Gpr156 gene encodes a wild type human GPR156 protein or a mutant human

GPR156 protein comprising an E533D variation, and wherein expression of the humanized Gpr156 gene is under control of the rodent Gpr156 promoter at the endogenous rodent Gpr156 locus; and obtaining a genetically modified rodent comprising the modified genome.

[0058] In some embodiments, the humanized *Gpr156* gene encodes a mutant human GPR156 protein comprising an E533D variation. In some embodiments, the humanized *Gpr156* gene encodes a wild type human GPR156 protein.

[0059] In some embodiments, the rodent genome is modified by (a) introducing a targeting vector into a rodent ES cell to obtain a modified rodent ES cell whose genome comprises a humanized *Gpr156* gene; and (b) making a rodent using the modified rodent ES cell of (a).

[0060] In some embodiments, the targeting vector comprises a human *GPR156* nucleic acid which is integrated into an endogenous rodent *Gpr156* locus thereby forming a humanized *Gpr156* gene an endogenous rodent *Gpr156* locus. The human *GPR156* nucleic acid can be a genomic DNA or a cDNA.

[0061] In some embodiments, the human *GPR156* nucleic acid comprises a coding sequence (e.g., a genomic or cDNA sequence) comprising the ATG start codon in the first coding exon through the stop codon in the last coding exon of a human *GPR156* gene, wherein the human *GPR156* gene comprises nucleotides (in the last coding exon) that encode either Glu (wild type) or an E533D variation (mutant). In some embodiments, the human *GPR156* nucleic acid further comprises a 5' and/or a 3' non-coding exonic sequence of a human *GPR156* gene. In some embodiments, the human *GPR156* nucleic acid comprises the 5' non-coding sequence of the first coding exon or the 3' UTR of a human *GPR156* gene, or a combination thereof. In specific embodiments, the human *GPR156* nucleic acid comprises the ATG start codon in the first coding exon through the last exon (i.e., including the 3' UTR) of a human *GPR156* gene, with human *GPR156* gene comprising nucleotides that encode either Glu (wild type) or an E533D variation (mutant).

[0062] In some embodiments, the rodent genomic fragment being replaced comprises the ATG start codon in the first coding exon through the stop codon in the last coding exon of the

endogenous rodent *Gpr156* gene. In some embodiments, the rodent genomic fragment being replaced further comprises a 5' an/or a 3' non-coding exonic sequence of the endogenous rodent *Gpr156* gene. In embodiments where the rodent is a mouse, the mouse genomic fragment being replaced may comprise exon 1, the 5' non-coding sequence in exon 2, or the 3' UTR of exon 10 of the endogenous mouse *Gpr156* gene, or a combination thereof.

[0063] In some embodiments, a humanized *Gpr156* gene encodes a mutant human GPR156 protein comprising an E533D variation, wherein the mutant protein is a full length GPR156 protein of 814 amino acids. In some embodiments, the mutant human GPR156 protein comprises the amino acid sequence set forth in SEQ ID NO: 5. In some embodiments, the mutant human GPR156 protein comprises the amino acid sequence set forth in SEQ ID NO: 6. In some embodiments, the mutant human GPR156 protein comprises an amino acid sequence that is substantially identical to SEQ ID NO: 5 or SEQ ID NO: 6.

[0064] In some embodiments, a humanized *Gpr156* gene encodes a mutant human GPR156 protein that is a short isoform GPR156 protein of 810 amino acids and comprises an E533D variation. In some embodiments, the mutant human GPR156 protein comprises the amino acid sequence set forth in SEQ ID NO: 7. In some embodiments, the mutant human GPR156 protein comprises the amino acid sequence set forth in SEQ ID NO: 8. In some embodiments, the mutant human GPR156 protein comprises an amino acid sequence that is substantially identical to SEQ ID NO: 7 or SEQ ID NO: 8.

[0065] In further embodiments, a humanized *Gpr156* gene encodes a wild type human GPR156 protein, wherein the wild type protein is a full length wild type human GPR156 protein of 814 amino acids. In some embodiments, the wild type human GPR156 protein comprises the amino acid sequence set forth in SEQ ID NO: 1. In some embodiments, the full length wild type human GPR156 protein comprises the amino acid sequence set forth in SEQ ID NO: 2. In some embodiments, the full length wild type human GPR156 protein comprises an amino acid sequence that is substantially identical to SEQ ID NO: 1 or SEQ ID NO: 2, and comprises Glu at position 533.

[0066] In some embodiments, a humanized *Gpr156* gene encodes a wild type human GPR156 protein that is a short isoform wild type human GPR156 protein of 810 amino acids. In some embodiments, the wild type human GPR156 protein comprises the amino acid sequence set forth in SEQ ID NO: 3. In some embodiments, the wild type human GPR156 protein comprises the amino acid sequence set forth in SEQ ID NO: 4. In some embodiments, the wild type human GPR156 protein comprises an amino acid sequence of 810 amino acids that is substantially identical to SEQ ID NO: 3 or SEQ ID NO: 4 and that comprises Glu at position 529.

[0067] In some embodiments, a rodent made in accordance with the present method is heterozygous for a humanized *Gpr156* gene. In some embodiments, a rodent made in accordance with the present method is homozygous for a humanized *Gpr156* gene.

[0068] In some embodiments, a rodent made in accordance with the present method is incapable of expressing an endogenous rodent Gpr156 protein.

[0069] In some embodiments, a rodent made in accordance with the present method is a mouse or a rat.

[0070] In an embodiment is a targeting nucleic acid vector which comprises a human *GPR156* nucleic acid to be integrated into a rodent *Gpr156* gene at an endogenous rodent *Gpr156* locus, flanked by a 5' nucleotide sequence and a 3' nucleotide sequence that are homologous to nucleotide sequences at the rodent *Gpr156* locus, wherein integration of the human *GPR156* nucleic acid into the rodent *Gpr156* gene results in a replacement of a rodent genomic fragment of an endogenous rodent *Gpr156* gene at the endogenous rodent *Gpr156* locus with the human *GPR156* nucleic acid to form a humanized *Gpr156* gene, and wherein expression of the humanized *Gpr156* gene is under control of the rodent *Gpr156* promoter at the endogenous rodent *Gpr156* locus. The human *GPR156* nucleic acid (and the humanized *Gpr156* gene) encodes either a wild type human GPR156 protein or a mutant human GPR156 protein comprising an E533D variation.

[0070a] Disclosed herein is a targeting vector, comprising a human GPR156 nucleic acid to

be integrated into a rodent Gpr156 gene at an endogenous rodent Gpr156 locus, flanked by a 5' nucleotide sequence and a 3' nucleotide sequence that are homologous to nucleotide sequences at the rodent Gpr156 locus, wherein integration of the human GPR156 nucleic acid into the rodent Gpr156 gene results in a replacement of a rodent genomic fragment of an endogenous rodent Gpr156 gene at the endogenous rodent Gpr156 locus with the human GPR156 nucleic acid to form a humanized Gpr156 gene, wherein the human GPR156 nucleic acid encodes either a wild type human GPR156 protein or a mutant human GPR156 protein comprising an E533D variation, wherein expression of the humanized Gpr156 gene is under control of the rodent Gpr156 promoter at the endogenous rodent Gpr156 locus, and wherein the rodent is a mouse or a rat.

[0071] In some embodiments, the human *GPR156* nucleic acid in a targeting vector comprises a coding sequence (e.g., a genomic or cDNA sequence) comprising the ATG start codon in the first coding exon through the stop codon in the last coding exon of a human *GPR156* gene, wherein the human *GPR156* gene comprises nucleotides that encode either Glu (wild type) or Asp (mutant) at an amino acid position corresponding to position 533 in a full length wild type human GPR156 protein (e.g., SEQ ID NO: 1 or 2). In some embodiments, the human *GPR156* nucleic acid in a targeting vector comprises the ATG start codon in the first coding exon through the last exon of the human *GPR156* gene.

[0072] In some embodiments, the targeting vector is designed as such that the rodent genomic fragment of the endogenous rodent *Gpr156* gene being replaced comprises the ATG start codon in the first coding exon through the stop codon in the last coding exon of the endogenous rodent *Gpr156* gene. In some embodiments, the rodent genomic fragment being replaced further comprises a 5' an/or a 3' non-coding exonic sequence of the endogenous rodent *Gpr156* gene. In embodiments where the rodent is a mouse, the targeting vector is designed as such that the mouse genomic fragment being replaced may comprise exon 1, the 5' non-coding sequence in exon 2, or the 3' UTR of exon 10 of the endogenous mouse *Gpr156* gene, or a combination thereof.

[0073] In some embodiments, the targeting vector is designed to integrate a human *GPR156* nucleic acid into the genome of a rodent, wherein the rodent is a mouse or a rat.

[0074] In another embodiment is a method comprising breeding a first rodent whose genome comprises a humanized *Gpr156* gene at an endogenous rodent *Gpr156* locus with a second rodent, resulting in a progeny rodent whose genome comprises the a humanized *Gpr156* gene, wherein expression of the humanized *Gpr156* gene is under control of the rodent *Gpr156* promoter at the endogenous rodent *Gpr156* locus.

[0075] In some embodiments, the humanized *Gpr156* gene encodes a mutant human GPR156 protein comprising an E533D variation. In some embodiments, the humanized *Gpr156* gene encodes a wild type human GPR156 protein.

[0076] In some embodiments, the first rodent and the second rodent are mice. In some embodiments, the first rodent and the second rodent are rats.

[0077] In a further embodiment a progeny rodent whose genome comprises a humanized *Gpr156* gene at an endogenous rodent *Gpr156* locus, wherein the progeny rodent is produced by a method comprising breeding a first rodent whose genome comprises the humanized *Gpr156* gene with a second rodent, wherein expression of the humanized *Gpr156* gene is under control of the rodent *Gpr156* promoter at the endogenous rodent *Gpr156* locus.

[0078] In some embodiments, the humanized *Gpr156* gene encodes a mutant human GPR156 protein comprising an E533D variation. In some embodiments, the humanized *Gpr156* gene encodes a wild type human GPR156 protein.

[0079] In some embodiments, the progeny rodent is heterozygous for the humanized *Gpr156* gene. In some embodiments, the progeny rodent is homozygous for the humanized *Gpr156* gene.

[0080] In some embodiments, the progeny rodent is a mouse. In some embodiments, the progeny rodent is a rat.

[0081] In one embodiment is a rodent whose genome comprises a genetically modified *Gpr156* locus, wherein the genetic modification comprises a deletion in an endogenous rodent *Gpr156* gene, and optionally also comprises an insertion of a reporter gene, wherein the reporter gene is operably linked to the endogenous rodent *Gpr156* promoter at the locus.

[0081a] Disclosed herein is a rodent, whose genome comprises genetically modified Gpr156 locus, wherein the genetic modification comprises a deletion in an endogenous rodent Gpr156 gene and an insertion of a reporter gene, wherein the reporter gene is operably linked to the endogenous rodent Gpr156 promoter at the locus, wherein the genomic fragment beginning from the nucleotide after the start codon through a subsequent coding exon has been deleted, and wherein the reporter gene is operably linked to the start codon of the endogenous rodent Gpr156 gene, and wherein the rodent is a rat or a mouse.

[0082] In some embodiments, a genomic fragment beginning from the nucleotide after the start codon in the first coding exon through a subsequent coding exon (e.g., the second, third, fourth, fifth, sixth, seventh, eighth, or ninth coding exon) has been deleted; and in some such embodiments, a reporter gene is inserted and is operably linked to the start codon in the first coding exon of the endogenous rodent *Gpr156* gene. In these embodiments, expression of the reporter gene resembles the expression pattern of an unmodified endogenous rodent *Gpr156* gene.

[0083] In some embodiments, the reporter gene is *LacZ*, or a gene encoding a protein selected the group consisting of luciferase, green fluorescent protein (GFP), enhanced GFP (eGFP), cyan fluorescent protein (CFP), yellow fluorescent protein (YFP), enhanced yellow fluorescent protein (eYFP), blue fluorescent protein (BFP), enhanced blue fluorescent protein (eBFP), DsRed, and MmGFP.

[0084] In some embodiments, the rodent is homozygous for the genetic modification. In some embodiments, the rodent is heterozygous for the genetic modification.

[0085] In some embodiments, the rodent is a mouse. In some embodiments, the rodent is a rat.

[0086] In another aspect, disclosed is a rodent model of mood disorders such as unipolar depression or anxiety disorders.

