Epilepsy Research (2012) **99**, 21—27

journal homepage: www.elsevier.com/locate/epilepsyres

Early clinical features in Dravet syndrome patients with and without *SCN1A* mutations

Cristina Petrelli[a,*], **Claudia Passamonti**[b], **Elisabetta Cesaroni**[b], **Davide Mei**[c], **Renzo Guerrini**[c], **Nelia Zamponi**[b], **Leandro Provinciali**[a]

[a] *Neurology Clinic, Polytechnic University of Marche, Ancona, Italy*
[b] *Child Neurology Unit Department, Ospedali Riuniti, Ancona, Italy*
[c] *Pediatric Neurology Unit and Laboratories, Children's Hospital A. Meyer — University of Florence,Italy*

Received 15 December 2010; received in revised form 3 October 2011; accepted 9 October 2011
Available online 8 November 2011

KEYWORDS
Dravet syndrome;
SCN1A mutations;
Seizures

Summary
Background: SCN1A is the most clinically relevant epilepsy gene, most mutations causing Dravet syndrome (also known as severe myoclonic epilepsy of infancy or SMEI). We evaluated clinical differences, if any, between young patients with and without a *SCN1A* mutations and a definite clinical diagnosis of Dravet syndrome.
Methods: Twenty-five patients with a diagnosis of Dravet Syndrome (7 males, 18 females; mean age at inclusion: 10.3; median: 9 ± 7; range: 18 months—30 years) were retrospectively studied. A clinical and genetic study focusing on *SCN1A* was performed, using DHPLC, gene sequencing and MLPA to detect genomic deletions/duplications. A formal cognitive and behavioral assessment was available for all patients.
Results: Analysis revealed *SCN1A* mutations comprising missense, truncating mutations and genomic deletions/duplications in eighteen patients and no mutation in seven. The phenotype of mutation positive patients was characterized by a higher number of seizures/month in the first year of life, an earlier seizure onset and a higher frequency of episodes of status epilepticus. The cognitive and behavioral profile was slightly worst in mutation positive patients.
Conclusions: These findings confirm that *SCN1A* gene mutations are strongly associated to a more severe phenotype in patients with Dravet syndrome.

Introduction

Dravet syndrome is a rare and distinct epileptic encephalopathy (Commission on Classification and terminology of the International League Against Epilepsy, 1989), which begins with infantile onset of febrile hemiclonic status epilepticus and evolves to a pattern of multiple seizure types including focal, myoclonic, absence,

* Corresponding author at: Neurology Clinic, Polytechnic University of Marche Ospedali Riuniti, Via Conca, Ancona, Italy. Tel.: +39 0715962484; fax: +39 0715962502.
E-mail address: cristinapetrelli84@alice.it (C. Petrelli).

0920-1211/$ — see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.eplepsyres.2011.10.010

and atonic seizures, associated with marked slowing or stagnation of psychomotor development, often accompanied by behavioral disturbances (Wolf et al., 2006; Riva et al., 2009; Ragona et al., 2010; Chieffo et al., 2010). Neuroimaging studies and metabolic investigations are not contributory, suggesting no structural or interposed metabolic abnormality. The *SCN1A* gene, which encodes the α1 subunit of the neuronal sodium channel, is the most relevant epilepsy gene with the largest number of epilepsy-related mutations (Escayg et al., 2001; Wallace et al., 2001; Marini et al., 2007; Harkin et al., 2007) including genetic (generalized) epilepsy with febrile seizures plus (GEFS+) and Dravet syndrome. In the mouse genetic model of Dravet syndrome a selective failure of excitability of γ-aminobutyric acid (GABA)ergic neurons, resulting from *SCN1A* mutations, has been shown (Yu et al., 2006). The *SCN1A* gene has also been involved in other phenotypes such as familial hemiplegic migraine and other non-epileptic disorders (Gambardella and Marini, 2009). The *SCN1A* frequency of missense and truncating mutations is approximately equal, both accounting for 40% of all mutation-positive patients (Scheffer, 2011). Interestingly, the large majority of the *SCN1A* mutations causing Dravet syndrome results in loss of function, whereas the GEFS+ phenotype is usually observed in patients harboring missense substitutions. *SCN1A* mutations can be identified in about 70—80% of patients with Dravet syndrome (Claes et al., 2001, 2003; Sun et al., 2008; Harkin et al., 2007; Mulley et al., 2005; Kanai et al., 2004; Fujiwara et al., 2003; Sugawara et al., 2002; Ohmori et al., 2002). Furthermore, recent studies have demonstrated that about 12% of mutation-negative Dravet syndrome patients have *SCN1A* genomic rearrangements detectable by multiplex ligation-dependent probe amplification (MLPA) and/or by Array-CGH (Marini et al., 2010). Some authors (Depienne et al., 2009; Marini et al., 2010) identified alterations in the *PCDH19* gene (encoding protocadherin 19) in patients who were negative for mutation or rearrangements of the *SCN1A* gene. Those patients had very similar clinical features to *SCN1A*-positive patients, indicating that the two clinical spectra largely overlap.

