

# Genetic insight into sick sinus syndrome

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## Aims

The aim of this study was to use human genetics to investigate the pathogenesis of sick sinus syndrome (SSS) and the role of risk factors in its development.

## Methods and results

We performed a genome-wide association study of 6469 SSS cases and 1 000 187 controls from deCODE genetics, the Copenhagen Hospital Biobank, UK Biobank, and the HUNT study. Variants at six loci associated with SSS, a reported missense variant in *MYH6*, known atrial fibrillation (AF)/electrocardiogram variants at *PITX2*, *ZFHX3*, *TTN1/CCDC141*, and

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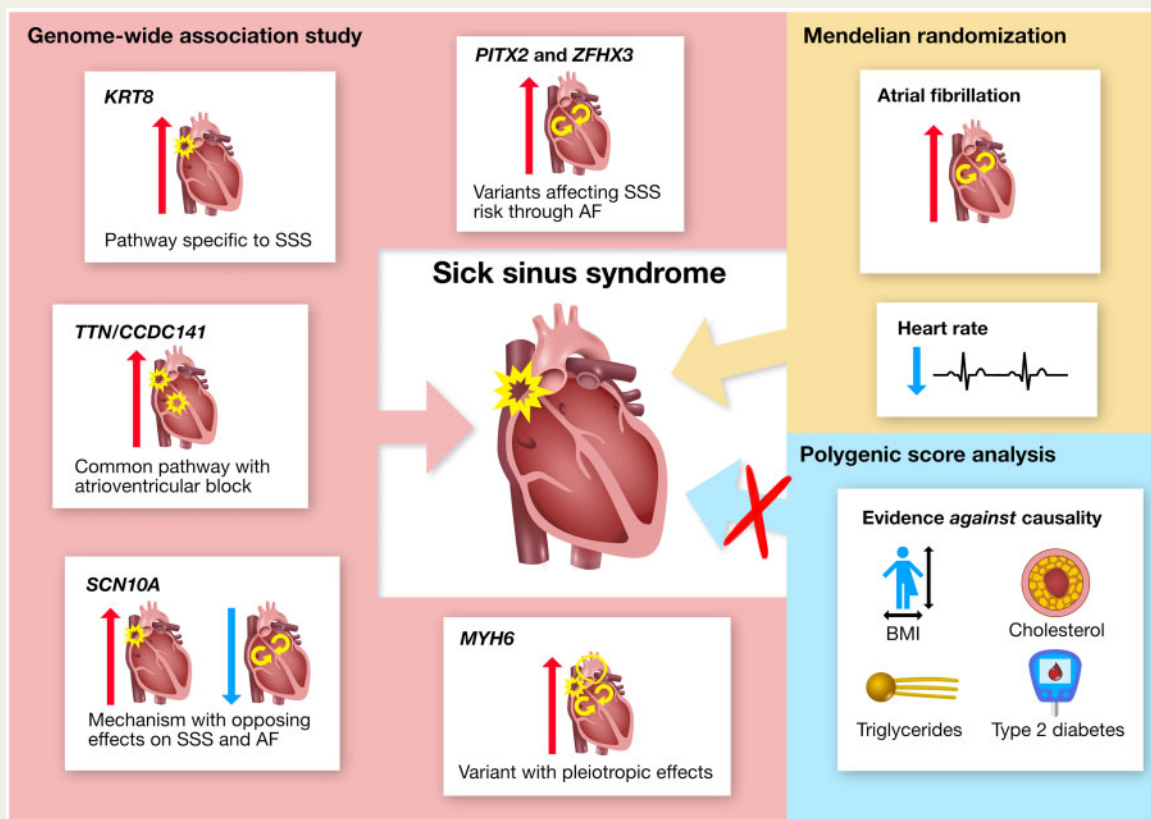
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*SCN10A* and a low-frequency (MAF = 1.1–1.8%) missense variant, p.Gly62Cys in *KRT8* encoding the intermediate filament protein keratin 8. A full genotypic model best described the p.Gly62Cys association ( $P = 1.6 \times 10^{-20}$ ), with an odds ratio (OR) of 1.44 for heterozygotes and a disproportionately large OR of 13.99 for homozygotes. All the SSS variants increased the risk of pacemaker implantation. Their association with AF varied and p.Gly62Cys was the only variant not associating with any other arrhythmia or cardiovascular disease. We tested 17 exposure phenotypes in polygenic score (PGS) and Mendelian randomization analyses. Only two associated with the risk of SSS in Mendelian randomization, AF, and lower heart rate, suggesting causality. Powerful PGS analyses provided convincing evidence against causal associations for body mass index, cholesterol, triglycerides, and type 2 diabetes ( $P > 0.05$ ).

## Conclusion

We report the associations of variants at six loci with SSS, including a missense variant in *KRT8* that confers high risk in homozygotes and points to a mechanism specific to SSS development. Mendelian randomization supports a causal role for AF in the development of SSS.

## Graphical Abstract



Summary of genetic insight into the pathogenesis of SSS and the role of risk factors in its development. Variants at six loci (named by corresponding gene names) were identified through GWAS and their unique phenotypic associations provide insight into distinct pathways underlying SSS. Investigation of the role of risk factors in SSS development supported a causal role for AF and heart rate and provided convincing evidence against causality for BMI, cholesterol (HDL and non-HDL), triglycerides and T2D. Mendelian randomization did not support causality for CAD, ischaemic stroke, heart failure, PR interval or QRS duration (not shown in figure). Red and blue arrows represent positive and negative associations, respectively.

