Human Molecular Genetics, 2015, Vol. 24, No. 10 2757–2763

doi: 10.1093/hmg/ddv036
Advance Access Publication Date: 3 February 2015
Original Article

ORIGINAL ARTICLE

Testing the burden of rare variation in arrhythmia-susceptibility genes provides new insights into molecular diagnosis for Brugada syndrome

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Abstract

The Brugada syndrome (BrS) is a rare heritable cardiac arrhythmia disorder associated with ventricular fibrillation and sudden cardiac death. Mutations in the *SCN5A* gene have been causally related to BrS in 20–30% of cases. Twenty other genes have been described as involved in BrS, but their overall contribution to disease prevalence is still unclear. This study aims to estimate the burden of rare coding variation in arrhythmia-susceptibility genes among a large group of patients with BrS. We have developed a custom kit to capture and sequence the coding regions of 45 previously reported arrhythmia-susceptibility genes and applied this kit to 167 index cases presenting with a Brugada pattern on the electrocardiogram as well as 167 individuals aged over 65-year old and showing no history of cardiac arrhythmia. By applying burden tests, a significant enrichment in rare coding variation (with a minor allele frequency below 0.1%) was observed only for *SCN5A*, with rare coding variants carried by 20.4% of

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Received: November 18, 2014. **Revised and Accepted:** January 31, 2015

cases with BrS versus 2.4% of control individuals ($P = 1.4 \times 10^{-7}$). No significant enrichment was observed for any other arrhythmia-susceptibility gene, including *SCN10A* and *CACNA1C*. These results indicate that, except for *SCN5A*, rare coding variation in previously reported arrhythmia-susceptibility genes do not contribute significantly to the occurrence of BrS in a population with European ancestry. Extreme caution should thus be taken when interpreting genetic variation in molecular diagnostic setting, since rare coding variants were observed in a similar extent among cases versus controls, for most previously reported BrS-susceptibility genes.

Introduction

The Brugada syndrome (BrS, MIM #601144) is a rare cardiac arrhythmia disorder associated with syncope and sudden cardiac death, which is characterized by a coved ST-segment and J-point elevation ≥0.2 mV, followed by a negative T wave in the right precordial leads of the electrocardiogram (ECG) (1).

Mutations in the *SCN5A* gene, which encodes the major cardiac sodium channel $Na_v1.5$, have been causally related to BrS in 20–30% of cases (2). Twenty other genes have been described as involved in BrS, mostly by candidate gene approaches. These genes encode subunits of cardiac sodium channels (*SCN1B*, *SCN2B*, *SCN3B*, *SCN10A*), L-type calcium channels (*CACNA1C*, *CACNB2*, *CACNA2D1*), potassium channels (*KCNH2*, *KCNE3*, *KCNE1L/KCNE5*, *KCND3*, *KCNJ8*, *ABCC9*), other channels (*TRPM4*, *HCN4*), channel-interacting proteins (*GPD1L*, *RANGRF/MOG1*, *SLMAP*), a desmosomal protein (*PKP2*) and a fibroblast growth factor (*FGF12*) (2–8). However, the prevalence of mutations in these other genes among BrS cases is still unclear. For example, the reported mutation prevalence of the *CACNA1C* gene ranges from 2 to 12% in the literature (2). Noteworthy, in a report describing the presence of putative pathogenic mutations among 12 of these genes in 20% of BrS cases, the authors showed that the mutations in all genes except *SCN5A* account for <5% of cases in total (9). Conversely, mutations in the *SCN10A* gene were recently reported as carried by 16.7% of patients with BrS (4).

The emergence of next-generation sequencing (NGS) technologies enables to screen multiple genes simultaneously and identify multiple rare genetic variants in patients. As a consequence, since rare variants are a common feature in the general population, there is an increasingly recognized risk of false-positive reports of causality (10). Studies are now required to determine which genes are significantly associated with BrS and interpret genetic data accurately in the context of molecular diagnosis. In this study, we have tested by targeted sequencing 45 genes previously involved in cardiac arrhythmias (including 21 BrS-susceptibility genes) in 167 index cases with BrS as well as 167 ethnically matched control individuals, in order to determine which genes carry a burden of rare genetic variants in cases versus controls and therefore contribute significantly to BrS.

