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Molecular pathological study on LRRC10 in sudden unexplained nocturnal death syndrome in the Chinese Han population

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Abstract

Sudden unexplained nocturnal death syndrome (SUNDS) is a perplexing disorder to both forensic pathologists and clinic physicians. Clinical features of SUNDS survivors suggested that SUNDS is similar to Brugada syndrome (BrS). Leucine-rich repeat containing 10 (*LRRC10*) gene was a newly identified gene linked to dilated cardiomyopathy, a disease associated with sudden cardiac death. To investigate the prevalence and spectrum of genetic variants of *LRRC10* gene in SUNDS and BrS, the coding regions of LRRC10 were genetically screened in 113 sporadic SUNDS victims (from January 2005 to December 2015, 30.7 ± 7.5 years) and ten BrS patients (during January 2010 to December 2014, 38.7 ± 10.3 years) using direct Sanger sequencing. Afterwards, LRRC10 missense variant carriers were screened for a panel of 80 genes known to be associated with inherited cardiac arrhythmia/cardiomyopathy using target-captured next-generation sequencing. In this study, an in silico-predicted malignant LRRC10 mutation p.E129K was detected in one SUNDS victim without pathogenic rare variant in a panel of 80 arrhythmia/cardiomyopathy-related genes. We also provided evidence to show that rare variant p.P69L might contribute to the genetic cause for one SUNDS victim and two BrS family members. This is the first report of genetic screening of LRRC10 in Chinese SUNDS victims and BrS patients. *LRRC10* may be a new susceptible gene for SUNDS, and LRRC10 variant was initially and genetically linked to BrS-associated arrhythmia.

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Compliance with ethical standards The patients or legal representatives of the victims gave written informed consent, and the principles outlined in the Declaration of Helsinki were followed. The project was approved for human study by the Ethics Committee of Sun Yat-sen University.

Conflict of interest The authors declare that they have no conflict of interest.

Keywords

Forensic pathology; Sudden cardiac death; Genetics; Leucine-rich repeat containing 10 (*LRRC10*) gene

Introduction

Sudden unexplained nocturnal death syndrome (SUNDS), also called Lai Tai (died during sleep) in Thailand [1], Pokkuri death syndrome (sudden unexplained death at night) in Japan [2], and Bangungut (moaning and dying during sleep) in the Philippines [3], is most prevalent in Southeast Asia and is characterized by sudden unexplained death during sleep or at rest in apparently healthy young people, most of whom are males [4]. Forensic autopsy, histopathology examination, toxicological analysis, and death-scene investigation have no identifiable abnormalities to explain the cause of death. In southern China, the annual incidence of SUNDS is as high as 2 per 100,000 person-years [4].

Clinical phenotype and functional characterization of SUNDS survivors suggest that some, but not all, SUNDS are most likely the same allelic disorder as Brugada syndrome (BrS), affecting younger males with structurally normal hearts [1, 5]. Similarly, our molecular autopsy of the BrS-associated genes (*SCN5A*, *SCN1B-4B*, *SCN10A*, *MOG1*, and *GPD1-L*) could only account for a small part of the genetic cause of SUNDS victims [6, 7], indicating that a majority of SUNDS may be due to other genetic bases.

Leucine-rich repeat containing 10 (LRRC10) is a cardiac-specific and highly conserved protein exclusively expressed in cardiomyocytes [8, 9].Deletion of LRRC10 in mice results in dilated cardiomyopathy (DCM) [9], and mutations in *LRRC10* gene have been recently linked to human DCM [10]. Because mutations causing cardiomyopathy (such as arrhythmogenic right ventricular cardiomyopathy (ARVC) and DCM) have been shown to cause arrhythmogenic heart disease without overt structural defects (such as BrS), and vice versa. For example, *PKP2* (gene related to ARVC) missense mutations that cause sodium current deficit could yield a BrS phenotype, even in the absence of overt structural feature characteristic of ARVC [11], and *SCN5A* (gene linked to BrS) missense mutations have been reported in DCM patients [12, 13]. Moreover, LRRC10 has been recognized as a cardiac-specific transcriptional target gene of Nkx2.5 [14], which transcriptionally regulates cardiac ion channels, including Nav1.5, Cav1.2, and ERG [15]. Dysfunction of these cardiac ion channels are all associated with BrS and even sudden cardiac death [16–21]. Here, we tested the hypothesis that genetic variants of *LRRC10* may underlie some cases of SUNDS and BrS.