## Keywords

Sick sinus syndrome • GWAS • *KRT8* • Mendelian randomization • Atrial fibrillation

## Introduction

Sick sinus syndrome (SSS) is a complex cardiac arrhythmia and the leading indication for permanent pacemaker implantation worldwide.<sup>1</sup> It is characterized by pathological sinus bradycardia, sinoatrial

block, or alternating atrial brady- and tachyarrhythmias. Symptoms include fatigue, reduced exercise capacity, and syncope.<sup>2,3</sup> Few studies have been conducted on the basic mechanisms of SSS and therapeutic limitations reflect an incomplete understanding of the pathophysiology. No specific treatment option is aimed at underlying pathways

and trying to determine who benefits from cardiac pacing can be challenging.<sup>4</sup> A better understanding of mechanisms leading to SSS is crucial for improvements in treatment and prevention and genetic studies provide an opportunity to gain such insight.

Coding variants in several genes, including *HCN4*, *SCN5A*, and *GNB2*, have been implicated in rare familial SSS through linkage analysis and candidate gene methods.<sup>5–15</sup> In the only published genome-wide association study (GWAS) of SSS to date, we identified a strong association with a missense variant in *MYH6*, encoding the alpha-heavy chain subunit of cardiac myosin. This was the first GWAS discovery implicating a cardiac structural or contractile unit as a primary cause of arrhythmia.<sup>16</sup>

Age is the strongest risk factor for SSS,<sup>17</sup> potentially reflecting degenerative fibrosis and electrical remodelling of the SA node and the atria in general.<sup>18–20</sup> SSS often coexists with the more common atrial fibrillation (AF)<sup>21</sup> and the two arrhythmias are thought to predispose to each other.<sup>22</sup> We have previously examined the role of AF in SSS development through Mendelian randomization,<sup>23</sup> a method using sequence variants associated with a risk factor (AF) as unbiased proxy indicators to determine whether the risk factor can cause a disease (SSS).<sup>24,25</sup> The study supported causality,<sup>23</sup> likely mediated through atrial remodelling.<sup>26,27</sup> Several other traits have been associated with SSS while the nature of the associations has not been ascertained. These include body mass index (BMI), height, heart rate, and cardiovascular diseases such as hypertension, myocardial infarction, heart failure, and stroke.<sup>17</sup>

To gain insight into the pathogenesis of SSS, we performed a large GWAS of over 6000 cases and one million controls of European descent. We used polygenic scores (PGSs) and Mendelian randomization to determine the nature of the associations of risk factors with SSS and in particular whether they contribute to the cause of the disease.

## Methods

The study design is two-fold (Supplementary material online, Figure S1). First, we performed a meta-analysis of GWASs, searching for associations of sequence variants with SSS. In total 6469 SSS cases were compared to 1 000 187 controls in a meta-analysis of material from deCODE genetics Iceland, the Copenhagen Hospital Biobank Cardiovascular Study (CHB-CVS)/Danish Blood Donor Study (DBDS), and the UK Biobank,<sup>28</sup> with follow-up in the Norwegian Nord-Trøndelag Health Study (HUNT).<sup>29</sup> Second, we performed PGS analysis and Mendelian randomization to examine the role of putative risk factors in SSS development (for detailed methods, see Supplementary material online).

### Sick sinus syndrome study populations

The deCODE genetics SSS sample consisted of 3577 Icelanders diagnosed with SSS and 347 764 controls. The CHB-CVS included 2209 SSS cases. The control group included blood donors from the DBDS ( $N > 99\,000$ )<sup>30</sup> and SSS cases were also compared to subjects in CHB-CVS with other cardiovascular conditions ( $N > 89\,000$ ). The SSS population from the UK Biobank consisted of 403 cases and 403 181 controls.<sup>28</sup> The HUNT cohort consisted of 280 Norwegian SSS cases and 69 141 controls recruited through a population-based health survey conducted in the Nord-Trøndelag County, Norway.<sup>29</sup> In all four cohorts, SSS diagnosis was based on International Classification of Diseases 9th (ICD-9: 427.8) or 10th revision (ICD-10: I49.5) from hospitals and/or outpatient clinics. The cohorts are described in more detail in Supplementary material online, Methods and Supplementary material online, Table S1.

## Genotyping

The deCODE study was based on whole-genome sequence data from 28 075 Icelanders participating in various disease projects. The 32.9 million variants that passed quality threshold were imputed into 127 175 Icelanders who had been genotyped using Illumina SNP chips. Finally, genotype probabilities for untyped relatives were calculated based on Icelandic genealogy.<sup>31</sup> Genotyping for CHB-CVS and DBDS was performed at deCODE using a north European sequencing panel of 15 576 individuals (including 8429 Danes) for imputation into those chip typed (methods manuscript in preparation). Genotyping in the UK Biobank has been described in detail elsewhere.<sup>32–35</sup> Illumina HumanCoreExome arrays were used for genotyping in the HUNT cohort.

## Genome-wide association study: statistical analysis

We performed a meta-analysis of GWAS of 6189 SSS cases and 931 046 controls from deCODE genetics, CHB-CVS/DBDS, and the UK Biobank. We then tested genome-wide significant and suggestive variants (Supplementary material online, Methods) among 280 cases and 69 141 controls from the Norwegian HUNT study. We used logistic regression to test for association between sequence variants and SSS and other case-control phenotypes, treating phenotype status as the response and allele count as a covariate. Other available individual characteristics that correlate with phenotype status were included in the model as nuisance variables. SSS associations were tested under additive, recessive, and full genotypic models. The full genotypic model includes separate parameters for heterozygotes and homozygotes. We corrected the threshold for genome-wide significance for multiple testing with a weighted Bonferroni adjustment using as weights the enrichment of variant classes with predicted functional impact among association signals estimated from the Icelandic data.<sup>36</sup> Significance thresholds were  $1.3 \times 10^{-7}$  for variants with high impact,  $2.6 \times 10^{-8}$  for variants with moderate impact (including missense),  $2.4 \times 10^{-9}$  for low-impact variants,  $1.2 \times 10^{-9}$  for other variants in DNase I hypersensitivity sites, and  $7.5 \times 10^{-10}$  for all other variants.<sup>36</sup> We used linear regression to test variant associations with quantitative phenotypes, treating the quantitative measurement as response and the genotype as covariate. These included endophenotypes of SSS, chronotropic response to exercise ( $N = 7746$ ), and electrocardiogram (ECG) measurements ( $N \sim 73\,000$ ) (see Supplementary material online, Methods for detail).