[0087] In some embodiments, a rodent model of mood disorders comprises a genetically modified rodent whose genome comprises a humanized *Gpr156* gene at an endogenous rodent *Gpr156* locus, wherein the humanized *Gpr156* gene encodes a mutant human GPR156 protein comprising an E533D variation, and wherein expression of the humanized *Gpr156* gene is under control of the rodent *Gpr156* promoter at the endogenous rodent *Gpr156* locus. The rodent can be heterozygous or homozygous for the humanized *Gpr156* gene.

[0088] In some embodiments, a rodent model of mood disorders further comprises a genetically modified rodent whose genome comprises a humanized *Gpr156* gene at an endogenous rodent *Gpr156* locus, wherein the humanized *Gpr156* gene encodes a wild type human GPR156 protein, and wherein expression of the humanized *Gpr156* gene is under control of the rodent *Gpr156* promoter at the endogenous rodent *Gpr156* locus. The rodent can be heterozygous or homozygous for the humanized *Gpr156* gene.

[0089] In some embodiments, a rodent model of mood disorders further comprises a genetically modified rodent whose genome comprises a genetically modified *Gpr156* locus, wherein the genetic modification comprises a deletion in an endogenous rodent *Gpr156* gene and optionally an insertion of a reporter gene, and wherein the reporter gene is operably linked to the endogenous rodent *Gpr156* promoter at the locus. The rodent can be heterozygous or homozygous for the genetic modification.

[0090] In accordance with this disclosure, a rodent homozygous for a humanized *Gpr156* gene that encodes a mutant human GPR156 protein comprising an E533D variation, or homozygous for a deletion in an endogenous rodent *Gpr156* gene, is unable to complete a forced swim test, whereas a control rodent (a rodent homozygous for a humanized *Gpr156* gene that encodes a wild type human GPR156 protein, or a rodent without genetic modification or humanization) is able to perform a forced swim test. The ability to complete the forced swim test is improved in the E533D mutant rodents or the *Gpr156* deletion rodents after treatment with an anti-depression/anti-anxiety drug. Accordingly, the inability to

complete a forced swim test in rodents homozygous for a humanized *Gpr156* gene encoding an E533D variant, or homozygous for a deletion in an endogenous rodent *Gpr156* gene, can serve as a feature or indication of mood disorders, and the rodents can be used to screen or test a therapeutic agent for the treatment of mood disorders such as unipolar depression and anxiety disorders.

[0091] In a further embodiment is a method of screening or testing a therapeutic agent for the treatment of unipolar depression or anxiety disorders, the method comprising administering an agent to a rodent disclosed herein, performing one or more assays (e.g., a forced swim test or marble burying test) to determine if the agent has an effect on the rodent's performance in the one or more assays. An agent may be identified as a therapeutic candidate when the agent has a therapeutic effect on the rodent as evaluated by, e.g., reducing, eliminating or preventing the impairment in a forced swim test, or by improving the ability to perform and complete a forced swim test; or by reducing, eliminating or preventing the increase in the number of buried marbles.

[0091a] Disclosed herein is a method of testing the effect of an agent for treating unipolar depression or an anxiety disorder, comprising subjecting a rodent to a test to assess behavior in the rodent indicative of unipolar depression or the anxiety disorder, wherein the rodent has been administered the agent as defined herein, and determining whether the agent has any effect on the behavior in the rodent.

BRIEF DESCRIPTION OF THE DRAWINGS

[0092] FIG. 1. Top, an endogenous mouse *Gpr156* locus (with the coding region depicted in strips); middle, a genetically modified mouse *Gpr156* locus with a deletion of mouse *Gpr156* exon 2 (in part, from the codon after ATG) and exons 3-5, and insertion of a *LacZ* coding sequence; and bottom, a genetically modified mouse *Gpr156* locus with a replacement of a mouse genomic fragment from the ATG start codon in exon 2 through the stop codon in exon 10 of the mouse *Gpr156* gene, with a human genomic fragment from the ATG start codon in the first coding exon through the stop codon in the last coding exon(also the last exon) of a

human *GPR156* gene (either a wild type human *GPR156* gene or a mutant human *GPR156* gene encoding the E533D variant). The exon ("Ex") numbering for the humanized *Gpr156* gene at the mouse *Gpr156* locus depicted in this figure follows the mouse exon numbering.

[0093] FIG. 2. Total time of swim test (in seconds). Mice were tested for a 6 minute forced swim test; not all mice were able to perform the task. The graph shows the duration of the swim test for different *Gpr156* genotypes tested, from left to right, male $Gpr156^{+/+}$, male $Gpr156^{-/-}$, male $GPR156^{hum/hum}$ (homozygous for wild type human *GPR156*), male $GPR156^{E533D/E533D}$, female $Gpr156^{+/+}$, female $Gpr156^{-/-}$, female $GPR156^{hum/hum}$, and female $GPR156^{E533D/E533D}$. Data are expressed as mean ± SEM for each animal group.

[0094] FIG. 3A. Left panel: Total time of swim test (in seconds) before and after daily treatment with intraperitoneal injection of Fluoxetine 10 mg/kg for 9 weeks (6 days per week daily injection). Mice were tested for a 6 minute forced swim every 3 weeks. The graph shows the average ± SEM duration of the swim test for different *Gpr156* genotypes tested: treated $GPR156^{E533D/E533D}$ mice, treated male $GPR156^{hum/hum}$, treated female $GPR156^{E533D/E533D}$, treated female $GPR156^{hum/hum}$, and untreated female $GPR156^{E533D/E533D}$. Treatment with Fluoxetine improves the impaired swim phenotype in $GPR156^{E533D/E533D}$ mice. Right panel: Percentage of mice achieving the swim test for different GPR156 genotypes tested at 3 weeks, 6 weeks and 9 weeks after intraperitoneal injection of Fluoxetine. While none (0/13) of the $GPR156^{E533D/E533D}$ mice were able to perform the swim test the first time, following a 9 weeks treatment with Fluoxetine, 53% (7/13) of the $GPR156^{E533D/E533D}$ mice achieved the test (with an 8th mouse failing the test at 350 seconds).

[0095] FIG. 3B. Performances in swim test plotted over the 9 weeks from individual $GPR156^{E533D/E533D}$ mice studied in the experiments in FIG. 3A, showing that treatment with Fluoxetine improves the impaired swim phenotype in the $GPR156^{E533D/E533D}$ mice. Following a 9 weeks treatment with Fluoxetine, 53% of the mice achieved the test. On the other hand, in the untreated group, while some mice improved their performance, only 1/11 mice achieved the swim test at 9 weeks.

[0096] FIGS. 3C-3D. Treatment with imipramine improves the impaired swim phenotype in *GPR156*$^{E533D/E533D}$ mice. FIG. 3C: Total time of swim test (in seconds) for different *Gpr156* genotypes tested before treatment with imipramine, and at 9 weeks after intraperitoneal injection of imipramine 15 mg/kg for 6 days/week (Monday through Saturday), with male and female mice separately plotted. FIG. 3D: Total time of swim test (in seconds) for different GPR156 genotypes tested before and after the treatment with imipramine for 9 weeks, with male and female mice combined for each genotype.

[0097] FIG. 4. Number of marbles buried in 10 minutes by mice of different genotypes, from left to right: male *Gpr156*$^{+/+}$, male *Gpr156*$^{-/-}$, male *GPR156* $^{hum/hum}$, male *GPR156* $^{E533D/E533D}$, female *Gpr156*$^{+/+}$, female *Gpr156*$^{-/-}$, female *GPR156* $^{hum/hum}$, and female *GPR156* $^{E533D/E533D}$. Male mice genetically modified and homozygous for the humanized *GPR156* gene encoding an E533D variation or homozygous for the deletion of the mouse *Gpr156* gene showed an increase in the number of marbles buried in 10 minutes. Data are expressed as mean ± SEM for each animal group.

DETAILED DESCRIPTION

[0098] Disclosed herein are genetically modified rodents suitable for use as an animal model of mood disorders such as unipolar depression and anxiety disorders. In particular, disclosed herein are genetically modified rodent animals (e.g., mouse or rat) that carry a humanized *Gpr156* gene at an endogenous rodent *Gpr156* locus, wherein the humanized *Gpr156* gene encodes a wild type human GPR156 protein or a mutant human GPR156 protein comprising Asp at an amino acid position corresponding to position 533 in a full length wild type human GPR156 protein (also referred to herein as an "E533D variation"). Also disclosed herein are methods and targeting vectors for making a genetically modified rodent, methods of breeding, and use of a genetically modified rodent as an animal model of mood disorders for screening and testing therapeutic agents.

[0099] Various aspects of the present disclosure are described in detail in the following sections.

GPR156

[0100] *GPR156* (G protein-coupled receptor 156) encodes a protein that belongs to group C G protein-coupled receptor family, and is most closely related to the $GABA_B$ receptor subfamily (Calver A, et al., Brain Res. Mol. Brain Res. 110: 305-307 (2003)). The GPR156 protein is predicted to contain seven transmembrane domains, an N-terminal extracellular domain, and a C-terminal intracellular domain, without a signal peptide (Calver et al., *supra*). The C-terminal sequence of GPR156 contains a putative coiled-coil domain, di-leucine and several RXR(R) ER retention motifs, all of which have been shown to be critical in $GABA_B$ receptor subunit function (Calver et al., *supra*). In addition, the distribution of GPR156 in the central nervous system is reminiscent of that of the other known $GABA_B$ subunits (Calver et al., *supra*).

[0101] The human *GPR156* gene (available, e.g., in GenBank under Accession No. NC_000003.12) is located on chromosome 3, between 3q13 and 3q22, and is about 120 kb in length. The human gene has been predicted to have 10 exons, with exon 2 being the first coding exon, although the canonical transcript does not appear to include the 5' non-coding exon present in the predicted transcript, and has 9 exons, with the first exon also being the first coding exon. The homologous mouse gene (available, e.g., in GenBank under Accession No. NC_000082.6) is located on chromosome 16, and is documented as having 10 exons, with exon 2 being the first coding exon. The homologous rat gene (available, e.g., in GenBank under Accession No. NC_005110.4) is located on chromosome 11, and is documented as having 9 exons, with exon 1 being the first coding exon. Exemplary human genomic DNA sequences encoding either a full length or short isoform, a wild type or a E533D variant, human GPR156 protein are set forth in SEQ ID NOS: 9-16. Exemplary rat and mouse transcript cDNA and protein sequences are set forth in SEQ ID NO: 25 (NM_153295.1), SEQ ID NO: 26 (NM_153394.2), SEQ ID NO: 27 (NP_695207.1), and SEQ ID NO: 28 (NP_700443.2).

[0102] GPR156 is highly conserved across species, with the mouse and rat proteins being 87.5% identical, and with the mouse and rat proteins being 69.3% and 69.5% identical to the human protein, respectively (Calver et al., *supra*).

[0103] Two wild type isoforms of human GPR156 protein have been reported. The longer isoform, also referred to herein as a full length wild type human GPR156 protein, consists of 814 amino acids. The shorter isoform contains a deletion of 4 amino acids at positions corresponding to positions 198 to 201 of the longer isoform, as a result of an in-frame deletion of 12 nucleotides. For each isoform, two variants exist and have either a glutamic acid or an aspartic acid at an amino acid position corresponding to position 516 in the longer isoform, or corresponding to position 512 in the shorter isoform.

[0104] In some embodiments, a full length wild type human GPR156 protein is represented by the amino acid sequence as set forth in SEQ ID NO: 1. In some embodiments, a full length wild type human GPR156 protein is represented by the amino acid sequence as set forth in SEQ ID NO: 2. SEQ ID NO: 1 and SEQ ID NO: 2 both consist of 814 amino acids and differ only in position 516, with SEQ ID NO; 1 having a glutamic acid and SEQ ID NO: 2 having an aspartic acid at this position. In some embodiments, a full length wild type human GPR156 protein may be represented by an amino acid sequence that is substantially identical to the amino acid sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 2.

[0105] In referring to a given amino acid sequence as being "substantially identical" to a reference sequence, it includes embodiments where the given amino acid sequence is at least 98% identical, at least 98.5%, at least 99% identical, or at least 99.5% identical, to a reference sequence; for example, a given amino acid sequence differs from a reference sequence by 1, 2, 3, 4, or 5 amino acids, or differs by not more than 5, 4, 3, 2, or 1 amino acid(s).