Results

Prevalence of rare variants in BrS susceptibility genes

The 167 patients with BrS included in this study were sequenced on average to a mean coverage depth of 749× per sample—with 98% of the targeted regions covered at least 10 times—in order to ensure the detection of most heterozygous variants (Supplementary Material, Table S1). Variants were considered as relevant if rare—i.e. reported with a minor allele frequency (MAF) below 0.1% in the European population—and if predicted to alter the protein sequence (see Supplementary Material, Methods for details). All relevant variants following these criteria—hereafter called 'functional' variants—are listed in Supplementary Material, Table S2.

Rare functional variants in *SCN5A* were identified in 20.4% of our BrS population (34 out of 167 cases, 35 variants; see Fig. 1, Table 1 and Supplementary Material, Table S2), in accordance with previous reports (2,9,11). Among these 35 variants, one is non-sense (NM_198056.2:c.535C>T, R179Ter), two are frameshift deletions (NM_198056.2:c.2559delT, F853LfsTer16 and NM_198056.2:c.5314delC, L1772WfsTer15) and one affects a splice site (NM_198056.2:c.3963+2T>C). In accordance with previous reports (9,12–14), we found that rare functional variation in *SCN5A* is associated with longer PR interval (197 ± 21 versus 174 ± 25 ms; $P < 0.001$) and longer QRS duration (109 ± 13 versus 101 ± 12 ms; $P = 0.005$). In our study, spontaneous BrS ECG pattern (67 versus 36%; $P = 0.002$) was more prevalent in *SCN5A* rare variant carriers (Table 2).

Interestingly, three patients carry two rare missense variants in *SCN5A*: BrS_15 (NM_198056.2:c.1003T>A, C335S and c.3121G>A, D1041N), BrS_27 (NM_198056.2:c.1844G>A, G615E and c.4247C>A, A1416E), and BrS_28 (NM_198056.2:c.1333C>G, H445D and c.5339A>G, E1780G). The six variants are highly conserved between species, with every GERP (Genomic Evolutionary Rate Profiling) conservation score above 4. The three patients present with a spontaneous Brugada pattern, one case being symptomatic (unexplained syncope without any arrhythmia in the follow up). The mean PR interval in the presence of two *SCN5A* variants [213 (190–230) ms] tends to be longer than among all *SCN5A* variant carriers, while the mean QRS interval is similar [110 (109–111) ms].

Rare functional variants in *SCN10A*, *CACNA1C*, *PKP2*, *CACNB2*, *KCNH2* and *TRPM4* were found in 1–6% of BrS cases (Table 1). One missense variant in *SCN10A* (NM_006514.2:c.3674T>C, I1225T) was previously reported in a BrS case (4). Only one rare variant (1/167 patients, 0.6% of the BrS population) was identified in each of the *KCND3*, *CACNA2D1*, *HEY2*, *SCN2B* and *SCN3B* genes. Note that the *SCN3B* missense variant (NM_018400.3:c.29T>C, L10P) was previously reported in one unrelated patient with BrS of German, Swedish and Native American descent (15) and is absent from the 1000 Genomes and ESP (NHLBI GO Exome Sequencing Project) European populations. No rare functional variant was identified in *ABCC9*, *SCN1B*, *RANGRF*, *FGF12*, *GPD1L*, *HCN4*, *KCNE1L/KCNE5*, *KCNE3* and *KCNJ8*.

