

# Andersen–Tawil syndrome

## New potassium channel mutations and possible phenotypic variation

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**Abstract—Objective:** To evaluate clinical, genetic, and electrophysiologic features of patients with Andersen–Tawil syndrome (ATS) in the United Kingdom. **Methods:** Clinical and neurophysiologic evaluation was conducted of 11 families suspected to have ATS. Molecular genetic analysis of each proband was performed by direct DNA sequencing of the entire coding region of *KCNJ2*. Control samples were screened by direct DNA sequencing. The electrophysiologic consequences of several new mutations were studied in an oocyte expression system. **Results:** All 11 ATS families harbored pathogenic mutations in *KCNJ2* with six mutations not previously reported. Some unusual clinical features including renal tubular defect, CNS involvement, and dental and phonation abnormalities were observed. Five mutations (T75M, D78G, R82Q, L217P, and G300D) were expressed, all of which resulted in nonfunctional channels when expressed alone, and co-expression with wild-type (WT) *KCNJ2* demonstrated a dominant negative effect. **Conclusion:** Six new disease-causing mutations in *KCNJ2* were identified, one of which was in a PIP<sub>2</sub> binding site. Molecular expression studies indicated that five of the mutations exerted a dominant negative effect on the wild-type allele. *KCNJ2* mutations are an important cause of ATS in the UK.

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Andersen–Tawil syndrome (ATS) is a multisystem channelopathy characterized by periodic paralysis, ventricular arrhythmias, and distinctive physical features.<sup>1–3</sup> Hypoplastic kidney and valvular heart defects have also been reported.<sup>4</sup> The periodic paralysis may be hypo-, hyper-, or normokalemic.<sup>5,6</sup> EKG abnormalities are common in ATS and include bidirectional ventricular tachycardia, long QT interval, and bigeminy. Life-threatening cardiac arrhythmias may occur, and patients require cardiologic follow-up.<sup>4,6,7</sup>

ATS is autosomal dominant with marked intrafamilial phenotypic variation and evidence of incomplete penetrance.<sup>5</sup> Mutations in the gene encoding an inward-rectifying potassium channel (Kir2.1) located

on chromosome 17q have been reported in association with ATS.<sup>4–10</sup> However, in one series, only 68% of kindreds with the ATS phenotype harbored a *KCNJ2* mutation, suggesting genetic heterogeneity.<sup>7</sup> This potassium channel belongs to a large family of Kir channels whose biophysical characteristic property, although described as inward rectification, also results in limiting the K<sup>+</sup> efflux during depolarization. The Kir2.1 channel therefore plays a major role in setting the resting membrane potential, buffering extracellular potassium, and modulating the action potential waveform.

In the current study, we describe the first molecular genetic study of ATS in the United Kingdom. We studied 22 affected individuals from 11 unrelated families. All affected genotyped individuals harbored pathogenic mutations. We observed possible new phenotypic variation in certain affected individuals and describe six new pathogenic *KCNJ2* mutations.

Additional material related to this article can be found on the *Neurology* Web site. Go to [www.neurology.org](http://www.neurology.org) and scroll down the Table of Contents for the October 11 issue to find the title link for this article.

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**Table** Clinical details of patients with Andersen–Tawil syndrome

Family	Member	Mutation	Abnormal physical features	Onset of PP, y	Ictal K <sup>+</sup>	Attack frequency	Attack duration	Effective therapy	Cardiac onset
1	II:5	D78G	+	8	Low	Monthly	3–8 d	Acetaz	17
2	II:1	R67W	+	9	Low	2 Monthly	1–4 d	Acetaz	13
3	II:1	G300D	+	1.5	Low	3 Monthly	2–5 d	Dichl/K <sup>+</sup>	32
	III:1		+	8	N/A	Monthly	1–2 wk	N/A	20
	III:3		+	15	N/A	2–3 Weekly	2–7 d	N/A	15
4	III:5	R218W	+	10	Low	Monthly	1–3 wk	K <sup>+</sup>	None
	I:2		+	17	N/A	Monthly	1–2 wk	N/A	8
	II:1		+	15	N/A	2 Monthly	7–10 d	None	9
	II:2		+	16	Low	3 Monthly	1–2 d	None	
5	II:3	R82Q	+	14	Low	2 Monthly	1–3 wk	K <sup>+</sup> /Acetaz	No symptoms
	II:2		+	15	Low	2 Monthly	5–7 d	Acetaz	None
6	II:2	L217P	+	5	N	Monthly	5–7 d	Acetaz	32
7	II:2	T75M	+	19	N/A	Monthly	3–7 d	None	7
8	I:2	R67W	N/A	10	N/A	Monthly	2–7 d	N/A	N/A
	II:2		+	13	Low	Monthly	7–10 d	K <sup>+</sup>	None
	III:1		+	9	N/A	Monthly	4–5 d	N/A	None
9	I:1	R218W	+	24	Low	3 Monthly	1–3 d	Acetaz	None
	II:1		+	14	N/A	Monthly	2–7 d	Acetaz	None
	II:2		+	13	N/A	Monthly	1–7 d	Acetaz	None
10	I:1	Y68D	N/A	N/A	N/A	N/A	N/A	N/A	None
	II:1		+	6	N/A	1–2 Monthly	Minutes–hours	Acetaz	No symptoms
11	II:2	V123G	+	18	N/A	Monthly	1 wk	N/A	None