Materials and methods

Study population

From January 2005 to December 2015, 113 sporadic SUNDS cases were enrolled at the Department of Forensic Pathology, Zhongshan School of Medicine, Sun Yat-sen University, in this study. The inclusion criteria for SUNDS are as follows [6]: (1) a southern Han Chinese (most of whom are male) older than or equal to 15 years of age who was (2) healthy

without any significant disease (3) prior to experiencing a sudden unexplained death during sleep or at rest and (4) had a negative forensic autopsy, toxicological analysis, and death-scene investigation, resulting in an unexplained death.

Ten BrS patients collected during January 2010 to December 2014 from the Department of Cardiology at the First Affiliated Hospital of Sun Yat-sen University were included. The inclusion criteria for BrS in this study were patients with (1) a basal ECG showing a BrS type I pattern, (2) at least one clinical criterion (documented family history of sudden cardiac death or BrS, and/or symptoms secondary to arrhythmia), and (3) no structural heart disease.

A control population of 220 age- and ethnic-matched unrelated healthy southern Chinese (440 alleles) were provided by the First Affiliated Hospital of Sun Yat-sen University. None of the control subjects had a history of syncope or cardiovascular disease. Genomic data of East Asian (EAS) from the 1000 Genomes Project Phase 3 (1000G, http://browser.1000genomes.org/) and Exome Aggregation Consortium (ExAC, http://exac.broadinstitute.org/) were also used as a population control.

Genomic DNA extraction

Genomic DNA was extracted from blood samples using DNA IQ™ Casework Pro Kit for Maxwell® 16 (Promega Corporation, Madison, WI, USA), according to standard procedures.

Genetic screening

Genetic screening of the *LRRC10* gene (Ensembl Transcript ID: ENST00000361484) was performed on all SUNDS cases and BrS patients using direct Sanger sequencing. PCR products were then direct sequenced on a 3730XL DNA Analyzer with the use of BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). Sequence chromatograms were analyzed by Sequencing Analysis 5.2 software (Applied Biosystems, Foster City, CA, USA). To investigate the importance of missense variants in *LRRC10* gene, a total of 80 genes associated with inherited cardiac arrhythmia/cardiomyopathy were genetically screened in LRRC10 missense variant carriers using target-captured next-generation sequencing, and then the identified variants were filtered for candidate pathogenic rare variants and confirmed by direct Sanger sequencing as we previously described [22].

Genetic variant analysis

The sequences were compared with the corresponding reference cDNA sequence of the *LRRC10* gene using SeqMan™I I exper t sequence analysis software (DNASTAR, Inc., Madison, WI, USA). All suspicious variants were sequenced in both sense and antisense directions. Any variant observed in only SUNDS cases or BrS patients and absent in EAS population from 1000G and ExAC was termed as a mutation. Variant identified in EAS population was annotated as a polymorphism. Polymorphism identified with a minor allele frequency (MAF) less than 0.01 was termed as a rare polymorphism. If the MAF were higher than 0.01, the polymorphism was regarded as a common polymorphism.

Bioinformatic analyses

PROVEAN (http://provean.jcvi.org/index.php) and PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) were used to evaluate the impact of amino acid substitutions on the structure and function of LRRC10. Evolutionary conservation of P69 and E129 residues of LRRC10 was generated by MUSCLE [23] among different species.

Statistical analysis

MAF were calculated for each SNP site by the allele counting method using the data from direct Sanger sequencing of SUNDS, control, 1000G, and ExAC.

Results

Demographics of study population

The average death age of 113 unrelated SUNDS victims (three cases were females) was 30.7 ± 7.5 years (range 15–50 years). There was no clinic record for all these apparently healthy SUNDS cases. After comprehensive forensic pathological examination, there were no significant morphological changes found to explain the sudden death of SUNDS victims.

All but two of the ten BrS cases were unrelated males; the related cases were from the same family (father and son). The average age at the time of diagnosis was 38.7 ± 10.3 years (range 25–58 years). Seven of ten BrS had suffered previous syncope and seizures. Six of ten BrS cases had suffered an episode of spontaneous ventricular fibrillation and had an implantable cardioverter defibrillator implanted. Eight of ten BrS had a previous family history of sudden unexpected death or BrS. The blood sample and detailed clinic information of family member of all 113 SUNDS and eight BrS cases were not available.

