A novel mutation in the human voltage-gated potassium channel gene (Kv1.1) associates with episodic ataxia type 1 and sometimes with partial epilepsy

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Summary
Episodic ataxia type 1 (EA1) is a rare autosomal dominant disorder characterized by brief episodes of ataxia associated with continuous interattack myokymia. Point mutations in the human voltage-gated potassium channel (Kv1.1) gene on chromosome 12p13 have recently been shown to associate with EA1. A Scottish family with EA1 harbouring a novel mutation in this gene is reported. Of the five affected individuals over three generations, two had partial epilepsy in addition to EA1. The detailed clinical, electrophysiological and molecular genetic findings are presented. The heterozygous point mutation is located at nucleotide position 677 and results in a radical amino acid substitution at a highly conserved position in the second transmembrane domain of the potassium channel. Functional studies indicated that mutant subunits exhibited a dominant negative effect on potassium channel function and would be predicted to impair neuronal repolarization. Potassium channels determine the excitability of neurons and blocking drugs are proconvulsant. A critical review of previously reported EA1 families shows an over-representation of epilepsy in family members with EA1 compared with unaffected members. These observations indicate that this mutation is pathogenic and suggest that the epilepsy in EA1 may be caused by the dysfunctional potassium channel. It is possible that such dysfunction may be relevant to other epilepsies in man.

Keywords: episodic ataxia type 1; potassium channel; epilepsy
Abbreviations: bp = base pair; EA1 = episodic ataxia type 1; EA2 = episodic ataxia type 2; PCR = polymerase chain reaction

Introduction
Episodic ataxia type 1 (EA1, MIM 160120) is a rare autosomal dominant disorder in which patients develop sudden episodes of ataxia precipitated by movement, startle or emotion and which last for a time ranging from seconds to minutes (Van Dyke et al., 1975). In addition, there is continuous myokymia which may be clinically evident or only detectable by EMG. In episodic ataxia type 2 (EA2, MIM 108500) affected individuals experience prolonged attacks of ataxia lasting hours, often accompanied by nausea, vomiting and headache but not associated with myokymia. EA2 subjects frequently develop a progressive cerebellar ataxia accompanied by cerebellar atrophy (Gancher et al., 1986). Point mutations in the voltage-gated potassium channel gene (Kv1.1) on chromosome 12p13 have been shown to be associated with EA1 while mutations in the P/Q-type voltage-gated calcium channel gene (CACNA4) on chromosome 19p13 underlie EA2 (Browne et al., 1994; Litt et al., 1994; Ophoff et al., 1996). There is increasing evidence from animal studies that dysfunctional ion channels are responsible for the commonest episodic neurological disorder, epilepsy (Noebels, 1996). We describe a Scottish family with EA1 in whom a point mutation has been identified in the voltage-gated potassium channel...
gene *Kv1.1*. In addition to EA1, two members had partial epilepsy and we discuss the possibility that dysfunction of this channel is associated with epilepsy in humans.

**Subjects and methods**

**Clinical study**

The family is of Scottish descent except for the mother of case I1, who was of German descent. The pertinent part of the pedigree is illustrated in Fig. 1. The disease was documented over three generations. The diagnosis of EA1 was based on the appropriate clinical history and the presence of myokymia on clinical and/or EMG examination. The diagnosis of epilepsy was based on the clinical history and witness account, and was supported by ictal EEG recordings in case III2. The spouse (I2) of the oldest affected family member (case I1) was healthy and did not have epilepsy. Members of his family were interviewed and the general practice records of his four siblings were reviewed to confirm there was no family history of epilepsy, particularly during early childhood.

EMG was performed on four patients (Synergy EMG/EP; Medelec, UK). EEG was carried out on four individuals (Nicolet Voyageur 1.4; Nicolet Biomedical, Madison, Wis., USA). Three individuals had a CT brain scan and one had a 1.5 T MRI brain scan. Video recordings were made of all individuals who were examined. Ataxic episodes were induced and recorded on videotape in two cases.

