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A rare variant in *MYH6* is associated with high risk of sick sinus syndrome

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Abstract

Through complementary application of SNP genotyping, whole-genome sequencing and imputation in 38,384 Icelanders, we have discovered a previously unidentified sick sinus syndrome susceptibility gene, *MYH6*, encoding the alpha heavy chain subunit of cardiac myosin. A missense variant in this gene, c.2161C>T, results in the conceptual amino acid substitution p.Arg721Trp, has an allelic frequency of 0.38% in Icelanders and associates with sick sinus syndrome with an odds ratio = 1 2.53 and $P = 1.5 \times 10^{-29}$. We show that the lifetime risk of being diagnosed with sick sinus syndrome is around 6% for non-carriers of c.2161C>T but is approximately 50% for carriers of the c.2161C>T variant.

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Note: Supplementary information is available on the Nature Genetics website.

AUTHOR CONTRIBUTIONS

The study was designed and results interpreted by H. Holm, D.F.G., D.O.A., P.S., U.T. and K.S. O.M., J.S., A.J., A.S., G.B.W. and H. Helgadottir managed and contributed to sequencing and genotyping. Data alignment, imputation and statistical analysis was carried out by D.F.G., G.M., A.G., P.S., G. Thorleifsson and A.K. Additional analyses were performed by A.H. and C.Z. D.O.A., H. Holm, G. Thorgeirsson, S.E.M. and H.S. collected the Icelandic data. Foreign data was collected and supervised by H.S., T.W., T.R., L.A.K., B.P., R.M., D.M.R. and D.D. H. Holm, D.F.G., D.O.A., U.T. and K.S. wrote the first draft of the paper. All authors contributed to the final version of the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare competing financial interests: details accompany the full-text HTML version of the paper at http://www.nature.com/naturegenetics/.

Sick sinus syndrome (SSS), or sinus node dysfunction, is a common clinical disorder first described in 1968 (ref. 1) that is characterized by pathological sinus bradycardia (slow heart rate), sinus arrest and/or chronotropic incompetence (attenuated heart rate response to exercise). The syndrome comprises a wide range of electrophysiological abnormalities, including failure of the sinus node and atrial impulse formation or propagation, as well as susceptibility to atrial tachyarrhythmias, particularly atrial fibrillation2. Although encountered at any age, SSS is primarily a disease of the elderly and is often secondary to other cardiac disorders when diagnosed in younger individuals. Symptoms are often intermittent and/or nonspecific and include dizziness, syncope and heart failure. The only effective treatment for symptomatic and irreversible sinus node dysfunction is permanent cardiac pacing3, and SSS remains the most common indication for permanent pacemaker implantation4.

Several studies have uncovered gene mutations in sporadic cases and kindreds with familial SSS, both with and without other concomitant cardiac conditions, mainly through candidate gene approaches. Implicated genes have been ion channel or ion channel–associated genes, including *SCN5A* (ref. 5), *HCN4* (refs. 6,7) and *ANK2* (ref. 8). Similarly, knockout mouse models of various ion-channel and gapjunction subunits have been shown to result in the SSS phenotype9.

With the aim of searching for sequence variants that predispose to SSS, we performed a genome-wide association study (GWAS) on 792 Icelandic individuals with SSS and 37,592 Icelandic population controls (Supplementary Table 1). All cases had received a diagnosis of SSS at Landspitali University Hospital (LUH) in Reykjavik, Iceland, the only tertiary care hospital in Iceland. Of the 792 SSS cases, 627 had undergone pacemaker implantation.

Initially, we tested a total of 7.2 million SNPs either directly genotyped with the Illumina HumanHap300 or CNV370 chips or imputed from one or more of four sources: the HapMap2 European CEU sample10 (60 triads), the 1000 Genomes Project data[11] (179 individuals) and Icelandic samples genotyped with the Illumina Human1 M-Duo (123 triads) or the HumanOmni1-Quad chips (505 individuals). Imputations were based on the IMPUTE model12 and long range phasing of chip-typed Icelandic samples13.

