International Journal of Cardiology 170 (2014) e63–e65

Contents lists available at ScienceDirect

International Journal of Cardiology

journal homepage: www.elsevier.com/locate/ijcard

Letter to the Editor

Genetic analysis in a family affected by sick sinus syndrome may reduce the sudden death risk in a young aspiring competitive athlete

Nicola Detta [a,1], Giulia Frisso [a,b,1], Giuseppe Limongelli [c], Michele Marzullo [d], Raffaele Calabrò [c], Francesco Salvatore [a,e,*]

[a] CEINGE-Biotecnologie Avanzate s.c.ar.l., Naples, Italy
[b] Dipartimento di Medicina Molecolare e Biotecnologie Mediche, Università di Napoli Federico II, Naples, Italy
[c] Unità Operativa Clinica di Cardiologia, Ospedale dei Colli, Seconda Università di Napoli, Naples, Italy
[d] Medicina dello Sport, Dipartimento di Scienze Biomediche Avanzate, University of Naples Federico II, Naples, Italy
[e] IRCCS-Fondazione SDN, Naples, Italy

ARTICLE INFO

Article history:
Received 21 August 2013
Accepted 2 November 2013
Available online 12 November 2013

Keywords:
Sick sinus syndrome
Genetic analysis of SSS
Electrophysiological cell measurements
Sudden death prevention
Athletes' sudden death risk
Mutation functional analysis

Sudden cardiac death is an unexpected event that sometimes occurs in young, apparently healthy subjects. Inherited alterations in cardiomyopathy-related genes may determine predisposition for arrhythmic events, particularly during or after strenuous physical activity. Indeed, by provoking acute or chronic hemodynamic changes or electrolytic imbalance, intense, competitive athletic exercise may trigger arrhythmic events leading to sudden death. However, the phenotypic expression of signs and symptoms of inherited heart disease is so heterogeneous that it is difficult to distinguish between pathology and physiology [1]. In fact, electrocardiography (ECG) and echocardiography, and other clinical signs or symptoms, may remain in the "gray zone" thereby leaving doubts about the presence of a critical heart alteration that could lead to sudden cardiac death.

Here we report the case of an adolescent athlete, who, as required by Italian law, underwent a sports eligibility consultation before commencing elite training. The consulting sports physician referred the boy to our Cardiology Division because of bradycardia. This 12-year-old boy was the second of two children of non-consanguineous parents (Fig. 1, III-2). The parents referred he had not experienced episodes of syncope; physical examination revealed no major signs of cardiovascular diseases.

During our observations carried out over several months, electrocardiography revealed sinus bradycardia (heart rate 50 bpm) with phases of sinus atrial blocks. The echocardiogram was normal. Twenty-four hour Holter ECG revealed many episodes of sinus atrial blocks with 16 asymptomatic pauses (longest 2.6 ms, at 8.58 a.m.). His sister (16 years old) referred two episodes of syncope during physical effort. Her ECG revealed normal PR and QTc intervals, and heart rate was 75 bpm. The echocardiogram was normal. The Holter ECG revealed frequent (n = 848), isolated and monomorphic supraventricular ectopic beats. The head-up tilt test was normal. The mother (48 years old, Fig. 1, II-2) was affected by SSS and underwent pacemaker implantation at the age of 42 years for symptomatic, intense bradycardia with episodes of low heart rate atrial fibrillation. The grandmother was affected by II degree atrioventricular block, Mobitz I. Cardiovascular examination of the father was unremarkable.

Given this picture, we obtained informed consent for genetic studies from all subjects. The proband was screened for mutations in the SCN5A gene. This revealed a novel SCN5A mutation (c.C1126T) that causes substitution of arginine 376 with a cysteine (p.R376C). The mutation is located in the highly conserved pore-forming loop between segments S5–S6 in domain I of the protein channel. It was absent from more than 500 chromosomes from ethnically matched control subjects. Familial genetic analysis revealed the mutation in the proband's mother and sister. Screening of other SSS-related gene exons (LMNA A/C, EMD, GJ5A and HCN4) was negative.

