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

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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 to determine *SCN5A* exon copy numbers. Mutation analysis of the candidate genes was performed by direct sequencing of polymerase chain reaction–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

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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 electrocardiogram (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 sodium channel membrane expression, nonfunctional channels, or channels that inactivate rapidly (see Tan et al for review[4]), 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 sodium 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[6] excluded *SCN5A* as the gene causing Brugada syndrome in a large family, confirming the genetic heterogeneity of the disorder. 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 also 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 sodium 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*).[29]

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Materials and methods

Patients

Thirty-eight Dutch Caucasian probands with a definite Brugada syndrome phenotype were ascertained at the Academic Medical Center, 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 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 the 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 elsewhere.[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 polymerase chain reaction (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 on request. Seventy-five Caucasian controls (150 alleles) were used to investigate whether identified novel nucleotide changes predicting a nonsynonymous amino acid substitution occurred in the general population. This was done by direct sequencing or restriction enzyme analysis.

Results

The study included 38 patients, 33 of whom were men. Thirty patients showed spontaneous ST-segment elevation on baseline ECG, while eight 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 or in the candidate genes tested. We identified 52 polymorphisms (of which 11 were nonsynonymous), 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 nonsynonymous 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).

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

Until 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*, which hypothetically lead to loss of sodium channel function, could also cause Brugada syndrome. Such mutations were, however, not detected in our Brugada syndrome patients who 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.

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 with endo-