Testing significant enrichment in rare functional variants

A substantial amount of rare functional variation was observed in arrhythmia-susceptibility genes when analysing a group of 167 ethnicity-matched individuals with no history of cardiac arrhythmia on the same targeted sequencing system (Supplementary Material, Table S2). Therefore, a burden test was applied to detect genes with a significant enrichment in rare variation in cases versus controls. *SCN5A* was the only BrS-susceptibility gene showing a significant enrichment associated with BrS with 20.4% of cases, compared with 2.4% of controls, carrying at least one rare functional variant in this gene ($P = 1.4 \times 10^{-7}$, Table 1). To reproduce this observation with an independent control set and evaluate if a set of external data could be used for

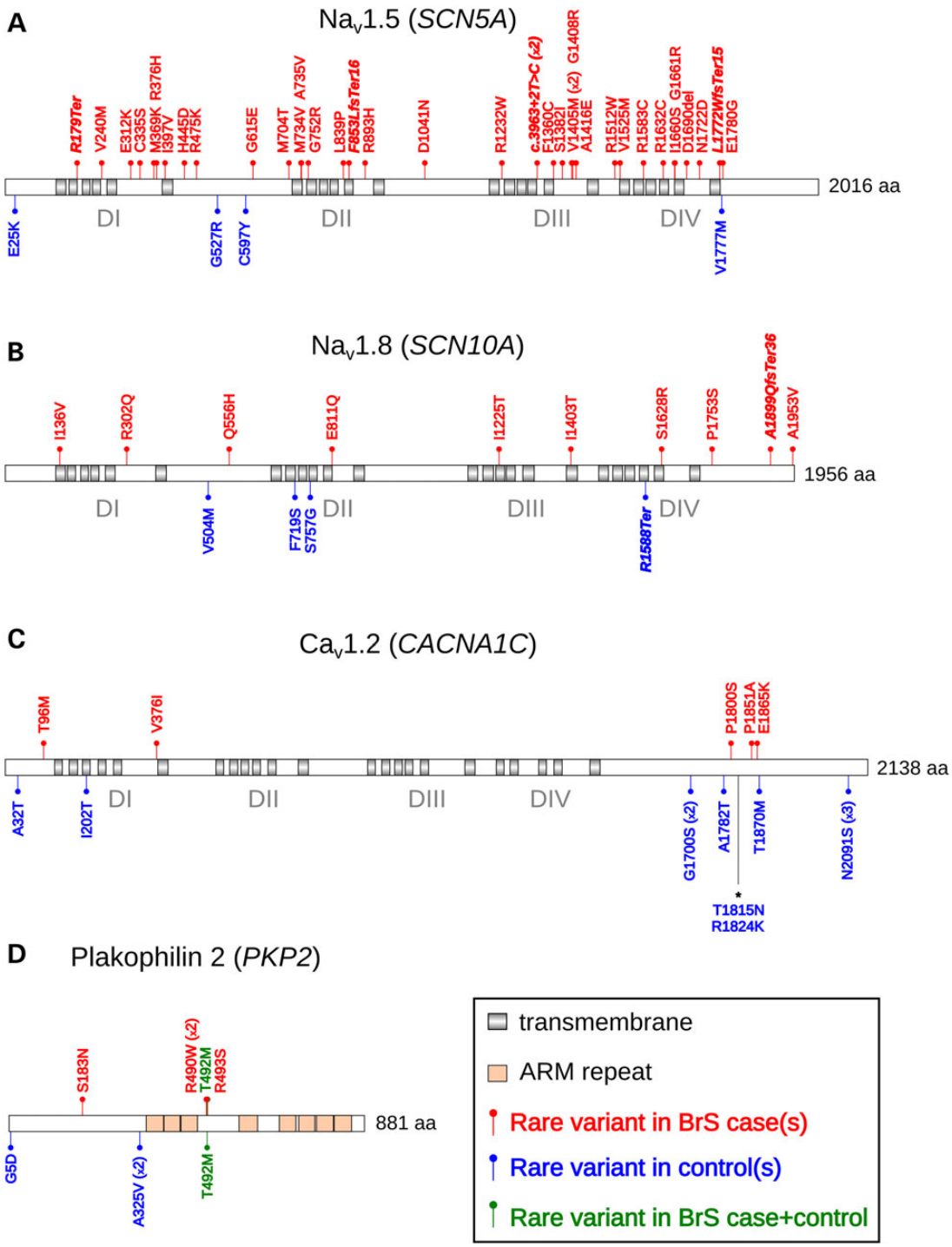

Figure 1. Location of the rare functional variants detected among cases and controls in four previously reported BrS-susceptibility genes: *SCN5A*, *SCN10A*, *CACNA1C* and *PKP2*. Rare functional variants (with an MAF <0.1%) detected in cases (red, above), controls (blue, below) or both cases and controls (green) are represented. If a rare variant was found in more than one case or control, the number of subjects is indicated in brackets. Domain annotations were obtained from UniProt. Non-sense, frameshift and splice site variants (four in *SCN5A* and two in *SCN10A*) are shown in bold/italics. (**A**) *SCN5A* (sodium channel, Na$_v$1.5), NCBI: NP_932173.1, UniProtKB: Q14524. (**B**) *SCN10A* (sodium channel, Na$_v$1.8), NCBI: NP_006505.2, UniProtKB: Q9Y5Y9. (**C**) *CACNA1C* (calcium channel, Ca$_v$1.2), NCBI: NP_000710.5, UniProtKB: Q13936–12. The asterisk points to two rare missense variants located in an alternative exon in isoform NP_001161097.1 (p.Thr1815Asn and p.Arg1824Lys). (**D**) *PKP2* (plakophilin-2), NCBI: NP_004563.2, UniProtKB: Q99959.

future studies, access was requested to the variant calls from 881 whole-exome sequences generated by the UK10K consortium. The significant association of *SCN5A* with BrS was confirmed with this independent control set: 20.4% of cases versus 2.4% of controls ($P = 1.7 \times 10^{-15}$, Table 1). *SCN5A* was again the only gene showing significant enrichment in rare functional variants in cases versus controls (Table 1).

Although not significant, a slight tendency to enrichment in rare functional variants in cases was also observed with both control sets for *SCN10A* (internal: 6 versus 2.4%, $P = 0.170$;

