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Novel Deletion Mutation in the Cardiac Sodium Channel Inactivation Gate Causes Long QT Syndrome

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Keywords

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Disturbances in cardiac sodium channel function are associated with inherited arrhythmia susceptibility. Mutations in *SCN5A*, which encodes the cardiac sodium channel ($Na_V1.5$), cause congenital long QT syndrome type 3 (LQT3), Brugada syndrome (BrS) and a variety of cardiac conduction disorders (CCD) [1,2]. These disorders have can have complex genotype-phenotype relationships [3,4]. Here we report the clinical features of an LQTS family segregating a novel amino acid deletion mutation (N1472del) in *SCN5A* that produces a unique pattern of biophysical disturbances consistent with the clinical phenotype.

Nine members of a three-generation Italian LQTS family were evaluated clinically by the Cardiology Division of the Monaldi Hospital (Naples, Italy) or in other hospitals (Fig. 1A). Investigations included a complete medical history, physical examination, 12-lead ECG recording, 24-hour Holter recording and echocardiogram at different times during a follow-up of at least 3 years. Informed consent for genetic studies was obtained from all subjects.

The proband (III-3, Fig. 1A) was a 27-year-old female who had postpartum cardiac arrest shortly after an uncomplicated Caesarian section while receiving erythromycin. An ECG performed during adolescence reportedly exhibited normal QT intervals, but her QTc was

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prolonged (480 ms) following successful resuscitation (Fig. 1B). She was treated with metoprolol, mexiletine and had an ICD implanted. During the following 8 years, she remained asymptomatic with a QTc ranging between 410 and 445 ms without conduction disturbances. There was a significant family history of unexplained syncope and sudden death. Particularly, the proband's uncle (II-6, Fig. 1A) experienced syncope at age 12, then died suddenly overnight at age 13. In retrospect, an ECG obtained at age 12 demonstrated a prolonged QTc and periods of 2:1 AV block (Fig. 1B).

The proband was screened for mutations in *SCN5A*, *KCNQ1*, *KCNH2*, *KCNE1* and *KCNE2* genes. Genetic analysis revealed a novel *SCN5A* in-frame deletion mutation (c. 4414-4416delAAC) predicting the deletion of asparagine-1472 (p.N1472del). The mutation deletes a conserved residue within the DIII-DIV cytoplasmic loop, which is an essential structural determinant of sodium channel fast inactivation [5,6]. The mutation was present in all clinically affected relatives who were genotyped (Fig. 1A) but was absent in 500 chromosomes from control subjects.

Mutation N1472del was generated by site-directed mutagenesis of recombinant human $Na_V1.5$ (GenBank M77235) inserted into the expression vector pRc/CMV. To determine the functional consequence of the mutant channel, sodium current was recorded by whole cell patch-clamp in transiently transfected tsA201 cells as previously described [7]. Peak current density recorded from cells expressing N1472del was ~50% smaller than that of cells expressing WT-$Na_V1.5$ (p.N1472del: 160.5 ± 21 pA/pF, n = 25; WT-$Na_V1.5$: 344.8 ± 52 pA/pF, n = 21; $p<0.05$) and the peak current of N1472del was shifted to a more positive potential (Fig. 2A). Correspondingly, there was a +15 mV depolarizing shift in the voltage dependence of activation observed for mutant channels (N1472del, $V_{½}$: −28.9±0.7 mV, n=23; WT-$Na_V1.5$, $V_{½}$: −44.4±1.1 mV, n=21; $p<0.001$) (Fig. 2B). Mutant channels also exhibited a +12 mV depolarizing shift in steady-state inactivation (N1472del, $V_{½}$: −72.6±0.5 mV, n=21; WT-NaV1.5, $V_{½}$: −85.0±2.2 mV, n=21; $p<0.001$) (Fig. 2C). In addition, the time course of recovery from fast inactivation observed for WT-$Na_V1.5$ was monoexponential described by a single time constant (6.8 ± 0.01 ms, n = 10), whereas recovery for N1472del was biexponential characterized by fast (4.3 ± 0.4 ms, 60%; n=20) and slow time constants (200 ± 33 ms, 38%; n = 21). The additional slow component explains the significantly slower recovery from inactivation exhibited by the mutant channel (Fig. 2D). Finally, the N1472del channel showed a greater level of TTX-sensitive persistent sodium current compared with WT-$Na_V1.5$ (N1472del: 2.6±0.2%, n=5; WT-$Na_V1.5$: 0.1±0.02, n=5; $p<0.001$) (Fig. 2E), a common feature of *SCN5A* mutations associated with LQT3 [8].