PP = periodic paralysis; K<sup>+</sup> = potassium; CK = creatine kinase; N/A = data not available; Acetaz = acetazolamide; QT<sub>c</sub> = corrected QT interval; dichl = dichlorphenamide; N = normal; SVT = supraventricular tachycardia.

**Methods.** *Patients.* As part of a UK government–funded national service, the Centre for Neuromuscular Disease at the National Hospital for Neurology receives referrals for clinical and genetic evaluation of patients with suspected skeletal muscle channelopathies. From our large cohort of cases confirmed to have periodic paralysis (currently >140 subjects), we identified 11 families suspected to have ATS. Most index cases had been confirmed to have hypokalemic periodic paralysis but did not harbor pathogenic mutations in either the *SCN4A* or the *CACNA1S* genes. In addition, these index cases were noted to exhibit either distinctive facial and bony features or cardiac abnormalities. The table summarizes the clinical features of the affected patients from these families. Two of the 11 kindreds are discussed in detail below.

In Family 2 (figure 1), the 25-year-old female proband (II:1) developed focal episodic weakness from age 9, typically presenting with an intermittent limp. By age 12, she was having weekly episodes of leg weakness with muscle pain particularly following vigorous exercise or fasting. Episodes lasted for 1 to 4 days and varied in severity from a mild limp to being bed-bound. The frequency of attacks increased in her late teens and then improved from age 18. The patient had noticed a mildly progressive limb weakness over the years. At age 13, the patient reported frequent palpitations without syncope. No other family member had similar symptoms. Examination revealed mild facial abnormalities (hypertelorism, broad-based nose, hypoplastic mandible) and mild persistent proximal muscle weakness (Medical Research Council grade 4). Investigations revealed an elevated creatine kinase (CK) activity at 350 U/L (normal <170 U/L) and a low serum potassium of 3.1 mmol/L was noted in one attack (normal 3.5 to 5.1 mmol/L). Routine EKG showed a slightly prolonged corrected QT interval (QT<sub>c</sub>) of 0.48 seconds (upper limit of normal for females 0.46 seconds). On exercise, she developed a bidirectional ventricular

tachycardia. Quadriceps muscle biopsy showed myopathic features, and 30% of muscle fibers contained tubular aggregates.

The parents and three siblings of the proband had normal physical examinations and normal routine EKGs. Acetazolamide 250 mg b.d. reduced the frequency and severity of the muscle pain and weakness. No treatment has been necessary for the cardiac arrhythmia to date.

In Family 3, the 32-year-old female proband (III:3) was noted to have mild learning difficulties in primary school. At age 15, she developed intermittent chest pain, recurrent palpitations, and syncope. She was initially thought to have a cardiomyopathy. At around the same time, she started to get attacks of weakness affecting the legs. The attacks varied in severity, the worst rendering her unable to walk. The episodes could last from 2 to 7 days. Exercise precipitated the attacks.

On examination, she was found to have a short stature, hypertelorism, low-set ears, hypoplastic mandible, clinodactyly, and overriding toes. She had a distinctive high-pitched voice without palatal deformity. There was no evidence of muscle weakness.

Investigations demonstrated a normal CK level, but no serum potassium level was available during an attack. Routine EKG showed a prolonged QT<sub>c</sub> of 0.55 seconds, and there were frequent ventricular ectopics. Twenty-four-hour EKG recording revealed runs of nonsustained ventricular tachycardia.

Between ages 18 and 26, she had short-lived symptomatic improvement of her palpitations with disopyramide but no change in the recorded arrhythmias. She has to date not had treatment for the paralytic episodes.

