Methods Of Treating Headaches And Migraines With Sodium Voltage-Gated Channel Alpha Subunit 11 (SCN11A) Inhibitors

Field

The present disclosure relates generally to the treatment of subjects having a headache or migraine with Sodium Voltage-Gated Channel Alpha Subunit 11 (SCN11A) inhibitors, and methods of identifying subjects having an increased risk of developing a headache or migraine.

Background

Headaches are a very common condition that most people will experience many times during their lives. The main symptom of a headache is a pain in the head or face. The pain can be throbbing, constant, sharp or dull. Headaches can be treated with medication, stress management and biofeedback. Headache pain results from signals interacting among the brain, blood vessels and surrounding nerves. During a headache, an unknown mechanism activates specific nerves that affect muscles and blood vessels. These nerves send pain signals to the brain.

Migraines are the second most common type of primary headaches. Symptoms of migraines include: moderate to severe pain, nausea or vomiting, pounding or throbbing pain, pain that lasts four hours to three days, sensitivity to light, noise or odors, and/or stomach upset or abdominal pain. The cause of migraines is not clearly understood.

Voltage-gated sodium channels are membrane protein complexes that play a fundamental role in the rising phase of the action potential in most excitable cells. Alpha subunits mediate voltage-dependent gating and conductance, while auxiliary beta subunits regulate the kinetic properties of the channel and facilitate membrane localization of the complex. Each alpha subunit consists of 4 domains connected by 3 intracellular loops; each domain consists of 6 transmembrane segments and intra- and extracellular linkers. SCN11A is a gene that codes for the alpha subunit of a voltage-gated sodium channel found on nociceptive (painsensing) neurons of the dorsal root ganglia and the trigeminal ganglia.

Summary

The present disclosure provides methods of treating a subject having a headache or at risk of developing a headache, the methods comprising administering an SCN11A inhibitor to the subject.

The present disclosure also provides method of treating a subject having a migraine or at risk of developing a migraine, the methods comprising administering an SCN11A inhibitor to the subject.

Disclosed herein is the use of a SCN11A inhibitor in the preparation of a medicament for treating a subject having a headache or migraine or at risk of developing a headache or migraine.

The present disclosure also provides methods of treating a subject with a therapeutic agent that treats or inhibits a headache or a migraine, wherein the subject has a headache or a migraine or is at risk of developing a headache or a migraine by administering a therapeutic agent that prevents a headache or a migraine, the methods comprising: determining whether the subject has an SCN11A predicted loss-of-function variant nucleic acid molecule by: obtaining or having obtained a biological sample from the subject; and performing or having performed a sequence analysis on the biological sample to determine if the subject has a genotype comprising the SCN11A predicted loss-of-function variant nucleic acid molecule; and administering or continuing to administer the therapeutic agent that treats, prevents, or inhibits the headache or the migraine in a standard dosage amount to a subject that is SCN11A reference, and/or administering an SCN11A inhibitor to the subject; administering or continuing to administer the therapeutic agent that treats, prevents, or inhibits the headache or the migraine in an amount that is the same as or less than a standard dosage amount to a subject that is heterozygous for the SCN11A predicted loss-of-function variant nucleic acid molecule, and/or administering an SCN11A inhibitor to the subject; or administering or continuing to administer the therapeutic agent that treats, prevents, or inhibits the headache or the migraine in an amount that is the same as or less than a standard dosage amount to a subject that is homozygous for the SCN11A predicted loss-of-function variant nucleic acid molecule; wherein the presence of a genotype having the SCN11A predicted loss-of-function variant nucleic acid molecule indicates the subject has a decreased risk of developing a headache or a migraine.

Disclosed herein is the use of a therapeutic agent that treats or inhibits a headache or a migraine in the preparation of a medicament for treating a subject that has a headache or a

migraine or is at risk of developing a headache or a migraine, wherein the subject has been determined to have or not have a SCN11A predicted loss-of-function variant nucleic acid molecule by: performing or having performed a sequence analysis on a biological sample obtained or having been obtained from the subject to determine if the subject has a genotype comprising the SCN11A predicted loss-of-function variant nucleic acid molecule; and wherein the therapeutic agent that treats, prevents, or inhibits the headache or the migraine is to be administered or continued to be administered in a standard dosage amount to a subject that is SCN11A reference, and/or an SCN11A inhibitor is to be administered to the subject; wherein the therapeutic agent that treats, prevents, or inhibits the headache or the migraine is to be administered or continued to be administered in an amount that is the same as or less than a standard dosage amount to a subject that is heterozygous for the SCN11A predicted loss-of-function variant nucleic acid molecule, and/or an SCN11A inhibitor is to be administered to the subject; or wherein the therapeutic agent that treats, prevents, or inhibits the headache or the migraine is to be administered or continued to be administered in an amount that is the same as or less than a standard dosage amount to a subject that is homozygous for the SCN11A predicted loss-of-function variant nucleic acid molecule; wherein the presence of a genotype having the SCN11A predicted loss-of-function variant nucleic acid molecule indicates the subject has a decreased risk of developing a headache or a migraine.

Disclosed herein is a method of identifying a subject having an increased risk of developing a headache or a migraine, the method comprising: determining or having determined the presence or absence of an SCN11A predicted loss-of-function variant nucleic acid molecule in a biological sample obtained from the subject; wherein: when the subject is SCN11A reference, then the subject has an increased risk of developing a headache or a migraine; and when the subject is heterozygous or homozygous for the SCN11A predicted loss-of-function variant nucleic acid molecule, then the subject has a decreased risk of developing a headache or a migraine.

The present disclosure also provides therapeutic agents that treat, prevent, or inhibit a headache or a migraine for use in the treatment and/or prevention of a headache or a migraine in a subject having an SCN11A predicted loss-of-function variant nucleic acid molecule.

Disclosed herein is the use of a therapeutic agent that treats, prevents, or inhibits a headache or a migraine in the preparation of a medicament for treating or preventing a

headache or a migraine in a subject having a SCN11A predicted loss-of-function variant nucleic acid molecule.

The present disclosure also provides SCN11A inhibitors for use in the treatment and/or prevention of a headache or a migraine in a subject that is SCN11A reference, or is heterozygous for SCN11A predicted loss-of-function variant nucleic acid molecule.

Disclosed herein is the use of a SCN11A inhibitor in the preparation of a medicament for treating or preventing a headache or a migraine in a subject that is SCN11A reference, or is heterozygous for SCN11A predicted loss-of-function variant nucleic acid molecule, a Cas protein and guide RNA (gRNA) that hybridizes to a gRNA recognition sequence within an SCN11A genomic nucleic acid molecule, a small molecule, or an antibody.

Description

Various terms relating to aspects of the present disclosure are used throughout the specification and claims. Such terms are to be given their ordinary meaning in the art, unless otherwise indicated. Other specifically defined terms are to be construed in a manner consistent with the definitions provided herein.

Unless otherwise expressly stated, it is in no way intended that any method or aspect set forth herein be construed as requiring that its steps be performed in a specific order. Accordingly, where a method claim does not specifically state in the claims or descriptions that the steps are to be limited to a specific order, it is in no way intended that an order be inferred, in any respect. This holds for any possible non-expressed basis for interpretation, including matters of logic with respect to arrangement of steps or operational flow, plain meaning derived from grammatical organization or punctuation, or the number or type of aspects described in the specification.

As used herein, the singular forms “a,” “an” and “the” include plural referents unless the context clearly dictates otherwise.

As used herein, the term “about” means that the recited numerical value is approximate and small variations would not significantly affect the practice of the disclosed embodiments. Where a numerical value is used, unless indicated otherwise by the context, the term “about” means the numerical value can vary by ±10% and remain within the scope of the disclosed embodiments.

As used herein, the term “comprising” may be replaced with “consisting” or “consisting essentially of” in particular embodiments as desired.

As used herein, the term “isolated”, in regard to a nucleic acid molecule or a polypeptide, means that the nucleic acid molecule or polypeptide is in a condition other than its native environment, such as apart from blood and/or animal tissue. In some embodiments, an isolated nucleic acid molecule or polypeptide is substantially free of other nucleic acid molecules or other polypeptides, particularly other nucleic acid molecules or polypeptides of animal origin. In some embodiments, the nucleic acid molecule or polypeptide can be in a highly purified form, i.e., greater than 95% pure or greater than 99% pure. When used in this context, the term “isolated” does not exclude the presence of the same nucleic acid molecule or polypeptide in alternative physical forms, such as dimers or Alternately phosphorylated or derivatized forms.

As used herein, the terms “nucleic acid”, “nucleic acid molecule”, “nucleic acid sequence”, “polynucleotide”, or “oligonucleotide” can comprise a polymeric form of nucleotides of any length, can comprise DNA and/or RNA, and can be single-stranded, double-stranded, or multiple stranded. One strand of a nucleic acid also refers to its complement.

As used herein, the term “subject” includes any animal, including mammals. Mammals include, but are not limited to, farm animals (such as, for example, horse, cow, pig), companion animals (such as, for example, dog, cat), laboratory animals (such as, for example, mouse, rat, rabbits), and non-human primates. In some embodiments, the subject is a human. In some embodiments, the human is a patient under the care of a physician.

It has been observed in accordance with the present disclosure that SCN11A predicted loss-of-function variant nucleic acid molecules (whether these variants are homozygous or heterozygous in a particular subject) associate with a decreased risk of developing a headache or a migraine. It is believed that SCN11A predicted loss-of-function variant nucleic acid molecules have not been associated with a headache or a migraine. Therefore, subjects that are SCN11A reference or heterozygous for an SCN11A predicted loss-of-function variant nucleic acid molecule may be treated with an SCN11A inhibitor such that a headache or a migraine is inhibited or prevented, the symptoms thereof are reduced or prevented, and/or development of symptoms is repressed or prevented. It is also believed that such subjects having a headache or a migraine may further be treated with therapeutic agents that treat or inhibit a headache or a migraine.

For purposes of the present disclosure, any particular subject, such as a human, can be categorized as having one of three SCN11A genotypes: i) SCN11A reference; ii) heterozygous for an SCN11A predicted loss-of-function variant nucleic acid molecule; or iii) homozygous for an SCN11A predicted loss-of-function variant nucleic acid molecule. A subject is SCN11A reference when the subject does not have a copy of an SCN11A predicted loss-of-function variant nucleic acid molecule. A subject is heterozygous for an SCN11A predicted loss-of-function variant nucleic acid molecule when the subject has a single copy of an SCN11A predicted loss-of-function variant nucleic acid molecule. An SCN11A predicted loss-of-function variant nucleic acid molecule is any nucleic acid molecule (such as, a genomic nucleic acid molecule, an mRNA molecule, or a cDNA molecule) encoding a variant SCN11A polypeptide having a partial loss-of-function, a complete loss-of-function, a predicted partial loss-of-function, or a predicted complete loss-of-function. An SCN11A predicted loss-of-function variant nucleic acid molecule can also be any nucleic acid molecule (such as, a genomic nucleic acid molecule, an mRNA molecule, or a cDNA molecule) resulting in complete loss or decreased or aberrant expression of SCN11A mRNA or polypeptide. A subject who has an SCN11A polypeptide having a partial loss-of-function (or predicted partial loss-of-function) is hypomorphic for SCN11A. A subject is homozygous for an SCN11A predicted loss-of-function variant nucleic acid molecule when the subject has two copies (same or different) of an SCN11A predicted loss-of-function variant nucleic acid molecule.

For subjects that are genotyped or determined to be SCN11A reference, such subjects have an increased risk of developing a headache or a migraine. For subjects that are genotyped or determined to be either SCN11A reference or heterozygous for an SCN11A predicted loss-of-function variant nucleic acid molecule, such subjects or subjects can be treated with an SCN11A inhibitor.

In any of the embodiments described herein, the subject in whom a headache or a migraine is prevented by administering the SCN11A inhibitor can be anyone at risk for developing a headache or a migraine including, but not limited to, subjects with a head injury (post-traumatic headaches), subjects with a genetic predisposition for developing headaches or migraines, or subjects having a family history of migraines or headaches. In addition, subjects may have lifestyle risk factors such as stress, lack of sleep, depression, anxiety, and the like. In addition, in some embodiments, any subject can be at risk of developing a headache or a migraine. In some embodiments, administering an SCN11A inhibitor may be carried out to

prevent development of an additional a headache or a migraine in a subject who has already had a headache or a migraine.

In any of the embodiments described herein, the SCN11A predicted loss-of-function variant nucleic acid molecule can be any nucleic acid molecule (such as, for example, genomic nucleic acid molecule, mRNA molecule, or cDNA molecule) encoding an SCN11A variant polypeptide having a partial loss-of-function, a complete loss-of-function, a predicted partial loss-of-function, or a predicted complete loss-of-function. In some embodiments, the SCN11A predicted loss-of-function variant nucleic acid molecule is any nucleic acid molecule (such as, a genomic nucleic acid molecule, an mRNA molecule, or a cDNA molecule) resulting in decreased or aberrant expression of SCN11A mRNA or polypeptide. In some embodiments, the SCN11A predicted loss-of-function variant nucleic acid molecule is associated with a reduced *in vitro* response to SCN11A ligands compared with reference SCN11A. In some embodiments, the SCN11A predicted loss-of-function variant nucleic acid molecule is an SCN11A variant nucleic acid molecule that results or is predicted to result in a premature truncation of an SCN11A polypeptide compared to the human reference genome sequence. In some embodiments, the SCN11A predicted loss-of-function variant nucleic acid molecule is a variant that is predicted to be damaging by *in vitro* prediction algorithms such as Polyphen, SIFT, or similar algorithms. In some embodiments, the SCN11A predicted loss-of-function variant nucleic acid molecule is a variant that causes or is predicted to cause a nonsynonymous amino-acid substitution in an SCN11A nucleic acid molecule and whose allele frequency is less than 1/100 alleles in the population from which the subject is selected. In some embodiments, the SCN11A predicted loss-of-function variant nucleic acid molecule is any rare missense variant (allele frequency < 0.1%; or 1 in 1,000 alleles), or any splice-site, stop-gain, start-loss, stop-loss, frameshift, or in-frame indel, or other frameshift SCN11A variant.

In any of the embodiments described herein, the SCN11A predicted loss-of-function polypeptide can be any SCN11A polypeptide having a partial loss-of-function, a complete loss-of-function, a predicted partial loss-of-function, or a predicted complete loss-of-function.

In any of the embodiments described herein, the SCN11A predicted loss-of-function variant nucleic acid molecule can include variations at positions of chromosome 3 using the nucleotide sequence of the SCN11A reference genomic nucleic acid molecule (see, ENST00000302328.9 at chr3:38845764-39051944 in the UCSC Genome Browser on Human Dec. 2013 (GRCh38/hg38) Assembly) as a reference sequence. The sequence provided in

ENST00000302328.9 at chr3:38845764-39051944 in the UCSC Genome Browser on Human Dec. 2013 (GRCh38/hg38) Assembly for the SCN11A genomic nucleic acid molecule is only an exemplary sequence. Other sequences for the SCN11A genomic nucleic acid molecule are also possible. Exemplary SCN11A predicted loss-of-function variant nucleic acid molecules include, but are not limited to the following:

Variants Associated with Migraines

Name	Rsid	variantEffects	hgvsc	hgvsp
3:38846799:ACCTTGGTCAC:A	rs774589923	frameshift	c.5261_5270delGTGACCAAGG:c.5147_5156delGTGACCAAGG	p.Gly1754fs:p.Gly1716fs
3:38945410:C:T		splice_donor	c.488+1G>A:c.488+1G>A:c.488+1G>A	
3:38904092:AT:A		frameshift	c.1614delA:c.1614delA:c.1614delA	p.Lys538fs:p.Lys538fs:p.Lys538fs
3:38950176:GCAAC:G		frameshift	c.183_186delGTTG:c.183_186delGTTG:c.183_186delGTTG	p.Lys61fs:p.Lys61fs:p.Lys61fs
3:38926846:G:A	rs747189580	stop_gained	c.574C>T:c.574C>T:c.574C>T	p.Arg192*:p.Arg192*:p.Arg192*
3:38846759:G:A	rs552852063	stop_gained	c.5311C>T:c.5197C>T	p.Gln1771*:p.Gln1733*
3:38946897:AC:A		frameshift	c.277delG:c.277delG:c.277delG	p.Val93fs:p.Val93fs:p.Val93fs
3:38879949:C:T	rs768826766	splice_donor	c.3393+1G>A:c.3279+1G>A:c.3393+1G>A	
3:38926918:C:CAGTGAAGA	rs780673867	frameshift	c.494_501dupTCTTCACT:c.494_501dupTCTTCACT:c.494_501dupTCTTCACT	p.Gly168fs:p.Gly168fs:p.Gly168fs
3:38905311:AG:A	rs763788482	frameshift	c.1483delC:c.1483delC:c.1483delC	p.Leu495fs:p.Leu495fs:p.Leu495fs
3:38896897:G:C		stop_gained	c.2351C>G:c.2351C>G:c.2351C>G	p.Ser784*:p.Ser784*:p.Ser784*

3:38905243:G:A	rs1168883338	stop_gained	c.1552C>T:c.1552C>T:c.1552C>T	p.Gln518*:p.Gln518*:p.Gln518*
3:38847656:G:A	rs371178341	stop_gained	c.4414C>T:c.4300C>T	p.Arg1472*:p.Arg1434*
3:38879949:C:A	rs768826766	splice_donor	c.3393+1G>T:c.3279+1G>T:c.3393+1G>T	
3:38867450:TTGTTCTTTC:T		Frameshift	c.3814-1_3821delGAAAGAACA:c.3700-1_3707delGAAAGAACA:c.3814-1_3821delGAAAGAACA	p.Lys1272fs:p.Lys1234fs:p.Lys1272fs
3:38847386:G:A	rs777094246	stop_gained	c.4684C>T:c.4570C>T	p.Arg1562*:p.Arg1524*
3:38847200:CAA:C		Frameshift	c.4868_4869delTT:c.4754_4755delTT	p.Phe1623fs:p.Phe1585fs
3:38850748:T:A		stop_gained	c.4060A>T:c.3946A>T:c.4060A>T	p.Lys1354*:p.Lys1316*:p.Lys1354*
3:38867363:G:GT	rs762160601	Frameshift	c.3908_3909insA:c.3794_3795insA:c.3908_3909insA	p.Phe1303fs:p.Phe1265fs:p.Phe1303fs
3:38883237:GC:G		Frameshift	c.3214delG:c.3100delG:c.3214delG	p.Ala1072fs:p.Ala1034fs:p.Ala1072fs
3:38886130:G:A	rs145290679	stop_gained	c.2944C>T:c.2944C>T	p.Arg982*:p.Arg982*
3:38847629:G:A	rs150141467	stop_gained	c.4441C>T:c.4327C>T	p.Arg1481*:p.Arg1443*
3:38867455:C:A		stop_gained	c.3817G>T:c.3703G>T:c.3817G>T	p.Glu1273*:p.Glu1235*:p.Glu1273*
3:38850602:GT:G	rs781141787	Frameshift	c.4205delA:c.4091delA:c.4205delA	p.Asn1402fs:p.Asn1364fs:p.Asn1402fs
3:38850480:C:T	rs1314199333	splice_donor	c.4327+1G>A:c.4213+1G>A	