[0106] In some embodiments, a short wild type human GPR156 protein isoform is represented by the amino acid sequence as set forth in SEQ ID NO: 3. In some embodiments, a short wild type human GPR156 protein isoform is represented by the amino acid sequence as set forth in SEQ ID NO: 4. SEQ ID NO: 3 and SEQ ID NO: 4 both consist of 810 amino acids and differ only in position 512, with SEQ ID NO; 3 having a glutamic acid and SEQ ID

NO: 4 having an aspartic acid at this position. In some embodiments, a short wild type human GPR156 protein isoform may be represented by an amino acid sequence that is substantially identical to the amino acid sequence set forth in SEQ ID NO: 3 or SEQ ID NO: 4.

[0107] All wild type human GPR156 proteins in accordance with this disclosure, both a full length and a short isoform proteins, have a Glu residue at the position corresponding to position 533 in a full length wild type human GPR156 protein (e.g., SEQ ID NO: 1 or SEQ ID NO: 2).

[0108] As used herein, the phrase "corresponding to" or grammatical variations thereof when used in the context of the numbering of positions in a given polypeptide or nucleic acid molecule refers to the numbering of a specified reference polypeptide or nucleic acid molecule when the given amino acid or nucleic acid molecule is compared to the reference molecule (e.g., with the reference molecule herein being the polypeptide or nucleic acid molecule of a full length wild type human GPR156). In other words, the position of an amino acid residue or nucleotide in a given polymer is designated with respect to the reference molecule rather than by the actual numerical position of the amino acid residue or nucleotide within the given polymer. For example, a given amino acid sequence can be aligned to a reference sequence by introducing gaps to optimize residue matches between the two sequences. In these cases, although the gaps are present, the numbering of the residue in the given amino acid or nucleic acid sequence is made with respect to the reference sequence to which it has been aligned.

[0109] For example, the phrase "GPR156 protein, wherein the protein comprises a glutamic acid at the position corresponding to position 533 of SEQ ID NO: 1" means that, if the amino acid sequence of the GPR156 protein is aligned to the sequence of SEQ ID NO: 1, the GPR156 protein has a glutamic acid at the position that corresponds to position 533 of SEQ ID NO: 1. As an example, the position in the short isoform of wild type GPR156 protein of SEQ ID NO: 3 that correspond to position 533 of SEQ ID NO: 1 is position 529.

[0110] As described above, a position within a GPR156 protein that corresponds to position 533 of a full length wild type human GPR156 protein can easily be identified by performing a

sequence alignment between the given GPR156 protein and the amino acid sequence of a full length wild type human GPR156 protein (e.g., SEQ ID NO: 1 or SEQ ID NO: 2). A variety of computational algorithms exist that can be used for performing a sequence alignment in order to identify an amino acid position that corresponds to position 533 in SEQ ID NO: 1 or 2. For example, by using the NCBI BLAST algorithm (Altschul et al. 1997 Nucleic Acids Res. 25: 3389-3402) or CLUSTALW software (Sievers and Higgins 2014 Methods Mol. Biol. 1079: 105-116.) sequence alignments may be performed. However, sequences can also be aligned manually.

Alteration/Mutation in Human *GPR156* Associated with Unipolar Depression and Anxiety Disorder

[0111] A rare variant in the human *GPR156* gene segregating with the phenotype of unipolar depression in affected family members has been reported in U.S. Published Application 2018/0030114 A1. More specifically, a genetic alteration in the human *GPR156* gene that changes the amino acid at the position corresponding to position 533 in a full length human GPR156 protein (e.g., SEQ ID NO: 1 or 2) from Glu to Asp has been identified in US 2018/0030114 A1 as segregating with the phenotype of unipolar depression in affected family members. Such a protein is also referred to herein as a mutant human GPR156 protein with an "E533D mutation" or "E533D variation." Additional family members who carried an E533D variation had confirmed diagnoses of anxiety disorder. Thus, it has been proposed that an E533D variation in human GPR156 is indicative of an increased susceptibility to developing mood disorders such as unipolar depression and an anxiety disorder.

[0112] In accordance with this disclosure, an endogenous Gpr156 locus in a rodent has been modified (or humanized) to carry a nucleic acid encoding and expressing a mutant human GPR156 protein comprising an E533D variation, i.e., comprising Asp at a position corresponding to position 533 in a full length wild type human GPR156 protein. As demonstrated herein, such genetically modified rodent exhibits features reflective of mood disorders such as unipolar depression and anxiety disorders, and thus can serve as a rodent model of mood disorders.

Rodents Comprising a Humanized *Gpr156* Gene

[0113] This disclosure provides genetically modified rodents whose genome comprises a humanized *Gpr156* gene at an endogenous rodent *Gpr156* locus, wherein the humanized *Gpr156* gene encodes a human GPR156 protein and is under control of the rodent *Gpr156* promoter at the endogenous rodent *Gpr156* locus.

[0114] A "humanized *Gpr156* gene" as used herein includes a *Gpr156* gene that comprises both a human *GPR156* nucleic acid portion and a rodent *Gpr156* nucleic portion. For example, a rodent *Gpr156* gene can be modified to have a portion of the rodent *Gpr156* gene replaced by a human *GPR156* nucleic acid (e.g., a portion of a human *GPR156* gene). In some embodiments, an endogenous rodent *Gpr156* gene at an endogenous rodent *Gpr156* locus has been modified such that a portion of the endogenous rodent *Gpr156* gene at the endogenous rodent *Gpr156* locus is replaced by a human *GPR156* nucleic acid (e.g., a portion of a human *GPR156* gene).

[0115] A "portion" of a gene is used herein interchangeably with a "fragment" of a gene, which includes references to contiguous nucleotide sequence portions of a gene, including, for example, a 5' regulatory region (e.g., promoter), a 5' non-coding exonic sequence, a 3' non-coding exonic sequence, a 5' or 3' untranslated region (UTR), an exon in full or in part, an intron in full or in part, a 3' region downstream of the last exon, or combinations thereof. In some embodiments, a portion of a gene refers to the coding region of the gene, e.g., a nucleic acid (genomic DNA or cDNA) comprising the ATG start codon through the stop codon of the gene.

[0116] A "nucleic acid," a "nucleic acid molecule," a "nucleic acid sequence," a "nucleotide sequence, "polynucleotide," or "oligonucleotide" can comprise a polymeric form of nucleotides of any length, may include RNA (e.g., mRNA) or DNA (e.g., genomic DNA or cDNA), and can be single-stranded, double-stranded, or multiple stranded. Thus, references to "a human *GPR156* nucleic acid" can include, for example, both genomic and cDNA forms of a human *GPR156* gene, in full or in part.

[0117] In some embodiments, the humanized *Gpr156* gene encodes a GPR156 protein that is a wild type human GPR156 protein.

[0118] In some embodiments, the wild type human GPR156 protein is a full length wild type human GPR156 protein. In some embodiments, the full length wild type human GPR156 protein is represented by the amino acid sequence as set forth in SEQ ID NO: 1. In some embodiments, the full length wild type human GPR156 protein is represented by the amino acid sequence as set forth in SEQ ID NO: 2. In some embodiments, a full length wild type human GPR156 protein may be represented by an amino acid sequence substantially identical to the amino acid sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 2.

[0119] In some embodiments, the wild type human GPR156 protein is a short isoform wild type human GPR156 protein. In some embodiments, the short isoform wild type human GPR156 protein is represented by the amino acid sequence as set forth in SEQ ID NO: 3. In some embodiments, the short isoform wild type human GPR156 protein is represented by the amino acid sequence as set forth in SEQ ID NO: 4. In some embodiments, a short isoform wild type human GPR156 protein may be represented by an amino acid sequence substantially identical to the amino acid sequence set forth in SEQ ID NO: 3 or SEQ ID NO: 4.

[0120] In some embodiments, the humanized *Gpr156* gene encodes a full length GPR156 protein of 814 amino acids, wherein the GPR156 protein comprises a Glu residue at a position corresponding to position 533 in a full length wild type human GPR156 protein (e.g., SEQ ID NO: 1 or SEQ ID NO: 2), and wherein the GPR156 protein comprises an amino acid sequence substantially identical to the amino acid sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 2.

[0121] In some embodiments, the humanized *Gpr156* gene encodes a short isoform GPR156 protein of 810 amino acids, wherein the GPR156 protein comprises Glu at the position corresponding to position 533 in a full length wild type human GPR156 protein (e.g., SEQ ID NO: 1 or SEQ ID NO: 2), and wherein the GPR156 protein comprises an amino acid sequence

substantially identical to the amino acid sequence set forth in SEQ ID NO: 3 or SEQ ID NO: 4.

[0122] In some embodiments, the humanized *Gpr156* gene encodes a mutant human GPR156 protein comprising an E533D variation, i.e., a mutant human GPR156 protein comprising Asp at an amino acid position corresponding to position 533 in a full length wild type human GPR156 protein (e.g., SEQ ID NO: 1 or 2). In some embodiments, an E533D variation is the only variation in a mutant human GPR156 protein as compared to a wild type human GPR156 protein, in which case the mutant GPR156 protein is also referred to as an E533D variant.

[0123] In some embodiments, the mutant human GPR156 protein is a full length human GPR156 protein. In some embodiments, the full length mutant human GPR156 protein is represented by the amino acid sequence as set forth in SEQ ID NO: 5. In some embodiments, the full length mutant human GPR156 protein is represented by the amino acid sequence as set forth in SEQ ID NO: 6. In some embodiments, a full length mutant human GPR156 protein may be represented by an amino acid sequence substantially identical to the amino acid sequence set forth in SEQ ID NO: 5 or SEQ ID NO: 6.

[0124] In some embodiments, the mutant human GPR156 protein is a short isoform human GPR156 protein. In some embodiments, the short isoform mutant human GPR156 protein is represented by the amino acid sequence as set forth in SEQ ID NO: 7. In some embodiments, the short isoform mutant human GPR156 protein is represented by the amino acid sequence as set forth in SEQ ID NO: 8. In some embodiments, a short isoform mutant human GPR156 protein may be represented by an amino acid sequence substantially identical to the amino acid sequence set forth in SEQ ID NO: 7 or SEQ ID NO: 8.

[0125] In some embodiments, the humanized *Gpr156* gene encodes a full length mutant GPR156 protein of 814 amino acids, wherein the full length mutant GPR156 protein comprises an E533D variation, and comprises an amino acid sequence substantially identical to the amino acid sequence set forth in SEQ ID NO: 5 or SEQ ID NO: 6.

[0126] In some embodiments, the humanized *Gpr156* gene encodes a short isoform mutant GPR156 protein of 810 amino acids, wherein the GPR156 protein comprises an E533D variation, and wherein the mutant GPR156 protein comprises an amino acid sequence substantially identical to the amino acid sequence set forth in SEQ ID NO: 7 or SEQ ID NO: 8.

[0127] As stated above, a humanized *Gpr156* gene can include a human *GPR156* nucleic acid portion and a rodent *Gpr156* nucleic acid portion.

[0128] In some embodiments, the human *GPR156* nucleic acid portion in a humanized *Gpr156* gene comprises a coding sequence for a GPR156 protein as described hereinabove, e.g., a wild type human GPR156 protein (either a full length or short isoform), or a mutant human GPR156 protein (either a full length or short isoform).

[0129] In some embodiments, the coding sequence is in the form of a genomic DNA. In some embodiments, the coding sequence is in the form of a cDNA (i.e., without intronic sequences). Examples of nucleic acid sequences suitable for use in providing a coding sequence are set forth in SEQ ID NOS: 9-24, with their correspondence to protein sequences being summarized below.

