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Clinical and Molecular Heterogeneity in the Brugada Syndrome

A Novel Gene Locus on Chromosome 3

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Background—Brugada syndrome is a form of idiopathic ventricular fibrillation characterized by a right bundle-branch block pattern and ST elevation (STE) in the right precordial leads of the ECG. Sodium channel blockers increase STE. Mutations of the cardiac sodium channel SCN5A cause the disorder, and an implantable cardioverter-defibrillator is often recommended for affected individuals. Mutations in other genes have not been identified, and it is not known if the efficacy of drug testing or the malignancy of arrhythmias correlates to the gene defect.

Methods and Results—We performed histories, physical examinations, ECGs, and drug testing on a large multigenerational family with Brugada syndrome. DNA isolated from blood samples, polymorphic genomic markers, and polymorphisms within candidate sodium channels were used for a genome-wide screen, fine mapping, and linkage analysis. We identified 12 affected individuals (right bundle-branch block, ≥1-mm STE) with an autosomal dominant inheritance pattern characterized by incomplete penetrance that appeared to be dependent on age and sex. Four affected individuals had syncope and 2 had documented ventricular arrhythmias, but there was minimal family history of sudden death. Procainamide infusions did not identify additional affected individuals. Linkage was present to an ∼15-cM region on chromosome 3p22-25 (maximum LOD score = 4.00). The sodium channel genes SCN5A, SCN10A, and SCN12A on chromosome 3 were excluded as candidates (LOD scores ≤ -2).

Conclusions—A Brugada syndrome locus distinct from SCN5A is associated with progressive conduction disease, a low sensitivity to procainamide testing, and a relatively good prognosis in a single large pedigree. (Circulation. 2002;105:707-713.)

Key Words: arrhythmia ■ ion channels ■ genetics ■ death, sudden

Syncope, ventricular fibrillation (VF), and sudden cardiac death have been reported in patients with no overt structural heart disease in association with a right bundle-branch block pattern (RBBB) and ST elevation (STE) in the right precordial leads of the ECG.1-3 This condition has come to be known as Brugada syndrome.4 Brugada syndrome is inherited in an autosomal dominant manner, is diagnosed predominantly in men, and is rare in the United States, although the exact prevalence is unknown and it may be considerably more common in southeast Asia.5,6 Sudden death is common, may be the first manifestation of disease, and is not prevented by antiarrhythmic drug therapy.3 The surface ECG manifestations of the syndrome can transiently disappear in affected individuals, although the risk of arrhythmias remains.3,7 This had led to the recommendation for implantable cardioverter-defibrillator (ICD) placement in affected individuals with inducible VF during cardiac electrophysiology study.8

Mutations of the cardiac sodium channel SCN5A cause some cases of the Brugada syndrome.9 A decrease in the depolarizing inward sodium current is thought to lead to early repolarization in the right ventricular epicardium, where the transient outward K" current (Ito) is large.10 This would cause a voltage gradient from endocardium to epicardium, STE on the ECG, and susceptibility to arrhythmias caused by phase 2 reentry. In support of this theory, sodium channel blocking agents unmask the ECG phenotype and are used clinically for the diagnosis of Brugada syndrome.7 Other genetic causes of the Brugada syndrome have not been reported, although <20% of affected patients have detectable SCN5A mutations.11 It is not known if the utility of provocative drug testing with sodium channel blockers or the incidence of arrhythmias and sudden death depend on the genetic defect.

In the present report, we describe a large multigenerational family with an autosomal dominant form of the Brugada
syndrome that is age and sex dependent. Linkage to a locus on chromosome 3p22-25 distinct from SCN5A is identified, confirming genetic heterogeneity of the disorder. The low incidence of sudden death in this family and the relative resistance to sodium channel–blocking agents raise the possibility that the efficacy of diagnostic and therapeutic interventions may be gene dependent.

**Methods**

**Clinical Testing**

The Institutional Review Board at the University of Pittsburgh approved all human studies. The proband was referred to our institution after a syncopal event. At-risk family members were asked to participate in this study. For those who agreed, a brief clinical history (including previously diagnosed cardiac conditions, syncope, palpitations, and medications), a physical examination, a 12-lead ECG, a 24-hour Holter monitor, an echocardiogram, and cardiac MRI were performed and evaluated by the investigators. Serial ECGs were obtained every 6 months when possible and from prior hospital records when available. For family members who had died, surviving relatives were questioned and hospital records were examined when available. A single ECG was performed on spouses to exclude inherited cardiac arrhythmopathies.

