Genetic basis of cardiac ion channel diseases

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Publication date
2008

Chapter 7

Exclusion of multiple candidate genes and large genomic rearrangements in *SCN5A* in a Dutch Brugada Syndrome cohort

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Heart Rhythm. 2007;4(6):752-5

Editorial comment by C. Antzelevitch: Genetic basis of Brugada syndrome.
Heart Rhythm.2007;4(6):756-7

Abstract

Background The Brugada syndrome is an inherited cardiac electrical disorder associated with a high incidence of life-threatening arrhythmias. Screening for mutations in the cardiac Na^+ channel encoding gene *SCN5A* uncovers a mutation in approximately 20% of Brugada syndrome cases. Genetic heterogeneity and/or undetected *SCN5A* mutations, such as exon duplications and deletions, could be involved in the remaining 80% mutation-negative patients.
Objectives Thirty-eight *SCN5A* mutation-negative Dutch Brugada syndrome probands were studied. The *SCN5A* gene was investigated for exon duplication and deletion and a number of candidate genes (Caveolin-3, *Irx-3*, *Irx-4*, *Irx-5*, *Irx-6,* Plakoglobin, Plakophilin-2, *SCN1B*, *SCN2B*, *SCN3B* and *SCN4B*) were tested for the occurrence of point mutations and small insertions/deletions.
Methods We used a quantitative multiplex approach (MLPA) to determine *SCN5A* exon copy numbers. Mutation analysis of the candidate genes was performed by direct sequencing of PCR-amplified coding regions.
Results No large genomic rearrangements in *SCN5A* were identified. No mutations were found in the candidate genes. Twenty novel polymorphisms were identified in these genes.
Conclusion Large genomic rearrangements in *SCN5A* are not a common cause of Brugada syndrome. Similarly, the studied candidate genes are unlikely to be major causal genes of Brugada syndrome. Further studies are required to identify other genes responsible for this syndrome.

Keywords

Brugada syndrome, genetics, mutation, polymorphism, sodium channels, iroquois homeobox transcription factors, adherens junctions, caveolin-3, GPD1L

7.1 Introduction

The Brugada syndrome (MIM 601144), with an estimated 5–50 cases per 10,000 individuals (with a higher incidence in Asia than in the United States and Europe),[1] is characterized by sudden cardiac death from ventricular tachyarrhythmias, in combination with a typical ECG pattern of ST-segment elevation in leads V1-V3.[2] Brugada syndrome is associated with mutations in the gene encoding the cardiac sodium (Na^+) channel pore-forming subunit *SCN5A*.[3] The mutations described in this gene result in reduced Na^+ channel membrane expression, nonfunctional channels or channels that inactivate rapidly (see Tan et al.[4] for review), causing a reduction in available Na^+ current during the upstroke of the action potential. Mutations in *SCN5A* are found in approximately 20% of Brugada syndrome patients. Until now, mutation screening of this gene was focused on finding point mutations and small deletions or insertions. Screening for large rearrangements such as large duplications or deletions, which could also cause loss of Na^+ channel function has not yet been investigated. The first aim of this study was to analyze *SCN5A* exon copy numbers in mutation-negative Brugada syndrome probands by Multiplex Ligation-Dependent Probe Amplification (MLPA), a quantitative multiplex approach to determine the relative copy number of gene exons.[5]

Furthermore, other genes are very likely involved in the pathogenesis of Brugada syndrome. Weiss et al. excluded *SCN5A* as the gene causing Brugada syndrome in a large family, confirming genetic heterogeneity of the disorder.[6] Linkage to chromosome 3p22-25 close to *SCN5A* was found in this family. A mutation in the glycerol-3-phosphate dehydrogenase 1-like gene (*GPD1L*), was found in this family.[7] The second aim of this study was to screen the *GPD1L* gene as well as a number of candidate genes in Brugada syndrome. The candidate genes included Na^+ channel β-subunits (*SCN1B*, *SCN2B*, *SCN3B*, *SCN4B*),[8-14] caveolin 3 (*CAV3*),[15,16] members of the Iroquois family of transcription factors 3-6 (*Irx-3*, *Irx-4, Irx-5, Irx-6*),[17,18] and the adherens junction proteins Plakophilin-2 (*PKP2*) and Plakoglobin (*PKGB*).[19]