Table 1

Human GPR156	Amino Acid Sequence	Genomic Sequence	cDNA Sequence
Wild type full length	SEQ ID NO: 1, 814 amino acids, Glu at position 516.	SEQ ID NO: 9, 120450 nt, Codon GAA or GAG at 117164-117166 encodes Glu.	SEQ ID NO: 17
Wild type full length	SEQ ID NO: 2, 814 amino acids, Asp at position 516.	SEQ ID NO: 10, 120450 nt, Codon GAT or GAC at 117164-117166	SEQ ID NO: 18

		encodes Asp.	
Wild type short isoform	SEQ ID NO: 3, 810 amino acids, Glu at position 512.	SEQ ID NO: 11, 120438 nt, Codon GAA or GAG at 117152-117154 encodes Glu.	SEQ ID NO: 19
Wild type short isoform	SEQ ID NO: 4, 810 amino acids, Asp at position 512.	SEQ ID NO: 12, 120438 nt, Codon GAT or GAC at 117152-117154 encodes Asp.	SEQ ID NO: 20
Mutant full length	SEQ ID NO: 5, 814 amino acids, Glu at position 516, and E533D at position 533.	SEQ ID NO: 13, 120450 nt, Codon GAA or GAG at 117164-117166 encodes Glu, and codon GAT or GAC at 117,215-117,217 encodes Asp (E533D).	SEQ ID NO: 21
Mutant full length	SEQ ID NO: 6, 814 amino acids, Asp at position 516, and E533D at position 533.	SEQ ID NO: 14, 120450 nt, Codon GAT or GAC at 117164-117166 encodes Asp, and codon GAT or GAC at 117,215-117,217 encodes Asp (E533D).	SEQ ID NO: 22
Mutant short isoform	SEQ ID NO: 7, 810 amino acids,	SEQ ID NO: 15, 120438 nt,	SEQ ID NO: 23

	Glu at position 512, and E533D at position 529.	Codon GAA or GAG at 117152-117154 encodes Glu, and codon GAT or GAC at 117,203-117,205 encodes Asp (E533D).	
Mutant short isoform	SEQ ID NO: 8, 810 amino acids, Asp at position 512, and E533D at position 529.	SEQ ID NO: 16, 120438 nt, Codon GAT or GAC at 117152-117154 encodes Asp, and codon GAT or GAC at 117,203-117,205 encodes Asp (E533D).	SEQ ID NO: 24

[0130] In some embodiments, the human *GPR156* nucleic acid portion in a humanized *Gpr156* gene comprises a coding sequence comprising the ATG start codon in the first coding exon through the stop codon in the last coding exon (also the last exon) of a human *GPR156* gene. The human *GPR156* gene can be a wild type gene with the last coding exon encoding Glu at the amino acid position corresponding to position 533 of a full length wild type GPR156 protein, or a mutant gene with the last coding exon encoding Asp at the amino acid position corresponding to position 533 of a full length wild type GPR156 protein.

[0131] In some embodiments, the human *GPR156* nucleic acid portion in a humanized *Gpr156* gene further comprises, in addition to a coding sequence for a GPR156 protein, a 5' non-coding exonic sequence (e.g., the 5' portion of the first coding exon upstream of the ATG start), a 3' non-coding exonic sequence (e.g., the 3' portion of the last exon downstream of the stop codon, i.e., the 3' UTR), a nucleotide sequence downstream of the last exon of a human *GPR156* gene, or a combination thereof.

[0132] In a humanized *Gpr156* gene of this disclosure, the human *GPR156* nucleic acid portion is operably linked to the rodent *Gpr156* nucleic acid portion. In some embodiments, the rodent *Gpr156* nucleic acid portion in a humanized *Gpr156* gene can include a 5' non-coding exonic sequence or a 3' non-coding exonic sequence of a rodent *Gpr156* gene, or a combination thereof. In embodiments where a rodent *Gpr156* gene is a mouse *Gpr156* gene, a 5' non-coding exonic sequence can include, for example, exon 1, the 5' non-coding portion of exon 2 (the first coding exon), or a combination thereof, of the mouse *Gpr156* gene; and a 3' non-coding exonic sequence can include, for example, the 3' UTR of exon 10 (the 9th and last coding exon) of the mouse *Gpr156* gene. In embodiments where a rodent *Gpr156* gene is a rat *Gpr156* gene, a 5' non-coding exonic sequence can include, for example, the 5' non-coding portion of exon 1 (also being the first coding exon) of the rat *Gpr156* gene; and a 3' non-coding exonic sequence can include, for example, the 3' UTR of exon 9 (also being last coding exon and last exon) of the rat *Gpr156* gene.

[0133] In some embodiments, a humanized *Gpr156* gene comprises the 3' UTR of a human *GPR156* gene. In some embodiments, a humanized *Gpr156* gene comprises a 3' non-coding exonic sequence (e.g., the 3' UTR) of a rodent *Gpr156* gene. In some embodiments, a humanized *Gpr156* gene comprises both the 3' UTR of a human *GPR156* gene and the 3' UTR of a rodent *Gpr156* gene, with the rodent 3' UTR placed downstream of the 3' UTR of the human *GPR156* gene.

[0134] In some specific embodiments, a humanized *Gpr156* gene comprises a 5' non-coding exonic sequence of a rodent *Gpr156* gene, operably linked to a human *GPR156* nucleic acid comprising the ATG start codon in the first coding exon through the last exon (i.e., including the 3' UTR) of a human *GPR156* gene, following by a 3' non-coding exonic sequence of a rodent *Gpr156* gene. In particular embodiments, a humanized *Gpr156* gene comprises exon 1 and the 5' non-coding portion of exon 2 (the first coding exon) of a mouse *Gpr156* gene, operably linked to a human *GPR156* nucleic acid comprising the ATG start codon through the last exon (i.e., including the 3' UTR) of a human *GPR156* gene, following by the 3' UTR of exon 10 of a mouse *Gpr156* gene.

[0135] In some embodiments, a humanized *Gpr156* gene is formed from a replacement of a rodent genomic fragment of an endogenous rodent *Gpr156* gene at an endogenous rodent *Gpr156* locus with a human *GPR156* nucleic acid encoding either a wild type human GPR156 protein or a mutant human GPR156 protein comprising an E533D variation. The human *GPR156* nucleic acid portion can be a genomic DNA or a cDNA.

[0136] In some embodiments, the human *GPR156* nucleic acid encoding a human GPR156 protein comprises a human genomic fragment comprising the ATG start codon in the first coding exon through the stop codon in the last coding exon of a human *GPR156* gene, wherein the human *GPR156* gene comprises nucleotides that encode either a Glu residue (wild type) or an E533D variation. In some embodiments, the human *GPR156* nucleic acid further comprises a 5' non-coding exonic sequence (e.g., the 5' non-coding sequence in the first coding exon), or a 3' non-coding exonic sequence (e.g., the 3' UTR), of a human *GPR156* gene, or a combination thereof. In specific embodiments, the human *GPR156* nucleic acid comprises the ATG start codon the first coding exon through the last exon (i.e., including the 3' UTR) of a human *GPR156* gene.

[0137] In some embodiments, the rodent genomic fragment being replaced comprises the ATG start codon in the first coding exon through the stop codon in the last coding exon of the endogenous rodent *Gpr156* gene. In some embodiments, the rodent genomic fragment being replaced further comprises a 5' and/or a 3' non-coding exonic sequence of the endogenous rodent *Gpr156* gene. In embodiments where the rodent is a mouse, the mouse genomic fragment being replaced may also comprise exon 1, the 5' non-coding sequence in exon 2, or the 3' UTR of exon 10 of the endogenous mouse *Gpr156* gene, or a combination thereof.

[0138] In some embodiments, a rodent animal disclosed herein that comprises a humanized *Gpr156* gene in an endogenous rodent *Gpr156* locus can be heterozygous or homozygous for the humanized *Gpr156* gene.

[0139] In some embodiments, a rodent animal disclosed herein is incapable of expressing an endogenous rodent Gpr156 protein. For example, a rodent is provided where the endogenous rodent *Gpr156* gene is disrupted or deleted or modified or replaced with a humanized *Gpr156*

gene. In some embodiments, a genomic fragment in each of the two endogenous rodent *Gpr156* alleles has been replaced with a human *GPR156* nucleic acid, resulting in two humanized *Gpr156* alleles and a rodent animal incapable of expressing an endogenous rodent Gpr156 protein. In some embodiments, a genomic fragment of one endogenous rodent *Gpr156* allele has been replaced with a human *GPR156* nucleic acid, and the other endogenous rodent *Gpr156* allele has been modified to contain a disruption or deletion, resulting in a rodent animal incapable of expressing an endogenous rodent Gpr156 protein.

[0140] Also provided herein are rodents whose genome comprises a genetically modified *Gpr156* locus, wherein the genetic modification comprises a deletion in an endogenous rodent *Gpr156* gene, and optionally also comprises an insertion of a reporter gene, and wherein the reporter gene is operably linked to the endogenous rodent *Gpr156* promoter at the locus. The rodent can be heterozygous or homozygous for the genetic modification.

[0141] In some embodiments, a genomic fragment beginning from the nucleotide immediately after the start codon in the first coding exon through a subsequent coding exon (e.g., the second, third, fourth, fifth, sixth, seventh, eighth, or ninth coding exon) has been deleted, and the reporter gene is inserted immediately downstream of the start codon of the endogenous rodent *Gpr156* gene. In such linkage, expression of the reporter gene is expected to resemble the expression pattern of an unmodified endogenous rodent *Gpr156* gene.

[0142] Multiple reporter genes are known in the art and are suitable for use herein. In some embodiments, the reporter gene is a *LacZ* gene. In some embodiments, the reporter gene is a gene encoding a protein selected the group consisting of luciferase, green fluorescent protein (GFP), enhanced GFP (eGFP), cyan fluorescent protein (CFP), yellow fluorescent protein (YFP), enhanced yellow fluorescent protein (eYFP), blue fluorescent protein (BFP), enhanced blue fluorescent protein (eBFP), DsRed, and MmGFP.

[0143] For any of the embodiments described herein, the rodents can include, for example, mice, rats, and hamsters.

[0144] In some embodiments, the rodent is a mouse. In some embodiments, the rodent is a mouse of a C57BL strain, for example, a C57BL strain selected from C57BL/A, C57BL/An, C57BL/GrFa, C57BL/KaLwN, C57BL/6, C57BL/6J, C57BL/6ByJ, C57BL/6NJ, C57BL/10, C57BL/10ScSn, C57BL/10Cr, and C57BL/Ola. In other embodiments, the rodent is a mouse of a 129 strain, for example, a 129 strain selected from the group consisting of 129P1, 129P2, 129P3, 129X1, 129S1 (e.g., 129S1/SV, 129S1/SvIm), 129S2, 129S4, 129S5, 129S9/SvEvH, 129/SvJae, 129S6 (129/SvEvTac), 129S7, 129S8, 129T1, 129T2 (see, e.g., Festing et al. (1999), *Mammalian Genome* 10:836; Auerbach et al. (2000), *Biotechniques* 29(5):1024-1028, 1030, 1032). In some embodiments, the rodent is a mouse that is a mix of an aforementioned 129 strain and an aforementioned C57BL/6 strain. In certain embodiments, the mouse is a mix (i.e., hybrid) of aforementioned 129 strains, or a mix of aforementioned C57BL strains, or a mix of a C57BL strain and a 129 strain. In certain embodiments, the mouse is a mix of a C57BL/6 strain with a 129 strain. In specific embodiments, the mouse is a VGF1 strain, also known as F1H4, which is a hybrid of C57BL/6 and 129. In other embodiments, the mouse is a BALB strain, e.g., BALB/c strain. In some embodiments, the mouse is a mix of a BALB strain and another aforementioned strain.

[0145] In some embodiments, the rodent is a rat. In certain embodiments, the rat is selected from a Wistar rat, an LEA strain, a Sprague Dawley strain, a Fischer strain, F344, F6, and Dark Agouti. In other embodiments, the rat is a mix of two or more strains selected from the group consisting of Wistar, LEA, Sprague Dawley, Fischer, F344, F6, and Dark Agouti.

Targeting Vectors, Methods for Making a Rodent Comprising a Humanized *Gpr156* Gene, and Methods of Breeding

[0146] Rodents comprising a humanized Gpr156 gene can be made using various methods. In some embodiments, a targeting nucleic acid construct (i.e., a targeting vector) carrying a human *GPR156* nucleic acid is constructed. The human *GPR156* nucleic acid can include a coding sequence for a GPR156 protein as described hereinabove, e.g., a wild type human GPR156 protein (either a full length or a short isoform), or a mutant human GPR156 protein (either a full length or a short isoform). Depending on size (e.g., whether a genomic DNA or

cDNA is used), a human *GPR156* nucleic acid can be cloned directly from cDNA sources or synthetically made. Alternatively, bacterial artificial chromosome (BAC) libraries can provide human *GPR156* nucleic acid sequences.

[0147] The targeting vector can include, in addition to a human *GPR156* nucleic acid, flanking nucleic acid sequences that are of suitable lengths and homologous to rodent *Gpr156* gene sequences at an endogenous rodent *Gpr156* locus so as to be capable of mediating homologous recombination and integration of the human *GPR156* nucleic acid into the endogenous rodent *Gpr156* gene, forming a humanized *Gpr156* gene at an endogenous rodent *Gpr156* locus.

