Analysis of ankyrin-B gene mutations in patients with long QT syndrome

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Abstract: **Objectives** To identify the ankyrin-B gene mutations that cause long QT syndrome (LQTS) and determine the prevalence of such mutations in Japanese patients with LQTS. **Methods** We conducted a search for ankyrin-B gene mutation in 78 unrelated patients with LQTS (28 males and 50 females, aged 2 to 89 years). With informed consent from all the subjects and/or their parents, genomic DNA was purified from the white blood cells of the patients and amplified using polymerase chain reaction (PCR). Single-strand conformational polymorphism (SSCP) analysis of the amplified DNA was performed to screen for mutations and aberrant SSCP products were isolated and sequenced by dye terminator cycle sequencing method using an automated fluorescent sequencer. PCR and restriction fragment length polymorphism (PCR-RFLP) analysis was carried out to further confirm the missense mutations by comparison with samples from 150 normal healthy individuals. **Results** We identified a T to A transition mutation at position 4 603 in exon 40, resulting in the substitution of arginine for a tryptophan at amino acid residue 1 535 (W1535R) in the regulatory domain of 220-kD ankyrin-B, which is a highly conserved domain shared by different species. **Conclusions** This novel missense mutation in the ankyrin-B gene may be a cause of type 4 LQTS. Ankyrin-B gene mutation might not play the major role in LQTS in Japanese.

Key words: missense mutation; long QT syndrome; ankyrin-B gene

Long QT syndrome (LQTS) is a cardiac disorder characterized by prolongation of the QT-interval on the electrocardiogram (ECG). Patients are susceptible to a specific ventricular tachycardia (VT), torsade de pointes (TdP), and ventricular fibrillation (VF), from which syncope and sudden death often precipitate [1]. LQTS-associated mutations have been identified in 9 genes: *KCNQ*1, previously designated *KVLQT*1, on chromosome 11p15.1 (LQT1), *KCNH*2 or *HERG* on 7q35-36 (LQT2), *SCN5A* on 3p21 (LQT3), ankyrin-B on 4q25-27 (LQT4), *KCNE*1 (LQT5) and *KCNE*2 (LQT6) on 21q22, *KCNJ*2 on 17q23 (LQT7), *HCN*4 on 15q24-q25 (LQT8), and *CACNA1A* on 12p13.3 (LQT9) [2]. Ankyrin-B is a member of a family of versatile membrane adapters, and its mutation was reported to cause type 4 LQTS [3, 4]. However, the prevalence and clinical features of ankyrin-B gene mutations in patients with LQTS remain undefined. The purpose of this study was to screen for ankyrin-B gene mutation in patients with LQTS, and clarify the prevalence of such gene mutation in LQTS patients.

MATERIALS AND METHODS

Patients and controls

The subjects enrolled in this study consisted of 78 unrelated probands with LQTS (28 males and 50 females, aged 2 to 89 years). The diagnosis of LQTS was established on the basis of the criteria proposed by Priori *et al* [5]. All probands were identified at the Kanazawa University Hospital and the affiliated hospitals. DNA samples from 150 healthy Japanese blood donors served as non-mutant controls for ankyrin-B allele screening. Informed consent was obtained from all the subjects or their guardians in accordance with the guidelines of the Bioethical Committee on Medical Researches, School of Medicine, Kanazawa University.

Detection of gene mutation

The entire coding region of the ankyrin-B gene was amplified with polymerase chain reaction (PCR) using 45 primer pairs [4]. Single-strand conformational polymorphism (SSCP) analysis of the amplified DNA was performed to screen for mutations in the ankyrin-B gene. For abnormal SSCP patterns, the nucleotide sequences of the PCR products were determined on both strands by the dye terminator cycle sequencing method using an automated fluorescent sequencer (ABI PRISM 310 Genetic Analyzer, PE Biosystems). The sequence variation in the ankyrin-B gene was confirmed by restriction enzyme digestion using the method of restriction fragment length polymorphism (RFLP) [6]. The same method was applied to determine the genotype in 150 healthy individuals.

Clinical evaluation

Evaluation of phenotype was completed before

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determination of the genotype. Each patient underwent a clinical evaluation and cardiovascular examination, including a 12-lead electrocardiogram (ECG)(Fig.5) and 24-hour Holter recording.

RESULTS

Genetic results

We screened all 45 exons of the ankyrin-B gene of each proband by SSCP, and 6 abnormalities of SSCP were found in exons 9, 26, 30, 40 (Fig.1), 41, and 44, respectively. Only one missense mutation was identified in the DNA sequence as a T to A transition at position 4 603 in exon 40, resulting in the substitution of arginine for a tryptophan at the amino acid residue 1 535 (W1535R) (Fig.2). The other sequence variations were nonsense mutations. For affected individuals, other known LQTS genes, including *KCNQ*1, *KCNH*2, *KCNE*1, *KCNE*2, *SCN*5*A*, *KCNJ*2, *HCN*4 and *CACNA1A* were also screened by SSCP and/or DNA sequence analysis, and no mutations were identified.