## Analysis of genetic risk shared by sick sinus syndrome and putative risk factors

We used PGSs for 17 exposure phenotypes to examine their correlation with SSS (Supplementary material online, Table S2). The phenotypes were chosen because of reported epidemiological associations with SSS or other cardiovascular conditions (see Supplementary material online, Methods). The PGSs were generated using summary statistics from the largest available GWASs (training sample) for each phenotype that do not include deCODE data (Supplementary material online, Table S2 and Supplementary material online, Figure S1). Subsequently, they were tested for association with the risk of SSS among 2556 chip-typed individuals from deCODE (target sample). *P*-values were corrected using genomic control.<sup>37</sup>

The use of PGSs to detect association between exposure and outcome is a robust method such that the absence of association in a well-powered PGS analysis provides strong evidence against causality.<sup>25</sup> However, finding association in PGS analysis does not confirm causality.<sup>38,39</sup> For the eight PGSs that associated with SSS, we performed a 2-sample Mendelian randomization analysis, equivalent to a fixed effect

**Table 1** Association results for lead variants at loci reaching genome-wide significance in a meta-analysis of sick sinus syndrome including 6469 cases and 1 000 187 controls

Locus number	Rs-name/Chr: position (hg38)	Effect allele/other	EAF <sup>a</sup> (%)	Variant annotation	Coding change	Closest gene	OR (95% CI)	P-value	P-value threshold
1	rs387906656 <sup>b</sup> /chr14:23396970	A/G	0.34	Missense	p.Arg721Trp	MYH6	8.88 (6.97–11.32)	$7.5 \times 10^{-70}$	$2.6 \times 10^{-8}$
2	rs7689774/chr4:110782354	T/G	20.07	Intergenic	–	MIR297, PITX2	1.21 (1.15–1.26)	$2.0 \times 10^{-15}$	$1.2 \times 10^{-9}$
3	rs11554495/chr12:52904798	A/C	1.64	Missense	p.Gly62Cys	KRT8	1.62 (1.43–1.84)	$9.4 \times 10^{-14}$	$2.6 \times 10^{-8}$
4	rs12932445/chr16:73035989	C/T	20.10	Intronic	–	ZFX3	1.16 (1.11–1.21)	$8.1 \times 10^{-10}$	$1.2 \times 10^{-9}$
5	rs35813871 <sup>c</sup> /chr2:178785681	A/G	26.63	Missense	p.Thr811Ile	TTN	1.13 (1.09–1.18)	$5.7 \times 10^{-9}$	$2.6 \times 10^{-8}$
5	rs34883828 <sup>c</sup> /chr2:178905448	A/C	15.23	Missense	p.Glu382Asp	CCDC141	1.15 (1.09–1.21)	$1.1 \times 10^{-7}$	$2.6 \times 10^{-8}$
6	rs6795970/chr3:38725184	A/G	35.78	Missense	p.Val1073Ala	SCN10A	1.12 (1.07–1.16)	$2.5 \times 10^{-8}$	$2.6 \times 10^{-8}$

CI, confidence interval; OR, odds ratio.

<sup>a</sup>Effect allele frequency in Iceland (deCODE).<sup>b</sup>Variant exclusive to Iceland.<sup>c</sup>p.Thr811Ile in TTN and p.Glu382Asp in CCDC141 are weakly correlated:  $R^2 = 0.20$ ,  $D' = 0.64$ .

MR-Egger<sup>40</sup> using published genome-wide significant SNPs from the largest available GWAS on each exposure phenotype as genetic instruments.<sup>41–49</sup> To estimate the causal effect, we regressed the published effects of the SNPs on the respective exposure phenotype against their SSS effects in deCODE, CHB-CVS/DBDS, and the UK Biobank, using minor allele frequency (MAF)  $\times$  (1 - MAF) as weights. For the ECG measurements PR interval and QRS duration, effects in milliseconds were converted to standard deviations (SDs) according to one SD in the Icelandic data so that all causal estimates in a Mendelian randomization forest plot correspond to equal odds ratio (OR) or SD changes for binary and quantitative traits, respectively. For significant associations, we performed further sensitivity analysis to detect outliers (funnel plots) and tested for directional pleiotropy using the MR-Egger intercept test (see [Supplementary Material online, Methods](#)).<sup>40</sup>