Table 1 Burden tests results for 45 genes linked to cardiac arrhythmias

Gene	BrS cases ($n = 167$)	Internal controls ($n = 167$)	P-value 1	UK10K controls ($n = 881$)	P-value 2
BrS-susceptibility genes					
SCN5A	20.4% (34)	2.4% (4)	1.4×10^{-7}[a]	2.4% (21)	1.7×10^{-15}[a]
SCN10A	6% (10)	2.4% (4)	0.170	3.5% (31)	0.131
CACNA1C	3% (5)	6.6% (11)	0.199	2% (18)	0.395
PKP2	3% (5)	2.4% (4)	1	1.7% (15)	0.348
CACNB2	1.8% (3)	1.2% (2)	1	0.9% (8)	0.396
KCNH2	1.2% (2)	3.6% (6)	0.283	1.6% (14)	1
TRPM4	1.2% (2)	3% (5)	0.448	1.9% (17)	0.754
KCND3	0.6% (1)	1.2% (2)	1	1.6% (14)	0.488
CACNA2D1	0.6% (1)	0.6% (1)	1	3.3% (29)	0.072
HEY2	0.6% (1)	0.6% (1)	1	0.1% (1)	0.293
SCN2B	0.6% (1)	0.6% (1)	1	0.5% (4)	0.581
SCN3B	0.6% (1)	0.6% (1)	1	0.5% (4)	0.581
ABCC9	—	3% (5)	0.061	1.1% (10)	0.379
SCN1B	—	1.8% (3)	0.248	0.3% (3)	1
RANGRF	—	0.6% (1)	1	0.2% (2)	1
FGF12	—	—	—	0.7% (6)	0.597
GPD1L	—	—	—	0.1% (1)	1
HCN4	—	—	—	1.6% (14)	0.144
KCNE1L	—	—	—	1% (9)	0.369
KCNE3	—	—	—	0.1% (1)	1
KCNJ8	—	—	—	0.5% (4)	1
Other susceptibility genes					
AKAP9	6% (10)	4.2% (7)	0.620	7.9% (70)	0.431
ANK2	4.2% (7)	3.6% (6)	1	5% (44)	0.844
RYR2	3.6% (6)	4.8% (8)	0.786	4.9% (43)	0.417[b]
TRDN	3.6% (6)	1.8% (3)	0.502	2.5% (22)	0.431
CASQ2	2.4% (4)	4.2% (7)	0.542	1.7% (15)	0.526
CACNA1D	2.4% (4)	1.8% (3)	1	5.1% (45)	0.161
NUP155	2.4% (4)	1.2% (2)	0.685	1.9% (17)	0.761
KCNQ1	1.8% (3)	—	0.248	1% (9)	0.691[b]
SNTA1	1.2% (2)	1.8% (3)	1	1.2% (11)	1
KCNJ5	1.2% (2)	—	0.498	0.6% (5)	0.310
DPP6	0.6% (1)	2.4% (4)	0.371	0.2% (2)	0.406
KCNA5	0.6% (1)	2.4% (4)	0.371	4.3% (38)	0.014
NKX2-5	0.6% (1)	1.8% (3)	0.623	0.5% (4)	0.581
GJA1	0.6% (1)	1.2% (2)	1	0.2% (2)	0.406
EMD	0.6% (1)	0.6% (1)	1	1.1% (10)	1
KCNE1	0.6% (1)	0.6% (1)	1	0.5% (4)	0.581
KCNJ2	0.6% (1)	0.6% (1)	1	0.6% (5)	1
CAV3	0.6% (1)	—	1	0.6% (5)	1
GATA4	0.6% (1)	—	1	0.2% (2)	0.406
NPPA	0.6% (1)	—	1	0.3% (3)	0.501
SCN4B	—	1.8% (3)	0.248	0.5% (4)	1
LMNA	—	0.6% (1)	1	0.8% (7)	0.605
GJA5	—	0.6% (1)	1	0.2% (2)	1
KCNE2	—	—	—	0.3% (3)	1