Video EEG (Oxford Medilog System 9200, Oxford, UK with Video Interface Processor; Oxford Medical Systems, Oxford, UK) was performed on two patients, capturing epileptic seizures in one and an ataxic episode in another.

**Genetic study**

Informed consent was obtained for DNA analysis from all nine individuals shown in the pedigree in Fig. 1. DNA was extracted from blood using standard methods. Three sets of oligonucleotide primers were designed to amplify all 1448 base pairs (bp) of the single-exon *Kv1.1* gene located on chromosome 12p13 (Table 1). The primers employed were tagged with M13 tails to facilitate subsequent DNA sequencing. The sequences of the primers used are shown in Table 1 without the M13 tails. Conditions for the initial polymerase chain reaction (PCR) were as follows: an initial denaturing step at 94°C for 3 min followed by 30 cycles of the following: 92°C for 30 s, 65°C for 30 s and 72°C for 30 s. A final extension step of 72°C for 10 min was used.

The products were cleaned using Centricon filters and both strands were sequenced using a Dye Primer Taq cycle sequencing kit (Applied Biosystems, Foster City, Calif., USA; ABI). The sequencing products were separated on 10% polyacrylamide–urea gels in a 373A automated DNA sequencer (ABI). The sequence data were analysed using Seq Ed software (ABI).

In order to screen control chromosomes and other family members for the C→G point mutation at position 677, a mismatch PCR technique was employed since this mutation neither created nor abolished a natural restriction site. This PCR reaction creates a restriction site for *DdeI* only in the presence of a G nucleotide at position 677. The two primers used were the forward (mismatch) primer (nucleotide positions 647–676): 5′-TCTTTCAGAGACCCCTTTCTCA-TCGTGCTAA3′ (mismatch nucleotides shown bold underlined), and the reverse primer (nucleotide positions 806–787): 5′-AGGGTGATGAAATAAGGAT3.

The wild-type sequence between positions 672 and 677 is GGAAAC. Following amplification with the mismatch primer, the following sequence is generated in the wild type: GCTAAC. In the presence of the C677G mutation the sequence GCTAAG is generated, which contains the recognition site for *DdeI* (C/TNAG). The conditions for the mismatch PCR were identical to those for the PCR described above. After digestion with *DdeI* according to the manufacturer’s recommendations, the fragments were separated on a 3.2% agarose gel stained with ethidium bromide and visualized on an ultraviolet light-box.

The 159 bp product is digested into two fragments of 131 bp.
and 28 bp in mutant but not wild-type DNA. The lower panel in Fig. 1 shows the products of digestion for individuals in the pedigree. The upper band is the undigested 159-bp product and the lower band is the digested 131-bp product. The 28-bp band has migrated off the gel. Unaffected individuals have a single 159-bp band while affected individuals are heterozygous for the mutant and have two bands (159 and 131 bp).

Functional study
Human Kv1.1 wild-type and mutant genes were amplified using PCR on genomic DNA extracted from a blood sample of one of the affected family members. PCR products were subcloned into the vector pSGEM (courtesy of Dr M. Hollmann, Göttingen, Germany), which provides a sample of one of the affected family members. PCR products were used to amplify the wild-type and mutant genes in vitro using T7-RNA polymerase (Boehringer Mannheim, Germany). *Xenopus* oocytes were defolliculated manually after collagenase treatment and injected with 0.2–1.4 ng mRNA using a Nanoject automatic injector (Drummond). Injected oocytes were kept in Barth’s medium for 3–5 days at 18°C prior to recording in a solution containing (in mM): NaCl, 115; KCl, 2.5; CaCl2, 1.8; HEPES, 10; pH = 7.4. Two-electrode voltage-clamp recording was performed at 22°C using a GeneClamp 500 amplifier, and data were acquired and analysed using pClamp6 (Axon Instruments, Foster City, Calif., USA). Leak and capacitative currents were subtracted applying a -P/4 protocol.

