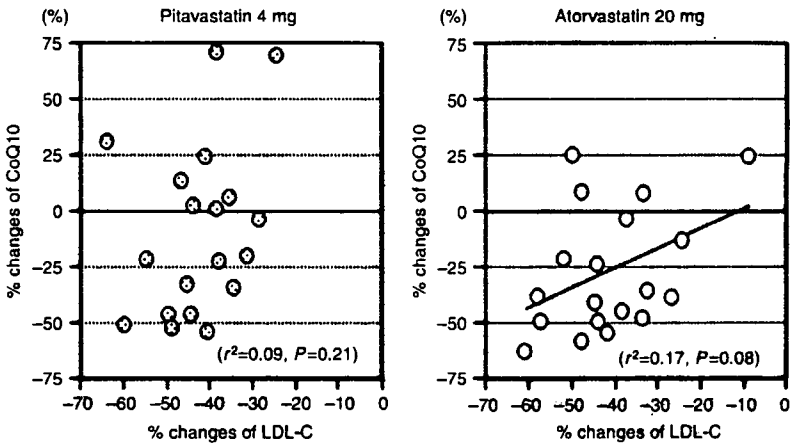

Figure 4 Correlations between percent changes of plasma CoQ10 and those of serum LDL cholesterol. LDL-C, low-density lipoprotein cholesterol.

Of 19 subjects, 13 were found to have mutations in the LDL receptor gene.[27] Exclusion criteria were a history of adverse events with a statin and taking probucol, a potent antioxidant, whose cholesterol-lowering effects last for more than 12 weeks, and antioxidants, including CoQ10. Previous lipid-lowering therapies with atorvastatin monotherapy or atorvastatin plus colestimide were acceptable (**Table 1**).

Written informed consent to participate in this study was obtained from each patient before entry into the study, and the ethical committee of Kanazawa University Hospital had approved the study protocol.

Study protocol. This study was conducted as an open randomized crossover study to compare the efficacy and the safety of pitavastatin and atorvastatin. All the patients were outpatients at the beginning of the study. Any lipid-lowering agents had been discontinued at least for 4 weeks before the study. Non-statin therapy was held constant during the course of the study.

Study subjects were divided into two groups by the envelope method. Group I (male/female = 5/5, average age = 51.6 years) began with 4 mg/day of pitavastatin, whereas group II (male/female = 2/7, average age = 64.9 years) began with 20 mg/day of atorvastatin. The first phase was continued for 8 weeks, and then pitavastatin and atorvastatin were discontinued for 4 weeks. Subsequently, each patient was switched to and maintained on the other statin for an additional 8 weeks. This protocol permitted 3 days' tolerance for each period.

Measurement of the laboratory data. Blood samples were obtained after an overnight fasting before and 56 days after each statin treatment and centrifuged at 4°C immediately. Serum cholesterol and triglycerides were determined by an enzymatic method, and HDL-cholesterol levels were measured by a polyamine polymer/detergent method (Daiichi, Tokyo, Japan), as described elsewhere.[28] LDL-cholesterol levels were determined by a direct method (Daiichi).[29] Apolipoproteins A-I, A-II, B, C-II, C-III, and E were determined as described previously.[30] The plasma levels of ubiquinol-10 and ubiquinone-10 were determined by an HPLC method.[11,31] Briefly, human plasma was mixed with 5 vols. of methanol and 10 vols. of hexane. After vigorous shaking and centrifugation, an aliquot of the hexane phase (5 µl) was injected immediately and directly onto a reverse-phase HPLC to minimize the oxidation of ubiquinol-10 to ubiquinone-10. Total CoQ10 refers to the sum of oxidized form (ubiquinone-10) and reduced form (ubiquinol-10).

Lipoprotein subfraction analysis. Serum lipoproteins were separated by two independent methods based on their densities and size. First, serum lipoproteins were separated by ultracentrifugation using the following densities: $d < 1.006$ g/ml for very LDL, $d = 1.006$–1.019 g/ml for intermediate-density lipoprotein, $d = 1.020$–1.063 g/ml for LDL, $d = 1.064$–1.125 g/ml for HDL2, and $d = 1.125$–1.21 g/ml for HDL3.

Second, the same samples were separated into 20 different lipoprotein subclasses by newly developed HPLC (LipoSEARCH, Skylight Biotech, Akita, Japan), as described elsewhere.[15] Briefly, 5 µl of whole plasma samples were injected into two connected columns of TSKgel LipopropakXL (Tosoh, Tokyo, Japan) and eluted by TSKeluent Lp-1 (Tosoh). The effluent from the columns was continuously monitored at 550 nm after an online enzymatic reaction with a commercial kit, DetermineL TC (Kyowa Medix, Tokyo, Japan). The cholesterol concentration in major lipoproteins and their subclasses was calculated by a computer program, which was designed to process complex chromatograms with a modified Gaussian curve-fitting function.

Statistical analyses. Values are expressed as mean ± SD unless otherwise stated. Effects of drug therapy on each variable were compared by means of a paired t-test. The percent changes of each variable after pitavastatin and atorvastatin treatment were compared by Wilcoxon's signed-rank test. All statistical analyses were performed with the Prism 4.0a system (GraphPad Software, San Diego, CA). A P value < 0.05 was considered significant.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

1. Scandinavian Simvastatin Survival Study Group. Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). *Lancet* **344,** 1383–1389 (1994).
2. Shepherd, J. *et al.* Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. *N. Engl. J. Med.* **333,** 1301–1307 (1995).
3. Sacks, F.M. *et al.* The effect of pravastatin on coronary events after myocardial infarction in patients with average cholesterol levels.

Cholesterol and Recurrent Events Trial investigators. *N. Engl. J. Med.* **1335,** 1001–1009 (1996).
4. Downs, J.R. *et al.* Primary prevention of acute coronary events with lovastatin in men and women with average cholesterol levels: results of AFCAPS/TexCAPS. Air Force/Texas Coronary Atherosclerosis Prevention Study. *JAMA* **279,** 1615–1622 (1998).
5. The Long-Term Intervention with Pravastatin in Ischaemic Disease (LIPID) Study Group. Prevention of cardiovascular events and death with pravastatin in patients with coronary heart disease and a broad range of initial cholesterol levels. *N. Engl. J. Med.* **339,** 1349–1357 (1998).
6. Cannon, C.P. *et al.* Pravastatin or Atorvastatin Evaluation and Infection Therapy—Thrombolysis in Myocardial Infarction 22 Investigators. Intensive versus moderate lipid lowering with statins after acute coronary syndromes. *N. Engl. J. Med.* **350,** 1495–1504 (2004).
7. Nissen, S.E. *et al.* REVERSAL Investigators. Effect of intensive compared with moderate lipid-lowering therapy on progression of coronary atherosclerosis: a randomized controlled trial. *JAMA* **291,** 1071–1080 (2004).
8. LaRosa, J.C. *et al.* Treating to New Targets (TNT) Investigators. Intensive lipid lowering with atorvastatin in patients with stable coronary disease. *N. Engl. J. Med.* **352,** 1425–1435 (2005).
9. Kovanen, P.T., Bilheimer, D.W., Goldstein, J.L., Jaramillo, J.J. & Brown, M.S. Regulatory role for hepatic low density lipoprotein receptors *in vivo* in the dog. *Proc. Natl. Acad. Sci. USA* **78,** 1194–1198 (1981).
10. Bliznakov, E.G. & Wilkins, D.J. Biochemical and clinical consequences of inhibiting coenzyme Q 10 biosynthesis by lipid-lowering HMG-CoA reductase inhibitors (statins): a critical overview. *Adv. Ther.* **15,** 213–228 (1998).
11. Mabuchi, H. *et al.* Reduction of serum ubiquinol-10 and ubiquinone-10 levels by atorvastatin in hypercholesterolemic patients. *J. Atheroscler. Thromb.* **12,** 111–119 (2005).
12. Berbel-Garcia, A. *et al.* Coenzyme Q 10 improves lactic acidosis, strokelike episodes, and epilepsy in a patient with MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis, and strokelike episodes). *Clin. Neuropharmacol.* **27,** 187–191 (2004).
13. Kajinami, K., Takekoshi, N. & Saito, Y. Pitavastatin: efficacy and safety profiles of a novel synthetic HMG-CoA reductase inhibitor. *Cardiovasc. Drug Rev.* **21,** 199–215 (2003).
14. Morikawa, S. *et al.* Relative induction of mRNA for HMG CoA reductase and LDL receptor by five different HMG-CoA reductase inhibitors in cultured human cells. *J. Atheroscler. Thromb.* **7,** 138–144 (2000).
15. Usui, S., Hara, Y., Hosaki, S. & Okazaki, M. A new on-line dual enzymatic method for simultaneous quantification of cholesterol and triglycerides in lipoproteins by HPLC. *J. Lipid Res.* **43,** 805–814 (2002).
16. Grundy, S.M. *et al.* National Heart, Lung, and Blood Institute; American College of Cardiology Foundation; American Heart Association. Implications of recent clinical trials for the National Cholesterol Education Program Adult Treatment Panel III Guidelines. *Circulation* **110,** 227–239 (2004).
17. Shin, M.J. *et al.* Effects of simvastatin on plasma antioxidant status and vitamins in hypercholesterolemic patients. *Int. J. Cardiol.* **118,** 173–177 (2007).
18. Hargreaves, I.P., Duncan, A.J., Heales, S.J. & Land, J.M. The effect of HMG-CoA reductase inhibitors on coenzyme Q10: possible biochemical/clinical implications. *Drug Saf.* **28,** 659–676 (2005).
19. Tomasetti, M., Alleva, R., Solenghi, M.D. & Littarru, G.P. Distribution of antioxidants among blood components and lipoproteins: significance of lipids/CoQ10 ratio as a possible marker of increased risk for atherosclerosis. *BioFactors* **9,** 231–240 (1999).
20. Berthold, H.K. *et al.* Effect of ezetimibe and/or simvastatin on coenzyme Q10 levels in plasma: a randomised trial. *Drug Saf.* **29,** 703–712 (2006).
21. Elmberger, P.G. *et al.* Effects of pravastatin and cholestyramine on products of the mevalonate pathway in familial hypercholesterolemia. *J. Lipid Res.* **32,** 935–940 (1991).
22. Furberg, C.D. & Pitt, B. Withdrawal of cerivastatin from the world market. *Curr. Control. Trials Cardiovasc. Med.* **2,** 205–207 (2001).
23. Yamazaki, H. *et al.* Influence of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors on ubiquinone levels in rat skeletal muscle and heart: relationship to cytotoxicity and inhibitory activity for cholesterol synthesis in human skeletal muscle cells. *J. Atheroscler. Thromb.* **13,** 295–307 (2006).
24. Farswan, M., Rathod, S.P., Upaganlawar, A.B. & Semwal, A. Protective effect of coenzyme Q10 in simvastatin and gemfibrozil induced rhabdomyolysis in rats. *Indian J. Exp. Biol.* **43,** 845–848 (2005).
25. Chu, C.S. *et al.* Effects of atorvastatin withdrawal on circulating coenzyme Q10 concentrations in patients with hypercholesterolemia. *BioFactors* **28,** 177–184 (2006).
26. Mabuchi, H., Koizumi, J., Shimizu, M. & Takeda, R. Development of coronary heart disease in familial hypercholesterolemia. *Circulation* **79,** 225–232 (1989).
27. Yu, W., Nohara, A., Higashikata, T., Lu, H., Inazu, A. & Mabuchi, H. Molecular genetic analysis of familial hypercholesterolemia: spectrum and regional difference of LDL receptor gene mutations in Japanese population. *Atherosclerosis* **165,** 335–342 (2002).
28. Huang, Y.C., Kao, J.T. & Tsai, K.S. Evaluation of two homogeneous methods for measuring high-density lipoprotein cholesterol. *Clin. Chem.* **43,** 1048–1055 (1997).
29. Usui, S., Kakuuchi, H., Okamoto, M., Mizukami, Y. & Okazaki, M. Differential reactivity of two homogeneous LDL-cholesterol methods to LDL and VLDL subfractions, as demonstrated by ultracentrifugation and HPLC. *Clin. Chem.* **48,** 1946–1954 (2002).
30. Kawashiri, M. *et al.* Efficacy of colestimide coadministered with atorvastatin in Japanese patients with heterozygous familial hypercholesterolemia. *Circ. J.* **69,** 515–520 (2005).
31. Yamashita, S. & Yamamoto, Y. Simultaneous detection of ubiquinol and ubiquinone in human plasma as a marker of oxidative stress. *Anal. Biochem.* **250,** 66–73 (1997).

Novel *SCN5A* mutation (Q55X) associated with age-dependent expression of Brugada syndrome presenting as neurally mediated syncope

Naomasa Makita, MD, PhD,* Naokata Sumitomo, MD, PhD,† Ichiro Watanabe, MD, PhD,‡ Hiroyuki Tsutsui, MD, PhD*

*From the *Department of Cardiovascular Medicine, Hokkaido University Graduate School of Medicine, Sapporo, Japan, †Division of Pediatrics, Department of Medicine, Nihon University School of Medicine, Tokyo, Japan, and ‡Division of Cardiovascular Disease, Department of Medicine, Nihon University School of Medicine, Tokyo, Japan.*

Introduction

Brugada syndrome is an autosomal dominant disorder characterized by ST elevation in the right precordial leads and syncope or sudden death due to malignant ventricular arrhythmias.[1] Brugada syndrome typically predisposes men aged 30 to 50 years to syncope. Mutations in the gene encoding the cardiac sodium channel α-subunit *SCN5A* have been identified in 20% to 30% of Brugada syndrome patients. The ECG signature of Brugada syndrome is dynamic and often concealed, and the prevalence of a Brugada-type ECG in the juvenile population is very low.[2] The most common cause of syncope in young individuals is neurally mediated syncope, a disorder of regulation of autonomic tone triggered by a wide variety of stimuli, including orthostatic and emotional stress. The prognosis of neurally mediated syncope in young individuals is generally benign. By definition, Brugada syndrome and neurally mediated syncope are distinct causes of syncope; however, several case reports have suggested an association between these conditions.[3–6] Furthermore, a clinical study showed that the head-up tilt test was positive in 35% of patients with a coved-type ST elevation.[7] In this report, we present the first case of neurally mediated syncope with a subclinical *SCN5A* mutation that manifested as Brugada syndrome during adolescence. This report emphasizes the genetic and clinical heterogeneity of Brugada syndrome and suggests that some individuals with neurally mediated syncope manifest Brugada syndrome in an age-dependent manner, possibly influenced by multiple factors, including hormonal and genetic substrates.