The proband's mother (II:1) developed intermittent leg weakness at age 18 months. In early adulthood, these were described as lasting up to a week but later persisted for up to 3 weeks. Exercise did not seem to provoke an attack, but mild exercise

Table Continued

Type of arrhythmia	Additional features	Muscle Biopsy	CK	McManis test
Ventricular	Painful paralysis	Vacuolar myopathy	230	Positive
Ventricular	Long QTc, painful paralysis	Tubular aggregates	350	N/A
Ventricular	Long QTc, high-pitched voice	Myopathic	48	N/A
Ventricular	High-pitched voice, learning disability	N/A	N/A	N/A
Ventricular	Long QTc, high-pitched voice, learning disability	N/A	110	N/A
None	Cleft palate, high-pitched voice, abnormal dentition, learning disability, renal tubular defect	N/A	N/A	N/A
Ventricular	None	N/A	N/A	N/A
Ventricular	Abnormal dentition, painful paralysis, mild ataxia	N/A	120	Positive
None	None	Myopathic	N/A	N/A
Ventricular	Painful paralysis	Vacuolar myopathy	154	N/A
None	None	N/A	N/A	Positive
Ventricular and SVT	None	N/A	204	Positive
Ventricular	None	N/A	240	Positive
N/A	N/A	N/A	N/A	N/A
None	None	Myopathic	N/A	Positive
None	None	N/A	N/A	Positive
None	Painful paralysis	Myopathic	184	N/A
None	None	N/A	N/A	Positive
None	None	N/A	N/A	N/A
None	None	N/A	N/A	N/A
None	Long QTc	N/A	672	N/A
None	Presented with muscle weakness and CK 2301	Normal	1,000–1,500	N/A

could sometimes improve the symptoms. At age 32, she developed atypical chest pain, palpitations with infrequent syncope, shortness of breath on exertion, and orthopnoea. She was diagnosed as having myopericarditis.

Examination revealed short stature, hypertelorism, low-set ears, and clinodactyly. She had the same high-pitched voice as her daughter but no palatal deformity. There was a mild persistent proximal weakness (Medical Research Council grade 4+).

Investigations demonstrated a normal CK level, and serum potassium was documented to be low (3.1 mmol/L) during an attack. Glucose and insulin challenge precipitated an attack of weakness. EKG showed frequent ventricular ectopics and a prolonged QT<sub>c</sub> of 0.57 seconds. Cardiac catheterization showed mild left ventricular dilatation with an ejection fraction of 50%. Quadriceps muscle biopsy showed nonspecific myopathic features (variation in fiber diameter, internal nuclei, but no tubular aggregates or vacuoles).

Treatment with oral potassium supplements improved the episodes of weakness, and dichlorphenamide resulted in further improvement. Disopyramide alleviated the cardiac symptoms but exacerbated the periodic paralysis. Amiodarone was ineffective. At age 50, the patient developed septicemia and required ventilation. She did not recover from this and died soon after.

The younger brother of the proband (III:5) was diagnosed with renal tubular acidosis at age 7 months after investigations for vomiting, irritability, and food refusal. Despite treatment with Albright mixture, development was delayed. Initially he was given the label of Russell–Silver syndrome due to short stature and triangular facies. He required surgery for a cleft lip and palate. At age 10, he developed episodes of paralysis affecting predominantly his upper limbs. These would occur about monthly and last 1 to 2 weeks. His legs were affected on one occasion. Episodes could be precipitated by strenuous exercise (e.g., weight lifting) but

