**SCN5A Mutation Associated With Dilated Cardiomyopathy, Conduction Disorder, and Arrhythmia**

William P. McNair, BA; Lisa Ku, MS; Matthew R.G. Taylor, MD; Pam R. Fain, PhD; Dmi Dao, MBA; Eugene Wolfel, MD; Luisa Mestroni, MD; and the Familial Cardiomyopathy Registry Research Group

**Background**—We studied a large family affected by an autosomal dominant cardiac conduction disorder associated with sinus node dysfunction, arrhythmia, and right and occasionally left ventricular dilatation and dysfunction. Previous linkage analysis mapped the disease phenotype to a 30-cM region on chromosome 3p22-p25 (CMD1E). This region also contains a locus for right ventricular cardiomyopathy (ARVD5) and the cardiac sodium channel gene (SCN5A), mutations that cause isolated progressive cardiac conduction defect (Lenègre syndrome), long-QT syndrome (LQT3), and Brugada syndrome.

**Methods and Results**—Family members were studied, and the positional candidate gene SCN5A was screened for mutations. We identified, by direct sequencing, a heterozygous G-to-A mutation at position 3823 that changed an aspartic acid to asparagine (D1275N) in a highly conserved residue of exon 21. This mutation was present in all affected family members, was absent in 300 control chromosomes, and predicted a change of charge within the S3 segment of domain III.

**Conclusions**—Our findings expand the clinical spectrum of disorders of the cardiac sodium channel to include cardiac dilatation and dysfunction and support the hypothesis that genes encoding ion channels can be implicated in dilated cardiomyopathies. (Circulation. 2004;110:2163-2167.)

Key Words: genetics • cardiomyopathy • conduction • arrhythmia • heart block

N 1986, Greenlee et al1 reported a peculiar form of dilated cardiomyopathy (DCM) after studying a large pedigree of German and Swiss ancestry. The affected phenotype included sinus node dysfunction in adolescence, supraventricular tachyarrhythmia, and a progressive atrioventricular (AV) and intraventricular conduction delay that led to permanent pacing in most cases. The phenotype was also characterized by a progression toward atrial dilatation, frequently followed by right ventricular dilatation and, in some cases, left ventricular dilatation and dysfunction.1 Subsequent linkage analysis mapped the disease locus to chromosome 3p22-p25 (CMD1E; OMIM %601154), which contains the voltage-gated α-subunit of the cardiac sodium channel (SCN5A) gene.2 SCN5A mutations have been associated with progressive cardiac conduction defect (Lenègre syndrome), isolated cardiac conduction disease, AV conduction block, sick sinus syndrome, sudden infant death syndrome, long-QT syndrome, and Brugada syndrome.3-9 This chromosomal region also contains a locus for right ventricular cardiomyopathy (ARVD5).10 We hypothesized that SCN5A mutations might be responsible for causing the conduction-related phenotype within this family (pedigree DN-ADFDC3; Figure 1A) and report the identification of an SCN5A mutation associated with the disease phenotype.

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**Clinical Investigation**

Informed written consent was obtained from participants according to our protocol approved by the Colorado Multiple Institutional Review Board. The proband (IV-2 in Figure 1A) in the present study and his first-degree relatives (III-4, III-5, IV-1, and IV-4 in Figure 1A) were evaluated at the University of Colorado Health Sciences Center. A complete medical and family history, physical examination, ECG, and echocardiography were performed (Table).11 Additional family members were interviewed, and their medical histories and medical records were reviewed to allow an accurate analysis of the phenotype (Table) (Figure 2).