KEYWORDS Brugada syndromeNeurally mediated syncope*SCN5A*Tilt test (Heart Rhythm 2007;4:516-519)

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Case report

A 17-year-old male patient was referred for evaluation of recurrent syncope. Medical examination performed when the patient was 8 years old revealed nonspecific intraventricular conduction delay and first-degree AV block (Figure 1A). Physical examination, chest x-ray film, echocardiography, and treadmill exercise testing were normal, and no ST elevation or arrhythmias were observed at that time. Six months later, he experienced the first episode of syncope during prolonged standing. Because the syncopal attacks typically occurred while the patient was in an upright posture or was under emotional stress, his condition was diagnosed as neurally mediated syncope, although it was not proved at that time. At age 11 years, saddleback ST elevation became apparent in leads V_2–V_3 (Figure 1B). At age 17 years, a coved-type ST elevation was recorded from the third intercostal space (Figure 1C).

Upon admission, a provocation test using intravenous administration of the sodium channel blocker pilsicainide 50 mg was performed to test for conversion of saddleback ST elevation to coved-type elevation in the standard right precordial leads. Pilsicainide significantly augmented the J wave and ST elevation in lead V_2 (0.2 mV) but did not convert the ECG to coved-type (Figure 2A). Signal-averaged ECG was positive for late potentials. The patient experienced an 8.3-second episode of sinus arrest during venipuncture before electrophysiologic study (EPS). EPS showed sinus nodal dysfunction (corrected sinus node recovery time = 833 ms, sinoatrial conduction time measured by Narula method = 473.5 ms) and AV nodal dysfunction (Figure 2B). Ventricular fibrillation was induced by double extrastimuli from the right ventricular apex (Figure 2C). Head-up tilt test provoked hypotension followed by 12 seconds of sinus arrest, indicating a mixed type I neurally mediated syncope.

The patient had no family history of sudden cardiac death, but his mother had sick sinus syndrome with first-degree AV block, and his asymptomatic brother had first-degree AV block and nonspecific intraventricular conduction delay (Figure 3A). Genetic screening revealed a novel *SCN5A* mutation at exon 2 resulting in a premature stop

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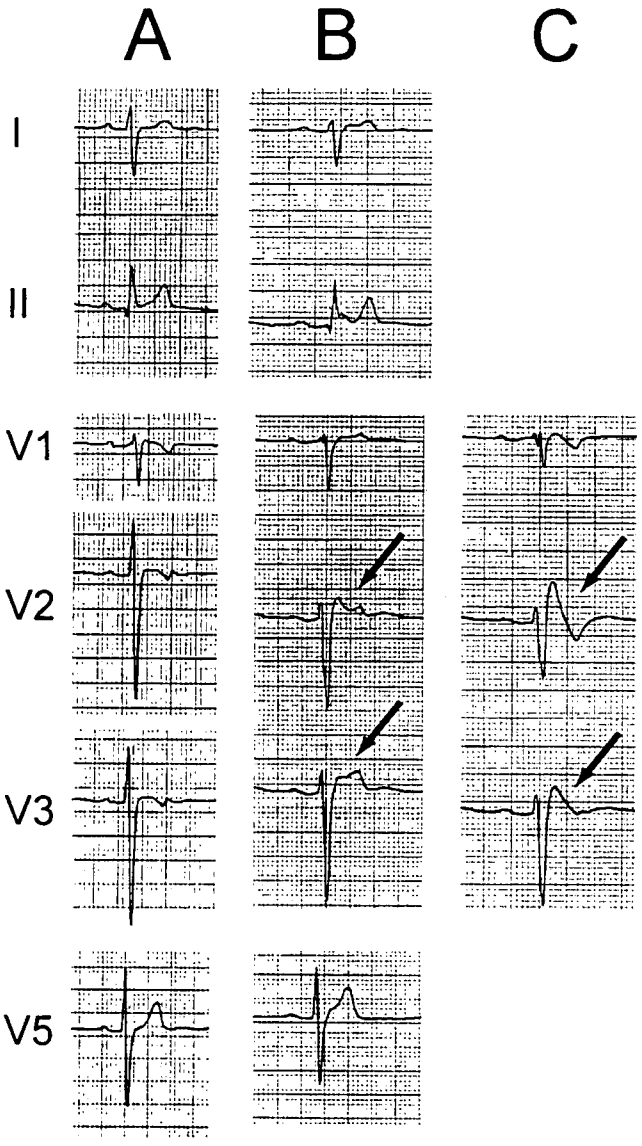

Figure 1 Age-dependent ECG changes in the proband. **A:** ECG recorded at age 8 years showed nonspecific intraventricular conduction delay without ST elevation. **B:** Saddleback ST elevation was evident in leads V_2–V_3 at age 11 years. **C:** Coved-type ST elevation was observed in leads V_1–V_3 recorded from the third intercostal space at age 17 years.

codon (Q55X) in the proband, his mother, and his brother (Figures 3A and 3B). This mutation is predicted to prematurely truncate the N-terminal of the sodium channel (Figure 3C). The heterologously expressed mutant Q55X channel showed no observable sodium current (Figure 3D).

An implantable cardioverter-defibrillator was recommended to the proband, but the patient declined. He has been treated with cilostazol, a phosphodiesterase inhibitor, to prevent severe bradycardia[8] and possible arrhythmias due to Brugada syndrome.[9] The proband's mother and brother did not agree to undergo EPS or pharmacologic testing.

Discussion

In the present case, the mode of syncope and the results of clinical examination, which included head-up tilt test, strongly favored the diagnosis of neurally mediated syncope before age 11 years. However, the clinical manifestation gradually changed to Brugada syndrome during adolescence. In this case, spontaneous coved-type ST elevation, inducible VF, and loss-of-function *SCN5A* mutation all are consistent with the diagnosis of Brugada syndrome that potentially could give rise to lethal arrhythmias. Some patients with Brugada syndrome, like the present case, exhibit only a coved-type ST elevation when the ECG is recorded from a higher intercostal space in the presence or absence of sodium channel blockers.[10,11] Therefore, the proband suffered from two apparently distinct conditions: neurally mediated syncope and Brugada syndrome. The autonomic nervous system has been implicated in both diseases,[12,13] and several case reports have described patients exhibiting clinical phenotypes of both neurally mediated syncope and Brugada syndrome.[3–5] Furthermore, a study demonstrated that 12 (35%) of 34 patients with a coved-type ST elevation showed a vasovagal response to head-up tilt test.[7] These observations suggest an association between neurally mediated syncope and Brugada syndrome rather than a simple coincidence. Identification of the causes of syncope in such patients often is difficult, therefore, treatment of these patients remains a therapeutic challenge.[5] Our report provides for the first time a genetic and biophysical basis that supports an association between neurally mediated syncope and Brugada syndrome.

The ECG signature of Brugada syndrome is dynamic and often concealed. The age-dependent manifestation of Brugada syndrome correlates well with previous observations that the ECG penetrance in mutation carriers of Brugada syndrome is considerably lower in children than in adults (17% vs 100%, respectively).[14] In a large family with overlapping phenotypes of Brugada syndrome and long QT syndrome due to the *SCN5A* mutation 1795insD, QT prolongation was recognized from birth onward, whereas ST elevation became apparent only after 5 years.[15] We speculate that at least some episodes of syncope that occurred before age 11 years in the present case were caused by arrhythmias due to the concealed Brugada syndrome substrate. Autonomic and hormonal influences, including testosterone, may be other candidates for age-dependent manifestation of the Brugada-syndrome phenotype, as indicated by a case report of the disappearance of the Brugada-type ECG pattern after surgical castration for prostate cancer.[16] Moreover, based on the observation that *SCN5A* is expressed not only in the myocardial cells but also in intracardiac ganglia,[17] it is speculated that the nonsense mutation of *SCN5A* provides not only the substrate for Brugada syndrome in the myocardium but also an imbalance in intracardiac ganglia activity, which in turn results in autonomic dysfunction implicated in both Brugada syndrome and neurally mediated syncope. Alternatively, genetic modifiers other than *SCN5A* may contribute to the age-dependent manifestation of the Brugada syndrome phenotype as well as the apparent phenotype–genotype dissociation observed between the proband and two mutation carriers.

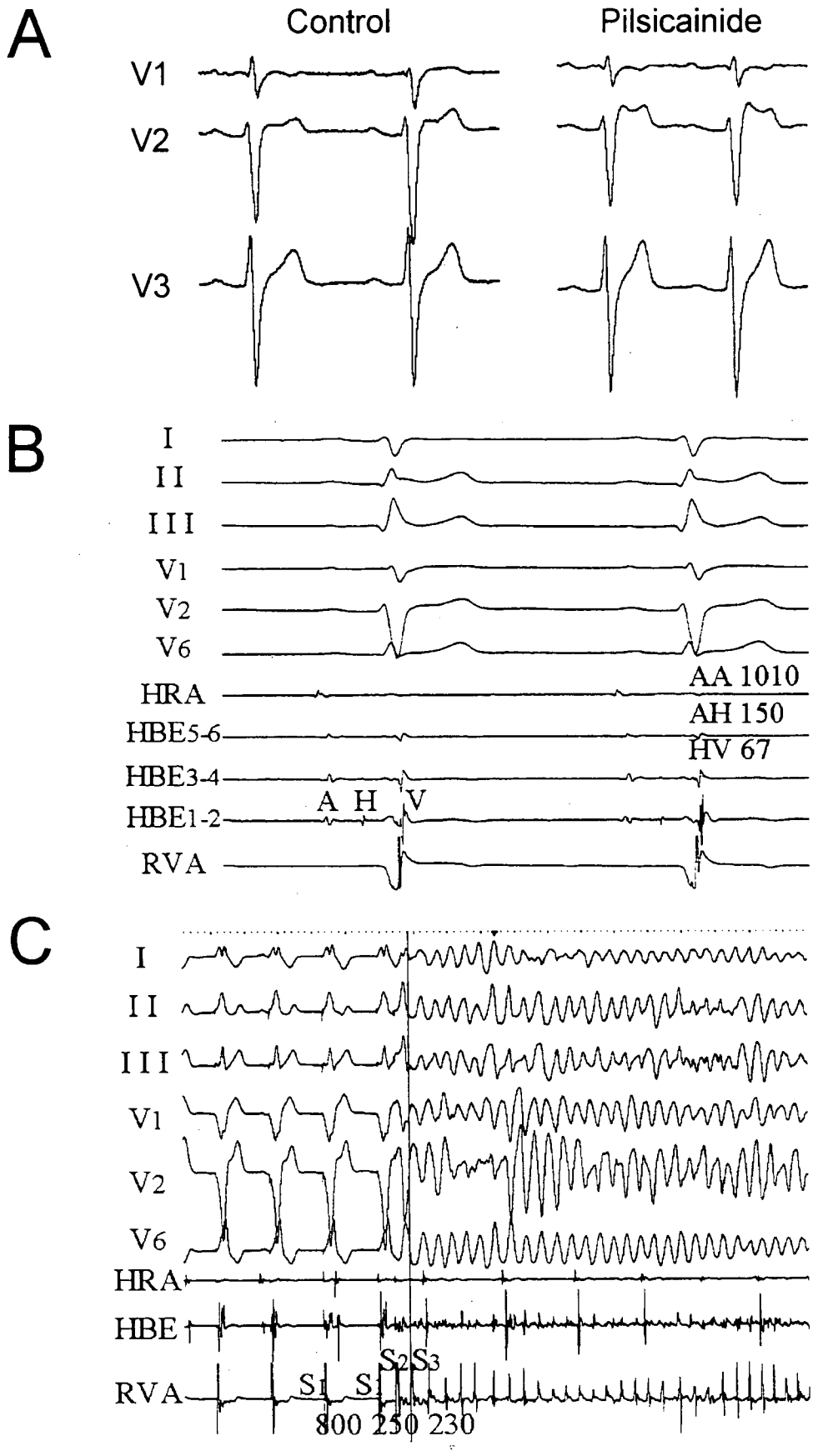

Figure 2 Provocation test with a sodium channel blocker and electrophysiologic study. **A:** Intravenous administration of pilsicainide 50 mg augmented the J wave and induced ST elevation in lead V_2. However, none of the standard right precordial leads converted to a coved-type. **B:** Intracardiac recording showing prolonged AA, AH, AV, and PR intervals. **C:** Ventricular fibrillation induced from the right ventricular apex by double extrastimuli (S1S1 = 800 ms, S1S2 = 250 ms, S2S3 = 230 ms).

