Nongenetic factors influence severity of episodic ataxia type 1 in monozygotic twins

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ABSTRACT

Objective: Episodic ataxia type 1 (EA1) is a monogenic channelopathy caused by mutations of the potassium channel gene *KCNA1*. Affected individuals carrying the same mutation can exhibit considerable variability in the severity of ataxia, neuromyotonia, and other associated features. We investigated the phenotypic heterogeneity of EA1 in 2 sets of identical twins to determine the contribution of environmental factors to disease severity. One of the mutations was also found in a distantly related family, providing evidence of the influence of genetic background on the EA1 phenotype.

Methods: We evaluated 3 families with an EA1 phenotype, 2 of which included monozygotic twins. We sequenced the *KCNA1* gene and studied the biophysical consequences of the mutations in HEK cells.

Results: We identified a new *KCNA1* mutation in each pair of twins. Both pairs reported striking differences in the clinical severity of symptoms. The F414S mutation identified in one set of twins also occurred in a distantly related family in which seizures complicated the EA1 phenotype. The other twins had an R307C mutation, the first EA1 mutation to affect an arginine residue in the voltage-sensor domain. Both mutants when expressed exerted a dominant-negative effect on wild-type channels.

Conclusion: These results broaden the range of *KCNA1* mutations and reveal an unexpectedly large contribution of nongenetic factors to phenotypic variability in EA1. The occurrence of epilepsy in 1 of 2 families with the F414S mutation suggests an interplay of *KCNA1* with other genetic factors. ***Neurology*® 2010;75:367–372**

GLOSSARY

EA1 = episodic ataxia type 1.

Episodic ataxia type 1 (EA1) is characterized by brief paroxysms of ataxia and interictal myokymia.[1] It is caused by heterozygous point mutations in the voltage-gated $K_V1.1$ potassium channel α-subunit, encoded by *KCNA1*.[2] Scrutiny of genetically confirmed kindreds reveals considerable phenotypic heterogeneity with respect to severity of ataxia and presence of additional features such as epilepsy.[1] Hitherto, attempts to account for phenotypic variability have concentrated on differences among distinct *KCNA1* mutations with respect to their functional consequences when expressed in vitro. All EA1 mutations cause a loss of function, typically measured as a decrease in current density.[1] Mutations associated with a severe phenotype have a dominant negative effect on wild-type channel function.[3-5] A recent study in neurons has shown that *KCNA1* mutations differentially affect action potential generation and neurotransmitter release.[3] Despite attempts to correlate phenotypic severity with functional studies, even within affected kindreds there is