A different mutation, p.R376H, was previously described at the same codon in a 17-year-old male who suddenly died during a soccer match [2]. Therefore, we studied the functional effects of both the novel mutation (p.R376C) and of p.R376H. We generated both mutations by site-directed mutagenesis of recombinant human $Na_V1.5$ (GenBank M77235) inserted into the expression vector pRc/CMV. Sodium current was recorded by whole cell patch-clamp in transiently transfected tsA201 cells as previously described [3]. Whole cell recordings showed reduction of the inward sodium current in cells expressing R376C or R376H compared with the WT channel (Fig. 2A). Peak current density recorded from cells expressing R376C and R376H was respectively

* Corresponding author at: CEINGE-Biotecnologie Avanzate s.c.ar.l., Naples, Italy.
E-mail address: salvator@unina.it (F. Salvatore).
[1] These authors contributed equally to the study.

http://dx.doi.org/10.1016/j.ijcard.2013.11.013

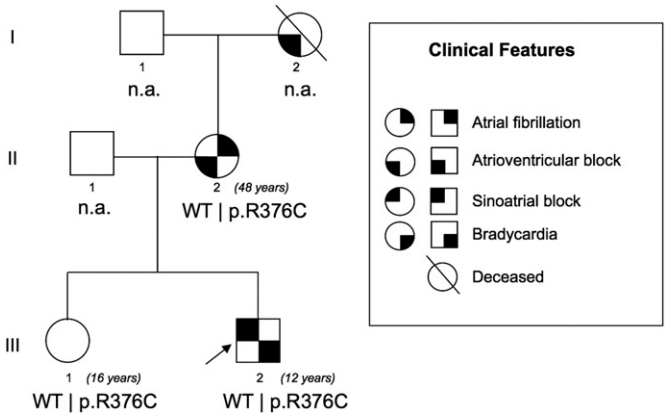

Fig. 1. Pedigree of the members of the family studied and their phenotypic spectrum and genotype. Phenotype is defined as shown in the figure inset. Open symbols represent subjects with a negative phenotype. The arrow indicates the proband (III-2). The ages of subjects are reported in brackets. n.a. not analyzed. □, ○, apparently clinically healthy.

about 95% and 70% lower compared with cells expressing the WT gene (R376C: −13.29 ± 1.7 pA/pF, n = 15; R376H: −71 ± 20 pA/pF, n = 6; WT-$Na_v1.5$: −264.95 ± 46 pA/pF, n = 11; $p < 0.05$; Fig. 2B). Analysis of activation of the mutant channel R376C showed a significant depolarizing shift in the voltage-dependence of activation versus both $Na_v1.5$-WT and R376H (R376C, $V_{1/2}$: −28.8 ± 1.31 mV, k: −14.5 ± 1.04, n = 15; R376H: $V_{1/2}$: −38.8 ± 0.53 mV, k: −9.26 ± 0.45, n = 6; WT-$Na_v1.5$: $V_{1/2}$: −40.3 ± 0.46 mV, k: −9.1 ± 0.4, n = 11; $p < 0.05$) (Fig. 2C). On the contrary, the steady-state of inactivation did not differ between wild-type and mutant channels (Fig. 2D).

The three subjects of the family carrying the R376C mutation manifested different phenotypes of the same disease spectrum. The mother was affected by atrial fibrillation with II degree atrioventricular block that required pacemaker implantation, whereas the proband and his sister did not manifest overt signs of SSS. Although SSS can occur in pediatric age [4,5], more than half the SSS patients are older than 50 years at diagnosis. Therefore, it is feasible that the overt pathological phenotype had not yet manifested in our proband and his sister because of their age.