3:38894817:CAA:C		Frameshift	c.2549_2550delTT:c.2549_2550delTT:c.2549_2550delTT	p.Leu850fs:p.Leu850fs:p.Leu850fs
3:38897227:T:C	rs1270488392	splice_acceptor	c.2023-2A>G:c.2023-2A>G:c.2023-2A>G	
3:38925414:C:T	rs768647693	splice_donor	c.712+1G>A:c.712+1G>A:c.712+1G>A	
3:38847143:ATTTGATAAATTGTG:A		Frameshift	c.4913_4926delCACAATTTATCAAA:c.4799_4812delCACAATTTATCAAA	p.Thr1638fs:p.Thr1600fs
3:38894696:G:C	rs368987172	stop_gained	c.2672C>G:c.2672C>G:c.2672C>G	p.Ser891*:p.Ser891*:p.Ser891*
3:38897085:C:CTGCA	rs1380972219	Frameshift	c.2159_2162dupTGCA:c.2159_2162dupTGCA:c.2159_2162dupTGCA	p.Gln721fs:p.Gln721fs:p.Gln721fs
3:38872295:C:T	rs1374951928	splice_acceptor	c.3394-1G>A:c.3280-1G>A:c.3394-1G>A	
3:38894798:C:T		stop_gained	c.2570G>A:c.2570G>A:c.2570G>A	p.Trp857*:p.Trp857*:p.Trp857*
3:38872240:G:A	rs762833274	stop_gained	c.3448C>T:c.3334C>T:c.3448C>T	p.Arg1150*:p.Arg1112*:p.Arg1150*
3:38894856:G:A	rs749301699	stop_gained	c.2512C>T:c.2512C>T:c.2512C>T	p.Arg838*:p.Arg838*:p.Arg838*
3:38894858:TCCAGTGCTAA:T		Frameshift	c.2500_2509delTTAGCACTGG:c.2500_2509delTTAGCACTGG:c.2500_2509delTTAGCACTGG	p.Leu834fs:p.Leu834fs:p.Leu834fs
3:38867452:GTTCT:G	rs773570414	Frameshift	c.3816_3819delAGAA:c.3702_3705delAGAA:c.3816_3819delAGAA	p.Lys1272fs:p.Lys1234fs:p.Lys1272fs
3:38850694:TG:T	rs1425003912	Frameshift	c.4113delC:c.3999delC:c.4113delC	p.Ile1372fs:p.Ile1334fs:p.Ile1372fs
3:38910209:T:C	rs559628396	splice_acceptor	c.960-2A>G:c.960-2A>G:c.960-2A>G	

3:38894672:AT:A	rs1064793260	Frameshift	c.2695delA:c.2695delA:c.2695delA	p.Ile899fs:p.Ile899fs:p.Ile899fs
3:38870745:C:A		splice_acceptor	c.3760-1G>T:c.3646-1G>T:c.3760-1G>T	
3:38870746:T:G		splice_acceptor	c.3760-2A>C:c.3646-2A>C:c.3760-2A>C	
3:38879977:C:T		stop_gained	c.3366G>A:c.3252G>A:c.3366G>A	p.Trp1122*:p.Trp1084*:p.Trp1122*
3:38921179:GC:G		Frameshift	c.788delG:c.788delG:c.788delG	p.Cys263fs:p.Cys263fs:p.Cys263fs
3:38863236:A:AT	rs1016939517	Frameshift	c.4014dupA:c.3900dupA:c.4014dupA	p.Leu1339fs:p.Leu1301fs:p.Leu1339fs
3:38919984:TC:T		Frameshift	c.909delG:c.909delG:c.909delG	p.Glu305fs:p.Glu305fs:p.Glu305fs
3:38926802:C:T	rs754708932	splice_donor	c.617+1G>A:c.617+1G>A:c.617+1G>A	
3:38880016:C:T		stop_gained	c.3327G>A:c.3213G>A:c.3327G>A	p.Trp1109*:p.Trp1071*:p.Trp1109*
3:38883285:CTG:C		Frameshift	c.3165_3166delCA:c.3051_3052delCA:c.3165_3166delCA	p.His1055fs:p.His1017fs:p.His1055fs
3:38910208:CTAGA:C		splice_acceptor	c.960-5_960-2delTCTA:c.960-5_960-2delTCTA:c.960-5_960-2delTCTA	
3:38850479:A:C	rs1271092061	splice_donor	c.4327+2T>G:c.4213+2T>G	
3:38894772:G:A	rs768683402	stop_gained	c.2596C>T:c.2596C>T:c.2596C>T	p.Gln866*:p.Gln866*:p.Gln866*
3:38894874:CT:C		Frameshift	c.2493delA:c.2493delA:c.2493delA	p.Val832fs:p.Val832fs:p.Val832fs
3:38909038:TC:T	rs1269760710	Frameshift	c.1257delG:c.1257delG:c.1257delG	p.Met420fs:p.Met420fs:p.Met420fs

3:38883320:GT:G		Frameshift	c.3131delA:c.3017delA:c.3131delA	p.Asn1044fs:p.Asn1006fs:p.Asn1044fs
3:38863301:T:C	rs1288853770	splice_acceptor	c.3952-2A>G:c.3838-2A>G:c.3952-2A>G	
3:38850472:TTTAC:T		Frameshift	c.4332_4335delGTAA	p.Lys1444fs
3:38846749:CAA:C		Frameshift	c.5319_5320delTT:c.5205_5206delTT	p.Cys1774fs:p.Cys1736fs
3:38897209:A:C		stop_gained	c.2039T>G:c.2039T>G:c.2039T>G	p.Leu680*:p.Leu680*:p.Leu680*
3:38900001:G:A	rs770933380	stop_gained	c.1915C>T:c.1915C>T:c.1915C>T	p.Arg639*:p.Arg639*:p.Arg639*
3:38921130:TCA:T	rs1169508336	Frameshift	c.836_837delTG:c.836_837delTG:c.836_837delTG	p.Leu279fs:p.Leu279fs:p.Leu279fs
3:38910065:C:T		splice_donor	c.1101+1G>A:c.1101+1G>A:c.1101+1G>A	
3:38863301:T:A		splice_acceptor	c.3952-2A>T:c.3838-2A>T:c.3952-2A>T	
3:38950362:T:C		start_lost	c.1A>G:c.1A>G:c.1A>G	p.Met1?:p.Met1?:p.Met1?
3:38885338:C:T	rs41285132	stop_gained	c.3014G>A:c.2900G>A:c.3014G>A	p.Trp1005*:p.Trp967*:p.Trp1005*
3:38905230:G:GCT		Frameshift	c.1563_1564dupAG:c.1563_1564dupAG:c.1563_1564dupAG	p.Ala522fs:p.Ala522fs:p.Ala522fs
3:38894886:TG:T		Frameshift	c.2481delC:c.2481delC:c.2481delC	p.Arg828fs:p.Arg828fs:p.Arg828fs
3:38909083:C:A	rs1274914399	stop_gained	c.1213G>T:c.1213G>T:c.1213G>T	p.Glu405*:p.Glu405*:p.Glu405*
3:38879942:AAACTTACAAT:A	rs757147946	splice_donor	c.3391_3393+7delATTGTAAGTT:c.3277_3279+7delATTGTAAGTT:c.3391	p.Ile1131del:p.Ile1093del:p.Ile1131del

			_3393+7delATTGTAAGT T	
3:38846837: G:A	rs747783198	stop_gained	c.5233C>T:c.5119C>T	p.Arg1745*:p.Arg1707*
3:38908051: C:CT	rs1483842671	Frameshift	c.1370dupA:c.1370dupA:c.1370dupA	p.Arg458fs:p.Arg458fs:p.Arg458fs
3:38850599: C:T	rs760853230	stop_gained	c.4209G>A:c.4095G>A:c.4209G>A	p.Trp1403*:p.Trp1365*:p.Trp1403*
3:38896844: C:T		splice_donor	c.2403+1G>A:c.2403+1G>A:c.2403+1G>A	
3:38905297: G:A	rs367770852	stop_gained	c.1498C>T:c.1498C>T:c.1498C>T	p.Arg500*:p.Arg500*:p.Arg500*
3:38926903: CA:C		Frameshift	c.516delT:c.516delT:c.516delT	p.Phe172fs:p.Phe172fs:p.Phe172fs
3:38871444: C:A		splice_donor	c.3759+1G>T:c.3645+1G>T:c.3759+1G>T	
3:38950345: G:C		stop_gained	c.18C>G:c.18C>G:c.18C>G	p.Tyr6*:p.Tyr6*:p.Tyr6*
3:38850644: G:C		stop_gained	c.4164C>G:c.4050C>G:c.4164C>G	p.Tyr1388*:p.Tyr1350*:p.Tyr1388*
3:38870712: A:T	rs960753751	stop_gained	c.3792T>A:c.3678T>A:c.3792T>A	p.Tyr1264*:p.Tyr1226*:p.Tyr1264*
3:38883272: GCT:G		Frameshift	c.3178_3179delAG:c.3064_3065delAG:c.3178_3179delAG	p.Ser1060fs:p.Ser1022fs:p.Ser1060fs
3:38946838: C:CA	rs780082897	Frameshift	c.336dupT:c.336dupT:c.336dupT	p.Gly113fs:p.Gly113fs:p.Gly113fs
3:38910073: TA:T		Frameshift	c.1093delT:c.1093delT:c.1093delT	p.Tyr365fs:p.Tyr365fs:p.Tyr365fs
3:38950114: TG:T		Frameshift	c.248delC:c.248delC:c.248delC	p.Pro83fs:p.Pro83fs:p.Pro83fs
3:38910125: ACCAG:A		Frameshift	c.1038_1041delCTGG:c.1038_1041delCTGG:c.1038_1041delCTGG	p.Trp347fs:p.Trp347fs:p.Trp347fs

3:38846888:TGGTGGTGACTATG:T		Frameshift	c.5169_5181delCATAGTCACCACC:c.5055_5067delCATAGTCACCACC	p.Ile1724fs:p.Ile1686fs
3:38847605:CAA:C		Frameshift	c.4463_4464delTT:c.4349_4350delTT	p.Phe1488fs:p.Phe1450fs
3:38880016:C:CCATTTTA		Frameshift	c.3320_3326dupTAAAATG:c.3206_3212dupTAAAATG:c.3320_3326dupTAAAATG	p.Trp1109fs:p.Trp1071fs:p.Trp1109fs
3:38926878:C:CT		Frameshift	c.541dupA:c.541dupA:c.541dupA	p.Arg181fs:p.Arg181fs:p.Arg181fs
3:38894532:C:T		splice_donor	c.2835+1G>A:c.2835+1G>A:c.2835+1G>A	
3:38908047:TTC:T		Frameshift	c.1373_1374delGA:c.1373_1374delGA:c.1373_1374delGA	p.Arg458fs:p.Arg458fs:p.Arg458fs
3:38871640:GA:G		Frameshift	c.3563delT:c.3449delT:c.3563delT	p.Phe1188fs:p.Phe1150fs:p.Phe1188fs
3:38909114:TAA:T		Frameshift	c.1180_1181delTT:c.1180_1181delTT:c.1180_1181delTT	p.Leu394fs:p.Leu394fs:p.Leu394fs
3:38863231:TC:T		Frameshift	c.4019delG:c.3905delG:c.4019delG	p.Gly1340fs:p.Gly1302fs:p.Gly1340fs
3:38909123:CA:C		Frameshift	c.1172delT:c.1172delT:c.1172delT	p.Leu391fs:p.Leu391fs:p.Leu391fs
3:38909181:GTAGT:G	rs779375851	Frameshift	c.1111_1114delACTA:c.1111_1114delACTA:c.1111_1114delACTA	p.Thr371fs:p.Thr371fs:p.Thr371fs
3:38908053:T:A		stop_gained	c.1369A>T:c.1369A>T:c.1369A>T	p.Lys457*:p.Lys457*:p.Lys457*
3:38872195:T:A		stop_gained	c.3493A>T:c.3379A>T:c.3493A>T	p.Lys1165*:p.Lys1127*:p.Lys1165*
3:38871626:CA:C		Frameshift	c.3577delT:c.3463delT:c.3577delT	p.Cys1193fs:p.Cys1155fs:p.Cys1193fs

3:38950094:A:G		splice_donor	c.267+2T>C:c.267+2T>C:c.267+2T>C	
3:38904036:C:T		stop_gained	c.1671G>A:c.1671G>A:c.1671G>A	p.Trp557*:p.Trp557*:p.Trp557*
3:38885336:AC:A		Frameshift	c.3015delG:c.2901delG:c.3015delG	p.Trp1005fs:p.Trp967fs:p.Trp1005fs
3:38847458:CAA:C	rs750015613	Frameshift	c.4610_4611delTT:c.4496_4497delTT	p.Phe1537fs:p.Phe1499fs
3:38894829:T:A		stop_gained	c.2539A>T:c.2539A>T:c.2539A>T	p.Arg847*:p.Arg847*:p.Arg847*
3:38908995:A:G		splice_donor	c.1299+2T>C:c.1299+2T>C:c.1299+2T>C	
3:38885387:TG:T		Frameshift	c.2964delC:c.2850delC:c.2964delC	p.Ser989fs:p.Ser951fs:p.Ser989fs
3:38885375:C:A		stop_gained	c.2977G>T:c.2863G>T:c.2977G>T	p.Glu993*:p.Glu955*:p.Glu993*
3:38908091:G:T		stop_gained	c.1331C>A:c.1331C>A:c.1331C>A	p.Ser444*:p.Ser444*:p.Ser444*
3:38872201:C:A		stop_gained	c.3487G>T:c.3373G>T:c.3487G>T	p.Gly1163*:p.Gly1125*:p.Gly1163*
3:38886203:CG:C		Frameshift	c.2870delC:c.2870delC	p.Thr957fs:p.Thr957fs
3:38850752:C:T		splice_acceptor	c.4057-1G>A:c.3943-1G>A:c.4057-1G>A	
3:38850521:C:T		stop_gained	c.4287G>A:c.4173G>A:c.4287G>A	p.Trp1429*:p.Trp1391*:p.Trp1429*
3:38926853:AG:A		Frameshift	c.566delC:c.566delC:c.566delC	p.Ser189fs:p.Ser189fs:p.Ser189fs
3:38925413:A:T		splice_donor	c.712+2T>A:c.712+2T>A:c.712+2T>A	
3:38925510:C:T	rs754798838	splice_acceptor	c.618-1G>A:c.618-1G>A:c.618-1G>A	
3:38847073:TG:T		Frameshift	c.4996delC:c.4882delC	p.Gln1666fs:p.Gln1628fs

3:38863294:AC:A	rs1347075787	Frameshift	c.3956delG:c.3842delG:c.3956delG	p.Gly1319fs:p.Gly1281fs:p.Gly1319fs
3:38870733:CT:C		Frameshift	c.3770delA:c.3656delA:c.3770delA	p.Lys1257fs:p.Lys1219fs:p.Lys1257fs
3:38899979:CT:C		Frameshift	c.1936delA:c.1936delA:c.1936delA	p.Ser646fs:p.Ser646fs:p.Ser646fs
3:38847155:G:A		stop_gained	c.4915C>T:c.4801C>T	p.Gln1639*:p.Gln1601*
3:38847412:C:T		stop_gained	c.4658G>A:c.4544G>A	p.Trp1553*:p.Trp1515*
3:38847257:C:A		stop_gained	c.4813G>T:c.4699G>T	p.Glu1605*:p.Glu1567*
3:38867329:G:A	rs1356435144	stop_gained	c.3943C>T:c.3829C>T:c.3943C>T	p.Gln1315*:p.Gln1277*:p.Gln1315*
3:38909019:TG:T		Frameshift	c.1276delC:c.1276delC:c.1276delC	p.Gln426fs:p.Gln426fs:p.Gln426fs
3:38863300:G:T		splice_acceptor	c.3952-1C>A:c.3838-1C>A:c.3952-1C>A	
3:38897093:CAA:C		Frameshift	c.2153_2154delTT:c.2153_2154delTT:c.2153_2154delTT	p.Val718fs:p.Val718fs:p.Val718fs
3:38899918:C:T		stop_gained	c.1998G>A:c.1998G>A:c.1998G>A	p.Trp666*:p.Trp666*:p.Trp666*
3:38885335:A:T		stop_gained	c.3017T>A:c.2903T>A:c.3017T>A	p.Leu1006*:p.Leu968*:p.Leu1006*
3:38904037:C:T		stop_gained	c.1670G>A:c.1670G>A:c.1670G>A	p.Trp557*:p.Trp557*:p.Trp557*
3:38900074:C:T		splice_acceptor	c.1843-1G>A:c.1843-1G>A:c.1843-1G>A	
3:38950361:A:G	rs764704628	start_lost	c.2T>C:c.2T>C:c.2T>C	p.Met1?:p.Met1?:p.Met1?
3:38867320:T:A		splice_donor	c.3951+1A>T:c.3837+1A>T:c.3951+1A>T	