Criteria for affected status were based on the scoring system proposed by Olson and Keating.2 In brief, points were assigned as follows: asymptomatic sinus bradycardia (heart rate ≤50 bpm), 1 point; incomplete bundle-branch block age ≤30 years (QRS interval 0.10 to 0.12 second), 1 point; symptomatic supraventricular tachycardia at ≤30 years of age, 2 points; first-degree AV block ≤30 years of age (PR interval >0.22 second), 2 points; clinical heart failure ≤55 years of age, 2 points; echocardiographic atrial dilation, 2 points; echocardiographic ventricular dilation, 2 points; ventricular dilation (cross-sectional dimension >95% for body surface area), 2 points; left ventricular systolic dysfunction (shortening fraction <28%), 3 points; complete bundle-branch block at ≤30 years of age (QRS interval >0.12 second), 3 points; symptomatic second-degree AV block at ≤30 years of age, 4 points; symptomatic sinus node dysfunction (sinus pause or arrest), 4 points; and stroke at ≤40 years

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From the University of Colorado Cardiovascular Institute (W.P.M., L.K., M.R.G.T., P.R.F., D.D., E.W., L.M.), Human Medical Genetics Program (W.P.M., M.R.G.T., P.R.F., L.M.), and Division of Cardiology (E.W., L.M.), University of Colorado Health Sciences Center, Denver, Colo.

Reprint requests to Dr Luisa Mestroni, University of Colorado Cardiovascular Institute, Bioscience Park Center, 12635 E Montview Blvd, Suite 150, Aurora, CO 80010-7116. E-mail Luisa.Mestroni@UCHSC.edu

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of age, 5 points. Patient status was considered affected with a score of 5 or greater, uncertain with 1 to 4 points, and unaffected with 0 points.²

Molecular Genetic Studies
Genotyping of family members was conducted with genomic DNA extracted from either peripheral blood leukocytes or buccal swab sampling (Epitope, Inc) according to standard protocols. Using publicly available genetic maps (NCBI, http://www.ncbi.nlm.nih.gov/), we localized positional candidate genes to the 3p22-p25 genomic region shared by affected individuals. Current contig maps place SCN5A ~7.5 megabases centromeric to the “affected” haplotype marker D3S1211, in a region with a multipoint lod score >3.² We hypothesized that SCN5A was a likely candidate because of the conduction-related component of this family’s phenotype. Primers for all of the protein-coding exons of the SCN5A gene were designed from a prior report or optimized with Primer 3 Input software (http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi) based on the NCBI and Celera (http://publication.celera.com/human-pub/index.jsp) human sequence databases. The proband’s genomic DNA was amplified by polymerase chain reaction (PCR) followed by bidirectional sequencing with the ABI 377 DNA Sequencer (Applied Biosystems). Sequencing results were compared with wild-type sequences published in NCBI.

Results
Characteristics of the phenotypes of 19 affected or uncertain family members of the 21 subjects investigated in family DN-ADFDC3 are reported in the Table. The affected status of individuals from generations I to III was consistent with the original report by Olson and Keating² and remain unchanged. New family members (Figure 1A: generation IV) were included in the present study and scored appropriately. We identified 4 new affected individuals within generation IV (IV-2, IV-8, IV-9, IV-10) and 2 obligate carriers (III-13 and III-17). None of the patients showed evidence of long-QT or Brugada syndromes; however, they frequently showed an
enlarged right ventricle at early stages of the disease and occasionally biventricular enlargement and clinical features of DCM in later stages (Table). Criteria for ARVD diagnosis were not fulfilled by the current clinical data. All but one of the affected family members currently have pacemakers as a result of conduction disease.

A heterozygous mutation (G3823A) was found in exon 21 of SCN5A, which led to the substitution of an aspartic acid (acidic) by an asparagine (polar, hydrophilic; D1275N) in the first third of the S3 transmembrane region of domain III (Figure 1B).12 This mutation cosegregates within the family with the affected phenotype and with the haplotype characterized by Olson and Keating.2 No recombination event was observed, and the mutation was absent in more than 300 control chromosomes. This amino acid residue is highly conserved among voltage-gated sodium channels and in calcium channels, potassium channels, and across species.15

The reported −44 (G→A) and +71 (A→G) allelic variations in the promoter region and first exon of the Cx40 gene were screened in 11 members of the pedigree, as shown in the Table. Three affected individuals, including the proband (II-4, III-10, and IV-2), were heterozygous, carrying the wild-type and the Cx40 polymorphic alleles. Five affected individuals were homozygous for the wild-type sequence at these positions. None of the tested pedigree members proved to be homozygous for the polymorphic allele.