Individuals were considered affected if they had at least a single ECG with an RBBB pattern (RSR' in leads V1 or V2) and ≥1 mm of J-point elevation and STE (measured 80 ms after the J-point) in the right
precordial leads V1 through V3. Individuals were considered to be "probable affecteds" if they had an intraventricular conduction delay and ≥1 mm of STE or J-point elevation in the right precordial leads. Individuals were classified as "unaffected" only if all ECGs were within normal limits, had a QRS duration of ≤0.09 seconds, and lacked any STE or J-point elevation. All other individuals not meeting these strict criteria were classified as uncertain.

Drug testing with intravenous procainamide (14 mg/kg) was performed in a subset of family members. ECGs were obtained before and at 5-minute intervals during and for 30 minutes after completion of drug infusion. An increase in STE of 0.1 mV after initiation of drug or a change from a saddleback to a coved type of STE was considered a positive test.

Data are presented as mean±SEM. A value of P<0.05 is considered significant.

**Linkage Analysis**

Blood samples (~10 mL) were obtained from participating family members and spouses, and genomic DNA was isolated (Puregene, GENTRA Systems, Inc, Minneapolis, Minn). Polynucleotide repeats were amplified by polymerase chain reaction with the use of 32P end-labeled primers and run on 6% denaturing polyacrylamide gels. Initially, linkage to the candidate sodium channel SCN5A on chromosome 3 was excluded by means of MapPairs D3S1100 and D3S1298 (Research Genetics) and primers for a polymorphic dinucleotide repeat in intron 16 of SCN5A. If end-labeled primers and run on 6% denaturing polyacrylamide gels. Initially, linkage to the candidate sodium channel SCN5A on chromosome 3 was excluded by means of MapPairs D3S1100 and D3S1298 (Research Genetics) and primers for a polymorphic dinucleotide repeat in intron 16 of SCN5A. Single-stranded conformational polymorphism was also used to identify exonic mutations in SCN5A as previously described. A 10-cM genome-wide scan was then performed by the Mammalian Genotyping Service of the Marshfield Medical Research Foundation. Fine mapping was performed on areas of chromosome 3 and 10, where LOD scores exceeded 1.5.

To characterize linkage to the other sodium channels on chromosome 3, clones were isolated by screening a human genomic library (Stratagene, La Jolla, Calif), and polymorphic dinucleotide repeats were identified in the introns adjacent to exon 5 of SCN10A and exon 8 of SCN12A. The sense and antisense primers used to amplify the segments were SCN10A: 5'-GCAGGTTATACC-TTCTTTCG-3' and 5'-GCATCTGTCTTCGACATGC-3' and SCN12A: 5'-GTCTTTACTTAGGCACAGCAAG-3' and 5'-GCAA-CTGGGAAAGGAGGAAAG-3'.

Linkage was assessed by means of a parametric analysis and the VITESSE program, assuming an autosomal dominant inheritance with (1) a rare disease allele frequency (1/10 000) and (2) liability classes based on age that were estimated from ECG characteristics in the family. For age ≤30 years, penetrance=80% and phenocopy rate=1/1000. For age >30 years, penetrance=90% and phenocopy rate=1/100. Multipoint analysis was performed for adjacent loci. Unaffected individuals of age <20 years were excluded from the analysis because of concern over penetrance of the phenotype. An LOD score ≥3.0 was considered indicative of linkage to a given locus, and a LOD score ≤−2 was considered to exclude linkage.

**Results**

**Clinical History of the Proband**

The proband was a 56-year-old, previously healthy man of Italian descent with a history of cigarette use but no other cardiac risk factors who went to his local emergency room after a witnessed, unheralded syncopal episode while sitting. On presentation, his ECG showed sinus rhythm, first degree AV block, a normal QT interval, a RBBB, a leftward axis, and STE in leads V1 through V3 consistent with the Brugada syndrome (Figure 1A). Cardiac enzymes were normal, and his ECG did not evolve. A cardiac catheterization showed a left dominant system, no coronary artery disease, normal filling pressures, possible mild hypokinesis of his mid-left anterolateral wall, and a preserved right and left ventricular ejection fraction. Infusion of procainamide increased the baseline STE. Electrophysiological testing demonstrated borderline prolongation of the HV interval (60 ms), and ventricular fibrillation was induced with 3 extrastimuli. He was referred to our hospital for further evaluation and treatment.