Supplemental data at www.neurology.org

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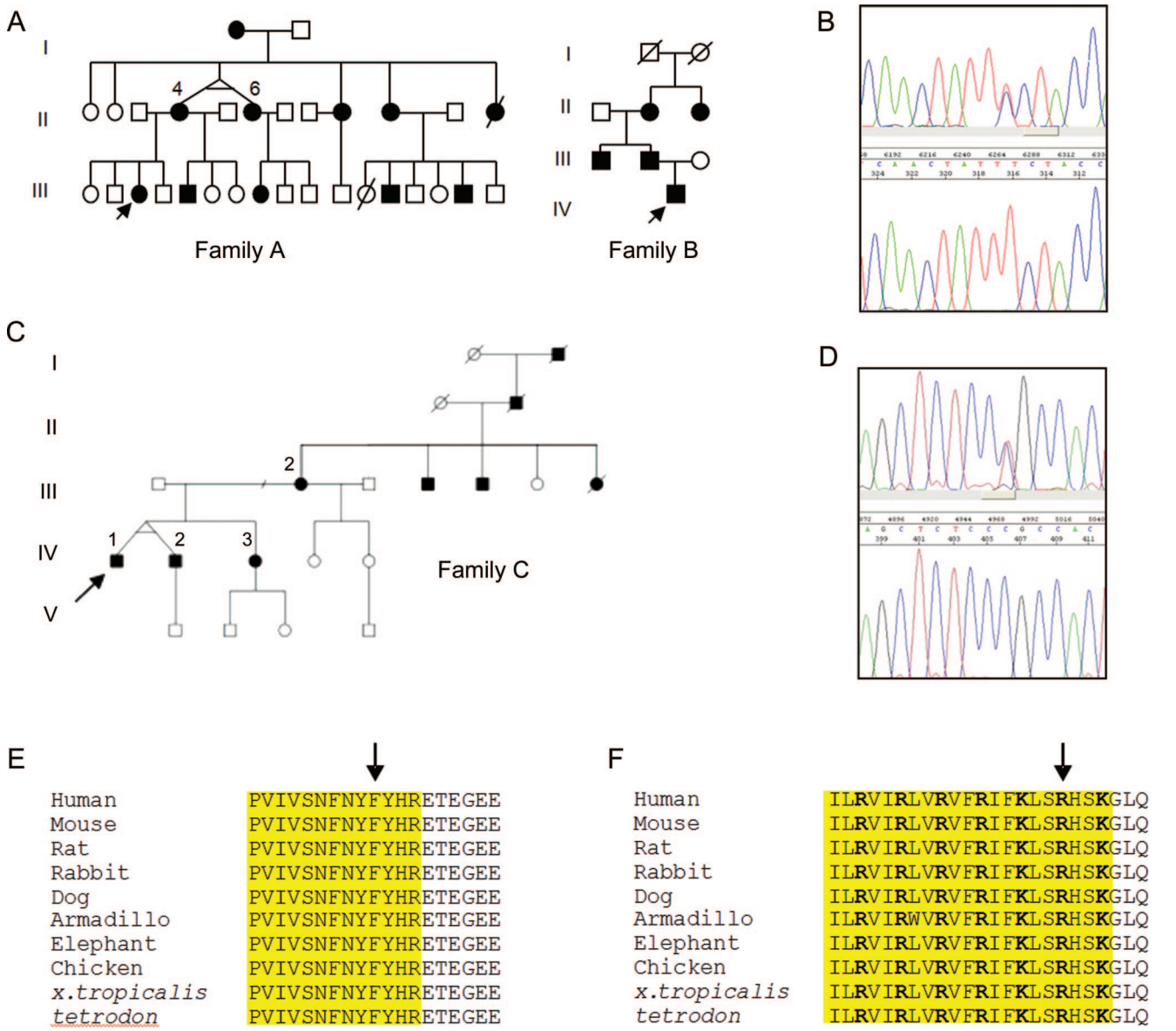
Figure 1 Families A and B



(A) Pedigrees of families. Filled boxes represent affected individuals. Arrow denotes proband. (B) Electropherogram showing T-to-C transversion at nucleotide 1,241. Upper panel, proband; lower panel, control. (C) Filled boxes represent affected individuals. Arrow denotes proband. (D) Electropherogram showing T-to-C transversion at nucleotide 919. Upper panel, proband; lower panel, control. (E) Conservation of F414 in *KCNA1*. Arrow denotes mutation position, yellow box represents S6 segment. (F) Conservation of R307 in *KCNA1*. Arrow denotes mutation position, yellow box represents S4 segment, positively charged voltage-sensor residues shown in bold.

frequently considerable heterogeneity in symptom severity. Monozygotic twins potentially provide an insight into the relative importance of modifier genes compared to lifestyle or environmental factors. Here we report 2 families where EA1 affects identical twins, and observe a surprising degree of discordance in symptom severity.

METHODS See e-Methods on the *Neurology*® Web site at www.neurology.org.

Standard protocol approvals, registrations, and patient consents. Ethical approval was obtained from the UCLH ethics committee. Written informed consent was obtained from all patients participating in the study. Consent to disclose was obtained for the video.

