

# Clinical and genetic factors predicting Dravet syndrome in infants with *SCN1A* mutations

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Supplemental data  
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## ABSTRACT

**Objective:** To explore the prognostic value of initial clinical and mutational findings in infants with *SCN1A* mutations.

**Methods:** Combining sex, age/fever at first seizure, family history of epilepsy, EEG, and mutation type, we analyzed the accuracy of significant associations in predicting Dravet syndrome vs milder outcomes in 182 mutation carriers ascertained after seizure onset. To assess the diagnostic accuracy of all parameters, we calculated sensitivity, specificity, receiver operating characteristic (ROC) curves, diagnostic odds ratios, and positive and negative predictive values and the accuracy of combined information. We also included in the study demographic and mutational data of the healthy relatives of mutation carrier patients.

**Results:** Ninety-seven individuals (48.5%) had Dravet syndrome, 49 (23.8%) had generalized/genetic epilepsy with febrile seizures plus, 30 (14.8%) had febrile seizures, 6 (3.5%) had focal epilepsy, and 18 (8.9%) were healthy relatives. The association study indicated that age at first seizure and frameshift mutations were associated with Dravet syndrome. The risk of Dravet syndrome was 85% in the 0- to 6-month group, 51% in the 6- to 12-month range, and 0% after the 12th month. ROC analysis identified onset within the sixth month as the diagnostic cutoff for progression to Dravet syndrome (sensitivity = 83.3%, specificity = 76.6%).

**Conclusions:** In individuals with *SCN1A* mutations, age at seizure onset appears to predict outcome better than mutation type. Because outcome is not predetermined by genetic factors only, early recognition and treatment that mitigates prolonged/repeated seizures in the first year of life might also limit the progression to epileptic encephalopathy. *Neurology*® 2017;88:1-8

## GLOSSARY

**CI** = confidence interval; **DOR** = diagnostic odds ratio; **FS** = febrile seizures; **GEFS+** = generalized/genetic epilepsy with febrile seizures plus; **OR** = odds ratio; **RESIDRAS** = Italian National Registry for Dravet Syndrome and *SCN1A*-Related Conditions; **ROC** = receiver operating characteristic.

The voltage-gated sodium channel *SCN1A* gene is, among all the known epilepsy genes, the most clinically relevant, with the largest number of epilepsy-related mutations characterized.<sup>1</sup> Epilepsy phenotypes associated with *SCN1A* mutations include familial febrile seizures (FS), GEFS+ (generalized or, as more recently proposed, genetic epilepsy with FS plus), and Dravet syndrome, the last representing by far the most severe phenotype (Online Mendelian Inheritance in Man No. 182389). Observations that in Dravet syndrome, but not in the other *SCN1A*-associated phenotypes, early normal development is followed by severe cognitive impairment and additional neurologic features<sup>2</sup> suggest that early epileptic activity contributes to impaired brain function, resulting in an epileptic encephalopathy.<sup>3</sup> This causal link has not yet been demonstrated, however.

At present, most pediatric epilepsy specialists suggest mutation screening of the *SCN1A* gene soon after an infant experiences prolonged/repeated fever-associated seizures because they

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suspect that these seizures may represent the initial manifestations of Dravet syndrome.<sup>4</sup> However, early detection of an *SCN1A* mutation leaves important practical questions unanswered concerning management, prognosis, and counseling in that genotype-phenotype correlations are loose and after a common early clinical presentation the phenotypic spectrum may vary considerably in severity.

We studied 200 individuals with *SCN1A* mutations and explored the prognostic value of mutational data and early clinical findings that may help clinicians to set up management choices adapted to individuals at higher risk of progressing to Dravet syndrome, without delaying them until the epileptic encephalopathy has become obvious.