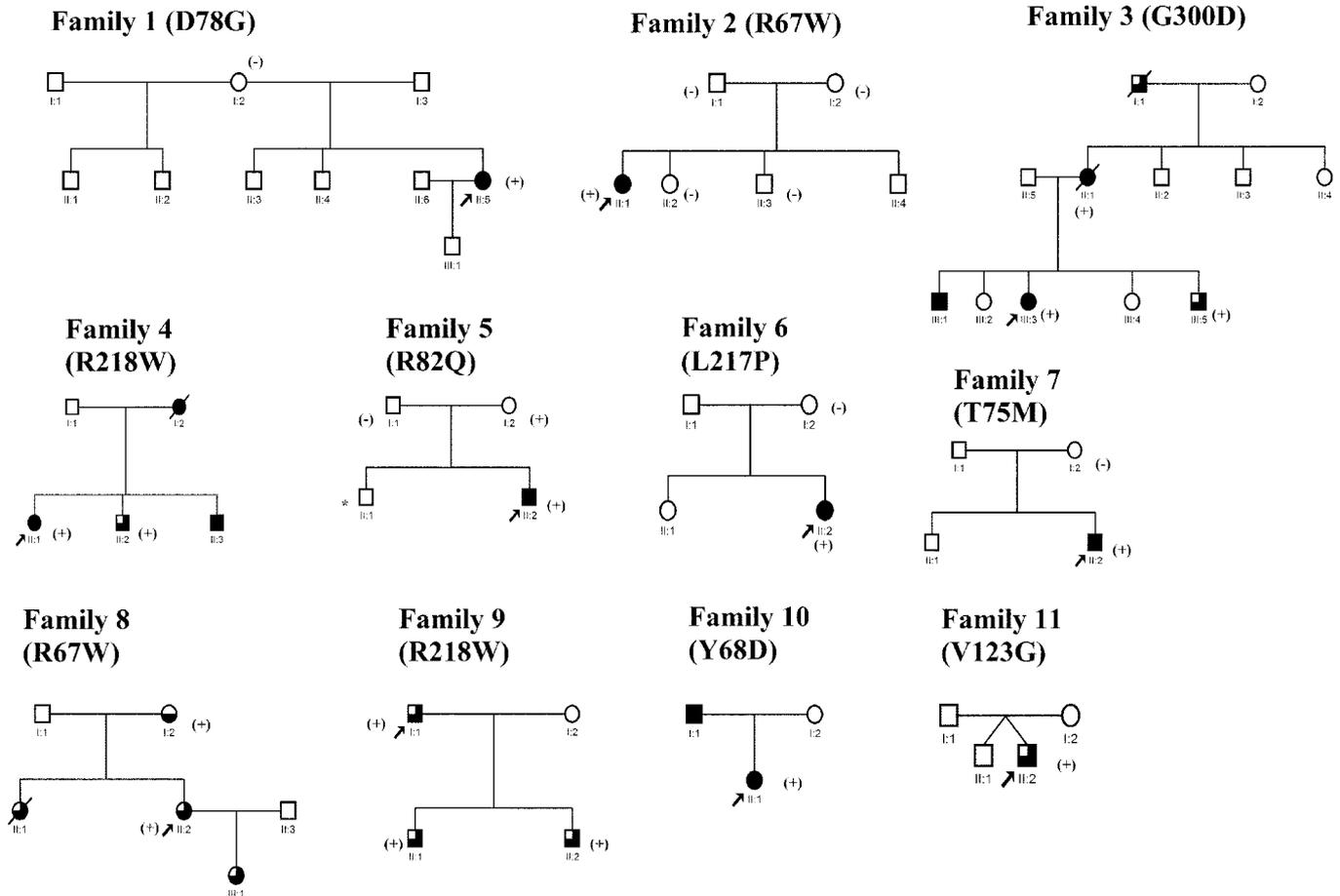
were prevented by gentle exercise if warning symptoms were experienced.

Examination revealed him to be of short stature with low-set ears, a hypoplastic mandible, and broad nasal base. He had evidence of a repaired cleft lip and palate and abnormal dentition with markedly discolored teeth. He had a very high-pitched voice with no abnormality of the hard palate. He had clinodactyly and syndactyly. There was minimal upper limb distal weakness (Medical Research Council grade 4+) but no proximal weakness.

Investigations showed a low serum potassium level during an attack but a normal EKG and QT<sub>c</sub>. Paralytic attacks were reduced in frequency and duration by oral potassium supplements.

**Procedure.** Neurophysiology. Routine nerve conduction studies and electromyography were performed. In addition, the long exercise test protocol as described was followed.<sup>11</sup>

**Molecular genetics.** Extraction of DNA from blood samples was performed using standard methods. Written informed consent was obtained for DNA analysis from all subjects. Direct sequence analysis of the *KCNJ2* gene was performed in all available family members. Four pairs of overlapping M13 tagged oligonucleotide primers were used to amplify the entire coding region of *KCNJ2* (primer sequence and PCR conditions available on request). The products were cleaned using Qiagen PCR purification kits, and both strands were sequenced using a Big Dye Terminator sequencing kit (ABI Applied Biosystems, Foster City, CA). The sequencing products were run on a 3700 or a 3100 automated DNA sequencer (ABI). Any sequence variants identified were sought in other family members by direct DNA sequencing. Screening of 80 control samples was performed by direct DNA sequencing. Paternity testing was performed by genotyping using 14 highly polymorphic fluorescent microsatellite markers on 10 different autosomes (sequences of oligonucleotide primers and PCR conditions are available from the authors).



**Figure 1.** Pedigree data of the 11 Andersen-Tawil syndrome (ATS) families.  $\circ/\square$  = unaffected;  $\bullet/\blacksquare$  = full ATS triad;  $\bullet/\blacksquare$  = facial and bony abnormalities and periodic paralysis without cardiac abnormalities;  $\circ/\square$  = periodic paralysis alone. Genotyped patients are marked with (+) = mutation positive and (-) = mutation negative. The following affected subjects were clinically examined but not genotyped: Family 3, II:1; Family 4, I:2 and II:3; Family 8, III:1; Family 10, I:1. Subjects I:1 from Family 3 and II:1 from Family 8 were identified by history. \*Subject II:1 from Family 5 is said to be affected with periodic paralysis but has never been examined.