RESULTS **Clinical.** ***Family A.*** Affected members of this pedigree (figure 1A) show a classical EA1 phenotype (summarized in the table). In the more severe cases, dysarthria and gait disturbance are also present during an attack. Attack precipitants include sudden movements or fever, although sev-

Table Clinical summary of patients

Family	Pedigree no.	Age at onset, y	Age when seen, y	Symptoms	Triggers	Frequency	Length of attack	Neuromyotonia on EMG	Response to carbamazepine
A	I-1[a]	12	42	Dizziness, slurred speech, unsteady gait	Paroxysmal or on sudden movement	Were daily, now weekly	Seconds to minutes, longest 10 min	No	Not tested
A	II-4 twin	14	NA	Dizziness, slurred speech, nausea, unsteady gait, incoordination of hands, weakness, myokymia	Paroxysmal or on sudden movement, stress, startle, fever	Daily, but weekly on carbamazepine	10–15 min	No	Effective
A	II-6[a] twin	16	39	Dizziness, slurred speech, incoordination of hands, tremors	Sudden movement, startle, stress, fever, sleep	Weekly	5–10 min	No	Not tested
A	II-9[a]	15	36	Dizziness, slurred speech, vertigo, nausea, unsteady gait, headache, incoordination of hands, weakness, myokymia	Sudden movement, startle, stress, alcohol, menstruation, fever	Several per week	Seconds to 10 min	No	Ineffective
A	II-10[a]	12	35	Dizziness, slurred speech, vertigo, tremor, weakness, myokymia, nausea, unsteady gait	Paroxysmal or on sudden movement, startle, fever, stress, alcohol, pregnancy	2 per week	Seconds to minutes	No	Not tested
A	II-12	Childhood	NA	Dizziness	Unknown	Unknown	Unknown	Unknown	Unknown
A	III-3 proband[a]	12	16	Dizziness, slurred speech, nausea, unsteady gait, incoordination of hands, weakness, myokymia	Paroxysmal or on sudden movement, fever, startle, stress, alcohol	Daily, up to 30/d	Seconds to 20 min	No	Ineffective (400 mg)
A	III-5	Childhood	NA	Dizziness	Only when systemically unwell	NA	Seconds	No	Not tested
A	III-8	Unknown	NA	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
A	III-13[a]	Infancy	14	Dizziness, slurred speech, unsteady gait	Paroxysmal or on sudden movement, fever	Daily, almost continuous during intercurrent illness	Up to 2 min	Yes	Not tested
A	III-16	4–5	NA	Dizziness	Unknown	Unknown	Unknown	Unknown	Unknown
C	III-2[a]	5	50	Imbalance, weakness, blurred vision, slurred speech, myokymia, incoordination of hands, vertigo, headache, nausea, tremor	Startle, fever, alcohol, caffeine, stress, sudden movement, exercise, heat, menstruation	Daily	Seconds to 10 min	Yes	No
C	IV-1[a] twin	6	23	Imbalance, weakness, blurred vision, slurred speech, myokymia, incoordination of hands	Startle, fever, food, smell, alcohol, caffeine, stress, sudden movement, exercise	Daily	Seconds to 3 min	No	Ineffective (400 mg)
C	IV-2[a] twin	1	23	Imbalance, weakness, blurred vision, slurred speech, myokymia, incoordination of hands, vertigo, headache, nausea	Alcohol, caffeine, stress exercise	Weekly	Seconds to 10 min	No	No
C	IV-3[a]	8	21	Imbalance, weakness, blurred vision, slurred speech, incoordination of hands	Startle, fever, food, caffeine, stress, sudden movement, menstruation	Daily	Seconds to 3 min	No	No

Individuals are identified by generation (roman numerals) and position in each generation in figure 1 numbered from left to right.
Abbreviation: NA = not available.
[a] Patients examined for the study.

eral patients have also had unprovoked attacks. The proband, who experiences up to 30 attacks a day, is the most severely affected, and has experienced no benefit from carbamazepine, in contrast to her mother, who reports a marked effect on attack frequency. Interictal myokymia has been observed in affected individuals. The proband's mother (II-4) has an identical twin (II-6; figure 1A). Although case II-4 is not available for objective evaluation, she and her twin sister report that their symptoms emerged at similar ages. However, their phenotypes differ with respect to attack frequency, duration, and severity. Indeed, while case II-4 has been maintained on carbamazepine, her identical sister, II-6, has not required medication.