RESULTS

A rare missense variant in *MYH6* associates with sick sinus syndrome

The genome-wide association analysis yielded significant association between SSS and three correlated SNPs at chromosome 14q11 (between 22.40 Mb and 22.94 Mb in build 36; Supplementary Table 2): rs2231801 (imputed from the Human1M-Duo and HumanOmni1-Quad chips), rs28730774 (imputed from the HumanOmni1-Quad chip) and 14-22399934 (imputed from the 1000 Genomes Project) (most significant $P = 1.3 \times 10^{-13}$ for rs2231801). For all three SNPs, the imputed minor allele frequency was low (0.010–0.026), and the minor allele was the risk allele.

In an effort to search for additional signals associating with SSS and to explore the observed association at 14q11, we selected seven chip-typed SSS cases for whole-genome sequencing, four of whom carried the rs28730774[T] variant (Fig. 1). The selection was based on this variant rather than rs2231801 because its association with SSS was stronger in the imputations based on the HumanOmni1-Quad chips where both SNPs were genotyped (Supplementary Table 2). We also whole-genome sequenced another 80 chip-typed Icelanders not diagnosed with SSS (see Online Methods for selection criteria) for a total of 87 samples that we sequenced to a mean mapped sequencing depth of 10× (Supplementary Fig. 1). Based on these sequencing data, a total of ~11 million SNPs were called and

imputed into chip-typed cases and controls, applying the long range phased haplotypes, and tested for association with SSS (See Supplementary Table 3 for exact SNP count and overlap with SNPs from the 1000 Genome Project and dbSNP, Supplementary Table 4 for rediscovery rates of SNPs on the 300K Illumina chips and Supplementary Table 5 for whole-genome sequencing depth and chip genotype comparison per sample). We found no significant association outside the 14q11 region. In this region, the strongest association observed was with a missense mutation, c.2161C>T, in *MYH6*, a gene encoding the alpha heavy chain subunit of cardiac myosin (Fig. 2, and see Supplementary Fig. 2 for the sequencing depth over the 14q11 region). This mutation was present in all four sequenced SSS rs28730774[T] carrier cases but was absent from both the non-carrier cases and a single control carrier of rs28730774[T]. The c.2161C>T mutation is located in exon 18 of *MYH6* at position 22,936,019 bp (in build 36 assembly of the UCSC Genome Browser) and leads to a missense arginine to tryptophan alteration at amino acid 721, p.Arg721Trp. No significant association remained at 14q11 after accounting for the effect of c.2161C>T, and the association of c.2161C>T could not be accounted for by any other genotyped or imputed variant.

In order to validate the c.2161C>T mutation and to explore its relationship with rs28730774[T], we used Sanger sequencing to resequence exon 18 of *MYH6* in a set of 351 Icelanders (118 SSS cases and 233 controls) enriched for carriers of rs28730774[T]. We also genotyped the mutation directly with a Centaurus single SNP assay in the same sample set, and the two genotyping methods concurred for all 351 individuals. In this set, 82 individuals were called as heterozygous carriers of c.2161C>T and none was called as homozygous. To increase imputation accuracy, we subsequently genotyped an additional 523 chip-typed Icelanders, primarily controls (50 SSS cases and 473 controls), with the c.2161C>T Centaurus assay. Four of these individuals were called as heterozygous carriers for c. 2161C>T. Amongst the two sample sets combined, there were 130 carriers of rs28730774[T]. The c.2161C>T variant was only observed amongst carriers of rs28730774[T] and was found in 91% of SSS case carriers but only in 38% of control carriers ($P = 2.9 \times 10^{-9}$; Supplementary Table 6).

