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SHORT REPORT

Benign familial neonatal convulsions (BFNC) resulting from mutation of the *KCNQ2* voltage sensor

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Benign familial neonatal convulsions (BFNC) is a rare autosomal inherited epilepsy. We studied the KCNQ2 coding region in a large, four-generation, Italian family with BFNC. A missense mutation C686T predicting the change of one of the innermost arginine (R214W) of the key functional voltage sensor (S4 helix), has been found in all affected members. This substitution probably reduces the movement of the voltage sensor that precedes channel opening during voltage-dependent activation. Several mutations affecting the trans-membrane domain and the pore region of the K^+ channels belonging to the KQT-like family have been described in some human diseases associated with altered regulation of cellular excitability (ie BFNC, some LQT syndromes and DFNA2). R214W represents the first mutation involving the region of the voltage sensor. *European Journal of Human Genetics* (2000) **8**, 994–997.

Keywords: BFNC; potassium channel; idiopathic generalised epilepsy; mutation analysis; voltage sensor

Introduction

Benign familial neonatal convulsions (BFNC; OMIM 121200) is a rare autosomal inherited epilepsy characterised by unprovoked, generalised or multifocal, tonic–clonic convulsions. Symptoms occur within the first days of life and disappear spontaneously after weeks to months.

Mutations in the genes *KCNQ2* and *KCNQ3* have recently been shown to cause BFNC.[1–3] Together with *KCNQ1*, which is altered in long QT (LQT) syndromes[4,5] and with *KCNQ4*, which is mutated in some cases of hereditary hearing impairment, these genes constitute a new sub-family of voltage-gated potassium channel genes.[6] *KCNQ2* and *KCNQ3* assemble together to build a functional tetrameric channel in the central nervous system.[7,8] These channels are likely to contribute to the native M-current, a well known slowly activating and deactivating potassium conductance which represents one of the most important regulators of excitability in many neurons.[9] Each channel is made up of six conserved hydrophobic transmembrane spans, including the voltage sensor contained in the S4 helix, an ion channel pore formed partly by the loop between S5 and S6, and a long C-terminal domain.[10] Four BFNC-causing mutations lie in the conserved, carboxyl-terminal portion of the KCNQ2 protein.[11,12] In the remaining three BFNC cases identified mutations involve the pore region (two cases) or the transmembrane domain S6.[2] The functional expression of some of these mutants in *Xenopus oocytes* resulted in a 20% drop in repolarising potassium currents compared with the wild type. This reduction impairs repolarisation leading to hyperexcitability of the cell membrane in the regions of the brain where *KCNQ2* and *KCNQ3* are expressed.[8] Here we describe a large, four-generation Italian family with BFNC harbouring a novel missense mutation in the *KCNQ2* S4 helix.

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Materials and methods

Subjects

The BFNC kindred had eight affected individuals in four generations (Figure 1). The index patient (IV, 2) aged 12 months, had her first seizure on day 2 after birth, characterised by generalised hypertonia with apnea and cyanosis followed by clonic limb jerks. The seizure lasted about 30 s and recurred on days 4 and 5 with the same semiology; at the age of 3 months a new burst of generalised seizures occurred, but they were successfully controlled by phenobarbital. Her brother (IV, 1) manifested generalised seizures on day 2 and