7.2 Material and methods

Patients

Thirty-eight Dutch Caucasian probands with a definite Brugada syndrome phenotype were ascertained at the Academic Medical Center (AMC), Amsterdam. The study was performed according to a protocol approved by the local ethics committee. Informed consent was obtained from the patients. Coding region and splice site mutations in *SCN5A* had been previously excluded in all probands by SSCP-DNA sequencing, dHPLC-DNA sequencing or by direct sequencing using primers in flanking intronic sequences.[20]

MLPA analysis

Probes for MLPA analysis of *SCN5A* exons 1-4, 6, 7, 9, 11, 15, 17, 19, 21-23, 25, 27, 28 and intron 1 (exon and intron numbering according to transcript NM_000335) were developed by MRC Holland (Amsterdam) in close collaboration. The remaining exons of this gene were not probed since they are in very close proximity to exons that were probed. In probe design, polymorphic sequences were avoided, because they could hamper hybridization and quantification. An additional 13 control probes for unlinked loci were also included. The MLPA procedure and analysis were carried out according to the manufacturer's instructions and as described previously.[21]

Mutation analysis

Mutation screening of *GPD1L*, *SCN1B*, *SCN2B*, *SCN3B*, *SCN4B*, *CAV3*, *Irx-3*, *Irx-4*, *Irx-5*, *Irx-6*, *PKP2* and *PKGB* was performed by PCR amplification of coding regions and flanking intronic regions followed by direct sequencing of amplicons on an ABI prism 3730 DNA Sequence Detection System. All primer sequences and PCR conditions are available upon request. Seventy-five Caucasian controls (150 alleles) were used to investigate whether identified novel nucleotide changes predicting a non-synonymous amino acid substitution occurred in the general population. This was done by direct sequencing or restriction enzyme analysis.

7.3 Results

The study included 38 patients, of which 33 were male. Thirty patients showed spontaneous ST-segment elevation on baseline ECG, while 8 patients had ST-segment elevation after drug-challenge with flecainide.

MLPA analysis
No large exon duplications or exon deletions in *SCN5A* were detected by MLPA analysis.

Mutation analysis
No coding region mutations were found in the *GPD1L* gene as well as the candidate genes tested. We identified 52 polymorphisms (of which 11 were non-synonymous), including 20 novel ones (Table 1). In these patients, 34 polymorphisms were detected with a minor allele frequency of ≥5%. The most common polymorphism was a transition in intron 3 of *SCN2B* (rs8192613), which was present in 68% of patients. Novel variants predicting non-synonymous amino acid substitution (p.Val99Met in *SCN2B*; p.Arg142His and p.Val648Ile in *PKGB*; p.Asp26Asn, p.Ser70Ile and p.Ser140Phe in *PKP2*) were screened in 75 control individuals and were found to be rare polymorphisms (data not shown).

7.4 Discussion

Large genomic rearrangements in *SCN5A*, coding region mutations in *GPD1L*, and coding region mutations in a number of candidate genes (*SCN1B*, *SCN2B*, *SCN3B*, *SCN4B*, *CAV3*, *Irx-3*, *Irx-4*, *Irx-5*, *Irx-6*, *PKGB*, *PKP2*) were excluded in 38 patients with Brugada syndrome. Mutations in these candidate genes are therefore unlikely to be major causes of Brugada syndrome.

SCN5A gene rearrangements
Till now, mutations in *SCN5A* causing Brugada syndrome have included missense and nonsense mutations, small insertions and deletions, frameshift mutations, and mutations affecting splice sites. In this study we examined the possibility that large genomic rearrangements in *SCN5A* that hypothetically lead to loss of Na^+ channel function, could also cause Brugada syndrome. Such mutations were however not detected in our Brugada syndrome patients previously tested negative for *SCN5A* coding region and splice site mutations. With regards to the pathogenetic mechanism of *SCN5A* in Brugada syndrome, the possibility remains that mutations in intronic regions could also be responsible for the disorder in these patients.

