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Two Novel SCN1A Missense Mutations in Generalized Epilepsy with Febrile Seizures Plus

*Grazia Annesi, *†Antonio Gambardella, *Sara Carrideo, ‡Gemma Incorpora, *†Angelo Labate, *Angela Aurora Pasqua, *Donatella Civitelli, ‡Agata Polizzi, *Ferdinanda Annesi, *Patrizia Spadafora, ‡Patrizia Tarantino, *Innocenza C. Cirò Candiano, *Nelide Romeo, ‡Elvira Valeria De Marco, †Patrizia Ventura, §Emilio LePiane, *†Mario Zappia, §Umberto Aguglia, ‡Lorenzo Pavone, and *†Aldo Quattrone

**Institute of Neurological Sciences, National Research Council, Piano Lago di Mangone, Cosenza; †Institute of Neurology, University Magna Graecia Catanzaro, ‡Institute of Pediatrics, University of Catania; and §Regional Epilepsy Centre, Hospital of Reggio Calabria, Italy*

We screened nine Italian families with generalized epilepsy with febrile seizures plus (GEFS$^+$) for mutations in SCN1A, SCN1B, and GABRG2 genes (1–3). Probands were ascertained from the clinical practice in three epilepsy centers in southern Italy. Detailed family pedigrees were constructed, including maternal and paternal lines extending as far back as possible. In the nine families, we investigated 110 members of whom 37 individuals were determined to be affected. Most patients had febrile seizures (FSs) or FS plus (FS$^{+)}$ alone. A consistent number of patients also had only afebrile generalized seizures. Few patients had myoclonic seizures. One patient had a clinical picture of severe myoclonic epilepsy at infancy (SMEI).

Genomic DNA was extracted by standard methods. The 26 exons of SCN1A, five exons of SCN1B, and nine exons of GABRG2 were individually amplified by using primers based on an intronic sequence defined by comparison of cDNA (GenBank accession no. 043484). Polymerase chain reaction (PCR) products were analyzed by single-strand conformation polymorphism and sequence.

In two of the nine GEFS$^+$ families (families 5 and 8), we identified two novel mutations of the *SCN1A* gene that cosegregated with the disorder. Neither mutation was observed in any of the 100 control individuals (200 chromosomes) matched for ethnicity. Both these mutations changed highly conserved amino acids.

The first mutation is a heterozygous point mutation, A2336G, which was detected in the three affected members of family 5, whereas it was not detected in three unaffected relatives including the mother. This mutation in exon 13 of *SCN1A* results in a Tyr779Cys amino acid substitution that lies within the S1 segment of domain II. The two probands of family 5 had had FSs until age 5 years. These were followed by afebrile generalized seizures, which disappeared with phenobarbital (PB) or valproate (VPA), respectively. Their father had only four FSs around age 3 years.

The second mutation is a heterozygous point mutation, T5522C, which was detected in both affected members of family 8, but not in their mother, who was unaffected. This second mutation led to a Met1841Thr amino acid substitution within the intracellular C-terminal region. Notably, one of the affected members of this family is the one with SMEI. His sister, who carried the same mutation, had a milder phenotype of GEFS$^+$, whereas the father, who had died at the time of the study, had FS$^+$.

We have provided evidence of two novel disease mutations of the *SCN1A* gene in patients with GEFS$^+$, whereas no mutation was found within the SCN1B or GABGR2 genes. In this way, our results confirm that missense mutations in the *SCN1A* gene are the major causes of GEFS$^+$. The present study also extends the distribution of SCN1A mutations to populations of Mediterranean origin.

Of particular relevance is the Met1841Thr mutation, which is similar to those described by Ohmori et al. (4), in that it lies within the C-terminal region. Most interestingly, also their patients who carried mutations within the C-terminal region displayed a severe phenotype of SMEI (5). On this basis, it reasonable to speculate that this region itself might play a basic role in spite of its distal

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Address correspondence and reprint requests to Prof. A. Quattrone at Cattedra U.O. di Neurologia, Facoltà di Medicina e Chirurgia, Università Magna Graecia, Via T. Campanella 88100 Catanzaro, Italy. E-mail: a.quattrone@isn.cnr.it

region on the molecule. Moreover, these findings greatly strengthen the view that SMEI represents the very severe end of the spectrum within the GEFS$^+$ phenotype (5), rather than being considered a distinct entity, outside the GEFS$^+$ spectrum, that is related to de novo frameshift and nonsense mutations (6).

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