Discussion

We have identified the putative disease-causing mutation for DCM with conduction disease, CMD1E, in the SCN5A gene. CMD1E is characterized by autosomal dominant transmission, a penetrance around 75%, young age of onset, and arrhythmia and conduction disease that culminates, in most cases, in left or biatrial dilatation and right or biventricular dilatation. The mutation we report (D1275N), located in the S3 region of domain III of SCN5A, was recently reported by Groenewegen et al14 in connection with a phenotype of atrial standstill that they defined as a purely electrical disease with relatively early onset and low penetrance. The lack of long-term clinical data in that study makes an accurate comparison between the 2 phenotypes difficult. Groenewegen et al14 hypothesized that full penetrance for the atrial standstill phenotype occurs exclusively in conjunction with the cosegregation of the D1275N mutation in SCN5A with 2 polymorphisms discovered in the promoter region and first exon of the Cx40 gene. In the DN-ADFDC3 pedigree, there is no such correlation between the reported Cx40 polymorphisms and the occurrence of the CMD1E phenotype. These data suggest that the D1275N mutation is the primary cause of the CMD1E phenotype and that other genetic or nongenetic factors could modify the mutant gene expressivity.

Electrophysiological studies performed by Groenewegen et al14 in xenopus oocytes found that the D1275N mutation shifted the activation curve of the sodium channel conductance by 3.8 mV.14 The shift disappeared when the β-1 subunit was coexpressed. They surmise that this shift in the activation curve toward more positive voltages may result in reduced excitability of myocytes expressing the mutation. Studies in mammalian cells should also be performed owing to significant discrepancies among the electrophysiological profiles of the particular cellular backgrounds on which wild-type channels are expressed.

DCM due to SCN5A mutations has been reported in LQT316 and congenital conduction disease.17 DCM has also been shown to associate with mutations in other ion channel proteins; a patient with a homozygous HERG mutation was found to have DCM,18 and recently, Bienengraeber et al19 reported that mutations in the ABCC9 gene encoding the SUR2A subunit of the cardiac potassium channel conferred susceptibility to DCM. Furthermore, mutations in the ryanodine receptor (ion channel) have been shown to cause a phenotype related to DCM, arrhythmogenic right ventricular dysplasia type 2 (ARVD2).20 The association of an ion channel mutation with dilated cardiomyopathy seen in the DN-ADFDC3 pedigree, however, is particularly remarkable.

Figure 2. ECG of individual II-6 from Table and Figure 1A showing atrial flutter, variable AV block, and left anterior fascicular block. This individual does not carry Cx40 polymorphisms.
Phenotypic Data for Pedigree DN-ADFDC3