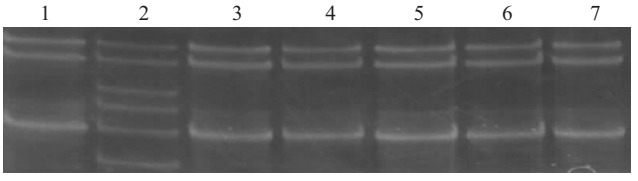


Fig.1 Results of SSCP analysis of a PCR product amplified from exon 40 of *ANKB* gene

The abnormal conformer of the PCR product potentially containing the mutation can be seen in the extra band in a proband with LQTS (lane 2). Lane 1: Normal healthy control; Lane 2: Affected individual with LQTS; Lanes 3-6: Unaffected individuals with LQTS

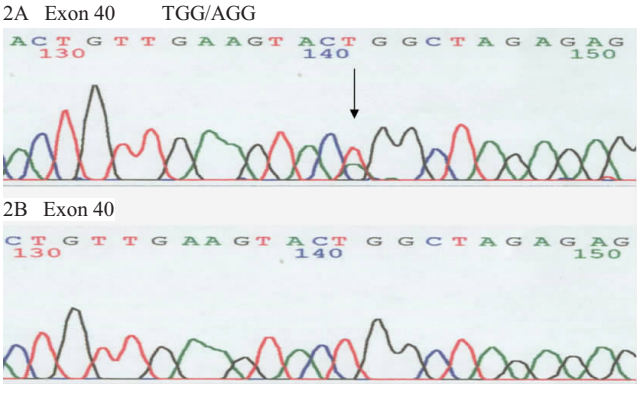


Fig.2 Sequence identification of the missense ankyrin-B gene mutation in exon 40

A: Sequence of mutant gene showing a single base T to A transition in one ankyrin-B allele at position 4 603 in an affected individual, causing replacement of Tryptophan by Arginine at the amino acid residue 1 535 (W1535R) in exon 40. B: Corresponding DNA sequence of ankyrin-B allele in a normal individual.

This missense mutation was then reconfirmed by RFLP analysis. This mutation was not identified in the 150 normal controls. Furthermore, the results of the DNA sequencing by PCR-RFLP analysis showed that this proband was a heterozygous carrier of this novel missense mutation (Fig.4), and the sequence alignment around the residue tryptophan 1 535 of human ankyrin-B gene, which showed identical sequences of other animal species to human ankyrin-B (Fig.3), indicates that the W1535R missense mutation occurred in a highly conservative domain of ankyrin-B gene.

Human Ankyrin-B	S	H	A	L	L	K	Y	W	L	E	R	D	G	K
Mutant	-	-	-	-	-	-	-	R	-	-	-	-	-	-
Rat	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rabbit	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fowl	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Canis	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cat	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Fig.3 Comparison of the sequence around the residue W1535R of ankyrin-B gene between different species

Dashes indicate sequences of ankyrin-B gene conserved in human and other animal species, showing that the W1535R missense mutation occurs in a highly conserved domain of the ankyrin-B gene.

Fig.4 RFLP confirmation of the gene mutation

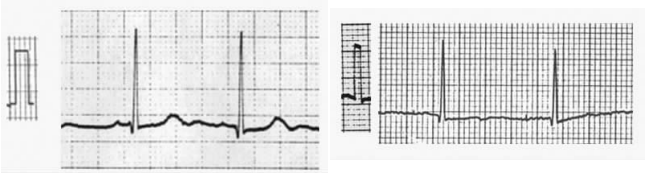
The blots from PCR-RFLP analysis show the heterozygous mutation in the ankyrin-B gene, which leads to the change in insusceptibility of DNA to *Sca* I, as shown by the appearance of the 300 bp band in lane 2. This PCR-RFLP change was observed in none of the DNA samples from the 150 control subjects. Lane 1: Size marker; Lane 2: Affected individual; Lanes 3-16: Normal healthy controls

Clinical manifestations

The proband, who has been identified as the carrier of the missense mutation in exon 40 of ankyrin-B gene, is a 72-year-old woman. Her QTc was 470 ms on ECG at age 65 (Fig.5). At 72 years of age, a 3.8 sec sinus arrest was found on Holter ECG and she underwent pacemaker implantation.