The proportion of BrS cases/controls (in %) carrying at least one rare 'functional' variant in each gene is shown. The corresponding number of cases/controls is indicated in brackets.

[a]Significant enrichment in rare 'functional' variants in BrS cases.

[b]One variant presenting two non-reference alleles was excluded for the CAST analysis.

UK10K: 6 versus 3.5%, $P = 0.131$), *PKP2* (internal: 3 versus 2.4%, $P = 1$; UK10K: 3 versus 1.7%, $P = 0.348$) and *CACNB2* (internal: 1.8 versus 1.2%, $P = 1$; UK10K: 1.8 versus 0.9%, $P = 0.396$). The same burden test was applied to 24 additional genes selected on the basis of their reported involvement in monogenic forms of cardiac arrhythmia and conduction defects: no significant enrichment was observed for any of these genes (Table 1). Rare variants were detected in large genes such as *ANK2*, *AKAP9* and *RYR2* in more than 3% of both case and control populations.

Distribution of rare variation across protein sequences

Four genes display rare functional variation in more than 2% of BrS cases: *SCN5A*, *SCN10A*, *CACNA1C* and *PKP2* (Fig. 1). Most variants identified in *SCN5A* among BrS cases alter the DI–DIV transmembrane domains of the $Na_v1.5$ protein. Conversely, three out of four rare variants identified among controls were located in the N-terminal domain or the DI–DII linker region known as harbouring variations with unlikely pathogenicity (16). The distribution of rare variants across *SCN10A* ($Na_v1.8$) seemed less predictive

Table 2. Genotype–phenotype correlations in relation to SCN5A status

	SCN5A positive	*SCN5A* negative	*P*-value
Number of BrS cases	34	133	—
Age	44 ± 14	49 ± 14	0.051
Symptoms	41% (14)	44% (59)	0.888
Male gender	65% (22)	82% (109)	0.051
Baseline BrS ECG pattern	67% (23)	36% (49)	0.002*
Heart rate (bpm)	70 ± 12	72 ± 12	0.260
PR interval (ms)	197 ± 21	174 ± 25	<0.001*
QRS duration (ms)	109 ± 13	101 ± 12	0.005*
QTc interval (ms)	410 ± 31	416 ± 32	0.304

*Significant at $P < 0.05$.

of their potential pathogenicity, three variants carried by control individuals being located in transmembrane domains.