**METHODS** We retrospectively analyzed 200 consecutive individuals with mutations in the *SCN1A* gene. Patients were enrolled from 6 Italian tertiary clinical centers with pediatric epilepsy expertise as part of a pilot study we conducted to preliminarily test the accuracy and feasibility of the data entry online form to adopt for the Italian National Registry for Dravet Syndrome and *SCN1A*-Related Conditions (RESIDRAS; <http://www.residras.com>). We included all patients and healthy carriers with *SCN1A* mutations who were consecutively observed in the participating centers and were >24 months of age when last seen because this is the age at which Dravet syndrome can usually be diagnosed.<sup>5</sup> Clinical data were collected through a standardized form including demographic data, family and personal history, age at duration of first seizure, presence of fever, and neurologic and neuropsychological outcome (figure e-1 at [Neurology.org](http://www.neurology.org)). The epilepsy phenotype was classified according to the International League Against Epilepsy criteria.<sup>6</sup> However, considering that such criteria predate the identification of *SCN1A* as the causative gene for Dravet syndrome and that some authors have subsequently described mutated patients with Dravet syndrome and seizure onset beyond the first year of life,<sup>7-9</sup> we did not firmly predefine age at first seizure as a cutoff time for diagnosis but relied on the clinical severity. We maintained the distinction between GEFS+ and focal epilepsy because some patients manifested focal seizures only and represented, in our opinion, a distinctive subgroup. We identified the following clinical subgroups: (1) Dravet syndrome (including the so-called borderline forms), (2) GEFS+, (3) focal epilepsy, (4) FS, and (5) *SCN1A* mutation carriers who had never experienced seizures. Definitions of the different clinical subgroups are provided in appendix e-1. To explore the value of early available parameters as prognostic indicators, we focused on patients' characteristics at seizure onset. Because Dravet syndrome is the most severe *SCN1A*-associated phenotype, with a constantly unfavorable outlook and requiring the most complex management choices, we analyzed data with patients divided into 2 groups: Dravet (also including borderline forms) and non-Dravet (including FS, GEFS+, focal epilepsy). Patients in the non-Dravet group were all free of seizures at last follow-up and exhibited normal or slightly delayed cognitive development.

**Standard protocol approvals, registrations, and patient consents.** Written informed consent was obtained for each individual. The study was approved by the Pediatric Ethics Committee of the Tuscany Region, in the context of both the EU Project DESIRE-602531 and the RESIDRAS initiative.

**Genetic analysis.** Methods for genetic analysis are provided in appendix e-1.

**Statistical analysis.** We used the STATA 13 (T Stat s.r.l.) for statistical analysis and descriptive statistics to describe the participants' main variables. For each Dravet/non-Dravet outcome, we performed the Pearson  $\chi^2$  test of independence on tables of frequency for each categorical variable of interest (mutation type, sex, type of first seizure, age at seizure onset, presence/absence of fever at onset, first seizure duration, familial epilepsy, EEG discharges) and Student *t* test for unequal variance for each continuous variable. Age at seizure onset was analyzed both as a continuous and as a categorical variable with individuals grouped into 3 classes: 0 to 6, 6 to 12, or >12 months at onset (table 1).

The studied population also included probands' relatives, as ascertained by familial segregation of clinical manifestations and mutations. Because of the hierarchical structure of data, we fit a standard logistic regression model that was amended to have random effects for each family. More formally put, we used a sandwich estimator for the variance-covariance matrix.<sup>10</sup> We performed a sensitivity analysis for a multilevel logistic model using a stepwise method.

To assess and compare the diagnostic accuracy of all the parameters we chose for discriminating at first seizure those patients who would develop Dravet syndrome from those who would face a less severe outcome, we calculated sensitivity, specificity, receiver operating characteristic (ROC) curves, diagnostic odds ratios (DORs), and positive and negative predictive values. Finally, we combined information from the different parameters. When appropriate, confidence intervals (CIs) were calculated with the use of exact likelihood.<sup>11</sup> Level of significance was set at 5% 2 sided.

**RESULTS Patients.** We analyzed 200 consecutive individuals carrying *SCN1A* mutations (109 male, 91 female carriers) with an average age of 18.58 years at last follow-up (SD 18.08, range 2.06–81.06 years). Seizures were the presenting symptom in 182 patients belonging to 139 unrelated families. In 33 instances, an *SCN1A* mutation was present in more than one family member. Of the 200 mutation carriers, 97 (48.5%) had Dravet syndrome, including borderline forms. In the non-Dravet group, distribution of phenotypes included 49 patients (23.8%) with GEFS+, 30 (14.8%) with FS, and 6 (3.5%) with focal seizures and 18 (8.9%) healthy individuals >18 years old who had undergone genetic testing during family studies for mutation confirmation and inheritance determination. The overall penetrance was 77%. Pedigrees with incomplete penetrance are shown in figure 1.