Family B. Another family with EA1 from the same geographic region is included here because the same mutation was identified. From 6 months to 5 years of age the proband had brief focal epileptic seizures with impairment of awareness and apnea. At age 5, he developed episodic ataxia. In addition, he has secondary generalized seizures once per month; these were not controlled by carbamazepine or lamotrigine, but have responded to acetazolamide, which has also reduced the frequency of episodic ataxia attacks. His father, paternal uncle, and paternal grandmother all have episodic ataxia and interictal myokymia.

Family C. Affected members of this pedigree have classic EA1 attacks (figure 1C, summarized in the table). The proband is one of confirmed identical twins (IV-1 and IV-2, figure 1C). His attacks began at the age of 6 and last up to 10 minutes. They consist of dizziness, imbalance, weakness, blurred vision, slurred speech, myokymia, and upper limb incoordination. His brother has less frequent, briefer, and less disabling attacks, which are associated with headache, vertigo, and nausea. The proband's mother (III-2) has a progressive interictal cerebellar syndrome, manifest as ataxia and dysarthria (see the video).

Genetics. A novel T-to-C substitution was identified at position nt1241 of *KCNA1* (figure 1B) in families A and B. This was not present in 128 control chromosomes. It leads to the substitution of a conserved phenylalanine (figure 1E) by serine at amino acid 414 (F414S) in the S6 transmembrane segment of the channel. Another mutation affecting the same residue (F414C) was recently identified in a large EA1 kindred.[4] Haplotype analysis between members of families A and B demonstrated that all except 1 marker was shared by 6 individuals (figure e-1), covering a chromosomal region of approximately 5.77 Mb. These results suggest a shared ancestral haplotype on which the mutation has arisen and that the 2 families are related.

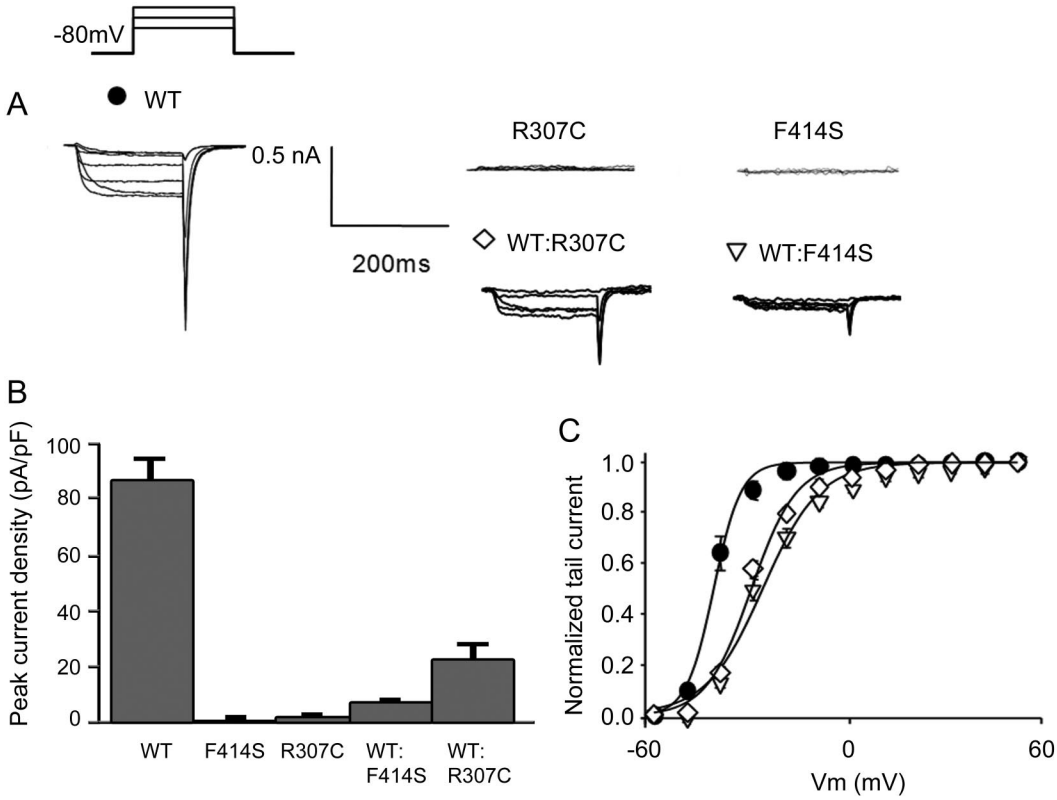
A novel T-to-C substitution was identified at position nt919 of *KCNA1* in family C (figure 1D). This was not present in 228 control chromosomes, and leads to the substitution of a highly conserved arginine (figure 1F), by cysteine at position 307 in the S4 segment of the channel (R307C).

