Repeated molecular genetic analysis in Brugada syndrome revealed a novel disease-associated large deletion in the SCN5A gene

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Introduction

Brugada syndrome (BrS) is a rare inherited cardiac disorder with a structurally normal heart.[1] The disease is associated with a severe outcome including syncope, cardiac arrest, and sudden cardiac death despite low penetrance and variable expressivity.[2] Electrocardiogram (ECG) characteristics of BrS are divided into 3 different types. Only type 1, with a prominent coved ST-segment elevation of ≥ 2 mm followed by a negative T wave in the right precordial leads (V_1–V_3), is diagnostic for BrS. Type 2 and 3 patterns demonstrate an ST-segment elevation of 2 mm and 1 mm, respectively, followed by a descending ST slope and a positive T wave, thereby forming a saddleback configuration. These ECG characteristics might be either concealed or intermittent.[3]

More than 300 mutations in 17 genes have been associated with BrS. The single most important gene is SCN5A, with an incidence of 11%–24%. BrS subtypes 2–17 are rare, with a very low incidence.[4] The molecular genetic diagnostic yield is therefore relatively low and only one third are successfully genotyped.[5] This case report underlines the importance of repeated genetic screening with multiplex ligation–dependent probe amplification (MLPA) in patients previously tested with routine Sanger DNA sequencing. Detection of large genomic rearrangements can be highly beneficial in further family evaluation of close relatives.

Case report

A 27-year-old man (II-1) was referred to the hospital in 2004 after a severe syncope during rest (Figure 1). Previously, he had experienced 2 episodes of syncope at rest, but without making contact to the health care system.

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The ECG demonstrated a Brugada type 1 pattern (Figure 2A). Transthoracic echocardiography showed a structurally normal heart and the coronary angiogram demonstrated normal coronary arteries. An electrophysiological study was performed without inducible sustained supraventricular or ventricular arrhythmias. There was no family history of arrhythmias, syncope, or sudden cardiac death. After informed consent an implantable cardioverter-defibrillator (ICD) was implanted. Within 6 months after ICD implantation 3 appropriate ICD therapies on ventricular fibrillation were observed (Figure 2B). At that time DNA Sanger sequencing revealed no mutations in the SCN5A gene.

Nine years later the index patient's 7-year-old son (III-2) was diagnosed with attention deficit–hyperactivity disorder (ADHD) and needed medical treatment (Figure 1). His baseline ECG showed a slight ST-segment elevation in leads V_1 and V_2, which raised the suspicion of BrS (Figure 2C). However, the observed ECG changes were not diagnostic for BrS. Before medical treatment for ADHD was initiated, we decided to repeat the molecular genetic analysis of the SCN5A gene, including MLPA for detection of large genomic deletions or insertions.

Surprisingly, the index patient (II-1) was now identified heterozygous for a deletion of exon 23, while the son (III-2) had 2 intact alleles. Mutations in the SCN5A gene that are linked to BrS are scattered throughout the entire gene. Exon 23 of the SCN5A gene encompasses bases 3841–3963 (NM_198056.2), corresponding to codons 1281–1321. The probe for exon 23 of the SCN5A gene binds at nucleotide 3872–3947, meaning that it spans almost the entire exon, and that breakpoints probably are located in the flanking introns. The exact locations of the breakpoints were not established. The deletion affects S3–S4/S5 of domain III of the protein. It is highly plausible that the transcripts that may be produced by the truncated allele are subject to nonsense-mediated RNA decay, leaving the affected patient with haploinsufficiency regarding SCN5A, causing BrS.

Cascade screening of relatives was established in accordance with international guidelines.[6] We found that the father (I-1) and both sisters (II-2 and II-3) carried the deletion,

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KEY TEACHING POINTS

- Molecular genetic testing including multiplex ligation–dependent probe amplification is important to identify large genomic rearrangements.
- Large genomic rearrangements in the SCN5A gene are very rare.
- Disease penetrance in Brugada syndrome is low.

while the mother (I-2) had 2 normal alleles (Figure 1). SCN5A deletion–positive relatives had normal ECGs and echocardiograms, and flecainide testing did not reveal a diagnostic Brugada pattern. All 3 relatives were offered annual follow-up without ICD and after 28 months of follow-up they remain asymptomatic.