[0148] In some embodiments, the targeting vector also includes a selectable marker gene (e.g., a self deleting cassette containing a selectable marker gene, as described in U.S. Patent Nos. 8,697,851, 8,518,392 and 8,354,389, all of which are incorporated herein by reference), which can be flanked by or comprises site-specific recombination sites (e.g., *lox*P, Frt, etc.). The selectable marker gene can be placed on the targeting vector adjacent to the human *GPR156* nucleic acid to permit easy selection of transfectants.

[0149] In some embodiments, a targeting vector (such as a BAC vector) carrying a human *GPR156* nucleic acid can be introduced into rodent embryonic stem (ES) cells by, e.g., electroporation. Both mouse ES cells and rat ES cells have been described in the art. See, e.g., US 7,576,259, US 7,659,442, US 7,294,754, and US 2008-0078000 A1 (all of which are incorporated herein by reference) describe mouse ES cells and the VELOCIMOUSE® method for making a genetically modified mouse; and US 2014/0235933 A1 and US 2014/0310828 A1 (all of which are incorporated herein by reference) describe rat ES cells and methods for making a genetically modified rat.

[0150] ES cells having the human *GPR156* nucleic acid integrated in the rodent genome can be selected. After selection, positive ES clones can be modified, e.g., to remove a self deleting cassette, if desired. ES cells having the human *GPR156* nucleic acid sequence integrated in the genome are then used as donor ES cells for injection into a pre-morula stage embryo (e.g., 8-cell stage embryo) by using the VELOCIMOUSE® method (see, e.g., US

7,576,259, US 7,659,442, US 7,294,754, and US 2008-0078000 A1), or methods described in US 2014/0235933 A1 and US 2014/0310828 A1. The embryo comprising the donor ES cells is incubated until blastocyst stage and then implanted into a surrogate mother to produce an F0 rodent fully derived from the donor ES cells. Rodent pups bearing the mutant allele can be identified by genotyping of DNA isolated from tail snips using a modification of allele (MOA) assay (Valenzuela *et al.*, *supra*) that detects the presence of the human *GPR156* nucleic acid sequence or a selectable marker gene.

[0151] Further provided herein are methods of breeding a genetically modified rodent as described herein with another rodent, as well as progenies obtained from such breeding.

[0152] In some embodiments, a method is provided which comprises breeding a first genetically modified rodent as described hereinabove (e.g., a rodent whose genome comprises a humanized *Gpr156* gene at an endogenous rodent *Gpr156* locus wherein expression of the humanized *Gpr156* gene is under control of the rodent *Gpr156* promoter at the endogenous rodent *Gpr156* locus), with a second rodent, resulting in a progeny rodent whose genome comprises the humanized *Gpr156* gene. As described above, a humanized *Gpr156* gene can encode a wild type human GPR156 protein, or a mutant human GPR156 protein comprising an E533D variation. The progeny may possess other desirable phenotypes or genetic modifications inherited from the second rodent used in the breeding. In some embodiments, the progeny rodent is heterozygous for the humanized *Gpr156* gene. In some embodiments, the progeny rodent is homozygous for the humanized *Gpr156* gene.

[0153] In some embodiments, a progeny rodent is provided whose genome comprises a humanized *Gpr156* gene at an endogenous rodent *Gpr156* locus, wherein the progeny rodent is produced by a method comprising breeding a first genetically modified rodent as described hereinabove (e.g., a rodent whose genome comprises a humanized *Gpr156* gene at an endogenous rodent *Gpr156* locus wherein expression of the humanized *Gpr156* gene is under control of the rodent *Gpr156* promoter at the endogenous rodent *Gpr156* locus), with a second rodent. In some embodiments, the progeny rodent is heterozygous for the humanized *Gpr156*

gene. In some embodiments, the progeny rodent is homozygous for the humanized *Gpr156* gene.

Rodent Model of Mood Disorders

[0154] Disclosed herein are rodent models of mood disorders such as unipolar depression and anxiety disorders.

[0155] According to the Diagnostic and Statistical Manual of Mental Disorders-Fifth Edition (DSM-V), published by the American Psychiatric Association, Washington D.C., 2013, unipolar depression consists of: major depressive disorder, dysthymic disorder, mixed depressive disorder, adjustment disorder with depressed mood, and depression not otherwise specified (NOS). DSMIV criteria for depression is for 2 weeks or more, five or more symptoms from the following: i) feeling depressed mood most of the day nearly every day; ii) markedly diminished interest or pleasure in all or almost all activities; iii) significant weight loss or decreased appetite; iv) insomnia or hypersomnia; v) psychomotor agitation or retardation; vi) fatigue or loss of energy; vii) feelings of worthlessness or excessive guilt; viii) diminished ability to think or concentrate or indecisiveness; and ix) recurrent thoughts of death. DSMIV criteria for dysthymic disorder is characterized by an overwhelming yet chronic state of depression, exhibited by a depressed mood for most of the days, for more days than not, for at least 2 years. The person who suffers from this disorder must not have gone for more than 2 months without experiencing two or more of the following symptoms: i) poor appetite or overeating; ii) insomnia or hypersomnia; iii) low energy or fatigue; iv) low self-esteem; v) poor concentration or difficulty making decisions; and vi) feelings of hopelessness.

[0156] Physiological symptoms of an anxiety disorder include, but are not limited to, muscle tension, heart palpitations, sweating, dizziness, and shortness of breath. Emotional symptoms include, but are not limited to, restlessness, a sense of impending doom, fear of dying, fear of embarrassment or humiliation, and fear of something terrible happening.

[0157] In some embodiments, a rodent model of mood disorders comprises a genetically modified rodent whose genome comprises a humanized *Gpr156* gene at an endogenous rodent *Gpr156* locus, wherein the humanized *Gpr156* gene encodes a mutant human GPR156 protein comprising an E533D variation, and wherein expression of the humanized *Gpr156* gene is under control of the rodent *Gpr156* promoter at the endogenous rodent *Gpr156* locus.

[0158] In some embodiments, a rodent model of mood disorders further comprises a genetically modified rodent whose genome comprises a humanized *Gpr156* gene at an endogenous rodent *Gpr156* locus, wherein the humanized *Gpr156* gene encodes a wild type human GPR156 protein, and wherein expression of the humanized *Gpr156* gene is under control of the rodent *Gpr156* promoter at the endogenous rodent *Gpr156* locus. Such rodent has been shown herein to be able to complete a forced swim test, similar to a wild type rodent (without any humanization), and is therefore useful as an additional control rodent to be compared to.

[0159] In some embodiments, a rodent model of mood disorders further comprises a genetically modified rodent whose genome comprises a genetically modified *Gpr156* locus, wherein the genetic modification comprises a deletion in an endogenous rodent *Gpr156* gene and optionally an insertion of a reporter gene, and wherein the reporter gene is operably linked to the endogenous rodent *Gpr156* promoter at the locus. The rodent can be heterozygous or homozygous for the genetic modification. A rodent homozygous for a deletion in an endogenous rodent *Gpr156* gene has been shown herein to be unable to complete a forced swim test and have an increase in the number of marbles buried within a specified time and is therefore useful as an additional control rodent to be compared to.

[0160] In some embodiments, a genetically modified rodent is assessed in a forced swim test. In exemplary embodiments of a forced swim test, rodents can be placed in a container or beaker filled with water to ensure rodents cannot touch the bottom of the container even with their tail. The water can be kept at room temperature or at 30°C. The test can last for about 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, or 10 minutes. In some embodiments, the test lasts about 6 min. If a rodent is unable to perform the test (e.g., unable to stay afloat before the test period

ends), it is pulled out of the water as soon as it shows signs of distress. As established herein, the inability to perform a forced swim test by a rodent homozygous for the humanized *Gpr156* gene encoding an E533D variation or homozygous for the deletion of the endogenous rodent *Gpr156* gene is a characteristic reflective of mood disorders such as depression or anxiety. A wild type rodent (expressing endogenous rodent Gpr156) and a genetically modified rodent homozygous for a humanized *Gpr156* gene (without the E533D mutation) have no difficulty in completing a swim test and can be used as controls. In some embodiments, an improvement in the ability to perform the swim test can be determined based on evaluating an individual rodent, e.g., at different time points, or before and after a treatment. In some embodiments, an improvement in the ability to perform the swim test is determined based on evaluating a group of rodents having the same genetic modification, e.g., at different time points, or before and after a treatment, and an increase in the percentage of rodents in the group that can successfully perform the test indicates an improved ability. A change in the ability to perform the swim test (e.g., an increase or improvement) can be determined based on comparison of a treated group of rodents relative to a separated untreated group of rodents having the same genotype, or based on comparison of a group of rodents before a treatment relative to the same group of rodents after the treatment.

[0161] The Forced Swim Test (or Porsolt Test, Porsolt et al., Arch Int Pharmacodyn Ther. 229(2):327-336 (1977)) is used in the art to assess helplessness based on how a rodent reacts to an unpleasant environment. Helplessness is a characteristic feature in depression. A rodent (e.g., a mouse) that is placed in water typically tries to escape. However, if the rodent exhibits a depressive behavior, the rodent will simply float without attempting to escape until rescued. Learned helplessness is a phenomenon discovered and explored initially by Seligman and his colleagues in the 1970s (Seligman, Annu Rev Med. 23(1):407-412 (1972)). One of the major attractions of this model is that it is derived from the cognitive view of depression in which events are viewed negatively and interpreted as not controllable, leading to feelings of anxiety and helplessness when dealing with them. There are many similarities between the learned helplessness in animal and human depression. For example, uncontrollable stressful events, which precipitate depression-like behaviors observed in

rodents, similarly precede the onset of some clinical depression in humans (Gold et al., N Eng J Med. 319(6):348-353 (1988); Lloyd, Arch Gen Psychiatry 37(5):541-548 (1980)). Moreover, exposure of animals to similar but controllable events does not produce relevant behavioral changes (Corum and Thurmond, Psychosom Med. 39(6):436-43 (1977); Weiss et al., A model for neurochemical study of depression, In M. Spiegelstein and A. Levy Eds., Elsevier, Amsterdam, 1982, pp. 195-223). As described in the art, the Porsolt Forced Swim test extends over a two-day period, with the first day being the training day and the second day being the testing day. During the training trial, animals learn that escape from the tank is not possible. On the test day (2nd day), animals are placed into the container of water for five minutes. During the second day, the amount of time the animals spend immobile is measured as the dependent variable.

[0162] It has been shown herein that genetically modified mice homozygous for the humanized *Gpr156* gene encoding an E533D variation and genetically modified mice homozygous for the deletion of the mouse *Gpr156* gene were unable to perform a 6 minute forced swim test corresponding to the training session. The mice were initially able to swim but after a couple of minutes of trying to find an escape, it appeared as if mice "panicked" in the water. These mice then started to display an abnormal behavior characterized by frenetic swimming, repetitive diving and spinning under water and become unable to stay afloat. If not quickly retrieved out of the water, these mice drown. In contrast, a wild type mouse (expressing endogenous mouse Gpr156) and a genetically modified mouse homozygous for the humanized *Gpr156* gene (without the mutation) had no difficulty completing the 6 minutes swim test.

[0163] The peculiar behavior displayed by genetically modified mice homozygous for the humanized *Gpr156* gene encoding an E533D variation or genetically modified mice homozygous for the deletion of the mouse GPR156 gene has not been described by others in the literature. Due to the fact that these mice are unable to complete the test, the inventors were not able to measure learned helplessness. However, the inventors observed that 90% of female and 60% of male genetically modified mice that are homozygous for the humanized *Gpr156* gene encoding an E533D variation failed the training swim session while genetically

modified mice homozygous for a humanized *Gpr156* gene encoding a wild type human GPR156 protein easily completed the test. Similarly, 90% of female and 53% of male genetically modified mice homozygous for a deletion of the mouse *Gpr156* gene failed the training swim session while their wild-type littermates completed the test. Similar results were obtained when the swim test training session was run in water heated at 30°C instead of room temperature (~23°C) or when using a water maze apparatus which is bigger and presents with a platform (escape option).

[0164] It has also been shown herein that rodents homozygous for the humanized *Gpr156* gene encoding an E533D variation improved on their ability to perform the swim test after treatment with fluoxetine (a selective Serotonin reuptake inhibitor) or with imipramine (an inhibitor of reuptake of norepinephrine, acetylcholine, dopamine and serotonine). Improvement in the ability to perform a 6-minute swim test has been observed for $GPR156^{E533D/E533D}$ mice after treatment with fluoxetine (10 mg/kg, i.p., daily) or imipramine (15 mg/kg, i.p., daily, six days/week) for 9 weeks.