Genetic variants of *LRRC10* in SUNDS and BrS

Four *LRRC10* genetic variants were found in SUNDS and BrS, including two missense variants, one noncoding region variant, and one synonymous variant (Table 1). The details of these variants are as follows (Fig. S1 in Electronic Supplementary Material online): c.−2G>T was a G to T transition at 5′ UTR of *LRRC10* gene and was identified in 1 of 88 SUNDS victims; c.206C>T was a C to T transition at the leucine-rich repeat 1 (LRR1) of LRRC10 leading to the missense exchange of a proline to a leucine residue (p.P69L) and was detected in 1 of 88 SUNDS cases and 2 of 10 BrS patients (Fig. 1); c.385G>A was a G to A transition at the LRR4 of LRRC10 leading to the missense exchange of a glutamic acid to a lysine residue (p.E129K) and was found in 1 of 113 SUNDS victims (Fig. 1); and c.453C>T was a synonymous variant (p.A151A) within the LRR5 of LRRC10 and was identified in 2 of 113 SUNDS victims.

Of these two missense variants, functional prediction by PROVEAN indicated that p.P69L may affect the function of LRRC10, which was not consistent with PolyPhen-2 (Table 1). p.E129K was predicted to be pathogenic by both PROVEAN and PolyPhen-2 (Table 1). Both P69 and E129 are evolutionarily conserved among different species (Fig. S2 in Electronic Supplementary Material online).

Target-captured next-generation sequencing of LRRC10 missense variant carriers

p.P69L was detected in one SUNDS and two BrS (Table 1). Of note, these two BrS patients (father and son) are from the same family. p.E129K was found only in one SUNDS victims (Table 1). To test the importance of p.P69L and p.E129K, the above carriers of LRRC10 missense variants were genetically screened using target-captured next-generation sequencing for pathogenic rare variants that might be involved in SUNDS and BrS.

In two BrS family members with p.P69L, only two genetic variants were identified among these candidate genes (Table 2). The first variant c.667C>G was a C to G transition in *SCN1B* gene leading to the missense exchange of a histidine to an aspartic acid residue (p.H223D) and it was detected in both father and son. The second variant c.536A>G was an A to G transition in *KCNJ5* gene leading to the missense exchange of a asparagine to a serine residue (p.N179S) and it was only detected in the son. Functional prediction in silico suggested that p.H223D may not alter the function of SCN1B, while p.N179S was predicted to alter the function of KCNJ5 (Table 2). The results suggested that these variants were unlikely underlying BrS. Moreover, there was no pathogenic rare variant found in SUNDS cases with p.P69L or p.E129K in all candidate genes.

Minor allele frequency of *LRRC10* genetic variants

Here, the MAF of four genetic variants in SUNDS were compared with our control and the population data from 1000G and ExAC (Table 3). c.−2G>Twas detected in 1 of 88 SUNDS victims (MAF = 0.00568) while it was either absent in our control, EAS, South Asian (SAS), African, and Latino population or rare in European (EUR) population (1 of 17,998, MAF = 0.00003). c.385G>A was identified in 1 of 113 SUNDS victims (MAF = 0.00442) while it was either absent in our control, EAS, EUR, African, and Latino population or detected in 1 (with unknown clinic phenotype) of 8241 SAS population (MAF = 0.00006). c. 206C>T was detected in 1 of 88 SUNDS victims (MAF = 0.00568) and 2 of 10 BrS patients. Its MAF was less than 0.01 in our control, EAS, EUR, and Latino but higher than 0.01 in SAS population. MAF of c.453C>T was 0.00885 (1 of 113) in SUNDS victims and higher than 0.01 in our control, EAS population. In contrast, the MAF of this variant was less than 0.01 in EUR and Latino population. Taken together, these four genetic variants were termed as mutations (c.−2G>T and c.385G>A), rare variant (c.206C>T), and common polymorphism (c.453C>T) in EAS population.

Discussion

LRRC10 is a cardiac-specific and highly conserved protein in embryonic and adult cardiomyocytes [8, 9]. Knockdown of LRRC10 caused severe cardiac morphological and functional defects [24]. Human LRRC10 contains no known functional domains other than its seven leucine-rich repeat (LRR) motifs (LRR1–7) [8], which function as protein interaction domains [25, 26]. LRRC10 can physically and endogenously interact with α-actinin and α-actin in the heart [9], forming a cytoskeletal complex possibly through its LRR motifs. Mutations in α-actinin and α-actin have been linked to human idiopathic DCM [27, 28]. In mice, deletion of LRRC10 results in DCM [9]. Most recently, the identification of rare variants of *LRRC10* in unrelated idiopathic DCM patients confirmed *LRRC10* as a

novel DCM-susceptibility gene [10]. LRRC10 is also needed to maintain cardiac function in response to pressure overload [29]. Taken together, LRRC10 is necessary for normal cardiac development and function.