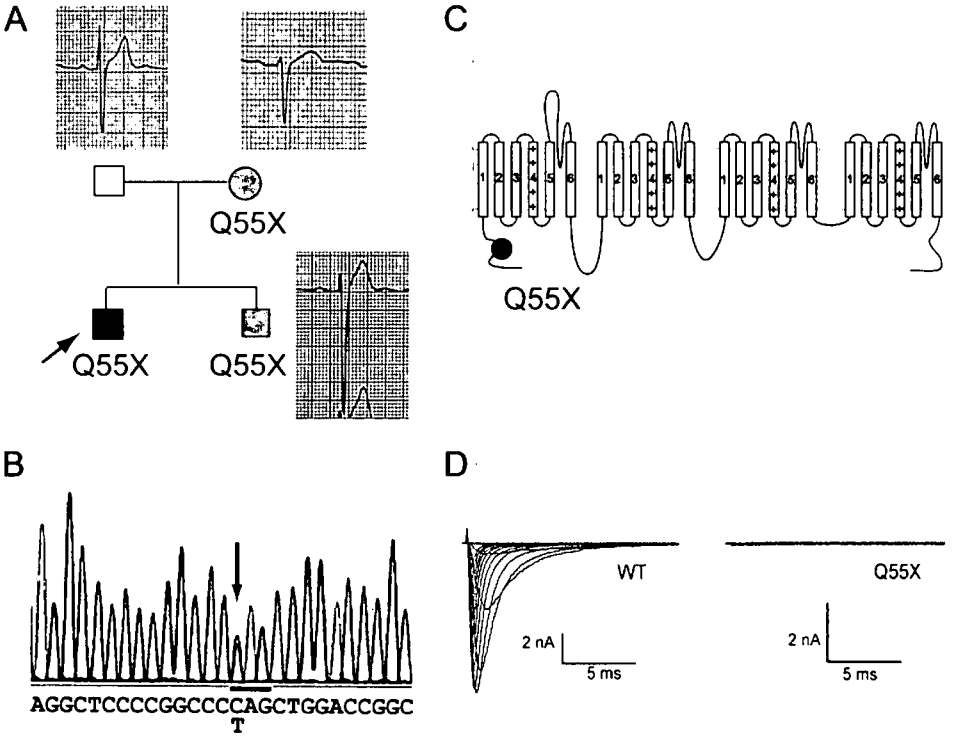

Figure 3 ECG and genetic evaluation of the pedigree and functional characterization of the mutation. **A:** Pedigree of the family with Brugada syndrome. *Arrow* indicates the proband. Lead V_2 ECGs are shown. *Open symbol* indicates the unaffected individual. *Closed* and *shaded symbols* indicate genetically affected individuals with and without symptoms, respectively. **B:** Sequence electropherogram of *SCN5A* of the proband shows heterozygous nonsense mutation *(arrow)* of a stop codon (TAG) for Gln-55 (CAG). **C:** Predicted topology of Nav1.5 and the location of Q55X. **D:** WT and Q55X channels were transiently transfected into tsA-201 cells, and the whole-cell sodium current was recorded as previously described.[18] Representative current traces elicited by test pulses (from −90 to +60 mV in 10-mV steps) from a holding potential of −120 mV are shown.

Conclusion

We demonstrated a novel nonsense *SCN5A* mutation in a patient with Brugada syndrome that previously was diagnosed as neurally mediated syncope. The functional consequences of *SCN5A* mutations are diverse, possibly influenced by multiple factors including age, hormones, and genetic modifiers. The prognosis of neurally mediated syncope may not necessarily be benign, because at least some patients with neurally mediated syncope, such as the present case, may also have Brugada syndrome due to a subclinical genetic substrate that may give rise to lethal arrhythmias.

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References

1. Antzelevitch C, Brugada P, Borggrefe M, Brugada J, Brugada R, Corrado D, Gussak I, LeMarec H, Nademanee K, Perez Riera AR, Shimizu W, Schulze-Bahr E, Tan H, Wilde A. Brugada syndrome: report of the second consensus conference: endorsed by the Heart Rhythm Society and the European Heart Rhythm Association. Circulation 2005;111:659–670.
2. Yoshinaga M, Anan R, Nomura Y, Tanaka Y, Tanaka Y, Sarantuya J, Oku S, Nishi S, Kawano Y, Tei C, Arima K. Prevalence and time of appearance of Brugada electrocardiographic pattern in young male adolescents from a three-year follow-up study. Am J Cardiol 2004;94:1186–1189.
3. Marquez MF, Rivera J, Hermosillo AG, Iturralde P, Colin L, Moragrega JL, Cardenas M. Arrhythmic storm responsive to quinidine in a patient with Brugada syndrome and vasovagal syncope. Pacing Clin Electrophysiol 2005;28:870–873.
4. Patruno N, Pontillo D, Anastasi R, Sunseri L, Giamundo L, Ruggeri G. Brugada syndrome and neurally mediated susceptibility. Ital Heart J 2005;6:761–764.
5. Samniah N, Iskos D, Sakaguchi S, Lurie KG, Benditt DG. Syncope in pharmacologically unmasked Brugada syndrome: indication for an implantable defibrillator or an unresolved dilemma? Europace 2001;3:159–163.
6. Patruno N, Pontillo D. Brugada syndrome and vasovagal syncope. Pacing Clin Electrophysiol 2006;29:215.
7. Yokokawa M, Sato Y, Kitamura S, Tanaka K, Nagai T, Noda T, Satomi K, Suyama K, Kurita T, Aihara N, Kamakura S, Shimizu W. Neurally-mediated syncope as a cause of syncope in patients with type 1 Brugada ECG. Heart Rhythm 2006;3:S58.
8. Atarashi H, Endoh Y, Saitoh H, Kishida H, Hayakawa H. Chronotropic effects of cilostazol, a new antithrombotic agent, in patients with bradyarrhythmias. J Cardiovasc Pharmacol 1998;31:534–539.
9. Tsuchiya T, Ashikaga K, Honda T, Arita M. Prevention of ventricular fibrillation by cilostazol, an oral phosphodiesterase inhibitor, in a patient with Brugada syndrome. J Cardiovasc Electrophysiol 2002;13:698–701.
10. Sangwatanaroj S, Prechawat S, Sunsaneewitayakul B, Sitthisook S, Tosukhowong P, Tungsanga K. New electrocardiographic leads and the procainamide test for the detection of the Brugada sign in sudden unexplained death syndrome survivors and their relatives. Eur Heart J 2001;22:2290–2296.
11. Shimizu W, Matsuo K, Takagi M, Tanabe Y, Aiba T, Taguchi A, Suyama K, Kurita T, Aihara N, Kamakura S. Body surface distribution and response to drugs of ST segment elevation in Brugada syndrome: clinical implication of eighty-seven-lead body surface potential mapping and its application to twelve-lead electrocardiograms. J Cardiovasc Electrophysiol 2000;11:396–404.
12. Miyazaki T, Mitamura H, Miyoshi S, Soejima K, Aizawa Y, Ogawa S. Autonomic and antiarrhythmic drug modulation of ST segment elevation in patients with Brugada syndrome. J Am Col Cardiol 1996;27:1061–1070.
13. Wichter T, Matheja P, Eckardt L, Kies P, Schafers K, Schulze-Bahr E, Haverkamp W, Borggrefe M, Schober O, Breithardt G, Schafers M. Cardiac autonomic dysfunction in Brugada syndrome. Circulation 2002;105:702–706.
14. Schulze-Bahr E, Eckardt L, Breithardt G, Seidl K, Wichter T, Wolpert C, Borggrefe M, Haverkamp W. Sodium channel gene (SCN5A) mutations in 44 index patients with Brugada syndrome: different incidences in familial and sporadic disease. Hum Mutat 2003;21:651–652.
15. Beaufort-Krol GCM, van den Berg MP, Wilde AAM, van Tintelen JP, Viersma JW, Bezzina CR, Bink-Boelkens MTE. Developmental aspects of long QT syndrome type 3 and Brugada syndrome on the basis of a single SCN5A mutation in childhood. J Am Coll Cardiol 2005;46:331–337.
16. Matsuo K, Akahoshi M, Seto S, Yano K. Disappearance of the Brugada-type electrocardiogram after surgical castration: a role for testosterone and an explanation for the male preponderance. Pacing Clin Electrophysiol 2003;26:1551–1553.
17. Scornik FS, Desai M, Brugada R, Guerchicoff A, Pollevick GD, Antzelevitch C, Perez GJ. Functional expression of "cardiac-type" Nav1.5 sodium channel in canine intracardiac ganglia. Heart Rhythm 2006;3:842–850.
18. Makita N, Sasaki K, Groenewegen WA, Yokota T, Yokoshiki H, Murakami T, Tsutsui H. Congenital atrial standstill associated with coinheritance of a novel SCN5A mutation and connexin 40 polymorphisms. Heart Rhythm 2005;2:1128–1134.

Circ J 2007; **Suppl A:** A-54–A-60

Genetic Polymorphisms and Arrhythmia Susceptibility

Naomasa Makita, MD; Hiroyuki Tsutsui, MD

Over the past 10 years, remarkable advances have been made in identifying the genes responsible for primary electrical heart diseases, such as congenital long QT syndrome and Brugada syndrome. Basic and clinical studies on these inherited arrhythmias have provided significant insight into the molecular basis of cardiac electrophysiology and the mechanisms of arrhythmias. However, many studies of genotype–phenotype relationships in these diseases have revealed considerable phenotypic variability in individuals from the same kindred carrying the identical disease-associated DNA variant, as is commonly observed in other polygenic disorders. Furthermore, despite rapid progress in understanding the molecular basis of primary electrical heart diseases, there is little insight into the genetics of acquired arrhythmias. Recently, it has been recognized that common genetic polymorphisms in cardiac ion channel and other genes may modify cardiac excitability, which in turn predisposes affected individuals to arrhythmias in the presence of triggering factors, such as electrolyte abnormalities or drugs. This paper reviews the current understanding of the contribution of genetic polymorphisms to the pathophysiology of cardiac arrhythmias. (*Circ J* 2007; **Suppl A:** A-54–A-60)

Key Words: Arrhythmia susceptibility; Common arrhythmias; Genetic modifier; Polymorphism; QT prolongation; Single nucleotide polymorphisms

Primary electrical heart disease refers to a disease entity of rare, often familial, cardiac arrhythmias in the absence of structural cardiac abnormalities. It includes several hereditary arrhythmias including long-QT syndrome (LQTS)[1] short-QT syndrome[2] Brugada syndrome (BS)[3] and cathecholaminergic polymorphic ventricular tachycardia[4,5] Most of these disorders are associated with mutations in the cardiac ion-channel genes, so they are referred to as cardiac ion channelopathies. Study of these rare diseases has been highly informative for basic and clinical electrophysiology, and a novel concept has recently emerged that common genetic variations might modify arrhythmia susceptibility in the general population. The finding that several drugs and electrolyte abnormalities are associated with development of cardiac arrhythmias has suggested a common genetic background in some individuals that predisposes them to arrhythmias in the presence of these triggering factors. With the availability of the human genome sequence, studies now focus on the identification of variations in the human genome and their contribution to arrhythmia predisposition.

Genetic Polymorphisms and Variable Penetrance

Mutations are generally defined as disease-associated alterations in DNA, occurring in less than 1% of the population. By contrast, polymorphisms are variations in the DNA sequence that have an allele frequency of at least 1% in a population, and are expected in approximately 1 in 1,000 base pairs of the human genome, differing in a polymorphic manner between 2 chromosomal homologues. As listed in Table 1, there are many different types of polymorphisms, including single nucleotide polymorphisms (SNPs), insertion/deletion variants, and microsatellite polymorphisms[6,7] Polymorphisms located within the coding region of a gene, such as non-synonymous SNPs, can directly influence the structure of its protein product, whereas others located within the regulatory region of a gene can influence the regulation of the expression levels of its protein product. These genetic variations may also alter phenotypic expression only under pathological conditions, such as during ischemia, or with the use of certain medications. However, a polymorphism does not necessarily mean that the variant is responsible for a clinical phenotype. Moreover, significant differences in polymorphic gene sites can be found in different ethnic backgrounds and may simply represent a genetic feature of a selected population rather than a susceptible allele.

In recent years, genetic approaches to understanding diversity in cardiac electrical function and susceptibility to cardiac arrhythmias have focused in particular on ion channels and gap junction proteins as key components in cardiac electrophysiology. Although mutations that cause the phenotype have been found in a single family or an individual in most cases, variations in genes linked to congenital arrhythmia syndromes may be relevant to more common acquired cardiac arrhythmias. Large population studies, in which cases of disease are compared with matched healthy controls from the same population, give a higher chance of detecting small genetic effects.

Systematic characterization of the clinical manifestations of genotyped families has revealed substantial intra- and interfamilial differences in phenotypic and disease expression, ranging from life-threatening arrhythmias to asymptomatic ECG changes. This phenomenon is referred to as "variable penetrance" and is typically observed in congenital LQTS[8] Variability elsewhere in the genome has been

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Department of Cardiovascular Medicine, Hokkaido University Graduate School of Medicine, Sapporo, Japan
Mailing address: Naomasa Makita, MD, Department of Cardiovascular Medicine, Hokkaido University Graduate School of Medicine, Kita-15, Nishi-7, Kita-ku, Sapporo 060-8638, Japan. E-mail: makitan@med.hokudai.ac.jp

Table 1 Types of Gene Polymorphisms[6,7]

Polymorphism type	*Sequence location*	*Predicted protein and potential functional effects*	*Occurrence in genome*	*Potential disease impact*
Nonsense	*Coding*	*Prematurely truncated, most likely loss of protein function*	*Very low*	*High*
Missense, non-synonymous	*Coding, non-conserved*	*Altered amino acid chain, mostly similar protein properties*	*Low*	*Low (to high)*
Missense, non-synonymous	*Coding, conserved*	*Altered amino acid chain, mostly different protein properties*	*Low*	*Medium to high*
Rearrangements (insertion/deletion)	*Coding*	*Altered amino acid chain, mostly different protein properties*	*Low*	*High*
Sense, synonymous	*Coding*	*Unchanged amino acid chain, rarely effect on exon splicing*	*Medium*	*Low (to medium)*
Promotor and regulatory sequences	*Non-coding, promotor/UTR*	*Unchanged amino acid chain, but may affect gene expression*	*Low to midium*	*Low to high, depending on site*
Intoronic nucleotide exchange (<40 bp)	*Non-coding, splice/lariat sites*	*Altered amino acid chain, failed recognition of exonic structure*	*Low*	*Low to high, depending on site*
Intoronic nucleotide exchange (>40 bp)	*Non-coding, between introns*	*Unchanged amino acid chain, rarely abnormal splicing or mRNA instability, site for gene rearrangements*	*Medium*	*Very low*
Intergenic nucleotide exchange	*Non-coding, between genes*	*Unchanged amino acid chain, may effect gene expression, site for gross rearrangements*	*High*	*Very low*

UTR, untranslated region (5' or 3' region of a gene); bp, base pairs.

