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(54) **Title**: ASSESSING AND TREATING HUMANS WITH LONG QT SYNDROME

(57) **Abstract**: This document provides methods and materials involved in assessing and treating humans with LQTS or with a potential mutation in a KCNQ1 nucleic acid that encodes a Kv7.1 potassium channel subunit. For example, methods and materials for determining if a human containing a mutation in a KCNQ1 nucleic acid that encodes a K_v7.1 potassium channel subunit on one allele also contains, on the same allele (a cis relationship) or on the other allele (a trans relationship), a genetic variation (e.g., a SNP) in a 3' UTR of KCNQ1 nucleic acid that creates a miR-378 binding site are provided.

ASSESSING AND TREATING HUMANS WITH LONG QT SYNDROME

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application Serial No. 61/480,962, filed April 29, 2011. The disclosure of the prior application is considered part of (and is incorporated by reference in) the disclosure of this application.

BACKGROUND

1. Technical Field

This document relates to methods and materials involved in assessing and treating humans with long QT syndrome (LQTS) or with a potential mutation in a KCNQ1 nucleic acid that encodes a $K_v7.1$ potassium channel subunit. For example, this document provides methods and materials for determining if a human containing a mutation in a KCNQ1 nucleic acid that encodes a $K_v7.1$ potassium channel subunit on one allele also contains, on the same allele (a cis relationship) or on the other allele (a trans relationship), a genetic variation (e.g., a SNP) in a 3' UTR of KCNQ1 nucleic acid that creates a new microRNA (e.g., miR-378) binding site.

2. Background Information

LQTS is a common heritable cardiac channelopathy that can cause premature sudden death due to life-threatening arrhythmias in relation to prolongation of the heart rate-corrected QT interval (QTc). The most prevalent form, LQT1, is caused by loss-of-function mutations in the KCNQ1-encoded $K_v7.1$ potassium channel (IKs). LQT1 is characterized by incomplete penetrance and variable expressivity whereby family members carrying identical mutations have profound differences in their QTc and clinical course. The cause for this heterogeneity remains largely elusive. $K_v7.1$ is a tetrameric channel derived from the post-translational assembly of four KCNQ1-encoded subunits. Therefore, patients heterozygous for an LQT1-causative mutation combine the translated products from normal and LQT1-mutation-containing alleles to form tetrameric channels. If both alleles are similarly expressed, one would predict that 1/16th of the $K_v7.1$ channels stem solely from the normal allele, and 1/16th of the $K_v7.1$ channels stem solely from the

mutated allele. The remaining channels would be hybrids of the mutated and healthy alleles.

SUMMARY

This document relates to methods and materials involved in assessing and treating humans with LQTS or with a potential mutation in a KCNQ1 nucleic acid that encodes a $K_v7.1$ potassium channel subunit. For example, this document provides methods and materials for determining if a human containing a mutation in a KCNQ1 nucleic acid that encodes a $K_v7.1$ potassium channel subunit on one allele also contains, on the same allele (a cis relationship) or on the other allele (a trans relationship), a genetic variation (e.g., a SNP) in the 3' UTR of KCNQ1 nucleic acid that creates a new microRNA (e.g., a miR-378) binding site.

For the purposes of this document, the term "KCNQ1 mutation" refers to a genetic variation (e.g., a substitution, deletion, or insertion of a nucleotide or nucleotides) that is present within a KCNQ1 nucleic acid that encodes a $K_v7.1$ potassium channel subunit, causes an alteration in the amino acid sequence of a $K_v7.1$ potassium channel subunit, and is associated with LQTS. The term "KCNQ1 UTR variation" refers to a genetic variation (e.g., a substitution, deletion, or insertion of a nucleotide or nucleotides) that is present within the 3' untranslated region of a KCNQ1 nucleic acid and creates a microRNA binding site (e.g., a miR-378 binding site). As described herein, humans with a KCNQ1 mutation that is present on the same allele that contains a KCNQ1 UTR variation can experience less severe symptoms of LQTS if the other allele lacks KCNQ1 mutations (e.g., is a wild-type KCNQ1 nucleic acid). Humans with KCNQ1 nucleic acid lacking KCNQ1 mutations (e.g., a wild-type KCNQ1 nucleic acid that encodes a $K_v7.1$ potassium channel subunit) that is present on the same allele that contains a KCNQ1 UTR variation can experience more severe symptoms of LQTS if the other allele contains a KCNQ1 mutation. Thus, the cis or trans relationship of KCNQ1 mutations and KCNQ1 UTR variations can be used to assess the severity of LQTS.

Having the ability to determine the cis or trans relationship of KCNQ1 mutations and KCNQ1 UTR variations within a human heterozygous for a KCNQ1 mutation and heterozygous for a KCNQ1 UTR variation can allow clinicians and patients to determine

appropriate levels of LQTS disease monitoring and care. For example, a patient identified as having increased LQTS severity as described herein can be selected for treatment with agents designed to inhibit microRNA activity.

In general, this document features a method for assessing the cis or trans nature of a human heterozygous for a KCNQ1 mutation and heterozygous for a KCNQ1 UTR variation. The method comprises, or consists essentially of, (a) determining if an allele of the human comprises (i) the KCNQ1 mutation and the KCNQ1 UTR variation, (ii) a lack of the KCNQ1 mutation and a lack of the KCNQ1 UTR variation, (iii) the KCNQ1 mutation and a lack of the KCNQ1 UTR variation, or (iv) the KCNQ1 UTR mutation and a lack of the KCNQ1 variation, (b) classifying the human as having the KCNQ1 mutation and the KCNQ1 UTR variation in cis if the allele comprises (i) or (ii), and (c) classifying the human as having the KCNQ1 mutation and the KCNQ1 UTR variation in trans if the allele comprises (iii) or (iv). The human can be an LQT1 patient. The allele can comprise the KCNQ1 mutation and the KCNQ1 UTR variation, and the human can be classified as having the KCNQ1 mutation and the KCNQ1 UTR variation in cis. The allele can comprise a lack of the KCNQ1 mutation and a lack of the KCNQ1 UTR variation, and the human can be classified as having the KCNQ1 mutation and the KCNQ1 UTR variation in cis. The allele can comprise the KCNQ1 mutation and a lack of the KCNQ1 UTR variation, and the human can be classified as having the KCNQ1 mutation and the KCNQ1 UTR variation in trans. The allele can comprise the KCNQ1 UTR variation and a lack of the KCNQ1 mutation, and the human can be classified as having the KCNQ1 mutation and the KCNQ1 UTR variation in trans. The KCNQ1 mutation can be L266P, R518X, G168R, or R594Q. The KCNQ1 UTR variation can be an rs2519184 SNP or rs8234 SNP.

In another aspect, this document features a method for treating a mammal having a KCNQ1 UTR variation that is part of a microRNA binding site, wherein the binding of a microRNA to the binding site reduces expression of a $K_v7.1$ potassium channel subunit lacking a mutation associated with LQTS. The method comprises, or consists essentially of, administering a miRNA inhibitor to the mammal under conditions wherein the miRNA inhibitor reduces the ability of the microRNA to reduce expression of the subunit.

In another aspect, this document features a method for treating a human comprising, or consisting essentially of, administering an agent that inhibits or mimics the activity of a microRNA that targets a KCNQ1 UTR variation to increase the ratio of expression of a non-mutated KCNQ1 allele to mutated KCNQ1 allele.

In another aspect, this document features a method for treating a human having long QT syndrome. The method comprises, or consists essentially of, (a) determining if an allele of the human comprises (i) the KCNQ1 mutation and the KCNQ1 UTR variation, (ii) a lack of the KCNQ1 mutation and a lack of the KCNQ1 UTR variation, (iii) the KCNQ1 mutation and a lack of the KCNQ1 UTR variation, or (iv) the KCNQ1 UTR mutation and a lack of the KCNQ1 variation, and (b) administering a beta blocker to the human, implanting an implantable cardioverter-defibrillator into the human, or performing a sympathetic denervation procedure on the human. The method can comprise administering the beta blocker to the human. The beta blocker can be metoprolol, carvedilol, bisoprolol, nebivolol, or propanolol. The method can comprise implanting the implantable cardioverter-defibrillator into the human. The method can comprise performing the sympathetic denervation procedure on the human.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

DESCRIPTION OF DRAWINGS

Figure 1. Genetic variation in the 3'untranslated region of *KCNQ1*. (A) Single nucleotide polymorphisms (SNPs) found in the study cohorts of the Academic Medical Center Amsterdam (AMC) and the Mayo Clinic (MC). Position of the nucleotide change

is starting from the ATG start codon (NCBI build 36, hg18). (B) Predicted binding between miR-378 and the 3'UTR of *KCNQ1* containing the minor A variant of SNP rs2519184 and the minor G variant of SNP rs8234. 'rs' numbers denote SNP identities from public database. Longer lightly shaded boxes represent exons, and darker shaded bars represent SNPs. (C) Relative expression of miR-378 in human donor hearts in comparison with other abundantly expressed cardiac miRNAs (miR-133b, miR-21, and miR-208b). Data are presented as mean ± standard error.

Figure 2. The functional effects of genetic variation in the 3'untranslated region (3'UTR) of *KCNQ1*. (A) Luciferase assays in neonatal rat heart-derived H10 cells transfected with two independent reporter plasmids containing either the major or the minor haplotype of SNPs in *KCNQ1*'s 3'UTR. Observed differences in luciferase activity between the major and minor haplotype may indicate that translation is inhibited by enhanced miRNA binding to the 3'UTR. (B) Luciferase assays in H10 cells transfected with a reporter plasmid containing either the major haplotype of the 3'UTR of *KCNQ1* or the same reporter where only one of the two SNPs predicted to affect miR-378 binding (rs2519184 or rs8234) was changed. Introducing either one of the minor SNP alleles was sufficient to decrease luciferase activity. (C) Luciferase assays in COS-7 cells cotransfected with the 3'UTR KCNQ1 reporter, containing either the major or the minor haplotype of SNPs in the 3'UTR of *KCNQ1*, and an expression vector for miR-378 or a control miRNA. Overexpression of miR-378 (but not the control miRNA) decreased luciferase activity of only the 3'UTR KCNQ1 reporter with the minor SNP haplotype. Data are presented as mean ± standard error. Darker shaded bar represents a major SNP variant, while the lighter shaded bar represents a minor SNP variant.

Figure 3. Allele-specific effects of SNPs rs2519184 and rs8234 on QTc. Allele-specific effects of SNP rs2519184 (A), SNP rs8234 (B), and haplotype of the two SNPs (C) on QTc duration for the AMC population. Allele-specific effects of SNP rs2519184 (D), SNP rs8234 (E), and haplotype of the two SNPs (F) on QTc duration for the MC population. The dashed line represents mean QTc of all individuals within the study population regardless of the specific LQT1-causative mutation and the 3'UTR SNP status. Numbers below genotypes denote group sizes. Data are presented as mean ± standard error. N, normal *KCNQ1* allele; M, mutant *KCNQ1* allele. Darker shaded box

represents a major SNP variant, while the lighter shaded box represents a minor SNP variant.

Figure 4. Allele-specific effects of SNPs rs2519184 and rs8234 on QTc in two single families. Pedigree structure and allele-specific haplotype analysis of SNPs rs2519184 and rs8234 with regard to QTc are displayed for the largest family from AMC (A) and MC (B). Numbers above genotypes denote QTc values. 'n.a.' means not available. N, normal *KCNQ1* allele; M, mutant *KCNQ1* allele. Darker shaded box represents a major SNP variant, while the lighter shaded box represents a minor SNP variant.

Figure 5. Allele-specific effects of SNPs rs2519184 and rs8234 on symptomatology. Allele-specific effects of SNPs rs2519184 and rs8234 with regard to the occurrence of cardiac symptoms (unexplained syncope, documented torsades de pointes ventricular tachycardia/ventricular fibrillation, and/or [aborted] sudden death) are shown in the total combined study population (AMC and MC). (A) Effects of SNP rs2519184. (B) Effects of SNP rs8234. (C) Allele-specific haplotype analysis of the two SNPs with regard to the occurrence of symptoms. Numbers below genotypes denote group sizes. Data are presented as mean ± standard error. N, normal *KCNQ1* allele; M, mutant *KCNQ1* allele. Darker shaded box represents a major SNP variant, while the lighter shaded box represents a minor SNP variant.

Figure 6. The functional effects of genetic variation in the 3'untranslated region (3'UTR) of *KCNQ1*. (A) Luciferase assays in neonatal rat cardiomyocytes transfected with two independent reporter plasmids containing either the major or the minor haplotype of SNPs in the 3'UTR of *KCNQ1*. Observed differences in luciferase activity between the major and minor haplotype may indicate that translation is inhibited by enhanced miRNA binding to the 3'UTR. (B) Luciferase assays in neonatal rat cardiomyocytes transfected with a reporter plasmid containing either the major haplotype of the 3'UTR of *KCNQ1* or the same reporter where only one of the two SNPs predicted to affect miR-378 binding (rs2519184 or rs8234) was changed. Introducing either one of the minor SNP alleles was sufficient to decrease luciferase activity. Data are presented as mean ± standard error. Darker shaded bars represent a major SNP variant, while the lighter shaded bars represent a minor SNP variant.