3:38904105:T:G		splice_acceptor	c.1604-2A>C:c.1604-2A>C:c.1604-2A>C	
3:38950291:CAG:C	rs1437845927	Frameshift	c.70_71delCT:c.70_71delCT:c.70_71delCT	p.Leu24fs:p.Leu24fs:p.Leu24fs
3:38897197:C:T		stop_gained	c.2051G>A:c.2051G>A:c.2051G>A	p.Trp684*:p.Trp684*:p.Trp684*
3:38880096:G:A	rs780255332	stop_gained	c.3247C>T:c.3133C>T:c.3247C>T	p.Gln1083*:p.Gln1045*:p.Gln1083*
3:38847161:CT:C		Frameshift	c.4908delA:c.4794delA	p.Ala1637fs:p.Ala1599fs
3:38946789:G:T		stop_gained	c.386C>A:c.386C>A:c.386C>A	p.Ser129*:p.Ser129*:p.Ser129*
3:38885286:A:G		splice_donor	c.3064+2T>C:c.2950+2T>C:c.3064+2T>C	
3:38863291:GC:G		Frameshift	c.3959delG:c.3845delG:c.3959delG	p.Gly1320fs:p.Gly1282fs:p.Gly1320fs
3:38879969:AG:A		Frameshift	c.3373delC:c.3259delC:c.3373delC	p.Asp1126fs:p.Asp1088fs:p.Asp1126fs
3:38871593:A:AAT		Frameshift	c.3609_3610dupAT:c.3495_3496dupAT:c.3609_3610dupAT	p.Phe1204fs:p.Phe1166fs:p.Phe1204fs
3:38896900:G:T		stop_gained	c.2348C>A:c.2348C>A:c.2348C>A	p.Ser783*:p.Ser783*:p.Ser783*
3:38863221:G:GT	rs1325097401	Frameshift	c.4029dupA:c.3915dupA:c.4029dupA	p.Pro1344fs:p.Pro1306fs:p.Pro1344fs
3:38894656:TG:T		Frameshift	c.2711delC:c.2711delC:c.2711delC	p.Pro904fs:p.Pro904fs:p.Pro904fs
3:38880125:T:C		splice_acceptor	c.3220-2A>G:c.3106-2A>G:c.3220-2A>G	
3:38904101:G:A		stop_gained	c.1606C>T:c.1606C>T:c.1606C>T	p.Gln536*:p.Gln536*:p.Gln536*
3:38894732:TCTTTG:T		Frameshift	c.2631_2635delCAAAG:c.2631_2635delCAAAG:c.2631_2635delCAAAG	p.Ser877fs:p.Ser877fs:p.Ser877fs

3:38871701:A:AC		Frameshift	c.3502dupG:c.3388dupG:c.3502dupG	p.Val1168fs:p.Val1130fs:p.Val1168fs
3:38847516:C:T		stop_gained	c.4554G>A:c.4440G>A	p.Trp1518*:p.Trp1480*
3:38850753:T:C		splice_acceptor	c.4057-2A>G:c.3943-2A>G:c.4057-2A>G	
3:38846711:T:A		stop_gained	c.5359A>T:c.5245A>T	p.Lys1787*:p.Lys1749*
3:38883232:C:A	rs1420432992	splice_donor	c.3219+1G>T:c.3105+1G>T:c.3219+1G>T	
3:38908123:C:G		splice_acceptor	c.1300-1G>C:c.1300-1G>C:c.1300-1G>C	
3:38907946:T:TA		splice_donor	c.1473+2_1473+3insT:c.1473+2_1473+3insT:c.1473+2_1473+3insT	
3:38847176:T:A		stop_gained	c.4894A>T:c.4780A>T	p.Lys1632*:p.Lys1594*
3:38885310:TTG:T		Frameshift	c.3040_3041delCA:c.2926_2927delCA:c.3040_3041delCA	p.Gln1014fs:p.Gln976fs:p.Gln1014fs
3:38894532:C:A		splice_donor	c.2835+1G>T:c.2835+1G>T:c.2835+1G>T	
3:38900055:TA:T		Frameshift	c.1860delT:c.1860delT:c.1860delT	p.Phe620fs:p.Phe620fs:p.Phe620fs
3:38894794:G:T		stop_gained	c.2574C>A:c.2574C>A:c.2574C>A	p.Cys858*:p.Cys858*:p.Cys858*
3:38899936:TAC:T		Frameshift	c.1978_1979delGT:c.1978_1979delGT:c.1978_1979delGT	p.Val660fs:p.Val660fs:p.Val660fs
3:38871566:G:C		stop_gained	c.3638C>G:c.3524C>G:c.3638C>G	p.Ser1213*:p.Ser1175*:p.Ser1213*
3:38909027:TTCCTGAAACA:T		frameshift	c.1259_1268delTGTTTCAGGA:c.1259_1268delT	p.Met420fs:p.Met420fs:p.Met420fs

			GTTTCAGGA:c.1259_1268delTGTTTCAGGA	
3:38846799:A:ACCTTGGTCAC		Frameshift	c.5261_5270dupGTGACCAAGG:c.5147_5156dupGTGACCAAGG	p.Asp1758fs:p.Asp1720fs
3:38894832:CA:C		Frameshift	c.2535delT:c.2535delT:c.2535delT	p.Phe845fs:p.Phe845fs:p.Phe845fs
3:38905309:CT:C		Frameshift	c.1485delA:c.1485delA:c.1485delA	p.Glu496fs:p.Glu496fs:p.Glu496fs
3:38926932:CT:C		splice_acceptor	c.489-2delA:c.489-2delA:c.489-2delA	
3:38950107:G:A	rs78812474	stop_gained	c.256C>T:c.256C>T:c.256C>T	p.Arg86*:p.Arg86*:p.Arg86*
3:38847213:AC:A		Frameshift	c.4856delG:c.4742delG	p.Gly1619fs:p.Gly1581fs
3:38925431:T:TG		Frameshift	c.695dupC:c.695dupC:c.695dupC	p.Ile233fs:p.Ile233fs:p.Ile233fs
3:38950173:TG:T	rs759790310	Frameshift	c.189delC:c.189delC:c.189delC	p.Lys64fs:p.Lys64fs:p.Lys64fs
3:38921185:GA:G	rs1064793259	Frameshift	c.782delT:c.782delT:c.782delT	p.Phe261fs:p.Phe261fs:p.Phe261fs
3:38894702:C:CT		Frameshift	c.2665dupA:c.2665dupA:c.2665dupA	p.Arg889fs:p.Arg889fs:p.Arg889fs
3:38883234:AGT:A		Frameshift	c.3216_3217delAC:c.3102_3103delAC:c.3216_3217delAC	p.Leu1073fs:p.Leu1035fs:p.Leu1073fs
3:38897074:CG:C		Frameshift	c.2173delC:c.2173delC:c.2173delC	p.Arg725fs:p.Arg725fs:p.Arg725fs
3:38863234:TA:T	rs746004883	Frameshift	c.4016delT:c.3902delT:c.4016delT	p.Leu1339fs:p.Leu1301fs:p.Leu1339fs
3:38847329:TG:T	rs762319868	Frameshift	c.4740delC:c.4626delC	p.Thr1581fs:p.Thr1543fs

Variants Associated with Migraines

Name	Rsid	variantEffects	hgvsc	hgvsp
3:38846888:TGGTGGTGACTATG:T		Frameshift	c.5169_5181delCATAGTCACCACC:c.5055_5067delCATAGTCACCACC	p.Ile1724fs:p.Ile1686fs
3:38850748:T:A		stop_gained	c.4060A>T:c.3946A>T:c.4060A>T	p.Lys1354*:p.Lys1316*:p.Lys1354*
3:38879969:AG:A		Frameshift	c.3373delC:c.3259delC:c.3373delC	p.Asp1126fs:p.Asp1088fs:p.Asp1126fs
3:38897085:C:CTGCA	rs1380972219	Frameshift	c.2159_2162dupTGCA:c.2159_2162dupTGCA:c.2159_2162dupTGCA	p.Gln721fs:p.Gln721fs:p.Gln721fs
3:38946789:G:T		stop_gained	c.386C>A:c.386C>A:c.386C>A	p.Ser129*:p.Ser129*:p.Ser129*
3:38850602:GT:G	rs781141787	Frameshift	c.4205delA:c.4091delA:c.4205delA	p.Asn1402fs:p.Asn1364fs:p.Asn1402fs
3:38846711:T:A		stop_gained	c.5359A>T:c.5245A>T	p.Lys1787*:p.Lys1749*
3:38908047:TTC:T		Frameshift	c.1373_1374delGA:c.1373_1374delGA:c.1373_1374delGA	p.Arg458fs:p.Arg458fs:p.Arg458fs
3:38872195:T:A		stop_gained	c.3493A>T:c.3379A>T:c.3493A>T	p.Lys1165*:p.Lys1127*:p.Lys1165*
3:38846749:CAA:C		Frameshift	c.5319_5320delTT:c.5205_5206delTT	p.Cys1774fs:p.Cys1736fs
3:38908051:C:CT	rs1483842671	Frameshift	c.1370dupA:c.1370dupA:c.1370dupA	p.Arg458fs:p.Arg458fs:p.Arg458fs
3:38897209:A:C		stop_gained	c.2039T>G:c.2039T>G:c.2039T>G	p.Leu680*:p.Leu680*:p.Leu680*
3:38867450:TTGTTCTTTC:T		Frameshift	c.3814-1_3821delGAAAGAACA:c.3700-1_3707delGAAAGAACA:	p.Lys1272fs:p.Lys1234fs:p.Lys1272fs

			c.3814-1_3821delGAAAGAACA	
3:38870712:A:T	rs960753751	stop_gained	c.3792T>A:c.3678T>A:c.3792T>A	p.Tyr1264*:p.Tyr1226*:p.Tyr1264*
3:38883232:C:A	rs1420432992	splice_donor	c.3219+1G>T:c.3105+1G>T:c.3219+1G>T	
3:38883285:CTG:C		Frameshift	c.3165_3166delCA:c.3051_3052delCA:c.3165_3166delCA	p.His1055fs:p.His1017fs:p.His1055fs
3:38847412:C:T		stop_gained	c.4658G>A:c.4544G>A	p.Trp1553*:p.Trp1515*
3:38910125:ACCAG:A		Frameshift	c.1038_1041delCTGG:c.1038_1041delCTGG:c.1038_1041delCTGG	p.Trp347fs:p.Trp347fs:p.Trp347fs
3:38847656:G:A	rs371178341	stop_gained	c.4414C>T:c.4300C>T	p.Arg1472*:p.Arg1434*
3:38950114:TG:T		Frameshift	c.248delC:c.248delC:c.248delC	p.Pro83fs:p.Pro83fs:p.Pro83fs
3:38900001:G:A	rs770933380	stop_gained	c.1915C>T:c.1915C>T:c.1915C>T	p.Arg639*:p.Arg639*:p.Arg639*
3:38880016:C:CCATTTTA		Frameshift	c.3320_3326dupTAAAATG:c.3206_3212dupTAAAATG:c.3320_3326dupTAAAATG	p.Trp1109fs:p.Trp1071fs:p.Trp1109fs
3:38847605:CAA:C		Frameshift	c.4463_4464delTT:c.4349_4350delTT	p.Phe1488fs:p.Phe1450fs
3:38925414:C:T	rs768647693	splice_donor	c.712+1G>A:c.712+1G>A:c.712+1G>A	
3:38846799:A:ACCTTGGTCAC		Frameshift	c.5261_5270dupGTGACCAAGG:c.5147_5156dupGTGACCAAGG	p.Asp1758fs:p.Asp1720fs
3:38885286:A:G		splice_donor	c.3064+2T>C:c.2950+2T>C:c.3064+2T>C	

3:38896897:G:C		stop_gained	c.2351C>G:c.2351C>G:c.2351C>G	p.Ser784*:p.Ser784*:p.Ser784*
3:38847155:G:A		stop_gained	c.4915C>T:c.4801C>T	p.Gln1639*:p.Gln1601*
3:38908995:A:G		splice_donor	c.1299+2T>C:c.1299+2T>C:c.1299+2T>C	
3:38885335:A:T		stop_gained	c.3017T>A:c.2903T>A:c.3017T>A	p.Leu1006*:p.Leu968*:p.Leu1006*
3:38846799:ACCTTGGTCAC:A	rs774589923	frameshift	c.5261_5270delGTGACCAAGG:c.5147_5156delGTGACCAAGG	p.Gly1754fs:p.Gly1716fs
3:38871566:G:C		stop_gained	c.3638C>G:c.3524C>G:c.3638C>G	p.Ser1213*:p.Ser1175*:p.Ser1213*
3:38909123:CA:C		frameshift	c.1172delT:c.1172delT:c.1172delT	p.Leu391fs:p.Leu391fs:p.Leu391fs
3:38904105:T:G		splice_acceptor	c.1604-2A>C:c.1604-2A>C:c.1604-2A>C	
3:38894817:CAA:C		frameshift	c.2549_2550delTT:c.2549_2550delTT:c.2549_2550delTT	p.Leu850fs:p.Leu850fs:p.Leu850fs
3:38863234:TA:T	rs746004883	frameshift	c.4016delT:c.3902delT:c.4016delT	p.Leu1339fs:p.Leu1301fs:p.Leu1339fs
3:38867385:G:T		stop_gained	c.3887C>A:c.3773C>A:c.3887C>A	p.Ser1296*:p.Ser1258*:p.Ser1296*
3:38926918:C:CAGTGAAGA	rs780673867	frameshift	c.494_501dupTCTTCACT:c.494_501dupTCTTCACT:c.494_501dupTCTTCACT	p.Gly168fs:p.Gly168fs:p.Gly168fs
3:38850694:TG:T	rs1425003912	frameshift	c.4113delC:c.3999delC:c.4113delC	p.Ile1372fs:p.Ile1334fs:p.Ile1372fs
3:38900055:TA:T		frameshift	c.1860delT:c.1860delT:c.1860delT	p.Phe620fs:p.Phe620fs:p.Phe620fs
3:38885387:TG:T		frameshift	c.2964delC:c.2850delC:c.2964delC	p.Ser989fs:p.Ser951fs:p.Ser989fs

3:38897093:CAA:C		frameshift	c.2153_2154delTT:c.2153_2154delTT:c.2153_2154delTT	p.Val718fs:p.Val718fs:p.Val718fs
3:38897099:CT:C		frameshift	c.2148delA:c.2148delA:c.2148delA	p.Val717fs:p.Val717fs:p.Val717fs
3:38870745:C:A		splice_acceptor	c.3760-1G>T:c.3646-1G>T:c.3760-1G>T	
3:38945410:C:T		splice_donor	c.488+1G>A:c.488+1G>A:c.488+1G>A	
3:38905243:G:A	rs1168883338	stop_gained	c.1552C>T:c.1552C>T:c.1552C>T	p.Gln518*:p.Gln518*:p.Gln518*
3:38899936:TAC:T		frameshift	c.1978_1979delGT:c.1978_1979delGT:c.1978_1979delGT	p.Val660fs:p.Val660fs:p.Val660fs
3:38908123:C:G		splice_acceptor	c.1300-1G>C:c.1300-1G>C:c.1300-1G>C	
3:38847629:G:A	rs150141467	stop_gained	c.4441C>T:c.4327C>T	p.Arg1481*:p.Arg1443*
3:38907946:T:TA		splice_donor	c.1473+2_1473+3insT:c.1473+2_1473+3insT:c.1473+2_1473+3insT	
3:38871626:CA:C		frameshift	c.3577delT:c.3463delT:c.3577delT	p.Cys1193fs:p.Cys1155fs:p.Cys1193fs
3:38894702:C:CT		frameshift	c.2665dupA:c.2665dupA:c.2665dupA	p.Arg889fs:p.Arg889fs:p.Arg889fs
3:38879942:AAACTTACAAT:A	rs757147946	splice_donor	c.3391_3393+7delATTGTAAGTT:c.3277_3279+7delATTGTAAGTT:c.3391_3393+7delATTGTAAGTT	p.Ile1131del:p.Ile1093del:p.Ile1131del
3:38909181:GTAGT:G	rs779375851	frameshift	c.1111_1114delACTA:c.1111_1114delACTA:c.1111_1114delACTA	p.Thr371fs:p.Thr371fs:p.Thr371fs

3:38904092:AT:A		frameshift	c.1614delA:c.1614delA:c.1614delA	p.Lys538fs:p.Lys538fs:p.Lys538fs
3:38850480:C:T	rs1314199333	splice_donor	c.4327+1G>A:c.4213+1G>A	
3:38909038:TC:T	rs1269760710	frameshift	c.1257delG:c.1257delG:c.1257delG	p.Met420fs:p.Met420fs:p.Met420fs
3:38872240:G:A	rs762833274	stop_gained	c.3448C>T:c.3334C>T:c.3448C>T	p.Arg1150*:p.Arg1112*:p.Arg1150*
3:38909019:TG:T		frameshift	c.1276delC:c.1276delC:c.1276delC	p.Gln426fs:p.Gln426fs:p.Gln426fs
3:38885375:C:A		stop_gained	c.2977G>T:c.2863G>T:c.2977G>T	p.Glu993*:p.Glu955*:p.Glu993*
3:38847213:AC:A		frameshift	c.4856delG:c.4742delG	p.Gly1619fs:p.Gly1581fs
3:38871701:A:AC		frameshift	c.3502dupG:c.3388dupG:c.3502dupG	p.Val1168fs:p.Val1130fs:p.Val1168fs
3:38883320:GT:G		frameshift	c.3131delA:c.3017delA:c.3131delA	p.Asn1044fs:p.Asn1006fs:p.Asn1044fs
3:38847257:C:A		stop_gained	c.4813G>T:c.4699G>T	p.Glu1605*:p.Glu1567*
3:38925510:C:T	rs754798838	splice_acceptor	c.618-1G>A:c.618-1G>A:c.618-1G>A	
3:38880125:T:C		splice_acceptor	c.3220-2A>G:c.3106-2A>G:c.3220-2A>G	
3:38894532:C:A		splice_donor	c.2835+1G>T:c.2835+1G>T:c.2835+1G>T	
3:38894874:CT:C		frameshift	c.2493delA:c.2493delA:c.2493delA	p.Val832fs:p.Val832fs:p.Val832fs
3:38946838:C:CA	rs780082897	frameshift	c.336dupT:c.336dupT:c.336dupT	p.Gly113fs:p.Gly113fs:p.Gly113fs
3:38897074:CG:C		frameshift	c.2173delC:c.2173delC:c.2173delC	p.Arg725fs:p.Arg725fs:p.Arg725fs
3:38879977:C:T		stop_gained	c.3366G>A:c.3252G>A:c.3366G>A	p.Trp1122*:p.Trp1084*:p.Trp1122*