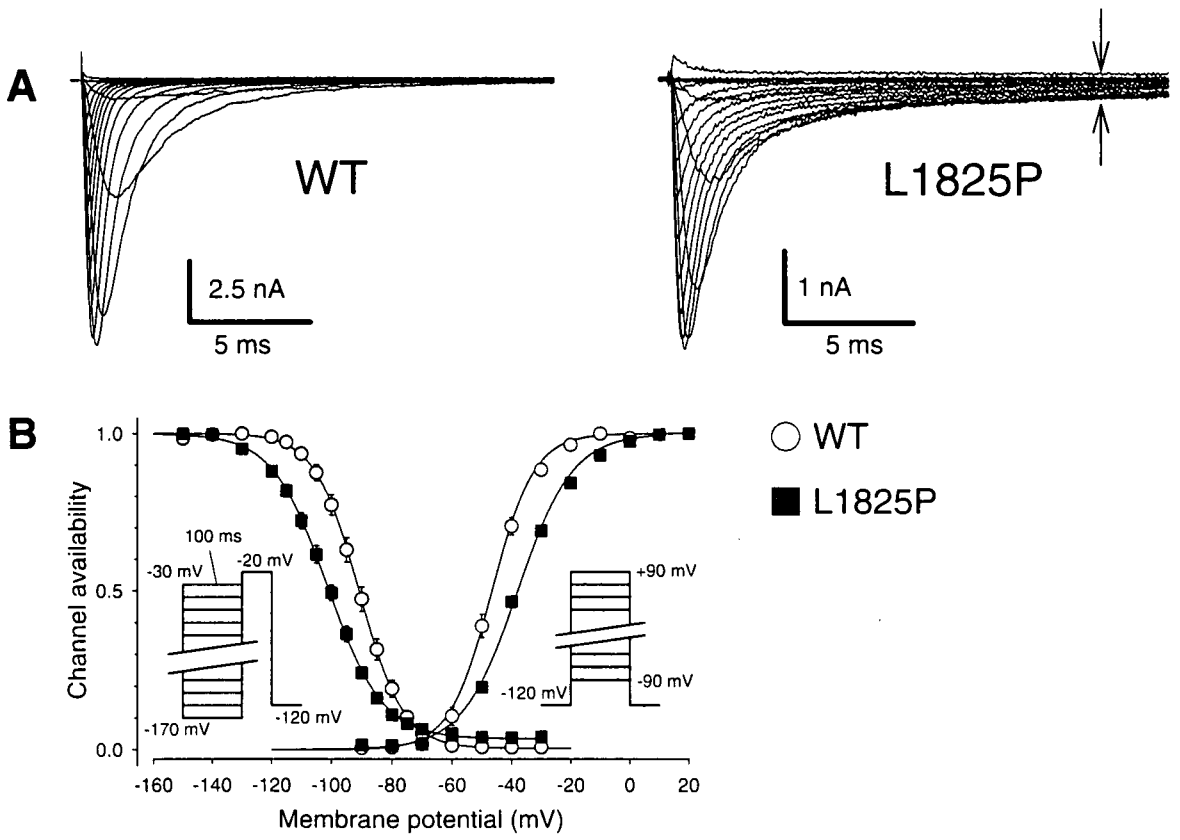

Fig 1. A mutation of *SCN5A*, L1825P, associated with cisapride-induced acquired long QT syndrome (LQTS). (A) Whole-cell Na current recorded from cells expressing wild-type (WT) or L1825P. Persistent non-inactivating Na current, characteristic of LQT3 mutations, is shown with an arrow. (B) Voltage-dependence of inactivation (Left) and activation (Right) is shifted in the hyperpolarizing and depolarizing directions, respectively. These biophysical abnormalities tend to reduce Na current. Therefore, L1825P exhibited the mixed channel dysfunction found in both LQT3 and Brugada syndrome.

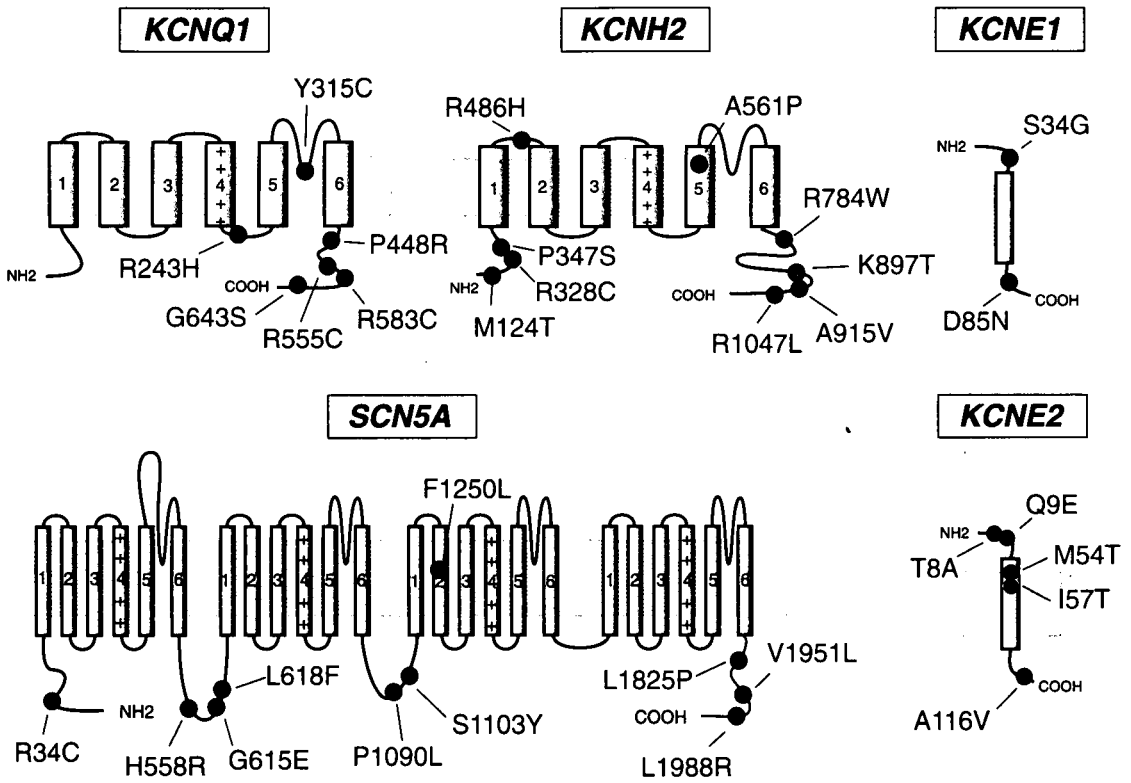

Fig 2. Mutations and non-synonymous single nucleotide polymorphisms (SNPs) of 5 long QT syndrome (LQTS) genes identified in drug-induced LQTS. Putative transmembrane domains of the 5 LQTS genes are shown with boxes and the locations of the SNPs are indicated with filled circles. Location of the *SCN5A* mutation, L1825P, is also shown.

assumed to contribute to this phenomenon. One possibility is that a modulator locus may reside in the same disease gene. Another possibility is that variability at 2 genetic loci might modulate an arrhythmogenic phenotype. One subset of such "double hit" cases is an especially severe or unusual phenotype in an individual with 2 abnormal alleles of the same gene, either by consanguinity (same abnormal allele) or by compound heterozygosity (different alleles).

Acquired LQTS

Acquired LQTS is often iatrogenic and associated with drugs, including familiar antibiotics, antihistamines, antipsychotics, and antiarrhythmics[9] LQTS can also be manifested by electrolyte imbalances or bradycardia, especially in combination with the aforementioned drugs. Patients with acquired drug-induced torsades de pointes (TdP) share a number of clinical features with the congenital form of LQTS: female preponderance, apparent increased risk with hypokalemia, QT prolongation and TdP, and evidence of adrenergic activation prior to TdP. These findings, as well as the relatively unpredictable nature of drug-associated QT prolongation, suggest that there may be a population at risk because of genetic factors, but whose phenotype remains subclinical until drug challenge. It has been proposed that repolarization in the heart is accomplished by multiple redundant mechanisms, and that each 1 of the risk factors impairs 1 or more of these mechanisms to a variable extent. However, because of redundancy in the system, there is considerable "repolarization reserve", and it is only when this reserve is exhausted by the presence of multiple risk factors that arrhythmias develop![10]

Following the concept of "repolarization reserve", it is likely that the occurrence of TdP would be independent of specific drugs and more linked to a drug's propensity to alter cardiac repolarization in individuals with a genetic predisposition to abnormal repolarization. Therefore, mutations can be identified not only in the I_{Kr} channel genes, but also in I_{Ks} or sodium channel genes in patients with drug-induced TdP. In fact, we found a novel *SCN5A* mutation, L1825P, in a patient with cisapride-induced acquired LQTS (Fig 1). The heterologously expressed mutant L1825P showed mixed biophysical abnormalities of the persistent Na current that is a hallmark of LQT3 mutations and loss-of-function properties characteristic of BS![11] Liu et al recently explained the mechanism of QT prolongation and TdP caused by an I_{Kr} blocker in a patient with the *SCN5A* mutation, L1825P![12] They showed that L1825P has a trafficking defect, and cisapride partially rescued the trafficking defect and the surface expression of the mutant channels, which led to an increase in the late Na current and QT prolongation.

The idea that common ion channel DNA variants may contribute to arrhythmia susceptibility has been driven by the identification of non-synonymous SNPs (Fig 2) and in vitro characterization showing subtle alterations differing from the wild type. In fact, genetic screening in 92 patients with drug-induced TdP demonstrated 6 genetic variants in 3 major LQTS genes (*KCNQ1*, *KCNH2*, and *SCN5A*)![13] including mutations with mild biophysical phenotypes, which possibly confer an increased risk of TdP in response to drug challenge. However, none of these variants showed statistically significant differences in the allele frequency between normal and TdP populations. Moreover, it should

be noted that functional studies of some ion channel SNPs are not comparable or may even produce contradictory results, probably because in vitro experiments are not standardized among different laboratories.

Genetic Variations in Cardiac Sodium Channel Genes

A recently identified SNP in the *SCN5A* gene, S1103Y, is associated with arrhythmia risk in African Americans.[14] The Y1103-allele, carried by 13% of African Americans and overrepresented among arrhythmia patients of African decent (56.5%), was also linked to prolongation of the QT interval in an African American family. This SNP is found in approximately 19% of West Africans and Caribbeans, but not in Caucasians or Asians. More recently, S1103Y has been predominantly found in victims of sudden infant death syndrome, and the heterologously expressed S1103Y channel exhibited higher sensitivity to a lower intracellular pH than the wild type. These data suggest that the variant appears to confer susceptibility to acidosis-induced arrhythmia, indicating a gene–environment interaction.[15]

H558R is one of the most prevalent SNPs of *SCN5A*. The electrophysiological characteristics of H558R do not differ from the wild type, but in vitro studies have reported that H558R modulates the trafficking of the *SCN5A* mutations T512I[16] (responsible for isolated cardiac conduction defects) and M1766L[17] (responsible for LQT3) when H558R and the mutations are on the same allele. More recent data show that H558R mitigates the trafficking abnormalities of a BS mutation, R282H, that is present on the non-mutant allele.[18] These studies demonstrate the effect of genetic background on the phenotypic expression of a disease-causing mutation. However, population studies of congenital or acquired arrhythmias have shown no association between H558R and arrhythmias. Thus, the pathophysiological relevance of this SNP needs further elucidation.

Bezzina et al recently found a haplotype variant in the promoter region of *SCN5A* consisting of 6 polymorphisms in near-complete linkage disequilibrium (LD);[19] an association of multiple loci on a chromosome caused by limited recombination between them. The allelic frequency of the variant haplotype (HapB) was 22% in the Asian population, but was absent in whites and blacks. Furthermore, HapB reduces transcription levels of *SCN5A*, and the promoter haplotype correlated well with PR and QRS durations. This study suggests that genetically-determined variable Na channel transcription is associated with variable conduction velocity, an important contributor to arrhythmia susceptibility.

Genetic Variations in Cardiac Potassium Channel Genes

Among 16 *KCNQ1* mutations responsible for LQT1,15 mutations were localized in the transmembrane domains and associated with a high percentage of symptomatic carriers and sudden deaths, whereas a missense mutation R555C at the C-terminal domain was associated with significantly less QT prolongation, and lower percentages of symptomatic carriers.[20] R555C appeared to represent a forme fruste phenotype, a factor favoring acquired LQT syndrome. Sesti et al identified a SNP of *KCNE2* T8A in a patient with sulfamethoxazole (SMZ)-associated LQTS (allelic frequency of 1.6% of the control population).[21] Functional studies revealed that T8A channels were normal at baseline, but inhibited by SMZ at therapeutic levels that did not affect the wild-type channels. That study demonstrates that clinically silent DNA variations can increase the risk of life-threatening arrhythmias after drug exposure. Kubota et al reported that G643S a SNP of *KCNQ1* (allelic frequency of 9% in the general Japanese population) decreased I_{Ks} current density in vitro.[22] This SNP was in the LQT families studied and was mostly associated with a rather mild phenotype. Prolongation of the QT interval was often precipitated by hypokalemia and bradyarrhythmias, implying that this polymorphism might be acting as a modifier gene in these LQT families.