Figure 7 is a diagram of a mechanism where SNPs in the 3'UTR of *KCNQ1* modulate the assembly of the $K_v7.1$ potassium channel in type 1 long QT syndrome. Individuals with type long QT syndrome (LQT1) are heterozygous for the disease-causing mutation in *KCNQ1*. Four *KCNQ1*-encoded subunits co-assemble post-translationally to form one tetrameric channel. Since the 3'UTR plays an important role in messenger-RNA translation, SNPs in this region may influence repolarization by altering the balance and composition of the $K_v7.1$ tetramers derived from translation of the normal allele and the mutant allele. If SNPs exert no effect on messenger-RNA translation, the balance between normal and mutant subunits within each channel is equal. However, if the minor genotypes of the SNPs suppress messenger-RNA translation (by creating target sites for microRNAs as predicted for SNPs rs2519184 and rs8234; see Figure 2), then the balance between normal and mutant subunits within each channel depends on whether the 'suppressive SNPs' reside on the normal allele or the mutant allele. If the 'suppressive SNPs' reside on the normal allele, the number of normal subunits in the channels would decrease. Inversely, if the 'suppressive SNPs' reside on the mutant allele, this would decrease the relative number of mutant subunits and shift the $K_v7.1$ tetramers to a greater percent of normal allele-derived monomeric subunits.

Figure 8 is a graph showing inhibition by a microRNA inhibitor (e.g., an anti-miR) designed to inhibit miR-378, at two different dosages.

Figure 9 is a graph showing that the inhibition shown in Figure 8 prevents the suppressive effects of UTR variants. The two bars on the right (antimiR54) represent a control demonstrating that the effect (i.e., lack of suppression) only occurs after using the specific antimir against 378.

Figure 10 is a graph of luciferase assay results of cardiac myocytes with knockdown of miR-378.

Figure 11 is a graph demonstrating an allelic imbalance on mRNA levels.

DETAILED DESCRIPTION

This document relates to methods and materials involved in assessing and treating humans with LQTS or with a potential mutation in a KCNQ1 nucleic acid that encodes a $K_v7.1$ potassium channel subunit. For example, this document provides methods and

materials for assessing LQTS severity by determining if a human containing a KCNQ1 mutation on one allele also contains, on the same allele (a cis relationship) or on the other allele (a trans relationship), a KCNQ1 UTR variation. Figure 7 provides several examples of how the cis and trans relationship of KCNQ1 mutations and KCNQ1 UTR variations can influence LQTS severity.

Examples of KCNQ1 mutations include, without limitation, those listed in Table A or described elsewhere (Kapplinger *et al.*, *Heart Rhythm.*, 6(9):1297-303 (2009)). Examples of KCNQ1 UTR variations include, without limitation, the rs2519184 SNP, rs8234 SNP, and rs10798 SNP. A KCNQ1 UTR variation can be any genetic variant present within the 3' untranslated region of a KCNQ1 nucleic acid that creates a microRNA binding site for a microRNA that is capable of reducing expression of mRNA to which it binds. Examples of such microRNA include, without limitation, miR-378, miR-328, and miR-220.

Table A. *KCNQ1* LQT1-associated mutations.

Region	Nucleotide	Variant	Mutation Type	Location	No. of patients
Exon 1	5 C>T	A2V*	Missense	N-Terminal	1
Exon 1	19 C>T	P7S*	Missense	N-Terminal	1
Exon 1	108insT	F36fs+247X*	Frame shift	N-Terminal	1
Exon 1	136 G>A	A46T	Missense	N-Terminal	2
Exon 1	153 C>G	Y51X	Nonsense	N-Terminal	1
Exon 1	176delC	A58fs+26X*	Frame shift	N-Terminal	1
Exon 1	190_210delCCTGCGTCCCCGGCCGCGCCC	64_70delPASPAAP*	In-frame del	N-Terminal	3
Exon 1	197 C>T	S66F*	Missense	N-Terminal	1
Exon 1	200_210delCGGCCGCGCCC	S66fs+213X*	Frame shift	N-Terminal	1
Exon 1	217 C>A	P73T	Missense	N-Terminal	4
Exon 1	242_264delCGCGGCCGCCGGTGAGCCTAGACinsGCGCCCGCGG	G80fs+151X*	Frame shift	N-Terminal	1

Exon 1	273_299delCTCC ATCTACAGCAC GCGCC GCCCGGTinsGG	V91fs+136X*	Frame shift	N-Terminal	1
Exon 1	332 A>G	Y111C	Missense	N-Terminal	5
Exon 1	350 C>T	P117L	Missense	N-Terminal	1
Exon 1	365insT	K121fs+162X*	Frame shift	N-Terminal	2
Exon 1	381 C>A	F127L*	Missense	S1 Domain	1
Intron 1	386+1 G>A		Splice site	S1 Domain	1
Exon 2	397 G>A	V133I	Missense	S1 Domain	1
Exon 2	401 T>C	L134P*	Missense	S1 Domain	1
Exon 2	403delG	L134fs+101X*	Frame shift	S1 Domain	1
Exon 2	430 A>G	T144A	Missense	S1/S2	1
Exon 2	451_452delCT	A150fs+132X	Frame shift	S2 Domain	1
Exon 2	458 C>T	T153M*	Missense	S2 Domain	1
Intron 2	477+1 G>A		Splice site	S2 Domain	1
Intron 2	477+5 G>C		Splice site	S2 Domain	1
Intron 2	477+5 G>A		Splice site	S2 Domain	4
Exon 3	479 A>T	E160V*	Missense/Splice	S2 Domain	1
Exon 3	484 G>A	V162M*	Missense	S2 Domain	1
Exon 3	488delT	V162fs+73X	Frame shift	S2 Domain	1
Exon 3	502 G>A	G168R	Missense	S2 Domain	15
Exon 3	502 G>C	G168R	Missense	S2 Domain	4
Exon 3	504delG	G168fs+67X*	Frame shift	S2 Domain	1
Exon 3	513 C>G	Y171X	Nonsense	S2/S3	1
Exon 3	514 G>A	V172M	Missense	S2/S3	2
Exon 3	520 C>T	R174C	Missense	S2/S3	1
Exon 3	521 G>A	R174H	Missense	S2/S3	1
Exon 3	524_534delTCTG GTCCGCC	R174fs+105X*	Frame shift	S2/S3	1
Exon 3	532 G>A	A178T	Missense	S2/S3	1
Exon 3	535 G>A	G179S	Missense	S2/S3	2
Exon 3	550 T>C	Y184H*	Missense	S2/S3	1
Exon 3	556 G>C	G186R*	Missense	S2/S3	1
Exon 3	564 G>A	W188X*	Nonsense	S2/S3	1
Exon 3	569 G>A	R190Q	Missense	S2/S3	3

Exon 3	569 G>T	R190L*	Missense	S2/S3	1
Exon 3	573_577delGCGCT	L191fs+90X	Frame shift	S2/S3	4
Exon 3	583 C>T	R195W*	Missense	S2/S3	2
Exon 3	585delG	R195fs+40X	Frame shift	S2/S3	4
Exon 3	592 A>G	I198V*	Missense	S3 Domain	1
Exon 3	595 T>G	S199A*	Missense	S3 Domain	1
Exon 3	604 G>A	D202N	Missense/Splice	S3 Domain	1
Intron 3	605-2 A>G		Splice site	S3 Domain	1
Exon 4	612 C>G	I204M	Missense	S3 Domain	1
Exon 4	643 G>A	V215M	Missense	S3 Domain	1
Exon 4	671 C>T	T224M*	Missense	S3/S4	1
Exon 4	674 C>T	S225L	Missense	S3/S4	8
Exon 5	691 C>T	R231C	Missense	S4 Domain	1
Exon 5	692 G>A	R231H	Missense	S4 Domain	1
Exon 5	704 T>A	I235N	Missense	S4 Domain	2
Exon 5	722 T>G	V241G*	Missense	S4 Domain	1
Exon 5	724 G>A	D242N	Missense	S4 Domain	4
Exon 5	727delC	D242fs+19X*	Frame shift	S4 Domain	1
Exon 5	727 C>T	R243C	Missense	S4 Domain	1
Exon 5	749 T>C	L250P*	Missense	S4/S5	1
Exon 5	760 G>A	V254M	Missense	S4/S5	10
Exon 5	775 C>T	R259C	Missense	S4/S5	5
Exon 5	776_780dupCCACC	H258fs+5X*	Frame shift	S4/S5	1
Exon 5	776 G>T	R259L	Missense	S4/S5	1
Exon 6	781 G>C	E261Q*	Missense/Splice	S4/S5	1
Exon 6	781 G>T	E261X*	Nonsense/Splice	S4/S5	1
Exon 6	784 C>G	L262V	Missense	S5 domain	1
Exon 6	796delC	T265fs+22X	Frame shift	S5 domain	3
Exon 6	797 T>C	L266P	Missense	S5 domain	30
Exon 6	803 T>G	I268S*	Missense	S5 domain	1
Exon 6	805 G>A	G269S	Missense	S5 domain	10
Exon 6	806 G>A	G269D	Missense	S5 domain	4
Exon 6	815 G>A	G272D	Missense	S5 domain	1
Exon 6	817 C>T	L273F	Missense	S5 domain	7
Exon 6	820 A>G	I274V	Missense	S5 domain	1

Exon 6	829 T>C	S277P*	Missense	S5 domain	1
Exon 6	830 C>T	S277L	Missense	S5 domain	2
Exon 6	839 T>A	V280E	Missense	S5 domain	1
Exon 6	842 A>G	Y281C	Missense	S5 domain	1
Exon 6	845 T>C	L282P*	Missense	S5 domain	1
Exon 6	848 C>G	A283G*	Missense	S5/pore	2
Exon 6	862_880delGTGA ACGAGTCAGGC CGCG	A287fs+59X*	Frame shift	S5/pore	2
Exon 6	875 G>A	G292D	Missense	S5/pore	1
Exon 6	877 C>T	R293C	Missense	S5/pore	4
Exon 6	905 C>T	A302V	Missense	Pore	1
Exon 6	905 C>A	A302E*	Missense	Pore	1
Exon 6	908 T>C	L303P*	Missense	Pore	1
Exon 6	913 T>C	W305R*	Missense	Pore	1
Exon 6	914 G>C	W305S	Missense	Pore	1
Exon 6	914 G>A	W305X	Nonsense	Pore	1
Exon 6	916 G>A	G306R	Missense	Pore	1
Exon 6	916 G>C	G306R	Missense	Pore	1
Exon 7	935 C>T	T312I	Missense	Pore	2
Exon 7	940 G>A	G314S	Missense	Pore	7
Exon 7	940 G>T	G314C	Missense	Pore	1
Exon 7	944 A>G	Y315C	Missense	Pore	4
Exon 7	947 G>T	G316V*	Missense	Pore	1
Exon 7	958 C>T	P320S*	Missense	Pore	1
Exon 7	964 A>G	T322A	Missense	Pore/S6	2
Exon 7	965 C>T	T322M	Missense	Pore/S6	4
Exon 7	973 G>A	G325R	Missense	Pore/S6	6
Exon 7	1016 T>A	F339Y*	Missense	S6	1
Exon 7	1017_1019delCTT	340delF	In-frame del	S6	1
Exon 7	1022 C>A	A341E	Missense	S6	4
Exon 7	1022 C>T	A341V	Missense	S6	8
Exon 7	1022 C>G	A341G*	Missense	S6	1
Exon 7	1024 C>T	L342F	Missense	S6	2
Exon 7	1028 C>T	P343L	Missense	S6	1
Exon 7	1031 C>T	A344V	Missense/Splice	S6	1
Exon 7	1032 G>A	A344A	Splice site	S6	10
Intron	1032+1 G>T		Splice site	S6	1

7					
Intron 7	1032+1 G>A		Splice site	S6	2
Intron 7	1032+2 T>C		Splice site	S6	1
Intron 7	1032+5 G>T		Splice site	S6	1
Exon 8	1046 C>A	S349X	Nonsense	C-Terminal	1
Exon 8	1048 G>A	G350R	Missense	C-Terminal	1
Exon 8	1052 T>C	F351S	Missense	C-Terminal	1
Exon 8	1061 A>G	K354R*	Missense	C-Terminal	1
Exon 8	1066 C>T	Q356X	Nonsense	C-Terminal	1
Exon 8	1075 C>T	Q359X*	Nonsense	C-Terminal	4
Exon 8	1079 G>T	R360M*	Missense	C-Terminal	2
Exon 8	1085 A>G	K362R	Missense	C-Terminal	5
Exon 8	1093 A>C	N365H*	Missense	C-Terminal	1
Exon 8	1096 C>T	R366W	Missense	C-Terminal	8
Exon 8	1097 G>A	R366Q	Missense	C-Terminal	1
Exon 8	1121 T>A	L374H	Missense	C-Terminal	1
Intron 8	1128+1 G>A		Splice site	C-Terminal	1
Intron 8	1128+1 G>T		Splice site	C-Terminal	1
Intron 8	1128+5 G>A		Splice site	C-Terminal	1
Exon 9	1135 T>G	W379G*	Missense	C-Terminal	1
Exon 9	1153 G>A	E385K*	Missense	C-Terminal	1
Exon 9	1165 T>C	S389P*	Missense	C-Terminal	1
Exon 9	1171_1173dupCTT	391dupS*	In-frame ins	C-Terminal	1
Exon 9	1177_1179dupTGG	393dupW*	In-frame ins	C-Terminal	3
Exon 9	1189 C>T	R397W	Missense	C-Terminal	3
Exon 9	1193 A>G	K398R*	Missense	C-Terminal	1
Exon 9	1196_1197delCCinsA	K398fs+19X*	Frame shift	C-Terminal	1
Exon 9	1202insC	P400fs+61X	Frame shift	C-Terminal	1
Intron 9	1251+2 T>C		Splice site	C-Terminal	1
Exon 10	1265delA	K421fs+9X*	Frame shift	C-Terminal	2
Exon 10	1338 C>G	D446E*	Missense	C-Terminal	2
Exon	1343 C>T	P448L*	Missense	C-Terminal	1