3:38950173:TG:T	rs759790310	frameshift	c.189delC:c.189delC:c.189delC	p.Lys64fs:p.Lys64fs:p.Lys64fs
3:38910209:T:C	rs559628396	splice_acceptor	c.960-2A>G:c.960-2A>G:c.960-2A>G	
3:38894858:TCCAGTGCTAA:T		frameshift	c.2500_2509delTTAGCACTGG:c.2500_2509delTTAGCACTGG:c.2500_2509delTTAGCACTGG	p.Leu834fs:p.Leu834fs:p.Leu834fs
3:38950176:GCAAC:G		frameshift	c.183_186delGTTG:c.183_186delGTTG:c.183_186delGTTG	p.Lys61fs:p.Lys61fs:p.Lys61fs
3:38894656:TG:T		frameshift	c.2711delC:c.2711delC:c.2711delC	p.Pro904fs:p.Pro904fs:p.Pro904fs
3:38921179:GC:G		frameshift	c.788delG:c.788delG:c.788delG	p.Cys263fs:p.Cys263fs:p.Cys263fs
3:38871593:A:AAT		frameshift	c.3609_3610dupAT:c.3495_3496dupAT:c.3609_3610dupAT	p.Phe1204fs:p.Phe1166fs:p.Phe1204fs
3:38879949:C:A	rs768826766	splice_donor	c.3393+1G>T:c.3279+1G>T:c.3393+1G>T	
3:38896962:GA:G		frameshift	c.2285delT:c.2285delT:c.2285delT	p.Phe762fs:p.Phe762fs:p.Phe762fs
3:38894696:G:C	rs368987172	stop_gained	c.2672C>G:c.2672C>G:c.2672C>G	p.Ser891*:p.Ser891*:p.Ser891*
3:38945449:AG:A		frameshift	c.449delC:c.449delC:c.449delC	p.Pro150fs:p.Pro150fs:p.Pro150fs
3:38867455:C:A		stop_gained	c.3817G>T:c.3703G>T:c.3817G>T	p.Glu1273*:p.Glu1235*:p.Glu1273*
3:38921130:TCA:T	rs1169508336	frameshift	c.836_837delTG:c.836_837delTG:c.836_837delTG	p.Leu279fs:p.Leu279fs:p.Leu279fs
3:38879949:C:T	rs768826766	splice_donor	c.3393+1G>A:c.3279+1G>A:c.3393+1G>A	

3:38903981:CA:C		frameshift	c.1725delT:c.1725delT:c.1725delT	p.Asp576fs:p.Asp576fs:p.Asp576fs
3:38880096:G:A	rs780255332	stop_gained	c.3247C>T:c.3133C>T:c.3247C>T	p.Gln1083*:p.Gln1045*:p.Gln1083*
3:38946909:T:C	rs1206610725	splice_acceptor	c.268-2A>G:c.268-2A>G:c.268-2A>G	
3:38885336:AC:A		frameshift	c.3015delG:c.2901delG:c.3015delG	p.Trp1005fs:p.Trp967fs:p.Trp1005fs
3:38910073:TA:T		frameshift	c.1093delT:c.1093delT:c.1093delT	p.Tyr365fs:p.Tyr365fs:p.Tyr365fs
3:38863291:GC:G		frameshift	c.3959delG:c.3845delG:c.3959delG	p.Gly1320fs:p.Gly1282fs:p.Gly1320fs
3:38950362:T:C		start_lost	c.1A>G:c.1A>G:c.1A>G	p.Met1?:p.Met1?:p.Met1?
3:38883272:GCT:G		frameshift	c.3178_3179delAG:c.3064_3065delAG:c.3178_3179delAG	p.Ser1060fs:p.Ser1022fs:p.Ser1060fs
3:38897227:T:C	rs1270488392	splice_acceptor	c.2023-2A>G:c.2023-2A>G:c.2023-2A>G	
3:38919984:TC:T		frameshift	c.909delG:c.909delG:c.909delG	p.Glu305fs:p.Glu305fs:p.Glu305fs
3:38894772:G:A	rs768683402	stop_gained	c.2596C>T:c.2596C>T:c.2596C>T	p.Gln866*:p.Gln866*:p.Gln866*
3:38872277:GA:G		frameshift	c.3410delT:c.3296delT:c.3410delT	p.Leu1137fs:p.Leu1099fs:p.Leu1137fs
3:38894886:TG:T		frameshift	c.2481delC:c.2481delC:c.2481delC	p.Arg828fs:p.Arg828fs:p.Arg828fs
3:38910208:CTAGA:C		splice_acceptor	c.960-5_960-2delTCTA:c.960-5_960-2delTCTA:c.960-5_960-2delTCTA	
3:38886130:G:A	rs145290679	stop_gained	c.2944C>T:c.2944C>T	p.Arg982*:p.Arg982*

3:38894672:AT:A	rs1064793260	frameshift	c.2695delA:c.2695delA:c.2695delA	p.Ile899fs:p.Ile899fs:p.Ile899fs
3:38871640:GA:G		frameshift	c.3563delT:c.3449delT:c.3563delT	p.Phe1188fs:p.Phe1150fs:p.Phe1188fs
3:38847458:CAA:C	rs750015613	frameshift	c.4610_4611delTT:c.4496_4497delTT	p.Phe1537fs:p.Phe1499fs
3:38850473:T:A		stop_lost	c.4335A>T	p.Ter1445Tyrext*?
3:38908053:T:A		stop_gained	c.1369A>T:c.1369A>T:c.1369A>T	p.Lys457*:p.Lys457*:p.Lys457*
3:38885338:C:T	rs41285132	stop_gained	c.3014G>A:c.2900G>A:c.3014G>A	p.Trp1005*:p.Trp967*:p.Trp1005*
3:38850521:C:T		stop_gained	c.4287G>A:c.4173G>A:c.4287G>A	p.Trp1429*:p.Trp1391*:p.Trp1429*
3:38863221:G:GT	rs1325097401	frameshift	c.4029dupA:c.3915dupA:c.4029dupA	p.Pro1344fs:p.Pro1306fs:p.Pro1344fs
3:38872209:TG:T		frameshift	c.3478delC:c.3364delC:c.3478delC	p.Gln1160fs:p.Gln1122fs:p.Gln1160fs
3:38883234:AGT:A		frameshift	c.3216_3217delAC:c.3102_3103delAC:c.3216_3217delAC	p.Leu1073fs:p.Leu1035fs:p.Leu1073fs
3:38847200:CAA:C		frameshift	c.4868_4869delTT:c.4754_4755delTT	p.Phe1623fs:p.Phe1585fs
3:38847143:ATTTGATAAATTGTG:A		frameshift	c.4913_4926delCACAATTTATCAAA:c.4799_4812delCACAATTTATCAAA	p.Thr1638fs:p.Thr1600fs
3:38909027:TTCCTGAAACA:T		frameshift	c.1259_1268delTGTTTCAGGA:c.1259_1268delTGTTTCAGGA:c.1259_1268delTGTTTCAGGA	p.Met420fs:p.Met420fs:p.Met420fs
3:38926846:G:A	rs747189580	stop_gained	c.574C>T:c.574C>T:c.574C>T	p.Arg192*:p.Arg192*:p.Arg192*
3:38863301:T:A		splice_acceptor	c.3952-2A>T:c.3838-2A>T:c.3952-2A>T	

3:38899993:C:T		stop_gained	c.1923G>A:c.1923G>A:c.1923G>A	p.Trp641*:p.Trp641*:p.Trp641*
3:38870746:T:G		splice_acceptor	c.3760-2A>C:c.3646-2A>C:c.3760-2A>C	
3:38846795:G:A	rs756161746	stop_gained	c.5275C>T:c.5161C>T	p.Gln1759*:p.Gln1721*
3:38894648:AGGGTCTTTGG:A		frameshift	c.2710_2719delCCAAAGACCC:c.2710_2719delCCAAAGACCC:c.2710_2719delCCAAAGACCC	p.Pro904fs:p.Pro904fs:p.Pro904fs
3:38870733:CT:C		frameshift	c.3770delA:c.3656delA:c.3770delA	p.Lys1257fs:p.Lys1219fs:p.Lys1257fs
3:38883237:GC:G		frameshift	c.3214delG:c.3100delG:c.3214delG	p.Ala1072fs:p.Ala1034fs:p.Ala1072fs
3:38894856:G:A	rs749301699	stop_gained	c.2512C>T:c.2512C>T:c.2512C>T	p.Arg838*:p.Arg838*:p.Arg838*
3:38850599:C:T	rs760853230	stop_gained	c.4209G>A:c.4095G>A:c.4209G>A	p.Trp1403*:p.Trp1365*:p.Trp1403*
3:38850472:TTTAC:T		frameshift	c.4332_4335delGTAA	p.Lys1444fs
3:38846759:G:A	rs552852063	stop_gained	c.5311C>T:c.5197C>T	p.Gln1771*:p.Gln1733*
3:38850644:G:C		stop_gained	c.4164C>G:c.4050C>G:c.4164C>G	p.Tyr1388*:p.Tyr1350*:p.Tyr1388*
3:38894832:CA:C		frameshift	c.2535delT:c.2535delT:c.2535delT	p.Phe845fs:p.Phe845fs:p.Phe845fs
3:38847176:T:A		stop_gained	c.4894A>T:c.4780A>T	p.Lys1632*:p.Lys1594*
3:38863301:T:C	rs1288853770	splice_acceptor	c.3952-2A>G:c.3838-2A>G:c.3952-2A>G	
3:38925413:A:T		splice_donor	c.712+2T>A:c.712+2T>A:c.712+2T>A	
3:38847386:G:A	rs777094246	stop_gained	c.4684C>T:c.4570C>T	p.Arg1562*:p.Arg1524*

3:38905309:CT:C		frameshift	c.1485delA:c.1485delA:c.1485delA	p.Glu496fs:p.Glu496fs:p.Glu496fs
3:38894862:GT:G		frameshift	c.2505delA:c.2505delA:c.2505delA	p.Leu836fs:p.Leu836fs:p.Leu836fs
3:38846837:G:A	rs747783198	stop_gained	c.5233C>T:c.5119C>T	p.Arg1745*:p.Arg1707*
3:38899979:CT:C		frameshift	c.1936delA:c.1936delA:c.1936delA	p.Ser646fs:p.Ser646fs:p.Ser646fs
3:38867452:GTTCT:G	rs773570414	frameshift	c.3816_3819delAGAA:c.3702_3705delAGAA:c.3816_3819delAGAA	p.Lys1272fs:p.Lys1234fs:p.Lys1272fs
3:38905311:AG:A	rs763788482	frameshift	c.1483delC:c.1483delC:c.1483delC	p.Leu495fs:p.Leu495fs:p.Leu495fs
3:38926853:AG:A		frameshift	c.566delC:c.566delC:c.566delC	p.Ser189fs:p.Ser189fs:p.Ser189fs
3:38863231:TC:T		frameshift	c.4019delG:c.3905delG:c.4019delG	p.Gly1340fs:p.Gly1302fs:p.Gly1340fs
3:38926903:CA:C		frameshift	c.516delT:c.516delT:c.516delT	p.Phe172fs:p.Phe172fs:p.Phe172fs
3:38899983:CA:C		frameshift	c.1932delT:c.1932delT:c.1932delT	p.Phe644fs:p.Phe644fs:p.Phe644fs
3:38926802:C:G		splice_donor	c.617+1G>C:c.617+1G>C:c.617+1G>C	
3:38926802:C:T	rs754708932	splice_donor	c.617+1G>A:c.617+1G>A:c.617+1G>A	
3:38950094:A:G		splice_donor	c.267+2T>C:c.267+2T>C:c.267+2T>C	
3:38847323:AG:A		frameshift	c.4746delC:c.4632delC	p.Tyr1583fs:p.Tyr1545fs
3:38899918:C:T		stop_gained	c.1998G>A:c.1998G>A:c.1998G>A	p.Trp666*:p.Trp666*:p.Trp666*
3:38910065:C:T		splice_donor	c.1101+1G>A:c.1101+1G>A:c.1101+1G>A	

3:38894732:TCTTTG:T		frameshift	c.2631_2635delCAAAG:c.2631_2635delCAAAG:c.2631_2635delCAAAG	p.Ser877fs:p.Ser877fs:p.Ser877fs
3:38863236:A:AT	rs1016939517	frameshift	c.4014dupA:c.3900dupA:c.4014dupA	p.Leu1339fs:p.Leu1301fs:p.Leu1339fs
3:38946897:AC:A		frameshift	c.277delG:c.277delG:c.277delG	p.Val93fs:p.Val93fs:p.Val93fs
3:38867363:G:GT	rs762160601	frameshift	c.3908_3909insA:c.3794_3795insA:c.3908_3909insA	p.Phe1303fs:p.Phe1265fs:p.Phe1303fs
3:38894798:C:T		stop_gained	c.2570G>A:c.2570G>A:c.2570G>A	p.Trp857*:p.Trp857*:p.Trp857*
3:38950345:G:C		stop_gained	c.18C>G:c.18C>G:c.18C>G	p.Tyr6*:p.Tyr6*:p.Tyr6*
3:38880016:C:T		stop_gained	c.3327G>A:c.3213G>A:c.3327G>A	p.Trp1109*:p.Trp1071*:p.Trp1109*
3:38847329:TG:T	rs762319868	frameshift	c.4740delC:c.4626delC	p.Thr1581fs:p.Thr1543fs
3:38905297:G:A	rs367770852	stop_gained	c.1498C>T:c.1498C>T:c.1498C>T	p.Arg500*:p.Arg500*:p.Arg500*
3:38867329:G:A	rs1356435144	stop_gained	c.3943C>T:c.3829C>T:c.3943C>T	p.Gln1315*:p.Gln1277*:p.Gln1315*
3:38850752:C:T		splice_acceptor	c.4057-1G>A:c.3943-1G>A:c.4057-1G>A	
3:38926878:C:CT		frameshift	c.541dupA:c.541dupA:c.541dupA	p.Arg181fs:p.Arg181fs:p.Arg181fs
3:38872201:C:A		stop_gained	c.3487G>T:c.3373G>T:c.3487G>T	p.Gly1163*:p.Gly1125*:p.Gly1163*
3:38921185:GA:G	rs1064793259	frameshift	c.782delT:c.782delT:c.782delT	p.Phe261fs:p.Phe261fs:p.Phe261fs
3:38847516:C:T		stop_gained	c.4554G>A:c.4440G>A	p.Trp1518*:p.Trp1480*
3:38909083:C:A	rs1274914399	stop_gained	c.1213G>T:c.1213G>T:c.1213G>T	p.Glu405*:p.Glu405*:p.Glu405*

3:38905230:G:GCT		frameshift	c.1563_1564dupAG:c.1563_1564dupAG:c.1563_1564dupAG	p.Ala522fs:p.Ala522fs:p.Ala522fs
3:38850479:A:C	rs1271092061	splice_donor	c.4327+2T>G:c.4213+2T>G	
3:38872259:CA:C		frameshift	c.3428delT:c.3314delT:c.3428delT	p.Leu1143fs:p.Leu1105fs:p.Leu1143fs

Any one or more (i.e., any combination) of the SCN11A predicted loss-of-function variant nucleic acid molecules described herein can be used within any of the methods described herein to determine whether a subject has an increased or decreased risk of developing a headache or a migraine. The combinations of particular variants can form a mask used for statistical analysis of the particular correlation of SCN11A and an increased or decreased risk of developing a headache or a migraine. In some embodiments, the mask used for statistical analysis of the particular correlation of SCN11A and an increased or decreased risk of developing a headache or a migraine can exclude any one or more of these SCN11A predicted loss-of-function variant nucleic acid molecules described herein.

In any of the embodiments described herein, the subject can have a headache. In any of the embodiments described herein, the subject can be at risk of developing a headache. In some embodiments, the headache is a tension headache, a sinus headache, an ice-pick headache, a hunger and/or thirst headache, a cluster headache, a hormonal headache, a caffeine headache, an exertion headache, a hypertension headache, a post-traumatic headache, an episodic headache, or a chronic headache. In some embodiments, the headache is a tension headache. In some embodiments, the headache is a sinus headache. In some embodiments, the headache is an ice-pick headache. In some embodiments, the headache is a hunger and/or thirst headache. In some embodiments, the headache is a cluster headache. In some embodiments, the headache is a hormonal headache. In some embodiments, the headache is a caffeine headache. In some embodiments, the headache is an exertion headache. In some embodiments, the headache is a hypertension headache. In some embodiments, the headache is a post-traumatic headache. In some embodiments, the headache is an episodic headache. In some embodiments, the headache is a chronic headache.

In any of the embodiments described herein, the subject can have a migraine. In any of the embodiments described herein, the subject can be at risk of developing a migraine. Migraines are a neurological disorder that can be identified by pain along one side of the head as well as nausea and sensitivity to light and sound. A sign of an oncoming migraine is the aura that precedes it. A migraine aura can be identified by numbness as well as visual disturbances such as flashes of light or spots in the field of vision. In some embodiments, the migraine is a retinal migraine, an ocular migraine, a migraine with aura, a migraine without aura, a hemiplegic migraine, an episodic migraine, a chronic migraine, or status migrainosus. In some embodiments, the migraine is a retinal migraine. In some embodiments, the migraine is an ocular migraine. In some embodiments, the migraine is a migraine with aura. In some embodiments, the migraine is a migraine without aura. In some embodiments, the migraine is a hemiplegic migraine. In some embodiments, the migraine is an episodic migraine. In some embodiments, the migraine is a chronic migraine. In some embodiments, the migraine is status migrainosus.

The present disclosure provides methods of treating a subject having a headache or at risk of developing a headache, the methods comprising administering an SCN11A inhibitor to the subject.