Does LRRC10 also play role in maintaining normal cardiac rhythm? LRRC10 has been recognized as a cardiac-specific transcriptional target gene of Nkx2.5 [14], which regulates cardiac ion channels, including Nav1.5 (encoded by *SCN5A* gene), Cav1.2 (encoded by *CACNA1C* gene), and ERG (encoded by *KCNH2* gene) [15]. Dysfunction of these cardiac ion channels are all linked to BrS and even sudden cardiac death [16–21]. Based on these facts, we propose that LRRC10 may be associated with cardiac ion channel disease. Here, we identified two rare *LRRC10* missense variants in SUNDS and BrS, which linked LRRC10 to primary arrhythmia-associated diseases for the first time.

c.385G>A (p.E129K) was detected in one SUNDS and absent in EAS population and, herein, was annotated as a mutation in EAS population. p.E129K was found in a 26-year-old male who died suddenly during sleep with moaning, which was confirmed by his family member. The main features of the heart of this case were heart weight 320 g, left ventricular wall 1.1 cm thick, right ventricular wall 0.2 cm thick, and no identifiable abnormalities (including luminal narrowing and coronary atherosclerosis) in coronary arteries and cardiac electrical conduction system. Forensic autopsy and histopathology examination of this case revealed a structurally normal heart. Additional toxicological analysis and death-scene investigation provided no apparent abnormalities to explain the cause of death, supporting the forensic pathologic diagnosis of SUNDS. Functional prediction in silico by PROVEAN and PolyPhen-2 suggest that this mutation is deleterious to the function of LRRC10, which was also confirmed by Mutation Taster (http://www.mutationtaster.org/) and SIFT (http://sift.jcvi.org/). Additionally, the altered amino acid of p.E129K is conserved evolutionarily among different species in LRR4 motif, which function as protein interaction domain. What is more, we found no reported or in silico-predicted pathogenic rare variants in candidate genes in this p.E129K carrier. Although we cannot exclude the possibility that pathogenic mutation(s) in genes not included in this screen may be the genetic cause of this SUNDS case, our analysis strongly suggests the pathogenic potential of p. E129K, which mechanism warrants further investigation.

c.206C>T (p.P69L) was detected not only in one SUNDS (MAF < 0.01) and two BrS but also in EAS population; therefore, it was termed as a rare variant in EAS population. p.P69L was found in a 37-year-old male who died suddenly during sleep, which was confirmed by his wife. The main features of the heart of this case were heart weight 360 g, left ventricular wall 1.3 cm thick, right ventricular wall 0.3 cm thick, and no identifiable abnormalities (including luminal narrowing and coronary atherosclerosis) in coronary arteries and cardiac electrical conduction system. Forensic autopsy, microscopic examination, toxicological analysis, and death-scene investigation all support the forensic pathologic diagnosis of SUNDS. Functional prediction by PROVEAN indicated that p.P69L may affect the function of LRRC10, which was not consistent with PolyPhen-2. Although in silico prediction produced inconsistent results, we cannot exclude the potentially deleterious effect of this p.P69L variant for (1) this variant is absent in our control (440 alleles) and rare in EAS population (MAF < 0.01), (2) there is no pathogenic rare variant found in candidate genes in

this p.P69L carrier, (3) the altered amino acid of p.P69L is completely conserved evolutionarily among different species in LRR1 motif, which function as protein interaction domain, and (4) there is no cellular electrophysiological evidence showing p.P69L exerts a benign effect.

Furthermore, p.P69L was also found in two BrS family members (father and son) with a history of syncope and implantable cardioverter defibrillator implanted. Target-captured next-generation sequencing in these two BrS family members identified one genetic variant each in *SCN1B* and *KCNJ5*, which are genes associated with BrS and long QT syndrome (LQTS), respectively. However, our analysis suggested that neither variant could be the cause of BrS in these patients: p.H223D in *SCN1B* was predicted to be benign by in silico analysis. On the other hand, although p.N179S may alter the function of KCNJ5, it was found only in the son. Moreover, *KCNJ5* is a LQTS but not BrS susceptible gene. Combining the finding in SUNDS, p.P69L was evaluated to be likely contributed to one SUNDS and two BrS family members. The likely pathogenic potential and mechanism of p.P69L warrants further investigation.