<table>
<thead>
<tr>
<th>Table 1 Three oligonucleotide primer pairs used to amplify the Kv1.1 gene</th>
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<td>(1) Forward (nucleotides 1–20)</td>
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<td>Reverse (nucleotides 550–531)</td>
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<td>(2) Forward (nucleotides 481–500)</td>
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<td>Reverse (nucleotides 983–974)</td>
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<td>(3) Forward (nucleotides 931–950)</td>
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<td>Reverse (nucleotides 1469–1488)</td>
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Results
Case reports
Case III2
The proband is a 3-year-old boy who presented aged 7 weeks with recurrent apnoeic episodes associated with cyanosis. Pregnancy and delivery had been unremarkable and the only abnormality noted prior to these episodes was a tendency to keep his fists clenched. Neurological examination was normal. Interictal EEG was normal. A 24-h video EEG study was performed during which several episodes were captured. These were shown to be complex partial seizures. Recordings showed the child staring forward or with his head turning to the left with his eyes deviating to the left, the eyelids flickering, lip-smacking and the development of cyanosis. The episodes lasted up to 2 min and terminated with the infant fixing on his mother and the onset of regular respiration. EEG changes during the episode consisted of rhythmical slow-wave activity over the right hemisphere (possible right temporal lobe onset), becoming spike and slow-wave complexes (Fig. 2). This activity then spread to the left hemisphere, and asynchronous flattening of rhythms occurred periodically over both hemispheres. A CT scan with angled cuts through the temporal lobes was normal. Plasma electrolytes, calcium and magnesium were normal. The patient was treated with carbamazepine and had no further epileptic seizures. Subsequent developmental progress was normal and antiepileptic medication was stopped at the age of 2 years. A diagnosis of benign partial epilepsy of infancy was made.

At 20 months the patient developed irritability and swelling of his hands and feet. He had clenched fists and flexion of the toes. The dorsum of his hands and feet appeared swollen and oedematous and he refused to walk. Investigations for renal and joint disorders were negative and the problems resolved within a few days.

At the age of 2 years episodes of unsteadiness when walking developed. His legs would appear to buckle under him. There were several months between attacks and interictal general and neurological examination were normal. From the age of 3 years he has had episodes of ataxia at least once a week lasting seconds to minutes, precipitated by startle, exercise and sudden movement. Consciousness is preserved in the attacks. Examination shows periorbital and finger myokymia. EMG shows continuous motor unit activity (Fig. 3). EEG at 3 years of age is normal.

Case II
The 50-year-old maternal grandmother of the proband has had symptoms of intermittent ataxia since early childhood. They are precipitated by sudden movements, exercise, anxiety or loud noises, or occur spontaneously and last seconds to a few minutes. During the episodes she becomes ataxic, has dysarthria but retains full consciousness. Phenobarbitone therapy as a child was not helpful. She attended a school for children with mild to moderate learning difficulties. Clinical examination is normal apart from periorbital and finger myokymia. Acetazolamide was unhelpful.
Case II2
This 31-year-old male was noted to have postural abnormalities of his upper and lower limbs at the age of 3 months when admitted with bronchiolitis. His wrists were flexed, the thumbs were adducted across his palms and his feet were held in equinovarus. There was no spasticity on examination. He was treated with splints. During this acute illness he developed transient oedema of his extremities. He was noted to have twitching of his eyelids but an EEG was normal. At 5 months he had surgery for a strangulated inguinal hernia. He walked at 14 months and his foot deformity had resolved, but he had a continued tendency to adduct his thumbs across his palms.

Episodes of ataxia began at the age of 4 years. They occur spontaneously, after a sudden movement or are precipitated by exercise. Attacks last seconds to minutes. Clinical examination showed myokymia, which was most prominent in the fingers and periorbital area. A typical ataxic episode, which lasted ~2 min, was recorded on video after he had walked briskly up a flight of stairs. He was markedly ataxic with dysarthria but eye movements were normal. EMG showed continuous motor unit activity consistent with myokymia. An interictal EEG was normal apart from showing prominent muscle activity with EMG at a frequency of ~10/s, which was more prominent with hyperventilation (Fig. 4). A 1.5 T MRI and cerebral perfusion SPECT scan were normal. Acetazolamide produced resolution of attacks for 3 months but they returned at a reduced frequency. Attacks tend to cluster.