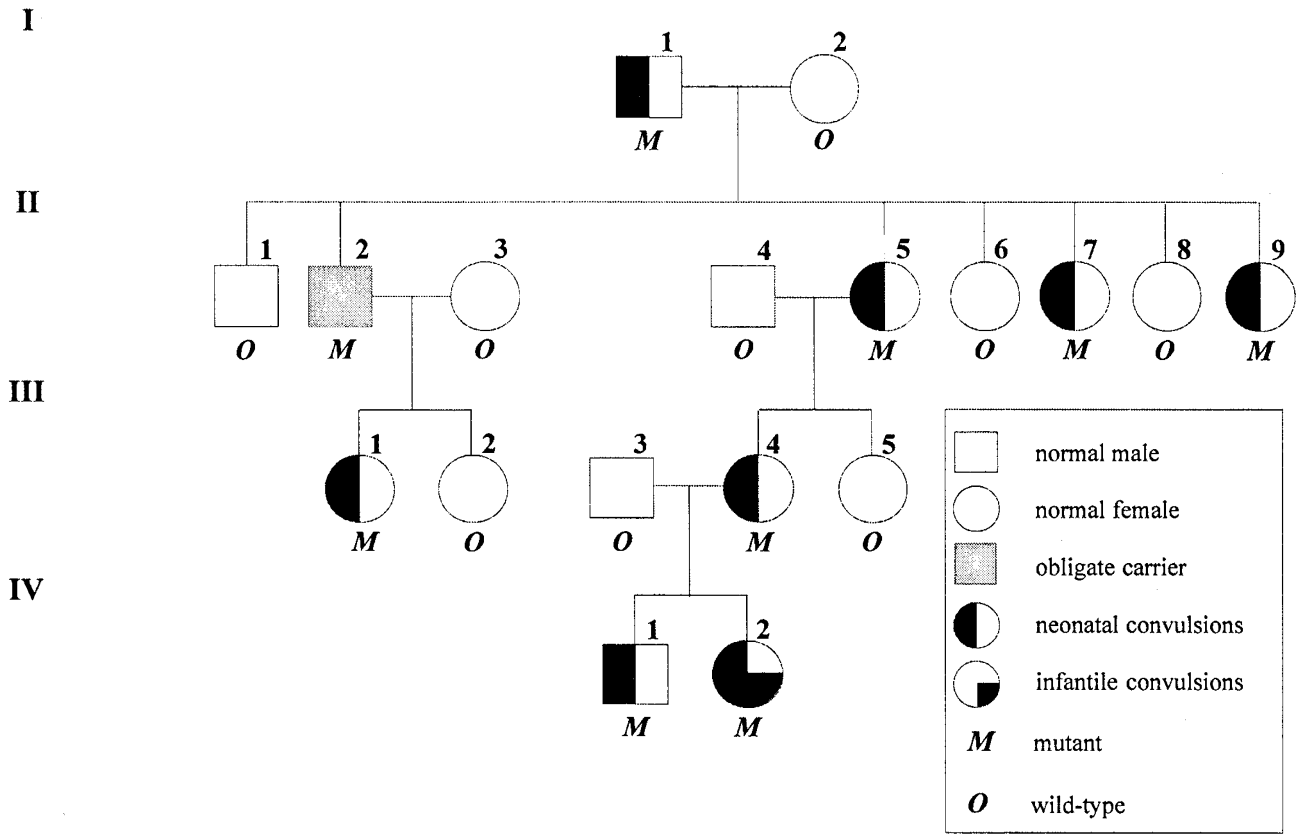

Figure 1 Pedigree of the examined Italian four-generation family with BFNC. Clinical and genetic data are given in the key (*M*: heterozygous for the C/T mutation in *KCNQ2* exon 3; *O*: homozygous for the wild type).

up to day 10; other sporadic seizures appeared at two months of age and no treatment was started. Her mother (III, 4) and the other affected relatives also manifested a few, mild seizures during the first 10 days of life. Collection and analysis of blood samples from all individuals included in this report was performed after appropriate informed consent.

Molecular studies

Segregation of the polymorphic marker D8S558 closely linked to *KCNQ3* and of the Thr752Asn polymorphism located on exon 16 of the *KCNQ2* gene[9] was studied in the members of the index family (II-4-5, III-3-4-5, IV-1-2).

The KCNQ2 coding region including the exon–intron boundaries of patients I-1, II-5-7-9, III-1-4, IV-1-2 and of the unaffected members of the kindred, I-2, II-2-4-6-8, III-2-3-5, were amplified using the primers and the conditions described by Biervert and Steinlein[11] and directly sequenced using an ABI PRISM 310 sequencer and the Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystem, Foster City, CA, USA).