**Association of clinical and mutational data with Dravet syndrome.** We used the Pearson  $\chi^2$  independence test to analyze the distribution of categorical clinical

**Table 1** Characteristics of the study population

	Total, n	%	Non-Dravet (n = 103), n	Dravet (n = 97), n	Missing data, n	p Value ( $\chi^2$ )
<b>Sex</b>					0	0.596
Male	109	54.5	58	51		
Female	91	45.5	45	46		
<b>Mutation type</b>					0	<0.001
Missense	129	64.5	78	51		
Splicing	19	9.5	13	6		
Nonsense	19	9.5	4	15		
Fs/rearrangements	33	16.5	9	24		
<b>Family history of seizures</b>					1	<0.001
Yes	111	55.8	78	33		
No	88	44.2	25	63		
<b>Age at seizure onset, mo</b>					47	<0.001
0-6	81	52.9	12	69		
6-12	37	24.2	18	19		
>12	35	22.9	35	0		
Mean (SD)	10.80 (14.48)		18.4 (19.51)	5.19 (2.233)		<0.001
<b>Fever at first seizure</b>					33	0.019
Yes	134	80.2	67	67		
No	33	19.8	9	24		
<b>Seizure types</b>					32	0.003
Clonic-tonic/clonic	142	84.5	73	69		
Focal	20	11.9	2	18		
Myoclonic	5	3.0	1	4		
Absence	1	0.6	0	1		
<b>Seizure duration, min</b>					74	0.001
0-5	65	51.6	37	28		
5-30	37	29.4	9	28		
>30	24	19.0	5	19		
<b>EEG abnormalities</b>					112	0.207
Yes	38	43.2	11	27		
No	50	56.8	21	29		

Abbreviation: Fs = frameshift mutations.