Case II4
This 30-year-old woman has EA1 and epilepsy. She presented in early infancy with more prominent postural deformities of
her limbs than in her brother. Her wrists were flexed to 50°, her thumbs were held in partial adduction, her knees were flexed to 20° and her feet were in an equinovarus posture. Serum creatine phosphokinase was normal. An EEG in infancy was normal. A diagnosis of atypical familial arthrogryposis was made. She also required surgery for an inguinal hernia. She walked late at 2 years of age and by 3 years the postural abnormalities had almost completely resolved. At the age of 9 years she was diagnosed as having stage IV Hodgkin’s disease. She responded to chemotherapy and localized radiotherapy and since then she has been disease-free.

At the ages of 9 and 10 years she began to have epileptic seizures and episodic ataxia, respectively. The clinical features of the seizures suggest that they are complex partial followed by secondary generalization. There is no warning and the onset involves turning the head to the right accompanied by impaired awareness lasting up to 30 s followed by a generalized tonic clonic seizure. EEG and CT brain were normal at the age of 9 years. The ataxic episodes are clearly distinguished from the seizures and are similar to those described in her mother and brother. Consciousness is fully preserved. Neither the seizures nor the ataxic episodes have responded to phenytoin or sodium valproate. On examination she has periorbital and finger myokymia. EEG in 1997 was normal apart from prominent muscle activity artefact.

**Case III1**

This 10-year-old boy was also born with postural abnormalities as described in other family members. At 8 months his fingers were held flexed and this would interfere with attempts to grasp and transfer objects. His knees were held slightly flexed and his feet were plantar-flexed (Fig. 5). At 10 months, during an intercurrent illness, he developed peripheral pitting oedema of his hands and feet, which resolved. By 14 months these postural deformities had resolved and he was able to cruise around furniture. At the age of 4 years he began to have typical ataxic episodes lasting from seconds to a few minutes. EEG shows regular muscle activity more prominent after hyperventilation but no other abnormality. EMG shows myokymia. Carbamazepine produced an initial decrease in ataxic episodes but this was not sustained.

**Genetic results**

Direct DNA sequence analysis of all 1488 bp of the *Kv1.1* gene revealed three heterozygous changes. These were C677G, C684T and C804G. In addition, our data indicate that the consensus nucleotide at position 1355 is C and not A (Browne et al., 1994). The C684G and C804G changes were silent and were observed in 5% of control chromosomes analysed. However, the C→G transition at position 677 was not detected in 200 control chromosomes and segregated with the disease in the family, suggesting that it is likely to be pathogenic (Figs 1 and 6). Furthermore, this mutation results in a radical amino acid substitution (threonine→arginine) at position 226, a highly conserved position in the second transmembrane segment of the channel, and is therefore likely to have functional consequences (Figs 7 and 8).
**Fig. 6** Electropherogram of Kv1.1 gene DNA sequence. *(Upper panel)* Wild-type DNA sequence. *(Lower panel)* Sequence from the proband (case II2). The silent polymorphism at position 684 (indicated by X) is present in both the control and the proband. The C→G mutation at position 677 indicated by the double arrowhead is present only in the proband.

**Fig. 7** Schematic representation of one subunit of Kv1.1. The site of the T226R amino acid substitution resulting from the C677G point mutation in the second transmembrane domain is indicated.

**Functional studies**

In order to investigate the possible consequences of the T226R mutation on ion channel function we heterologously expressed wild-type and mutant Kv1.1 subunits in Xenopus laevis oocytes. Whole-cell current amplitudes were assessed after 3–5 days. Oocytes injected with mutant mRNA showed a significant decrease in voltage-activated current to ~3% of that of wild type (Fig. 9, solid bars). Since heterozygous patients express both alleles, we simulated this situation by co-injecting constant amounts of wild-type mRNA and increasing amounts of mutant mRNA to yield ratios as indicated in Fig. 9 (hatched bars). We found that increasing the ratio of mutant to wild-type mRNA reduced the current amplitude, consistent with a dominant negative effect of the mutant allele. Wild-type mRNA was also injected at higher concentrations on its own to exclude the possibility that reduced whole-cell currents were due to saturation of the translation machinery (data not shown).