## Results

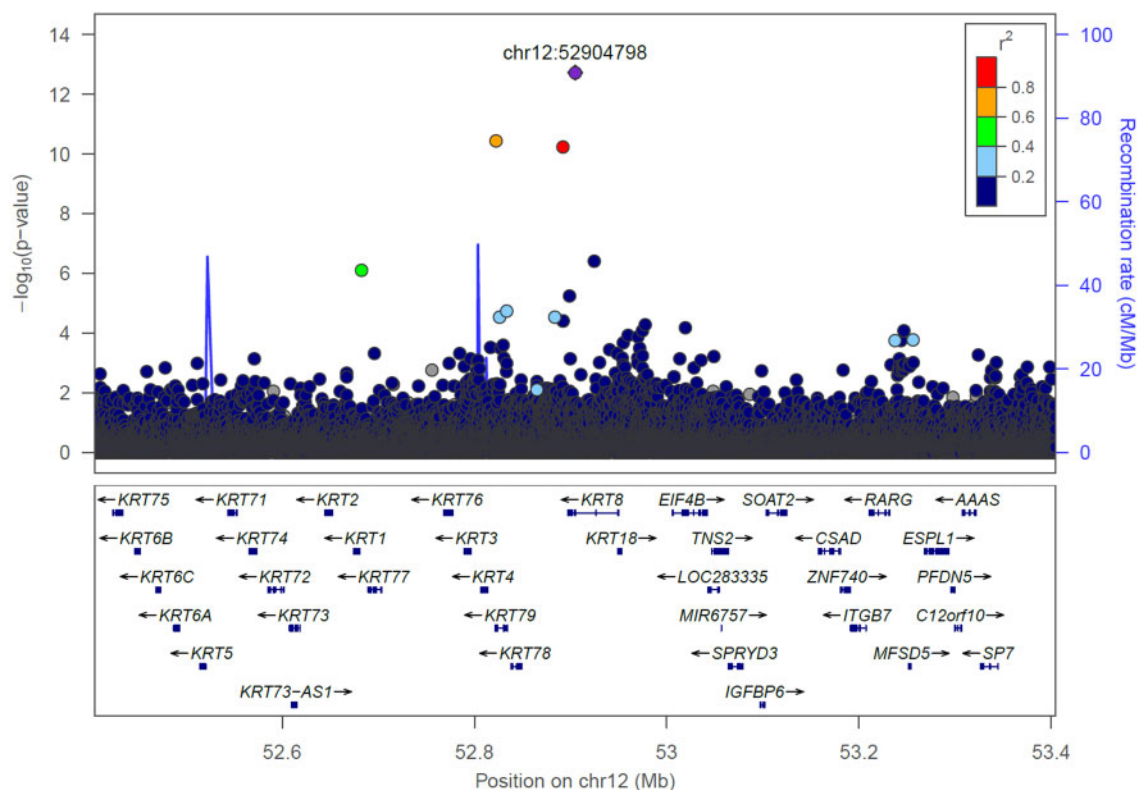
### Six sick sinus syndrome loci

In a GWAS comparing 6469 SSS cases to 1 000 187 controls, variants at six loci satisfied our criteria for genome-wide significance ([Table 1](#), [Supplementary material online, Table S3](#), [Figure 1](#), and [Supplementary material online, Figure S2](#)), with ORs ranging from 1.12 to 8.88. One is at a novel SSS locus, a low-frequency missense variant, p.Gly62Cys (MAF = 1.1–1.8% in the four cohorts) in the gene *KRT8* on chromosome 12. This variant has not been associated with arrhythmias or ECG traits before. The other variants are the rare Icelandic SSS missense variant p.Arg721Trp in the sarcomere gene *MYH6*,<sup>16</sup> common variants at two AF loci, *PITX2*<sup>50</sup> and *ZFX3*,<sup>51</sup> and two ECG loci, *TTN/CCDC141*<sup>52</sup> and *SCN10A*.<sup>52–54</sup> We have previously reported secondary associations of variants at the four AF and ECG loci with SSS.<sup>23,52</sup> At the *TTN/CCDC141* locus, we report associations of two missense variants, p.Thr811Ile in *TTN* and p.Glu382Asp in *CCDC141*, that are weakly correlated ( $R^2 = 0.20$ ,  $D' = 0.64$ , [Supplementary material online, Table S4](#)).

### High penetrance of sick sinus syndrome among homozygotes for p.Gly62Cys in *KRT8*

The SSS association of p.Gly62Cys in *KRT8* was discovered with the additive model ([Table 1](#) and [Supplementary material online, Tables S5 and S6](#)). However, homozygotes of p.Gly62Cys have a higher risk of SSS than assumed under the additive model ([Table 2](#), homozygous genotypic OR  $> 1.62^2 = 2.62$ ,  $P_{full \text{ vs. additive}} = 2.1 \times 10^{-8}$ ). We observed an OR of 1.44 for heterozygotes (95% CI = 1.26–1.65) and 13.99 for homozygotes (95% CI = 8.16–23.98), compared to non-carriers ( $P$ -value for the full genotypic model =  $1.6 \times 10^{-20}$ , [Table 2](#)). None of the other SSS associations deviated from the additive model ( $P > 0.05$ ). *KRT8* encodes the intermediate filament keratin 8 (K8, [Supplementary material online, Figure S3](#)) which is widely expressed, including in the heart,<sup>55</sup> and we detected its expression ( $52.1 \pm 30.2$  transcripts per million) in our cardiac samples from right atria ( $n = 169$ , [Supplementary material online, Methods](#)). Neither p.Gly62Cys nor two correlated variants ( $R^2 > 0.6$ ) associated with the expression of *KRT8* or nearby genes (1 Mb) in our cardiac samples ([Supplementary material online, Table S7](#)) or in GTEX.<sup>55</sup>

About 1 in 5000 individuals from the four European populations in this study is homozygous for p.Gly62Cys in *KRT8*, consistent with the



**Figure 1** Regional plot of the *KRT8* locus on chromosome 12q13. The plot depicts association results ( $P$ -values) with SSS ( $N = 6189$ ) in a meta-analysis with data from deCODE, the CHB-CVS/DBDS, and the UK Biobank. The  $y$ -axis shows the  $-\log_{10}$   $P$ -value and  $x$ -axis shows the genomic position (hg38). The lead variant of the signal (p.Gly62Cys) is labelled as a diamond and coloured purple. Other variants are coloured according to correlation ( $R^2$ ) with the lead variant in the deCODE data. The plot includes variants common to the three datasets as well as variants specific to the Icelandic deCODE data.

Hardy–Weinberg equilibrium ( $P = 0.071$ – $0.82$ ). To evaluate the penetrance of SSS among homozygous carriers of p.Gly62Cys, we analysed the two largest SSS datasets in the study, deCODE and CHB-CVS/DBDS. Considering that SSS is a late onset disease, the observed penetrance is relatively high although incomplete (Figure 2). Eight out of 36 genotyped Icelandic homozygotes (aged 23–88 years, mean 59.9) had SSS (Supplementary material online, Table S8) and 10 out of 79 homozygotes (aged 27–103 years, mean 62.1) in CHB-CVS/DBDS. Homozygotes were not significantly younger at SSS diagnosis than heterozygotes or non-carriers ( $P = 0.53$  in deCODE and  $P = 0.26$  in CHB-CVS/DBDS, Supplementary material online, Table S9).