This family exhibited a heterogeneous phenotype with variability in severity and clinical presentation. Among the four documented and one presumed mutation carrier (Fig. 1A), the presentation ranged from late onset syncope (subject II-1) to sudden unexplained death during adolescence (subject II-6) or post-partum cardiac arrest (proband, III-3). The sudden death victim and presumed mutation carrier also exhibited ECG evidence of cardiac conduction system disease in the form of 2:1 AV block (Fig. 1B) that has been reported as a condition with higher life-threatening risk [9]. None of the mutation carriers exhibited bradycardia. Clinical variability is typical of LQTS and may be related to the variable exposure of individuals to circumstances promoting arrhythmia or co-inheritance of genetic factors other than the primary mutation that either potentiate or reduce the risk of arrhythmic events. The absence of bradycardia and the favorable response to monotherapy with β-blockers observed in some *SCN5A* mutation carriers (III-1, IV-2) are atypical of LQT3. Exposure of LQT3 subjects to β-blockers carries risk of evoking bradycardia that then potentiates arrhythmia risk [10].

There have been reports of other *SCN5A* deletion mutations within the DIII-DIV cytoplasmic loop but not all of these cause LQTS. In particular, delKPQ 1505–1507 and delQKP 1507–1509 are associated with LQTS [11,12], while K1479del and K1493del have been reported in subjects with BrS [13,14]. These two lysine mutations have not been functionally characterized. Furthermore, K1500del mutation has been reported in a family that exhibits features of LQTS, BrS and conduction disease in the same subjects [4]. These reports demonstrate the difficulty in predicting phenotype from genotype when considering only the structural domain involved.

The biophysical properties of the novel *SCN5A* N1472del mutation provided insight into the cause of LQTS in this family and might help explain other clinical features. This mutation elicits a defect in channel inactivation leading to increased persistent current, a hallmark of $Na_V1.5$ dysfunction in LQT3 (Fig. 2E). Increased persistent sodium current causes a delay in repolarization that, in turn, elicits prolonged ventricular action potential duration. The pathogenic effects of increased persistent sodium current in LQTS have been strongly supported by computer simulations of cardiac action potentials [15,16] and direct electrophysiological investigations of native cardiac myocytes from genetically engineered mice carrying LQTS mutations [17,18]. Increased persistent current can be considered a ‘gain-of-function’ that accounts for the dominant nature of genetic arrhythmia risk in heterozygous mutation carriers. Potentially compounding this defect in N1472del channels is a depolarizing shift in voltage dependence of steady-state inactivation that increases channel availability (Fig. 2D), however this is offset by other functional abnormalities.

In addition to the ‘gain-of-function’ defect, our experiments revealed other biophysical properties that may impair channel function under physiological conditions (reduced peak current density, depolarized conductance-voltage relationship and slower recovery from inactivation) (Fig. 2A,B,D). The impact of these ‘loss-of-function’ effects on the clinical phenotype observed in mutation carriers cannot be known for certain, but we speculate that these features may have contributed to AV conduction block in subject II-6. The combination of ‘gain-of-function’ and ‘loss-of-function’ features has been reported with *SCN5A* mutations associated with mixed phenotypes in ‘overlap syndromes’ (e.g., LQT3 with BrS) [3,4]. Although the family we present did not have an overlap syndrome, provocative testing, to expose Brugada syndrome for example, was not performed and therefore we cannot exclude an additional latent phenotype.

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ABBREVIATIONS

AV block	Atrio-ventricular block
Bpm	Beats per minute
BrS	Brugada syndrome
CCD	Cardiac conduction defects
ECG	Electrocardiogram

HR	Heart rate
ICD	Implantable cardioverter defibrillator
K	Slope factor
LQTS	Long QT syndrome
QTc	QT interval corrected for heart rate
TTX	Tetrodotoxin
$V_{½}$	Membrane potential at half-maximal activation or inactivation

