Mutations of *KCNJ2* gene associated with Andersen–Tawil syndrome in Korean families

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Abstract Mutations of the *KCNJ2* gene are a major underlying cause of Andersen–Tawil syndrome (ATS), a rare autosomal dominant inherited disorder that is characterized by periodic paralysis, cardiac arrhythmias, and developmental dysmorphic features. The *KCNJ2* gene encodes an inward rectifying K⁺ channel protein, Kir2.1, which plays an important role in maintaining the homeostasis of channel current in various cell types. We have identified two missense mutations of *KCNJ2* (R218Q and M307I) in two Korean families diagnosed with ATS. The M307I mutation is a novel mutation, located at the intracellular C-terminal domain, which is known to be concerned with putative phosphatidylinositol 4,5-bisphosphate (PIP₂) binding and channel trafficking.

Keywords Andersen–Tawil syndrome (ATS) · *KCNJ2* · Kir2.1 · Mutation · Korea

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Abbreviations

ATS Andersen–Tawil syndrome
CMAP Compound muscle action potential
PCR Polymerase chain reaction
PIP₂ Phosphatidylinositol 4,5-bisphosphate

Introduction

Andersen–Tawil syndrome (ATS; also known as Andersen syndrome) is a rare inherited disorder caused by ion channel dysfunction, which is recognized as a typical channelopathy. ATS is characterized clinically by the triad of periodic paralysis, cardiac arrhythmia, and dysmorphic features, including clinodactyly, hypertelorism, micrognathia, low-set ears, a broad forehead and cleft plate (Andersen et al. 1971; Tawil et al. 1994; Sansone et al. 1997; Plaster et al. 2001).

Since the first report by Andersen et al. (1971), more than two-thirds of the reported ATS cases have been identified as having been caused by mutations in the *KCNJ2* gene, which encodes an inward rectifying K⁺ channel protein, Kir2.1 (Plaster et al. 2001; Ai et al. 2002; Tristani-Firouzi et al. 2002; Donaldson et al. 2003; Davies et al. 2005). The Kir family maintains K⁺ level homeostasis in various cell types and regulates membrane potential (Jongsma and Wilders 2001). The *KCNJ2* mutations in ATS patients are concentrated at highly conserved regions, including the two transmembrane segments (M1 and M2) that are separated by an extracellular pore loop, and intracellular amino and carboxyl termini (Yang et al. 1995; Tristani-Firouzi et al. 2002). As the channel consists of homo-tetramers

of Kir2.1 protein, a mutation in one subunit may be enough to disrupt its whole function. This was indeed evidenced by expression experiments with *Xenopus* oocytes and mammalian cultured cells, where a mutant form of Kir2.1 showed a dominant-negative effect (Ai et al. 2002; Tristani-Firouzi et al. 2002).

In this study we identified two causative mutations in the *KCNJ2* gene in Korean families with ATS, as confirmed through clinical and electrophysiological findings. One of them, M307I, was a novel mutation located in the intracellular C-terminal domain that is involved in putative phosphatidylinositol 4,5-bisphosphate (PIP₂) binding and channel trafficking.

Patients and methods

Subjects

This study involved samples from two Korean ATS familial members (five affected and four unaffected) and a group of 109 non-ATS healthy controls. All patients in this study exhibited typical ATS phenotypes of dysmorphic facial features, including hypertelorism and micrognathia (Fig. 1). All participants provided written informed consent according to the protocol approved by the Ethics Committee of Ewha Womans University Hospital (Seoul, Korea).

Clinical assessment

KC-1 family

A 19-year-old man (Fig. 2a, III-5 of KC-1 family) was admitted to our hospital due to intermittent quadriplegia. At 5 years of age, he experienced a first episode of weakness and, thereafter, two to three attacks per year during elementary school years; the rate of episodes had increased to two to three per month at the time of hospital admission. His motor weakness occurred frequently after specific conditions, e.g., the next morning after vigorous exercise, several hours in an uncomfortable yogi-like position, sleeping in a cold environment. The duration of weakness varied from 1 day to 10 days. The weakness was symmetrical in

both legs. His younger sister (III-6) had a cardiac pacemaker due to arrhythmia, and his maternal aunt (II-5) had died of a sudden cardiac attack while in her fourth decade. They said that several other family members, including his mother (II-3), an older maternal aunt (II-1) and his maternal grandfather (I-1), had similar intermittent episodes of weakness. The proband and his mother presented some dysmorphic features of hypertelorism, a broad-based nose, low-set ears and micrognathia (Fig. 1a, b). Weakness reported by his mother was found in all limbs, with that of the proximal part of the leg most prominent. Somatic sensation and deep tendon reflexes were normal.

Serum creatine kinase (CK) was mildly increased to 279 IU/l (normal 44–245 IU/l) and serum potassium mildly decreased to 3.4 mmol/l (normal 3.5–5.5 mmol/l). The results of the thyroid function test were normal. Conventional electrocardiography showed a left ventricular hypertrophy, but 24-h Holter monitoring indicated premature ventricular contractions and ventricular tachycardia during sleep. The compound muscle action potential (CMAP) was decreased on routine nerve conduction study, but there were no abnormal spontaneous activities, including myotonic discharges, during needle electromyography. We checked nerve conduction and serum potassium levels during the study and noted a tendency for the CMAPs to be low in proportion to muscle weakness, but the relationship between serum potassium levels and the degree of weakness was indefinite.