### Diverse associations of sick sinus syndrome variants with other phenotypes

We tested the associations of the SSS variants with other phenotypes in deCODE, CHB-CVS/DBDS, and the UK Biobank datasets (Supplementary material online, Tables S10–S14). In addition to SSS, p.Gly62Cys in *KRT8* associated with pacemaker implantation (OR heterozygotes = 1.28, 95% CI = 1.13–1.45, OR homozygotes = 9.17, 95% CI = 2.89–29.09,  $P = 1.9 \times 10^{-8}$ ). The variant did not associate with other arrhythmias or cardiovascular diseases, with electrolyte

or hormonal disturbances that are linked to the development of SSS (potassium, calcium, thyroid stimulating hormone)<sup>57</sup> or with the suggested consequence of p.Gly62Cys in candidate gene studies<sup>58</sup> (pancreatitis/lipase, liver disease, Bonferroni-adjusted significance threshold of  $P = 0.05/23 = 0.0022$ , Supplementary material online, Tables S10 and S11). Furthermore, p.Gly62Cys did not associate with any of the 122 ECG parameters ( $N$  up to 72 825, Supplementary material online, Table S13).

The associations of SSS variants with AF varied. p.Gly62Cys in *KRT8* (Supplementary material online, Tables S10 and S11) and the missense variants in *TTN/CCDC141* (Supplementary material online, Table S12) did not associate with the risk of AF, applying a Bonferroni-corrected significance threshold of  $P < 0.05/14 = 0.0036$ . The *TTN/CCDC141* variants are located within 300 kbp from previously reported AF variants<sup>41</sup> and are independent of them ( $R^2 < 0.2$ , Supplementary material online, Figure S4). The other four SSS loci have been reported to affect AF but their pattern of association with the two phenotypes differ. p.Arg721Trp in *MYH6*<sup>23</sup> associates stronger with SSS than AF but the reverse applies to variants at *PITX2*. At *ZFH3*, the effects on AF and SSS are comparable.<sup>41,50,51,59,60</sup> p.Val1073Ala in *SCN10A* is one of the top AF variants at this locus (Supplementary material online, Figure S5), but the allele that

**Table 2** Association of p.Gly62Cys in *KRT8* with sick sinus syndrome in the deCODE, CHB-CVS/DBDS, UK Biobank, and HUNT samples under the full genotypic model, calculating independent risk among heterozygotes and homozygotes

Rs-name/Pos (hg38)	Effect allele/other	Coding change	Gene	Cohort	N	EAF (%)	Risk for heterozygous carriers, OR (95% CI)	Risk for homozygous carriers, OR (95% CI)	P-value for full genotypic model <sup>a</sup>
rs11554495(chr12:52904798)	A/C	p.Gly62Cys	KRT8	deCODE	3577	1.64	1.42 (1.17–1.72)	12.95 (4.58–36.63)	$1.5 \times 10^{-9}$
				CHB-CVS	2209	1.77	1.34 (1.08–1.66)	16.13 (6.24–41.67)	$2.6 \times 10^{-10}$
				UK Biobank	403	1.05	2.35 (1.42–3.88)	17.90 (0.74–435.16)	0.00012
				HUNT	280	1.15	1.55 (0.77–2.91)	0.00 <sup>b</sup>	0.50
				Combined	6469	–	1.44 (1.26–1.65)	13.99 (8.16–23.98)	$1.6 \times 10^{-20}$

CI, confidence interval; EAF, effect allele frequency; OR, odds ratio; Pos, position; SSS, sick sinus syndrome.

<sup>a</sup>The full genotypic model ( $P_{full} = 1.6 \times 10^{-20}$ ) deviates significantly from the additive model ( $P_{additive} = 9.4 \times 10^{-14}$ ),  $P$  for deviation from the additive model =  $2.1 \times 10^{-6}$ .

<sup>b</sup>Among 14 homozygotes in the HUNT study, none had SSS ( $P$ -value for homozygotes = 0.97).

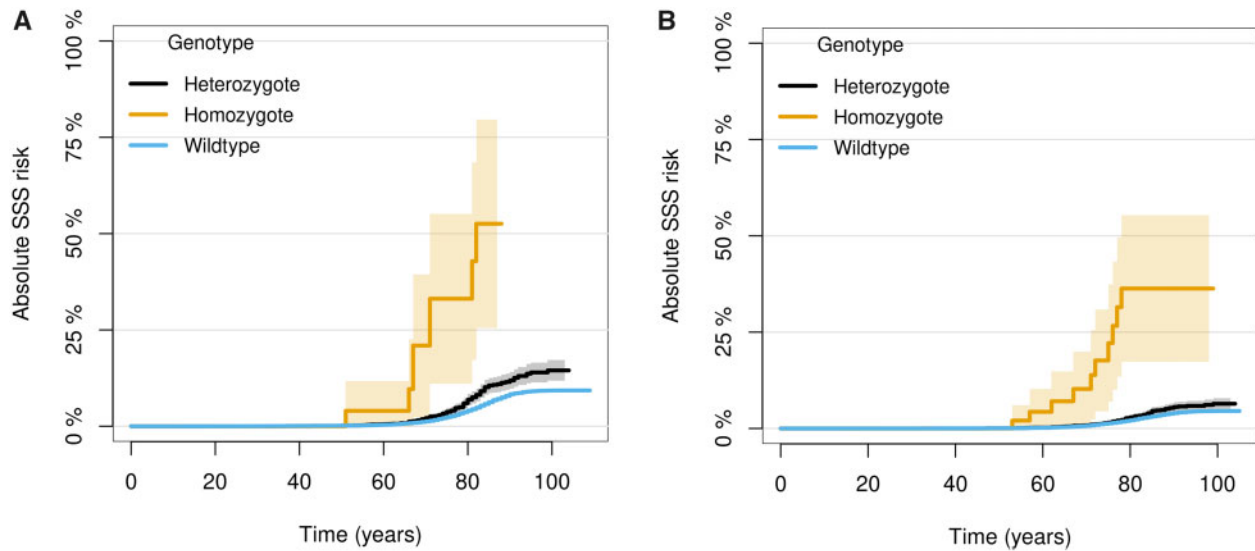
increases the risk of SSS protects against AF (Supplementary material online, Table S12).<sup>53,61</sup>