In present study, we identified mutation p.E129K in 1 of 113 SUNDS, revealing the prevalence of mutation was approximately 0.89%. Rare polymorphism p.P69L was detected in 1 of 88 SUNDS with prevalence of 1.14% and two BrS family members. Besides p.P69L in two BrS family members, missense variants (p.E129K and p.P69L) with prevalence around 1% in SUNDS cases, revealing *LRRC10* gene variant may contribute to the genetic cause of some SUNDS victims and BrS patients. The electrophysiological study of these plausible pathogenic variants and the genetic studies with larger SUNDS cases and healthy controls are in the process by our group.

For forensic pathological investigation, forensic autopsy, histopathology examination, toxicological analysis, and death-scene investigation are all extremely important to establish the cohort of SUNDS. Unfortunately, absence of clinical data and family disease history often made it difficult to explain the cause of death. With the development of genomics, molecular autopsy using Sanger direct sequencing or with next-generation sequencing can be a very powerful technique in forensic pathological investigation of sudden unexplained death with negative autopsy, including SUNDS. Reasonably, a combination of current forensic pathological investigation, molecular autopsy, and cautious forensic pathologic evaluation could provide a reliable forensic diagnosis for SUNDS. Thus, molecular autopsy should be involved in all SUNDS cases as a routine postmortem genetic testing, and the victim’s or survivor’s genetic information could help the family members or survivors to prevent sudden cardiac death.

Conclusion

In summary, we identified p.E129K might be a potential pathogenic mutation underlying the death of one SUNDS. We also provide evidence to show that rare variant in East Asia, p.P69L, might contribute to the genetic cause for one SUNDS and BrS for two family members. This is the first report of genetic screening of LRRC10 in Chinese SUNDS and BrS. Our investigation implies that *LRRC10* may be a new susceptible gene for SUNDS and

genetically links LRRC10 variants to primary arrhythmia-associated BrS and SUNDS. We are currently carrying out electrophysiological study to test the functional impact of these putative pathogenic mutations of LRRC10 and confirm and support their role as pathogenic variants in Chinese SUNDS and BrS.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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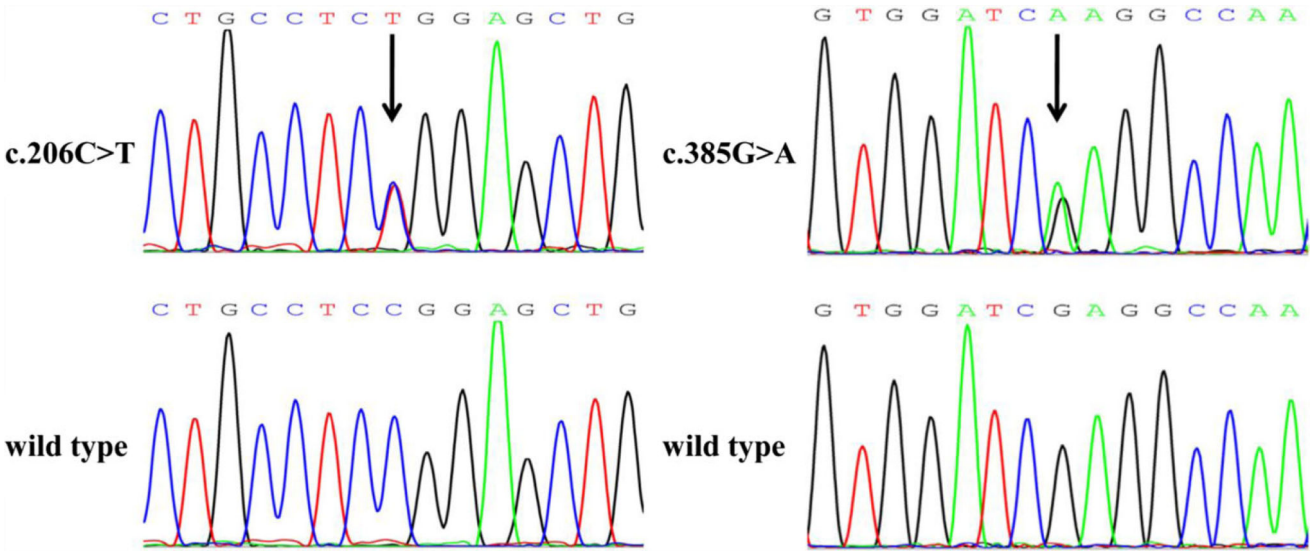

Fig. 1.