[0165] In some embodiments, a genetically modified rodent is assessed in a marble burying test. Marble burying task is used as an indicator of obsessive compulsive-like behavior and/or anxiety-like behavior. Rodents with OCD-like symptoms tend to engage in a high degree of repetitive behavior (including digging) and rodents with a high degree of anxiety tend to engage in a high degree of digging in novel contexts.

[0166] In some embodiments, a marble burying test can be performed as follows. Rodent cages can be evenly filled with corn cob bedding and marbles are evenly spaced on top of the bedding. Each rodent is placed at the center of the cage and the test is run for about 10 minutes. After 10 min, the animals are removed from cages and the marbles at least two-thirds buried are counted.

[0167] As examples, it has been shown herein that genetically modified male mice homozygous for the humanized *Gpr156* gene encoding an E533D variation or homozygous for the deletion of the mouse GPR156 gene exhibited a trend toward an increase in the number of marbles buried in 10 minutes (see e.g., FIG. 4).

[0168] The rodents and models disclosed herein permit a better understanding of the function of the GPR156 protein and the development of mood disorders. In addition, such rodents may be used in the screening, testing and development of therapeutic agents for the treatment of mood disorders such as unipolar depression or anxiety disorders.

[0169] In some embodiments, an effect of a therapeutic agent for treating unipolar depression or an anxiety disorder is determined herein by administering the agent to a rodent disclosed herein, e.g., a rodent carrying a humanized *Gpr156* gene encoding an E533D variant, subjecting the rodent to one or more tests (e.g., a forced swim test or marble burying test), and determining whether the candidate therapeutic agent has any effect on the performance of the rodent in the test, e.g., any improvement on the ability to complete the swim test, or any decrease in the number of marbles buried in a marble burying test.

[0170] Therapeutic agents that can be tested in a rodent disclosed herein include both commercially available antidepressants and anxiolytics, and candidate compounds under development for treating unipolar depression and anxiety disorders. Both small molecule chemical compounds and nucleic acids (e.g., gene therapy drugs) are included.

[0171] In some embodiments, the antidepressant is a selective serotonin reuptake inhibitor (SSRI). Suitable examples of SSRIs include, but are not limited to, Citalopram (Celexa®), Escitalopram (Lexapro®, Cipralex®), Paroxetine (Paxil®, Seroxat®), Fluoxetine (Prozac®), Fluvoxamine (Luvox®), and Sertraline (Zoloft®, Lustral®).

[0172] In some embodiments, the antidepressant is a serotonin-norepinephrine reuptake inhibitor (SNRI). Suitable examples of SNRIs include, but are not limited to, Desvenlafaxine (Pristiq®), Duloxetine (Cymbalta®), Levomilnacipran (Fetzima®), Milnacipran (Ixel®, Savella®), Tofenacen (Elamol®,Tofacine®), and Venlafaxine (Effexor®).

[0173] In some embodiments, the antidepressant is a norepinephrine reuptake inhibitor (NRI). Suitable examples of NRIs include, but are not limited to, Reboxetine (Edronax®), Viloxazine (Vivalan®), and Atomoxetine (Strattera).

[0174] In some embodiments, the antidepressant is lithium.

[0175] In some embodiments, the antidepressant is a serotonin modulator and stimulator (SMS). Suitable examples of SMSs include, but are not limited to, Vilazodone (Viibryd®) and Vortioxetine (Trintellix®).

[0176] In some embodiments, the antidepressant is a serotonin antagonist and reuptake inhibitor (SARI). Suitable examples of SARIs include, but are not limited to, Etoperidone (Axiomin®, Etonin®), Nefazodone (Nefadar®, Serzone®), and Trazodone (Desyrel®).

[0177] In some embodiments, the antidepressant is a tricyclic antidepressant (TCA). Suitable examples of TCAs include, but are not limited to, Amitriptyline (Elavil®, Endep®), Amitriptylinoxide (Amioxid®, Ambivalon®, Equilibrin®), Clomipramine (Anafranil®), Desipramine (Norpramin®, Pertofrane®), Dibenzepin (Noveril®, Victoril®), Dimetacrine (Istonil®), Dosulepin (Prothiaden®), Doxepin (Adapin®, Sinequan®), Imipramine (Tofranil®), Lofepramine (Lomont®, Gamanil®), Melitracen (Dixeran®, Melixeran®, Trausabun®), Nitroxazepine (Sintamil®), Nortriptyline (Pamelor®, Aventyl®), Noxiptiline (Agedal®, Elronon®, Nogedal®), Pipofezine (Azafen®/Azaphen®), Protriptyline (Vivactil®), and Trimipramine (Surmontil®). Also included are Butriptyline (Evadyne®), demexiptiline (Deparon®, Tinoran®), Imipraminoxide (Imiprex®, Elepsin®), iprindole (Prondol®, Galatur®, Tetran®), metapramine (Timaxel®), propizepine (Depressin®, Vagran®), and quinupramine (Kinupril®, Kevopril®). Also included are Opipramol (Insidon®) and Tianeptine (Stablon®).

[0178] In some embodiments, the antidepressant is a tetracyclic antidepressants (TeCA). Suitable examples of TeCAs include, but are not limited to, Amoxapine (Asendin®), Maprotiline (Ludiomil®), Mianserin (Bolvidon®, Norval®, Tolvon®), Mirtazapine (Remeron®), and Setiptiline (Tecipul®).

[0179] In some embodiments, the antidepressant is a monoamine oxidase inhibitors (MAOI). Suitable examples of MAOIs include, but are not limited to, Iproniazid (Marsilid®), Isocarboxazid (Marplan®), Phenelzine (Nardil®), Selegiline (Eldepryl®, Zelapar®,

Emsam®), Tranylcypromine (Parnate®), Metralindole (Inkazan®), Moclobemide (Aurorix®, Manerix®), Pirlindole (Pirazidol®), and Toloxatone (Humoryl®). Others include, for example, benmoxin (Neuralex®), Caroxazone (Surodil®, Timostenil®), iproclozide (Sursum®), mebanazine) (Actomol®), nialamide (Niamid®), octamoxin (Ximaol®), pheniprazine (Catron®), phenoxypropazine (Drazine®), pivhydrazine (Tersavid®), safrazine (Safra®), Eprobemide (Befol®), and minaprine (Brantur®, Cantor®).

[0180] In some embodiments, the antidepressant is an atypical antipsychotic. Suitable examples of atypical antipsychotic include, but are not limited to, Amisulpride (Solian®), Lurasidone (Latuda®), and Quetiapine (Seroquel®).

[0181] In some embodiments, the antidepressant is Agomelatine (Valdoxan®), Bifemelane (Alnert®, Celeport®), Bupropion (Wellbutrin®), Ketamine (Ketalar®), Tandospirone (Sediel®), or Teniloxazine (Lucelan®, Metatone®).

[0182] In some embodiments, the anxiolytic agent is a benzodiazepine, including but not limited to, Alprazolam (Xanax®), Bromazepam (Lectopam®, Lexotan®), Chlordiazepoxide (Librium®), Clonazepam (Klonopin®, Rivotril®), Clorazepate (Tranxene®), Diazepam (Valium®), Flurazepam (Dalmane®), Lorazepam (Ativan®), Oxazepam (Serax®, Serapax®), Temazepam (Restoril®), Triazolam (Halcion®), and Tofisopam (Emandaxin®, Grandaxin®).

[0183] In some embodiments, the anxiolytic agent is a carbamate, including but not limited to, meprobamate (Miltown®, Equanil®).

[0184] In some embodiments, the anxiolytic agent is an antihistamine, including but not limited to, Hydroxyzine (Atarax®), Chlorpheniramine (Chlor-Trimeton®), and diphenhydramine (Benadryl®).

[0185] In some embodiments, the anxiolytic agent is an azapirone, including but not limited to, Buspirone (Buspar®) and Tandospirone (Sediel®).

[0186] In some embodiments, the anxiolytic agent is an SSRI, SNRI, TCA, TeCA, or MAOI, as described herein.

[0187] In some embodiments, the anxiolytic agent is Mebicar (Mebicarum®), Fabomotizole (Afobazole®), Selank, Bromantane, Emoxypine, Pregabalin, Menthyl isovalerate, or Menthyl isovalerate (Validol®).

[0188] Administration of the antidepressant or anxiolytic agents to a rodent can be by any suitable route including, but not limited to, parenteral, intravenous, oral, subcutaneous, intra-arterial, intracranial, intrathecal, intraperitoneal, topical, intranasal, or intramuscular. Administration may also be by continuous infusion, local administration, sustained release from implants (gels, membranes or the like), and/or intravenous injection.

[0189] The agents can be given to a rodent for a period of time appropriate, for example, 3, 4, 5, 6, 7, 8, 9, or 10 weeks, or longer if needed.

[0190] The present description is further illustrated by the following examples, which should not be construed as limiting in any way. The contents of all cited references (including literature references, issued patents, and published patent applications as cited throughout this application) are hereby expressly incorporated by reference.

EXAMPLES

Example 1. Generation of Genetically Modified Mice.

[0191] *Gpr156* targeting constructs were designed as follows. For knockout of mouse *Gpr156,* a bacterial artificial chromosome (BAC) containing mouse *Gpr156* genomic sequence was modified such that a floxed *lacZ* reporter cassette containing a neomycin resistance gene under the control of the human *UBC* (ubiquitin) promoter replaced *Gpr156* exon 2 (or coding exon 1, i.e., the first coding exon) just after the start ATG codon to the end of exon 5 (or coding exon 4). The cassette was cloned such that the *lacZ* coding sequence was in frame with the start ATG codon. This construct was electroporated into100% C57Bl/6NTac mouse embryonic stem cells. To humanize the *Gpr156* locus, the mouse ATG to stop genomic sequence was replaced with human *GPR156* ATG to 100 bp beyond the end of the 3' untranslated region. This human *GPR156* DNA encodes the long isoform (814

amino acids) with Glu (E) at position 516. A self-deleting neomycin resistance cassette was placed just after the humanization, at the 3' end of the *Gpr156* locus. This targeting construct was then further modified by adding a single A to T base pair substitution in human *GPR156* coding exon 9 to generate the E533D human mutation and a puromycin resistance cassette replaced the neomycin version. These constructs were electroporated into a 50% C57Bl/6NTac/50% 129SvEvTac mouse embryonic stem cell line. Successfully targeted clones from all electroporations were identified by TAQMAN analysis. *Gpr156*$^{-/+}$, *Gpr156*$^{humIn/+}$, and *Gpr156*$^{humIn\ G533D/+}$ mice were generated using the VelociGene© method (Valenzuela 2003 Nat Biotech PMID:12730667; Poueymirou 2007 Nat Biotech PMID:17187059) and backcrossed to C57Bl/6NTac as needed.

Example 2. Assessing Expression of Gpr156 Through A Reporter

[0192] Using genetically modified mice which comprise a deletion in an endogenous mouse *Gpr156* gene and an insertion of a reporter gene, wherein the reporter gene is operably linked to the endogenous mouse *Gpr156* promoter at the endogenous mouse *Gpr156* locus, the inventors were able to confirm expression of Gpr156 in the brain of adult mice with significant expression in olfactory bulb, hippocampus, habenula, fasciculus retroflexus, and the colliculus. These regions of the brain have been associated with depression and anxiety in humans and animal models of depression. The strongest expression was detected in the habenula; medial and lateral habenula show reduced volume in patients having a depressive disorder. Functional imaging studies in humans highlighted the lateral habenula as selectively activated by negative outcomes (Shepard et al., Schiz Bull. 32:417-421 (2006), Ullsperger and von Cramon, J Neurosci. 23:4308-4314 (2003)). In addition, accumulating evidence in rodents and humans demonstrate that the lateral habenula is hyperactive in several models of major depression, with increased activity linked to exposure to different kinds of stressors (Li et al., Nature 470:535-539 (2011), Mirrione et al., Front. Hum. Neurosci. 8:29 (2014), Morris et al., NeuroImage 10:163-172 (1999), Shumake et al., Brain Res. 963:274-281 (2003), Wirtshafter et al., Brain Res. 633:21-26 (1994)). The habenula has also been successfully tested as a novel target for deep brain stimulation in the treatment of depression (Sartorius et al., Biol. Psychiatry 67:e9-e11 (2010)).

Example 3. Methods Used to Assess Genetically Modified Rodents

Animals

[0193] All procedures were conducted in compliance with protocols approved by Regeneron Pharmaceuticals Institutional Animal Care and Use Committee (IACUC). All mice are housed on 12 hours on off light cycle (7am to 7pm) with free access to water and food.