All the SSS variants increased the risk of pacemaker implantation (Supplementary material online, Tables S10–S12), as expected since SSS is the most common reason for this procedure. We have previously described in detail the associations of p.Arg721Trp in *MYH6* with coarctation of the aorta, other congenital malformations of the heart, aortic valve stenosis, and heart failure<sup>23,62,63</sup> (Supplementary material online, Table S12). We note that p.Glu382Asp in *CCDC141* associated with both second- and third-degree atrioventricular block (AVB)<sup>52</sup> and the association with third-degree AVB was genome-wide significant ( $P = 1.3 \times 10^{-14}$ , OR = 1.27, 95% CI = 1.20–1.35) and more significant than the SSS association. Another notable association is that of the *PITX2* variant with heart failure (Supplementary material online, Table S12) as recently reported in a GWAS of heart failure.<sup>47</sup> None of the SSS variants associated with chronotropic response to exercise (Supplementary material online, Tables S15 and S16) in our dataset. However, the *SCN10A* and *CCDC141* loci have been reported to associate with heart rate profile during exercise in larger studies of UK Biobank data.<sup>64,65</sup>

## Genetically predicted atrial fibrillation and lower heart rate associate with sick sinus syndrome

PGSs are powerful tools for detecting associations between phenotypes.<sup>38</sup> We generated PGSs for 17 exposure phenotypes using summary statistics from the largest available GWASs (Supplementary material online, Table S2) and tested them for association with SSS in deCODE data. Eight PGSs, for AF, heart rate, coronary artery disease (CAD), height, QRS duration, PR interval, ischaemic stroke, and heart failure, associated with SSS while those for BMI, type 2 diabetes (T2D), non-HDL cholesterol, HDL cholesterol, triglycerides, and hypertension/blood pressure traits did not (Bonferroni-adjusted significance threshold of  $P = 0.05/17 = 0.0029$ , Supplementary material online, Table S17). The PGS for heart rate was the only one to inversely associate with SSS risk.

We then performed Mendelian randomization to determine the causality of the associations between the eight exposures and SSS, using genome-wide significant SNPs from GWAS of the exposure phenotypes as instruments (see Methods). Genetic predisposition to AF and genetically determined heart rate and height associated with the risk of SSS but the height association did not remain after accounting for AF in a multivariate analysis (Figures 3 and 4A and B).<sup>66–68</sup> With few exceptions, the effects of AF variants on SSS are proportional to their effects on AF ( $P = 7.8 \times 10^{-14}$ , Figure 4A and Supplementary material online, Figure S6A). The greatest deviation from the expected SSS effect was for the association at *SCN10A* (tagged by rs6790693, OR for AF = 1.06, OR for SSS = 0.89, Supplementary material online, Figure S6A). Mendelian randomization did not support causality for CAD, ischaemic stroke, heart failure, PR interval, or QRS duration (Figure 3 and Supplementary material online, Figure S7).



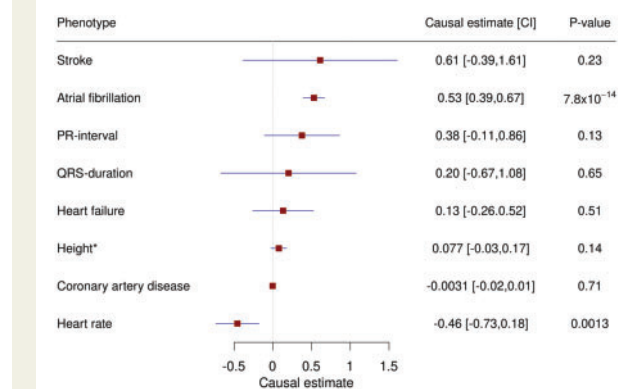
**Figure 2** Cumulative incidence curves for SSS age of onset based on *KRT8* p.Gly62Cys genotype (A) in deCODE and (B) in CHB-CVS/DBDS. Aalen–Johansen estimator was used, treating death as a competing risk.<sup>56</sup> Age of onset was determined by the first registered ICD diagnosis of SSS available to us and thus represents an upper range (Supplementary material online, Methods).

## Discussion

Traditionally, SSS has been considered as a collection of conditions with a variety of causes, intrinsic and extrinsic to the SA node.<sup>69</sup> In line with this notion, we identified sequence variants associating with SSS at six loci implicating several distinct pathways in SSS development. One of the SSS associations is novel, with a low-frequency missense variant, p.Gly62Cys in *KRT8*. One is with the reported p.Arg721Trp in *MYH6*,<sup>16</sup> and four confirm loci previously implicated in SSS secondarily to AF, at *PITX2*,<sup>50</sup> and *ZFH3*,<sup>51</sup> or ECG traits, at *SCN10A*<sup>53</sup> and *TTN/CCDC141*.<sup>52</sup> The leading variants at four of the SSS loci are missense.