We combined the 87 whole-genome sequenced samples and the 874 Centaurus single-SNP assay and chip-genotyped individuals into a set of 952 individuals (five individuals overlapped and nine could not be phased accurately in the region around *MYH6*), of which 73 were carriers of the c.2161C>T mutation, to create a new reference set for imputation. Based on this imputation set, the association was stronger than when it was based on the initial imputation set. The c.2161C>T missense mutation in *MYH6* resulting in p.Arg721Trp (estimated allelic frequency in Iceland = 0.38%) associates with SSS with an odds ratio (OR) = 12.53 (95% CI 8.08–19.44) and a corresponding $P = 1.5 \times 10^{-29}$.

We genotyped c.2161C>T in a second set of 469 Icelandic SSS cases and 1,185 controls who were not chip genotyped and therefore unavailable for the haplotype-based imputation of c.2161C>T (Supplementary Tables 1,7). Among the SSS cases, we identified 20 heterozygous carriers of c.2161C>T (allelic frequency of 2.1%), whereas we observed only five heterozygous carriers among the controls (allelic frequency of 0.21%). This replicates the original association with SSS based on imputation (OR = 12.95, $P = 3.8 \times 10^{-5}$).

The c.2161C>T variant is neither present in the available 1000 Genomes Project data nor in the HapMap samples according to our own genotyping effort. We also tested for the presence of this variant in 184 Danish, 94 Dutch and 1,498 US controls as well as 135 US SSS cases without observing a single carrier of c.2161C>T.

The sibling recurrence risk ratio ($\lambda_{sibling}$) is a commonly used measure of the strength of familial aggregation. Based on the allelic frequency of c.2161C>T of 0.38% and an OR for SSS of 12.53, we estimated the $\lambda_{sibling}$ for SSS accounted for by c.2161C>T to be 1.52. This contrasts with most of the sequence variants that in recent years have been found to associate with common complex disorders and create $\lambda_{sibling}$ values that that are substantially less than 1.1. Despite the high OR, the c.2161C>T mutation is also distinct from most Mendelian disorder alterations because of its higher population frequency. There is, however, a striking similarity with the Icelandic c.999del5 *BRCA2* breast cancer mutation[14], which is almost exclusive to Iceland and which we estimate to have an allelic frequency of 0.43% (based on 20,635 males) and an OR of 11.4 (based on 2,466 female cases and 25,357 female controls) leading to a $\lambda_{sibling}$ of 1.47.

The 73 chip-typed individuals who were shown to carry the c.2161C>T mutation through direct genotyping all share a 26-SNP haplotype spanning 230 kb, or 0.51 cM. Using surrounding SNPs, we estimated the age of the introduction of the c.2161C>T mutation to Iceland, either by mutation or by immigration, to be 29 generations, or approximately 870 years[15,16].

Carriers of the c.2161C>T variant have a high lifetime risk of sick sinus syndrome

Based on data from the 38,384 chip-typed Icelanders, including both SSS cases and controls, the lifetime risk of being diagnosed with SSS is ~6% for non-carriers of c.2161C>T but is ~50% for carriers of the c.2161C>T mutation (Fig. 3). Notably, the chip-typed Icelanders were not selected for genotyping based on SSS or any other phenotype related to SSS and can therefore be used to assess the probability of developing SSS depending on genotype and age. The effect of c.2161C>T as estimated by our logistic model shows a trend toward increasing with age by a factor of 1.06 per 10 years ($P = 0.042$). We note that the cases and controls in our study are not age matched, but that the frequency of c.2161C>T is independent of year of birth (Supplementary Fig. 3; correlation $r = -0.0043$, $P = 0.41$).

To explore the relationship between SSS, genotype and syncope, a common but non-specific sign of SSS, we obtained a list of individuals who had received the discharge diagnosis of syncope (syncope, convulsions or dizziness; Online Methods) at the LUH between the years 1987 and 2010 either through a visit to the emergency room or hospital admission. For our analysis, we used data from the 916 individuals who had previously been chip-typed at deCODE (mean year of birth = 1929.7). We observed that amongst individuals with syncope, 50% of carriers ($N = 16$) of the c.2161C>T mutation had been diagnosed with SSS compared to 19% of the non-carriers ($N = 900$, $P = 0.0050$).