Sequence electropherogram of missense variants in *LRRC10* gene. Numbering of the nucleotide starts at the ATG codon. Genetic variants are indicated by an *arrow*

Table 1

Genetic variants of *LRRC10* gene in southern Han Chinese sporadic SUNDS cases and BrS patients

Region	Nucleotide change[a]	Amino acid change	Disease	Cases harboring variants/all[b]	dbSNP	Tape of variant	Position	PROVEAN	PolyPhen-2
5′ UTR	c.−2G>T	Noncoding	SUNDS	1/88	rs762951776	Noncoding	–	Unavailable	Unavailable
Exon	c.206C>T	p.Pro69Leu (p.P69L)	SUNDS	1/88	rs140389574	Missense	LRR1	Deleterious	Benign
Exon	c.206C>T	p.Pro69Leu (p.P69L)	BrS	2/10	rs140389574	Missense	LRR1	Deleterious	Benign
Exon	c.385G>A	p.Glu129Lys (p.E129K)	SUNDS	1/113	rs762144273	Missense	LRR4	Deleterious	Probably damaging
Exon	c.453C>T	p.Ala151Ala (p.A151A)	SUNDS	2/113	rs12229290	Synonymous	LRR5	Neutral	Unavailable

LRR leucine-rich repeat

[a] Nucleotide number relative to the translation start site

[b] Due to the quality and quantity limit of the SUNDS samples, the sample size of each site that can be successfully detected is different

Table 2

Genetic variants in two p.P69L BrS family members

Patient	Age	Gene	Gene-associated disease	Nucleotide change[a]	Amino acid change	dbSNP	Tape of variant	PROVEAN	PolyPhen-2
Father	58 years	SCN1B	BrS	c.667C>G	p.His223Asp (p.H223D)	rs754870200	Missense	Neutral	Benign
Son	40 years	SCN1B	BrS	c.667C>G	p.His223Asp (p.H223D)	rs754870200	Missense	Neutral	Benign
		KCNJ5	LQTS	c.536A>G	p.Asn179Ser (p.N179S)	rs147070381	Missense	Deleterious	Probably damaging

[a]Nucleotide number relative to the translation start site

Table 3

Minor allele frequency of genetic variants in *LRRC10* gene

Nucleotide change	MAF (M/m) SUNDS	Control	EAS-CHS[a]	EAS[a]	EAS[b]	SAS[a]	SAS[b]
Mutations in EAS							
c.−2G>T (rs762951776)	0.00568 (175/1)	0.00000 (440/0)	Undetected	Undetected	0.00000 (4028/0)	Undetected	0.00000 (6256/0)
c.385G>A (rs762144273)	0.00442 (225/1)	0.00000 (440/0)	Undetected	Undetected	0.00000 (8640/0)	Undetected	0.00006 (16,481/1)
Rare polymorphism in EAS							
c.206C>T (rs140389574)	0.00568 (175/1)	0.00000 (440/0)	0.00000 (210/0)	0.00000 (1008/0)	0.00208 (8630/18)	0.02249 (956/22)	0.01836 (16,197/303)
Common polymorphism in EAS							
c.453C>T (rs12229290)	0.00885 (224/2)	0.02045 (431/9)	0.01905 (206/4)	0.02778 (980/28)	0.02953 (8379/255)	0.00000 (978/0)	0.00000 (16,470/0)
Nucleotide change	MAF (M/m) EUR[a]		EUR[b]	AFR[a]	AFR[b]	AMR[a]	Latino[b]
Mutations in EAS							
c.−2G>T (rs762951776)	Undetected		0.00003 (35,995/1)	Undetected	0.00000 (5452/0)	Undetected	0.00000 (4998/0)
c.385G>A (rs762144273)	Undetected		0.00000 (73,188/0)	Undetected	0.00000 (10,324/0)	Undetected	0.00000 (11,560/0)
Rare polymorphism in EAS							
c.206C>T (rs140389574)	0.00000 (1006/0)		0.00014 (73,176/10)	0.00000 (1322/0)	0.00000 (10,376/0)	0.00000 (694/0)	0.00009 (11,551/1)
Common polymorphism in EAS							
c.453C>T (rs12229290)	0.00000 (1006/0)		0.00001 (73,131/1)	0.00000 (1322/0)	0.00000 (10,284/0)	0.00000 (694/0)	0.00017 (11,548/2)

MAF minor allele frequency, *M* major allele, *m* minor allele

[a] 1000 Genomes Project Phase 3

[b] ExAC