The biophysical study of the novel R376C mutation in this family provided meaningful insights into the cause of SSS. In fact, the mutation determines severe loss-of-function of the cardiac sodium channel protein thereby resulting in a significant reduction of the inward sodium current, which is consistent with a diagnosis of SSS. We also compared the biophysical behavior of mutation R376C with that of mutation R376H previously identified in a young man who suddenly died during sustained physical effort [2]. Given this fatal event and the worst electrophysiological behavior of R376C with respect to R376H, and

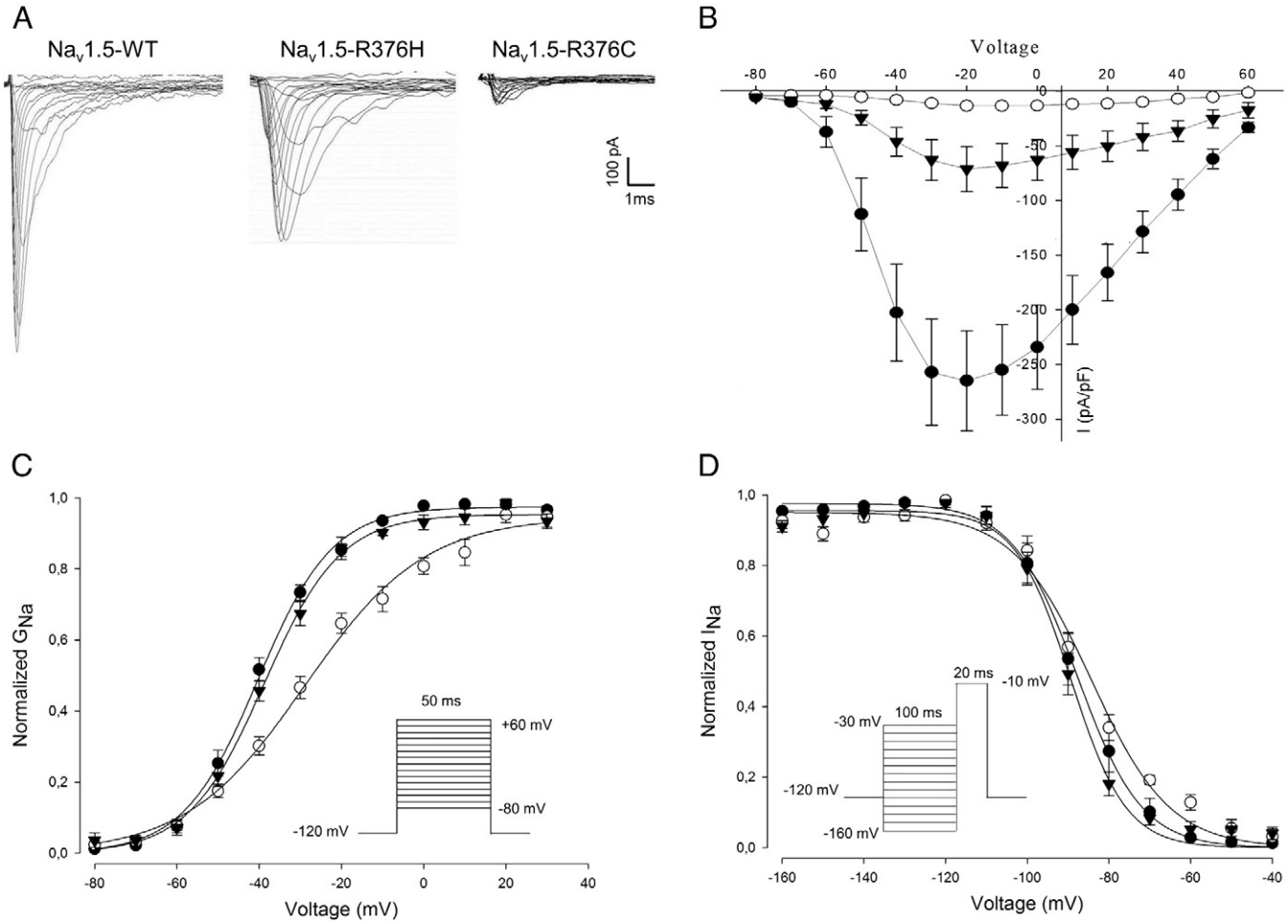

Fig. 2. Biophysical properties of $Na_v1.5$-WT, R376C and R376H sodium channels. (A) Representative whole-cell current traces obtained from tsA201 cells transfected with $Na_v1.5$-WT, R376C or R376H sodium channels. All studies were performed in cells co-transfected with the sodium channel β1 subunit. Recordings were performed from a holding potential of −120 mV and then stepped up from −80 mV to +60 mV in 10 mV increments. (B) Current–voltage relationships for $Na_v1.5$-WT (filled circles), R376C (open circles) and R376H (filled triangles). Currents were normalized for cell capacitance to obtain a measure of sodium current density. (C) Voltage-dependence of activation for $Na_v1.5$-WT (filled circles) R376C (open circles) and R376H (filled triangles) assessed using the voltage protocol illustrated as inset. Conductance–voltage curves were fit with a Boltzmann distribution (solid lines). (D) Voltage-dependence of steady-state inactivation for $Na_v1.5$-WT (filled circles), R376C (open circles) and R376H (filled triangles). Currents were normalized to the peak current amplitude. Lines represent Boltzmann fits to the data.

also considering that the proband was a young aspiring athlete, competitive activity was discouraged, and the family dissuaded him from undertaking a career in sports.

Familial SSS is usually associated to compound heterozygosity for mutations in the SCN5A gene. Rarely, it may be transmitted as dominant trait (which is the case of HCN4 gene mutations) or may show digenic inheritance, involving the LMNA A/C, EMD and GJ5A genes. In our family, we found only one SCN5A mutation, which suggests autosomal dominant inheritance, thereby prompting the search for more complex pathogenetic events which may also include epistatic genes.