Results

Preliminary study of the index family excluded linkage of BFNC to *KCNQ3*, whilst linkage to *KCNQ2* was not excluded. Therefore, the coding region of *KCNQ2* was analysed. A missense mutation in exon 3 C686T predicting the change of arginine of codon 214 to tryptophane (CGG→-TGG) was found in all affected members (Figure 2a). The same mutation has also been found in the unaffected subject II-2 (the father of patient III-4). He was considered an obligate carrier. The finding is not unexpected because the overall penetrance in BFNC family studies, as determined by the rate of obligate carriers with no clinical manifestations, is 0.8–0.85.[13]

The mutation abolishes an *Age*I restriction site. None of the 150 Italian independent controls screened showed this mutation. It lies in the highly conserved, key functional S4 domain (Figure 2b).

Discussion

Voltage-dependent ion channels are regulated by membrane potential. To achieve this regulation, charged voltage sensors in the channel protein respond to changes in the membrane potential and initiate conformational changes that lead to

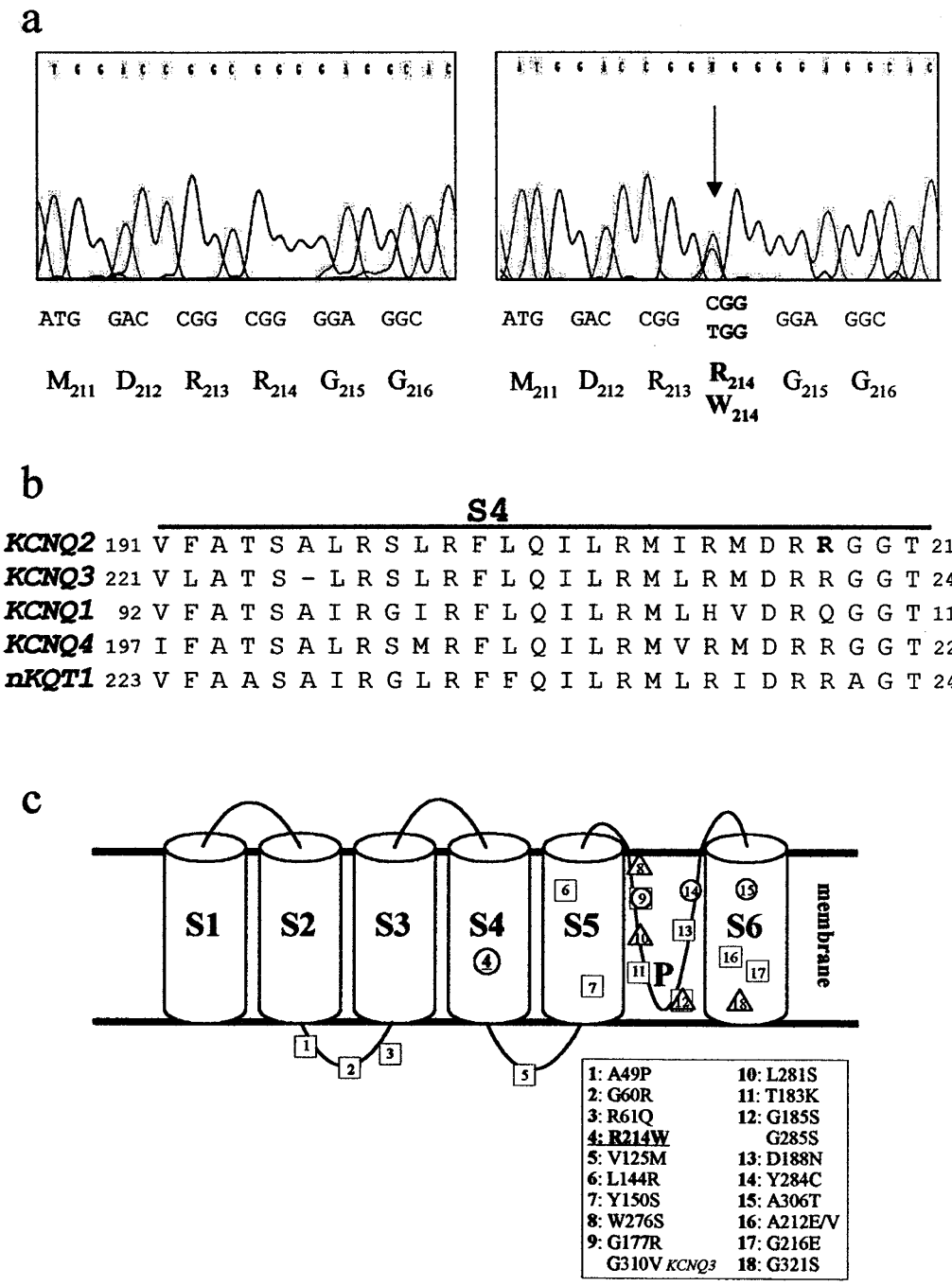

Figure 2 **a** *KCNQ2* exon 3 partial double-stranded sequencing and deduced amino acid sequences of individuals III-3 (left) and IV-2 (right). The arrow indicates the position of the C/T mutation leading to the arginine (R)/tryptophane (W) substitution at codon 214 **b** Alignment of the wild-type voltage sensor domains (S4) of the human potassium channel proteins KCNQ2, KCNQ3, KCNQ1 and KCNQ4 and of the *C. elegans* potassium channel protein nKQT1. Amino acid numbering is given according to Wang *et al*.[4] The arginine (R) mutation is shown in bold **c** Transmembrane structural organization of the K^+ channel proteins, showing the S1–S6 transmembrane domains and the P pore region. Amino acids substituted in this region and causing BFNC (*KCNQ2* or *KCNQ3*), LQT syndromes (*KCNQ1*) and DFNA2 (*KCNQ4*) are distinguished by circles, squares and triangles, respectively. C-terminal mutations are omitted. *KCNQ3* mutation causing BFNC is specified. No mutation lies in the S4 domain where the R214W substitution (in bold and underlined) has been found.