**Discussion**

**Clinical features and investigations**

We have reported a family with the rare autosomal dominant disorder EA1 in which two individuals have epilepsy. As in other paroxysmal conditions due to ion channel dysfunction, there was a long delay in making the correct diagnosis. This was appreciated only when the family history became apparent. A factitious disorder had been considered in case II2. The initial presentation with postural abnormalities in infancy in all cases except case II1 has been described in one family previously (Hanson et al., 1977). This is presumed to be a manifestation of the continuous muscle fibre activity at this age. Inguinal hernias were present in two siblings in generation II, and these may also be secondary to myokymia of the abdominal wall musculature. Case II4 had the most severe postural deformities and a diagnosis of arthrogryposis was considered. It is notable that the postural deformities gradually resolve and are not a significant long-term problem. It is therefore important to avoid surgical intervention. The postural abnormalities were more pronounced during acute illnesses in three of our cases. This may be due to acid–base or electrolyte imbalance exacerbating ion channel dysfunction. The age at onset of the ataxic episodes was between 2 and 4 years except in case II4, whose attacks started at the age of 9 years. The ataxia was precipitated by similar stimuli of sudden movement, startle or exercise in each case. The episodes never lasted for more than a few minutes. In contrast to EA2, eye movements remain normal during the attacks and there is no evidence of progressive interictal cerebellar dysfunction. Myokymia was present in all individuals. Clinically this was evident as small semirhythmic irregular lateral finger movements and rippling of muscles below the eyelids.

The most helpful clinical investigation was EMG, which showed typical rhythmic continuous motor unit activity in all individuals (Fig. 3). The observation of a continuous rhythmic muscle discharge artefact on EEG recordings was also a clue to the correct diagnosis (Fig. 4). The muscle activity on the EEG became more prominent with hyperventilation, which may reflect the effect of pH or CO₂ changes on ion channel function. Acetazolamide, a carbonic anhydrase inhibitor, which may be effective in reducing ataxic episodes in some individuals, has been partly effective in only one of our patients (Lubbers et al., 1995). The precise therapeutic mechanism of acetazolamide is not known. It may act by increasing the CO₂ concentration in the vicinity of the ion channel, causing hyperpolarization of the cell membrane and thereby reducing neuronal excitability (Brunt et al., 1990). Some of the cases reported here had a partial response to carbamazepine. Other reported cases have responded to antiepileptic medication such as carbamazepine, phenytoin and phenobarbitone. These observations indicate that the treatment response in EA1 varies, and it is possible that it may be mutation-specific.
Molecular genetic and functional studies

In view of the reported association between EA1 and mutations in the human voltage-gated potassium channel gene \(Kv1.1\), we sequenced the entire gene from genomic DNA in the proband. The heterozygous C→G change identified at position 677 had characteristics that suggested it was likely to be of pathogenic relevance. Further evidence in favour of pathogenicity was obtained from expression studies. These indicated that the mutant allele is correctly translated and processed to the cell membrane, but that it yields currents with a largely reduced amplitude compared with wild-type channels. In co-expression studies the mutant allele seemed to dominate over the wild type with respect to current amplitude. Thus, heterotetrameric channels, as might be expressed in neurons of heterozygous patients, would be predicted to have a reduced potassium efflux during action potentials. A similar degree of current amplitude reduction was observed in co-expression studies (ratio 1 : 1) of another pathogenic \(Kv1.1\) point mutation at amino acid position 408 (Adelman et al., 1995). Such studies have shown a greater degree of current amplitude reduction in association with other pathogenic \(Kv1.1\) mutations (Adelman et al., 1995). However, the relationship between the degree of current amplitude reduction in such co-expression studies and functional impairment of neuronal repolarization is unknown. It is possible that even a relatively small reduction in potassium current amplitude could disturb the complex equilibrium between a number of conductances in the membrane of affected neurons and account for a delay in repolarization, thereby facilitating the generation and spread of action potentials. This delayed repolarization is likely to be the basis of the neuromyotonia and episodes of ataxia. In addition, it is possible that such a defect in neuronal repolarization could lower the seizure threshold. To date, nine point mutations in \(Kv1.1\) have been reported in association with the EA1 phenotype. Expression studies have identified two broad mechanisms by which such mutations induce channel dysfunction and would be predicted to impair neuronal repolarization. The first group of mutations form homomeric channels with altered gating properties. The second group of mutations do not produce functional homomeric channels but co-expression studies with wild-type channels indicate that these mutations may induce a dominant negative effect (Adelman et al., 1995; Zerr et al., 1998a, b).