A gated MRI showed mild fatty infiltration in the right ventricle (RV) (usually considered a nonspecific finding) but no fibrofatty infiltration or RV dilation diagnostic of arrhythmogenic right ventricular dysplasia (ARVD). An ICD was implanted. Several weeks later, 2 syncopal events occurred while...
ECGs were diagnostic for the Brugada syndrome. PR interval of 12 (42%) affected individuals, only a fraction of the available Eleven of the 12 affected individuals were male (Table 1). For 5 Clinical Characteristics of Affected Individuals

Clinical Analysis of the Pedigree

The proband was the youngest of 13 children and part of a large multigenerational family (Figure 2). The proband’s father died at age 80 years of a stroke. He had known conduction system disease and a pacemaker, and review of his ECG classified him as a probable affected individual. Two of the proband’s siblings died of noncardiac causes (sister as an infant with pneumonia and a brother at age 21 years in the military). One nephew of the proband had a history of chest pain and alcohol use and died in his sleep at age 50 years; no ECG was available. One niece of the proband died at birth. There was no other family history of sudden death.

Serial ECGs and blood draws were performed on 72 at-risk members of the family at age ≥18 years. A total of 12 members of the family were classified as affected (age range, 22 to 75 years), 4 as probably affected (age range, 26 to 78 years), and 7 as unaffected (age range, 24 to 50 years). The remaining family members were classified as uncertain, predominantly because of J-point elevation and mild degrees of QRS widening. Three affected brothers of the proband had a history of syncope or near-syncope and were advised to have ICDs placed. Two agreed, and electrophysiologic studies before ICD placement showed normal HV intervals and reproducibly inducible sustained polymorphic ventricular tachycardia or VF. Intracardiac electrograms from one examined during a routine follow-up visit documented an asymptomatic 26-beat run of ventricular tachycardia at a cycle length of 240 to 270 ms that self-terminated.

Clinical Characteristics of Affected Individuals

Eleven of the 12 affected individuals were male (Table 1). For 5 of 12 (42%) affected individuals, only a fraction of the available ECGs were diagnostic for the Brugada syndrome. PR interval was prolonged on at least one ECG in 7 of 12 (58%), with a mean PR interval for affected individuals of 188±24 ms. QRS duration of the affected individuals tended to increase as a function of age (0.55 ms/y, r=0.46), and affected individuals with serial ECGs demonstrated progressive QRS widening (Figure 3). The degree of J-point elevation, on the other hand, did not increase with age (−0.01 mm/y, r=0.16).

Ten of the affected individuals underwent clinical testing including echocardiography, gated MRI, and Holter monitoring (Table 1). Left ventricular function was normal in all of the affected individuals tested. Physical examination revealed a murmur suggestive of mitral regurgitation in 2 affected individuals, and 4 of the older affected individuals had echocardiographically documented mitral valve abnormalities (mild or moderate mitral regurgitation, myxomatous leaflets, mitral valve prolapse). One affected individual (III-55) had mild right ventricular dilatation by echocardiogram. No changes typical of ARVD (fibrofatty infiltration) were seen by MRI in him or in any other affected family members. No ventricular arrhythmias were documented on 24-hour Holter monitors.

Drug testing with intravenous procainamide was performed on 15 at-risk members of the family (7 affected, 2 probably affected, 6 uncertain). Intravenous procainamide increased the precordial STE by at least 0.1 mV in 4 of 7 affected individuals (Table 1). Of note, the pattern of STE

![Figure 3. QRS duration as function of age for 4 affected individuals with serial ECGs over a period of at least 5 years.](http://circ.ahajournals.org/)
never changed from saddleback to coved, none of the probably affected or uncertain family members developed the typical Brugada ECG pattern, and no individuals were reclassified on the basis of this testing.

**Linkage of This Family to a Locus on Chromosome 3p22–25**

A genome-wide screen at a density of 10 cM (Marshfield Medical Research Foundation, Marshfield, Wis) yielded a region on chromosome 3 and another on chromosome 10 with LOD scores >1.5. Fine mapping on chromosome 10 yielded no LOD scores >2.0, whereas fine mapping on chromosome 3 demonstrated linkage with a maximum LOD score of 4.0 at markers D3S3047, D3S1283, and D3S3547 (Table 2). Limiting the analysis to affected individuals yielded a maximum LOD score of 3.0. Multipoint and haplotype analyses localized the region of interest to 3p22–25 (Figures 4 and 5).

**Discussion**

We have identified a large multigenerational family with ventricular tachyarrhythmias and STE in the right precardial leads similar to those described in prior reports of the Brugada syndrome. We excluded them as candidates by using polymorphic dinucleotide repeats within each gene (2-point and multipoint LOD scores < −2.5). We also excluded a number of candidates that map to this area of chromosome 3 (OMIM database, NCBI) by linkage analysis with intragenic polynucleotide repeats. These include (1) the plasma membrane Ca2+-ATPase, Type 2 (PMCA2), (2) the inositol 1,4,5-triphosphate receptor, type 1 (ITPR1), and (3) caveolin-3 (CAV3, LGMD1C), a cause of limb-girdle muscular dystrophy (data not shown).