channel opening. In voltage gated K^+ channels, the fourth transmembrane segment (S4) contains conserved, regularly spaced, positively charged residues, making it a strong candidate for the voltage sensor. Analysis of the atomic scale movement of the S4 domain in the *Shaker* potassium channel measured by spectroscopy has recently clarified the mechanism by which this region functions as a voltage sensor. In the closed state of this model, charged arginines lie within a narrow internal crevice that opens into the intracellular solution. In response to voltage, the S4 undergoes a rotation and tilt, and the charged residues became accessible to the extracellular solution via a narrow external crevice.[14] The *R214W* mutation neutralises one of the innermost positive charge of the S4 segment. This substitution probably reduces the movement of the voltage sensor that precedes channel opening during voltage-dependent activation. Alternatively, it might have indirect effects on contiguous charged residues, affecting their movement or the electric field that they pass through, or both, in this way altering the voltage sensitivity of channel opening.

There are four members belonging to the KQT-like family; two of them (*KCNQ2* and *KCNQ3*) are expressed in the brain and their mutations produce BFNC;[7] *KCNQ1* is expressed in heart and the inner ear; mutations affecting this gene cause the Romano-Ward syndrome (the long-QT syndrome linked to chromosome 11) and the Jervell and Lange-Nielsen cardioauditory syndrome.[15–17] Mutations in *KCNQ4*, which is expressed in sensory outer hair cells of the cochlea, cause an autosomal dominant non-syndromic deafness (DFNA2).[18–20] Although several mutations affecting the trans-membrane domains and the pore region have been described in these syndromes none of them involve the S4 domain (Figure 2c). R214W represents the first description of a mutation involving the K^+ channel voltage sensor in a human disease associated with altered regulation of cellular excitability. Interestingly, the clinical features of BFNC patients with mutation on the voltage sensor overlap with the phenotypes of BFNC patients with mutations in the pore or in the other transmembrane regions, suggesting a similar effect on current decline.

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