10					
Exon 10	1351 C>T	R451W*	Missense	C-Terminal	1
Exon 10	1378 G>A	G460S	Missense	C-Terminal	1
Exon 11	1430 C>T	P477L*	Missense	C-Terminal	1
Exon 11	1462delG	E487fs+9X*	Frame shift	C-Terminal	1
Exon 11	1486_1487delCT	T495fs+18X	Frame shift	C-Terminal	1
Exon 11	1513 C>T	Q505X*	Nonsense/Splice	C-Terminal	1
Intron 11	1515-2 del AG		Splice site	C-Terminal	1
Exon 12	1531 C>T	R511W*	Missense	C-Terminal	1
Exon 12	1552 C>T	R518X	Nonsense	C-Terminal	24
Exon 12	1553 G>A	R518Q*	Missense	C-Terminal	1
Exon 12	1559 T>G	M520R	Missense	C-Terminal	3
Exon 12	1565 A>C	Y522S*	Missense	C-Terminal	1
Exon 12	1571 T>G	V524G	Missense	C-Terminal	1
Exon 12	1573 G>A	A525T	Missense	C-Terminal	1
Exon 12	1574 C>T	A525V	Missense	C-Terminal	1
Exon 12	1588 C>T	Q530X	Nonsense	C-Terminal	10
Exon 13	1591 C>T	Q531X*	Nonsense/Splice	C-Terminal	1
Exon 13	1597 C>T	R533W	Missense	C-Terminal	2
Exon 13	1615 C>T	R539W	Missense	C-Terminal	6
Exon 13	1616 G>A	R539Q*	Missense	C-Terminal	1
Exon 13	1621 G>A	V541I*	Missense	C-Terminal	1
Exon 13	1627 G>A	E543K*	Missense	C-Terminal	1
Exon 13	1637 C>T	S546L	Missense	C-Terminal	4
Exon 13	1640 A>G	Q547R*	Missense	C-Terminal	1
Exon 13	1663 C>A	R555S*	Missense	C-Terminal	1

Exon 13	1663 C>T	R555C	Missense	C-Terminal	4
Exon 13	1664 G>A	R555H	Missense	C-Terminal	1
Exon 13	1669 A>G	K557E	Missense	C-Terminal	1
Intron 13	1686-1 G>T		Splice site	C-Terminal	1
Exon 14	1696 T>C	S566P*	Missense	C-Terminal	1
Exon 14	1697 C>T	S566F	Missense	C-Terminal	5
Exon 14	1697 C>A	S566Y	Missense	C-Terminal	2
Exon 14	1700 T>C	I567T	Missense	C-Terminal	3
Exon 14	1702 G>A	G568R	Missense	C-Terminal	7
Exon 14	1705 A>G	K569E*	Missense	C-Terminal	1
Exon 14	1712 C>T	S571L*	Missense	C-Terminal	1
Exon 15	1760 C>T	T587M	Missense	C-Terminal	2
Exon 15	1766 G>A	G589D	Missense	SAR	1
Exon 15	1771 C>T	R591C	Missense	SAR	1
Exon 15	1772 G>A	R591H	Missense	SAR	7
Exon 15	1781 G>A	R594Q	Missense	SAR	15
Exon 15	1781 G>C	R594P	Missense	SAR	1
Exon 15	1786_1788delAGA	596delE*	In-frame del	SAR	1
Exon 15	1786 G>A	E596K*	Missense	SAR	1
Exon 15	1794 G>A	K598K*	Splice site	SAR	2
Intron 15	1794+1 G>T		Splice site	SAR	1
Exon 16	1799 C>T	T600M	Missense	SAR	3
Exon 16	1811insC	D603fs+47X*	Frame shift	SAR	1
Exon 16	1831 G>A	D611N*	Missense	SAR	1
Exon 16	1842_1844delCCA	614delH*	In-frame del	SAR	1
Exon 16	1876 G>A	G626S	Missense	C-Terminal	1

Exon 16	1894insC	P631fs+19X	Frame shift	C-Terminal	1
Exon 16	1903 G>A	G635R*	Missense	C-Terminal	2
Exon 16	1986 C>G	Y662X*	Nonsense	C-Terminal	1

* denotes a novel variant, unique to this cohort. Deletion variants are indicated as del, insertions as ins, duplications as dup, and frameshift mutations are annotated for example as R174fs+105X format, where R174 represents the last properly encoded amino acid followed by a frameshift (fs) in the coding sequence resulting in 105 miscoded amino acids leading up to a premature stop codon (X). SAR = subunit assembly region.

Any appropriate method can be used to assess the cis or trans relationship of KCNQ1 mutations, KCNQ1 UTR variation, or wild-type sequences. For example, cDNA generated from KCNQ1 mRNA (e.g., KCNQ1 cDNA generated by RT-PCR) can be sequenced to determine the cis or trans relationship of KCNQ1 mutations, KCNQ1 UTR variations, or wild-type sequences. Any appropriate biological sample such as a blood lymphocytes, skin biopsy, or myocardial biopsy can be used as a source for KCNQ1 mRNA.

In some cases, a person identified as having a more severe form of LQTS can be at an increased risk for sudden death. In such cases, the person can be treated prophylactically with, for example, beta blocker therapy, ICD implantation, and/or sympathetic denervation.

In some cases, a human determined to have a cis or trans relationship of KCNQ1 mutations, KCNQ1 UTR mutations, or wild-type sequences indicative of more severe LQTS can be treated with one or more microRNA inhibitors (e.g., anti-miRs) designed to reduce the ability of a microRNA (e.g., miR-378) to inhibit the expression of a wild-type KCNQ1 allele. A microRNA inhibitor can be designed to inhibit or reduce the activity of a particular microRNA (e.g., a miR-378). Examples of anti-miRs designed to inhibit or reduce the activity of miR-378 include, without limitation, cholesterol-based antagomiR agents and locked nucleic acid-based anti-miRs. In some cases, an anti-miR can have various backbone and/or sugar modifications such as 2′-O-methyl (2′-OMe), 2′-fluoro (2′-F), phosphothioate linkages, and/or cholesterol conjugations.

The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims.

EXAMPLES

Example 1 – Functional variants in KCNQ1's 3'UTR modify disease severity in patients with Type 1 LQTS through miRNA-dependent mechanisms

Genetic Analysis

LQT1-associated KCNQ1 mutations were identified previously using standard protocols (Bhuiyan *et al.*, *Prog. Biophys. Mol. Biol.*, 98(2-3):319-327 (2008)). For this analysis, the final exon and the 3'UTR of KCNQ1 were sequenced. The region to be analyzed included 3 amplicons and was amplified with polymerase chain reaction using oligonucleotide pairs: 5'-GGCACCTTCCCTTCTCTGG-3' (SEQ ID NO:1) with 5'-ACC-ACCATGCCAGTGATGTC-3' (SEQ ID NO:2); 5'-CACAGCCTGCACTTGGG-3' (SEQ ID NO:3) with 5'-CAGGGCTCCTCTCCAGC-3' (SEQ ID NO:4); and 5'-CAGTCTCA-CCATTTCCCCAG-3' (SEQ ID NO:5) with 5'-GCCCAGAACAGGAGCGAC -3' (SEQ ID NO:6).

In Silico Analyses of Interactions Between miRNA and KCNQ1 3'UTR SNPs

The KCNQ1 3'UTR mRNA sequence was retrieved from the Ensemble Genome Browser (NCBI build 36, hg18), and SNPs identified experimentally in a KCNQ1 mutation carrier were mapped on the KCNQ1 mRNA sequence. For each SNP, two sequences centered on the major or minor allele with 30 nucleotides flanking each side were assembled. The search by sequence option of www.mirbase.org was used to identify potential miRNA binding sites within these sequences using an E-value cut-off of 1000. This option used the miRanda algorithm to predict targeting of all miRNAs in the database to the input sequence. Comparison of the major and minor allele for miRNA target site predictions for each SNP was used to determine potential miRNA target sites that were created by the presence of the minor allele.

miRNA Expression in Human Hearts

Post-mortem myocardial samples were obtained from subjects who had died due to non-cardiac causes. Total RNA was extracted using Trizol (Invitrogen), and real-time PCR was used to assess expression levels of miR-378, miR-21, miR-208b, and miR-133.

miRNA expression was normalized for expression of U6 to evaluate the relative myocardial expression levels.

Luciferase Reporter Assay – Plasmid Construction

Using polymerase chain reaction, a luciferase reporter plasmid was constructed by amplifying the 3'UTR of KCNQ1 from a patient heterozygous for the SNPs rs2519184, rs8234, and rs10798 with the following primers: 5'-ACTGACTAGTCATGGACC-ATGCTGTCTG-3' (SEQ ID NO:7) and 5'-ACTGGAGCTCCAGCCTGTGATTCTC-CACG-3' (SEQ ID NO:8). This 878 base pair fragment was cloned into the pMIR-REPORT™ Luciferase vector (Ambion) downstream of the luciferase coding region, creating luciferase-KCNQ1-3'UTR-G-A-A (major haplotype) and luciferase-KCNQ1-3'UTR-A-G-G (minor haplotype). The miR-378 overexpression vector, pCDH1-miR-378, was constructed by PCR-amplification of miR-378 precursor DNA form human genomic DNA using the following primers: 5'-ACTGGAATTCAGAAAGAGGCTG-CGAGGAG-3' (SEQ ID NO:9) and 5'-ACTGGGATCCGGAACAACCAGAAC-ATCTCAC-3' (SEQ ID NO:10). This 306 base pair fragment was cloned into the pCDH1-MCS1-EF1-Puro vector (System Biosciences) under the control of a CMV promotor. The negative control miRNA overexpression vector, PCDH1-control-miRNA, was based on the pcDNATM6.2-GW/miR-neg control plasmid (Invitrogen), which contained an insert that can form a hairpin structure that is processed into mature miRNA but is predicted not to target any known vertebrate gene. All generated constructs were verified by sequencing.

Cell Isolation and Preparation

Neonatal rat cardiac myocytes, immortalized with a temperature-sensitive SV40 T antigen (H10 cells) as described elsewhere (Jahn *et al.*, *J. Cell Sci.*, 109(Pt 2):397-407 (1996)), were cultured in Dulbecco's Modified Eagles Medium supplemented with 10% fetal calf serum (Gibco-BRL) and glutamine at 33 °C. One day prior to transfection, cells were seeded in a 24-well plate at a density of $1.4x10^6$ cells per plate. Cardiomyocytes from 1-2-day-old Lewis neonatal rats were isolated and cultured as described elsewhere (Leenders *et al.*, *J. Biol. Chem.*, 285(35):27449-27456 (2010)).

Transfection and Luciferase Assay

H10 cells were transiently transfected per well with 5 ng Renilla luciferase plasmid, phRL vector (Promega), and 5 ng or 10 ng of either luciferase-KCNQ1-3'UTR-G-A-A, luciferase-KCNQ1-3'UTR-A-A-A, luciferase-KCNQ1-3'UTR-G-G-A, or luciferase-KCNQ1-3'UTR-A-G-G using GeneJammer (Agilent Technologies). Neonatal rat cardiomyocytes were transfected with 50 ng Renilla luciferase plasmid, phRL vector, and 100 ng of either luciferase-KCNQ1-3'UTR-G-A-A, luciferase-KCNQ1-3'UTR-A-A-A, luciferase-KCNQ1-3'UTR-G-G-A, or luciferase-KCNQ1-3'UTR-A-G-G using lipofectamine 2000 reagent (Invitrogen). COS-7 cells were transiently transfected per well with 5 ng Renilla luciferase plasmid, phRL vector, 100 ng of either luciferase-KCNQ1-3'UTR-G-A-A or luciferase-KCNQ1-3'UTR-A-G-G, 100 ng of miR-378 or negative control miRNA overexpression construct, and empty pCDH1 as filler using polyethylenimine (PEI). At 48 hours after transfection, cells were lysed and assayed for luciferase and Renilla luciferase activity with a luminometer (Glomax multi detection system, Promega) by using the Renilla reporter assay system (Promega). Renilla luciferase activity was assayed to normalize luciferase results for cell densities and transfection efficiency.