DISCUSSION

Primary arrhythmogenic disorders of the heart are a major cause of sudden cardiac death in otherwise healthy, and frequently young individuals. These arrhythmias are likely to be caused by aberrant function of the ion channels that lead to abnormal electrical



A B

Fig.5 ECG (V6) evaluation of the old women with the missense ankyrin-B gene mutation

ECGs of the proband before (A) and after (B) implantation of pacemaker show a prolonged QTc (0.47s and 0.48s, respectively)

properties of the heart. Mutations in the ion channels involved in the generation and termination of the action potentials constitute a family of molecular defects that underlie fatal cardiac arrhythmias such as inherited LQTS [7]. Mutations in the genes encoding the ion channels have emerged in the last decade as the basis for a variety of inherited arrhythmias, including LQTS types 1-3 and 5-9, Brugada syndrome, and catecholaminergic polymorphic ventricular tachycardia [1]. However, 30% -45% of the cases with arrhythmia can not be explained by mutation of currently identified genes [2]. Mutation of ankyrin-B results in disruption in the cellular organization of the sodium pump, the sodium/calcium exchanger, and inosital-1,4,5-trisphosphate receptors (all ankyrin-B-binding proteins), which reduces the targeting of the these proteins to the transverse tubules as well as reducing overall protein level. Ankyrin-B mutation also leads to altered Ca^{2+} signaling in adult cardiomyocytes that results in extrasystoles, and provides a rationale for arrhythmia [3, 8]. In this study, we identified a novel missense mutation in exon 40 of ankyrin-B gene from an elderly patient, who has been diagnosed to have LQTS.

Analysis of the data suggests that this newly identified missense mutation in exon 40 (W1535R) of ankyrin-B gene might be responsible for causing type 4 LQTS. One reason lies in the fact that several missense mutations, including E1425G, L1622I, T1626N, R1788W, and E1813K mutations which have been identified recently in different studies, all occur in or near the regulatory and functional domains of exons 36 to 46 [9]. The W1535R mutation is also located in this domain, which is generally regarded as the so-called mutation-prone domain, suggesting that this novel mutation could also cause type 4 LQTS as others five did, on the basis of exclusion of any mutation happened in other several LQTS-associated genes. Furthermore, this study shows that the W1535R mutation occurs in a conserved domain shared by other animal species, suggesting that this newly identified missense mutation would bring about molecular defect, leading to loss of function of ankyrin-B protein, which is widely believed to have intimate involvement in regulating the coordinated expression of such ion channel-associated proteins as noted above and possibly others. Therefore, it is reasonable to suggest that this mutation would cause dysfunction in humans leading to type 4 LQTS [10], or even lethal cardiac arrhythmias. The study might serve as a clue for further study to unfold the mechanism.

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对其进行酶切收获纯化的 LvgA-Hsp60 蛋白,进而可对其的结构及免疫学特性等进行多方面研究；最终,要将该融合基因亚克隆入真核表达质粒并进行表达,以一定的包封形式制成疫苗进行动物实验检测相应的免疫学指标,比较评估其免疫原性和保护性,为探寻新的军团菌疫苗作尝试性研究,为军团菌感染的防治做深入有益的探索。

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QT间期延长综合征病人 ankyrin-B 基因突变的解析

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摘要：目的 确定 ankyrin-B 基因突变在日本人群 QT 间期延长综合征中的发病率以及 ankyrin-B 基因突变与 QT 间期延长综合征之间的关系。**方法** 我们对相互之间无血缘关系的日本人群已确诊的 QT 间期延长综合征患者 78 例(男 28 例,女 50 例)进行了研究。在征得患者的同意之后,从其白细胞中提取纯化基因组 DNA 并行多聚酶链式反应(PCR)扩增。随即对扩增的 DNA 行单链构像多态性(SSCP)分析。而后将突变或异常的 SSCP 产物分离后,采用自动荧光测序仪检测 DNA 序列。最后再用 150 例正常健康人的 DNA 作为对照,对上述经测序确认的误义点突变进行 PCR- 限制性片段长度多态性分析,以进一步证实误义点突变的真实性和可靠性。**结果** 我们在一例确诊的 QT 间期延长综合征患者的 ankyrin-B 基因的 4 603 碱基位点上发现了从 T 到 A 的突变(T4603A)。出现于基因的第 40 号外显子上的该误义点突变，导致了氨基酸序列第 1 535 位点上的色氨酸残基被精氨酸残基所取代 (W1535R)。而该氨基酸序列正位于 ankyrin-B 基因高度保守的调节区域。**结论** 新发现的位于 ankyrin-B 基因调节区域的误义点突变可能是导致 4 型 QT 间期延长综合征的原因之一,而该突变似乎并不是导致日本人群 4 型 QT 间期延长综合征的主要病因。

关键词：误义突变; QT 间期延长综合征; ankyrin-B 基因

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