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Characterization of two *de novo KCNT1* mutations in children with malignant migrating partial seizures in infancy

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ABSTRACT

The *KCNT1* gene encodes for subunits contributing to the Na^+-activated K^+ current (K_{Na}), expressed in many cell types. Mutations in *KCNT1* have been found in patients affected with a wide spectrum of early-onset epilepsies, including Malignant Migrating Partial Seizures in Infancy (MMPSI), a severe early-onset epileptic encephalopathy characterized by pharmacoresistant focal seizures migrating from one brain region or hemisphere to another and neurodevelopment arrest or regression, resulting in profound disability. In the present study we report identification by whole exome sequencing (WES) of two *de novo*, heterozygous *KCNT1* mutations (G288S and, not previously reported, M516V) in two unrelated MMPSI probands. Functional studies in a heterologous expression system revealed that channels formed by mutant *KCNT1* subunits carried larger currents when compared to wild-type *KCNT1* channels, both as homo- and heteromers with these last. Both mutations induced a marked leftward shift in homomeric channel activation gating. Interestingly, the *KCNT1* blockers quinidine (3–1000 μM) and bepridil (0.03–10 μM) inhibited both wild-type and mutant *KCNT1* currents in a concentration-dependent manner, with mutant channels showing higher sensitivity to blockade. This latter result suggests two genotype-tailored pharmacological strategies to specifically counteract the dysfunction of *KCNT1* activating mutations in MMPSI patients.

1. Introduction

Malignant Migrating Partial Seizures in Infancy (MMPSI; EIEE14, OMIM #614959) is a rare (~100 cases described) early-onset epileptic encephalopathy (EOEE) characterized by polymorphous focal seizures and cognitive, sensory and motor impairment, with arrest of psychomotor development in the first 6 months of life (Coppola et al., 1995; Coppola et al., 2006). Seizures are pharmacoresistant, and ictal electroencephalogram (EEG) discharges arise randomly from various areas of both brain hemispheres and seem to migrate from one region to another.

Growing evidence favours a genetic aetiology of MMPSI. First genetic studies in MMPSI patients failed to reveal genetic abnormalities in the coding regions of known epilepsy ion channel genes such as *KCNQ2*, *KCNQ3*, *SCNA2*, *SCN2A* or *CLCN2* (Coppola et al., 2006). More recently, *de novo* mutations in the *KCNT1* gene (also known as Slo2.2 or Slack) were described as disease-causing in about 50% of all MMPSI cases (Barcia et al., 2012; De Filippo et al., 2014; Ishii et al., 2013). *KCNT1* encodes for Na^+-activated K^+ channel subunits having a topological arrangement similar to that of classical voltage-gated K_v channel subunits, with six transmembrane segments (S_1–S_6), and a pore-lining loop between S_5 and S_6; *KCNT1* subunits assemble as homo- or hetero-tetramers with highly-homologous KCNT2 (Slo2.1 or Slick) subunits to form functional, voltage-dependent channels (Kaczmarek, 2013). Additional genetic causes for MMPSI include missense mutations and one deletion in the sodium channel gene *SCN1A* (Carranza Rojo et al., 2011; Freilich et al., 2011), a duplication within the 16p11.2 chromosomal region (Bedoyan et al., 2010), a homozygous deletion in *PLCB1* (Poduri et al., 2012), and compound heterozygosity of the gene encoding the *TBC1* domain protein

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TBC1D24 (Milh et al., 2013). Interestingly, *KCNT1* has been implicated also in autosomal-dominant and sporadic cases of nocturnal frontal lobe epilepsy (Heron et al., 2012) and in few cases of Ohtahara syndrome (Martin et al., 2014), multifocal epilepsy, West (Moller et al., 2015; Ohba et al., 2015) and Brugada (Juang et al., 2014) syndromes.

In this work, we analysed by whole exome sequencing (WES) the genetic basis for MMPSI in two probands, and identified two *de novo* heterozygous mutations in *KCNT1* (one new and one previously reported). Furthermore, we investigated the biochemical and electrophysiological characteristics of channels carrying these mutations, as well as their pharmacological sensitivity to the *KCNT1* blockers bepridil and quinidine.

2. Materials and methods

2.1. Case description

2.1.1. Proband 1

D.R., male, with a family history negative for seizures, developmental delay, psychiatric disorders, and mental retardation. He is the first of two children of healthy parents, conceived by intrauterine sperm injection. He exhibited seizure activity starting on the second day of life characterized by apnoea and cyanosis, which lasted 30 s to 1 min. The day after, focal motor seizures started with clonic movements involving the right hand and the left foot. His first electroencephalogram (EEG) showed frequent independent sharp waves involving the centro-temporal regions. Treatment with phenobarbital, midazolam, phenytoin, vitamin B6, folinic acid, and pyridoxalphosphate did not improve seizure recurrence. At 3 months of age, the child showed an inconstant gaze fixation, absent visual tracking, axial hypotonia, bilateral shifting esotropia, no major craniofacial dysmorphisms. Ictal EEGs were recorded with multifocal paroxysmal activity as uniform finding. They demonstrated either shifting areas of ictal onset (migrating spikes) between hemispheres within the same EEG recording or overlapping seizures with different areas of ictal onset in differing hemispheres (Fig.1A, left panel). At 36 months (last follow-up), he developed severe axial hypotonia without gaze fixation, and poor spontaneous motor activity, with clonus at the lower extremities, minimal head and trunk control and decline in head growth with resultant microcephaly. He did not exhibit