Statistical Methods

QTc was calculated using Bazett's formula and compared between the various LQT1 genotype and 3'UTR SNP haplotype combinations. A linear mixed effect regression model from the kinship package in R was used (R Development Core Team. R: a language and environment for statistical computing. The R Project for Statistical Computing Web Site. (World Wide Web at "r-project.org") (2009)). Differences in symptom prevalence were analyzed with a logistic regression model using generalized estimation equations. In both models, a correction for the relatedness among individuals was applied. Furthermore, age at the time of ECG, gender, and proband status were included as covariates, and genotype effects were modeled as additive effects. p-values <0.05 was regarded as statistically significant.

Results

SNPs are present in the 3'UTR of KCNQ1

From two institutions (Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands (AMC) and Mayo Clinic, Rochester, Minnesota, USA (MC)), families were included where a mutation in *KCNQ1* was identified in at least three members, and at least one affected member displayed QTc prolongation. Individuals with acquired cardiac diseases, electrolyte abnormalities, or use of medications known to prolong repolarization were excluded. From the AMC, 84 LQT1-positive subjects from 24 families were included. From the MC, 84 LQT1-positive subjects from 17 families were included. Informed written consent was obtained from all subjects and demographic data (Table 1) and details of the identified mutations (Table 2) were collected. In all subjects, the final exon and the complete 3'UTR of *KCNQ1* were sequenced. In the AMC cohort, seven SNPs were found of which six SNPs were in the 3'UTR. SNPs at nucleotide positions 403092 and 403641 were not reported previously (NCBI build 36, hg18). Except for the SNP at nucleotide position 403092, all SNPs (and no additional SNPs) were also found in the MC population (Figure 1A; Table 3 for SNP frequencies).

Table 1. Clinical characteristics, mutation types, and ECG parameters of the study populations from AMC and MC.

Variable	**AMC**	**MC**
Clinical characteristics		
Patients, n (%)	84 (100)	84 (100)
Male/female, n	34/50	38/46
Age, years	34±3	27±2
Proband, n (%)	22 (26)	13 (15)
Family history for sudden death, n (%)	40 (48)	58 (69)
Syncope, n (%)	18 (21)	18 (21)
Documented TdP/VF, n (%)	5 (6)	1 (1)

Mutation type		
Missense, n (%)	63 (75)	67 (80)
Frameshift, n (%)	7 (8)	1 (1)
Splice site, n (%)	11 (13)	4 (5)
Deletion, n (%)	3 (4)	12 (14)
ECG parameters		
RR interval (msec)	859±19	917±24
Males	879±26	968±41
Females	844±28	875±27
QT duration (msec)	411±7	426±6
Males	405±8	429±10
Females	416±10	425±8
QTc (msec)	446±7	451±4
Males	435±6	445±6
Females	454±7*	457±4*

Values are expressed as number of patients (percentage of total), or as mean ± standard error of the mean (SEM). * denotes statistical significance compared to males.

Table 2. Mutations in *KCNQ1* found in the study populations from AMC and MC.

Region	**Nucleotide change**	**Mutation**	**Mutation type**	**Location**	**Cr.**	**#**	**Ref.**
Exon 1	360 G>C	W120C	Missense	N-terminus	A	2	1
Exon 1	365 G>A	C122Y	Missense	N-terminus	M	1	2
Exon 2	387-?_1393+?del		Deletion	S1	A	3	-
Intron 2	477+1 G>C		Splice site	S2	A	3	3
Exon 3	551 A>C	Y184S	Missense	S2/S3	A	7	4
Exon 3	739 T>C	F193L	Missense	S2-S3	A	1	5*
Exon 4	674 C>T	S225L	Missense	S3/S4	A, M	1, 3	6*

Exon 5	704 T>A	I235N	Missense	S4	M	20	7*
Exon 5	727 C>T	R243C	Missense	S4	A, M	14, 3	8*
Exon 5	775 C>T	R259C	Missense	S4/S5	A	2	9*
Exon 5	776 G>T	R259L	Missense	S4/S5	M	1	2
Exon 6	797 T>C	L266P	Missense	S5	M	3	10
Exon 6	805 G>A	G269S	Missense	S5	M	3	11*
Exon 6	806 G>A	G269D	Missense	S5	M	2	12*
Exon 6	820 A>G	I274V	Missense	S5	A	4	1
Exon 6	875 G>A	G292D	Missense	S5/pore	A	2	1
Exon 6	887 T>C	F296S	Missense	S5/pore	A	9	13*
Exon 7	940 G>A	G314S	Missense	Pore	A	1	14*
Exon 7	941 G>A	G314D	Missense	Pore	A	1	15
Exon 7	944 A>G	Y315C	Missense	Pore	M	7	6*
Exon 7	964 A>G	T322A	Missense	Pore/S6	M	9	15
Exon 7	1015_1017delTTC	339delF	Deletion	S6	M	12	16*
Exon 7	1031 C>T	A344V	Missense	S6	A	4	17*
Intron 7	1032+5 G>A		Splice site	S6	A, M	8, 4	18*
Exon 9	1189 C>T	R397W	Missense	C-terminus	A	6	1
Exon 10	1265 A>C	K422T	Missense	C-terminus	A	3	19
Exon 10	1343 ins C	P448fsX13	Frameshift	C-terminus	A, M	7, 1	-
Exon 12	1571 T>G	V524G	Missense	C-terminus	M	2	1
Exon 13	1615 C>T	R539W	Missense	C-terminus	M	4	20*
Exon 14	1700 T>G	I567S	Missense	C-terminus	M	4	15
Exon 15	1771 C>T	R591C	Missense	SAD	A	2	1
Exon 15	1772 G>A	R591H	Missense	SAD	M	5	21*
Exon 15	1781 G>A	R594Q	Missense	SAD	A	4	22*

Del, deletion; ins, insertions; P448fsX13, frameshift mutation whereby P448 represents the last normally encoded amino acid followed by a frameshift (fs) in the coding sequence generating 13 miscoded amino acids leading up to a premature stop codon (X); 387-?_1393+?del, mutation leading to the deletion of exon 2 (starting from nucleotide 387) to exon 10 (ending at nucleotide 1393), but whereby the exact nucleotide change is not yet determined (?); N-terminus, amino-terminus; S, transmembrane segment; C-terminus, carboxyl-terminus; SAD, subunit assembly domain; Cr., center in which the mutation was found (A: Academic Medical Center Amsterdam, M: Mayo Clinic); #, number of individuals within each study population affected with the mutation; Ref., reference linking the mutation with long QT syndrome; *, biophysical/loss-of-function properties of the mutation are described in the reference.

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Table 3. Single nucleotide polymorphisms found in the 3'untranslated region of *KCNQ1*.

					AMC (n = 84)		MC (n = 84)	
Genetic Location	**Nucleotide change position**	**Allele (Major/ minor)**	**SNP ID**	**Genotype**	**N (%)**	**Minor allele frequency**	**N (%)**	**Minor allele frequency**
Exon 16	402865	C/T	rs11601907	CC	38 (45)	0.327	50 (60)	0.214
				CT	37 (44)		32 (38)	
				TT	9 (11)		2 (2)	
3'UTR	403092	G/A	not available	GG	81 (96)	0.018	84 (100)	0.000
				GA	3 (4)		0	
				AA	0		0	
3'UTR	403321	C/T	rs45460605	CC	81 (96)	0.018	83 (99)	0.006
				CT	3 (4)		1 (1)	
				TT	0		0	
3'UTR	403389	G/A	rs2519184	GG	78 (93)	0.036	50 (60)	0.202
				GA	6 (7)		34 (40)	
				AA	0		0	

3'UTR	403641	G/A	not available	GG	83 (99)	0.006	83 (99)	0.006
				GA	1 (1)		1 (1)	
				AA	0		0	
3'UTR	403785	A/G	rs8234	AA	52 (62)	0.208	31 (37)	0.351
				AG	29 (34)		47 (56)	
				GG	3 (4)		6 (7)	
3'UTR	403842	A/G	rs10798	AA	52 (62)	0.208	31 (37)	0.351
				AG	29 (34)		47 (56)	
				GG	3 (4)		6 (7)	

Position of the nucleotide change is starting from the ATG start codon (NCBI build 36, hg18). The SNP ID denotes single nucleotide polymorphism identity from public databases. N (%) denotes number of patients (percentage of total).

Minor variants of SNPs rs2519184 and rs8234 in KCNQ1's 3'UTR create binding sites for miRNA-378

The mirbase algorithm (World Wide Web at "mirbase.org") predicted that the minor (A) variant of SNP rs2519184 and the minor (G) variant of SNP rs8234 create binding sites for a number of miRNAs including two binding sites for miRNA-378 (miR-378; Figure 1B). miR-378 was found to be abundantly expressed in the human left ventricle of four healthy donor hearts, at levels comparable to miR-208b (miR-208b is expressed in heart tissue and a low levels in skeletal muscle) (Figure 1C).

Minor variants of SNPs rs2519184 and rs8234 repress mRNA expression in the presence of miR-378

To assess the functional effects of SNPs rs2519184 and rs8234 on mRNA expression, the 3'UTR of *KCNQ1* were cloned with either the major 3'UTR SNP haplotype (G-A-A) or the minor haplotype (A-G-G) of SNPs rs2519184, rs8234 including rs10798 as the latter two SNPs were in complete linkage disequilibrium in all patients in this study. Next, these 3'UTRs were placed downstream of the luciferase coding region in the pMIR-REPORT™ plasmid (Ambion) and transfected into both heart-derived H10 cells (Figure 2A) and primary neonatal rat cardiomyocytes (Figure 6A). The plasmid containing the minor 3'UTR SNP haplotype (A-G-G), which harbors two created binding sites for miR-378, had significantly lower luciferase activity in both cardiac cell types.

Moreover, introduction of either minor allele of SNP rs2519184 or rs8234 into the major allele was sufficient to decrease luciferase activity, indicating that those two nucleotides control translation of *KCNQ1* (Figure 2B for H10 cells, and Figure 6B for neonatal rat cardiomyocytes). To assess the specific effect of miR-378 on mRNA expression, miR-378 was overexpressed in COS-7 cells transfected with a reporter containing either the major 3'UTR SNP haplotype (G-A-A) or the minor SNP haplotype (A-G-G). Overexpression of miR-378, but not of a control miRNA, repressed luciferase activity from the reporter containing the minor 3'UTR SNP haplotype (A-G-G), but not from the reporter containing the major SNP haplotype (G-A-A; Figure 2C).

Inhibition of microRNA-378 by a specific anti-miR solely directed against microRNA-378 inhibited this microRNA and abolished the suppressive effects of the SNPs (Figures 8 and 9).

The experiment of Figure 9 was repeated several times, and the combined results are presented in Figure 10.

An additional experiment was performed to show the allelic imbalance on mRNA levels, thereby confirming that there is imbalance on both the polypeptide and mRNA levels. The allelic imbalance on mRNA level was demonstrated using an allele-specific qPCR approach. In this experiment, primers were designed to specifically amplify and detect the KCNQ1 3'UTR depending on the variant in the 3'UTR. Briefly, for the allele-specific qPCR, 400 ng of input RNA was used in the reverse transcription reaction. Input RNA was reverse transcribed using superscript II Reverse transcriptase (Invitrogen) with oligo-dT primers according to the manufacturer's protocol.

The quantitative PCR was performed using LightCycler 480 sybr green I master (Roche). For SNP rs8234, allele-specific reverse primers (5'-ACCACAAAT-TATTGATTTCTATGCGAT-3' (SEQ ID NO:11) for amplifying the major A allele or 5'-ACCACAAATTATTGATTTCTATGCGAC-3' (SEQ ID NO:12) for amplifying the minor G allele) and a general forward primer (5'-AGCCAGCCAAACACACAG-3' (SEQ ID NO:13)) were used. Quantitative PCR reactions were performed on a lightCycler480 system II (Roche) using the following program: 5 minutes pre-incubation at 95°C and 40 cycles of 10 seconds of denaturation at 95°C, 20 seconds of annealing at 60°C, and 20 seconds of elongation at 72°C. Data were analyzed using LinRegPCR quantitative PCR

data analysis software. The starting concentrations of transcripts estimated by this software were corrected for the estimated starting concentrations of the housekeeping gene GAPDH (5'-ACCCACTCCTCCACCTTTGAC-3' (SEQ ID NO:14) and 5'-ACCCTGTTGCTGTAGCCAAATT-3' (SEQ ID NO:15)) and for the estimated starting concentration of a total KCNQ1 amplicon closely resembling the allele-specific amplicons (5'-GAAGTGACGGTTCCTACAC-3' (SEQ ID NO:16) and 5'-AGCTTGCACAATTAATAATCAAAATC-3' (SEQ ID NO:17)).

By directly comparing the amplification of the major and minor variant, it was possible to calculate the relative expression. A 10% difference in expression between major and minor allele was detected, where the minor allele was less abundant (Figure 11). This demonstrates that the minor variants were suppressive variants.