**Mutagenesis and in vitro transcription.** Human *KCNJ2* complementary DNA (cDNA) in the plasmid pSGEM was used. Mutagenic primers were designed to include each of the mutations identified with 15 base pairs on either side. Site-directed mutagenesis was then performed using a PCR-based strategy with proofreading Pfu DNA polymerase (Stratagene, LaJolla, CA). Mutated clones were identified by direct sequencing. Complementary RNA (cRNA) was prepared following linearization of the construct and in vitro transcription using T7-RNA polymerase (Boehringer Mannheim UK, East Sussex, UK). The integrity and concentration of the transcript were determined by denaturing gel electrophoresis and ethidium bromide staining as well as by spectrophotometry.

**Preparation and injection of oocytes.** Female *Xenopus laevis* frogs were killed by 0.5% tricaine, decapitation, and pithing. Stage V or VI oocytes were isolated and stored at 4 to 18 °C in ND96 medium containing the following (mM): NaCl 96, KCl 2, MgCl<sub>2</sub> 1, CaCl<sub>2</sub> 1.8, HEPES 5, gentamicin 50 μg/mL (pH 7.4; sterilized by filtration). The follicular cell layer was removed from the oocytes using 2 mg/mL collagenase (type IA; Sigma Aldrich, Dorset, UK) in a calcium-free ND96 solution. WT and mutant RNA were injected into the cytoplasm of the oocytes (Nanoject, Drummond, Broomall, PA) at a concentration of 9.2 ng/oocyte. For co-expression experiments, WT and mutant were injected in a ratio of 1:1 (4.6 ng/oocyte each).

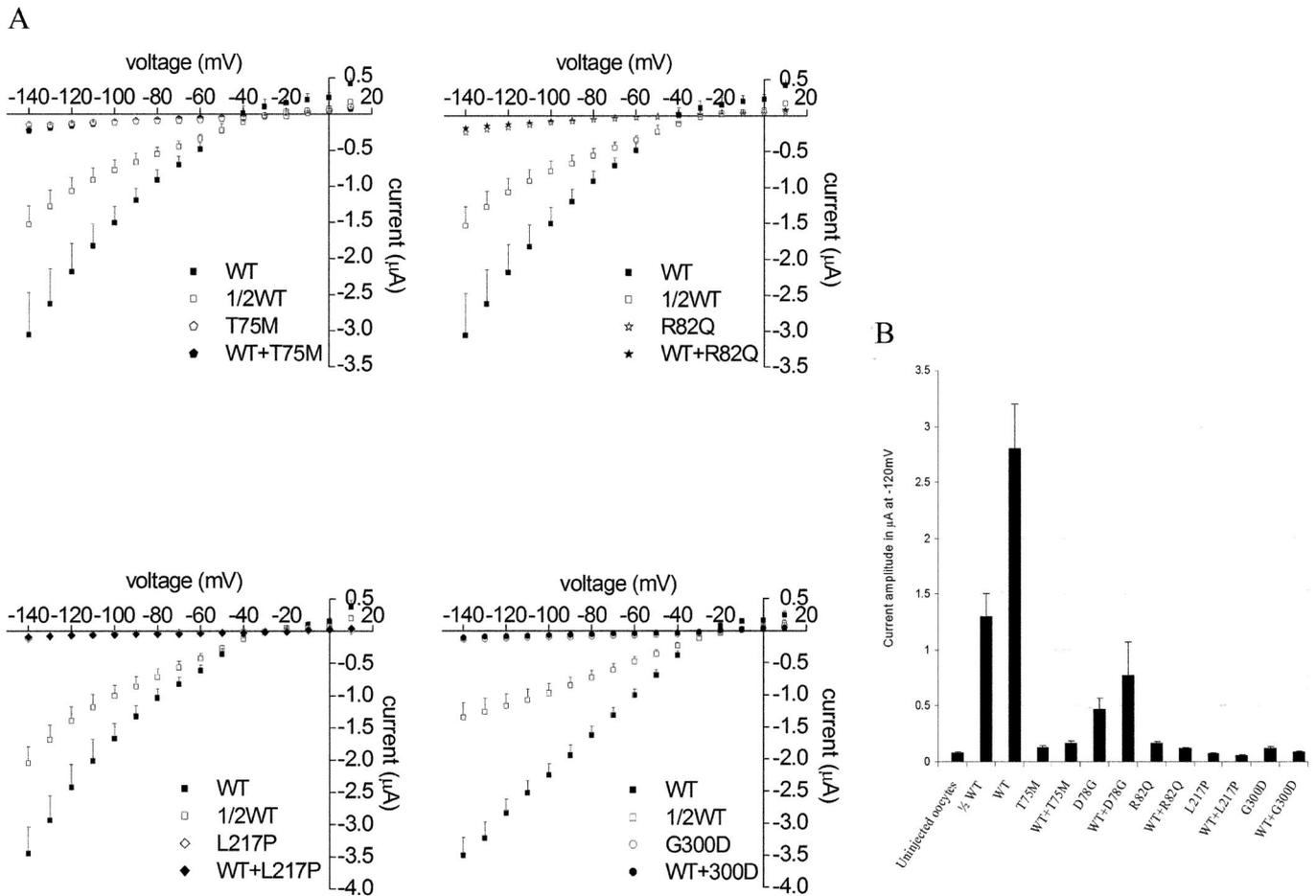
**Electrophysiology.** Whole-cell potassium currents were recorded 3 days after RNA injection (Geneclamp 500 amplifier and DigiData 1200 interface; Axon Instruments, Union City, CA) using electrodes with a tip resistance of 0.5 to 1 MΩ and standard two-electrode voltage-clamp techniques. Oocytes were bathed at