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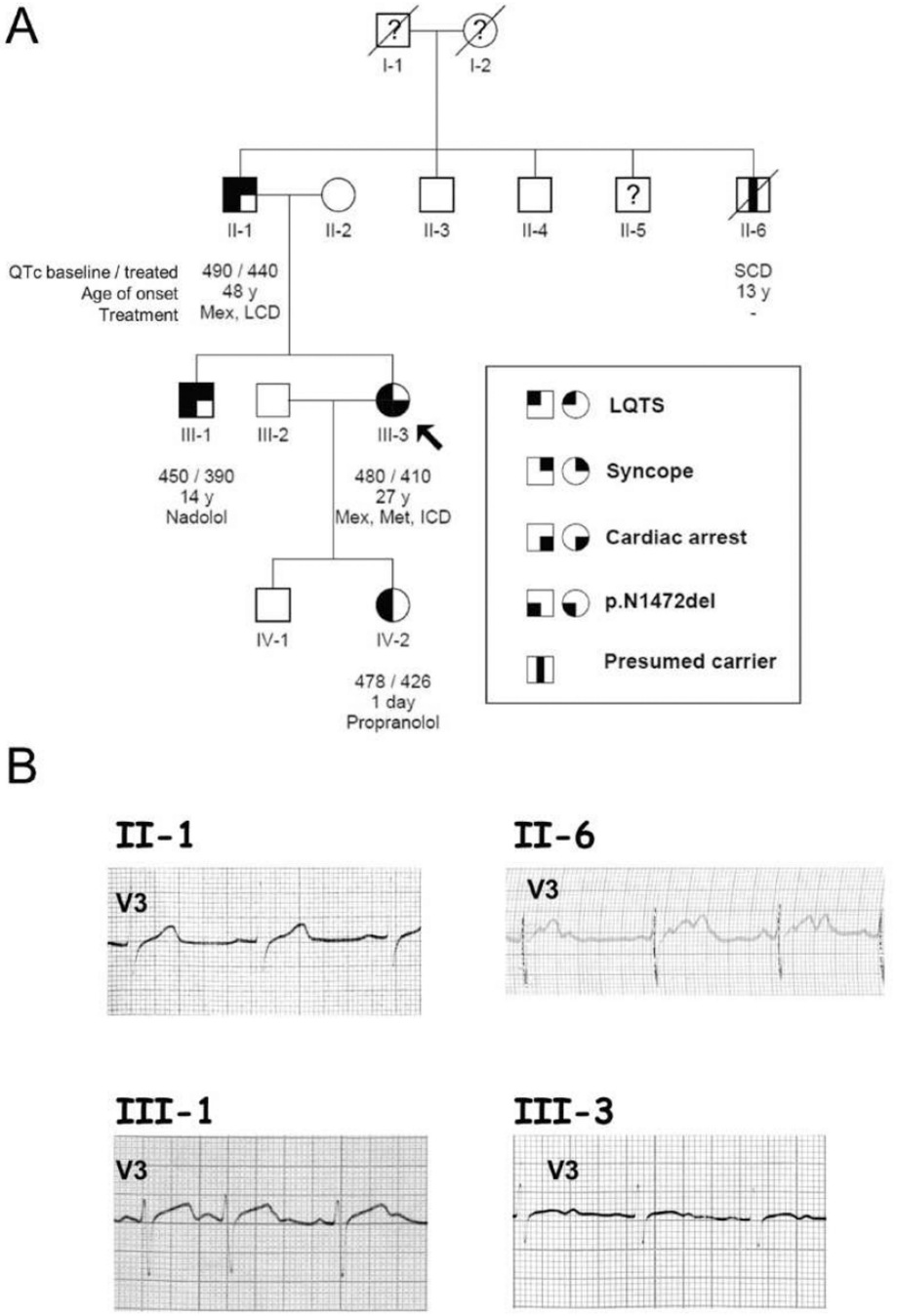

Figure 1. Pedigree of a LQTS family with representative ECG traces

(**A**) Genotype and phenotype is defined according to the legend inset. Open symbols represent subjects with a negative genotype and phenotype. An arrow marks the proband (III-3). Symbols with a question mark represent subjects with unknown phenotype. The QTc interval, the age of onset and the treatment are reported below each subject symbol (abbreviations: Mex, mexiletine; Met, Metoprolol; LCD, left cardiac sympathetic denervation; ICD, implantable cardioverter defibrillator; SCD, sudden cardiac death). (**B**) Representative ECG traces from four genotype positive family members.