KC-2 family

A 16-year-old man (Fig. 2a, III-1 of KC-2 family) visited our hospital due to a 5-year history of intermittent quadriplegia. The conditions that seemed to be conducive to his reported weakness included the morning after vigorous exercise, a large meal, or sleeping while in an uncomfortable posture. The usual duration of weakness was 2–3 days, but, on occasion, it could be as long as 1 week. Weakness was dominant proximally and not so severe as to disturb unaided walking.

The patient's mother (II-2) had a history of intermittent quadriplegia, just as the proband had, until her college years; routine electrocardiography revealed

Fig. 1 Dysmorphic facial features of ATS patients. All patients exhibited developmental defects of hypertelorism and microgna-

thia. **a** III-5 in KC-1, **b** II-3 in KC-1, **c** III-1 in KC-2, and **d** II-2 in KC-2

Fig. 2 Pedigrees and mutational analysis of *KCNJ2* in ATS families. **a** Pedigrees of two ATS families. The available DNA samples are indicated by asterisks. Unaffected samples are represented by open squares and open circles, and affected samples are depicted by filled squares and filled circles. The half-filled symbol (I-2 in KC-2) indicates a possibly affected female. Arrows indicate probands. **b** Chromatograms of mutant alleles.

ventricular arrhythmia. Both the proband and his mother had certain dysmorphic features in common: hypertelorism, a broad-based nose, low-set ears and micrognathia (Fig. 1c, d). Clinodactyly was observed, and they said that his maternal grandmother also showed the same dysmorphic features.

At the time of first visit of our hospital, no definitive weakness could be documented. The results of the thyroid function test for the proband were normal, and serum potassium was measured at 4.1 mmol/l (normal 3.5–5.5 mmol/l). The 24-h Holter monitoring showed asymptomatic premature ventricular and atrial contractions. At the age of 19 years he was admitted to our hospital due to severe weakness (lower extremity MRC grade II) after sleeping while on a school trip. The serum CK level was elevated at 307 IU/l (normal 44–245 IU/l), and the CMAP decreased on a nerve conduction study (CMAP of right median nerve, 1.6 mV; CMAP of right ulnar nerve, 1.3 mV).

Mutation screening

Genomic DNA was extracted from whole-blood samples using a DNA extraction kit (SolGent, Korea). Mutations in the *KCNJ2* gene were searched for by direct DNA sequencing of the entire coding region. DNA fragments were amplified by a standard polymerase chain reaction (PCR) method. PCR amplification was carried out in a total volume of 20 μ l containing 50 ng of genomic DNA, 0.5 pmol of each primer, 200 μ mol of each dNTP, 2 mmol of $MgCl_2$, 0.6 U of *Taq* DNA

Exons were amplified by standard polymerase chain reaction (PCR) and sequenced with an ABI 3100 automatic sequencer (Applied Biosystems, USA). **c** Conservation of amino acid at the M307I mutation site in different members of the Kir family. The mutation site and surrounding amino acid sequences were aligned with those of other members of the Kir family

polymerase and 1 $\times$ of the supplied buffer (Promega, USA), using a thermal cycler (Perkin-Elmer 9700, USA). Primer sequences and PCR conditions are available upon request. The PCR products were purified with a PCR product purification kit (SolGent). Sequences were determined by sequencing purified PCR products using an ABI 3100 automatic sequencing analyzer (Applied Biosystems, USA). We confirmed sequence variations by analyzing both strands of DNA.

Results and discussion

From mutation screening of the *KCNJ2* gene in two Korean families with ATS, we were able to identify two causative missense mutations, R218Q (c.653G > A) and M307I (c.921G > A). Of the two, the M307I mutation was an unreported novel mutation. These two mutations were not found in the 218 control chromosomes.

In the KC-1 family, a heterozygous G to A transition (c.653G > A) was observed in all the affected individuals examined in the pedigree, II-3, III-5 and III-6 (Fig. 2a). This nucleotide transition resulted in a missense mutation substitution of arginine to glutamine (Fig. 2b). The R218Q mutation is located within the C-terminal region of the putative PIP_2 interaction, and was previously reported by Plaster et al. (2001) and Tristani-Firouzi et al. (2002).

In the KC-2 family a novel G to A transition mutation (c.921G > A) was found, which altered the amino acid from methionine to isoleucine (M307I).

The mutation was present in the proband (III-1) and his mother (II-2), but not in the proband's father (II-1) and his sibling (III-2), nor in any of the 218 control chromosomes (Fig. 2b). Multiple alignments of amino acid sequences demonstrated that the mutation site is conserved between several other Kir families, with the exception of Kir6.1, an ATP-sensitive inward rectifier K⁺ channel 8 (Fig. 2c). Although the defective role of this mutation for the function of inward rectifying K⁺ channel protein was not characterized, the C-terminal region has been suggested to be an essential motif of PIP₂ binding and channel trafficking (Huang et al. 1998; Zhang et al. 1999; Lopes et al. 2002; Bendahhou et al. 2003). In addition, a neighboring V302M mutation has also shown the defect of channel trafficking (Bendahhou et al. 2003). Frequent mutations in the C-terminal region of putative PIP₂ interactions, including this novel mutation, suggest that PIP₂ binding is very important for the functions of Kir2.1 channel protein. The mutation co-segregated with the affected KC-2 family members and was not present in unaffected family members or the controls. Collectively, these results suggest that the novel M307I missense mutation in the *KCNJ2* gene would be responsible for the observed ATS phenotype in the KC-2 family. This study is the first report of the causative mutations of the *KCNJ2* gene in Korean ATS patients.

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