In conclusion, this case illustrates the importance of early genetic screening, coupled with the biophysical characterization of the mutant protein, in families in which at least one member is affected by a heart condition that entails a risk of sudden death. Indeed, this approach may indicate the need for long-term surveillance and to discourage from competitive sports to prevent sudden death in carriers of the mutation without overt pathology.

Acknowledgments

The work was supported by grant from the Regione Campania (Convenzione CEINGE-Regione Campania, G.R. 27/12/2007), the Ministero dell'Istruzione, dell'Università e della Ricerca-Rome PS35-126/IND and from IRCCS-Fondazione SDN, and the Ministero della Salute, Rome, Italy. We thank Angela Cordella for her skillful assistance in mutation screening, Jean Ann Gilder (Scientific Communication srl., Naples, Italy) for text editing and Vittorio Lucignano, CEINGE-Biotecnologie Avanzate for technical assistance. The authors of this manuscript certify that they comply with the Principles of Ethical Publishing in the International Journal of Cardiology.

References

[1] Pelliccia A, Maron MS, Maron BJ. Assessment of left ventricular hypertrophy in a trained athlete: differential diagnosis of physiologic athlete's heart from pathologic hypertrophy. Prog Cardiovasc Dis 2012;54:387–96.
[2] Rossembacker T, Carroll SJ, Liu H, et al. Novel pore mutation in SCN5A manifests as a spectrum of phenotypes ranging from atrial flutter, conduction disease, and Brugada syndrome to sudden cardiac death. Heart Rhythm 2004;1:610–5.
[3] Detta N, Frisso G, Zullo A, et al. Novel deletion mutation in the cardiac sodium channel inactivation gate causes long QT syndrome. Int J Cardiol 2013;165:362–5.
[4] Benson DW, Wang DW, Dyment M, et al. Congenital sick soinus syndrome caused by recessive mutations in the cardiac sodium channel gene (SCN5A). J Clin Invest 2003;112:1019–28.
[5] Kodama T, Serio A, Disertori M, et al. Autosomal recessive paediatric sick sinus syndrome associated with novel compound mutations in SCN5A. Int J Cardiol 2013;167:3078–80.