Table 1: Polymorphisms identified during candidate gene screening in Brugada syndrome patients (n=38). Minor allele frequency (MAF) refers to the frequency in the 76 alleles tested.

Gene	Variant	rs number‡	Location	Minor allele	MAF
GPD1L **(NM_015141)**	c.111A>C*	NA	exon 2	C	0.01
	c.408C>T*	NA	exon 4	T	0.14
	c.506-124C>G*	NA	intron 4	G	0.05
	c.618+23delA	rs11351972	intron 5	del	0.03
CAV3 **(NM_033337)**	c.27C>T	rs1974763	exon 1	T	0.21
	c.99C>T	rs1008642	exon 1	T	0.21
	c.114+26G>A	rs11922879	intron 1	A	0.05
	c.11531_47delAGCGGGTGGCTTCTGTG*	NA	intron 1	del	0.22
	c.115-23G>C*	NA	intron 1	C	0.11
	c.123T>C	rs13087941	exon 2	C	0.37
Irx-3 **(NM_024336)**	c.1-160_172delCGTCGCCGCCGC	rs3833834	5'UTR	del	0.31
	c.1113G>T*	NA	exon 2	T	0.01
	p.Gln479His	rs1126960	exon 3	T	0.22
	c.1506+ 67del A	rs11448817	3'UTR	del	0.01
Irx-4 **(NM_016358)**	c.1-27G>A	rs2232372	5'UTR	A	0.05
	c.90A>C	rs2232374	exon 2	C	0.26
	c.297+6T>G	rs2307118	intron 2	G	0.25
	p.Ala119Thr	rs2232376	exon 3	A	0.21
	c.381A>G	rs4975753	exon 3	G	0.25
	c.1203G>A	rs1689717	exon 5	A	0.08
	c.1431C>T	rs2279589	exon 5	T	0.16
Irx-5 **(NM_005853)**	p.Pro225Thr	rs13336114	exon 3	A	0.45
Irx-6 **(NM_024335)**	c.291G>T*	NA	exon 2	T	0.03
	c.414-5C>T	rs31082	intron 3	T	0.33
	c.420C>T*	NA	exon 4	T	0.03
	c.1333+15G>A	rs31087	intron 5	A	0.34
PKGB **(NM_021991)**	c.-88A>G	rs4796702	5'UTR	A	0.34
	c.208+18_19delCG*	NA	intron 1	wt	0.34
	c.213T>C	rs7405731	exon 3	T	0.18
	p.Arg142His*	NA	exon 3	A	0.04
	c.909+17T>C	rs12942034	intron 5	T	0.14
	c.1774-34C>A	rs8067890	intron 10	C	0.28
	c.2046+22A>G	rs7216034	intron 12	A	0.28
	p.Val648Ile*	NA	exon 12	A	0.03
	p.Met697Leu	rs12937241	exon 14	A	0.28
PKP2 **(NM_004572)**	p.Asp26Asn*	NA	exon 1	A	0.01
	p.Ser70Ile*	NA	exon 1	T	0.03
	p.Ser140Phe*	NA	exon 3	T	0.01
	p.Leu366Pro	rs1046116	exon 4	C	0.16
	c.2299+7C>T*	NA	intron 11	T	0.01
	c.2489+14insC*	NA	intron 12	ins	0.22
	c.2578-69G>A	rs7956824	intron 13	A	0.31
	c.2578-60C>A*	NA	intron 13	A	0.03
SCN1B **(NM_001037)**	c.40+15G>T*	NA	intron 1	T	0.20
	c.351C>T	rs3746255	exon 3	T	0.02
	c.501T>C	rs16969930	exon 4	C	0.02
SCN2B **(NM_004588)**	c.237+27A>G	rs645675	intron 2	G	0.03
	p.Val99Met*	NA	exon 3	A	0.01
	c.449-12C>A	rs8192613	intron 3	A	0.43
SCN3B **(NM_018400)**	c.55 +44C>T	rs3851102	intron 1	T	0.18
	c.438C>T	rs1275085	exon 3	T	0.07
SCN4B **(NM_174934)**	c.174C>T*	NA	exon 2	T	0.04

*Novel variant; ‡data from the Ensembl database (www.ensembl.org); NA: not applicable.