The present disclosure provides methods of treating a subject having a migraine or at risk of developing a migraine, the methods comprising administering an SCN11A inhibitor to the subject.

In some embodiments, the SCN11A inhibitor comprises an inhibitory nucleic acid molecule. Examples of inhibitory nucleic acid molecules include, but are not limited to, antisense nucleic acid molecules, small interfering RNAs (siRNAs), and short hairpin RNAs (shRNAs). Such inhibitory nucleic acid molecules can be designed to target any region of an SCN11A nucleic acid molecule. In some embodiments, the antisense RNA, siRNA, or shRNA hybridizes to a sequence within an SCN11A genomic nucleic acid molecule or mRNA molecule and decreases expression of the SCN11A polypeptide in a cell in the subject. In some embodiments, the SCN11A inhibitor comprises an antisense molecule that hybridizes to an SCN11A genomic nucleic acid molecule or mRNA molecule and decreases expression of the SCN11A polypeptide in a cell in the subject. In some embodiments, the SCN11A inhibitor comprises an siRNA that hybridizes to an SCN11A genomic nucleic acid molecule or mRNA molecule and decreases expression of the SCN11A polypeptide in a cell in the subject. In some

embodiments, the SCN11A inhibitor comprises an shRNA that hybridizes to an SCN11A genomic nucleic acid molecule or mRNA molecule and decreases expression of the SCN11A polypeptide in a cell in the subject.

The inhibitory nucleic acid molecules can comprise RNA, DNA, or both RNA and DNA. The inhibitory nucleic acid molecules can also be linked or fused to a heterologous nucleic acid sequence, such as in a vector, or a heterologous label. For example, the inhibitory nucleic acid molecules can be within a vector or as an exogenous donor sequence comprising the inhibitory nucleic acid molecule and a heterologous nucleic acid sequence. The inhibitory nucleic acid molecules can also be linked or fused to a heterologous label. The label can be directly detectable (such as, for example, fluorophore) or indirectly detectable (such as, for example, hapten, enzyme, or fluorophore quencher). Such labels can be detectable by spectroscopic, photochemical, biochemical, immunochemical, or chemical means. Such labels include, for example, radiolabels, pigments, dyes, chromogens, spin labels, and fluorescent labels. The label can also be, for example, a chemiluminescent substance; a metal-containing substance; or an enzyme, where there occurs an enzyme-dependent secondary generation of signal. The term "label" can also refer to a "tag" or hapten that can bind selectively to a conjugated molecule such that the conjugated molecule, when added subsequently along with a substrate, is used to generate a detectable signal. For example, biotin can be used as a tag along with an avidin or streptavidin conjugate of horseradish peroxidate (HRP) to bind to the tag, and examined using a calorimetric substrate (such as, for example, tetramethylbenzidine (TMB)) or a fluorogenic substrate to detect the presence of HRP. Exemplary labels that can be used as tags to facilitate purification include, but are not limited to, myc, HA, FLAG or 3XFLAG, 6XHis or polyhistidine, glutathione-S-transferase (GST), maltose binding protein, an epitope tag, or the Fc portion of immunoglobulin. Numerous labels include, for example, particles, fluorophores, haptens, enzymes and their calorimetric, fluorogenic and chemiluminescent substrates and other labels.

The inhibitory nucleic acid molecules can comprise, for example, nucleotides or non-natural or modified nucleotides, such as nucleotide analogs or nucleotide substitutes. Such nucleotides include a nucleotide that contains a modified base, sugar, or phosphate group, or that incorporates a non-natural moiety in its structure. Examples of non-natural nucleotides include, but are not limited to, dideoxynucleotides, biotinylated, aminated, deaminated, alkylated, benzylated, and fluorophor-labeled nucleotides.

The inhibitory nucleic acid molecules can also comprise one or more nucleotide analogs or substitutions. A nucleotide analog is a nucleotide which contains a modification to either the base, sugar, or phosphate moieties. Modifications to the base moiety include, but are not limited to, natural and synthetic modifications of A, C, G, and T/U, as well as different purine or pyrimidine bases such as, for example, pseudouridine, uracil-5-yl, hypoxanthin-9-yl (I), and 2-aminoadenin-9-yl. Modified bases include, but are not limited to, 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl uracil and cytosine, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo (such as, for example, 5-bromo), 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine, 7-methyladenine, 8-azaguanine, 8-azaadenine, 7-deazaguanine, 7-deazaadenine, 3-deazaguanine, and 3-deazaadenine.

Nucleotide analogs can also include modifications of the sugar moiety. Modifications to the sugar moiety include, but are not limited to, natural modifications of the ribose and deoxy ribose as well as synthetic modifications. Sugar modifications include, but are not limited to, the following modifications at the 2’ position: OH; F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S- or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl, and alkynyl may be substituted or unsubstituted C_{1-10}alkyl or C_{2-10}alkenyl, and C_{2-10}alkynyl. Exemplary 2’ sugar modifications also include, but are not limited to, $-O[(CH_2)_nO]_mCH_3$, $-O(CH_2)_nOCH_3$, $-O(CH_2)_nNH_2$, $-O(CH_2)_nCH_3$, $-O(CH_2)_n$-ONH_2, and $-O(CH_2)_nON[(CH_2)_nCH_3)]_2$, where n and m, independently, are from 1 to about 10. Other modifications at the 2’ position include, but are not limited to, C_{1-10}alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH_3, OCN, Cl, Br, CN, CF_3, OCF_3, $SOCH_3$, SO_2CH_3, ONO_2, NO_2, N_3, NH_2, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an oligonucleotide, or a group for improving the pharmacodynamic properties of an oligonucleotide, and other substituents having similar properties. Similar modifications may also be made at other positions on the sugar, particularly the 3’ position of the sugar on the 3’ terminal nucleotide or in 2’-5’ linked oligonucleotides and the 5’ position of 5’ terminal nucleotide. Modified sugars can also include those that contain modifications at the bridging ring oxygen, such as CH_2 and S.

Nucleotide sugar analogs can also have sugar mimetics, such as cyclobutyl moieties in place of the pentofuranosyl sugar.

Nucleotide analogs can also be modified at the phosphate moiety. Modified phosphate moieties include, but are not limited to, those that can be modified so that the linkage between two nucleotides contains a phosphorothioate, chiral phosphorothioate, phosphorodithioate, phosphotriester, aminoalkylphosphotriester, methyl and other alkyl phosphonates including 3'-alkylene phosphonate and chiral phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates. These phosphate or modified phosphate linkage between two nucleotides can be through a 3'-5' linkage or a 2'-5' linkage, and the linkage can contain inverted polarity such as 3'-5' to 5'-3' or 2'-5' to 5'-2'. Various salts, mixed salts, and free acid forms are also included. Nucleotide substitutes also include peptide nucleic acids (PNAs).

In some embodiments, the antisense nucleic acid molecules are gapmers, whereby the first one to seven nucleotides at the 5' and 3' ends each have 2'-methoxyethyl (2'-MOE) modifications. In some embodiments, the first five nucleotides at the 5' and 3' ends each have 2'-MOE modifications. In some embodiments, the first one to seven nucleotides at the 5' and 3' ends are RNA nucleotides. In some embodiments, the first five nucleotides at the 5' and 3' ends are RNA nucleotides. In some embodiments, each of the backbone linkages between the nucleotides is a phosphorothioate linkage.

In some embodiments, the siRNA molecules have termini modifications. In some embodiments, the 5' end of the antisense strand is phosphorylated. In some embodiments, 5'-phosphate analogs that cannot be hydrolyzed, such as 5'-(E)-vinyl-phosphonate are used.

In some embodiments, the siRNA molecules have backbone modifications. In some embodiments, the modified phosphodiester groups that link consecutive ribose nucleosides have been shown to enhance the stability and *in vivo* bioavailability of siRNAs The non-ester groups (-OH, =O) of the phosphodiester linkage can be replaced with sulfur, boron, or acetate to give phosphorothioate, boranophosphate, and phosphonoacetate linkages. In addition, substituting the phosphodiester group with a phosphotriester can facilitate cellular uptake of siRNAs and retention on serum components by eliminating their negative charge. In some embodiments, the siRNA molecules have sugar modifications. In some embodiments, the sugars are deprotonated (reaction catalyzed by exo- and endonucleases) whereby the

2’-hydroxyl can act as a nucleophile and attack the adjacent phosphorous in the phosphodiester bond. Such alternatives include 2’-O-methyl, 2’-O-methoxyethyl, and 2’-fluoro modifications.

In some embodiments, the siRNA molecules have base modifications. In some embodiments, the bases can be substituted with modified bases such as pseudouridine, 5’-methylcytidine, N6-methyladenosine, inosine, and N7-methylguanosine.

In some embodiments, the siRNA molecules are conjugated to lipids. Lipids can be conjugated to the 5’ or 3’ termini of siRNA to improve their *in vivo* bioavailability by allowing them to associate with serum lipoproteins. Representative lipids include, but are not limited to, cholesterol and vitamin E, and fatty acids, such as palmitate and tocopherol.

In some embodiments, a representative siRNA has the following formula:

Sense: mN*mN*/i2FN/mN/i2FN/mN/i2FN/mN/i2FN/mN/i2FN/mN/i2FN/mN/i2FN/mN/i2FN/mN/i2FN/*mN*/32FN/

Antisense: /52FN/*/i2FN/*mN/i2FN/mN/i2FN/mN/i2FN/mN/i2FN/mN/i2FN/mN/i2FN/mN/i2FN/mN/i2FN/mN*N*N

wherein: “N” is the base; “2F” is a 2’-F modification; “m” is a 2’-O-methyl modification, “I” is an internal base; and “*” is a phosphorothioate backbone linkage.

In any of the embodiments described herein, the inhibitory nucleic acid molecules may be administered, for example, as one to two hour i.v. infusions or s.c. injections. In any of the embodiments described herein, the inhibitory nucleic acid molecules may be administered at dose levels that range from about 50 mg to about 900 mg, from about 100 mg to about 800 mg, from about 150 mg to about 700 mg, or from about 175 to about 640 mg (2.5 to 9.14 mg/kg; 92.5 to 338 mg/m^2 – based on an assumption of a body weight of 70 kg and a conversion of mg/kg to mg/m^2 dose levels based on a mg/kg dose multiplier value of 37 for humans).

The present disclosure also provides vectors comprising any one or more of the inhibitory nucleic acid molecules. In some embodiments, the vectors comprise any one or more of the inhibitory nucleic acid molecules and a heterologous nucleic acid. The vectors can be viral or nonviral vectors capable of transporting a nucleic acid molecule. In some embodiments, the vector is a plasmid or cosmid (such as, for example, a circular double-stranded DNA into which additional DNA segments can be ligated). In some embodiments, the vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Expression vectors include, but are not limited to, plasmids, cosmids, retroviruses, adenoviruses, adeno-associated viruses (AAV), plant viruses such as cauliflower mosaic virus and tobacco mosaic

virus, yeast artificial chromosomes (YACs), Epstein-Barr (EBV)-derived episomes, and other expression vectors known in the art.

The present disclosure also provides compositions comprising any one or more of the inhibitory nucleic acid molecules. In some embodiments, the composition is a pharmaceutical composition. In some embodiments, the compositions comprise a carrier and/or excipient. Examples of carriers include, but are not limited to, poly(lactic acid) (PLA) microspheres, poly(D,L-lactic-coglycolic-acid) (PLGA) microspheres, liposomes, micelles, inverse micelles, lipid cochleates, and lipid microtubules. A carrier may comprise a buffered salt solution such as PBS, HBSS, etc.

In some embodiments, the SCN11A inhibitor comprises a nuclease agent that induces one or more nicks or double-strand breaks at a recognition sequence(s) or a DNA-binding protein that binds to a recognition sequence within an SCN11A genomic nucleic acid molecule. The recognition sequence can be located within a coding region of the SCN11A gene, or within regulatory regions that influence the expression of the gene. A recognition sequence of the DNA-binding protein or nuclease agent can be located in an intron, an exon, a promoter, an enhancer, a regulatory region, or any non-protein coding region. The recognition sequence can include or be proximate to the start codon of the SCN11A gene. For example, the recognition sequence can be located about 10, about 20, about 30, about 40, about 50, about 100, about 200, about 300, about 400, about 500, or about 1,000 nucleotides from the start codon. As another example, two or more nuclease agents can be used, each targeting a nuclease recognition sequence including or proximate to the start codon. As another example, two nuclease agents can be used, one targeting a nuclease recognition sequence including or proximate to the start codon, and one targeting a nuclease recognition sequence including or proximate to the stop codon, wherein cleavage by the nuclease agents can result in deletion of the coding region between the two nuclease recognition sequences. Any nuclease agent that induces a nick or double-strand break into a desired recognition sequence can be used in the methods and compositions disclosed herein. Any DNA-binding protein that binds to a desired recognition sequence can be used in the methods and compositions disclosed herein.

Suitable nuclease agents and DNA-binding proteins for use herein include, but are not limited to, zinc finger protein or zinc finger nuclease (ZFN) pair, Transcription Activator-Like Effector (TALE) protein or Transcription Activator-Like Effector Nuclease (TALEN), or Clustered Regularly Interspersed Short Palindromic Repeats (CRISPR)/CRISPR-associated (Cas) systems.

The length of the recognition sequence can vary, and includes, for example, recognition sequences that are about 30-36 bp for a zinc finger protein or ZFN pair, about 15-18 bp for each ZFN, about 36 bp for a TALE protein or TALEN, and about 20 bp for a CRISPR/Cas guide RNA.

In some embodiments, CRISPR/Cas systems can be used to modify an SCN11A genomic nucleic acid molecule within a cell. The methods and compositions disclosed herein can employ CRISPR-Cas systems by utilizing CRISPR complexes (comprising a guide RNA (gRNA) complexed with a Cas protein) for site-directed cleavage of SCN11A nucleic acid molecules.

Cas proteins generally comprise at least one RNA recognition or binding domain that can interact with gRNAs. Cas proteins can also comprise nuclease domains (such as, for example, DNase or RNase domains), DNA binding domains, helicase domains, protein-protein interaction domains, dimerization domains, and other domains. Suitable Cas proteins include, for example, a wild type Cas9 protein and a wild type Cpf1 protein (such as, for example, FnCpf1). A Cas protein can have full cleavage activity to create a double-strand break in an SCN11A genomic nucleic acid molecule or it can be a nickase that creates a single-strand break in an SCN11A genomic nucleic acid molecule. Additional examples of Cas proteins include, but are not limited to, Cas1, Cas1B, Cas2, Cas3, Cas4, Cas5, Cas5e (CasD), Cas6, Cas6e, Cas6f, Cas7, Cas8a1, Cas8a2, Cas8b, Cas8c, Cas9 (Csn1 or Csx12), Cas10, Cas10d, CasF, CasG, CasH, Csy1, Csy2, Csy3, Cse1 (CasA), Cse2 (CasB), Cse3 (CasE), Cse4 (CasC), Csc1, Csc2, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1 , Cmr3, Cmr4, Cmr5, Cmr6, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csx1, Csx15, Csf1, Csf2, Csf3, Csf4, and Cu1966, and homologs or modified versions thereof. In some embodiments, a Cas system, such as Cas12a, can have multiple gRNAs encoded into a single crRNA. Cas proteins can also be operably linked to heterologous polypeptides as fusion proteins. For example, a Cas protein can be fused to a cleavage domain, an epigenetic modification domain, a transcriptional activation domain, or a transcriptional repressor domain. Cas proteins can be provided in any form. For example, a Cas protein can be provided in the form of a protein, such as a Cas protein complexed with a gRNA. Alternately, a Cas protein can be provided in the form of a nucleic acid molecule encoding the Cas protein, such as an RNA or DNA.

In some embodiments, targeted genetic modifications of SCN11A genomic nucleic acid molecules can be generated by contacting a cell with a Cas protein and one or more gRNAs that hybridize to one or more gRNA recognition sequences within a target genomic locus in the SCN11A genomic nucleic acid molecule. The gRNA recognition sequence can include or be

proximate to the start codon of an SCN11A genomic nucleic acid molecule or the stop codon of an SCN11A genomic nucleic acid molecule. For example, the gRNA recognition sequence can be located from about 10, from about 20, from about 30, from about 40, from about 50, from about 100, from about 200, from about 300, from about 400, from about 500, or from about 1,000 nucleotides of the start codon or the stop codon.

The gRNA recognition sequences within a target genomic locus in an SCN11A genomic nucleic acid molecule are located near a Protospacer Adjacent Motif (PAM) sequence, which is a 2-6 base pair DNA sequence immediately following the DNA sequence targeted by the Cas9 nuclease. The canonical PAM is the sequence 5'-NGG-3' where “N” is any nucleobase followed by two guanine (“G”) nucleobases. gRNAs can transport Cas9 to anywhere in the genome for gene editing, but no editing can occur at any site other than one at which Cas9 recognizes PAM. In addition, 5'-NGA-3' can be a highly efficient non-canonical PAM for human cells. Generally, the PAM is about 2-6 nucleotides downstream of the DNA sequence targeted by the gRNA. The PAM can flank the gRNA recognition sequence. In some embodiments, the gRNA recognition sequence can be flanked on the 3’ end by the PAM. In some embodiments, the gRNA recognition sequence can be flanked on the 5’ end by the PAM. For example, the cleavage site of Cas proteins can be about 1 to about 10, about 2 to about 5 base pairs, or three base pairs upstream or downstream of the PAM sequence. In some embodiments (such as when Cas9 from *S. pyogenes* or a closely related Cas9 is used), the PAM sequence of the non-complementary strand can be 5'-NGG-3', where N is any DNA nucleotide and is immediately 3' of the gRNA recognition sequence of the non-complementary strand of the target DNA. As such, the PAM sequence of the complementary strand would be 5'-CCN-3', where N is any DNA nucleotide and is immediately 5' of the gRNA recognition sequence of the complementary strand of the target DNA.