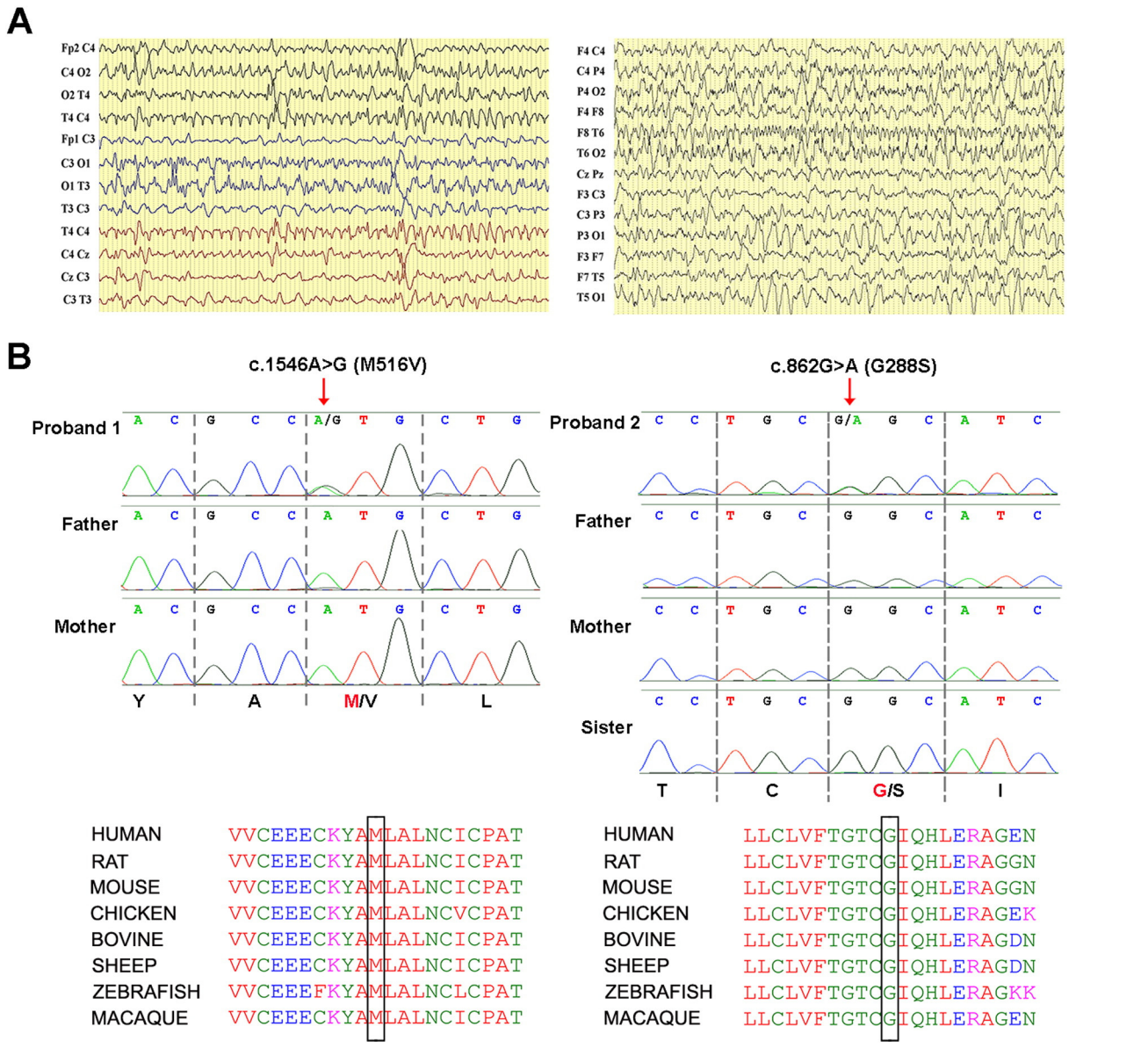

Fig. 1. EEG and genetic analysis in two MMPSI patients. (A) EEG recordings of case 1 (left panel) and 2 (right panel), respectively; (B) results of Sanger sequencing (left for Proband 1 and right for Proband 2) of the two *de novo* heterozygous mutations identified (upper panels) and alignment of *KCNT1* orthologous peptide sequences (lower panels) with boxes highlighting the amino acids influenced by these nucleotide mutations.