With the *KCNH2* (HERG) gene, several conflicting studies pertaining to K897T and the duration of QT interval in the healthy population have been reported. Pietila et al reported an association between the 897T-allele (allelic frequency of 16% in Finns) and prolongation of QTc interval in Finnish females.[23] In a LQT2 family with the *KCNH2* mutation A1116V, K897T is a genetic modifier that exaggerates the I_{Kr} reduction caused by A1116V; thus, only the individuals carrying both A1116V and K897T manifest QT prolongation.[24] Bezzina et al found a significant association between the 897T-allele and shorter QTc intervals in healthy Caucasian groups, and the electrophysiological characterization of the K897T channel revealed gain-of-function properties leading to a shorter QT interval.[25] More recently, a large LD-based SNP association study of LQTS genes showed that K897T is significantly associated with a shorter QTc interval.[26]

Genetic Variants of Other Genes

Gap junctions are clusters of connexin (Cx) channels that span the closely opposed plasma membranes forming cell-to-cell pathways and facilitate action potential conduction. Cx40 is a gap junction protein predominantly expressed in the atrium and the specialized conduction system. There are 2 closely linked SNPs in the promoter region of Cx40, which lead to a substantial reduction in promoter activity.[27] In 2 kindreds with atrial standstill, individuals with both the *SCN5A* mutation and the Cx40 promoter SNPs displayed the clinical phenotype, whereas individuals with a single defect are phenotypically indistinguishable from normal family members.[27,28] These findings support the proposed interaction of the polymorphism with the mutation. By extension, multiple common variants in ion channel genes, or other genes that modulate cardiac electrophysiology, can be logically proposed as candidate modulators of clinical arrhythmia phenotypes.

More generally, this is an example of biology informing the identification of new candidate loci in which DNA variants might modulate a clinical phenotype. As complex signaling pathways determining integrated biologic responses (such as normal cardiac rhythm) are unraveled, each element is defined as such a candidate. Importantly, such candidate loci may involve modifier genes or signaling pathways heretofore not associated with arrhythmia phenotypes. Predicting the behavior of complex systems that have been perturbed by disease, drug administration, or functionally significant DNA variants is a major challenge to contemporary biology.

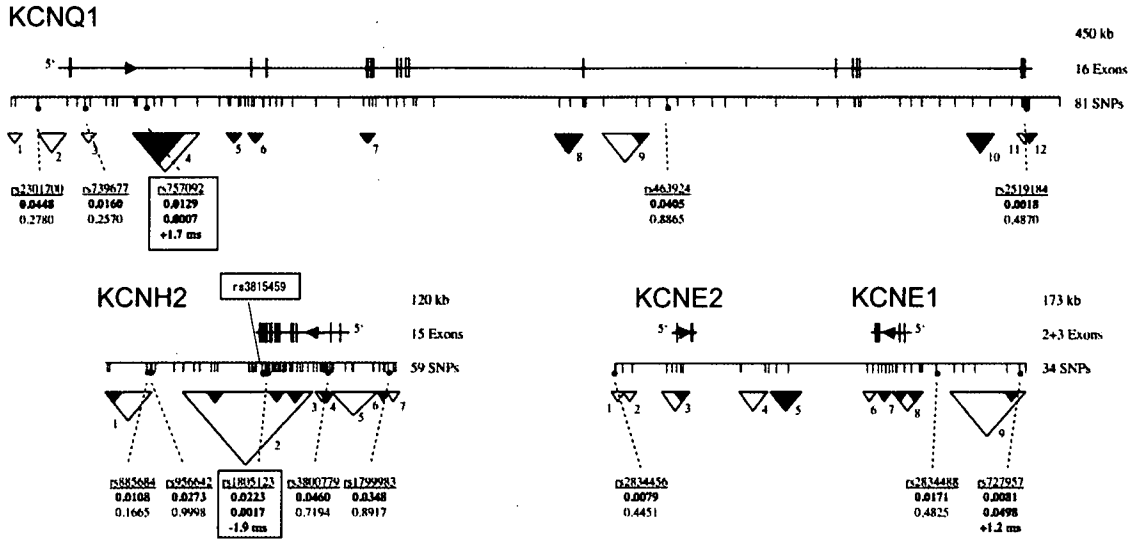

Fig 3. Genomic structure, linkage disequilibrium (LD)-structure, and genotyped single nucleotide polymorphisms (SNPs) in the investigated gene regions[26] 174 SNPs genotyped in the screening sample are denoted as (|), 13 SNPs genotyped in the confirmation sample are marked by ●. The regions of LD structure are marked by D'-based haplotype block boundaries (▽) and by neighboring SNPs exceeding r^2-values of 0.5 (▼). For SNPs genotyped in both samples, p-values for association with QTc_RAS in the screening (Top) and in the confirmation sample (Bottom) and for associated SNPs the effect of 1 minor allele on QTc_RAS in the entire sample is given.

Table 2 Genetic Polymorphisms

	0	*1*	*2*	*QTc_RAS±SD*	*From total sample (n=3,966), n*
KCNQ1 rs757092	*GG*	*GA*	*AA*		
KCNH2 rs1805123 (K879T)	*CC*	*CA*	*AA*		
KCNH2 rs3815459	*GG*	*GA*	*AA*		
QT-prolongation score					
0				*412.7±13.4*	*79*
1				*415.5±16.9*	*462*
2				*416.6±16.9*	*1,021*
3				*418.3±17.8*	*1,132*
4				*419.3±16.9*	*641*
5				*423.2±19.4*	*135*

Effect of the 5 genotypic changes significant in the multivariate regression analysis from the 3 confirmed single nucleotide polymorphisms KCNQ1-rs757092, KCNH2-K897T, and KCNH2-rs3815459 was determined by a QT-prolongation score (p<0.00005). For each score-class the average QTc_RAS, standard deviation and the number of individuals are given. Individuals harboring the maximum possible number of 5 QT-prolonging alleles had on average a 10.5-ms longer QTc_RAS than individuals that had no QT-prolonging allele (0.95% of variance; p<0.00005).

Genetic Factors Determining Cardiac Repolarization

Altered myocardial repolarization is one of the important substrates of malignant ventricular arrhythmias, and rare gene variants affect repolarization in congenital LQTS. To investigate the influence of common gene variants on the QT interval, Pfeufer et al performed an LD-based SNP association study of 4 candidate genes[26] (Fig 3). They initially genotyped 174 SNPs of 4 LQTS genes (*KCNQ1*, *KCNH2*, *KCNE1*, and *KCNE2*) in 689 individuals, and successively screened 14 SNPs with suggestive linkage in a confirmatory sample of 3,277 individuals (total 3,966 individuals). They showed association to 1 SNP of *KCNQ1* (rs757092: +1.7 ms/allele) and 2 SNPs of *KCNH2* (K897T, rs1805123, −1.9 ms/allele; and rs3815459, +1.7 ms) (Fig 3). These SNPs have additive effects on the QTc interval, showing a 10.5 ms difference in the QTc_RAS (QT interval corrected for rate, age and sex) between extreme-score groups (Table 2). This study is the first evidence that cardiac repolarization is heritable as a quantitative phenotypic trait.

To identify common genetic variants that modulate cardiac repolarization, Arking et al recently performed a genome-wide association study using 200 subjects at the extremes of a population-based QTc_RAS distribution of 3,966 subjects from the KORA cohort in Germany, with follow-on screening of selected markers in the remainder of the cohort[29] They identified *NOS1AP* (*CAPON*), a regulator of neuronal NOS, as a modulator of cardiac repolarization. Although the physiological roles of CAPON in the heart have not been extensively studied, a recent report showed that overexpression of CAPON in cardiomyocytes accelerated repolarization via a reduction of the L-type Ca current[30] As additional polymorphisms are elucidated, the true multigenic scope of arrhythmia susceptibility will emerge, but the clinical implication of individual polymorphisms will grow increasingly complex.

Atrial Fibrillation (AF)

AF is the most common cardiac arrhythmia, and it increases in prevalence with advancing age to approximately 6% in individuals older than 65 years. Since the initial identification of the locus of familial AF on chromosome 10q22-24,[31] 7 further loci, including 4 relevant K channel genes, have been mapped.[32] The *KCNQ1* mutation, S140G, was identified in Chinese familial AF, and the heterologously expressed S140G channel revealed gain-of-function properties on the I_{Ks} current, in contrast to the loss-of-function effects of the *KCNQ1* mutations previously described in LQT1 patients.[33] In addition to monogenic diseases, Lai et al reported a non-synonymous SNP (G38S) of *KCNE* as a risk factor for AF susceptibility.[34] The frequency of the 38G-allele was significantly higher in the AF group than in the control group (76.4 vs 63.0%), although the functional significance of this SNP remains to be elucidated. More recently, Zeng et al have shown that none of the *KCNQ1* and *KCNE1* non-synonymous SNPs was associated with AF in the Chinese population, but the *KCNE4* SNP E145D was associated with AF.[35]

Limitations of Association Studies and Functional Assessment of SNPs

There are a number of potential limitations before the results of association studies can be integrated into clinical practice. First, the biological effects of a single polymorphism may be undetected, especially in the setting of multifactorial diseases, where the small additive effects of many factors may contribute to the disease phenotype. Second, the genetic heterogeneity of the population studied is also a major issue. Efforts should be made to match the subjects carefully by ethnic–geographic origin and other confounding variables (age, sex, smoking, cardiovascular disease, etc); thus, large-scale studies are required in order to detect even moderate associations.

Identification of non-synonymous SNPs and their subtle biophysical alterations characterized in vitro have prompted the idea that a common ion channel sequence variance may also contribute to a common arrhythmia and arrhythmia susceptibility. However, the results of ion channel alterations may be variable or even contradictory, because in vitro experiments are not standardized among different laboratories. For instance, Baroudi et al proposed an interesting mechanism underlying the apparent discrepancy between the severe clinical phenotype of an individual with BS carrying a T1620M mutation and its relatively minor biophysical abnormalities when expressed in cultured cells.[36] Based on the fact that the proband carried an additional rare SNP R1232W, they constructed a double mutant (T1620M/R1232W) and characterized Na-channel function. In contrast to the relatively minor functional abnormalities of T1620M, they found that the double mutant channel molecule failed to reach the plasma membrane (defect of membrane trafficking), leading to total loss of the cardiac Na current. This observation supports the hypothesis that polymorphisms of ion channel genes can affect membrane trafficking or gating, thereby modulating the channel properties of the coexisting mutation. In order to confirm this observation, we constructed the same double mutation and performed patch clamp and confocal imaging to characterize its biophysical properties and subcellular distribution. To our surprise, the T1620M/R1232W channels elicited robust Na current, despite showing altered gating properties, and the subcellular distribution was nearly normal (data not shown). Unfortunately, such contradictions of in vitro experiments are common among different laboratories, and this represents a major hurdle that needs to be overcome. Standardization of in vitro functional assessment of non-synonymous SNPs is needed to compare lab-specific results.

Conclusions

An important focus of future efforts will be to determine the mechanisms that control the expression of ion channel genes and the modulating factors that determine normal cardiac electrophysiology. Furthermore, it will be important to determine how genetic variations may cause arrhythmias. The identification of common variants that cause a subtle increase in the risk of life-threatening arrhythmias will facilitate prevention of sudden cardiac death through the rapid identification of populations at risk. Moreover, large databases with well-characterized drug responses may help define new drug targets and develop expedited technology to screen individuals with potential arrhythmogenic substrates, thereby leading to new treatment strategies.