A gRNA is an RNA molecule that binds to a Cas protein and targets the Cas protein to a specific location within an SCN11A genomic nucleic acid molecule. An exemplary gRNA is a gRNA effective to direct a Cas enzyme to bind to or cleave an SCN11A genomic nucleic acid molecule, wherein the gRNA comprises a DNA-targeting segment that hybridizes to a gRNA recognition sequence within the SCN11A genomic nucleic acid molecule. Exemplary gRNAs comprise a DNA-targeting segment that hybridizes to a gRNA recognition sequence present within an SCN11A genomic nucleic acid molecule that includes or is proximate to the start codon or the stop codon. For example, a gRNA can be selected such that it hybridizes to a gRNA

recognition sequence that is located from about 5, from about 10, from about 15, from about 20, from about 25, from about 30, from about 35, from about 40, from about 45, from about 50, from about 100, from about 200, from about 300, from about 400, from about 500, or from about 1,000 nucleotides of the start codon or located from about 5, from about 10, from about 15, from about 20, from about 25, from about 30, from about 35, from about 40, from about 45, from about 50, from about 100, from about 200, from about 300, from about 400, from about 500, or from about 1,000 nucleotides of the stop codon. Suitable gRNAs can comprise from about 17 to about 25 nucleotides, from about 17 to about 23 nucleotides, from about 18 to about 22 nucleotides, or from about 19 to about 21 nucleotides. In some embodiments, the gRNAs can comprise 20 nucleotides.

The Cas protein and the gRNA form a complex, and the Cas protein cleaves the target SCN11A genomic nucleic acid molecule. The Cas protein can cleave the nucleic acid molecule at a site within or outside of the nucleic acid sequence present in the target SCN11A genomic nucleic acid molecule to which the DNA-targeting segment of a gRNA will bind. For example, formation of a CRISPR complex (comprising a gRNA hybridized to a gRNA recognition sequence and complexed with a Cas protein) can result in cleavage of one or both strands in or near (such as, for example, within 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 50, or more base pairs from) the nucleic acid sequence present in the SCN11A genomic nucleic acid molecule to which a DNA-targeting segment of a gRNA will bind.

Such methods can result, for example, in an SCN11A genomic nucleic acid molecule in which a region of the SCN11A genomic nucleic acid molecule is disrupted, the start codon is disrupted, the stop codon is disrupted, or the coding sequence is disrupted or deleted. Optionally, the cell can be further contacted with one or more additional gRNAs that hybridize to additional gRNA recognition sequences within the target genomic locus in the SCN11A genomic nucleic acid molecule. By contacting the cell with one or more additional gRNAs (such as, for example, a second gRNA that hybridizes to a second gRNA recognition sequence), cleavage by the Cas protein can create two or more double-strand breaks or two or more single-strand breaks.

In some embodiments, the methods of treatment and/or prevention further comprise detecting the presence or absence of an SCN11A predicted loss-of-function variant nucleic acid molecule in a biological sample from the subject. In some embodiments, the SCN11A predicted

loss-of-function variant nucleic acid molecule can be any of the SCN11A predicted loss-of-function variant nucleic acid molecules disclosed herein.

The present disclosure also provides methods of treating a subject with a therapeutic agent that treats or inhibits a headache or a migraine, wherein the subject has a headache or a migraine or is at risk of developing a headache or a migraine. In some embodiments, the methods comprise determining whether the subject has an SCN11A predicted loss-of-function variant nucleic acid molecule by obtaining or having obtained a biological sample from the subject, and performing or having performed a sequence analysis on the biological sample to determine if the subject has a genotype comprising the SCN11A predicted loss-of-function variant nucleic acid molecule. In some embodiments, the methods further comprise administering or continuing to administer the therapeutic agent that treats, prevents, or inhibits a headache or a migraine in a standard dosage amount to a subject that is SCN11A reference, and/or administering an SCN11A inhibitor to the subject. In some embodiments, the methods further comprise administering or continuing to administer the therapeutic agent that treats, prevents, or inhibits a headache or a migraine in an amount that is the same as or less than a standard dosage amount to a subject that is heterozygous for the SCN11A predicted loss-of-function variant nucleic acid molecule, and/or administering an SCN11A inhibitor to the subject. In some embodiments, the methods further comprise administering or continuing to administer the therapeutic agent that treats, prevents, or inhibits a headache or a migraine in an amount that is the same as or less than a standard dosage amount to a subject that is homozygous for the SCN11A predicted loss-of-function variant nucleic acid molecule. The presence of a genotype having the SCN11A predicted loss-of-function variant nucleic acid molecule indicates the subject has a decreased risk of developing a headache or a migraine. In some embodiments, the subject is SCN11A reference. In some embodiments, the subject is heterozygous for an SCN11A predicted loss-of-function variant nucleic acid molecule.

For subjects that are genotyped or determined to be either SCN11A reference or heterozygous for an SCN11A predicted loss-of-function variant nucleic acid molecule, such subjects can be administered an SCN11A inhibitor, as described herein.

Detecting the presence or absence of an SCN11A predicted loss-of-function variant nucleic acid molecule in a biological sample from a subject and/or determining whether a subject has an SCN11A predicted loss-of-function variant nucleic acid molecule can be carried out by any of the methods described herein. In some embodiments, these methods can be

carried out *in vitro*. In some embodiments, these methods can be carried out *in situ*. In some embodiments, these methods can be carried out *in vivo*. In any of these embodiments, the nucleic acid molecule can be present within a cell obtained from the subject.

In some embodiments, when the subject is SCN11A reference, the subject is administered a therapeutic agent that treats, prevents, or inhibits a headache or a migraine in a standard dosage amount. In some embodiments, when the subject is heterozygous for an SCN11A predicted loss-of-function variant nucleic acid molecule, the subject is administered a therapeutic agent that treats, prevents, or inhibits a headache or a migraine in a dosage amount that is the same as or less than a standard dosage amount.

In some embodiments, the treatment and/or prevention methods comprise detecting the presence or absence of an SCN11A predicted loss-of-function polypeptide in a biological sample from the subject. In some embodiments, when the subject does not have an SCN11A predicted loss-of-function polypeptide, the subject is administered a therapeutic agent that treats, prevents, or inhibits a headache or a migraine in a standard dosage amount. In some embodiments, when the subject has an SCN11A predicted loss-of-function polypeptide, the subject is administered a therapeutic agent that treats, prevents, or inhibits a headache or a migraine in a dosage amount that is the same as or less than a standard dosage amount.

The present disclosure also provides methods of treating a subject with a therapeutic agent that treats or inhibits a headache or a migraine, wherein the subject has a headache or a migraine or is at risk of developing a headache or a migraine. In some embodiments, the method comprises determining whether the subject has an SCN11A predicted loss-of-function polypeptide by obtaining or having obtained a biological sample from the subject, and performing or having performed an assay on the biological sample to determine if the subject has an SCN11A predicted loss-of-function polypeptide. When the subject does not have an SCN11A predicted loss-of-function polypeptide, the therapeutic agent that treats or inhibits a headache or a migraine is administered or continued to be administered to the subject in a standard dosage amount, and/or an SCN11A inhibitor is administered to the subject. When the subject has an SCN11A predicted loss-of-function polypeptide, the therapeutic agent that treats or inhibits a headache or a migraine is administered or continued to be administered to the subject in an amount that is the same as or less than a standard dosage amount, and/or an SCN11A inhibitor is administered to the subject. The presence of an SCN11A predicted loss-of-function polypeptide indicates the subject has a decreased risk of developing a headache or a

migraine. In some embodiments, the subject has an SCN11A predicted loss-of-function polypeptide. In some embodiments, the subject does not have an SCN11A predicted loss-of-function polypeptide.

The present disclosure also provides methods of preventing a subject from developing a headache or a migraine by administering a therapeutic agent that prevents a headache or a migraine. In some embodiments, the method comprises determining whether the subject has an SCN11A predicted loss-of-function polypeptide by obtaining or having obtained a biological sample from the subject, and performing or having performed an assay on the biological sample to determine if the subject has an SCN11A predicted loss-of-function polypeptide. When the subject does not have an SCN11A predicted loss-of-function polypeptide, the therapeutic agent that prevents a headache or a migraine is administered or continued to be administered to the subject in a standard dosage amount, and/or an SCN11A inhibitor is administered to the subject. When the subject has an SCN11A predicted loss-of-function polypeptide, the therapeutic agent that prevents a headache or a migraine is administered or continued to be administered to the subject in an amount that is the same as or less than a standard dosage amount, and/or an SCN11A inhibitor is administered to the subject. The presence of an SCN11A predicted loss-of-function polypeptide indicates the subject has a decreased risk of developing a headache or a migraine. In some embodiments, the subject has an SCN11A predicted loss-of-function polypeptide. In some embodiments, the subject does not have an SCN11A predicted loss-of-function polypeptide.

Detecting the presence or absence of an SCN11A predicted loss-of-function polypeptide in a biological sample from a subject and/or determining whether a subject has an SCN11A predicted loss-of-function polypeptide can be carried out by any of the methods described herein. In some embodiments, these methods can be carried out *in vitro*. In some embodiments, these methods can be carried out *in situ*. In some embodiments, these methods can be carried out *in vivo*. In any of these embodiments, the polypeptide can be present within a cell obtained from the subject.

In some embodiments, the SCN11A inhibitor is a small molecule. In some embodiments, the SCN11A inhibitor is a non-selective sodium blocker. In some embodiments, the non-selective sodium blocker is lignocaine, mexiletine, or carbamazepine. In some embodiments, the non-selective sodium blocker is lignocaine. In some embodiments, the non-selective sodium blocker is mexiletine. In some embodiments, the non-selective sodium blocker

is carbamazepine. In some embodiments, the SCN11A inhibitor is menthol, mibefradil, or an inorganic l(Ca) blocker.

In some embodiments, the SCN11A inhibitor is an antibody.

Examples of therapeutic agents that treat or prevent a headache include, but are not limited to, nonsteroidal anti-inflammatory drugs (NSAIDs) (such as, for example, aspirin, ibuprofen, fenoprofen, flurbiprofen, ketoprofen, and naproxen sodium), acetaminophen, celecoxib, diclofenac, indomethacin, ketorolac tromethamine, meclofenamate sodium, diflunisal, tolmetin, nabumetone, carisoprodol, orphenadrine citrate, methocarbamol, cyclobenzaprine hydrochloride, metaxalone, prednisone, ergotamine, lithium, propranolol, diltiazem, and an opioid, or any combination thereof.

Examples of therapeutic agents that treat or inhibit a migraine include, but are not limited to, nonsteroidal anti-inflammatory drugs (NSAIDs) (such as, for example, aspirin, ibuprofen, fenoprofen, flurbiprofen, ketoprofen, and naproxen sodium); acetaminophen; diclofenac; ketorolac; propofol; Lasmiditan; a combination of aspirin, acetaminophen, and caffeine) a combination of isometheptene, dichloralphenazone, and acetaminophen; a triptan (such as, for example, almotriptan, avitriptan, eletriptan, frovatriptan, naratriptan, rizatriptan, donitriptan, sumatriptan, zolmitriptan, LY-334370, and L-694247); an ergot (such as, for example, dihydroergotamine and ergotamine tartrate); a calcitonin gene-related peptide (CGRP) receptor antagonist (such as, for example, rimegepant, atogepant, ubrogepant, eptinezumab, erenumab, fremanezumab, and galcanezumab); an anti-nausea agent (such as, for example, chlorpromazine, droperidol, metoclopramide, and prochlorperazine); a high blood pressure medication (such as, for example, a beta-blocker (such as, for example, propranolol, timolol, and metoprolol) and a calcium channel blocker (such as, for example, verapamil)); an antidepressant (such as, for example, amitriptyline and nortriptyline); an antiseizure medication (such as, for example, gabapentin, topiramate, and valproic acid); an opioid; a barbiturate; feverfew; and botulinum toxin.

In some embodiments, the dose of the therapeutic agents that treat, prevent, or inhibit a headache or a migraine can be decreased by about 10%, by about 20%, by about 30%, by about 40%, by about 50%, by about 60%, by about 70%, by about 80%, or by about 90% for subjects that are heterozygous for an SCN11A predicted loss-of-function variant nucleic acid molecule (i.e., a less than the standard dosage amount) compared to subjects that are SCN11A reference (who may receive a standard dosage amount). In some embodiments, the dose of the

therapeutic agents that treat, prevent, or inhibit a headache or a migraine can be decreased by about 10%, by about 20%, by about 30%, by about 40%, or by about 50%. In addition, the subjects that are heterozygous for an SCN11A predicted loss-of-function variant nucleic acid molecule can be administered less frequently compared to subjects that are SCN11A reference.

In some embodiments, the dose of the therapeutic agents that treat, prevent, or inhibit a headache or a migraine can be decreased by about 10%, by about 20%, by about 30%, by about 40%, by about 50%, for subjects that are homozygous for an SCN11A predicted loss-of-function variant nucleic acid molecule compared to subjects that are heterozygous for an SCN11A predicted loss-of-function variant nucleic acid molecule. In some embodiments, the dose of the therapeutic agents that treat, prevent, or inhibit a headache or a migraine can be decreased by about 10%, by about 20%, by about 30%, by about 40%, or by about 50%. In addition, the dose of therapeutic agents that treat, prevent, or inhibit a headache or a migraine in subjects that are homozygous for an SCN11A predicted loss-of-function variant nucleic acid molecule can be administered less frequently compared to subjects that are heterozygous for an SCN11A predicted loss-of-function variant nucleic acid molecule.

Administration of the therapeutic agents that treat, prevent, or inhibit a headache or a migraine and/or SCN11A inhibitors can be repeated, for example, after one day, two days, three days, five days, one week, two weeks, three weeks, one month, five weeks, six weeks, seven weeks, eight weeks, two months, or three months. The repeated administration can be at the same dose or at a different dose. The administration can be repeated once, twice, three times, four times, five times, six times, seven times, eight times, nine times, ten times, or more. For example, according to certain dosage regimens a subject can receive therapy for a prolonged period of time such as, for example, 6 months, 1 year, or more.

Administration of the therapeutic agents that treat, prevent, or inhibit a headache or a migraine and/or SCN11A inhibitors can occur by any suitable route including, but not limited to, parenteral, intravenous, oral, subcutaneous, intra-arterial, intracranial, intrathecal, intraperitoneal, topical, intranasal, or intramuscular. Pharmaceutical compositions for administration are desirably sterile and substantially isotonic and manufactured under GMP conditions. Pharmaceutical compositions can be provided in unit dosage form (i.e., the dosage for a single administration). Pharmaceutical compositions can be formulated using one or more physiologically and pharmaceutically acceptable carriers, diluents, excipients or auxiliaries. The formulation depends on the route of administration chosen. The term “pharmaceutically

acceptable" means that the carrier, diluent, excipient, or auxiliary is compatible with the other ingredients of the formulation and not substantially deleterious to the recipient thereof.

The terms "treat", "treating", and "treatment" and "prevent", "preventing", and "prevention" as used herein, refer to eliciting the desired biological response, such as a therapeutic and prophylactic effect, respectively. In some embodiments, a therapeutic effect comprises one or more of a decrease/reduction in a headache or a migraine, a decrease/reduction in the severity of a headache or a migraine (such as, for example, a reduction or inhibition of development of a headache or a migraine), a decrease/reduction in symptoms and headache- or migraine-related effects, delaying the onset of symptoms and headache- or migraine-related effects, reducing the severity of symptoms of headache- or migraine-related effects, reducing the number of symptoms and headache or migraine-related effects, reducing the latency of symptoms and headache- or migraine-related effects, an amelioration of symptoms and headache- or migraine-related effects, reducing secondary symptoms, reducing secondary infections, preventing relapse to a headache or a migraine, decreasing the number or frequency of relapse episodes, increasing latency between symptomatic episodes, increasing time to sustained progression, speeding recovery, or increasing efficacy of or decreasing resistance to alternative therapeutics, and/or an increased survival time of the affected host animal, following administration of the agent or composition comprising the agent. A prophylactic effect may comprise a complete or partial avoidance/inhibition or a delay of a headache or a migraine development/progression (such as, for example, a complete or partial avoidance/inhibition or a delay), and an increased survival time of the affected host animal, following administration of a therapeutic protocol. Treatment of a headache or a migraine encompasses the treatment of a subject already diagnosed as having any form of a headache or a migraine at any clinical stage or manifestation, the delay of the onset or evolution or aggravation or deterioration of the symptoms or signs of a headache or a migraine, and/or preventing and/or reducing the severity of a headache or a migraine.

The present disclosure also provides methods of identifying a subject having an increased risk of developing a headache or a migraine. In some embodiments, the method comprises determining or having determined in a biological sample obtained from the subject the presence or absence of an SCN11A predicted loss-of-function variant nucleic acid molecule (such as a genomic nucleic acid molecule, mRNA molecule, and/or cDNA molecule). When the subject lacks an SCN11A predicted loss-of-function variant nucleic acid molecule (i.e., the

subject is genotypically categorized as SCN11A reference), then the subject has an increased risk of developing a headache or a migraine. When the subject has an SCN11A predicted loss-of-function variant nucleic acid molecule (i.e., the subject is heterozygous or homozygous for an SCN11A predicted loss-of-function variant nucleic acid molecule), then the subject has a decreased risk of developing a headache or a migraine.

Having a single copy of SCN11A predicted loss-of-function variant nucleic acid molecule is more protective of a subject from developing a headache or a migraine than having no copies of an SCN11A predicted loss-of-function variant nucleic acid molecule. Without intending to be limited to any particular theory or mechanism of action, it is believed that a single copy of an SCN11A predicted loss-of-function variant nucleic acid molecule (i.e., heterozygous for an SCN11A predicted loss-of-function variant nucleic acid molecule) is protective of a subject from developing a headache or a migraine, and it is also believed that having two copies of an SCN11A predicted loss-of-function variant nucleic acid molecule (i.e., homozygous for an SCN11A predicted loss-of-function variant nucleic acid molecule) may be more protective of a subject from developing a headache or a migraine, relative to a subject with a single copy. Thus, in some embodiments, a single copy of an SCN11A predicted loss-of-function variant nucleic acid molecule may not be completely protective, but instead, may be partially or incompletely protective of a subject from developing a headache or a migraine. While not desiring to be bound by any particular theory, there may be additional factors or molecules involved in the development of a headache or a migraine that are still present in a subject having a single copy of an SCN11A predicted loss-of-function variant nucleic acid molecule, thus resulting in less than complete protection from the development of a headache or a migraine.