Possible relationship between EA1 and epilepsy

This is the eleventh family with EA1 to be reported. Within this group, 90 individuals have been identified with the phenotype of episodic ataxia and myokymia (Van Dyke et al., 1975; Hanson et al., 1977; Gancher and Nutt 1986; Brunt and Van Weerden 1990; Vaamonde et al., 1991; Browne et al., 1994; Browne et al., 1995; Lubbers et al., 1995; Comu et al., 1996). Of these, eight individuals from three families, including our own, are reported to have epilepsy. In addition we have recently identified a further family with a previously undescribed \(Kv1.1\) mutation in which members affected with EA1 also have epilepsy (R. Liguori and M. G. Hanna, unpublished observations). In all these families none of the unaffected family members are reported to have epilepsy.
Table 2 summarizes the published information. These data suggest that patients with EA1 are ten times more likely to develop epilepsy than normal individuals. Assuming a general population risk of epilepsy of 1:200, the relative risk of epilepsy in EA1 is 17.8 (95% confidence interval 2.3–140.4).

In all reported cases the epileptic seizures were easily distinguished from the ataxic episodes. The seizure type was described only in one previous report, and was a ‘generalized motor seizure’ (Van Dyke et al., 1975). The first case (III2) with EA1 and epilepsy that we describe is difficult to classify using standard criteria (Commission on Classification and Terminology of the International League Against Epilepsy, 1989). His epilepsy is best described as a benign partial seizure disorder of infancy. He had onset of complex partial seizures in the neonatal period, normal interictal EEG, no identifiable lesion on imaging, an excellent response to antiepileptic medication and normal subsequent development (Okumura et al., 1996). In contrast, case II4 had clinical features which were compatible with a partial epilepsy with secondary generalization. It is possible that the mutation we have identified causes the different epilepsy phenotypes in our family and that other mutations in Kv1.1 may be associated with epilepsy. In support of this suggestion, a mouse knockout for Kv1.1 has been described to have a lethal epilepsy phenotype (Smart et al., 1999). It is also recognized that drugs which block Kv1.1 are proconvulsant in humans (Newsom-Davis, 1993; Morales-Villagran et al., 1996). The expression of Kv1.1 has not been studied in man, but in rats it is widely expressed throughout the nervous system, including the hippocampus (Beckh et al., 1990). It remains to be explained why only two of the five members of our family developed epilepsy. It is possible that other factors, which may be genetic or environmental, could be important in influencing the phenotype. In other ion channel disorders it is recognized that the same point mutation can be associated with phenotypic heterogeneity (Bulman, 1997).

Recently two new potassium genes have been cloned, and direct evidence has been provided that mutations in these genes are associated with epilepsy (Biervert et al., 1998; Charlier et al., 1998; Singh et al., 1998). It is of interest that in both cases these seizures were similar to those observed in case III2. They were benign seizures in the neonatal period which resolved and were classified as benign neonatal familial seizures.

In conclusion, we described a novel mutation in Kv1.1 in a family with episodic ataxia type I. Functional studies have shown that this mutation reduces potassium channel current amplitude in a dominant negative fashion. This is likely to be the basis of the neuromyotonia and episodic ataxia. We provide some evidence that dysfunction of this channel may be associated with epilepsy in man.

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