In LQT1, SNPs rs2519184 and rs8234 modify QTc in an allele-specific fashion

Since the functional analysis revealed that the minor alleles of SNPs rs2519184 and rs8234 suppress translation, it was hypothesized that this translational suppression would increase or decrease QTc depending on whether they occur *in trans* (opposite allele) or *in cis* (on the same allele) to the LQT1-causative *KCNQ1* mutation, as defined by analysis of phase in the families. Occurrence of 'suppressive 3'UTR SNPs' *in cis* to the LQT1 mutation would be expected to attenuate QTc prolongation by decreasing the abundance of mutant protein available for tetrameric assembly, while 'suppressive 3'UTR SNPs' *in trans* to the mutation would have the opposite effects by decreasing translation of the normal allele and thereby increasing the amount of mutant $K_V7.1$ sub-units resulting in greater QTc prolongation (Figure 7). The statistical approach to assess differences in QTc is described herein.

The presence of the suppressive minor (A) allele of SNP rs2519184 *in trans* to the mutated allele (i.e., on the normal allele) was associated with a marked increase in QTc by 46 ± 16 milliseconds in the initial AMC cohort (Figure 3A). This was corroborated by similar findings in the MC cohort where the presence of the minor allele *in trans* was associated with 60 ± 16 milliseconds longer QTc (Figure 3D). Notably, in the MC cohort, subjects were also identified where this 3'UTR minor allele SNP resided on the LQT1-mutation containing allele (i.e., *in cis* to the mutation). Here, location of this SNP

in cis was associated with 16 ± 8 milliseconds shorter QTc. The same effects were seen for the suppressive SNP rs8234. When the minor SNP rs8234 (G) was *in trans* to the mutated allele, QTc was increased by 26 ± 10 milliseconds (Figure 3B). Again, this was confirmed in the MC cohort where G located *in trans* increased the QTc by 33 ± 7 milliseconds (Figure 3E). The opposite location of these suppressive minor alleles (i.e., *in cis* to the mutation) was related with shorter QTc (minus 41 ± 25 milliseconds), which was confirmed in the MC population (reduction by 9 ± 9 milliseconds; not significant).

Allele-specific haplotype analysis of SNPs rs2519184 and rs8234 revealed that location of the suppressive minor alleles of the SNPs (A-G) *in trans* to the mutation was associated with 49 ± 16 milliseconds longer QTc in the AMC cohort and a 60 ± 14 milliseconds longer QTc in the MC cohort (Figure 3C and Figure 3F). Subjects where the A-G-G haplotype was *in cis* to the mutation were only present in the MC cohort. Indeed, in these subjects, the opposite effect on QTc was suggested, as the QTc was now attenuated by 12 ± 9 milliseconds. Various allele-specific haplotype combinations of *KCNQ1* and the minor alleles of the three SNPs, as shown in Figure 3C and Figure 3f, displayed an intermediate effect on QTc.

SNPs rs2519184 and rs8234 modify QTc in an allele-specific fashion in singles LQT1 families

The data provided herein demonstrates that testing for the suppressive 3'UTR SNPs can predict disease severity of a given *KCNQ1* mutation in one single family. To test this further, the allele-specific haplotype of SNPs rs2519184 and rs8234 with regard to QTc in the largest families in the AMC (14 affected) and MC (20 affected) cohorts were analyzed. Indeed, the KCNQ1-R243C carriers, where the minor suppressive alleles of one or two SNPs was found *in trans* to the mutation, exhibited markedly longer QTc (533 ± 24 and 489 ± 25 milliseconds, respectively) than their KCNQ1-R243C-positive relatives who did not carry these SNPs (441 ± 7 milliseconds; Figure 4A). The opposite was seen in the largest MC family where the 3'UTR SNPs resided on the mutation (KCNQ1-I235N)-containing allele. Family members with the suppressive SNPs *in cis* to the mutation displayed shorter QTc values (421 ± 6 milliseconds) than family members lacking these SNPs (458 ± 17; Figure 4B).

In LQT1, SNPs rs2519184 and rs8234 modify symptomatology in an allele-specific fashion

The following was performed to assess whether allele-specific location of these suppressive SNPs in the *KCNQ1*'s 3'UTR also modifies the known cardiac sequelae of LQT1 (unexplained syncope, documented torsades de pointes ventricular tachycardia/ventricular fibrillation, and/or [aborted] sudden death). Location of the suppressive allele of SNPs rs8234 *in trans* to the mutation was associated with a statistically significant increased occurrence of symptoms. Inversely, the opposite location of the minor suppressive SNP haplotype (A-G) (i.e., *in cis* to the mutation) tended to be related to fewer symptoms (Figure 5).

The effect of SNPs rs2519184, rs8234, and rs10798 on QTc in the general population

To study the effect of SNPs rs2519184, rs8234, and rs10798 in the general population, the following was performed using the KORA Study to evaluate whether the SNPs rs2519184, rs8234, or rs10798 are correlated with QTc duration in the general population. The KORA Study is a series of independent population-based epidemiological surveys of participants living in the region of Augsburg, Southern Germany. All survey participants were residents of German nationality identified through the registration office and were examined in 1994-95 (KORA S3) and 1999-2001 (KORA S4). In 2004-05, 3.006 subjects participated in a 10-year follow-up examination of S3 (KORA F3) and in 2006-08, 3.080 subjects participated in a 7-year follow-up examination of S4 (KORA F4). Individuals for genotyping in KORA F3 and KORA F4 were randomly selected.

The data about rs2519184 is described elsewhere (Pfeufer *et al.*, *Circ. Res.*, 96(6):693-701 (2005)). A correlation was found between the SNP and QTc duration in screening sample of 689 individuals, but this association was not found in a confirmation sample of 3277 individuals. An analysis of KORA reveals that rs8234 and rs10798 were not associated with QTc duration in two populations of the KORA study (Table 4), suggesting that the general suppressive effects of these SNPs does not lower KCNQ1 proteins sufficiently to induce QTc prolongation in healthy individuals. However, as

indicated herein, effects of the suppressive 3'UTR SNPs were pronounced when one of the two KCNQ1 alleles harbours a disease-causing mutation.

Table 4. The effect of SNPs rs8234 and rs10798 on QTc in the general population.

SNP	Population*	Number	Major allele/haplotype	Minor allele/haplotype	MAF	Effect on QTc Beta (msec)	*p*- value
rs8234	KORA F3	1459	A	G	0.3041	-0.573105	0.52
rs10798	KORA F3	1459	A	G	0.304	-0.572674	0.52
rs8234	KORA S4	975	A	G	0.3216	1.383768	0.13
rs10798	KORA S4	975	A	G	0.3209	1.42896	0.12
Haplotype	KORA F3	1459	AA	GG	0.3047	0.569323	0.52
Haplotype	KORA S4	975	AA	GG	0.3185	0.611701	0.42

Number: number of individuals within the screening sample; MAF: minor allele frequency. The effect on the QTc duration refers to the dosage (i.e., expected number of copies) of the major allele. The QTc duration is calculated by the Bazett's formula, and is corrected for age and gender.

The results provided herein demonstrate that SNPs in the 3'UTR of KCNQ1 (SNPs rs2519184 and rs8234) create binding sites for miR-378, a miRNA appreciably expressed in the human heart. Creation of these binding sites can allow miR-378 to suppress translation of the SNP-containing KCNQ1 allele. These results are clinically relevant when these suppressive 3'UTR SNPs occur in carriers of an LQT1-causing mutation. The allelic location of these suppressive SNPs can alter the balance between normal and mutated $K_v7.1$ channel subunits. When the SNPs are in cis to the mutation, the diseased allele can be suppressed and the expressed LQT1 phenotype can be less severe. However, when the suppressive SNPs reside on the normal non-mutated allele (in trans), then translation of the normal allele can be suppressed and the LQT1 phenotype can be more severe.

The results provided herein also demonstrate that LQT1 genetic testing can include an analysis and cis/trans phase determination of the suppressive 3'UTR SNPs in KCNQ1. More generally, the results provided herein demonstrate that genetic variation in the 3'UTR may be an important source for clinical variability by altering the balance of translation between the two alleles.

It is noteworthy that the size of the effects on QTc duration and symptoms that is described go well beyond what has been shown for modifiers described elsewhere (Pfeufer *et al.*, *Circ. Res.*, 96(6):693-701 (2005); and Crotti *et al.*, *Circulation*, 120(17):1657-1663 (2009)).

In summary, the results provided herein demonstrate that naturally occurring SNPs in KCNQ1's 3'UTR suppress translation by creating binding sites for miR-378. In KCNQ1-mutation carriers, these functional SNPs explain an important part of the incomplete penetrance and variable expressivity associated with LQT1, suggesting that the clinical effects of a pathogenic mutation may be determined by naturally occurring genetic variation in its 3'UTR that results in the miRNA-mediated alteration of normal or mutant allele expression. These results also suggest that in autosomal dominant diseases like long QT syndrome, disease severity can be importantly modified even by sequence variation in the 3'UTR stemming from the unaffected parent.

OTHER EMBODIMENTS

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

WHAT IS CLAIMED IS:

1. A method for assessing the cis or trans nature of a human heterozygous for a KCNQ1 mutation and heterozygous for a KCNQ1 UTR variation, wherein said method comprises:

(a) determining if an allele of said human comprises (i) said KCNQ1 mutation and said KCNQ1 UTR variation, (ii) a lack of said KCNQ1 mutation and a lack of said KCNQ1 UTR variation, (iii) said KCNQ1 mutation and a lack of said KCNQ1 UTR variation, or (iv) said KCNQ1 UTR mutation and a lack of said KCNQ1 variation,

(b) classifying said human as having said KCNQ1 mutation and said KCNQ1 UTR variation in cis if said allele comprises (i) or (ii), and

(c) classifying said human as having said KCNQ1 mutation and said KCNQ1 UTR variation in trans if said allele comprises (iii) or (iv).

2. The method of claim 1, wherein said human is an LQT1 patient.

3. The method of claim 1, wherein said allele comprises said KCNQ1 mutation and said KCNQ1 UTR variation, and said human is classified as having said KCNQ1 mutation and said KCNQ1 UTR variation in cis.

4. The method of claim 1, wherein said allele comprises a lack of said KCNQ1 mutation and a lack of said KCNQ1 UTR variation, and said human is classified as having said KCNQ1 mutation and said KCNQ1 UTR variation in cis.

5. The method of claim 1, wherein said allele comprises said KCNQ1 mutation and a lack of said KCNQ1 UTR variation, and said human is classified as having said KCNQ1 mutation and said KCNQ1 UTR variation in trans.

6. The method of claim 1, wherein said allele comprises said KCNQ1 UTR variation and a lack of said KCNQ1 mutation, and said human is classified as having said KCNQ1 mutation and said KCNQ1 UTR variation in trans.

7. The method of claim 1, wherein said KCNQ1 mutation is L266P, R518X, G168R, or R594Q.

8. The method of claim 1, wherein said KCNQ1 UTR variation is an rs2519184 SNP or rs8234 SNP.

9. A method for treating a mammal having a KCNQ1 UTR variation that is part of a microRNA binding site, wherein the binding of a microRNA to said binding site reduces expression of a $K_v7.1$ potassium channel subunit lacking a mutation associated with LQTS, wherein said method comprises administering an miRNA inhibitor to said mammal under conditions wherein said miRNA inhibitor reduces the ability of said microRNA to reduce expression of said subunit.

10. The method of claim 9, wherein said mammal is a human.

11. A method for treating a human comprising administering an agent that inhibits or mimics the activity of a microRNA that targets a KCNQ1 UTR variation to increase the ratio of expression of a non-mutated KCNQ1 allele to mutated KCNQ1 allele.

12. A method for treating a human having long QT syndrome, wherein said method comprises:

(a) determining if an allele of said human comprises (i) said KCNQ1 mutation and said KCNQ1 UTR variation, (ii) a lack of said KCNQ1 mutation and a lack of said KCNQ1 UTR variation, (iii) said KCNQ1 mutation and a lack of said KCNQ1 UTR variation, or (iv) said KCNQ1 UTR mutation and a lack of said KCNQ1 variation, and

(b) administering a beta blocker to said human, implanting an implantable cardioverter-defibrillator into said human, or performing a sympathetic denervation

procedure on said human.