Determining whether a subject has an SCN11A predicted loss-of-function variant nucleic acid molecule in a biological sample from a subject and/or determining whether a subject has an SCN11A predicted loss-of-function variant nucleic acid molecule can be carried out by any of the methods described herein. In some embodiments, these methods can be carried out *in vitro*. In some embodiments, these methods can be carried out *in situ*. In some embodiments, these methods can be carried out *in vivo*. In any of these embodiments, the nucleic acid molecule can be present within a cell obtained from the subject.

In some embodiments, when a subject is identified as having an increased risk of developing a headache or a migraine, the subject is administered a therapeutic agent that

treats, prevents, or inhibits a headache or a migraine, and/or an SCN11A inhibitor, as described herein. For example, when the subject is SCN11A reference, and therefore has an increased risk of developing a headache or a migraine, the subject is administered an SCN11A inhibitor. In some embodiments, such a subject is also administered a therapeutic agent that treats, prevents, or inhibits a headache or a migraine. In some embodiments, when the subject is heterozygous for an SCN11A predicted loss-of-function variant nucleic acid molecule, the subject is administered the therapeutic agent that treats, prevents, or inhibits a headache or a migraine in a dosage amount that is the same as or less than a standard dosage amount, and is also administered an SCN11A inhibitor. In some embodiments, such a subject is also administered a therapeutic agent that treats, prevents, or inhibits a headache or a migraine. In some embodiments, when the subject is homozygous for an SCN11A predicted loss-of-function variant nucleic acid molecule, the subject is administered the therapeutic agent that treats, prevents, or inhibits a headache or a migraine in a dosage amount that is the same as or less than a standard dosage amount. In some embodiments, the subject is SCN11A reference. In some embodiments, the subject is heterozygous for an SCN11A predicted loss-of-function variant nucleic acid molecule. In some embodiments, the subject is homozygous for an SCN11A predicted loss-of-function variant nucleic acid molecule.

The present disclosure also provides methods of determining a subject's aggregate burden, or risk score, of having two or more SCN11A variant nucleic acid molecules, and/or two or more SCN11A variant polypeptides associated with a decreased risk of developing a headache or migraine. The aggregate burden is the sum of two or more genetic variants that can be carried out in an association analysis with a headache or migraine. In some embodiments, the subject is homozygous for one or more SCN11A variant nucleic acid molecules associated with a decreased risk of developing a headache or migraine. In some embodiments, the subject is heterozygous for one or more SCN11A variant nucleic acid molecules associated with a decreased risk of developing a headache or migraine. When the subject has a lower aggregate burden, the subject has an increased risk of developing a headache or migraine, and the subject is administered or continued to be administered the headache or migraine therapeutic agent in an amount that is the same as or less than the standard dosage amount or headache or migraine therapy, and/or an SCN11A inhibitor. When the subject has a higher aggregate burden, the subject has a decreased risk of developing a headache or migraine and the subject is administered or continued to be administered the

headache or migraine therapeutic agent in a standard dosage amount or headache or migraine therapy. The higher the aggregate burden, the lower the risk of developing a headache or migraine.

In some embodiments, a subject's aggregate burden of having any two or more SCN11A variant nucleic acid molecules represents a weighted sum of a plurality of any of the SCN11A variant nucleic acid molecules. In some embodiments, the aggregate burden is calculated using at least about 2, at least about 3, at least about 4, at least about 5, at least about 10, at least about 20, at least about 30, at least about 40, at least about 50, at least about 60, at least about 70, at least about 80, at least about 100, at least about 120, at least about 150, at least about 200, at least about 250, at least about 300, at least about 400, at least about 500, at least about 1,000, at least about 10,000, at least about 100,000, or at least about or more than 1,000,000 genetic variants present in or around (up to 10 Mb) the SCN11A gene, where the genetic burden is the number of alleles multiplied by the association estimate with a headache or migraine or related outcome for each allele (e.g., a weighted polygenic burden score). In some embodiments, when the subject has an aggregate burden higher than a desired threshold score, the subject has a decreased risk of developing a headache or migraine. In some embodiments, when the subject has an aggregate burden lower than a desired threshold score, the subject has an increased risk of developing a headache or migraine.

In some embodiments, the aggregate burden may be divided into quintiles, e.g., top quintile, second quintile, intermediate quintile, fourth quintile, and bottom quintile, wherein the top quintile of aggregate burden corresponds to the lowest risk group and the bottom quintile of aggregate burden corresponds to the highest risk group. In some embodiments, a subject having a higher aggregate burden comprises the highest weighted aggregate burdens, including, but not limited to the top 10%, top 20%, top 30%, top 40%, or top 50% of aggregate burdens from a subject population. In some embodiments, the genetic variants comprise the genetic variants having association with a headache or migraine in the top 10%, top 20%, top 30%, top 40%, or top 50% of p-value range for the association. In some embodiments, each of the identified genetic variants comprise the genetic variants having association with a headache or migraine with p-value of no more than about 10^{-2}, about 10^{-3}, about 10^{-4}, about 10^{-5}, about 10^{-6}, about 10^{-7}, about 10^{-8}, about 10^{-9}, about 10^{-10}, about 10^{-11}, about 10^{-12}, about 10^{-13}, about 10^{-14}, about or 10^{-15}. In some embodiments, the identified genetic variants comprise the genetic variants having association with a headache or migraine with p-value of less than 5×10^{-8}. In

some embodiments, the identified genetic variants comprise genetic variants having association with a headache or migraine in high-risk subjects as compared to the rest of the reference population with odds ratio (OR) about 1.5 or greater, about 1.75 or greater, about 2.0 or greater, or about 2.25 or greater for the top 20% of the distribution; or about 1.5 or greater, about 1.75 or greater, about 2.0 or greater, about 2.25 or greater, about 2.5 or greater, or about 2.75 or greater. In some embodiments, the odds ratio (OR) may range from about 1.0 to about 1.5, from about 1.5 to about 2.0, from about 2.0 to about 2.5, from about 2.5 to about 3.0, from about 3.0 to about 3.5, from about 3.5 to about 4.0, from about 4.0 to about 4.5, from about 4.5 to about 5.0, from about 5.0 to about 5.5, from about 5.5 to about 6.0, from about 6.0 to about 6.5, from about 6.5 to about 7.0, or greater than 7.0. In some embodiments, high-risk subjects have aggregate burdens in the bottom decile, quintile, or tertile in a reference population. The threshold of the aggregate burden can be determined on the basis of the nature of the intended practical application and the risk difference that would be considered meaningful for that practical application.

In embodiments where the aggregate burden is determined for SCN11A genetic variants associated with a headache or migraine, then the aggregate burden represents a subject's risk score for developing a headache or migraine. In some embodiments, the aggregate burden or risk score includes the SCN11A variant genomic nucleic acid molecule that comprises any of the genetic variations described herein, or is an mRNA molecule produced therefrom, or is a cDNA molecule produced from the mRNA molecule. In some embodiments, a subject's aggregate burden can be determined for SCN11A genetic variants associated with a headache or migraine in combination with additional genetic variants for other genes also associated with a headache or migraine to produce a polygenic risk score (PRS) for developing a headache or migraine. In some embodiments, the PRS includes the SCN11A variant genomic nucleic acid molecule that comprises any of the genetic variations described herein, or is an mRNA molecule produced therefrom, or is a cDNA molecule produced from the mRNA molecule.

The present disclosure also provides methods of detecting the presence or absence of an SCN11A predicted loss-of-function variant nucleic acid molecule (i.e., a genomic nucleic acid molecule, an mRNA molecule, or a cDNA molecule produced from an mRNA molecule) in a biological sample from a subject. It is understood that gene sequences within a population and

mRNA molecules encoded by such genes can vary due to polymorphisms such as single-nucleotide polymorphisms.

The biological sample can be derived from any cell, tissue, or biological fluid from the subject. The biological sample may comprise any clinically relevant tissue, such as a bone marrow sample, a tumor biopsy, a fine needle aspirate, or a sample of bodily fluid, such as blood, gingival crevicular fluid, plasma, serum, lymph, ascitic fluid, cystic fluid, or urine. In some cases, the sample comprises a buccal swab. The biological sample used in the methods disclosed herein can vary based on the assay format, nature of the detection method, and the tissues, cells, or extracts that are used as the sample. A biological sample can be processed differently depending on the assay being employed. For example, when detecting any an SCN11A predicted loss-of-function variant nucleic acid molecule, preliminary processing designed to isolate or enrich the biological sample for the genomic DNA can be employed. A variety of techniques may be used for this purpose. When detecting the level of any an SCN11A predicted loss-of-function variant nucleic acid molecule, different techniques can be used enrich the biological sample with mRNA molecules. Various methods to detect the presence or level of an mRNA molecule or the presence of a particular variant genomic DNA locus can be used.

In some embodiments, detecting an SCN11A predicted loss-of-function variant nucleic acid molecule in a subject comprises performing a sequence analysis on a biological sample obtained from the subject to determine whether SCN11A genomic nucleic acid molecule in the biological sample, and/or an SCN11A mRNA molecule in the biological sample, and/or an SCN11A cDNA molecule produced from an mRNA molecule in the biological sample, comprises one or more variations that cause a loss-of-function (partial or complete) or are predicted to cause a loss-of-function (partial or complete).

In some embodiments, the methods of detecting the presence or absence of an SCN11A predicted loss-of-function variant nucleic acid molecule (such as, for example, a genomic nucleic acid molecule, an mRNA molecule, and/or a cDNA molecule produced from an mRNA molecule) in a subject, comprise performing an assay on a biological sample obtained from the subject. The assay determines whether a nucleic acid molecule in the biological sample comprises a particular nucleotide sequence.

In some embodiments, the biological sample comprises a cell or cell lysate. Such methods can further comprise, for example, obtaining a biological sample from the subject

comprising an SCN11A genomic nucleic acid molecule or mRNA molecule, and if mRNA, optionally reverse transcribing the mRNA into cDNA. Such assays can comprise, for example determining the identity of these positions of the particular SCN11A nucleic acid molecule. In some embodiments, the method is an *in vitro* method.

In some embodiments, the determining step, detecting step, or sequence analysis comprises sequencing at least a portion of the nucleotide sequence of the SCN11A genomic nucleic acid molecule, the SCN11A mRNA molecule, or the SCN11A cDNA molecule in the biological sample, wherein the sequenced portion comprises one or more variations that cause a loss-of-function (partial or complete) or are predicted to cause a loss-of-function (partial or complete).

In some embodiments, the assay comprises sequencing the entire nucleic acid molecule. In some embodiments, only an SCN11A genomic nucleic acid molecule is analyzed. In some embodiments, only an SCN11A mRNA is analyzed. In some embodiments, only an SCN11A cDNA obtained from SCN11A mRNA is analyzed.

Alteration-specific polymerase chain reaction techniques can be used to detect mutations such as SNPs in a nucleic acid sequence. Alteration-specific primers can be used because the DNA polymerase will not extend when a mismatch with the template is present.

In some embodiments, the nucleic acid molecule in the sample is mRNA and the mRNA is reverse-transcribed into a cDNA prior to the amplifying step. In some embodiments, the nucleic acid molecule is present within a cell obtained from the subject.

In some embodiments, the assay comprises contacting the biological sample with a primer or probe, such as an alteration-specific primer or alteration-specific probe, that specifically hybridizes to an SCN11A variant genomic sequence, variant mRNA sequence, or variant cDNA sequence and not the corresponding SCN11A reference sequence under stringent conditions, and determining whether hybridization has occurred.

In some embodiments, the determining step, detecting step, or sequence analysis comprises: a) amplifying at least a portion of the SCN11A nucleic acid molecule that encodes the SCN11A polypeptide; b) labeling the amplified nucleic acid molecule with a detectable label; c) contacting the labeled nucleic acid molecule with a support comprising an alteration-specific probe; and d) detecting the detectable label.

In some embodiments, the assay comprises RNA sequencing (RNA-Seq). In some embodiments, the assays also comprise reverse transcribing mRNA into cDNA, such as by the reverse transcriptase polymerase chain reaction (RT-PCR).

In some embodiments, the methods utilize probes and primers of sufficient nucleotide length to bind to the target nucleotide sequence and specifically detect and/or identify a polynucleotide comprising an SCN11A variant genomic nucleic acid molecule, variant mRNA molecule, or variant cDNA molecule. The hybridization conditions or reaction conditions can be determined by the operator to achieve this result. The nucleotide length may be any length that is sufficient for use in a detection method of choice, including any assay described or exemplified herein. Such probes and primers can hybridize specifically to a target nucleotide sequence under high stringency hybridization conditions. Probes and primers may have complete nucleotide sequence identity of contiguous nucleotides within the target nucleotide sequence, although probes differing from the target nucleotide sequence and that retain the ability to specifically detect and/or identify a target nucleotide sequence may be designed by conventional methods. Probes and primers can have about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or 100% sequence identity or complementarity with the nucleotide sequence of the target nucleic acid molecule.

Illustrative examples of nucleic acid sequencing techniques include, but are not limited to, chain terminator (Sanger) sequencing and dye terminator sequencing. Other methods involve nucleic acid hybridization methods other than sequencing, including using labeled primers or probes directed against purified DNA, amplified DNA, and fixed cell preparations (fluorescence in situ hybridization (FISH)). In some methods, a target nucleic acid molecule may be amplified prior to or simultaneous with detection. Illustrative examples of nucleic acid amplification techniques include, but are not limited to, polymerase chain reaction (PCR), ligase chain reaction (LCR), strand displacement amplification (SDA), and nucleic acid sequence based amplification (NASBA). Other methods include, but are not limited to, ligase chain reaction, strand displacement amplification, and thermophilic SDA (tSDA).

In hybridization techniques, stringent conditions can be employed such that a probe or primer will specifically hybridize to its target. In some embodiments, a polynucleotide primer or probe under stringent conditions will hybridize to its target sequence to a detectably greater degree than to other non-target sequences, such as, at least 2-fold, at least 3-fold, at least 4-

fold, or more over background, including over 10-fold over background. In some embodiments, a polynucleotide primer or probe under stringent conditions will hybridize to its target nucleotide sequence to a detectably greater degree than to other nucleotide sequences by at least 2-fold. In some embodiments, a polynucleotide primer or probe under stringent conditions will hybridize to its target nucleotide sequence to a detectably greater degree than to other nucleotide sequences by at least 3-fold. In some embodiments, a polynucleotide primer or probe under stringent conditions will hybridize to its target nucleotide sequence to a detectably greater degree than to other nucleotide sequences by at least 4-fold. In some embodiments, a polynucleotide primer or probe under stringent conditions will hybridize to its target nucleotide sequence to a detectably greater degree than to other nucleotide sequences by over 10-fold over background. Stringent conditions are sequence-dependent and will be different in different circumstances.

Appropriate stringency conditions which promote DNA hybridization, for example, 6X sodium chloride/sodium citrate (SSC) at about 45°C., followed by a wash of 2X SSC at 50°C, are known or can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. Typically, stringent conditions for hybridization and detection will be those in which the salt concentration is less than about 1.5 M Na^+ ion, typically about 0.01 to 1.0 M Na^+ ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (such as, for example, 10 to 50 nucleotides) and at least about 60°C for longer probes (such as, for example, greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. Optionally, wash buffers may comprise about 0.1% to about 1% SDS. Duration of hybridization is generally less than about 24 hours, usually about 4 to about 12 hours. The duration of the wash time will be at least a length of time sufficient to reach equilibrium.

In some embodiments, such isolated nucleic acid molecules comprise or consist of at least about 5, at least about 8, at least about 10, at least about 11, at least about 12, at least about 13, at least about 14, at least about 15, at least about 16, at least about 17, at least about 18, at least about 19, at least about 20, at least about 21, at least about 22, at least about 23, at least about 24, at least about 25, at least about 30, at least about 35, at least about 40, at least about 45, at least about 50, at least about 55, at least about 60, at least about 65, at least about 70, at least about 75, at least about 80, at least about 85, at least about 90, at least about 95, at least about 100, at least about 200, at least about 300, at least about 400, at least about 500, at

least about 600, at least about 700, at least about 800, at least about 900, at least about 1000, at least about 2000, at least about 3000, at least about 4000, or at least about 5000 nucleotides. In some embodiments, such isolated nucleic acid molecules comprise or consist of at least about 5, at least about 8, at least about 10, at least about 11, at least about 12, at least about 13, at least about 14, at least about 15, at least about 16, at least about 17, at least about 18, at least about 19, at least about 20, at least about 21, at least about 22, at least about 23, at least about 24, or at least about 25 nucleotides. In some embodiments, the isolated nucleic acid molecules comprise or consist of at least about 18 nucleotides. In some embodiments, the isolated nucleic acid molecules comprise or consists of at least about 15 nucleotides. In some embodiments, the isolated nucleic acid molecules consist of or comprise from about 10 to about 35, from about 10 to about 30, from about 10 to about 25, from about 12 to about 30, from about 12 to about 28, from about 12 to about 24, from about 15 to about 30, from about 15 to about 25, from about 18 to about 30, from about 18 to about 25, from about 18 to about 24, or from about 18 to about 22 nucleotides. In some embodiments, the isolated nucleic acid molecules consist of or comprise from about 18 to about 30 nucleotides. In some embodiments, the isolated nucleic acid molecules comprise or consist of at least about 15 nucleotides to at least about 35 nucleotides.

In some embodiments, such isolated nucleic acid molecules hybridize to SCN11A predicted loss-of-function variant nucleic acid molecules (such as genomic nucleic acid molecules, mRNA molecules, and/or cDNA molecules) under stringent conditions. Such nucleic acid molecules can be used, for example, as probes, primers, alteration-specific probes, or alteration-specific primers as described or exemplified herein, and include, without limitation primers, probes, antisense RNAs, shRNAs, and siRNAs, each of which is described in more detail elsewhere herein, and can be used in any of the methods described herein.