Association between the c.2161C>T variant in *MYH6* and heart rate

Although *MYH6* has not previously been associated with SSS or other arrhythmias in man, we recently established a link between a common variant in this gene and cardiac conduction. Through a large GWAS on electrocardiogram (ECG) measures in Icelanders[17], we discovered an association between a missense variant in exon 25 of *MYH6* (rs365990[G], p.Ala1101Val) and both heart rate and the PR interval. The G allele of rs365990 (frequency = 0.341) associates with a 0.91-beat decrease in heart rate per minute. The association between rs365990[G] and heart rate has since been replicated in a large meta-analysis including subjects of European ancestry from both the United States and Europe[18]. There is, however, not a significant association between this variant and SSS in our data. The c.2161C>T mutation occurs on the background of the G allele of rs365990, and when tested in our previously described ECG database17 (Supplementary Table 8), after exclusion of all cases with SSS and pacemakers, we found it to associate with a 4.16-beat decrease in heart rate per minute ($P = 0.0019$) and a prolongation of the PR interval (Table 1). The association

of c.2161C>T with lower heart rate in subjects who have not been diagnosed with SSS indicates a higher penetration of this mutation than suggested by purely assessing the association with the diagnosis of SSS, but with variation in expressivity.

Correlation between the c.2161C>T variant and other cardiac diseases

Given the fact that SSS is commonly associated with other cardiac diseases, particularly other conduction disorders, we tested for association between the c.2161C>T variant and several other cardiovascular diseases (Table 2). We analyzed Icelandic sample sets both before and after exclusion of known cases of SSS. Although these results can only be considered as suggestive, there was a residual association, after exclusion of SSS cases, with several diseases, including atrial fibrillation and thoracic aortic aneurysm.

DISCUSSION

Cardiac muscle myosin, along with actin, is one of the major components of the sarcomere, the building block of the contractile system of cardiac muscle[19]. Myosin is a hexamer consisting of two heavy chain subunits (alpha and beta), two light chain subunits and two regulatory subunits. *MYH6* encodes the alpha myosin heavy chain subunit (αMHC) that encompasses ~26,000 bp and consists of 39 exons, 37 of which contain coding information[20] (Supplementary Fig. 4). The beta myosin heavy chain subunit (βMHC), a relatively slow ATPase encoded by *MYH7*, is the heavy chain isoform predominantly expressed in human heart, with expression of αMHC, a fast ATPase, being primarily restricted to atrial tissue[21]. The two genes (*MYH6* and *MYH7*) are in a head-to-tail orientation on chromosome 14[22] and are regulated in an antithecal manner. In heart failure and other cardiac disorders in humans, βMHC is upregulated whereas αMHC is downregulated, resulting in diminution of cardiac performance[23], and it has been suggested that even minor shifts in the MHC composition of the cardiac muscle can markedly influence cardiac function. Mutations in *MYH7* are a well known cause of hypertrophic cardiomyopathy in man, and recently, mutations in *MYH6* were also linked to both cardiomyopathy (hypertrophic and dilated)[24] and a variety of congenital heart defects25.

The mutation resulting in p.Arg721Trp is in exon 18 that encodes part of the converter domain of αMHC (Supplementary Fig. 4). This domain functions as a socket for the C-terminal α-helical tail of the αMHC and plays a critical role in amplifying the structural rearrangements in the motor domain and transmitting them to the α-helical tail during movements of the myosin during contraction[26]. Based on PolyPhen[27], the p.Arg721Trp alteration is predicted to alter the structure of the converter. Whether and how this structural alteration may directly affect cardiac conduction is pending further structure-function analysis of the protein. Further, although the αMHC itself has not been directly linked to cardiac conduction, a cardiac-specific highly conserved microRNA, miR-208a, encoded by intron 27 of *MYH6* in both humans and mice, has recently been shown in mice to be necessary for maintenance of normal cardiac conduction[27]. Evidence suggests that miR-208a is required for expression of *Gja5* (also known as *Cx40*), which is of particular interest as mice lacking *Gja5* show cardiac conduction abnormalities, including both sinus node impulse formation and atrial propagation[28]–[30]. Thus, another potential functional consequence of the p.Arg721Trp alteration could be an effect on αMHC mRNA stability or processing, as previously documented for both synonymous and non-synonymous coding mutations[30]–[32], which would directly affect the amount of miR-208a present in the heart.