| Pedigree ID | Age at Onset, y | Age at Most Recent Evaluation, y | HR, bpm | PR Intervals | QRS Intervals | Age at Stroke, y | Age at CHF, y | Atrial Dilatation | Left Ventricular Internal Diastolic Dimension, mm | Left Ventricular Posterior Wall, mm | Ventricular Dilatation | Shortening Fraction | Total Points | Health Status | SCN5A Genotype | Cx40 Genotype |
|------------|----------------|----------------------------------|--------|--------------|---------------|----------------|--------------|----------------|----------------------------------------|-------------------------------|------------------|------------------|----------------|-------------|---------------|---------------|----------------|
| I-2        | N/A            | 88                               | AF     |              |               | B              | N/A          | N/A           | R > 28                   |                                |                  |                  |                |             |               |               |                |
| I-3        | N/A            | 60                               | SND    | AF           | LBBB          | 37             | 54           | B             | N/A 1.7§                | < 28             | 23               | A                | N/A          | N/A           |               |               |
| I-5        | N/A            | 58                               | SND    | AF           | RBBB          | 52             | B            | N/A           | N/A                    | 26               | 18               | A                | N/A          | N/A           |               |               |
| I-6        | N/A            | 62                               | 76     | 0.24         | L              | N/A           | N/A           | 24            | 5                      | A                | N/A             | N/A             | N/A          |               |               |               |
| I-7        | 15             | 68                               | AF     |              | LAFB          | 63             | 50           | B             | N/A 23                   | 15               | A                | +/-            | +/-         | +/-          |               |               |
| I-8        | 37             | 70                               | SVT*   | 0.18         | 0.08          | N/A           | N/A           | > 28          | 2                       |                  | U                | N/A             | +/-          | +/-         |               |               |
| I-9        | 53             | 60                               | Paced† |              |               | 47             | 9            | 41            | 0                       |                  |                  |                  | N/A          |               |               |               |
| I-10       | 23             | 51                               | Paced AF 3rd AVB | 0.18 | 38 | B | 55 | 7 | R | 34‡ | 14 | A | +/- | +/- |               |               |
| I-11       | 22             | 41                               | Paced SSS/AF 3rd AVB | 0.07 | 33 | L | 47 | 11 | R | 31 | 15 | A | +/- | +/- |               |               |
| I-12       | 46             | 82                               | Paced | 0.18        | RBBB          | 29             | 29           | 48            | 12                      | 20               | 23               | A                | N/A          | N/A           |               |               |
| I-13       | 34             | 47                               | Paced SSS/AF 1st AVB | 0.18 | 41 | B | 52 | 10 | 46 | 14 | A | +/- | +/- |               |               |
| I-14       | 24             | 36                               | Brady  | 0.19        | 0.09          | N/A           | N/A           | 30            | 1                      | UC               | +/-             | +/-             | N/A          |               |               |               |
| I-15       | 31             | 47                               | Paced | 0.14        | 0.08          | N/A           | N/A           | 35            | 2                      | U                | N/A             | N/A             |              |               |               |               |
| I-16       | 28             | 60                               | Paced PVCVT 3rd AVB | RBBB | 46 | 9 | R | 48 | 7 | A | +/- | +/- |               |               |
| I-17       | 19             | 78                               | Paced | 0.14        | 0.08          | N/A           | N/A           | 48            | 0                      |                  | +/-             | +/-             |              |               |               |               |
| I-18       | 23             | 90                               | Paced SSS/AFAS 1st AVB | N/A | 47 | 9 | 40 | 2 | U | N/A | N/A |               |               |
| I-19       | 14             | 20                               | Paced Brady/AFAS | N/A | 35 | 10 | 38 | 7 | A | +/- | +/- |               |               |
| I-20       | 16             | 20                               | Paced SSS | N/A | N/A | LVEF 50% | 7 | A | +/- | +/- |               |               |
| I-21       | 13             | 18                               | Paced SSS | N/A | N/A |                           | 47 | 4.1 | 32 | 7 | A | +/- | +/- |               |               |

ID indicates identification; Age at most recent evaluation, age at which last clinical data were available; HR, sinus heart rate; CHF, clinical heart failure; N/A, information unavailable; blank spaces, no abnormality; AF, atrial flutter/fibrillation; R, right; A, affected; SND, sinus node dysfunction; LBBB, left bundle-branch block; B, bilateral; RBBB, right bundle-branch block; L, left; LAFB, left anterior fascicular block; SVT, supraventricular tachycardia; U, uncertain; 3rd AVB, third-degree AV block; SSS, sick sinus syndrome; IRBBB, incomplete right bundle-branch block; UC, uncertain/obligate carrier; 2nd AVB, second-degree AV block; 1st AVB, first-degree AV block; Brady, bradycardia; PVC, premature ventricular contraction; VT, ventricular tachycardia; CHF, clinical heart failure; and LVEF, left ventricular ejection fraction. 
‡Diastolic dysfunction with restrictive physiology.
†Pacemaker at age >50 years, diagnosis unknown.
§Dimension acquired from autopsy report.

There is a strong correlation between the penetrance of a conduction disorder and the manifestation of dilatation in this pedigree. This correlation is 100% for affected individuals beyond the youngest generation. Overall, these data suggest that DCM can result not only from structural changes in the myocytes but also from altered ion homeostasis.

The findings of the present study expand the clinical spectrum of disorders of the cardiac sodium channel to include cardiac dilatation and dysfunction and support the hypothesis that genes encoding ion channels can be strongly implicated in DCMs.

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