Most variants affecting *CACNA1C* among cases (3/5) as well as controls (6/8) were located within the C-terminal tail of $Ca_v1.2$ (Fig. 1C), indicating that genetic variation may be extensive and benign in this region. Finally, one particular *PKP2* interval coding for four amino acids was the site of three rare variants detected among BrS patients: NM_004572.3:c.1468C>T (R490W, 2 cases), NM_004572.3:c.1475C>T (T492M, 1 case, 1 control) and NM_004572.3:c.1479G>C (R493S, 1 case). This interval, albeit exhibiting low evolutionary conservation, might be a preferential site for rare variants causally related to BrS (Supplementary Material, Table S2).

Discussion

Is *SCN5A* the only major BrS-susceptibility gene?

Three genes are currently considered as major susceptibility genes for BrS, possibly explaining 50% of cases: *SCN5A*, *SCN10A* and *CACNA1C* (17). However, given that the coding portions of these three genes are the largest among BrS-susceptibility genes, one could expect to find higher levels of variation affecting the corresponding transcripts. To take into account such biological parameters, burden tests were performed by comparing rare genetic variants detected in BrS cases with those identified in two independent control populations. In view of these burden tests, *SCN5A* appears to be the only gene—among the genes previously reported as involved in cardiac arrhythmia—to contribute significantly to the occurrence of BrS (~20% of BrS cases) in populations of European origin.

The prevalence of mutations/variants in BrS populations can vary greatly between studies in the absence of clear guidelines for the frequency definition of a 'rare' variant. In this study, we considered variants as 'rare' if the frequency observed in ethnically matched individuals from public databases was <0.1%. When considering only variants with an MAF below 0.1%, the proportion of *SCN10A* carriers among BrS cases reported by Hu *et al.* falls to 7.3% (versus 16.7% reported with a 0.5% threshold), a figure similar to the frequency of 6% reported in the present study. While 3.6% of BrS cases carried a rare functional variant in *CACNA1C*, rare variants were also identified in 2–6.6% of control individuals, and thus, no significant enrichment was observed among cases. In addition, rare genetic variation is extensive in the C-terminal region of $Ca_v1.2$, in both case and control groups. While this domain was previously reported as harbouring a high proportion of rare variants in BrS cases [50–66%, (18,19)], the observations reported here confirm that *CACNA1C* mutations in isolated BrS are unlikely and may be restricted to BrS patients also presenting with a short QT interval (9).

How to interpret rare genetic variation?

The burden test results demonstrate the usefulness of considering the extent of rare genetic variation in the general population when testing the contribution of rare alleles in the susceptibility to rare diseases. As discussed above, this strategy is particularly relevant for large genes or transcripts, such as *SCN5A*, *SCN10A*, *CACNA1C*, *PKP2*, *KCNH2* and *TRPM4*. Furthermore, several genes included in this study harbour rare variants in less than 1% of the control population (*HEY2, SCN2B*, *SCN3B*, *RANGRF*, *FGF12*, *GPD1L*, *KCNE3*, *KCNJ8*), indicating that although no statistical enrichment in rare variation could be observed for these genes, individual rare variants observed in patients with BrS might still be causally related to the cardiac electrical anomaly.