**Relation of Brugada Syndrome to Other Inherited Cardiac Disorders**

ARVD, another autosomal dominant syndrome, is characterized by both structural and electrophysiological abnormalities, including replacement of the right ventricular myocar-
dium by fibrofatty infiltrates, right ventricular dilation, and epsilon waves and QRS prolongation in the right precordial leads of the surface ECG. In at least one family with the Brugada ECG pattern, some individuals had RV structural changes suggestive of ARVD, whereas others did not. The family described here has Brugada syndrome and not ARVD, based on the clinical presentation, the typical ECG pattern, and the lack of RV structural abnormalities by MRI and echocardiography.

Affected individuals in the family reported here develop progressive conduction disease with PR prolongation and QRS widening. Some SCN5A mutations cause progressive conduction disease, although this has not been clearly demonstrated in the Brugada syndrome. In addition, 4 of 12 affected individuals have mild to moderate mitral regurgitation and/or mitral valve prolapse. It is not certain that these mitral valve abnormalities are related to the mutation that causes the Brugada ECG phenotype. It is possible, though, that patients with the Brugada syndrome comprise a part of a phenotypic spectrum of arrhythmopathies caused by multiple gene defects that can affect the electrical properties of the ventricle, the conduction system, the myocardium, and the heart valves to varying degrees.

**Molecular Basis of the Brugada Syndrome**

We have identified a novel locus for the Brugada syndrome on chromosome 3p22–25 and excluded the 3 previously identified sodium channels located nearby. It is possible that mutations in another as yet unidentified member of this sodium channel cluster could cause the Brugada syndrome in the family that we studied. Alternatively, a mutation in another ion channel or in a nearby gene that modulates sodium channel function is possible. Other potential candidate genes on chromosome 3 include the synaptic protein synapsin II (HGNC), the cardiac extracellular matrix protein fibulin-2 (FBLN2), and the oncogene/kinase RAF1.

The locus that we have identified overlaps with the previously reported ARVD5 locus at 3p23 and a dilated cardiomyopathy with conduction disease locus at 3p22–25. Although the phenotype reported here is quite distinct from that of ARVD and dilated cardiomyopathy, it is certainly possible that different mutations in the same gene could be responsible for more than one of these syndromes.

The locus identified at 3p22–25 is near the SCN5A locus at 3p21. In the large family that we studied, 2 obligate recombinant affected individuals excluded SCN5A as a candidate. In smaller families, interpretation of linkage to the area around SCN5A will require caution. In the absence of recombinant events between the 2 loci, it may not be possible to distinguish between the 2 genes.

It remains unclear why the ECG pattern of affected individuals with Brugada syndrome can vary from day to day and why most affected individuals are male. Sex and hormonal differences in ion channel distribution have been described in the heart and could cause alterations in disease penetrance. It is also not known if other clinical conditions...
that cause STE or J-point elevation (ischemia, hypothermia, acidosis) cause arrhythmias by mechanisms similar to the Brugada syndrome. The identification of novel genes that cause the Brugada syndrome and the correlation of phenotype to genotype may lead to improvements in diagnosis, assessment of prognosis, and choice of therapeutic alternatives for patients with this rare inherited propensity to arrhythmias. In addition, knowledge of the roles of these genes could have broader implications toward the understanding of the electrophysiological function of the heart.

**Diagnostic and Therapeutic Implications**

Patients with Brugada syndrome are thought to be at high risk for sudden cardiac death, leading to the recommendation for provocative drug testing of family members and ICD placement in asymptomatic individuals with electrophysiology studies positive for inducible VF. A recent study of 65 patients from 52 families with Brugada syndrome demonstrated a less malignant phenotype in asymptomatic patients and questioned the utility of programmed electrical stimulation and drug testing with sodium channel blockers. Only one member of the extended family presented here died suddenly despite the identification of 8 affected individuals over age 50 years. Thus, affected individuals in the family described here appear to have a not extremely malignant phenotype and a relatively good prognosis. Drug testing with intravenous procainamide, the only clinically available intravenous sodium channel blocker in the United States, did not improve the diagnostic accuracy in our extended family. We speculate that the prognostic and phenotypic differences between the family described here and previous reports may reflect the different underlying genetic defects. Other families with mutations at this genetic locus will be necessary to test this hypothesis.

**Limitations**

We did not perform programmed electrical stimulation in asymptomatic affected individuals. Thus, we do not know whether such studies play a role in predicting the onset of symptoms or in the risk stratification for sudden cardiac death.

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