13. The method of claim 12, wherein said method comprises administering said beta blocker to said human.

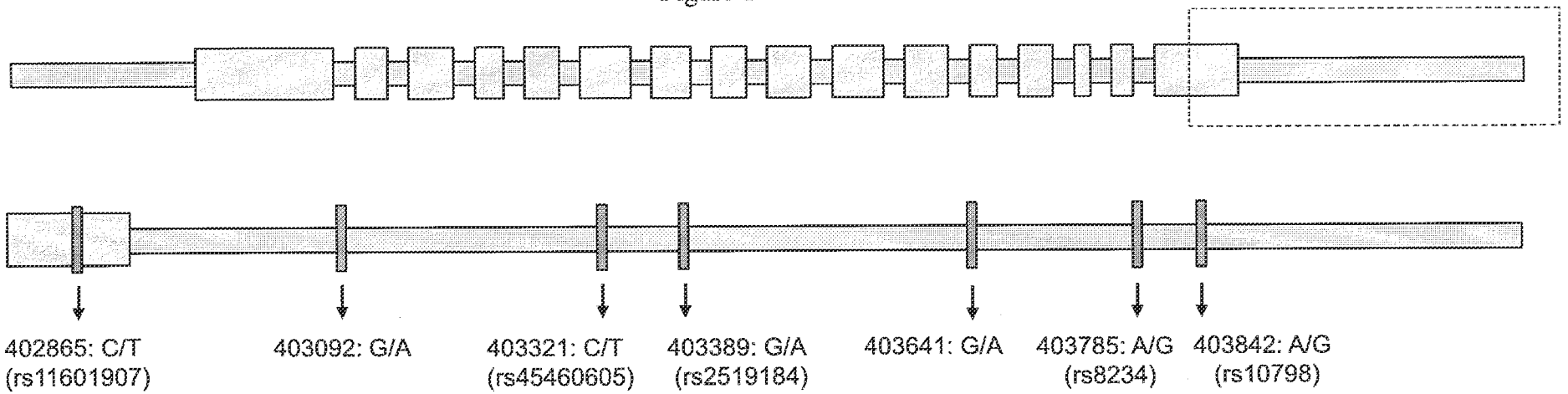
14. The method of claim 13, wherein said beta blocker is metoprolol, carvedilol, bisoprolol, nebivolol, or propanolol.

15. The method of claim 12, wherein said method comprises implanting said implantable cardioverter-defibrillator into said human.

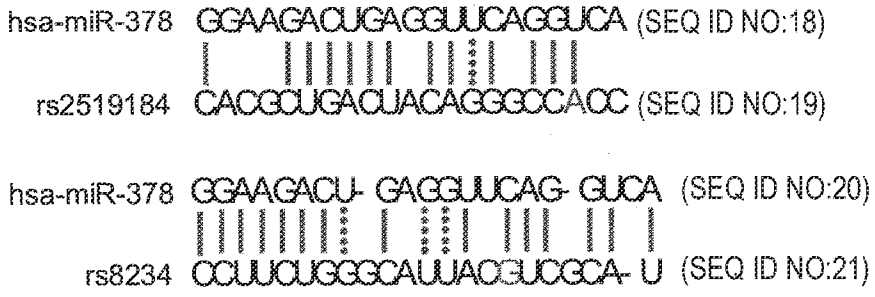
16. The method of claim 12, wherein said method comprises performing said sympathetic denervation procedure on said human.

Figure 1

a


402865: C/T
(rs11601907)
403092: G/A
403321: C/T
(rs45460605)
403389: G/A
(rs2519184)
403641: G/A
403785: A/G
(rs8234)
403842: A/G
(rs10798)

b


hsa-miR-378 GGAAGACUGAGGUUCAGGUCA (SEQ ID NO:18)
rs2519184 CACGCUGACUACAGGGCCACC (SEQ ID NO:19)
hsa-miR-378 GGAAGACU- GAGGUUCAG- GUCA (SEQ ID NO:20)
rs8234 CCUUCUGGGCAUUACGUCGCA- U (SEQ ID NO:21)

c

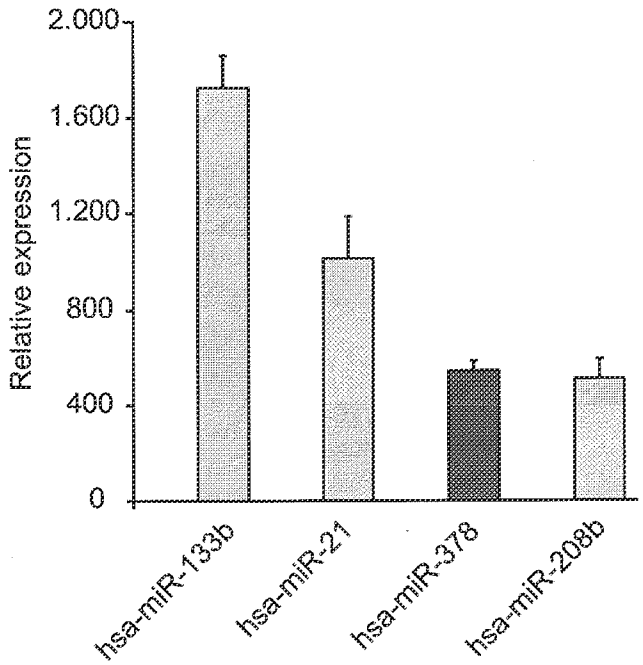

Relative expression
2.000
1.600
1.200
800
400
0
hsa-miR-133b
hsa-miR-21
hsa-miR-378
hsa-miR-208b

Figure 2

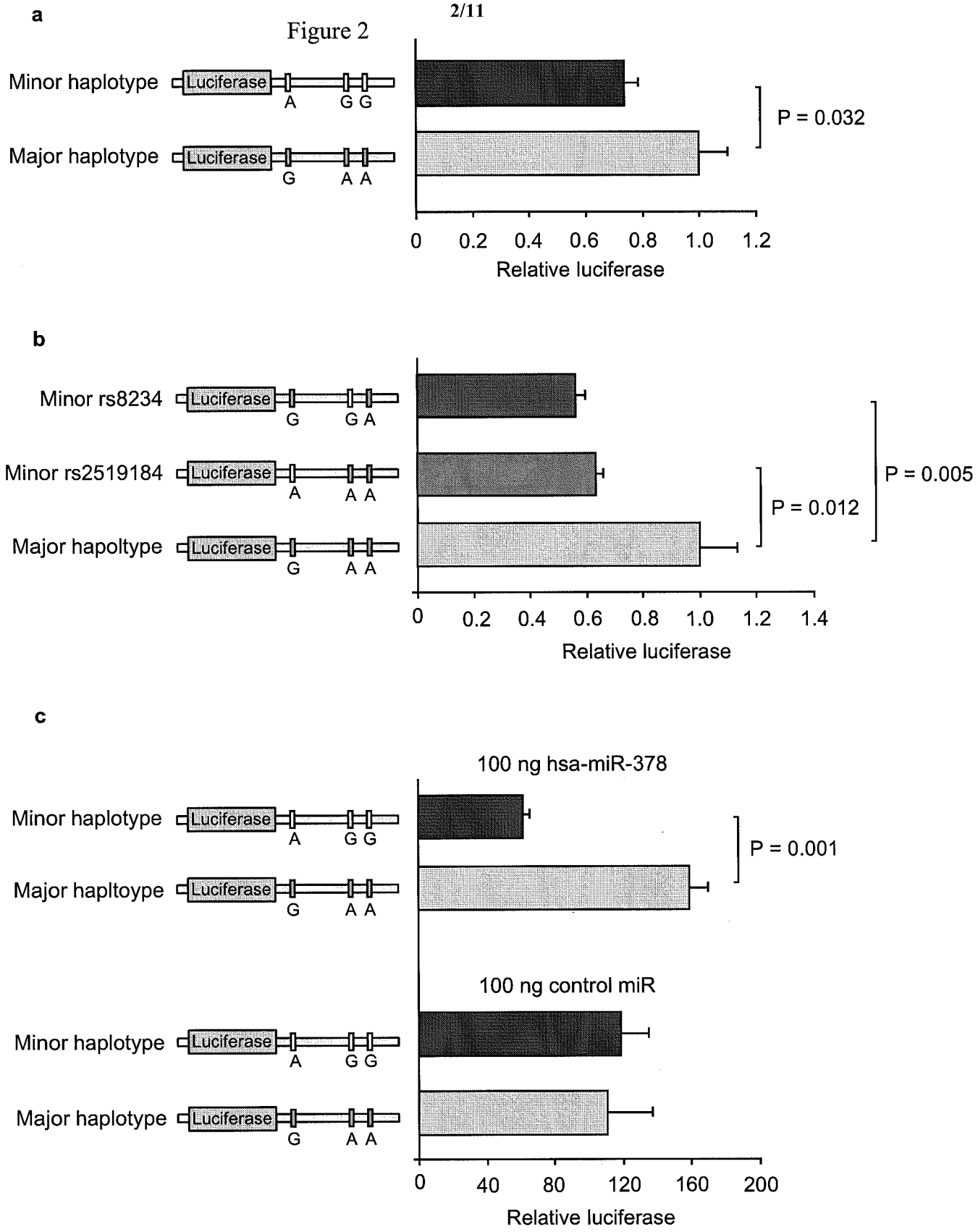

a
Minor haplotype
Luciferase
A G G
Major haplotype
Luciferase
G A A
P = 0.032
0 0.2 0.4 0.6 0.8 1.0 1.2
Relative luciferase
b
Minor rs8234
Luciferase
G G A
Minor rs2519184
Luciferase
A A A
Major hapoltype
Luciferase
G A A
P = 0.005
P = 0.012
0 0.2 0.4 0.6 0.8 1.0 1.2 1.4
Relative luciferase
c
100 ng hsa-miR-378
Minor haplotype
Luciferase
A G G
Major hapltoype
Luciferase
G A A
P = 0.001
100 ng control miR
Minor haplotype
Luciferase
A G G
Major haplotype
Luciferase
G A A
0 40 80 120 160 200
Relative luciferase

Figure 3

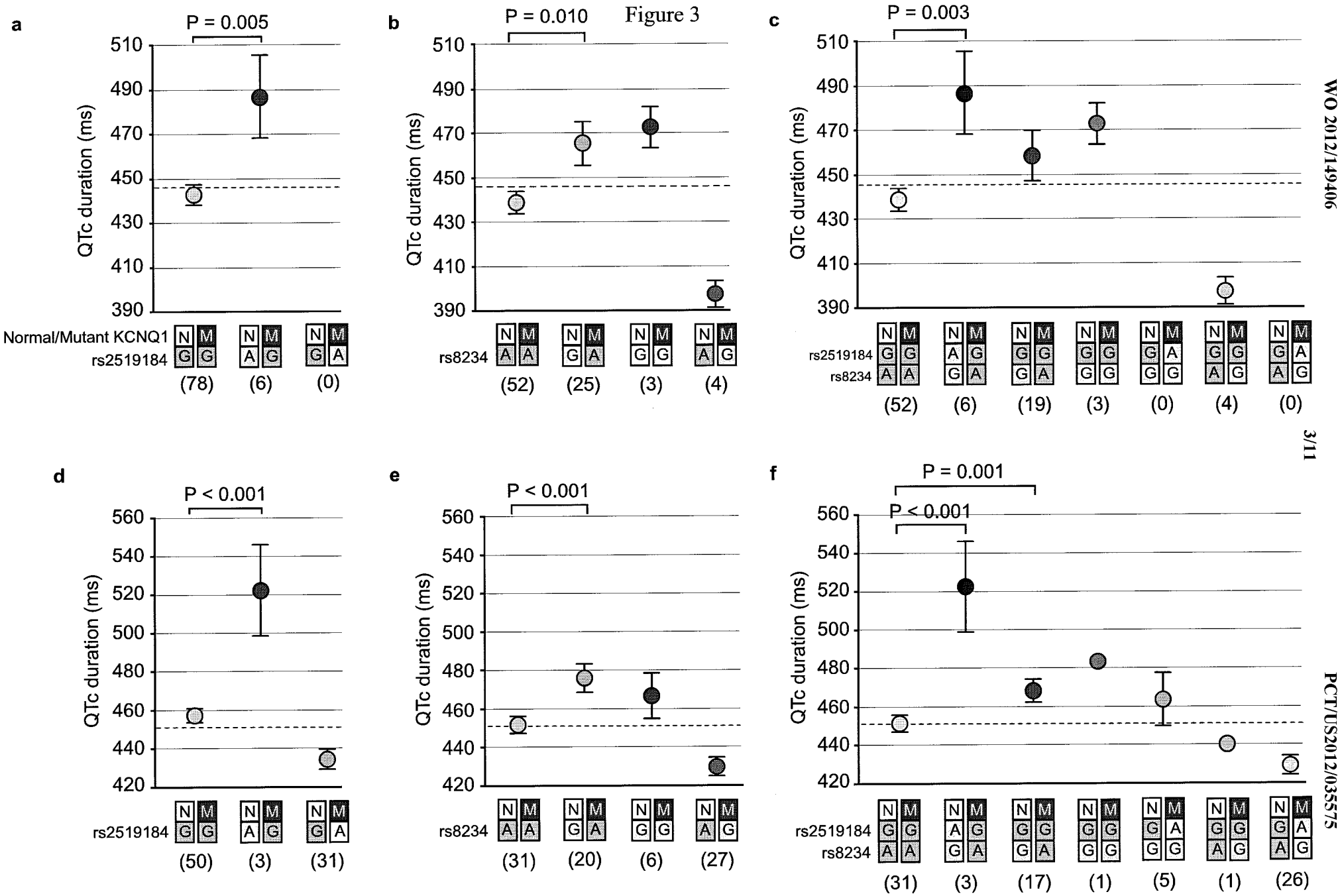

a
P = 0.005
QTc duration (ms)
510
490
470
450
430
410
390
Normal/Mutant KCNQ1
rs2519184
N M
G G
N M
A G
N M
G A
(78)
(6)
(0)
b
P = 0.010
QTc duration (ms)
510
490
470
450
430
410
390
rs8234
N M
A A
N M
G A
N M
G G
N M
A G
(52)
(25)
(3)
(4)
c
P = 0.003
QTc duration (ms)
510
490
470
450
430
410
390
rs2519184
rs8234
N M
G G
A A
N M
A G
G A
N M
G G
G A
N M
G G
G G
N M
G A
G G
N M
G G
A G
N M
G A
A G
(52)
(6)
(19)
(3)
(0)
(4)
(0)
d
P < 0.001
QTc duration (ms)
560
540
520
500
480
460
440
420
rs2519184
N M
G G
N M
A G
N M
G A
(50)
(3)
(31)
e
P < 0.001
QTc duration (ms)
560
540
520
500
480
460
440
420
rs8234
N M
A A
N M
G A
N M
G G
N M
A G
(31)
(20)
(6)
(27)
f
P = 0.001
P < 0.001
QTc duration (ms)
560
540
520
500
480
460
440
420
rs2519184
rs8234
N M
G G
A A
N M
A G
G A
N M
G G
G A
N M
G G
G G
N M
G A
G G
N M
G G
A G
N M
G A
A G
(31)
(3)
(17)
(1)
(5)
(1)
(26)