Marble burying task

[0194] Marble burying task is used as an indicator of obsessive compulsive-like behavior and/or anxiety-like behavior. When mice are placed in a novel cage, a common response is an increase in digging behavior. This test is used to evaluate the effect of drugs on the mice's natural and repetitive behavior of burying marbles, which is reduced by drugs with anti-compulsive property (Njung'e and Handley, Br J Pharmacol. 104:105-112 (1991b), Thomas et al., Psychopharmacol. 204:361-373 (2009)).

[0195] Tecniplast Mouse Cages (39 x 19 x 16 cm) were evenly filled with 5 cm of corn cob bedding. 24 clear glass marbles (15 cm diameter) were evenly spaced on top of the bedding in a 4 X 6 grid pattern. Each mouse was placed at the center of the cage and the test was run for 10 minutes. After 10 min, the animals were removed from cages and the number of marbles at least two-thirds buried was counted.

Forced swim test

[0196] Mice were placed in a 5 liter clear plastic beaker (25 x 18 cm diameter) filled with 4 liters of 23-25^{o}C tap water to ensure mice cannot touch the bottom of the flask even with their tail. Four animals were recorded simultaneously using an ANY-maze™ video-tracking system (Stoelting Co.). Data were recorded for 6 minutes. Flask were separated by 3 sided white plastic partitions (40 cm high x 25 cm wide). Swim test was recorded for 6 minutes. Recordings were set up to start automatically if there was motion in the flask so that recorded

times are specific for each individual mouse. If a mouse was unable to perform the test, it was pulled out of the water as soon as it showed signs of distress.

[0197] The 6 minutes recording was broken into three time segments (0-100 seconds, 101-200 second and last 158 seconds) to get a better sampling of behaviors across time.

[0198] Main data points analyzed for the FST were swim time, time mobile, time immobile, speed, distance traveled, rearing in the swim zone, mobile episodes and immobile episodes. Immobility is defined as 60% of total body mass being immobile for 2 seconds. This parameter allows for small movements of hind legs and feet to maintain balance and or to tread water without be considered actively swimming.

Balance beam

[0199] The balance beam (Maze Engineers) was used to assess coordination and sensorineural balance. Mice were trained to walk on a one meter long, 6 mm wide beam with an open platform at one end at its beginning and an open sided black box as a finish point. The beam was elevated above a cloth hammock to catch any mouse that slips off the beam. During the training session, mice were first placed right next to the end box and then placed progressively further away from it until the mouse could walk the beam. Then three subsequent 1-minute trials were run with a break of 30 second between runs. The mouse was place in front of the open platform facing finish point and is scored for number of slips and time taken to reach the finish point.

Rotarod test

[0200] Motor coordination and balance were evaluated by an accelerating Rotarod from IITC Life Science series 8. Mice were placed on a horizontally oriented, rotating cylinder suspended above a cage floor for 3 minutes. The rotating cylinder was low enough not to injure the animal but high enough to induce avoidance of fall. The length of time that a given animal stays on this rotating rod is a measure of their balance, coordination, physical condition, and motor-planning. Animals were first trained to stay on a rotarod (3.5 cm

diameter, 9 cm wide) rotating at progressively higher speeds (maximum speed 15 rpm, 3 trials). The next day, each mouse was placed on the rotarod at up to 15 rpm for 3 consecutive trials (30 minutes between trials) for 180 seconds per trial. The latency to fall off was recorded or a score of up to 180 was assigned if the animals did not fall off. The median score of 3 trials for each mouse was used in analysis.

Catwalk

[0201] To evaluate footfall and gait changes, mice were evaluated using the CatWalk™ XT (Noldus) video based automated free-walking gait analysis system which allows for quantitative assessment of up to 35 gait parameters (from footprint size to locomotion dynamics). This system allows for the animals to voluntarily move at preferred speeds in a similar fashion to clinical gait testing in humans. Animals were placed at the beginning of the runway, with the open end in front of them. Mice spontaneously run to the end of the runway to attempt to escape. Each mouse was evaluated until it had three successful catwalk runs and mice must travel the recording area of the walkway within 5 seconds for the run to be counted. The device consisted of a 130 cm long hardened glass platform with an adjustable alleyway to limit movements to straight lines, a red overhead light, a green LED light attached to the glass platform, and a high-speed color camera mounted below the platform. The green LED light attached to the apparatus emitted light into the glass plate, and this light was only refracted wherever rodent paws contacted the glass, allowing the high speed digital camera to capture precise rodent paw placement in real time. The overhead red light created contrast for recording of the body outline. The visual data was digitized and transferred to an attached computer where the CatWalk™ XT software could be used for semi-automated labeling and analysis of static and dynamic gait kinematics via distance, time, and intensity differences between paw prints. Gait data was then exported for data storage and subsequent analyses.

[0202] The camera recorded, and the software of the system measured the footprints. The footprints were analyzed for abnormalities in paw placement.

Open field

[0203] The open field is an empty relatively large, brightly lit rectangular shape arena, in which the animal's activity is measured. The assessment reflects novel environment exploration, general locomotor activity, and can provide an initial screen for anxiety-related behavior in rodents. Mice were placed in the open field apparatus (Kinder scientific; 16"Wx16"L x15"H chamber) and scored for 60 minutes. The chamber was set up as two zones; a center zone grid (6"x6") and an outer zone grid (16"x16") and animal movements are collected by 32 infra-red photobeams. A decrease in exploratory behavior into the center zone has been associated with an anxiety phenotype. The amount of times a mouse crosses a grid line (i.e. move from one grid to another) was counted, as well as number of times rearing, time spent immobile, and number of grooming events. In the Kinder Scientific apparatus, these events were automatically counted via computer software and infrared beams around the open field.

Tail twist

[0204] The tail twist is a simple method that can be used to screen for possible vestibular dysfunction. The mouse was placed onto a flat surface and grasping the mouse mid-tail twist the tails so that the mouse was turned onto it's back. If the animal had difficulties righting itself back onto its feet, this could indicate a vestibular deficit.

Righting reflex

[0205] Righting Reflex is a simple assay to assess motor coordination and vestibular dysfunction. Mice were placed on their back on a flat surface. The time taken to right itself (up to a maximum of 30 sec) was measured.

Wire hanging test

[0206] Wire hanging is an alternate test to measure grip strength of either the hind paws or fore paws. Slipping of the feet, or inability to hang can indicate a deficit. A wire cage top suspended at least 12 inches from table top with blue pad was used. Mice were allowed to grip the wire cage top with either their hind paws or fore paws, and the total time mice held onto the bar was measured during a 60 second interval. Three 60 second intervals per mouse was measured. Latency to fall was recorded.

Example 3. Assessing Mice in Various Behavioral Assays

Forced Swim Test

[0207] Mice were assessed in a 6 minute forced swim test; not all mice were able to perform the task. FIG. 2 shows the duration of the swim test for different *GPR156/Gpr156* genotypes tested. Data are expressed as mean ± SEM for each animal group.

[0208] The data show that genetically modified mice homozygous for the humanized *Gpr156* gene encoding an E533D variation and genetically modified mice homozygous for the deletion of the mouse *Gpr156* gene exhibited an inability to perform a forced swim test. The mice were initially able to swim but after a couple of minutes of trying to find an escape, it appeared as if mice "panicked" in the water. These mice then started to display an abnormal behavior characterized by frenetic swimming, repetitive diving and spinning under water and become unable to stay afloat. If not quickly retrieved out of the water, these mice drown. In contrast, a wild type mouse (expressing endogenous mouse *Gpr156*) and a genetically modified mouse homozygous for the humanized *Gpr156* gene (without the mutation) had no difficulty completing the 6 minutes swim test.

[0209] The peculiar behavior displayed by genetically modified mice homozygous for the humanized *Gpr156* gene encoding an E533D variation or genetically modified mice homozygous for the deletion of the mouse *Gpr156* gene has not been described by others in the literature. Due to the fact that these mice are unable to complete the test, the inventors were not able to measure learned helplessness. However, the inventors observed that 85% of female and 90% of male genetically modified mice that are homozygous for the humanized

Gpr156 gene encoding an E533D variation failed the training swim session while genetically modified rodent, homozygous for a humanized *Gpr156* gene encoding a wild type human GPR156 protein easily completed the test. Similarly, 90% of female and 53% of male genetically modified mice homozygous for a deletion of the mouse *Gpr156* gene failed the training swim session while their wild-type littermates completed the test. Similar results were obtained when the swim test training session was run in water heated at 30°C instead of room temperature (~ 23°C) or when using a water maze apparatus which is bigger and presents with a platform (escape option). The observations from the forced swim test suggest that the E533D point mutation leads to a deficit or a loss in the function of the GPR156 protein.

[0210] Mice were also assessed for their coordination, balance and motor functions, their general locomotion activity, and their vestibular functions in rotarod, catwalk, balance beam, open field, righting reflex, tail hanging reflex and wire hanging tests. Genetically modified mice homozygous for the humanized *Gpr156* gene encoding an E533D variation or wild type humanized *Gpr156* gene did not exhibit any gross deficits in balance, and coordination or motor deficits and/or vestibular dysfunction, as summarized in Table 2 below.

[0211] To investigate whether the inability to perform the swim test could be a feature of anxiety, mice were treated with intraperitoneal injection of Fluoxetine, a selective Serotonin Reuptake Inhibitor (SSRI, 10 mg/kg), daily for 4 weeks. Mice were tested for a 6 minute forced swim test weekly before and after daily treatment with intraperitoneal injection of Fluoxetine 10 mg/kg for 4 weeks. Total time of swim test (in seconds) before and after daily treatment with Fluoxetine was recorded. As shown in FIG. 3A, in the initial forced swim test (baseline, before treatment), none of the mice homozygous for the humanized *Gpr156* gene encoding an E533D variation tested were able to perform the 6 minutes swim test. After 4 weeks of treatment with fluoxetine, 62.5% of males (5 out of 8 mice) and 50% of female (5 out of 10) were able to successfully perform the 6 minutes swim test. A group of untreated females $GPR156^{E533D}$ was also run in parallel as control, after 4 weeks only 20% (1 out of 5) females achieved the swim test. Data were expressed as mean ± SEM for each animal group.

[0212] To test whether Fluoxetine could reverse the impaired swim test phenotype, GPR156$^{hum/hum}$ and GPR156$^{E533D/E533D}$ mice were treated with Fluoxetine 10 mg/kg (i.p) daily for 6 days/week for 9 weeks. Similar to GPR156$^{-/-}$ mice, GPR156$^{E533D/E533D}$ mice showed an impairment in the swim test with about 85 % of female and 90% of male failing to complete a 6 minutes swim test. Therefore, mice were subjected to a swim test (baseline) and animals able to perform the 6 minutes swim test were excluded from the study. The remaining mice were then split into 2 groups, treated and untreated with Fluoxetine. As shown in FIG. 3B, while none (0/13) of the GPR156^{E533D} mice were able to perform the swim test the first time, following a 9 weeks treatment with Fluoxetine, 61% (8/13) of the mice achieved the test. In contrast, in the untreated group, while some mice improved their performance, only 1/11 mice achieved the swim test at 9 weeks (see FIG. 3C; see also FIG. 3B).

[0213] Treatment with another antidepressant drug, imipramine (Tofranil), also improves the impaird swim phenotype in GPR156$^{E533D/E533D}$. Imipramine is a tricyclic antidepressant that inhibits reuptake of norepinephrine, acetylcholine, dopamine and serotonin. For this study, mice were subjected to a swim test (baseline) and animals able to perform the 6 minutes swim test were excluded from the study. Mice were treated with imipramine 15 mg/kg (i.p) daily for 6 days/week for 9 weeks. As shown in FIG. 3D, treatment with imipramine also induced an improvement in the performance of the GPR156$^{E533D/E533D}$ mice to complete the swim test.

[0214] Mice were additionally assessed in a marble burying test. As shown in FIG. 4, genetically modified male mice homozygous for the humanized *Gpr156* gene encoding an E533D variation or homozygous for the deletion of the mouse *Gpr156* gene exhibited a trend toward an increase in the number of marbles buried in 10 minutes. Data are expressed as mean ± SEM for each animal group.