Acknowledgments

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References

1. Keating MT, Sanguinetti MC. Molecular and cellular mechanisms of cardiac arrhythmias. *Cell* 2001; **104:** 569–580.
2. Gaita F, Giustetto C, Bianchi F, Wolpert C, Schimpf R, Riccardi R, et al. Short QT Syndrome: A familial cause of sudden death. *Circulation* 2003; **108:** 965–970.
3. Brugada P, Brugada J. Right bundle branch block, persistent ST segment elevation and sudden cardiac death: A distinct clinical and electrocardiographic syndrome: A multicenter report. *J Am Coll Cardiol* 1992; **20:** 1391–1396.
4. Lahat H, Pras E, Olender T, Avidan N, Ben-Asher E, Man O, et al. A missense mutation in a highly conserved region of CASQ2 is associated with autosomal recessive catecholamine-induced polymorphic ventricular tachycardia in Bedouin families from Israel. *Am J Hum Genet* 2001; **69:** 1378–1384.
5. Priori SG, Napolitano C, Tiso N, Memmi M, Vignati G, Bloise R, et al. Mutations in the cardiac ryanodine receptor gene (hRyR2) underlie catecholaminergic polymorphic ventricular tachycardia. *Circulation* 2001; **103:** 196–200.
6. Tabor HK, Risch NJ, Myers RM. Candidate-gene approaches for studying complex genetic traits: Practical considerations. *Nature Rev Genet* 2002; **3:** 391–397.
7. Kaab S, Schulze-Bahr E. Susceptibility genes and modifiers for cardiac arrhythmias. *Cardiovasc Res* 2005; **67:** 397–413.
8. Priori SG, Napolitano C, Schwartz PJ. Low penetrance in the long-QT syndrome: Clinical impact. *Circulation* 1999; **99:** 529–533.
9. Roden DM. Pharmacogenetics and drug-induced arrhythmias. *Cardiovasc Res* 2001; **50:** 224–231.
10. Roden DM. Taking the "idio" out of "idiosyncratic": Predicting torsades de pointes. *Pacing Clin Electrophysiol* 1998; **21:** 1029–1034.
11. Makita N, Horie M, Nakamura T, Ai T, Sasaki K, Yokoi H, et al. Drug-induced long-QT syndrome associated with a subclinical SCN5A mutation. *Circulation* 2002; **106:** 1269–1274.
12. Liu K, Yang T, Viswanathan PC, Roden DM. New mechanism contributing to drug-induced arrhythmia: Rescue of a misprocessed LQT3 mutant. *Circulation* 2005; **112:** 3239–3246.
13. Yang P, Kanki H, Drolet B, Yang T, Wei J, Viswanathan PC, et al. Allelic variants in long-QT disease genes in patients with drug-associated Torsades de Pointes. *Circulation* 2002; **105:** 1943–1948.
14. Splawski I, Timothy KW, Tateyama M, Clancy CE, Malhotra A, Beggs AH, et al. Variant of SCN5A sodium channel implicated in

risk of cardiac arrhythmia. *Science* 2002; **297:** 1333–1336.
15. Plant LD, Bowers PN, Liu Q, Morgan T, Zhang T, State MW, et al. A common cardiac sodium channel variant associated with sudden infant death in African Americans, SCN5A S1103Y. *J Clin Invest* 2006; **116:** 430–435.
16. Viswanathan PC, Benson DW, Balser JR. A common SCN5A polymorphism modulates the biophysical effects of an SCN5A mutation. *J Clin Invest* 2003; **111:** 341–346.
17. Ye B, Valdivia CR, Ackerman MJ, Makielski JC. A common human SCN5A polymorphism modifies expression of an arrhythmia causing mutation. *Physiol Genomics* 2003; **12:** 187–193.
18. Poelzing S, Forleo C, Samodell M, Dudash L, Sorrentino S, Anaclerio M, et al. SCN5A polymorphism restores trafficking of a Brugada syndrome mûtation on a separate gene. *Circulation* 2006; **114:** 368–376.
19. Bezzina CR, Shimizu W, Yang P, Koopmann TT, Tanck MW, Miyamoto Y, et al. Common sodium channel promoter haplotype in asian subjects underlies variability in cardiac conduction. *Circulation* 2006; **113:** 338–344.
20. Donger C, Denjoy I, Berthet M, Neyroud N, Cruaud C, Bennaceur M, et al. KVLQT1 C-terminal missense mutation causes a forme fruste long-QT syndrome. *Circulation* 1997; **96:** 2778–2781.
21. Sesti F, Abbott GW, Wei J, Murray KT, Saksena S, Schwartz PJ, et al. A common polymorphism associated with antibiotic-induced cardiac arrhythmia. *Proc Natl Acad Sci USA* 2000; **97:** 10613–10618.
22. Kubota T, Horie M, Takano M, Yoshida H, Takenaka K, Watanabe E, et al. Evidence for a single nucleotide polymorphism in the KCNQ1 potassium channel that underlies susceptibility to life-threatening arrhythmias. *J Cardiovasc Electrophysiol* 2001; **12:** 1223–1229.
23. Pietila E, Fodstad H, Niskasaari E, Laitinen PP, Swan H, Savolainen M, et al. Association between HERG K897T polymorphism and QT interval in middle-aged Finnish women. *J Am Coll Cardiol* 2002; **40:** 511–514.
24. Crotti L, Lundquist AL, Insolia R, Pedrazzini M, Ferrandi C, De Ferrari GM, et al. KCNH2-K897T is a genetic modifier of latent congenital long-QT syndrome. *Circulation* 2005; **112:** 1251–1258.
25. Bezzina CR, Verkerk AO, Busjahn A, Jeron A, Erdmann J, Koopmann TT, et al. A common polymorphism in KCNH2 (HERG) hastens cardiac repolarization. *Cardiovasc Res* 2003; **59:** 27–36.
26. Pfeufer A, Jalilzadeh S, Perz S, Mueller JC, Hinterseer M, Illig T, et al. Common variants in myocardial ion channel genes modify the QT interval in the general population: Results from the KORA study. *Circ Res* 2005; **96:** 693–701.
27. Groenewegen WA, Firouzi M, Bezzina CR, Vliex S, van Langen IM, Sandkuijl L, et al. A cardiac sodium channel mutation cosegregates with a rare connexin40 genotype in familial atrial standstill. *Circ Res* 2003; **92:** 14–22.
28. Makita N, Sasaki K, Groenewegen WA, Yokota T, Yokoshiki H, Murakami T, et al. Congenital atrial standstill associated with coinheritance of a novel SCN5A mutation and connexin 40 polymorphisms. *Heart Rhythm* 2005; **2:** 1128–1134.
29. Arking DE, Pfeufer A, Post W, Kao WHL, Newton-Cheh C, Ikeda M, et al. A common genetic variant in the NOS1 regulator NOS1AP modulates cardiac repolarization. *Nat Genet* 2006; **38:** 644–651.
30. Chan K-C, Barth A, Kizana E, Kashiwakura Y, Sasano T, Zhang Y, et al. CAPON, a nitric oxide synthase regulator associated with QT interval variation in humans, modulates cardiac repolarization in ventricular myocytes. *Circulation* 2006; **114:** II-167.
31. Brugada R, Tapscott T, Czernuszewicz GZ, Marian AJ, Iglesias A, Mont L, et al. Identification of a genetic locus for familial atrial fibrillation. *N Engl J Med* 1997; **336:** 905–911.
32. Roberts R. Mechanisms of disease: Genetic mechanisms of atrial fibrillation. *Nat Clin Pract Cardiovasc Med* 2006; **3:** 276–282.
33. Chen Y-H, Xu S-J, Bendahhou S, Wang X-L, Wang Y, Xu W-Y, et al. KCNQ1 gain-of-function mutation in familial atrial fibrillation. *Science* 2003; **299:** 251–254.
34. Lai L-P, Su M-J, Yeh H-M, Lin J-L, Chiang F-T, Hwang J-J, et al. Association of the human minK gene 38G allele with atrial fibrillation: Evidence of possible genetic control on the pathogenesis of atrial fibrillation. *Am Heart J* 2002; **144:** 485–490.
35. Zeng Z, Tan C, Teng S, Chen J, Su S, Zhou X, et al. The single nucleotide polymorphisms of I_{Ks} potassium channel genes and their association with atrial fibrillation in a Chinese population. *Cardiology* 2007; **108:** 97–103.
36. Baroudi G, Acharfi S, Larouche C, Chahine M. Expression and intracellular localization of an SCN5A double mutant R1232W/T1620M implicated in Brugada syndrome. *Circ Res* 2002; **90:** 11e–16e.

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SCN5A variants in Japanese patients with left ventricular noncompaction and arrhythmia

Lishen Shan [a,f], Naomasa Makita [b], Yanlin Xing [a,f], Sayaka Watanabe [a], Takeshi Futatani [a], Fei Ye [a], Kazuyoshi Saito [a], Keijiro Ibuki [a], Kazuhiro Watanabe [a], Keiichi Hirono [a], Keiichiro Uese [a], Fukiko Ichida [a,*], Toshio Miyawaki [a], Hideki Origasa [c], Neil E. Bowles [d], Jeffrey A. Towbin [e]

[a] *Department of Pediatrics, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan*
[b] *Department of Cardiovascular Medicine, Hokkaido University Graduate School of Medicine, Sapporo 060-8638, Japan*
[c] *Division of Biostatistics, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan*
[d] *Department of Pediatrics (Cardiology), University of Utah School of Medicine, Salt Lake City, UT, USA*
[e] *Department of Molecular and Human Genetics, College of Medicine, Houston, TX, USA*
[f] *Department of Pediatrics, The ShengJing Hospital affiliated to China Medical University, Shenyang, China*

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Abstract

Left ventricular noncompaction (LVNC) is a genetically heterogenous disorder. Mutations in the human cardiac sodium channel alpha-subunit gene (*SCN5A*) are involved in the pathophysiology of cardiac arrhythmias and cardiomyopathies. This study was performed to compare the frequency of *SCN5A* variants in LVNC patients with or without arrhythmias, and to investigate the relationship between variants and disease severity. DNA was isolated from the peripheral blood of 62 Japanese probands with LVNC, comprising 17 familial cases and 45 sporadic cases. Blood samples were screened for variants in *SCN5A* using single-strand conformational polymorphism analysis (SSCP) and DNA sequencing. Seven variants, rs6599230:G > A, c.453C > T, c.1141-3C > A, rs1805124:A > G (p.H558R), rs1805125:C > T (p.P1090L), c.3996C > T, and rs1805126:T > C were identified in 7 familial and 12 sporadic cases. The frequency of *SCN5A* variants was significantly higher in the patients with arrhythmias than those without (50% vs 7%: $P = 0.0003$), suggesting these variants represent a risk factor for arrhythmia and supporting the hypothesis that genes encoding ion channels are involved in LVNC pathophysiology. The LVNC patients with heart failure also had high occurence of *SCN5A* variants, suggesting the presence of *SCN5A* variants and/or arrhythmias increase the severity of LVNC.

Keywords: Noncompaction; *SCN5A* variants; Arrhythmia; Heart failure

Introduction

Left ventricular noncompaction (LVNC) is characterized by persistence of multiple prominent ventricular trabeculations and deep intertrabecular recesses in the left ventricle and is defined as an unclassified cardiomyopathy [1]. To date, this disorder is considered an arrest in the morphogenetic process of myocardial compaction [2]. The clinical manifestations are not specific for this form of cardiomyopathy, with clinical overlap with dilated and hypertrophic cardiomyopathies, and are highly variable, ranging from asymptomatic to severe cardiac dysfunction leading to heart transplantation or death. Most LVNC patients also present with some form of arrhythmia [2].

LVNC, without other morphologic cardiac abnormalities, was first described echocardiographically in 1984 [3].

* Corresponding author. Fax: +81 76 434 5029.
E-mail address: fukiko@med.u-toyama.ac.jp (F. Ichida).

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In recent years, defects in several genes have been associated with LVNC. Recently, we identified novel sequence variants in *DTNA*, *TAZ*, and *LDB3* in patients with LVNC [4–6]. However, like other forms of inherited cardiomyopathy, LVNC is a genetically heterogeneous disease and can be inherited as an autosomal dominant or X-linked recessive disorder [4–6].

Mutations in the human cardiac sodium channel alpha-subunit gene (*SCN5A*) have been identified in patients with a range of arrhythmias including the long QT syndrome (LQTS) [7], Brugada syndrome [8], sudden unexplained nocturnal death syndrome [9], idiopathic ventricular fibrillation [10], congenital sick sinus syndrome [11], and cardiac conduction defects (CCD) [12], as well as sudden infant death syndrome [13]. Recently, variants in *SCN5A* [14,15] and a cardiac K_{ATP} channel gene (*ABCC9*)[16] have been reported in patients with dilated cardiomyopathy (DCM). The sodium channel plays a central role in the excitability of myocardial cells, by establishing a subtle equilibrium between depolarizing and repolarizing currents determining the action potential (AP) duration. Thus, variations in *SCN5A* may influence this equilibrium, even by weak effects on activity and/or the expression level of channels subunits. Single nucleotide polymorphisms (SNPs) in *SCN5A* have been implicated not only as the causes of inherited arrhythmic syndromes, but also as genetic risk factors for some acquired arrhythmias [17–20].

Therefore, we hypothesized that variations in genes encoding ion channels are implicated in the pathophysiology of LVNC in relation to the development of arrhythmias and the severity of disease. Here we report the analysis of the *SCN5A* gene in a large cohort of Japanese patients with LVNC, and present data supporting this hypothesis.

Methods

Subjects and clinical diagnostic criteria

LVNC was diagnosed by echocardiographic criteria, including: (1) LV hypertrophy with deep endomyocardial trabeculations in ≥ 1 ventricular wall segments, (2) reduced LV systolic function, (3) a two-layered endocardium with a noncompacted to compacted ratio of >2.0, and (4) deep recesses filled with blood from the ventricular cavity visualized on color Doppler imaging (Fig. 1) [4].

Initial clinical evaluations were performed without knowledge of genotype status. Once a proband was identified, a family history was obtained, and all potentially informative family members underwent clinical evaluation, including physical examination, chest radiograph, electrocardiogram (ECG), echocardiogram (2 dimensional and color Doppler) was used to evaluate the cardiac structure, LV size and function (shortening fraction and ejection fraction), and valve regurgitation (Fig. 1).

Peripheral blood samples were collected after written informed consent. Lymphoblastoid cell lines were established from the peripheral blood samples and then genomic DNA was isolated using QIAamp DNA extraction kits (Qiagen: Valencia, CA). The study was approved by the Research Ethics Committee of Toyama University Hospital.