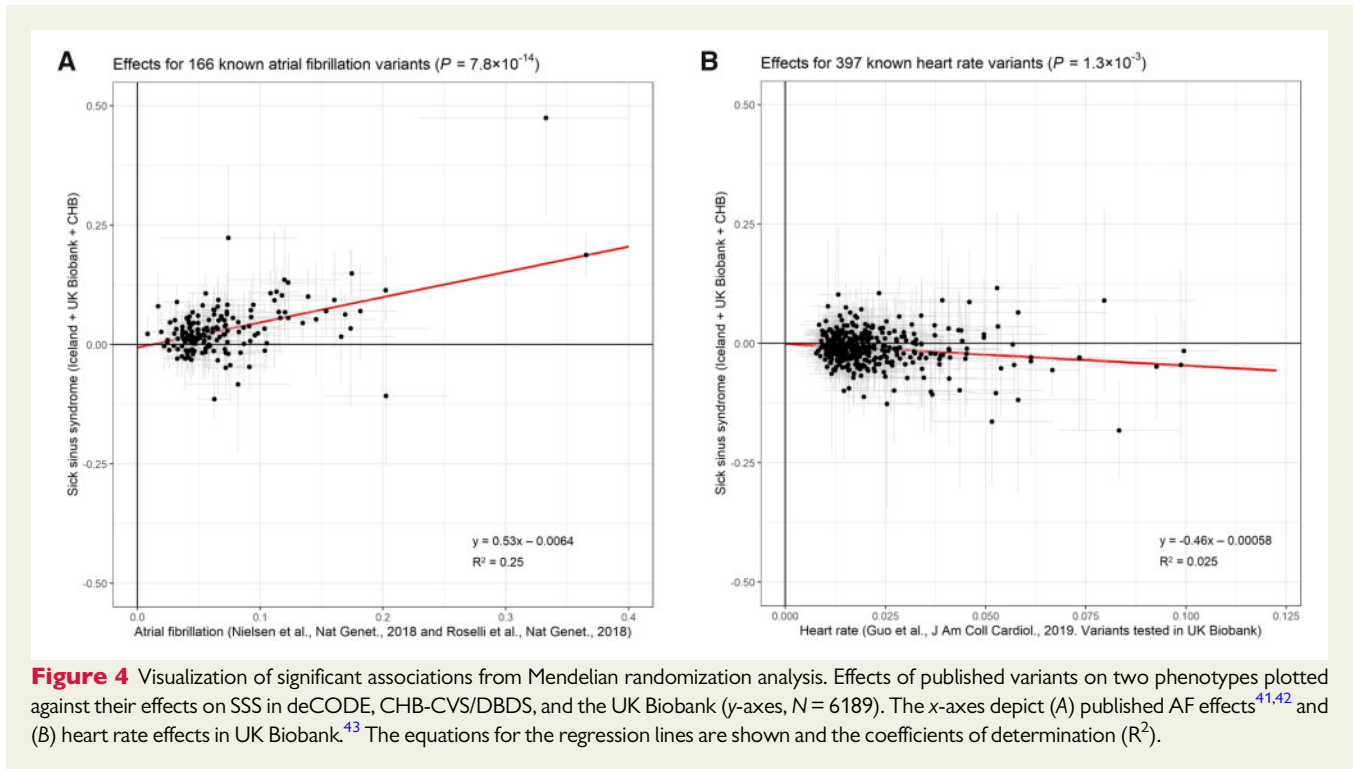
*KRT8* encodes K8, a cytoskeletal intermediate filament protein widely expressed, including in skeletal muscle and the heart.<sup>55</sup> Historically, keratins have been described as epithelial-specific intermediate filaments<sup>70</sup> and mutations affecting keratin function have almost exclusively been associated with diseases of the skin and other epithelial tissues.<sup>71,72</sup> The association of p.Gly62Cys in *KRT8* with SSS described here supports the notion of a role for K8 in the human heart, as previously suggested by animal studies.<sup>73–76</sup> Experiments have revealed loss-of-function effects of p.Gly62Cys, that is located in the head domain of K8 (Supplementary material online, Figure S3).<sup>77,78</sup> Glycine at position 62 is a conserved amino acid (GERP<sup>79</sup> score 1.73) and the variant introduces cysteine into a protein otherwise devoid of cysteine residues. This interrupts intermediate filament assembly *in vitro*<sup>77</sup> and prevents K8 from serving as a phosphate ‘sponge’ for stress-activated kinases that mediate apoptosis.<sup>78</sup> Thus, the variant could increase the risk of SSS either by affecting the structural or cardioprotective role of K8.<sup>77,78</sup>

In addition to p.Gly62Cys in *KRT8*, lead variants at two other SSS loci are missense variants in genes encoding structural components



**Figure 3** Forest plot showing causal effect estimates from a Mendelian randomization analysis of putative risk factors on SSS. We regressed published effects of SNPs on the respective exposure phenotype against their SSS effects in deCODE, CHB-CVS/DBDS, and the UK Biobank. The causal estimates are the slopes from the regression lines where a one unit change in the exposure phenotypes equals a one standard deviation change for quantitative traits and an odds ratio of 2.7 for binary exposure phenotypes. The forest plot shows the causal estimates, 95% confidence intervals, and corresponding P-values from the Mendelian randomization analysis. \*For height, the result from a multivariate analysis accounting for AF effects is shown.

of the heart. *MYH6* encodes the alpha-myosin heavy chain ( $\alpha$ MHC) and *TTN* encodes the giant protein titin, both of which are key components of the sarcomere.<sup>80,81</sup> Since the identification of *MYH6* in



the first GWAS of SSS,<sup>16</sup> structural components of cardiomyocytes have increasingly been implicated in atrioventricular conduction and arrhythmias, in particular AF.<sup>23,41,82,83</sup> However, the variants at *KRT8*, *MYH6* and *TTN* do not mediate their risk through AF because they either do not associate with AF (*KRT8* and *TTN*) or have a stronger effect on SSS risk (*MYH6*).

K8,  $\alpha$ MHC, and titin have a common role in cardiac adaptive responses to stress which may be of relevance to their association with SSS.  $\alpha$ MHC allows greater economy in force generation than the homologous beta chain and a shift in their relative expression plays a major role in regulating myocardial contractile activity.<sup>84,85</sup> Likewise, the isoform switch of titin alters ventricular filling<sup>81,86</sup> and K8 is upregulated under stress to maintain structural integrity.<sup>76</sup> The expression of all three genes is modified in human heart failure.<sup>76,84,87</sup> Thus, whether or not the SSS variants in *KRT8*, *MYH6* and *TTN* directly affect the SA node, it is possible that they could contribute to inadequate cardiac output and symptom development in SSS by interrupting adaptive increase in stroke volume.