In summary, we have discovered a rare variant in a previously unidentified SSS susceptibility gene that confers high risk of the disease. The same variant has a substantial effect on heart rate in subjects who have not been diagnosed with SSS. We have shown that common variants in this gene modulate cardiac conduction affecting heart rate and the PR

interval. To the best of our knowledge, this was the first time that cardiac myosin, a key component of the cardiac contractile system, was directly linked to cardiac conduction in man. We have now established the importance of cardiac myosin in the development of cardiac arrhythmias.

In the last few years, GWAS have proven highly effective in unraveling the genetic architecture of complex human traits, yielding robust associations with hundreds of genomic variants. By design, the GWAS methods capture a large fraction of the common variants in the human genome. However, it has become apparent that despite expanded GWAS that capture common variants with both moderate and small effects, a substantial fraction of the heritability of most traits remains unaccounted for. The question has thus been raised of whether uncommon variants with substantial or large effects contribute to the genetic component of complex traits. This possibility is supported by a recent study of hypertriglyceridemia in which resequencing of four genes associated with the trait through GWAS suggested an interplay between common and rare variants in the genetic architecture of this specific phenotype[33]. Through complementary application of SNP genotyping, whole-genome sequencing and imputation in the study of SSS, we now provide further evidence for the role of rare variants in the development of common complex diseases.

URLs. Picard, http://picard.sourceforge.net/.

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturegenetics/.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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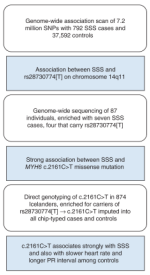

Figure 1.
Study design and outcomes. The white boxes describe study actions performed. The blue boxes describe results from preceding actions. SSS, sick sinus syndrome.

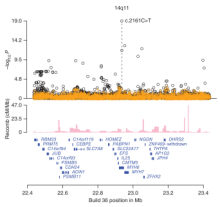

Figure 2.

An overview of the region around c.2161C>T. The black circles show $-\log_{10} P$ for association with sick sinus syndrome for imputed SNPs based on whole-genome sequencing as a function of their build 36 coordinates. The orange crosses show results conditional on the effect of c.2161C>T. Neighboring genes are shown in blue. Recombination rates are reported in cM/Mb.

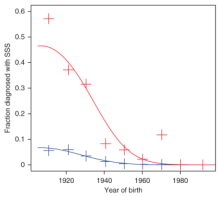

Figure 3.

Penetrance of sick sinus syndrome among carriers and non-carriers of c.2161C>T. The red crosses represent observed penetrance of sick sinus syndrome for 10-year birth cohorts among heterozygous carriers of c.2161C>T. The red line represents the fit of the logistic model to the c.2161C>T carrier data. The blue line and crosses represent the same information for non-carriers of c.2161C>T. SSS, sick sinus syndrome.