room temperature (22 to 25 °C) in a high-potassium solution containing the following (in mM): KCl 90, MgCl<sub>2</sub> 3, HEPES 10 (pH 7.4). Currents were evoked by voltage commands from a holding potential of -10 mV, delivered in -100-mV increments from +10 to -140 mV.

Data acquisition, analysis, fitting, averaging, and presentation were done with PClamp6 (Axon Instruments), Microcal Origin 6.0, and Microsoft PowerPoint.

**Results. Genetic studies.** The genetic results for all families are shown in the table. Five families harbored previously described *KCNJ2* mutations; Families 2 and 8 (R67W),<sup>4,6</sup> Families 4 and 9 (R218W),<sup>5,6</sup> and Family 3 (G300D).<sup>6</sup> The R67W mutation in Family 2 occurred de novo in the proband as no other family member harbored this mutation and there was no genetic evidence of nonpaternity. A previous study has also described de novo mutations in families with ATS.<sup>5</sup>

Six families harbored new pathogenic mutations (see figure E-1 in the supplementary material on the *Neurology* Web site; go to [www.neurology.org](http://www.neurology.org)). In Family 1, there was an A-to-G transition at position 233, resulting in the substitution of glycine for aspartate at codon 78 (D78G, c.233A→G). In Family 5, a glutamine was substituted for arginine at codon 82 (R82Q, c.245G→A). Family 6 harbored a mutation resulting in the substitution of proline



**Figure 2.** (A) Functional effects of T75M, R82Q, L217P, and G300D Kir2.1 mutations. Instantaneous current-voltage relationships for oocytes injected with wild type (WT), 1/2WT, mutants, and coinjected WT and mutants. Currents were elicited by step depolarizations from +10 to -140 mV, from a holding potential of -10 mV. Oocytes were injected with 9.2 ng of total cRNA, with the exception of 1/2WT that was injected with 4.6 ng of WT cRNA. Currents induced by injection of 4.6 ng of WT were approximately one-half that induced by 9.2 ng of WT Kir2.1. Symbols are shown in figure 2. Data are means  $\pm$  SEM for  $n = 8$  to 10 oocytes for each group. (B) Current amplitudes for WT and mutant Kir2.1 channels at -120 mV. Data represent means  $\pm$  SD for  $n = 6$  to 8 oocytes.

for leucine at codon 217 (L217P, c.650T→C). Family 7 had a substitution of methionine for threonine at codon 75 (T75M, c.224C→T). Family 10 showed a tyrosine-to-aspartic acid change at residue 68 (Y68D, c.202T→G). In Family 11, valine at position 123 was substituted by glycine (V123G, c.368T→G). These mutations were not identified in 80 control samples, occur at highly conserved sites within *KCNJ2* (see figure E-2 on the *Neurology* Web site), and were shown to segregate with disease.

**Expression studies.** We studied the functional consequences of the G300D mutation, which has only been characterized genetically previously<sup>6</sup> and the newly identified mutations T75M, D78G, R82Q, and L217P. To test whether these mutant Kir2.1 subunits were able to form functional homomultimeric channels, we injected 9.2 ng/oocyte WT and mutant RNA into *X. laevis* oocytes and recorded K<sup>+</sup> currents with the two-electrode voltage-clamp technique. Currents were evoked by depolarizing steps from +10 to -140 from a holding potential of -10 mV. Injection of WT Kir2.1-induced currents showing strong inward rectification, as previously described.<sup>12</sup> Small endogenous currents, almost identical to those in uninjected control oocytes, were recorded in oocytes injected with mu-

tant RNAs T75M, R82Q, L217P, and G300D (figure 2). A less marked current reduction was observed with the D78G mutant ( $0.47 \pm 0.1 \mu\text{A}$  for D78G compared with  $-2.8 \pm 0.4 \mu\text{A}$  for WT at -120 mV).