Molecular genetic analysis

DNA was extracted from leukocytes. Screening for mutations in the SCN5A gene including all exons and exon–intron boundaries was performed with the LightScanner (Biofire Diagnostic Inc., Salt Lake City, Utah), unlabeled primers, and the intercalating fluorescent dye LCGreen. Sanger sequencing of exons with aberrant melting patterns was performed using BigDye v1.1 (Applied Biosystems, Foster City, California) and Genetic Analyzer 3130XL (Applied Biosystems, Foster City, California). Sequences were interrogated/queried with Gensearch v4.0 (Phenosystems, Wallonia, Belgium) or Sequencer v5.0 (Gene Codes Corporation, Ann Arbor, Michigan) software. Primer sequences for Sanger sequencing were partly designed by Millat et al[7] and partly designed in house, and are available upon request.

For detection of large genomic deletions or insertions MLPA was performed with the SALSA MLPA probe mix P108-B2 SCN5A (MRC Holland, Amsterdam, The Netherlands), which holds probes for all exons from 1b through 29 (NM_198056.2). Results were analyzed with GeneMapper software (Applied Biosystems, Foster City, California), and deviations from 2 reference samples were assessed by MAQ-S software (Multiplicom, Niel, Belgium).

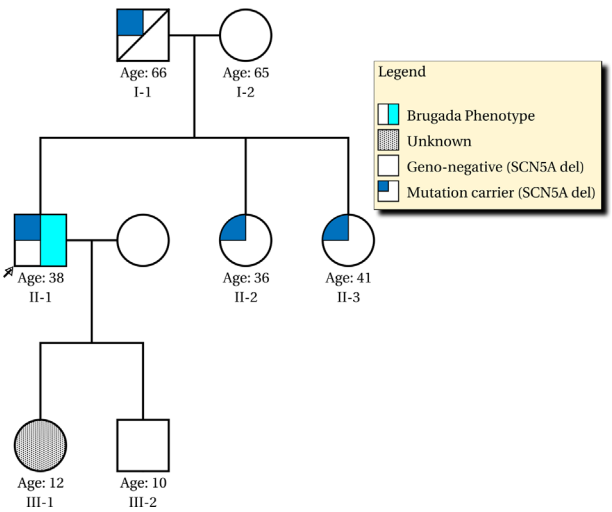

Figure 1 Family pedigree. Index patient is marked with an *arrow*.

Discussion

Genetic testing in cardiac arrhythmias has been widely used during the past couple of decades. MLPA, however, has not been part of the routine practice in most laboratories worldwide until recently. Since September 2009 we have tested all samples received for BrS testing as well as testing other inherited cardiac arrhythmias for large deletions/duplications, using MLPA. No large gene rearrangements in SCN5A were found until the present case.

The index patient presents a malignant Brugada phenotype, but the penetrance in the family is surprisingly very low. Despite carrying a large SCN5A deletion, which encompasses more than an entire exon, the relatives do not exhibit a Brugada phenotype. However, low disease penetrance is not a unique feature of BrS, and is also well known in other channelopathies like long QT syndrome (LQTS).[8]

Mutations in the alpha subunit of the voltage-gated sodium channel (SCN5A) are a well-known cause of both hereditary LQTS and BrS. Where the mutations causing LQTS induce gain of function of the protein, mutations causing BrS induce loss of function.[9] The most common genetic alterations in the SCN5A gene are missense mutations, followed by nonsense, frameshift, and splice-site mutations. Large deletions/insertions are extremely rare worldwide.[10]

Others have documented the rarity of large deletions/duplications. In a Dutch study, 38 SCN5A mutation–negative index patients were investigated for exon duplications and deletions without detection of large genomic rearrangements.[11] Recently, a similar study on 37 Spanish BrS patients was performed without discovering large gene rearrangements.[12] Other groups have documented the presence of large deletions in the SCN5A gene, but these cases are rare.[13–15]

Although the presence of large deletions/insertions in SCN5A has been reported before, this case emphasizes the importance of repeated genetic testing with MLPA in patients with obvious disease phenotypes, even though they previously have been tested with routine Sanger sequencing technique. The benefits are clear, and 50% of the relatives are exonerated from having a disease-causing variant, like the 7-year-old boy described here. Shortly after genetic testing, the boy could start his much-needed medication for ADHD without an increased risk of life-threatening arrhythmias.

Conclusion

If routine molecular genetic testing is limited to DNA sequencing, findings of large genomic deletions/insertions will continue to be rare, leading to under-diagnosis of hereditary arrhythmia cases as BrS. Repeated DNA analysis with addition of the MLPA technique is beneficial and increases the diagnostic yield of molecular genetic variants causing BrS.