Remarkable phenotypic variability exists between patients with SCN1A mutations, even within the same family (Suls et al., 2010; Guerrini et al., 2010). The occurrence of possible between *SCN1A* mutated and non mutated patients, as well as between Dravet patients with *SCN1A* mutations and Dravet patients with *PCDH19* alterations, has been the object of recent investigations (Depienne et al., 2009; Marini et al., 2007, 2010). A study of Marini et al. (2007) revealed that Dravet patients with *SCN1A* mutations had an earlier age of onset of febrile seizures. We evaluated whether clinical differences exists between Dravet syndrome patients with *SCN1A* alterations and those without. To this purpose, medical reports from twenty-five patients with Dravet syndrome have been retrospectively reviewed.

Methods

Subjects

Medical reports of twenty-five patients with Dravet syndrome (eighteen females and seven males), for whom clinical details and DNA were available, were reviewed from the Child Neurology Department of ''G. Salesi Hospital''. The research received prior approval by the local ethical committee and each parent/guardian signed an informed consent form. Mean age at the time of the study was 10.3 years (median: 9 ± 7, range: 18 months—30 years). All patients fulfilled the following diagnostic criteria established by the International League Against Epilepsy (Commission on Classification and terminology of the International League Against Epilepsy, 1989): normal development before seizure onset; occurrence of either generalized, unilateral, or partial seizures during the first year of life; seizures that were frequently provoked by fever; presence of myoclonic seizures with spike and wave-complex or segmental myoclonus, diffuse spike-waves or focal spikes on EEG during the clinical course; intractable epilepsy; gradual evidence of psychomotor delay after two years of age. For each patient the following clinical features were retrospectively studied: mean age at onset of first febrile or afebrile seizure, family history in first degree relatives, number of seizures before one year of age, seizure types (generalized tonic-clonic seizures, hemiconvulsion and focal seizure, status epileptic), epileptic discharges on EEG. Patients' demographical and clinical details are summarized in Table 1. Seizure type and number were established by reviewing seizure diaries given to patients and medical reports.

Genetic analysis

Molecular analysis was performed on genomic DNA extracted from blood using standard procedures. All 26 exons of *SCN1A* were amplified by polymerase chain reaction (PCR) and analyzed by DHPLC as previously described (Marini et al., 2007). Exons showing abnormal DHPLC chromatographic profiles were analyzed by direct sequencing. Patients negative to DHPLC screening were first sequenced to ensure that point mutations were not missed, and then screened by MLPA. When an alteration was found, genetic test of parents was requested in order to search for inherited mutations. The novel *SCN1A* missense substitutions were not found in a cohort of 95 control DNA (195 alleles). *SCN1A*-negative patients were screened by MLPA, using the P137-A2 *SCN1A* kit of MRC Holland (Wang et al., 2008; Marini et al., 2009), in order to detect genomic deletions/duplications. In *SCN1A*-negative female patients the six exons covering the coding regions of *PCDH19* and their intron—exon boundaries were PCR amplified and sequenced as previously described (Marini et al., 2010). Patients were divided in two groups on the basis of presence/absence of *SCN1A* mutations: the case group presented *SCN1A*-mutation (*SCN1A*+), while the control group had no *SCN1A*-mutation (*SCN1A*—) (Table 2).

Cognitive and behavioral evaluation

A formal cognitive and behavioral examination, including developmental quotient (DQ) and adaptive behavior was available for all patients. Assessment was performed at two sessions, i.e. at the time of diagnosis (T1), which was performed between 18 and 30 months of life (data available