In ATS, which is an autosomal dominant disorder, affected individuals possess one normal and one mutant *KCNJ2* allele. Four subunits are required to assemble a functional channel. To test whether the mutant Kir2.1 subunits were able to form functional heteromultimeric channels with WT subunits, we co-injected mutant (4.6 ng/oocyte) and WT (4.6 ng/oocyte) Kir2.1 cRNA in *X. laevis* oocytes and compared currents with those induced by injection of WT Kir2.1 cRNA alone (4.6 or 9.2 ng/oocyte). Co-expression of WT and mutated Kir2.1 subunits induced an almost complete suppression of the inwardly rectifying K<sup>+</sup> current at -120 mV in four of the five mutant Kir2.1 subunits studied, ranging from  $-0.059 \pm 0.003 \mu\text{A}$  (WT/L217P),  $-0.09 \pm 0.01 \mu\text{A}$  (WT/G300D),  $-0.12 \pm 0.01 \mu\text{A}$  (WT/T75M), to  $-0.16 \pm 0.02 \mu\text{A}$  (WT/R82Q) compared with WT only current at  $-2.8 \pm 0.4 \mu\text{A}$  (9.2 ng/oocyte) and  $-1.3 \pm 0.2 \mu\text{A}$  for 1/2WT (4.6 ng/oocyte) (see figure 2B). Again, the current reduction with the D78G mutant was

less marked than that seen with the other mutant subunits ( $-0.77 \pm 0.3 \mu\text{A}$  for WT/D78G).

These findings demonstrate that mutated subunits co-assemble with WT Kir2.1 subunits and cause variable degrees of dominant negative suppression of channel function.

**Discussion.** The majority of the clinical manifestations in the ATS families described herein conform to those reported previously.<sup>1-7,9</sup> It is notable that in all cases where potassium was documented during attacks of weakness, it was found to be low. This is consistent with previous observations that the paralysis is most commonly hypokalemic. Although renal hypoplasia has been described in ATS,<sup>4</sup> we identified one patient (Family 3, III:5) with a renal tubular defect, possibly related to Kir2.1 dysfunction. Kir2.1 expression has been demonstrated in rat metanephron,<sup>13</sup> in the proximal renal tubule system, and in isolated glomeruli in humans.<sup>14</sup> It is notable that another inward-rectifying potassium channel (Kir1.1) causes Bartter syndrome type II.<sup>15</sup> Our observation indicates that further study of renal tubular function in ATS patients should be considered.

In the same family, several affected members had a peculiarly high-pitched voice, the cause of which is unknown. Initially, it was considered that a structural palatal defect was responsible, but on further investigation, this was found not to be the case. The precise cause of this abnormality in phonation is uncertain, but it was a consistent observation in affected family members, all harboring the G300D mutation.

Previous authors have described learning disability and pyramidal signs in a single individual with ATS.<sup>2</sup> The possibility that these CNS features were related to the genetic abnormality was raised. However, this previously described individual experienced a nonfatal cardiac arrest at age 4. Three of our patients, all harboring the G300D mutation, had mild learning difficulties (Family 3). One patient in our series developed involuntary movements and gait ataxia at age 44 (Family 4, II:1). These observations raise the possibility that CNS involvement may be a feature in certain patients with ATS.

Many ATS patients studied here had cardiac abnormalities similar to those previously reported. Studies in guinea pig ventricular myocytes have shown that suppression of the cardiac inward-rectifying current decelerates repolarization, prolongs the action potential duration, and depolarizes and destabilizes the resting membrane potential in keeping with the long QT phenotype seen in ATS patients.<sup>16</sup> The majority of arrhythmias described in ATS seem to arise from the ventricle probably because of the higher Kir channel density in ventricular compared with atrial myocytes and their differing rectifying properties.<sup>17,18</sup> However, in this study, we identified an individual with prominent supraventricular tachycardia in addition to ventricular arrhythmias. This was associated with a new mutation

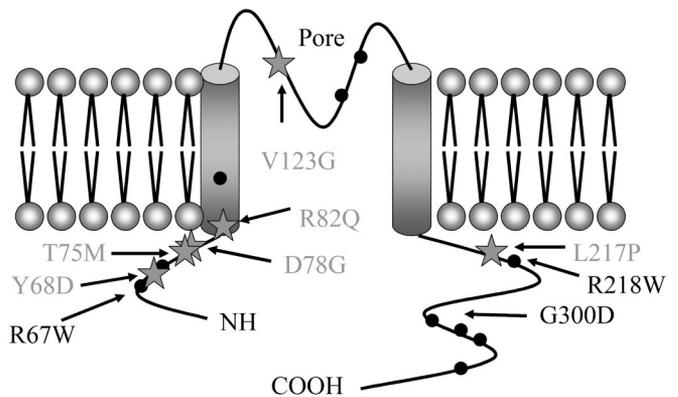


Figure 3. Position of *KCNJ2* mutations within the Kir2.1 subunit. Newly identified mutations are indicated with stars.