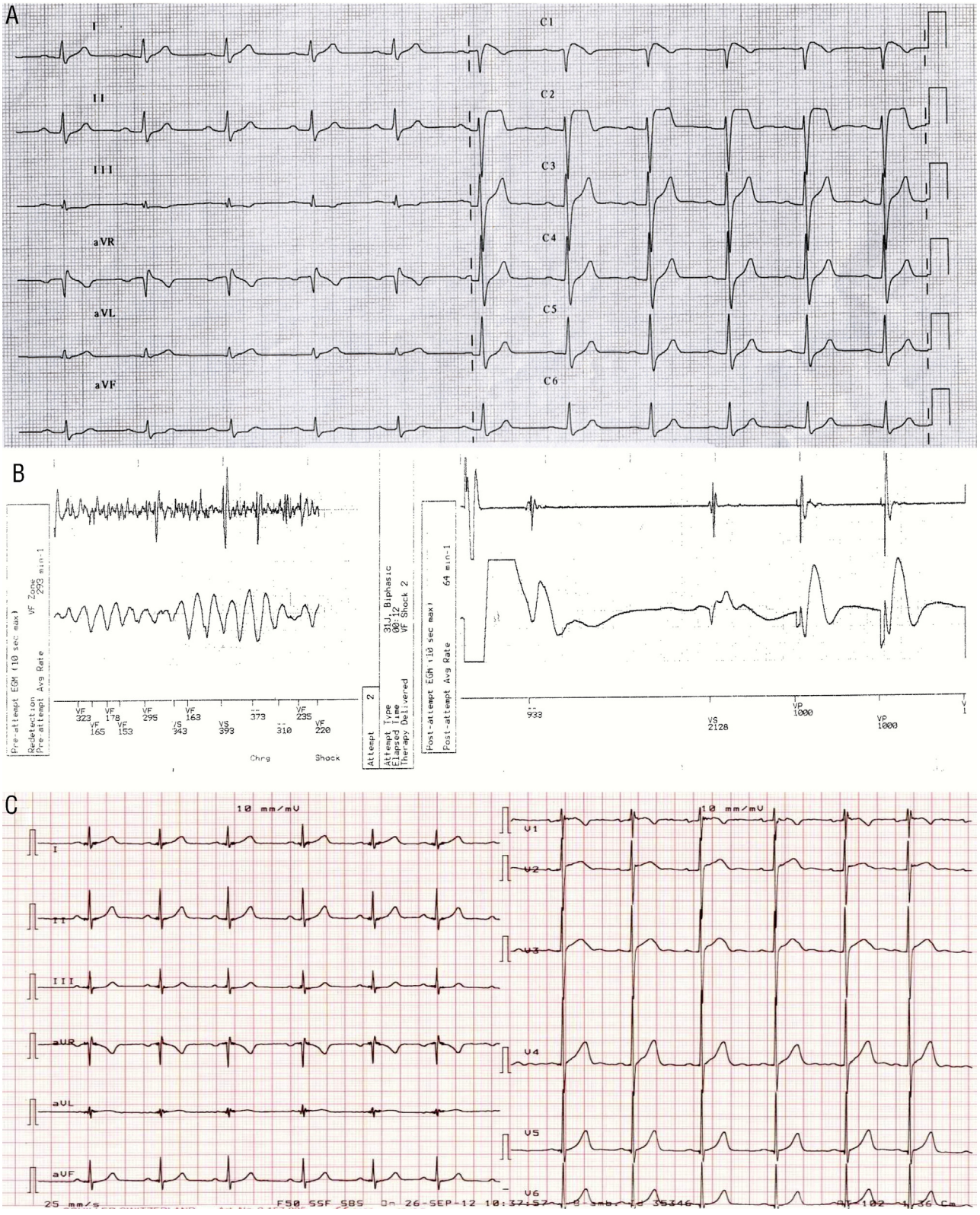

Figure 2 **A:** A 12-lead electrocardiogram (ECG) from the index patient with type 1 pattern diagnostic for Brugada syndrome. **B:** Implantable cardioverter-defibrillator (ICD) interrogation in the index patient, with ventricular fibrillation eliciting appropriate and effective ICD therapy. **C:** A 12-lead ECG from the son of the index patient.

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