Table 1

Association of c.2161C>T and rs365990[G] with electrocardiogram measures

		c.2161C>T		rs365990[G]	
Phenotype	***N***	**Effect (95% CI)**	***P***	**Effect (95% CI)**	***P***
Chip typed individuals (imputed c.2161C>T data)					
Heart rate[a]	10,849	−5.46 (−8.68 to −2.24)	0.0023	−0.94 (−1.34 to −0.54)	2.4×10^{-5}
PR interval[b]	10,687	7.64 (2.72 to 12.55)	0.010	0.95 (0.34 to 1.56)	0.010
QRS duration[b]	10,849	−0.58 (−3.73 to 2.57)	0.75	−0.33 (−0.72 to 0.06)	0.15
QT interval[b]	10,849	−2.41 (−7.82 to 3.01)	0.42	−0.78 (−1.45 to −0.10)	0.038
Single-assay typed individuals					
Heart rate[a]	7,909	−2.48 (−6.08 to 1.13)	0.22	−0.95 (−1.41 to −0.50)	0.00016
PR interval[b]	7,909	6.94 (1.37 to 12.51)	0.039	0.93 (0.22 to 1.63)	0.029
QRS duration[b]	7,909	−0.45 (−3.94 to 3.05)	0.83	−0.05 (−0.50 to 0.39)	0.84
QT interval[b]	7,908	−0.62 (−7.02 to 5.78)	0.86	−1.76 (−2.57 to −0.95)	8.6×10^{-5}
Combined					
Heart rate[a]	18,758	−4.16 (−6.57 to −1.75)	0.0019	−0.96 (−1.26 to −0.66)	1.0×10^{-8}
PR interval[b]	18,596	7.32 (3.63 to 11.00)	0.0010	0.95 (0.48 to 1.41)	0.00069
QRS duration[b]	18,758	−0.53 (−2.88 to 1.81)	0.70	−0.21 (−0.51 to 0.08)	0.22
QT interval[b]	18,757	−1.65 (−5.78 to 2.49)	0.47	−1.18 (−1.70 to −0.67)	3.8×10^{-5}

[b] Intervals and durations in milliseconds. Joint estimates of the association of c.2161C>T and the previously published rs365990[G] with electrocardiogram measures. Individuals known to have sick sinus syndrome, permanent pacemaker and/or atrial fibrillation are excluded from this analysis. c.2161C>T occurs on the background of the G allele of rs365990.

[a] Heart rate in beats per minute.

Table 2

Association of c.2161C>T with several cardiovascular diseases

	Including known SSS cases				Excluding known SSS cases			
Phenotype	N_{cases}	$N_{controls}$	**OR (95% CI)**	***P***	N_{cases}	$N_{controls}$	**OR (95% CI)**	***P***
Sick sinus syndrome	776	36,955	12.53 (8.08–19.44)	1.5×10^{-29}	–	–	–	–
Pacemaker implantation	906	36,825	10.17 (6.56–15.77)	3.6×10^{-25}	295	36,660	1.35 (0.30–6.16)	0.7
Atrial fibrillation	2,318	35,413	2.39 (1.54–3.71)	0.0001	2,020	34,935	2.03 (1.15–3.58)	0.015
Atrioventricular block	254	37,477	2.80 (0.97–8.10)	0.057	177	36,778	1.17 (0.15–9.31)	0.88
Sudden cardiac death	327	37,404	1.63 (0.55–4.80)	0.38	293	36,662	2.50 (0.79–7.90)	0.12
Aortic stenosis	565	37,166	3.11 (1.51–6.38)	0.002	497	36,458	2.48 (0.93–6.60)	0.069
Thoracic aortic aneurysm	55	37,676	7.85 (1.68–36.66)	0.0088	53	36,902	11.68 (2.33–58.57)	0.0028
Abdominal aortic aneurysm	438	37,293	0.90 (2.90–0.28)	0.86	414	36,541	1.38 (0.39–4.80)	0.62
Heart failure	654	37,077	3.01 (1.52–5.95)	0.0016	563	36,392	1.51 (0.50–4.52)	0.46
Coronary artery disease	6,683	31,048	1.11 (0.78–1.58)	0.57	6,274	30,681	1.07 (0.71–1.62)	0.75

This table shows association between c.2161C>T and several cardiovascular diseases in Icelandic case-control sample sets, both including and excluding known cases of sick sinus syndrome (SSS). Results are based on logistic regression using sex, year of birth and year of birth squared as covariates.