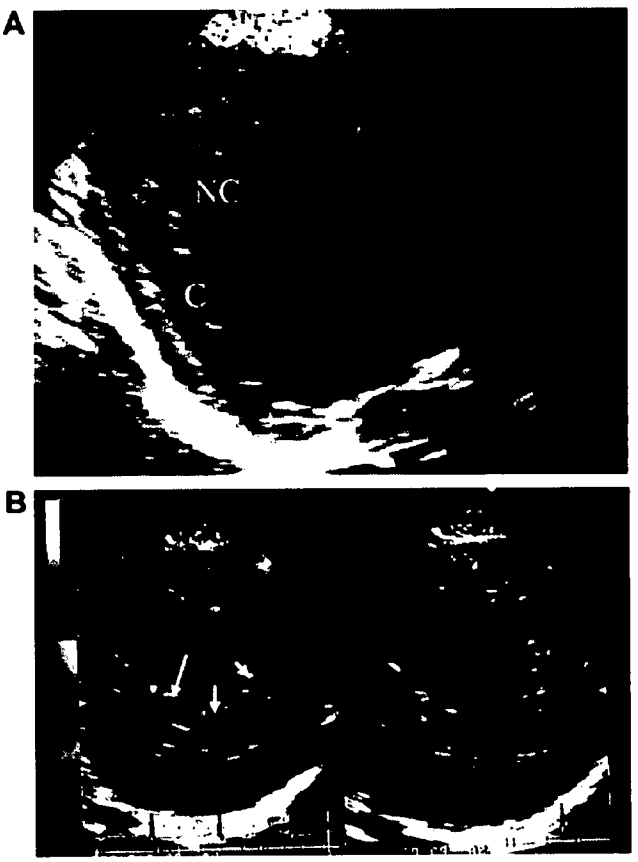

Fig. 1. (A) Two-dimensional echocardiogram of a patient with LVNC revealing noncompacted layer with intertrabecular recesses (NC) and outside the compacted layer (C), with an NC:C ratio >2.0. (B) Color Doppler echocardiogram of a LVNC patient demonstrating flow within deep intertrabecular recesses (arrow) in continuity with the left ventricular cavity. (For interpretation of the references to colours in this figure legend, the reader is referred to the web version of this paper.)

Molecular genetic studies

The *SCN5A* gene was amplified by PCR from the genomic DNA of each of the probands. The PCR primers were designed to amplify each of the coding exons, as well as flanking intronic sequences, using the online utility Primer 3 (http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi) and sequences from NCBI and Celera databases: the NCBI genomic DNA sequence file NT_022517.17 served as the primary source. PCR reactions were performed as previously described [6]: primer sequences and PCR conditions are available upon request. After PCR amplification, the samples were denatured by heating in a denaturing buffer and analyzed by SSCP [5].

Both normal and aberrant SSCP bands were cut directly from dried gels, purified, and sequenced according to the Applied Biosystems (ABI) Big Dye Terminator Cycle Sequencing protocol and analyzed using an ABI 310 Automated Sequencer. Sequencing results were compared with wild-type sequence published in NCBI by the BLAST comparative search algorithms (www.ncbi.nlm.nih.gov/BLAST/). Variations were confirmed by repeating the PCR from the genomic DNA template and sequencing the PCR products.

Statistical analysis

SNP frequencies in both of the groups were compared using the Chi square test. Relations between variants and arrhythmias were calculated by logistic regression. The differences were considered to be significant when $P < 0.05$.

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Results

Genetic studies

The study cohort comprised 62 unrelated Japanese LVNC probands, which included 45 sporadic cases (S) and 17 familial cases (F): a further 8 affected family members were also recruited for genotype-phenotype correlations. Table 1 summarizes the probands sex, age at diagnosis, and clinical phenotype, and the *SCN5A* variants identified.

Six heterozygous and one homozygous (rs6599230:G > A) variants were identified in 7 familial and 12 sporadic cases (Tables 1 and 2). Of these, 4 are reported in the NCBI SNP database for *SCN5A* (http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?geneId=6331&ctg=NT_022517.17&mrna=NM_198056.1&prot=NP_932173.1&orien=reverse), rs6599230:G > A, rs1805124:A > G (p.H558R), rs1805125: C > T (p.P1090L), and rs1805126:T > C. Two have been reported as SNPs (c.453C > T [21], c.1141-3C > A [22]). The last variant, c.3996C > T, was detected in a sporadic case of isolated LVNC with complete atrioventricular block (AVB): this variant has not been reported previously.

Association of SCN5A variants with arrhythmias

SCN5A variants were detected in 17 (50%) of the 34 probands with arrhythmias (7 familial cases and 10 sporadic cases) compared with 2 (7%) of the 28 probands without arrhythmias (Table 3): this difference was significant by Chi square analysis ($P = 0.0003$). In these 17 cases, a wide variety of arrhythmias were noted, including AVB ($n = 8$), atrial fibrillation (AF) ($n = 2$), LQTS ($n = 2$), Wolff-Parkinson-White syndrome (WPW) ($n = 5$), SSS ($n = 2$), ventricular tachycardia (VT) ($n = 7$), premature ventricular contraction (PVC) ($n = 10$), paroxysmal supraventricular tachycardia (PSVT) ($n = 3$).

While most of the variants were associated with multiple types of arrhythmia (Table 2), in the analysis of extended families multiple arrhythmia phenotypes were detected in the same family. For example for proband F6 (Fig. 2), who presented with LVNC with premature ventricular contraction (PVC), two variants were identified, rs1805126:T > C and p.P1090L. Both variants were inherited from his affected mother, who presented with LVNC with sick sinus syndrome (SSS). Using logistic regression analysis, patients who have the rs1805126:T > C variant have significantly high risk of arrhythmia (OR = 5.69 (1.05-30.84), $P = 0.044$).

We also investigated the correlation between the *SCN5A* variants and the severity of LVNC. Similar to the findings with arrhythmias, *SCN5A* variants were detected in 18 (53%) of the 34 patients who had developed heart failure, compared with just 1 (4%) of the 28 who had not. This was also a significant difference by Chi square analysis ($P = 0.0002$) (Table 4).

Discussion

During the more than ten years since mutations in the gene encoding the cardiac sodium channel, *SCN5A*, were first reported in 1995 [7], genetic alterations in *SCN5A* have been shown to influence the pathophysiology of cardiac arrhythmias, pharmacological sensitivities to antiarrhythmic drugs and recently cardiomyopathy [14]. Some common exonic SNPs were previously described as being able to produce subtle functional changes in channels physiology.

To date variations in three genes have been identified in patients with LVNC, *DTNA, TAZ* and *LDB3*.[4–6] Arrhythmias occur in more than 50% of patients with LVNC [23]. Since this phenotype does not correlate with the identification of gene variations, we hypothesized that variations in ion channel genes may contribute to the development of arrhythmias in LVNC patients regardless of the underlying cause of the disease. Therefore, we screened patients with LVNC for genetic variants in *SCN5A*. A significant number of genetic variations (both synonymous and non-synonymous nucleotide substitutions) were identified in the patient cohort. However, variations were detected significantly more frequently in patients who presented with arrhythmias than those who did not: 50% vs 7% ($P = 0.0003$).

Since LVNC patients with arrhythmias tend to have worse outcomes than patients without arrhythmias [23], we anticipated identifying a similar correlation between genetic variants and heart failure. Indeed *SCN5A* variants were detected in significantly more of the patients with heart failure, than this without: 53% vs 4% ($P = 0.0002$). Therefore, these data support the hypothesis that variations in ion channel encoding genes contribute to the underlying pathophysiology of LVNC and determining outcome.

The H558R substitution is located in the Na^+ channel I, II interdomain cytoplasmic linker and previous functional studies have shown that the R558-encoding minor allele can dramatically alter the phenotype of true disease-causing *SCN5A* mutations [24]. It has been suggested that it modulates Na^+ channel functional changes caused by other variations, and plays a role in intragenic complementation [25,26]. H558R expressed in the different variant backgrounds has profoundly different effectd on function, even "loss of function."[27] In our two cases with the H558R variant the QT interval was normal, but they both had severe arrhythmias, one with PVC and the other WPW. The P1090L substitution was identified in a sporadic case with AF and a familial case with PVC (proband) and SSS (the proband's mother). P1090 is localized in the II, III interdomain cytoplasmic linker. As a relatively Asian-specific common polymorphism, P1090L has been shown to result in a significant negative shift of activation midpoint in the Q1077del background [27].

With the exception of the c.3996C > T variant, all of the other variants are commonly detected in the normal popu-

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Table 1
Clinical and molecular data for probands with LVNC

ID	Sex	Age	Arrhythmia	Heart failure	SCN5A variant
F1	M	0y	PVC	+	No
F2	F	69y	—	+	No
F3	M	5y	AVB	+	No
F4*	F	1w	PVC	+	c.453C > T
F5	F	60y	AVB, VT	+	RS1805126:T > C
F6	M	4y	PVC, SSS	+	RS1805126:T > C, p.P1090L
F7	F	1y	—	—	No
F8	F	5y	AF	+	RS1805126:T > C
F9	M	3m	—	+	No
F10	M	13y	LQT	+	RS6599230:G > A, RS1805126:T > C
F11	F	29y	—	+	No
F12	F	1y	—	—	No
F13	F	3y	—	—	No
F14	M	4y	—	—	No
F15	F	N/A	PVC	+	RS1805126:T > C, RS6599230:G > A
F16	F	1m	—	—	No
F17*	M	13	WPW	+	RS1805124:A > G, c.1141-3C > A
S18	F	0.2y	PSVT, WPW	+	No
S19	M	12y	PVC	+	RS1805124:A > G, c.1141-3C > A, RS1805126:T > C
S20	F	12y	AVB	+	No
S21	M	7y	AVB	+	No
S22	F	1w	WPW	+	RS1805126:T > C
S23	N/A	N/A	WPW	—	No
S24	F	21y	VT	—	No
S25	M	3y	VT	+	No
S26	M	10d	PSVT, VT	+	No
S27	M	25y	AVB	—	No
S28	M	1y	AVB	+	No
S29	F	3m	PSVT	+	RS1805126:T > C
S30	M	10y	PVC	—	No
S31	F	12y	AF	+	p.P1090L, RS1805126:T > C
S32	F	1d	LQT	—	No
S33	F	14y	VT	—	No
S34	F	1y	PVC, VT	+	No
S35	N/A	N/A	—	—	No
S36	N/A	N/A	—	+	RS1805126:T > C
S37	N/A	N/A	—	—	No
S38	N/A	N/A	—	—	No
S39	N/A	N/A	—	+	RS1805126:T > C
S40	N/A	N/A	—	—	No
S41	M	1m	—	—	No
S42	M	2m	—	+	No
S43	M	0y	AVB	+	P1332P
S44	M	9m	PVC	+	c.1141-3C > A
S45	M	3y	—	+	No
S46	M	36y	VT	+	RS1805126:T > C
S47	M	N/A	SSS	+	RS1805126:T > C
S48	F	N/A	AVB	—	No
S49	F	1y	—	—	No
S50	M	8m	WPW	—	No
S51	M	0y	—	—	No
S52	M	8m	—	—	No
S53	F	22y	—	—	No
S54	F	4m	—	+	No
S55	M	15y	—	—	No
S56	M	5m	PVC	+	RS1805126:T > C
S57	M	17y	—	—	No
S58	M	1y	—	+	No
S59	F	1y	—	—	No
S60	F	0y	—	—	No
S61	M	43y	PVC	—	RS6599230:G > A
S62	F	16y	—	—	No

F, familial case; S, sporadic case; ID indicates identification; Age, proband's age at diagnosis; N/A, information unavailable; blank, no abnormality; +, the proband had heart failure; no, no variant was found. AVB AV block; AF, atrial fibrillation; LQT, long QT syndrome; WPW, Wolff-Parkinson-White syndrome; SSS, sick sinus syndrome; VT, ventricular tachycardia; PVC, premature ventricular contraction; PSVT, paroxysmal supraventricular tachycardia.

* Compound with *LDB3* variation [6].

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Table 2
Summary of *SCN5A* variants detected

DNA variant (dbSNP ID)	Protein change	Exon	Family/case	Types of arrhythmia
87G > A (rs6599230)		Exon 2	F10,F15,S61	PVC, LQT
453C > T		Exon 4	F4	PVC
1141-3C > A		Intron 9	F17, S19, S44	PVC, WPW
1673A > G (rs1805124)	H558R	Exon 12	F17, S19	PVC, WPW
3269C > T (rs1805125)	P1090L	Exon 18	F6, S31	AF, SSS, PVC
3996G > A		Exon23	S43	AVB
5457T > C (rs1805126)		Exon28	F5, F6, F8, F10, F15, S19, S22, S29, S31, S36,S39, S46, S47, S56	PSVT, VT, PVC, AVB, LQT, SSS, AF, WPW

Abbreviations are defined in Table 1.

Table 3
Incidence of *SCN5A* variants in the cases with arrhythmia and in those without arrhythmia

	SCN5A Variant			(%)	No *SCN5A* variant			(%)
	Familial cases	Sporadic cases	Total		Familial cases	Sporadic cases	Total	
Arrhythmia	7	10	17	(50)	2	15	17	(50)
No arrhythmia	0	2	2	(7)	8	18	26	(93)
Total	7	12	19	(31)	10	33	43	(69)

($P = 0.0003$).

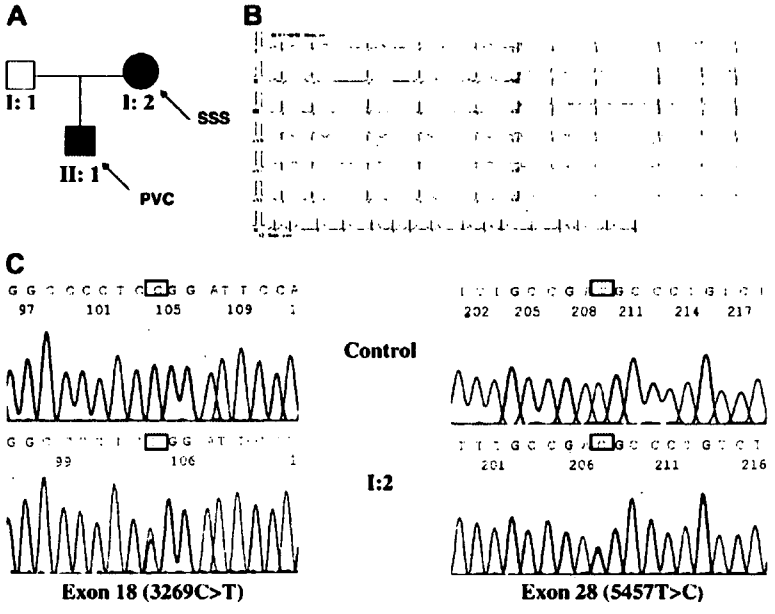

Fig. 2. (A) Pedigree of familial case 6. (B) ECG of the proband's mother who presented with SSS. (C) DNA sequence analysis of exon18 (left) and exon 28 (right) of *SCN5A* in a normal control (top) and in the proband's mother (I:2, bottom).