Figure 4

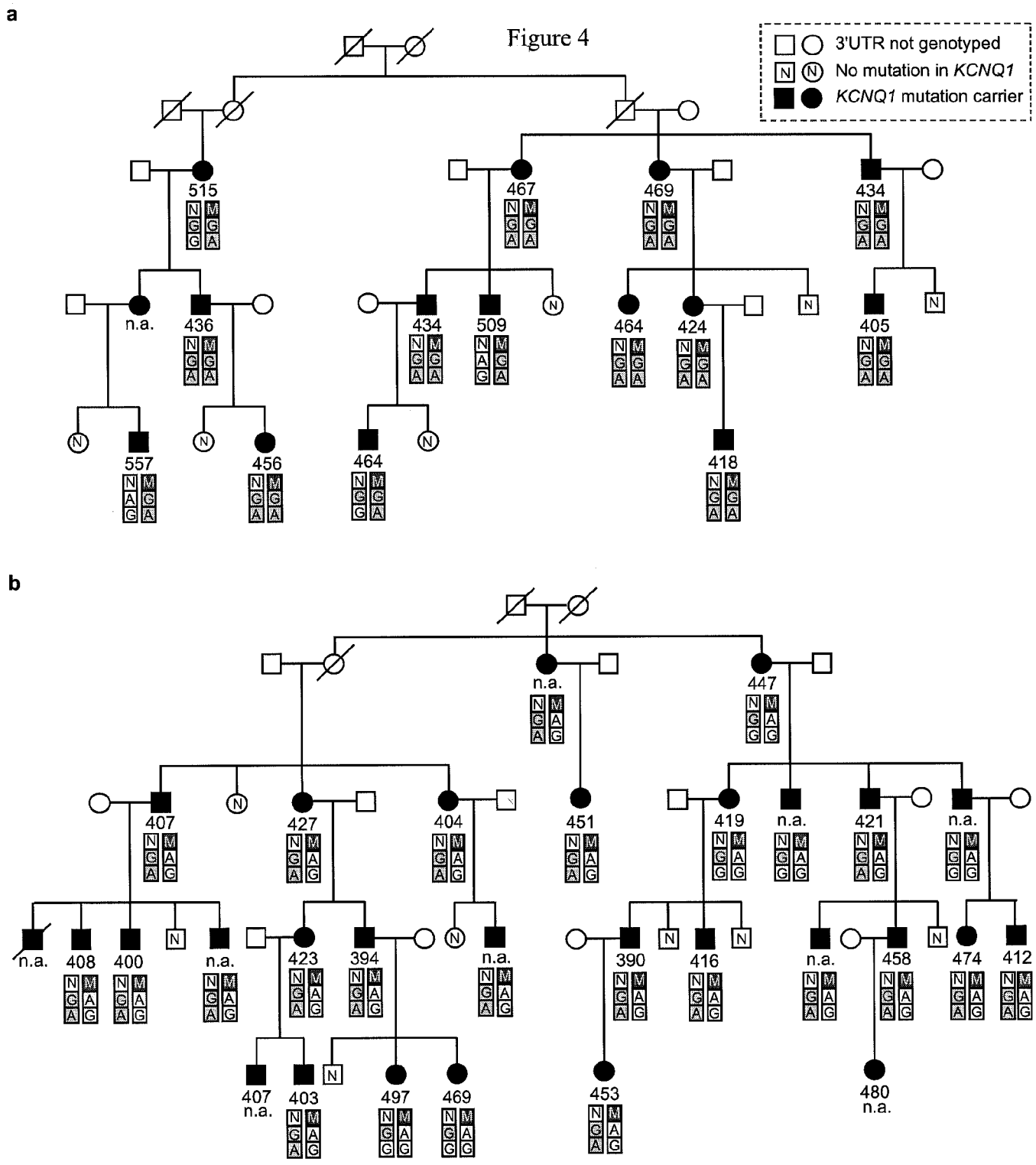

a
3'UTR not genotyped
No mutation in KCNQ1
KCNQ1 mutation carrier
515
467
469
434
n.a.
436
434
509
464
424
405
557
456
464
418
b
n.a.
447
407
427
404
451
419
n.a.
421
n.a.
n.a.
408
400
n.a.
423
394
n.a.
390
416
n.a.
458
474
412
407
n.a.
403
497
469
453
480
n.a.

Figure 5

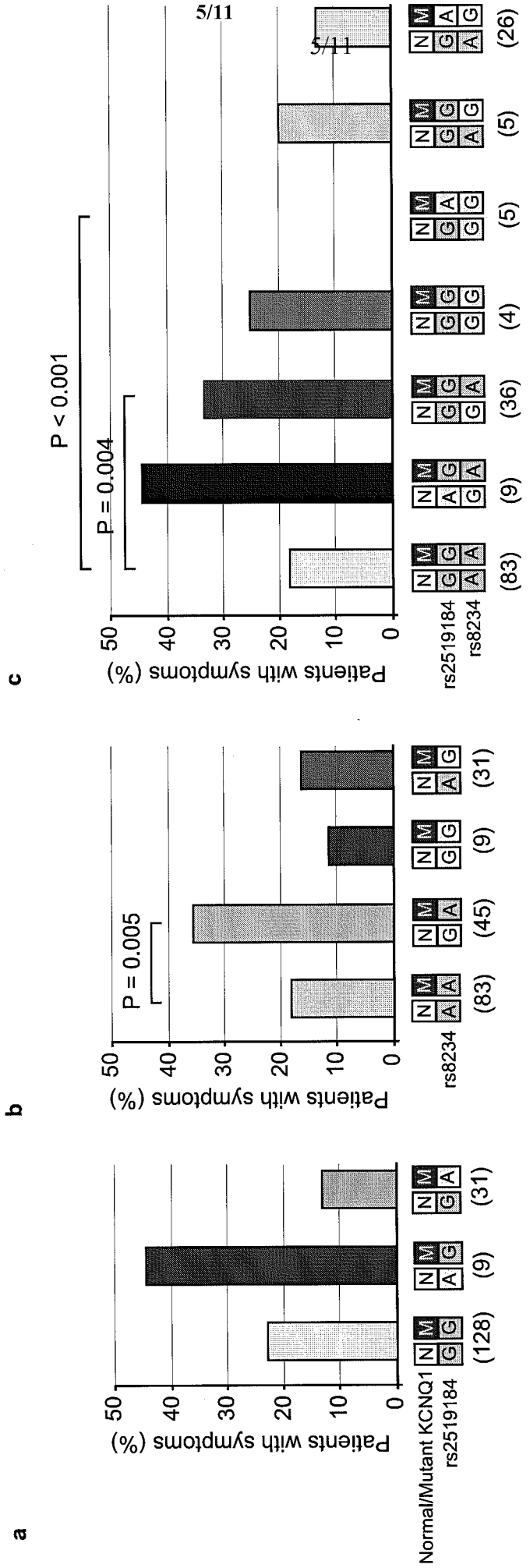

a
b
c
Patients with symptoms (%)
50
40
30
20
10
0
P = 0.005
P = 0.004
P < 0.001
Normal/Mutant KCNQ1
rs2519184
rs8234
(128)
(9)
(31)
(83)
(45)
(9)
(31)
(83)
(9)
(36)
(4)
(5)
(5)
(26)
5/11
5/11

Figure 6

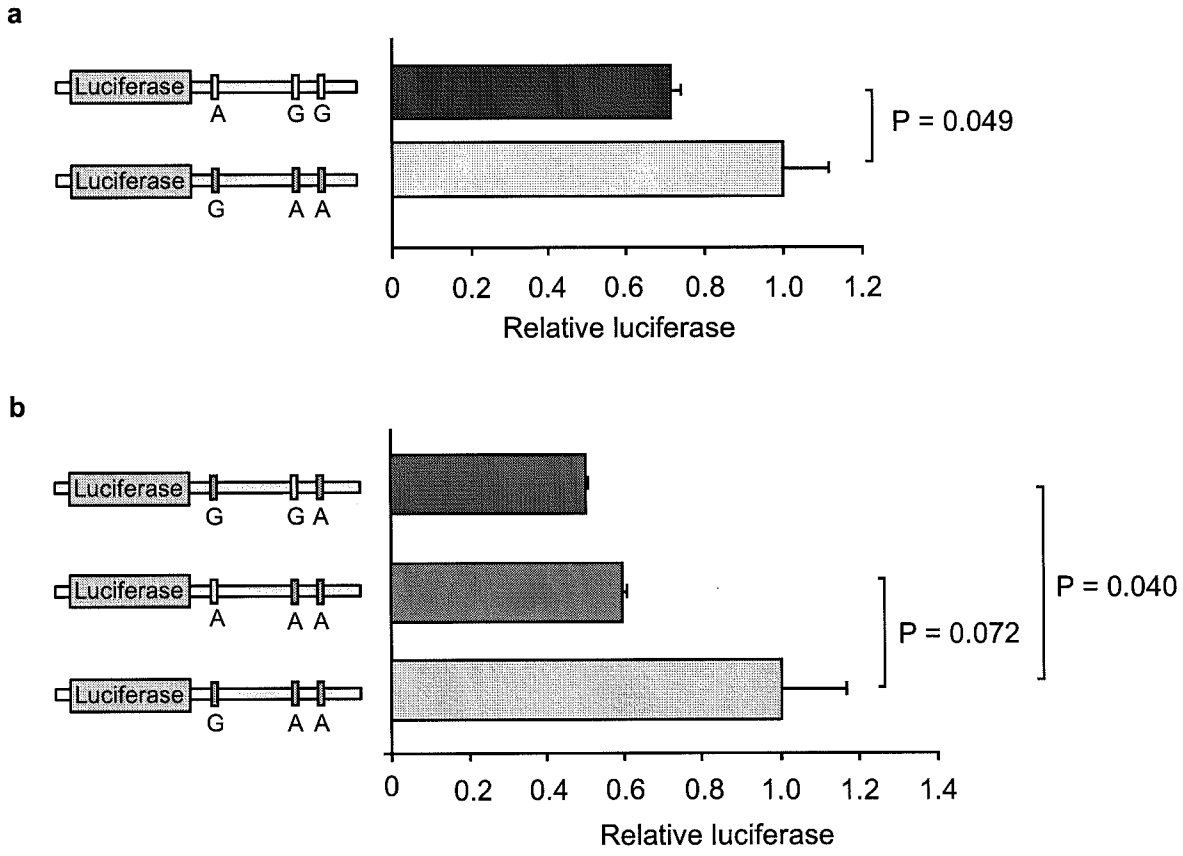

a
Luciferase
A
G G
Luciferase
G
A A
P = 0.049
0
0.2
0.4
0.6
0.8
1.0
1.2
Relative luciferase
b
Luciferase
G
G A
Luciferase
A
A A
Luciferase
G
A A
P = 0.072
P = 0.040
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
Relative luciferase

