IRX3 variant as a modifier of Brugada syndrome with frequent ventricular fibrillation

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Introduction

Brugada syndrome (BrS) is characterized by coved-type ST-segment elevation in the right precordial leads of electrocardiogram (ECG) and the development of ventricular fibrillation (VF) leading to sudden cardiac death.[1,2] Inheritance of BrS occurs via an autosomal-dominant mode of transmission. The first gene linked to BrS is *SCN5A*, the gene that encodes the alpha subunit of the cardiac sodium channel.[3] *SCN5A* mutations, mainly loss-of-function, have been identified in 10%–28% of BrS probands in 9 international centers around the world.[4] Over 300 mutations in *SCN5A* have been linked to BrS.[5]

SCN5A mutations are associated with both BrS and familial atrial fibrillation, progressive cardiac conduction defect, sick sinus syndrome, early repolarization syndrome, dilated cardiomyopathy, and sudden infant death syndrome.[6] Furthermore, BrS has been linked to mutations in 18 other genes, but they are rarer. Thus, BrS is often genetically undetermined and the genotype-phenotype correlations are not completely matched, even in *SCN5A* mutation–positive BrS families.[7,8]

Recently, we have identified *IRX3* mutation as a genetic risk factor of idiopathic VF, including BrS without *SCN5A* mutation.[9] Here we present the first case report demonstrating that both the *SCN5A* mutation and the *IRX3* variant accentuated the Brugada phenotype, inducing repetitive VF.

KEYWORDS Brugada syndrome; Genes; IRX3; SCN5A; Ventricular fibrillation
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Case report

Patient

A 35-year-old man was admitted to our hospital after successful resuscitation of cardiac arrest caused by VF in 1989. His past history was noncontributory, with no familial history involving sudden cardiac death. He was initially diagnosed with idiopathic VF, and there had been no episodes in 15 years following treatment with disopyramide. He was subsequently diagnosed with BrS on the basis of spontaneous type 1 Brugada ECG, which was more pronounced in the third intercostal space (Figure 1A), and received an implantable cardioverter-defibrillator in 1998. When he was 50 years old, he underwent 4 successful implantable cardioverter-defibrillator interventions for VF (Figure 1B). Denopamine was added and effectively reduced premature ventricular complexes, especially at nighttime. VF episodes then decreased to 0–2 times per year. At the age of 60, however, he experienced multiple VF episodes, including a VF storm, despite the combination therapy with disopyramide (450 mg/day), denopamine (15 mg/day), and cilostazol (200 mg/day). Quinidine was considered but not used, because disopyramide, which has a similar effect of suppressing the transient outward potassium (Ito) current, may have already been ineffective.

Genetic study

We examined the sequence of exons in 13 proposed BrS-related genes in humans (*SCN5A*, *GPD1-L*, *CACNA1C*, *CACNB2*, *KCNE3*, *SCN1B*, *SCN3B*, *KCNJ8*, *MOG1*, *HCN4*, *KCND3*, *KCNE5*, and *SLMAP*) and found 1 *SCN5A* mutation, 2205C > T (A735V), in the patient (proband) and

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KEY TEACHING POINTS

- *IRX3* gene, even if it is not a mutation but also a variant, may act as a modifier of Brugada syndrome (BrS) and ventricular fibrillation (VF) storm.
- *SCN5A* mutations probably act as a major modulating factor in revealing the BrS phenotype.
- BrS and its VF episodes are likely due to multiple mechanisms.

his father and son. The *SCN5A*-A735V has already been reported as causing BrS, a sudden unexplained nocturnal death syndrome.[10]

Even though the proband's father and son have the same *SCN5A* mutation, their ECGs did not show BrS and they had no symptoms or ventricular arrhythmias (his father had atrial fibrillation) (Figure 1C). Therefore, we further examined the *IRX3* gene, which we had reported as a risk factor of idiopathic VF[9] in this family. The proband carried the *IRX3* 2207G>T (Q479H) variant in addition to the *SCN5A* mutation, (A735V). The patient's father and son, who had the same *SCN5A*-A735V mutation, did not have the *IRX3* Q479H variant. In contrast, his mother and daughter, who did not have the *SCN5A* mutation, had the *IRX3* 2207G>T (Q479H) variant (Figure 2A). None of these 4 family members had the Brugada ECG or episodes of syncope, ventricular tachyarrhythmias, or sudden cardiac death. Thus, the *IRX3* 2207G>T (Q479H) variant appears to act as a modifier in BrS.