TABLE 2

Phenotype	Behavioral assay	GPR156$^{-/-}$	GPR156 humanized	GPR156E533D
Depression	Forced swim test	-90% of female and 67% of males failed the training swim session (not a known depression phenotype)	Similar to wildtype, all animals achieved the swim test	-85% of female and 90% of males failed the training swim session -Following 4–9 weeks of treatment with Fluoxetine (10 mg/kg, i.p., daily), 53% (7/13) of GPR156$^{E533D/E533D}$ mice achieved the test while none of the mice were able to perform the swim test prior to treatment,
Coordination, balance and motor deficits	Rotarod	No significant difference	No significant difference	Overall all mice completed the task but some males GPR156$^{E533D+/+}$ fell off in 1 of the 3 consecutive trials
	Catwalk	No significant difference	No significant difference	No significant difference
	Balance beam	No significant difference	No significant difference	No significant difference
Activity, general locomotion	Open field	- Slight increase in immobility and decrease in ambulation - Slight decrease in number of rears and rearing time compared to wild type mice littermates	No significant difference	No significant difference
Vestibular dysfunction	Righting reflex Tail hanging reflex Wire hanging	No significant difference	No significant difference	No significant difference
Anxiety	Marble burying	Males and females GPR156$^{-/-}$ may show increased number of marbles buried in 10 min	No significant difference	Males GPR156$^{E533D+/+}$ may show increased number of marbles buried in 10 min

WHAT IS CLAIMED IS:

1. A genetically modified rodent, whose genome comprises a humanized G protein-coupled receptor 156 (*GPR156*) gene at an endogenous rodent *Gpr156* locus, wherein the humanized *Gpr156* gene comprises a rodent *Gpr156* nucleic acid portion and a human *GPR156* nucleic acid portion, wherein the humanized *Gpr156* gene encodes a wild type human GPR156 protein or a mutant human GPR156 protein comprising Asp at an amino acid position corresponding to position 533 in a full length wild type human GPR156 protein (E533D variation), wherein expression of the humanized *Gpr156* gene is under control of the rodent *Gpr156* promoter at the endogenous rodent *Gpr156* locus, and wherein the rodent is a mouse or a rat.

2. The rodent of claim 1, wherein the humanized *Gpr156* gene encodes a mutant human GPR156 protein comprising an E533D variation.

3. The rodent of claim 2, wherein the human *GPR156* nucleic acid portion comprises the ATG start codon through the stop codon of a human *GPR156* gene, wherein the human *GPR156* gene comprises nucleotides that encode the E533D variation.

4. The rodent of claim 3, wherein

(i) the human *GPR156* nucleic acid portion further comprises the 3' UTR of the human *GPR156* gene; and/or

(ii) the rodent *Gpr156* nucleic acid portion comprises a 5' non-coding exonic sequence of a rodent *Gpr156* gene, optionally wherein the humanized *Gpr156* gene further comprises a 3' non-coding exonic sequence of a rodent *Gpr156* gene.

5. The rodent according to any one of claims 2-4, wherein the humanized *Gpr156* gene is formed from a replacement of a rodent genomic fragment of an endogenous rodent *Gpr156* gene at the endogenous rodent *Gpr156* locus with the human *GPR156* nucleic acid portion, optionally wherein the human *GPR156* nucleic acid portion comprises the ATG start codon through the stop codon of a human *GPR156* gene, wherein the human *GPR156* gene comprises nucleotides that encode the E533D variation.

6. The rodent according to any one of claims 2-5, wherein the mutant human GPR156 protein comprising an E533D variation comprises an amino acid sequence as set forth in SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, or SEQ ID NO: 8.

7. The rodent of claim 1, wherein the humanized *Gpr156* gene encodes a wild type human GPR156 protein, wherein the human *GPR156* nucleic acid portion comprises the ATG start codon through the stop codon of a human *GPR156* gene.

8. The rodent of claim 7, wherein

(i) the human *GPR156* nucleic acid portion further comprises the 3' UTR of the human *GPR156* gene; and/or

(ii) the rodent *Gpr156* nucleic acid portion comprises a 5' non-coding exonic sequence of a rodent *Gpr156* gene, optionally wherein the humanized *Gpr156* gene further comprises a 3' non-coding exonic sequence of a rodent *Gpr156* gene.

9. The rodent of claim 7 or 8, wherein the humanized *Gpr156* gene is formed from a replacement of a rodent genomic fragment of an endogenous rodent *Gpr156* gene at the endogenous rodent *Gpr156* locus with the human *GPR156* nucleic acid portion, optionally wherein the human *GPR156* nucleic acid portion comprises the ATG start codon through the stop codon of a human *GPR156* gene.

10. The rodent according to any one of claims 7-9, wherein the wild type human GPR156 protein comprises an amino acid sequence as set forth in SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, or SEQ ID NO: 4.

11. The rodent according to any one of claims 1-10, wherein the rodent is heterozygous for the humanized *Gpr156* gene.

12. The rodent according to any one of claims 1-10, wherein the rodent is homozygous for the humanized *Gpr156* gene.

13. An isolated rodent cell or tissue, whose genome comprises a humanized *Gpr156* gene at an endogenous rodent *Gpr156* locus, wherein the humanized *Gpr156* gene comprises a rodent *Gpr156* nucleic acid portion and a human *GPR156* nucleic acid portion, wherein the humanized *Gpr156* gene encodes a wild type human GPR156 protein or a mutant human GPR156 protein comprising an E533D variation, wherein expression of the humanized *Gpr156* gene is under control of the rodent *Gpr156* promoter at the endogenous rodent *Gpr156* locus, and wherein the rodent cell or tissue is a mouse cell or tissue, or a rat cell or tissue.

14. The isolated rodent cell or tissue of claim 13, wherein the human *GPR156* nucleic acid portion comprises the ATG start codon through the stop codon of a human *GPR156* gene, wherein the human *GPR156* gene comprises nucleotides that encode either Glu or Asp at a position corresponding to position 533 in a full length wild type human GRP156 protein.

15. The isolated rodent cell or tissue of claim 14, wherein

(i) the human *GPR156* nucleic acid portion further comprises the 3' UTR of the human *GPR156* gene; and/or

(ii) the rodent *Gpr156* nucleic acid portion comprises a 5' non-coding exonic sequence of a rodent *Gpr156* gene, and optionally further comprises further comprises a 3' non-coding exonic sequence of a rodent *Gpr156* gene.

16. The isolated rodent cell or tissue according to any one of claims 13-15, wherein the humanized *Gpr156* gene is formed from a replacement of a rodent genomic fragment of an endogenous rodent *Gpr156* gene at the endogenous rodent *Gpr156* locus with the human *GPR156* nucleic acid portion, optionally wherein the human *GPR156* nucleic acid portion comprises the ATG start codon through the stop codon of a human *GPR156* gene, wherein the human *GPR156* gene comprises nucleotides that encode either Glu or Asp at a position corresponding to position 533 in a full length wild type human GRP156 protein.

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17. The isolated rodent cell or tissue of any one of claims 13-16, wherein the humanized *Gpr156* gene encodes a mutant human GPR156 protein comprising an E533D variation, wherein the mutant human GPR156 protein comprises an amino acid sequence as set forth in SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, or SEQ ID NO: 8.

18. The isolated rodent cell or tissue of any one of claims 13-16, wherein the humanized *Gpr156* gene encodes a wild type human GPR156 protein, wherein the wild type human GPR156 protein comprises an amino acid sequence as set forth in SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, or SEQ ID NO: 4.

19. The isolated rodent cell or tissue according to any one of claims 13-18, wherein the isolated rodent cell or tissue is heterozygous for the humanized *Gpr156* gene.

20. The isolated rodent cell or tissue according to any one of claims 13-18, wherein the isolated rodent cell or tissue is homozygous for the humanized *Gpr156* gene.

21. The isolated rodent cell or tissue according to any one of claims 13-20, wherein the rodent cell is a rodent embryonic stem (ES) cell.

22. A method of making a genetically modified rodent, comprising:

modifying a rodent genome so that the modified genome comprises a humanized *Gpr156* gene at an endogenous rodent *Gpr156* locus, wherein the humanized *Gpr156* gene comprises a rodent *Gpr156* nucleic acid portion and a human *GPR156* nucleic acid portion, wherein the humanized *Gpr156* gene encodes a wild type human GPR156 protein or a mutant human GPR156 protein comprising an E533D variation, and wherein expression of the humanized *Gpr156* gene is under control of the rodent *Gpr156* promoter at the endogenous rodent *Gpr156* locus; and

obtaining a genetically modified rodent comprising the modified genome.

23. The method of claim 22, wherein the rodent genome is modified by a process comprising

(a) introducing a targeting vector into a rodent ES cell, wherein the targeting vector comprises the human *GPR156* nucleic acid portion to be integrated into an endogenous rodent *Gpr156* locus thereby forming a humanized *Gpr156* gene an endogenous rodent *Gpr156* locus;

(b) obtaining a genetically modified rodent ES cell whose genome comprises the humanized *Gpr156* gene; and

(c) making a rodent using the genetically modified rodent ES cell of (b).

24. The method of claim 23, wherein the human *GPR156* nucleic acid portion comprises a human genomic fragment comprising the ATG start codon through the stop codon of a human *GPR156* gene, wherein the human *GPR156* gene comprises nucleotides that encode either Glu or Asp at an amino acid position corresponding to position 533 in a full length wild type human GPR156 protein.

25. The method of claim 24, wherein

(i) the human *GPR156* nucleic acid portion further comprises the 3' UTR of the human *GPR156* gene; and/or

(ii) the rodent *Gpr156* nucleic acid portion comprises a 5' non-coding exonic sequence of the endogenous rodent *Gpr156* gene, optionally wherein the humanized *Gpr156* gene further comprises a 3' non-coding exonic sequence of a rodent *Gpr156* gene.

26. The method of claim 23, wherein the humanized *Gpr156* gene is formed from a replacement of a rodent genomic fragment of an endogenous rodent *Gpr156* gene at the endogenous rodent *Gpr156* locus with the human *GPR156* nucleic acid portion, optionally wherein the human *GPR156* nucleic acid portion comprises a human genomic fragment comprising the ATG start codon through the stop codon of a human *GPR156* gene, wherein the human *GPR156* gene comprises nucleotides that encode Glu or Asp at an amino acid position corresponding to position 533 in a full length wild type human GPR156 protein.

27. The method according to any one of claims 22-26, wherein the rodent is heterozygous for the humanized *Gpr156* gene.

28. The method according to any one of claims 22-26, wherein the rodent is homozygous for the humanized *Gpr156* gene.

29. A targeting vector, comprising

a human *GPR156* nucleic acid to be integrated into a rodent *Gpr156* gene at an endogenous rodent *Gpr156* locus, flanked by a 5' nucleotide sequence and a 3' nucleotide sequence that are homologous to nucleotide sequences at the rodent *Gpr156* locus,

wherein integration of the human *GPR156* nucleic acid into the rodent *Gpr156* gene results in a replacement of a rodent genomic fragment of an endogenous rodent *Gpr156* gene at the endogenous rodent *Gpr156* locus with the human *GPR156* nucleic acid to form a humanized *Gpr156* gene,

wherein the human *GPR156* nucleic acid encodes either a wild type human GPR156 protein or a mutant human GPR156 protein comprising an E533D variation,

wherein expression of the humanized *Gpr156* gene is under control of the rodent *Gpr156* promoter at the endogenous rodent *Gpr156* locus, and

wherein the rodent is a mouse or a rat.

30. The targeting vector of claim 29, wherein the human *GPR156* nucleic acid comprises the ATG start codon through the stop codon of a human *GPR156* gene, wherein the human *GPR156* gene comprises nucleotides that encode either Glu or Asp at an amino acid position corresponding to position 533 in a full length wild type human GPR156 protein, optionally wherein the human *GPR156* nucleic acid comprises the ATG start codon through the last exon of the human *GPR156* gene.

31. A rodent, whose genome comprises genetically modified *Gpr156* locus, wherein the genetic modification comprises a deletion in an endogenous rodent *Gpr156* gene and an insertion of a reporter gene, wherein the reporter gene is operably linked to the endogenous rodent *Gpr156* promoter at the locus, wherein the genomic fragment beginning from the nucleotide after the start codon through a subsequent coding exon has been deleted, and wherein the reporter gene is operably linked to the start codon of the endogenous rodent *Gpr156* gene, and wherein the rodent is a rat or a mouse.

32. A method of testing the effect of an agent for treating unipolar depression or an anxiety disorder, comprising

subjecting a rodent to a test to assess behavior in the rodent indicative of unipolar depression or the anxiety disorder, wherein the rodent has been administered the agent according to any one of claims 2-6 and 11-12, and

determining whether the agent has any effect on the behavior in the rodent.

33. The method of claim 32, wherein the test is a forced swim test.

34. The method of claim 32, wherein the test is a marble burying test.