In some embodiments, the isolated nucleic acid molecules hybridize to at least about 15 contiguous nucleotides of a nucleic acid molecule that is at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% identical to SCN11A predicted loss-of-function variant nucleic acid molecules. In some embodiments, the isolated nucleic acid molecules consist of or comprise from about 15 to about 100 nucleotides, or from about 15 to about 35 nucleotides. In some embodiments, the isolated nucleic acid molecules consist of or comprise from about 15 to about 100 nucleotides. In some

embodiments, the isolated nucleic acid molecules consist of or comprise from about 15 to about 35 nucleotides.

In some embodiments, the alteration-specific probes and alteration-specific primers comprise DNA. In some embodiments, the alteration-specific probes and alteration-specific primers comprise RNA.

In some embodiments, the probes and primers described herein (including alteration-specific probes and alteration-specific primers) have a nucleotide sequence that specifically hybridizes to any of the nucleic acid molecules disclosed herein, or the complement thereof. In some embodiments, the probes and primers specifically hybridize to any of the nucleic acid molecules disclosed herein under stringent conditions.

In some embodiments, the primers, including alteration-specific primers, can be used in second generation sequencing or high throughput sequencing. In some instances, the primers, including alteration-specific primers, can be modified. In particular, the primers can comprise various modifications that are used at different steps of, for example, Massive Parallel Signature Sequencing (MPSS), Polony sequencing, and 454 Pyrosequencing. Modified primers can be used at several steps of the process, including biotinylated primers in the cloning step and fluorescently labeled primers used at the bead loading step and detection step. Polony sequencing is generally performed using a paired-end tags library wherein each molecule of DNA template is about 135 bp in length. Biotinylated primers are used at the bead loading step and emulsion PCR. Fluorescently labeled degenerate nonamer oligonucleotides are used at the detection step. An adaptor can contain a 5'-biotin tag for immobilization of the DNA library onto streptavidin-coated beads.

The probes and primers described herein can be used to detect a nucleotide variation within any of the SCN11A predicted loss-of-function variant nucleic acid molecules disclosed herein. The primers described herein can be used to amplify any SCN11A predicted loss-of-function variant nucleic acid molecule, or a fragment thereof.

In the context of the disclosure “specifically hybridizes” means that the probe or primer (such as, for example, the alteration-specific probe or alteration-specific primer) does not hybridize to a nucleic acid sequence encoding an SCN11A reference genomic nucleic acid molecule, an SCN11A reference mRNA molecule, and/or an SCN11A reference cDNA molecule.

In some embodiments, the probes (such as, for example, an alteration-specific probe) comprise a label. In some embodiments, the label is a fluorescent label, a radiolabel, or biotin.

The present disclosure also provides supports comprising a substrate to which any one or more of the probes disclosed herein is attached. Solid supports are solid-state substrates or supports with which molecules, such as any of the probes disclosed herein, can be associated. A form of solid support is an array. Another form of solid support is an array detector. An array detector is a solid support to which multiple different probes have been coupled in an array, grid, or other organized pattern. A form for a solid-state substrate is a microtiter dish, such as a standard 96-well type. In some embodiments, a multiwell glass slide can be employed that normally contains one array per well.

The genomic nucleic acid molecules, mRNA molecules, and cDNA molecules can be from any organism. For example, the genomic nucleic acid molecules, mRNA molecules, and cDNA molecules can be human or an ortholog from another organism, such as a non-human mammal, a rodent, a mouse, or a rat. It is understood that gene sequences within a population can vary due to polymorphisms such as single-nucleotide polymorphisms.

Also provided herein are functional polynucleotides that can interact with the disclosed nucleic acid molecules. Examples of functional polynucleotides include, but are not limited to, antisense molecules, aptamers, ribozymes, triplex forming molecules, and external guide sequences. The functional polynucleotides can act as effectors, inhibitors, modulators, and stimulators of a specific activity possessed by a target molecule, or the functional polynucleotides can possess a *de novo* activity independent of any other molecules.

The isolated nucleic acid molecules disclosed herein can comprise RNA, DNA, or both RNA and DNA. The isolated nucleic acid molecules can also be linked or fused to a heterologous nucleic acid sequence, such as in a vector, or a heterologous label. For example, the isolated nucleic acid molecules disclosed herein can be within a vector or as an exogenous donor sequence comprising the isolated nucleic acid molecule and a heterologous nucleic acid sequence. The isolated nucleic acid molecules can also be linked or fused to a heterologous label. The label can be directly detectable (such as, for example, fluorophore) or indirectly detectable (such as, for example, hapten, enzyme, or fluorophore quencher). Such labels can be detectable by spectroscopic, photochemical, biochemical, immunochemical, or chemical means. Such labels include, for example, radiolabels, pigments, dyes, chromogens, spin labels, and fluorescent labels. The label can also be, for example, a chemiluminescent substance; a metal-containing substance; or an enzyme, where there occurs an enzyme-dependent secondary generation of signal. The term “label” can also refer to a “tag” or hapten that can

bind selectively to a conjugated molecule such that the conjugated molecule, when added subsequently along with a substrate, is used to generate a detectable signal. For example, biotin can be used as a tag along with an avidin or streptavidin conjugate of horseradish peroxidate (HRP) to bind to the tag, and examined using a calorimetric substrate (such as, for example, tetramethylbenzidine (TMB)) or a fluorogenic substrate to detect the presence of HRP. Exemplary labels that can be used as tags to facilitate purification include, but are not limited to, myc, HA, FLAG or 3XFLAG, 6Xhis or polyhistidine, glutathione-S-transferase (GST), maltose binding protein, an epitope tag, or the Fc portion of immunoglobulin. Numerous labels include, for example, particles, fluorophores, haptens, enzymes and their calorimetric, fluorogenic and chemiluminescent substrates and other labels.

Percent identity (or percent complementarity) between particular stretches of nucleotide sequences within nucleic acid molecules or amino acid sequences within polypeptides can be determined routinely using BLAST programs (basic local alignment search tools) and PowerBLAST programs (Altschul *et al.*, J. Mol. Biol., 1990, 215, 403-410; Zhang and Madden, Genome Res., 1997, 7, 649-656) or by using the Gap program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, Madison Wis.), using default settings, which uses the algorithm of Smith and Waterman (Adv. Appl. Math., 1981, 2, 482-489). Herein, if reference is made to percent sequence identity, the higher percentages of sequence identity are preferred over the lower ones.

The present disclosure also provides therapeutic agents that treat, prevent, or inhibit a headache or a migraine for use in the treatment and/or prevention of a headache or a migraine in a subject having an SCN11A predicted loss-of-function variant nucleic acid molecule. Any of the therapeutic agents that treat, prevent, or inhibit a headache or a migraine described herein can be used in these methods. Any of the SCN11A predicted loss-of-function variant nucleic acid molecules disclosed herein can be used in these methods.

The present disclosure also provides uses of therapeutic agents that treat, prevent, or inhibit a headache or a migraine for use in the preparation of a medicament for treating and/or preventing a headache or a migraine in a subject having an SCN11A predicted loss-of-function variant nucleic acid molecule. Any of the therapeutic agents that treat, prevent, or inhibit a headache or a migraine described herein can be used in these methods. Any of the SCN11A predicted loss-of-function variant nucleic acid molecules disclosed herein can be used in these methods.

The present disclosure also provides SCN11A inhibitors for use in the treatment and/or prevention of a headache or a migraine in a subject that is SCN11A reference, or is heterozygous for an SCN11A predicted loss-of-function variant nucleic acid molecule. Any of the SCN11A inhibitors described herein can be used in these methods. Any of the SCN11A predicted loss-of-function variant nucleic acid molecules disclosed herein can be used in these methods.

The present disclosure also provides SCN11A inhibitors in the preparation of a medicament for treating and/or preventing a headache or a migraine in a subject that is SCN11A reference, or is heterozygous for an SCN11A predicted loss-of-function variant nucleic acid molecule. Any of the SCN11A inhibitors described herein can be used in these methods. Any of the SCN11A predicted loss-of-function variant nucleic acid molecules disclosed herein can be used in these methods.

All patent documents, websites, other publications, accession numbers and the like cited above or below are incorporated by reference in their entirety for all purposes to the same extent as if each individual item were specifically and individually indicated to be so incorporated by reference. If different versions of a sequence are associated with an accession number at different times, the version associated with the accession number at the effective filing date of this application is meant. The effective filing date means the earlier of the actual filing date or filing date of a priority application referring to the accession number if applicable. Likewise, if different versions of a publication, website or the like are published at different times, the version most recently published at the effective filing date of the application is meant unless otherwise indicated. Any feature, step, element, embodiment, or aspect of the present disclosure can be used in combination with any other feature, step, element, embodiment, or aspect unless specifically indicated otherwise. Although the present disclosure has been described in some detail by way of illustration and example for purposes of clarity and understanding, it will be apparent that certain changes and modifications may be practiced within the scope of the appended claims.

The following examples are provided to describe the embodiments in greater detail. They are intended to illustrate, not to limit, the claimed embodiments. The following examples provide those of ordinary skill in the art with a disclosure and description of how the compounds, compositions, articles, devices and/or methods described herein are made and evaluated, and are intended to be purely exemplary and are not intended to limit the scope of

any claims. Efforts have been made to ensure accuracy with respect to numbers (such as, for example, amounts, temperature, etc.), but some errors and deviations may be accounted for. Unless indicated otherwise, parts are parts by weight, temperature is in °C or is at ambient temperature, and pressure is at or near atmospheric.

Examples

Example 1: Meta-Analysis of Headache and Migraine

A meta-analysis of headache and migraine was conducted across four cohorts (UK Biobank, Geisinger, Malmo diet and cancer study, and Mt. Sinai BioMe Biobank). An aggregate of rare, predicted loss-of-function variants (Minor allele frequency (MAF <1%)) in SCN11A were identified as associated with protection from headaches and migraines (see, Table 1), suggesting that downregulation or inhibition of SCN11A could protect from headaches and migraines.

Table 1

Trait	Odds Ration (LCI, UCI)	P-value	AAF	Cases RR|RA|AA	Controls RR|RA|AA
1	0.679 (0.578, 0.798)	2.51E-06	0.00121434	114,390 114,167|222|1	239,300 238,666|633|1
2	0.684 (0.542, 0.863)	1.36E-03	0.0013	37,009 36,934|75|0	334,901 333,992|908|1

Trait 1 = Self-reported + ICD (migraines/headaches).

Trait 2 = Self-reported + ICD (migraine).

Various modifications of the described subject matter, in addition to those described herein, will be apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims. Each reference (including, but not limited to, journal articles, U.S. and non-U.S. patents, patent application publications, international patent application publications, gene bank accession numbers, and the like) cited in the present application is incorporated herein by reference in its entirety and for all purposes.

What is Claimed is:

1. Use of a Sodium Voltage-Gated Channel Alpha Subunit 11 (SCN11A) inhibitor in the preparation of a medicament for treating a subject having a headache or migraine or at risk of developing a headache or migraine.

2. The use according to claim 1, wherein the SCN11A inhibitor comprises an inhibitory nucleic acid molecule that hybridizes to an SCN11A nucleic acid molecule, a Cas protein and guide RNA (gRNA) that hybridizes to a gRNA recognition sequence within an SCN11A genomic nucleic acid molecule, a small molecule, or an antibody.

3. The use according to claim 2, wherein the inhibitory nucleic acid molecule comprises an antisense nucleic acid molecule, a small interfering RNA (siRNA), or a short hairpin RNA (shRNA).

4. The use according to any one of claims 1 to 3, further comprising detecting the presence or absence of an SCN11A predicted loss-of-function variant nucleic acid molecule in a biological sample from the subject.

5. The use according to claim 4, wherein a therapeutic agent that treats, prevents, or inhibits a headache or a migraine is to be further administered in a standard dosage amount to a subject wherein the SCN11A predicted loss-of-function variant nucleic acid molecule is absent from the biological sample.

6. The use according to claim 4, wherein a therapeutic agent that treats, prevents, or inhibits a headache or a migraine is to be further administered in a dosage amount that is the same as or less than a standard dosage amount to a subject that is heterozygous for the SCN11A predicted loss-of-function variant nucleic acid molecule.

7. The use according to any one of claims 4 to 6, wherein the SCN11A predicted loss-of-function variant nucleic acid molecule is a splice-site variant, a stop-gain variant, a start-loss variant, a stop-loss variant, a frameshift variant, or an in-frame indel variant, or a variant that encodes a truncated SCN11A predicted loss-of-function polypeptide.

8. Use of a therapeutic agent that treats or inhibits a headache or a migraine in the preparation of a medicament for treating a subject that has a headache or a migraine or is at risk of developing a headache or a migraine, wherein the subject has been determined to have or not have a Sodium Voltage-Gated Channel Alpha Subunit 11 (SCN11A) predicted loss-of-function variant nucleic acid molecule by:

performing or having performed a sequence analysis on a biological sample obtained or having been obtained from the subject to determine if the subject has a genotype comprising the SCN11A predicted loss-of-function variant nucleic acid molecule; and

wherein the therapeutic agent that treats, prevents, or inhibits the headache or the migraine is to be administered or continued to be administered in a standard dosage amount to a subject that is SCN11A reference, and/or an SCN11A inhibitor is to be administered to the subject;

wherein the therapeutic agent that treats, prevents, or inhibits the headache or the migraine is to be administered or continued to be administered in an amount that is the same as or less than a standard dosage amount to a subject that is heterozygous for the SCN11A predicted loss-of-function variant nucleic acid molecule, and/or an SCN11A inhibitor is to be administered to the subject; or

wherein the therapeutic agent that treats, prevents, or inhibits the headache or the migraine is to be administered or continued to be administered in an amount that is the same as or less than a standard dosage amount to a subject that is homozygous for the SCN11A predicted loss-of-function variant nucleic acid molecule;

wherein the presence of a genotype having the SCN11A predicted loss-of-function variant nucleic acid molecule indicates the subject has a decreased risk of developing a headache or a migraine.

9. The use according to claim 8, wherein the subject is SCN11A reference, and the subject is to be administered or continued to be administered the therapeutic agent that treats, prevents, or inhibits the headache or the migraine in a standard dosage amount, and is to be administered the SCN11A inhibitor.

10. The use according to claim 8, wherein the subject is heterozygous for SCN11A predicted loss-of-function variant nucleic acid molecule, and the subject is to be administered or continued to be administered the therapeutic agent that treats, prevents, or inhibits the headache or the migraine in an amount that is the same as or less than a standard dosage amount, and is to be administered the SCN11A inhibitor.

11. The use according to any one of claims 8 to 10, wherein the SCN11A predicted loss-of-function variant nucleic acid molecule is a splice-site variant, a stop-gain variant, a start-loss

variant, a stop-loss variant, a frameshift variant, or an in-frame indel variant, or a variant that encodes a truncated SCN11A predicted loss-of-function polypeptide.

12. The use according to any one of claims 8 to 11, wherein the SCN11A inhibitor comprises an inhibitory nucleic acid molecule that hybridizes to an SCN11A nucleic acid molecule, a Cas protein and guide RNA (gRNA) that hybridizes to a gRNA recognition sequence within an SCN11A genomic nucleic acid molecule, a small molecule, or an antibody.

13. The use according to claim 12, wherein the inhibitory nucleic acid molecule comprises an antisense nucleic acid molecule, a small interfering RNA (siRNA), or a short hairpin RNA (shRNA).

14. The use according to any one of claims 8 to 13, wherein the therapeutic agent that treats or inhibits a headache is chosen from a nonsteroidal anti-inflammatory drug, acetaminophen, celecoxib, diclofenac, fenoprofen, indomethacin, ketorolac tromethamine, meclofenamate sodium, diflunisal, tolmetin, ketoprofen, and flurbiprofen, or any combination thereof, and the therapeutic agent that treats or inhibits a migraine is chosen from a nonsteroidal anti-inflammatory drug, acetaminophen, a triptan, an ergot, a calcitonin gene-related peptide (CGRP) receptor antagonist, an anti-nausea agent, a high blood pressure medication, an antidepressant, an antiseizure medication and botox, or any combination thereof.

15. A method of identifying a subject having an increased risk of developing a headache or a migraine, the method comprising:

determining or having determined the presence or absence of a Sodium Voltage-Gated Channel Alpha Subunit 11 (SCN11A) predicted loss-of-function variant nucleic acid molecule in a biological sample obtained from the subject;

wherein:

when the subject is SCN11A reference, then the subject has an increased risk of developing a headache or a migraine; and

when the subject is heterozygous or homozygous for the SCN11A predicted loss-of-function variant nucleic acid molecule, then the subject has a decreased risk of developing a headache or a migraine.

16. The method according to claim 15, wherein the SCN11A predicted loss-of-function variant nucleic acid molecule is a splice-site variant, a stop-gain variant, a start-loss variant, a

stop-loss variant, a frameshift variant, or an in-frame indel variant, or a variant that encodes a truncated SCN11A predicted loss-of-function polypeptide.

17. Use of a therapeutic agent that treats, prevents, or inhibits a headache or a migraine in the preparation of a medicament for treating or preventing a headache or a migraine in a subject having a Sodium Voltage-Gated Channel Alpha Subunit 11 (SCN11A) predicted loss-of-function variant nucleic acid molecule.

18. The use according to claim 17, wherein the SCN11A predicted loss-of-function variant nucleic acid molecule is a splice-site variant, a stop-gain variant, a start-loss variant, a stop-loss variant, a frameshift variant, or an in-frame indel variant, or a variant that encodes a truncated SCN11A predicted loss-of-function polypeptide.

19. Use of a Sodium Voltage-Gated Channel Alpha Subunit 11 (SCN11A) inhibitor in the preparation of a medicament for treating or preventing a headache or a migraine in a subject that is SCN11A reference, or is heterozygous for SCN11A predicted loss-of-function variant nucleic acid molecule, a Cas protein and guide RNA (gRNA) that hybridizes to a gRNA recognition sequence within an SCN11A genomic nucleic acid molecule, a small molecule, or an antibody.

20. The use according to claim 19, wherein the SCN11A inhibitor comprises an inhibitory nucleic acid molecule that hybridizes to an SCN11A nucleic acid molecule.

21. The use according to claim 20, wherein the inhibitory nucleic acid molecule comprises an antisense nucleic acid molecule, a small interfering RNA (siRNA), or a short hairpin RNA (shRNA).