The SSS variants at *PITX2* and *ZFX3* point to genes encoding transcription factors.<sup>88,89</sup> They likely mediate their effects on SSS through AF since these have consistently been the strongest AF loci in GWAS.<sup>42,50,51</sup> Conversely, p.Val1073Ala in *SCN10A* has opposite effects on the two arrhythmias. The allele that increases the risk of SSS protects against AF.<sup>53,61</sup> It also prolongs the PR interval<sup>53,54,61</sup> and duration of the QRS complex.<sup>52,54</sup> *SCN10A* encodes the neuronal sodium channel isoform Na<sub>v</sub>1.8. A plausible mechanism behind the unique phenotypic effects of p.Val1073Ala involves Na<sub>v</sub>1.8's role in the way in which the autonomic nervous system affects the heart.<sup>90–92</sup> In support of this, animal studies have shown that blockade of

Na<sub>v</sub>1.8 in intracardiac autonomic neurons in the ganglionated plexi (GP) suppresses AF inducibility.<sup>90,91</sup> The same applies to GP ablation in patients with AF.<sup>93–95</sup> However, GP ablation is controversial due to observed adverse events,<sup>96–98</sup> including development of SSS and pacemaker implantation.<sup>99</sup> The opposite effects of p.Val1073Ala on SSS and AF risk support concerns that GP ablation for AF treatment may cause SSS and links this adverse effect to Na<sub>v</sub>1.8 function specifically.

Our genetic analyses shed light on pathways contributing to the development of SSS and the role of risk factors. In particular, they provide insight into the intricate and debated relationship between SSS and AF.<sup>22</sup> Our Mendelian randomization analysis reveals a strong association between genetic predisposition for AF and risk of SSS. This is consistent with our previous report<sup>23</sup> and likely reflects causality. Pacing-induced AF has been shown to impair SA node function in dogs<sup>100</sup> and AF causes regional atrial substrate changes around the SA node in humans.<sup>27</sup> Pathobiological pathways common to AF and SSS, such as inflammation, interstitial atrial fibrosis, and alterations in intracellular Ca<sup>2+</sup> dynamics, may also contribute to this trend.<sup>22,26,101</sup>

Importantly, our results also show that SSS does not only occur as a consequence of AF or pathways common to AF. This is evident in the case of SSS variants at *KRT8* and *TTN/CCDC141* that do not associate with the risk of AF in a large dataset. p.Gly62Cys in *KRT8* is the only SSS variant that does not associate with other arrhythmias, ECG traits, or cardiovascular diseases in our dataset. Thus, this variant in particular may point to a mechanism that is specific to SSS development. We observe a genome-wide significant association of p.Glu382Asp in *CCDC141* with third-degree AVB, the first one reported for complete heart block. Therefore, this locus points to a



mechanism linking the functions of the AV and SA nodes and does not mediate the risk of SSS as a consequence of AF. The complexity of the relationship between SSS and AF is further evident by the opposite effects of the *SCN10A* locus on the two arrhythmias. Finally, since SSS variants do not consistently associate with AF, our data do not suggest a strong causal role for SSS in the development of AF.

Heart rate variants associate inversely with SSS, consistent with epidemiological observation,<sup>17</sup> potentially because bradycardia is an integral part of the SSS diagnosis. However, lower heart rate could also represent a direct marker of biological processes affecting SSS, such as fibrosis or altered autonomic neural input. Finally, lack of associations with powerful PGSs based on large datasets serves as convincing evidence against causality.<sup>25</sup> In particular, this applies to BMI, cholesterol, triglycerides, and T2D.

Several of our findings have direct clinical implications. In particular, the causal role of AF in SSS development emphasizes the need for heightened awareness of potential SSS among patients with AF, especially those with unspecific symptoms. Furthermore, the strong association of the SSS variant p.Glu382Asp in *CCDC141* with AVB encourages consideration of dual chamber pacemaker implantation in SSS patients carrying this variant. Lastly, the *SCN10A* locus points to SSS as a potential adverse effect of GP ablation therapy for AF.

The major strength of the study is the large SSS sample set and extensive phenotypic and genotypic data for the same datasets. Different proportions of comorbidities and drug use in SSS cases and controls could constitute confounding; however, the consistent associations of SSS variants with both SSS and pacemaker implantation across cohorts support their true effect on cardiac conduction. Furthermore, if a specific comorbidity would explain one of the SSS associations, this would be evident by a stronger effect on that phenotype. The strength of Mendelian randomization in determining causality includes less concern for confounding and reverse causation than in observational studies since sequence variants associated with the exposure (e.g. AF) are randomized during meiosis and this process is unaffected by the presence of the outcome (SSS) later in life.<sup>24</sup> For heart failure, a limitation of the Mendelian randomization analysis is that variants identified in GWAS are not ideal instruments because of their association with potential confounders (e.g. AF, CAD, and BMI).<sup>47</sup>

## Conclusion

In this large genetic study, we found six SSS loci, some of which also associate with other arrhythmias and cardiac electrical function. One of the associations is with a missense variant in the intermediate filament gene *KRT8* that confers high risk of SSS among homozygous carriers. Mendelian randomization analysis suggests that AF and lower heart rate are directly involved in SSS development. PGS analysis provides convincing evidence against causality for BMI, cholesterol, triglycerides, and T2D. On the other hand, the data also show that SSS can result from perturbation of pathways unrelated to AF. In particular, p.Gly62Cys at *KRT8* does not associate with any other cardiovascular traits and points to a mechanism that is specific to SSS development.

## Supplementary material

Supplementary material is available at *European Heart Journal* online.

### Data availability

The Icelandic population WGS data has been deposited at the European Variant Archive under accession code PRJEB15197. We declare that the data supporting the findings of this study are available within the article, its Supplementary material online and upon reasonable request. The genome-wide association scan summary data will be made available at <http://www.decode.com/summarydata>.

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