Table 4
Incidence of *SCN5A* variants in the cases with heart failure and in those without heart failure

	SCN5A Variant			(%)	No *SCN5A* variant			(%)
	Familial cases	Sporadic cases	Total		Familial cases	Sporadic cases	Total	
Heart failure	7	11	18	(53)	5	11	16	(47)
No heart failure	0	1	1	(4)	5	22	27	(96)
Total	7	12	19	(31)	10	33	43	(69)

($P = 0.0002$).

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lation. Therefore, the question remains, how do these variants, most of which are synonymous substitutions, lead to abnormal arrhythmogenesis in the population? Regardless of their effect on the amino acid sequence, a significant number of single nucleotide substitutions alter mRNA splicing efficiency or accuracy [5]. Exonic splicing enhancer (ESE) and silencer (ESS) elements are present in most exons. Since ESEs are often distant from the canonical splice junctions, point mutations that inactivate an ESE can result in partial or complete exon skipping, affecting the structure or amounts of the expressed protein product. These splicing alterations may be incomplete, resulting in subtle changes in sodium channel expression that do not manifest changes in function in the normal heart, but in a pathological environment result in changes in cardiac repolarization, which consequently could modify the clinical expression of a latent LVNC pathogenic mutation. Rs1805126:T > C has been reported to be associated with short QTc intervals [18]. In our study, it related with the significantly high risk of the arrhythmia in LVNC patients. However, analysis using splice site prediction software (http://www.fruitfly.org/seq_tools/splice.html) or exonic splice enhancer prediction (http://genes.mit.edu/burgelab/rescue-ese/) does not predict any differences in mRNA splicing due to this SNP (data not shown).

Further, subtle changes in ion channel expression could be exacerbated by the presence of variants in proteins that interact with the ion channel. In most of the patients studied here, disease causing mutations have not been identified. Interestingly, multiple proteins containing PDZ domains have been associated with ion channel localization and function [28]. One such protein, LIM Domain Binding Protein 3 (LDB3), has been implicated in the pathogenesis of LVNC [6]. The p.D626N LDB3 variant was identified in two of the probands studied here (F4 and F17). In the first family, both the proband (F4) and her 1week old maternal twin sister carried the *LDB3* variant and the c.453C > T *SCN5A* variation. Shortly after birth, both were diagnosed with LVNC by echocardiography and have severe PVC. In the second family, the proband (F17) was initially diagnosed with isolated LVNC and WPW syndrome in a routine physical examination when he was 13 years old. He had the same variant in *LDB3*, as well as the p.H558R and c.1141-3 C > A variants in *SCN5A*. Thus it is possible to speculate that the presence of the D626N LDB3 variant, irrespective of whether it is disease-causing per se, could alter sodium channel expression, localization and/or function.

Study limitations

Due to the sensitivity of the PCR-SSCP analysis it is possible that the frequencies of SNPs reported here are lower than actually occur. However, as both patient groups were screened simultaneously without knowledge of phenotype, there would be no bias between groups for the variants identified. However, other variants may not have been detected for technical reasons and, it is possible, that they could have occurred more frequently in the groups of patients without arrhythmias. The mechanistic basis of the effect of these variants is largely unknown. Therefore, additional studies in which events such as intracellular acidosis or Ca overload can be induced may result in novel findings.

Conclusion

The prevalence of *SCN5A* variants is significantly higher in LVNC patients with arrhythmias than those without, supporting that the hypothesis that *SCN5A* variants increase arrhythmia susceptibility in LVNC. Further, the increased susceptibility to arrhythmias in turns leads to a greater risk of heart failure. We speculate that drugs targeted to the restoration of sodium channel function may offer a novel therapeutic option for patients with LVNC.

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References

[1] P. Richardson, W. McKenna, M. Bristow, B. Maisch, B. Mautner, J. O'Connell, E. Olsen, G. Thiene, J. Goodwin, I. Gyarfas, I. Martin and P. Nordet, Report of the 1995 World Health Organization/International Society and Federation of Cardiology Task Force on the definition and classification of cardiomyopathies, Circulation 93 (1996) 841–842.

[2] T.K. Chin, J.K. Perloff, R.G. Williams, K. Jue, R. Mohrmann, Isolated noncompaction of left ventricular myocardium: a study of eight cases, Circulation 82 (1990) 507–513.

[3] R. Engberding, F. Bender, Identification of a rare congenital anomaly of the myocardium by two-dimensional echocardiography: persistence of isolated myocardial sinusoids, Am. J. Cardiol. 53 (1984) 1733–1734.

[4] F. Ichida, S. Tsubata, K.R. Bowles, N. Haneda, K. Uese, T. Miyawaki, W.J. Dreyer, J. Messina, H. Li, N.E. Bowles, J.A. Towbin, Novel gene mutations in patients with left ventricular noncompaction or Barth syndrome, Circulation 103 (2001) 1256–1263.

[5] R. Chen, T. Tsuji, F. Ichida, K.R. Bowles, X. Yu, S. Watanabe, K. Hirono, S. Tsubata, Y. Hamamichi, J. Ohta, Y. Imai, N.E. Bowles, T. Miyawaki, J.A. Towbin, Mutation analysis of the G4.5 gene in patients with isolated left ventricular noncompaction, Mol. Genet. Metab. 77 (2002) 319–325.

Please cite this article in press as: L. Shan et al., SCN5A variants in Japanese patients with left ventricular ..., Mol. Genet. Metab. (2007), doi:10.1016/j.ymgme.2007.10.009

[6] Y. Xing, F. Ichida, T. Matsuoka, T. Isobe, Y. Ikemoto, T. Higaki, T. Tsuji, N. Haneda, A. Kuwabara, R. Chen, T. Futatani, S. Tsubata, S. Watanabe, K. Watanabe, K. Hirono, K. Uese, T. Miyawaki, K.R. Bowles, N.E. Bowles, J.A. Towbin, Genetic analysis in patients with left ventricular noncompaction and evidence for genetic heterogeneity, Mol. Genet. Metab. 88 (2006) 71–77.

[7] Q. Wang, J. Shen, I. Splawski, D. Atkinson, Z. Li, J.L. Robinson, A.J. Moss, J.A. Towbin, M.T. Keating, SCN5A mutations associated with an inherited cardiac arrhythmia, long QT syndrome, Cell 80 (1995) 805–811.

[8] C. Bezzina, M.W. Veldkamp, M.P. van Den Berg, A.V. Postma, M.B. Rook, J.W. Viersma, I.M. van Langen, G. Tan-Sindhunata, M.T. Bink-Boelkens, A.H. van Der Hout, M.M. Mannens, A.A. Wilde, A single Na(+) channel mutation causing both long-QT and Brugada syndromes, Circ. Res. 85 (1999) 1206–1213.

[9] M. Vatta, R. Dumaine, G. Varghese, T.A. Richard, W. Shimizu, N. Aihara, K. Nademanee, R. Brugada, J. Brugada, G. Veerakul, H. Li, N.E. Bowles, P. Brugada, C. Antzelevitch, J.A. Towbin, Genetic and biophysical basis of sudden unexplained nocturnal death syndrome (SUNDS), a disease allelic to Brugada Syndrome, Hum. Mol. Genet. 11 (2002) 337–345.

[10] Q. Chen, G.E. Kirsch, D. Zhang, R. Brugada, J. Brugada, P. Brugada, D. Potenza, A. Moya, M. Borggrefe, G. Breithardt, R. Ortiz-Lopez, Z. Wang, C. Antzelevitch, R.E. O'Brien, E. Schulze-Bahr, M.T. Keating, J.A. Towbin, Q. Wang, Genetic basis and molecular mechanism for idiopathic-ventricular fibrillation, Nature 392 (1998) 293–296.

[11] D.W. Benson, D.W. Wang, M. Dyment, T.K. Knilans, F.A. Fish, M.J. Strieper, T.H. Rhodes, A.L. George, Congenital sick sinus syndrome caused by recessive mutations in the cardiac sodium channel gene (SCN5A), J. Clin. Invest. 112 (2003) 1019–1028.

[12] J.J. Schott, C. Alshinawi, F. Kyndt, V. Probst, T.M. Hoorntje, M. Hulsbeek, A.A. Wilde, D. Escande, M.M. Mannens, H. Le Marec, Cardiac conduction defects associate with mutations in SCN5A, Nat. Genet. 23 (1999) 20–21.

[13] D.W. Wang, Desai, R. Reshma, L. Crotti, M. Arnestad, R. Insolia, M. Pedrazzini, C. Ferrandi, A. Vege, T. Rognum, P.J. Schwartz, A.L. George, Cardiac sodium channel dysfunction in sudden infant death syndrome, Circulation 115 (2007) 368–376.

[14] W.P. McNair, L. Ku, M.R. Taylor, P.R. Fain, D. Dao, E. Wolfel, L. Mestroni, Familial Cardiomyopathy Registry Research Group, SCN5A mutation associated with dilated cardiomyopathy, conduction disorder, and arrhythmia, Circulation 110 (2004) 2163–2167.

[15] T.M. Olson, V.V. Michels, J.D. Ballew, S.P. Reyna, M.L. Karst, K.I. Herron, S.C. Horton, R.J. Rodeheffer, J.L. Anderson, Sodium channel mutations and susceptibility to heart failure and atrial fibrillation, JAMA 293 (2005) 447–454.

[16] M. Bienengraeber, T.M. Olson, V.A. Selivanon, E.C. Kathmann, F. O'Cochlain, F. Gao, A.B. Karger, J.D. Ballew, D.M. Hodgson, L.V. Zingman, Y. Pang, A.E. Alekseev, A. Terzic, ABCC9 mutations identified in human dilated cardiomyopathy disrupt catalytic K_{ATP} channel gating, Nat. Genet. 36 (2004) 382–387.

[17] I. Splawski, K.W. Timothy, M. Tateyama, C.E. Clancy, A. Malhotra, A.H. Beggs, F.P. Cappuccio, G.A. Sagnella, R.S. Kass, M.T. Keating, Variant of SCN5A sodium channel implicated in risk of cardiac arrhythmia, Science 297 (2002) 1333–1336.

[18] L. Gouas, V. Nicaud, M. Berthet, A. Forhan, L. Tiret, B. Balkau, P. Guicheney, D.E.S.I.R. Study Group, Association of KCNQ1, KCNE1, KCNH2 and SCN5A polymorphisms with QTc interval length in a healthy population, Eur. J. Hum. Genet. 13 (2005) 1213–1222.

[19] C.R. Bezzina, W. Shimizu, P. Yang, T.T. Koopmann, M.W. Tanck, Y. Miyamoto, S. Kamakura, D.M. Roden, A.A. Wilde, Common sodium channel promoter haplotype in asian subjects underlies variability in cardiac conduction, Circulation 113 (2006) 338–344.

[20] N. Makita, H. Tsutsui, Genetic polymorphisms and arrhythmia susceptibility, Circ. J. Suppl. A (2007) A54–A60.

[21] K. Maekawa, Y. Saito, S. Ozawa, S. Adachi-Akahane, M. Kawamoto, K. Komamura, W. Shimizu, K. Ueno, S. Kamakura, N. Kamatani, M. Kitakaze, J. Sawada, Genetic polymorphisms and haplotypes of the human cardiac sodium channel α subunit gene (SCN5A) in Japanese and their association with arrhythmia, Ann. Hum. Genet. 69 (2005) 413–428.

[22] E. Schulze-Bahr, L. Eckardt, G. Breithardt, K. Seidl, T. Wichter, C. Wolpert, M. Borggrefe, W. Haverkamp, Sodium channel gene (SCN5A) mutations in 44 index patients with Brugada syndrome: different incidences in familial and sporadic disease, Hum. Mutat. 6 (2003) 651–652. Erratum in: Hum. Mutat. 1 (2005) 61.

[23] F. Ichida, Y. Hamamichi, T. Miyawaki, Y. Ono, T. Kamiya, T. Akagi, H. Hamada, O. Hirose, T. Isobe, K. Yamada, S. Kurotobi, H. Mito, T. Miyake, Y. Murakami, T. Nishi, M. Shinohara, M. Seguchi, S. Tashiro, H. Tomimatsu, Clinical features of isolated noncompaction of the ventricular myocardium: long-term clinical course, hemodynamic properties, and genetic background, J. Am. Coll. Cardiol. 34 (1999) 233–240.

[24] T.J. Bunch, M.J. Ackerman, Promoting arrhythmia susceptibility, Circulation 113 (2006) 330–332.

[25] P.C. Viswanathan, D.W. Benson, J.R. Balser, A common SCN5A polymorphism modulates the biophysical effects of an SCN5A mutation, J. Clin. Invest. 111 (2003) 341–346.

[26] B. Ye, C.R. Valdivia1, M.J. Ackerman, J.C. Makielski, A common human SCN5A polymorphism modifies expression of an arrhythmia causing mutation, Physiol. Genomics 12 (2003) 187–193.

[27] B.H. Tan, C.R. Valdivia1, B.A. Rok, B. Ye, K.M. Ruwaldt, D.J. Tester, M.J. Ackerman, J.C. Makielski, Common human SCN5A polymorphisms have altered electrophysiology when expressed in Q1077 splice variants, Heart Rhythm 7 (2005) 741–747.

[28] O. Staub, D. Rotin, Regulation of ion transport by protein–protein interaction domains, Curr. Opin. Nephrol. Hypertens. 5 (1997) 447–454.

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