Discussion

Inheritance of BrS occurs via an autosomal-dominant mode of transmission, and more than 13 responsible genes have been associated with BrS. Genetic abnormalities are only found in one-third of BrS patients. Even *SCN5A*, the first and most well-documented gene, accounts for less than 30% of clinically diagnosed BrS patients.[4] The penetrance of BrS is thus considered to be low. *SCN5A* mutation carriers had, on average, longer PR and QRS intervals than noncarriers, demonstrating that these mutations exerted functional effects.[8] A recent genome-wide association study revealed the 3 genes, *SCN10A*, *SCN5A*, and *HEY2*, as risk factors of the Brugada phenotype.[11] Therefore, *SCN5A* mutations probably act as major modulating factors in revealing BrS. BrS is likely an oligogenic disease, and is not considered a monogenic Mendelian disease.[12]

A previous Xenopus oocytes patch clamp study demonstrated that *SCN5A* A735V mutant expressed current with steady-state activation voltage shifted to more positive potentials and that slower recovery from inactivation resulted in reduced sodium channel current.[10] These findings are consistent with the QRS interval prolongation in the proband and family members with *SCN5A* A735V (Figure 1A), although only the proband suffered lethal arrhythmias due to BrS. *Irx3/IRX3* encodes a transcription factor specifically

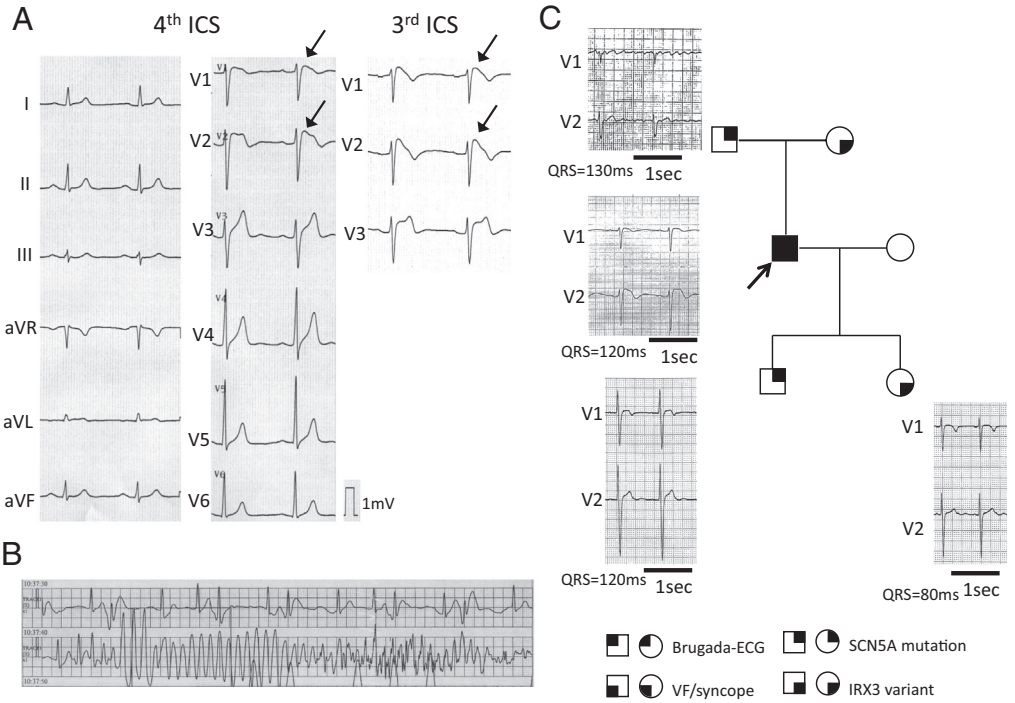

Figure 1 **A:** Surface electrocardiogram (ECG) of the proband in the fourth and the third intercostal spaces (ICS). ECG showed coved-type ST elevation in V1 and V2 (*arrows*), which was more pronounced in the third ICS than the fourth ICS. **B:** Monitor ECG of a spontaneous occurrence of ventricular fibrillation (VF) in the proband. Premature ventricular complexes developed immediately before the occurrence of VF. **C:** Family pedigree of Brugada syndrome patient with the *SCN5A* A735V mutation. In those with available ECG recordings, V1- and V2-lead ECGs are shown. *Arrows*: proband. *Square*: male subjects; *circle*: female subjects.