Functional characterization. When expressed in HEK cells, both F414S and R307C were detected with anti-K_V1.1 antibodies with similar distribution to WT K_V1.1, suggesting the mutant subunits were present in the cells (figure e-2). However, no potassium currents could be detected in cells transfected with only mutant subunits (figure 2), suggesting that they are not functional. Coexpression of each mutant with WT K_V1.1 produced potassium currents with a markedly reduced maximum current density compared to WT Kv1.1 channels alone (WT, 86.8 ± 7.5 pA/pF, n = 7; WT:R307C, 22.1 ± 5.4 pA/pF, n = 7, $p = 0.003$; WT:F414S, 6.9 ± 0.9 pA/pF, n = 7, $p = 0.002$). Both mutants also conferred a significant positive shift in the $V_{1/2MAX}$ of the voltage dependence of activation of K_v1.1 when coexpressed with WT channels (WT, $V_{1/2MAX} = -41.9 \pm 0.5$; WT:R307C, $V_{1/2MAX} = -30.5 \pm 0.9$, $p < 0.0001$; WT:F414S, $V_{1/2MAX} = -27.0 \pm 1.4$, $p < 0.0001$). These findings are consistent with both mutants exerting a dominant-negative effect on WT subunits.

DISCUSSION Both mutations were identified in families with symptoms that fall within the spectrum previously reported for *KCNA1* mutations. Carbamazepine, where tested, was not effective in all individuals. The occurrence of epilepsy in the proband of family B adds to the list of mutations associated with EA1 and epilepsy.[1] The absence of epilepsy in family A, who are related to family B, is consistent with a role for other genes interacting with *KCNA1* to determine seizure risk. An unusual feature of the R307C mutation identified in family C is the progressive ataxia in the proband's mother, which has not previously been reported in EA1.

Several observations point to causal roles for the F414S and R307C mutations in the disease. First, they lead to radical amino acid substitutions in the ion-conducting pore and voltage sensor, respectively. Second, the mutations were not detected in control chromosomes. Third, both the nucleotides and the amino acids are highly conserved through evolution. Fourth, functional expression revealed loss of function with a dominant-negative effect when coexpressed with

Figure 2 Electrophysiologic characterization of R307C and F414S $K_V1.1$



(A) Voltage protocol and current traces obtained from representative HEK cells expressing WT, homomeric mutant (R307C and F414S), and WT coexpressed with mutants (WT:R307C and WT:F414S). (B) Maximum tail current density (±SEM) measured at +60 mV in low intracellular potassium solutions (see Methods). (C) Normalized voltage dependence of activation (±SEM) for cells expressing WT, WT:R307C, and WT:F414S. Tail currents were sampled 1 msec after return to −80 mV. For all cells, n = 7.

wild type channels, consistent with previous functional studies on EA1 mutations. And finally, the residue mutated in families A and B is also affected in another family with EA1, who carry the F414C mutation, which decreases potassium currents.[4]

Both sets of identical twins reported unexpectedly large differences in severity and frequency of EA attacks. In both sets of twins, one has sought treatment, whereas the less severely affected twin has not required medication. The self-reported variability was as large among the identical twins as among other affected members of each family.

Few channelopathies have been studied in twins. One pair of identical twins with severe myoclonic epilepsy of infancy sharing an *SCNA1* mutation was reported to be concordant for phenotype.[5] However, discordant monozygotic twin pairs have also been reported in familial long QT syndrome[6] and in episodic ataxia type 2.[7] Although the numbers remain small, these findings suggest that symptom heterogeneity among individuals harboring the same mutation reflects the interplay not only of modifier genes, but also of nongenetic factors.

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