One common strategy to evaluate the potential contribution of any rare or private coding variant to disease is to interrogate functional prediction algorithms. However, a substantial proportion of the rare variants detected in controls are predicted to be deleterious or damaging with these tools (Supplementary Material, Table S2). Conversely, a subset of rare variants detected in BrS cases, although predicted as benign with the same tools, may impact the function of the encoded protein. For example, one variant in *SCN3B*, carried by one patient in our study, was previously reported in another unrelated case with BrS and is absent from European populations in public variation databases. Although this variant is predicted to be tolerated or benign by SIFT and PolyPhen-2 (20,21), it leads to a reduction in the sodium current density (15). This illustrates that prediction tools have limited power to predict the causality of rare missense variants and should be used with great caution, especially if used to guide clinical decision-making. In the absence of experimental data regarding the functional effect of one rare genetic variant, segregation analysis in relatives remains the strategy of choice to further estimate its causal relationship with disease.

Which insights into molecular diagnosis?

The current study indicates that *SCN5A* is the only major susceptibility gene for BrS identified so far, with rare coding variants in this gene accounting for ~20% of cases. Although additional genes such as *SCN10A*, *PKP2* and *CACNB2* may contribute to BrS susceptibility in small subsets of cases, the aetiology of this cardiac electrical anomaly remains largely unknown. Rare genetic variation identified in arrhythmia-susceptibility genes among patients with BrS should thus be interpreted with precaution, as the only criterion available so far to evaluate the arrhythmic risk is the presence of the Brugada ECG pattern. Genetic testing in BrS should thus be restricted to *SCN5A* in clinical diagnostic setting, as genetic counselling is unlikely to relieve patient distress under current knowledge on other reported susceptibility genes.

The authors of this study have recently reported that common genetic alleles at the *SCN5A-SCN10A* and *HEY2* loci are associated with BrS, with an unexpectedly high cumulative effect of the risk alleles on disease susceptibility (22). Further investigations addressing the combined effect of rare and common genetic variants on the BrS-specific ECG pattern are now required to shed light on the genetic aetiology of this complex arrhythmia disorder and better address risk stratification based on genetic information.

Limitations

NGS technologies allow high-throughput genetic screening in the context of molecular diagnosis, but some relevant variation may have been missed due to lower coverage depth for particular exon sequences. Controls have not undergone any pharmacological test to unmask a putative BrS-specific ECG pattern, and therefore, it is possible that the control sets are not devoid of such cases. We did not consider the possibility that rare alleles at risk and protective may occur in the same gene and therefore did not formally apply tests suited to this model.

Conclusions

This study demonstrates that, among the arrhythmia-susceptibility genes reported so far in the literature, only *SCN5A* accounts for a significant proportion of cases with BrS, with rare coding variants altering this gene in ~20% of cases versus <3% of individuals from a reference population. For every other gene, including *SCN10A* and *CACNA1C*, no enrichment of rare coding variation was observed. Extreme caution should thus be taken to avoid false-positive reports of causality in the context of genetic counselling, as rare coding variation with potential functional effect in arrhythmia-susceptibility genes is extensive among the general population.

Materials and Methods

This study was carried out in accordance with the ethical guidelines of the Declaration of Helsinki and with French guidelines for clinical and genetic research. Informed written consent was obtained from each individual who agreed to participate in the genetic study.

A full description of the methods is available as Supplementary Material.

Patient and control populations

A group of 167 index cases of European origin and diagnosed with BrS on the basis of the second consensus conference (1) was included in the study. Seventy-three cases (44%) were symptomatic and ECG examination revealed a baseline BrS pattern for 43% of the patients. The mean age at diagnosis was 48-year old (range: 38–58). The mean heart rate was 72 ± 12, with PR, QRS and QTc intervals of 179 ± 26, 104 ± 14 and 415 ± 32 ms, respectively. Two physicians reviewed the clinical and ECG data independently. The first control set ('internal') comprised 167 individuals of European origin aged over 65-year old and showing no history of cardiac arrhythmia. The second control set ('UK10K') included 881 whole-exome sequences released by the UK10K consortium (http://www.uk10k.org).