(L217P), and it is unclear whether this may be a specific manifestation of this defect.

Finally, in Families 3 and 4, poor dentition has been noted. Dental abnormalities such as delayed teeth development, missing teeth, and abnormal positioning have been noted previously including in the original description.<sup>1</sup> However, in our subjects, the change was that of discoloration, which did not seem to be related to poor oral hygiene but possibly due to abnormal dentine and enamel formation. Similar findings have been reported in Lowe syndrome, a disorder due to disruption of the  $\text{PIP}_2$  system.<sup>19,20</sup>

We have identified nine mutations in British families with ATS, in which probands presented with periodic paralysis. The mutations occur either in the N terminus (R67W, Y68D, T75M, D78G, R82Q), the extracellular loop (V123G), or the C terminus (L217P, R218W, G300D), but none affects the transmembrane segments or the pore-lining part of the loop connecting the transmembrane segments (figure 3).

We identified previously described *KCNJ2* mutations in five families. The sex-linked bias in clinical manifestations of ATS due to the R67W mutation previously suggested<sup>4</sup> was not present in either of our two families harboring this defect in keeping with observations by other authors.<sup>6</sup> We confirm the R218 residue as a hotspot for disease-causing mutations.

Our expression studies clearly showed a major effect of the mutations on Kir2.1 function. To date, all the mutations causing ATS exert a dominant negative effect on WT subunits when studied in functional expression systems. However, the degree of dominant negative effect in vitro does not seem to correlate with the severity of the disease. Approximately half the mutations described in the literature cause channel dysfunction by adversely affecting the binding of phosphatidylinositol 4,5-bisphosphate ( $\text{PIP}_2$ ).<sup>21</sup>  $\text{PIP}_2$ , a membrane-bound phospholipid that acts as a precursor for secondary messengers, is required to stabilize the open state.<sup>22</sup> It binds directly to Kir channels through interaction between positively charged amino acids of the Kir channel and

negatively charged phosphate groups of the lipid. Both the L217 and the R218 residues are located within one of the three putative PIP<sub>2</sub> binding sites in the C terminus of the Kir2.1 channel.<sup>23</sup> The L217P mutation identified in this study changes a leucine to the more bulky proline. It is adjacent to the residue R218 where mutations changing a basic amino acid to a nonpolar (R218W)<sup>5</sup> or an uncharged polar (R218Q)<sup>5</sup> residue have been identified in patients with ATS.

The mutations Y68D, T75M, and D78G occur in highly conserved but functionally undetermined regions within the N terminus of Kir2.1. The electrophysiologic studies of T75M and D78G clearly demonstrate a profound effect on channel function. Scanning cysteine mutagenesis of an amino-terminal segment immediately adjacent to M1 (from C54 to V86) revealed that the majority of amino acids, including residues Y68, T75, and D78, are water accessible and probably contribute to the formation of a long and wide intracellular pore vestibule that protrudes into the cytoplasm.<sup>24</sup> Comparison with the crystallographic structure of the prokaryotic Kir channel KirBac1.1 locates these residues within the slide helix, a transmembrane channel segment that has been suggested to play a role in the gating process.<sup>25</sup> The R82Q mutation involves one of the first residues of the M1 transmembrane domain. The mutation has been investigated previously via site-directed mutagenesis and expression studies, and the change from the basic arginine to the uncharged glutamine at codon 82 resulted in very little whole-cell current.<sup>21</sup> It appears its effect is independent of the PIP<sub>2</sub> system. The V123M mutation affects a residue of the extracellular part of the loop that connects the two transmembrane segments.

As with other potassium channels, the Kir2.1 subunit can form functional heteromers with members of the same family (e.g., Kir2.2 or 2.3).<sup>26</sup> In the same study, it was shown that mutant Kir2.1 could exert a dominant negative effect on both Kir2.2 and Kir2.3. This provides some insight into possible mechanisms explaining the marked intrafamilial variability in this disorder with interindividual variability of heteromer channel formation. Considering this hypothesis, it may be that the renal tubular abnormality in Case III:5 (Family 3) was caused by a dominant negative effect of mutant Kir2.1 on WT Kir1.1.

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