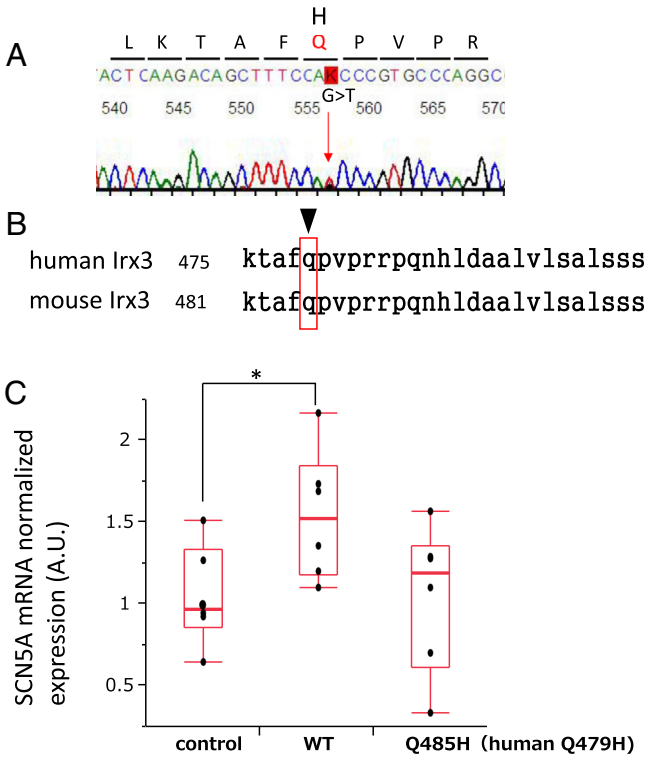

Figure 2 **A:** DNA sequence analysis results of the proband. The arrow indicates a heterozygous variant in the *IRX3* gene (Q479H). **B:** Homology of human *IRX3* and murine *Irx3*. Amino acids sequence conserved between human *IRX3* and mouse *Irx3* was highly conserved between human *IRX3* and mouse *Irx3*. **C:** Effects of transfection of HL-1 cells with *Irx3* in pcDNA3.1 vector on the expression of *SCN5A*. The expression of *SCN5A* was normalized to that of *Irx3*. Wild-type *Irx3* increased the mRNA expression of *SCN5A*, whereas Irx3-Q485H (human Q479H) did not increase the *SCN5A* mRNA expression. $*P < .05$ vs control.

expressed in the His-Purkinje system in the heart.[13] Genetic deletion of *Irx3* in a mouse model shows ventricular fast conduction disturbance without anatomical or contraction abnormalities. We recently reported the link between the perturbed His-Purkinje system and idiopathic VF in *Irx3*-null mice, and *IRX3* mutations are associated with idiopathic VF patients, including BrS without *SCN5A* or other Brugada-related mutations.[9] It remains unclear how *IRX3* variants contribute to the manifestation of BrS and the risk of VF or sudden cardiac death.

To show the functional differences of mRNA expression in *SCN5A* by *IRX3* variants, we performed transfection of wild-type (WT) or Q485H (human Q479H, Figure 2B) *Irx3* in pcDNA3.1 vector into HL-1 cells as shown previously.[9] The transfection of WT *Irx3* increased the expression of *SCN5A* mRNA; however, Q485H variant did not increase the *SCN5A* mRNA expression as much as the WT (Figure 2C). Thus, a common variant, *Irx3* Q479H, might be one of the modifiers for BrS as a cause of relative downregulation of mRNA expression in *SCN5A*.

The 2207G>T (Q479H) is a common variant in a chaperone binding domain of *IRX3* that was identified as "benign" by PolyPhen-2, found in 16.8% in the 1000 genome project and 22%–23% in Europeans but 2.1% in East Asians by ExAC. In the Japanese population, the frequency of the 2207 G>T variant was 3.1% (4/130) in idiopathic VF cases but 0.4% (1/250) in control subjects. Although the precise explanation for 2207G>T remains unknown, it may be due to ethnic differences. In this family, the only proband has both *SCN5A* mutation and *IRX3* variant, which is likely why he has the Brugada ECG with repetitive VF episodes. These findings suggest that the *IRX3* gene, even if it is also a variant and not a mutation, may act as a modifier of the BrS and VF storm. However, it might be specified to the group of the Japanese population but not a large population.

This study did not completely exclude all other possible modifiers; additional (or unknown) factors such as *SCN10A* or *HEY2* genes could be involved as well in the clinical presentation of the patient. However, the role of rare variants in *SCN10A* and *HEY2* on the BrS phenotype remain controversial.[14] The proband had suffered a VF storm when elderly. Age-dependent conduction abnormalities due to the patient's genetic background are strongly associated with the development of VF storm. Further genetic studies are necessary to investigate the role of the *IRX3* gene and other candidate genes in the risk of VF storm in BrS.

In conclusion, *IRX3* variant Q479H may play a critical role as a modifier for BrS-related frequent VF events in *SCN5A* mutation–positive subjects.

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