Targeted sequencing

We developed a custom design based on the HaloPlex™ technology (Agilent Technologies) to perform high-throughput sequencing of the coding regions of 45 genes previously linked to cardiac arrhythmias or conduction defects and/or sudden cardiac death, including 21 genes linked to BrS. Targeted coding regions (exons) ± 10 bp correspond to 141 kb of genomic sequence. Multiplex amplification and library preparation were performed following the manufacturer's instruction. Libraries were pooled to an equimolar concentration, then pools were diluted to a 4 pM final concentration before proceeding to 100 bp paired-end Illumina sequencing on MiSeq for the validation study (39 patients) and on HiSeq for the main study (167 cases/167 controls).

Variant calling

Raw sequence reads were aligned to the reference genome GRCh37 using BWA-MEM (version 0.7.5a) after removing adapter sequences with Cutadapt v1.2. GATK was used for indel realignment and base recalibration. Variants were called and considered for further analyses if found by both GATK UnifiedGenotyper (version 2.8) and Samtools mpileup (version 0.1.19), with a minimum quality score of 25. Variants were defined as rare if the MAF was <0.1% in the European population according to the 1000 genomes phase 1 data (379 individuals) and the NHLBI GO Exome Sequencing Project (ESP) data (4300 individuals). The potential pathogenicity of variants was determined using the Variant Effect Predictor (Ensembl) and filtering was performed using Knime4Bio (23).

Validation of the targeted sequencing system

Thirty-nine patients for whom genetic variation had previously been identified in arrhythmia-susceptibility genes were recruited to evaluate the performance of the targeted sequencing system. After DNA capture, sequencing on the Illumina MiSeq system resulted in a mean coverage depth of 141× per sample, with 94% of the targeted regions covered at least 10 times (Supplementary Material, Table S1). The variant-calling pipeline allowed the automatic detection of 67 out of the 68 (98.5%) genetic variants previously identified by capillary sequencing. The only undetected variant is a substitution located at a chromosomal position covered <10 times. In order to limit the risk of missing genetic variants, subsequent sequencing was performed on the Illumina HiSeq system, which enables higher coverage at reasonable cost, and only samples with a mean coverage depth above 100× were included in further analyses.

Burden tests

Burden tests were carried out to compare the proportion of cases and controls carrying at least one rare variant with potential functional consequence in a given gene. Two tests were performed for each gene: (i) 167 BrS cases versus 167 internal controls and (ii) 167 BrS cases versus 881 UK10K controls. First, we performed a Fisher's exact test for each variant, comparing allele counts in cases versus controls, to exclude variants with a *P*-value of <0.01, which would be potential false positives. We also excluded variants found in 5% or more of cases and/or controls but absent from European populations from the 1000 Genomes Project and/or ESP. For the second burden test (using UK10K data), only variants located in UK10K exome capture regions ± 100 bp were considered. Finally, the Cohort Allelic Sums Test (CAST) (24), based on a Fisher's exact test, was applied for each gene. Any *P*-value lower than 0.05 was considered as suggestive of association.

Supplementary material

Supplementary Material is available at *HMG* online.

Acknowledgements

We would like to thank the French clinical network against inherited cardiac arrhythmias, in particular the Hospitals of Tours, Brest, la Roche-sur-Yon, La Rochelle, Bayonne and la Réunion.

We are also grateful to the members of the Genomics and Bioinformatics Core Facility of Nantes (Biogenouest) for their technical expertise. This study makes use of data generated by the UK10K Consortium as controls. A full list of the investigators who contributed to the generation of these data is available from http://www.UK10K.org. The UK10K project was funded by the Wellcome Trust under award WT091310.

Conflict of Interest statement. None declared.

Funding

This work was supported by the Institut National de la Santé et de la Recherche Médicale (INSERM, ATIP-Avenir program to R.R.); the French Regional Council of Pays-de-la-Loire; and the Fondation pour la Recherche Médicale (FRM grant DEQ20140329545).

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