Figure 7

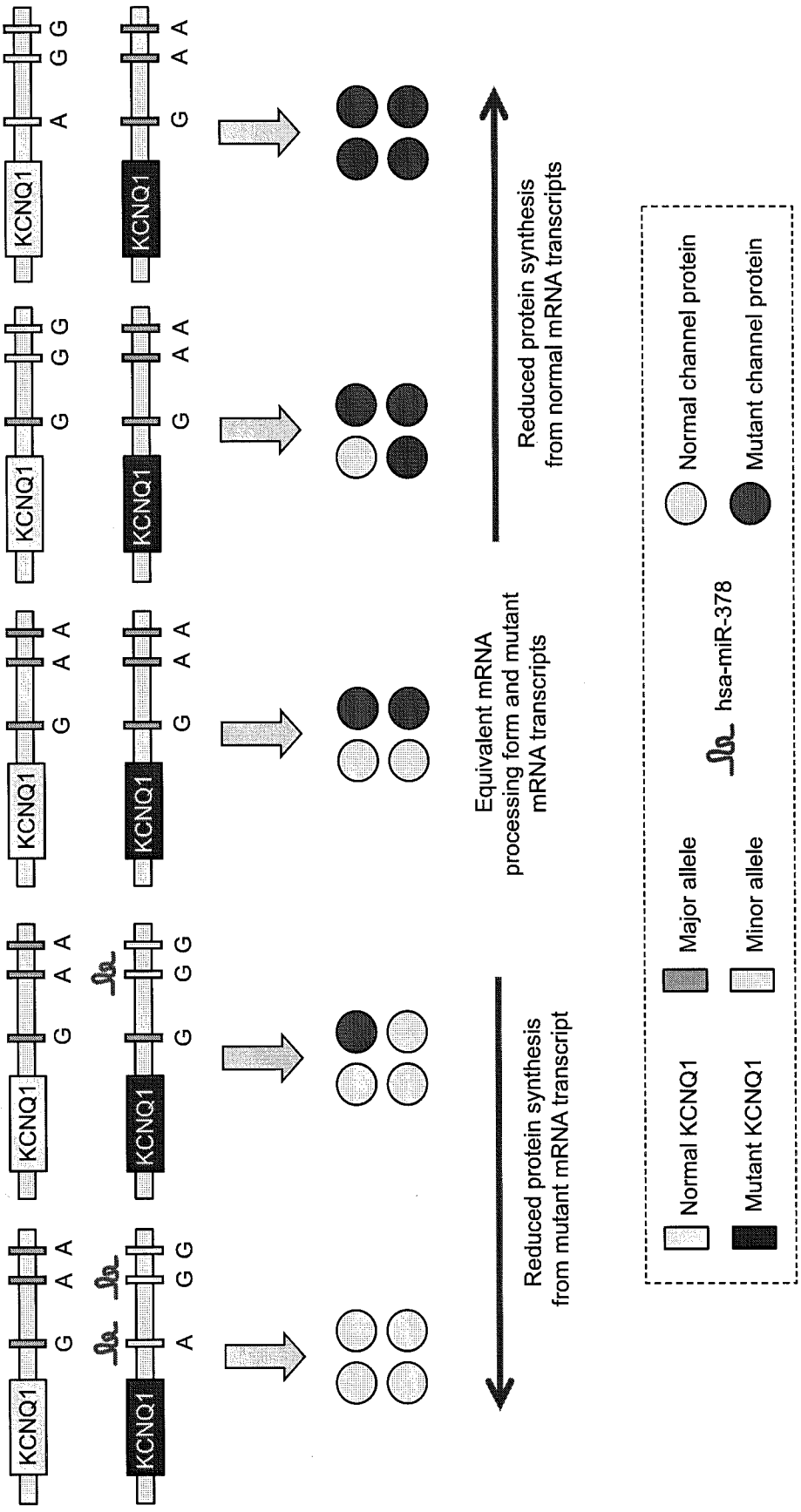

KCNQ1
G A A
A G G
G G G
G A A
Reduced protein synthesis from mutant mRNA transcript
Equivalent mRNA processing form and mutant mRNA transcripts
Reduced protein synthesis from normal mRNA transcripts
Normal KCNQ1
Mutant KCNQ1
Major allele
Minor allele
hsa-miR-378
Normal channel protein
Mutant channel protein

Figure 8

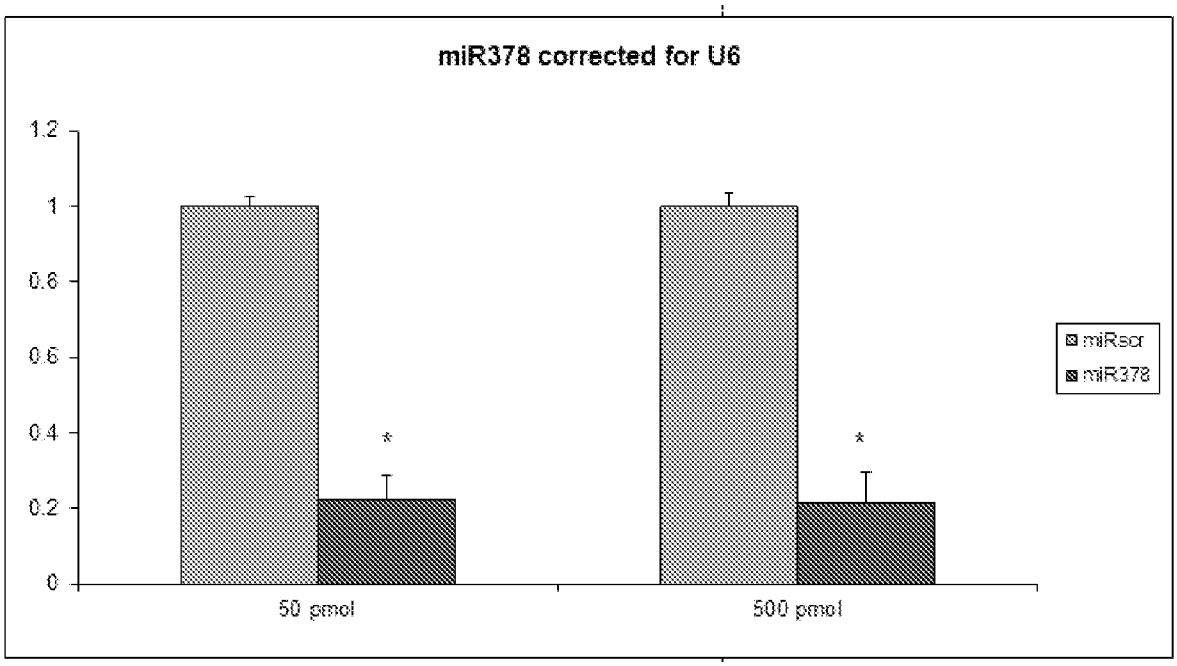

miR378 corrected for U6
1.2
1
0.8
0.6
0.4
0.2
0
*
*
50 pmol
500 pmol
miRscr
miR378

Figure 9

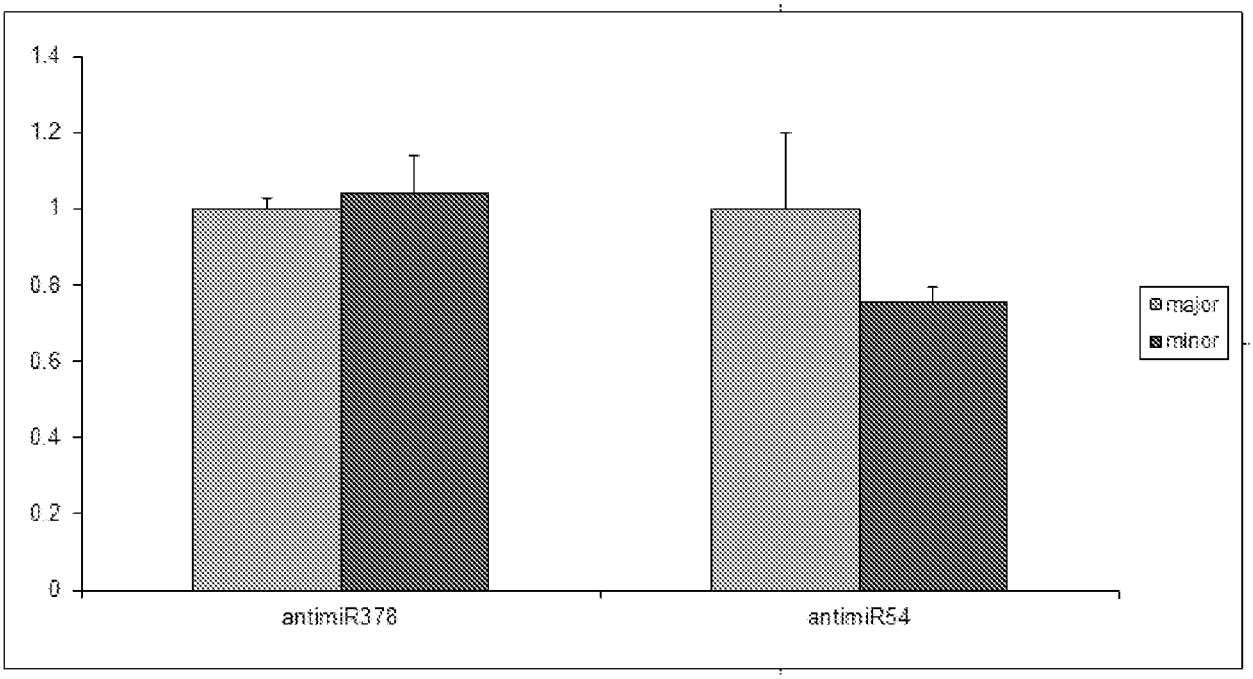


Figure 10

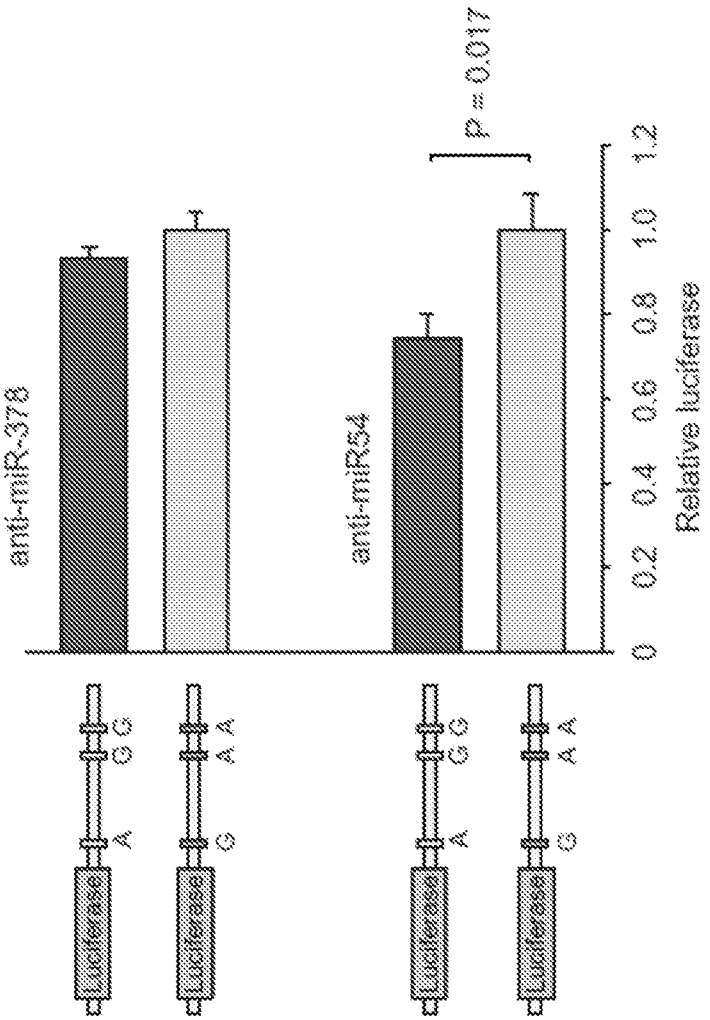
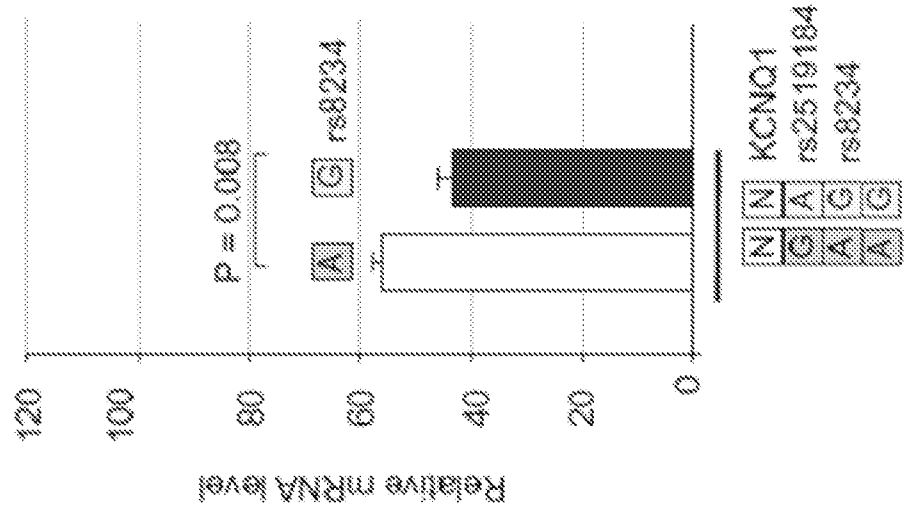

anti-miR-378
anti-miR54
P = 0.017
0
0.2
0.4
0.6
0.8
1.0
1.2
Relative luciferase
Luciferase
C C
A
Luciferase
A A
G
Luciferase
C C
A
Luciferase
A A
G

Figure 11


Relative mRNA level
120
100
80
60
40
20
0
P = 0.008
A
G
rs8234
KCNQ1
rs2519184
rs8234
N N A G G
N C A G A