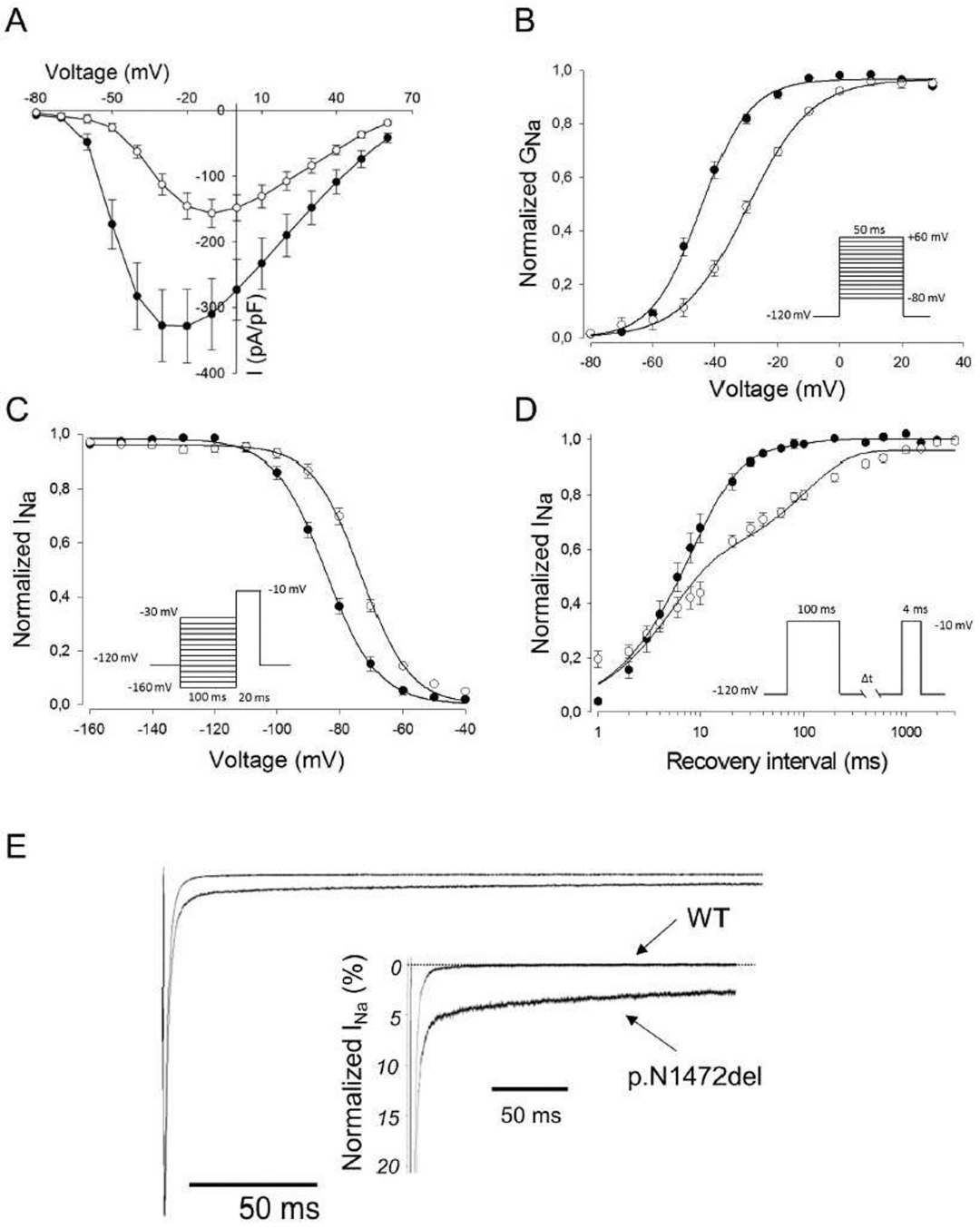

Figure 2. Biophysical properties of WT-Na$_V$1.5 and N1472del sodium channels

(**A**) Current-voltage relationships for WT-Na$_V$1.5 (filled circles) and N1472del (open circles). Currents were normalized for cell capacitance to give a measure of sodium current density. (**B**) Voltage dependence of activation for WT-Na$_V$1.5 (filled circles) and N1472del (open circles) assessed using the voltage protocol illustrated in panel A. Conductance-voltage curves were fit with a Boltzmann distribution (solid lines). (**C**) The voltage dependence of steady-state inactivation for WT-Na$_V$1.5 (filled circles) and N1472del (open circles). Currents were normalized to the peak current amplitude. Lines represent Boltzmann fits to the data. (**D**) Recovery from inactivation for WT-Na$_V$1.5 (filled circles) and N1472del

(open circles) measured with double-pulse protocol shown in the inset. The current-time curve was fit with a single (WT-$Na_V1.5$) or double-exponential equation (N1472del). (**E**) Increased TTX-sensitive persistent sodium current for N1472. Persistent sodium current was recorded during a 200 ms depolarizing voltage pulse to −30 mV from a holding potential of −120 mV in the absence then presence of 30 μM TTX followed by offline digital subtraction of the currents. Zero current level is indicated by the dotted line. The inset shows an expanded y-axis scale to emphasize the relative proportion of WT-$Na_V1.5$ and N1472del currents.