GPD1L

Mutations in the Brugada syndrome associated gene *GPD1L* were not found, implying that Brugada syndrome-causing mutations in this gene are rare.

Candidate genes

The multifunctional β-subunits *SCN1B*, *SCN2B*, *SCN3B* and *SCN4B* regulate the level of expression of voltage-gated Na^+ channels at the plasma membrane, control gating of these channels, and are involved in cell adhesion.[8-14] Recently, a nonsense mutation in *SCN4B* that functionally disturbed cardiac Na^+ channel function was reported in a patient with long QT syndrome,[22] a primary rhythm disorder associated with syncope and sudden death.[23]

Caveolae are involved in vesicular trafficking and serve as a platform to organize and regulate a variety of signal transduction pathways. Interestingly, caveolae have been described to colocalize with *SCN5A* and thereby may be involved in the formation of a Na^+ channel macromolecular complex.[16] Caveolins are the principal proteins of caveolae. *CAV3*-encoded caveolin-3 is specifically expressed in cardiomyocytes and skeletal muscle. Recently, mutations in *CAV3* altering cardiac Na^+ channel function have been described in Long QT syndrome.[15]

The I_{to}–mediated phase 1 repolarization which gives rise to a notched appearance of the action potential, is more prominent in ventricular epicardium compared to endocardium.[24,25] This transmural gradient of expression of I_{to} has been implicated in the pathogenesis of Brugada syndrome.[26] It is thought that the transcription factors *Irx-3* and *Irx-5* underlie this transmural gradient.[17,18] Furthermore, *Irx-4* and *Irx-5* interact with the cardiac transcriptional corepressor mBop[17,27] and thereby repress expression of *Kcnd2* which encodes the α-subunit of the voltage-gated K^+ channel Kv4.2 conducting I_{to}. Besides *Irx-3-5*, *Irx-6* was also considered a candidate gene in this study, since its expression pattern is similar to that of *Irx-5*.[28]

Mutations in *PKP2* and *PKGB*, components of adherens junctions, have been associated with arrhythmogenic right ventricular cardiomyopathy (ARVC, see Sen-Chowdhry et al.[29] for review). Like ARVC, Brugada syndrome affects primarily the right ventricle and a structural degeneration component is also increasingly recognized.[19]

Mutation screening of these candidate genes did not reveal any mutations in our Brugada syndrome patient cohort. We cannot however exclude the possibility of mutations in these genes in other cohorts. Similarly, mutations within promoter and intronic regions of these genes could still be involved.

In this study, numerous novel variants were uncovered in the candidate genes studied. The exact prevalence of these polymorphisms in Caucasians needs to be investigated further in a larger sample. These variants could be of interest in future studies interrogating the association between common genetic variation and modulation of arrhythmia susceptibility in various settings. In conclusion, we have excluded multiple candidate genes in a Dutch Brugada syndrome cohort. Further studies are required to identify novel gene(s) responsible for the Brugada syndrome.

Acknowledgements

The authors wish to thank I. van Twillert, D.H.M. van Gent and F. Asidah for technical assistance, and Dr. V.M. Christoffels for helpful discussions. This study was supported by Netherlands Heart Foundation grants 2003B195 (CRB) and 2003T302 (AAMW), and the Interuniversity Cardiology Institute of the Netherlands (project 27, AAMW). Dr. Bezzina is an Established Investigator of the Netherlands Heart Foundation (Grant 2005/T024).

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