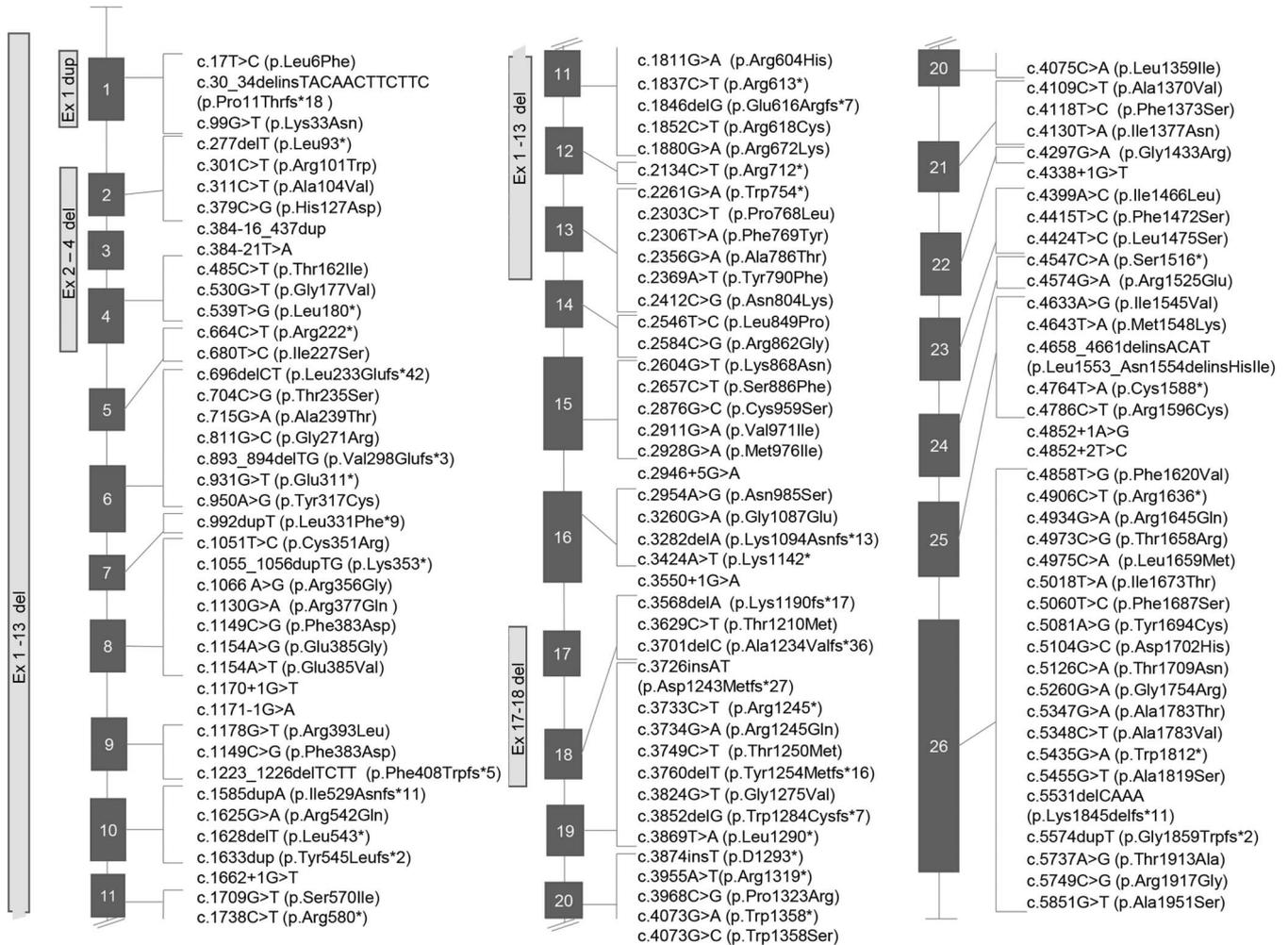
Seizure duration was grouped into 3 classes: 0 to 5, 5 to 30, and >30 minutes. Age at seizure onset was grouped into 3 classes: 0 to 6, 6 to 12, and >12 months. Seizure type, fever, seizure duration, age at seizure onset, and EEG discharges were calculated on 182 patients, excluding 18 individuals who did not experience seizures.

variables in the Dravet and non-Dravet groups and the Student *t* test for unequal variance for each continuous variable of interest. We analyzed mutation type and 7 parameters that are usually available at clinical presentation, including sex, family history of epilepsy, age at seizure onset, type and duration of first seizure, fever at first seizure, and epileptiform discharges (sharp waves, spikes, spikes and waves) at first EEG. Six of the analyzed parameters, with the exception of sex and EEG abnormalities, had a significantly different

distribution in the 2 groups (table 1). The Student *t* test showed a significantly different age at seizure onset in patients with Dravet syndrome vs individuals without Dravet ( $5.19 \pm 2.23$  vs  $18.4 \pm 19.51$  months;  $p < 0.001$ ).

We fit a multilevel logistic model with the significant parameters and adjusted for age at last follow-up (table 2). The multivariate model showed that age at seizure onset (OR = 0.647, 95% CI 0.541–0.774,  $p < 0.001$ ) and frameshift vs missense mutations (OR = 8.567, 95% CI 1.828–40.140,  $p = 0.006$ )

**Figure 1** Schematic representation of *SCN1A* mutations identified in this study



Nomenclature of mutations followed recommendations of the Human Genome Variation Society.

were significantly associated with Dravet syndrome. The sensitivity analysis confirmed the results obtained in the multilevel logistic models (table 2).

**Association of age at seizure onset with Dravet syndrome.**

Age at seizure onset, analyzed as both a continuous and a categorical variable, was distributed differently in patients with Dravet and those without Dravet syndrome. Mean age at first seizure was 5.19 months for patients with Dravet and 18.4 months for those without Dravet syndrome (table 1). Multilevel logistic regression analysis showed an OR of 0.647, meaning that an older age at seizure onset represents a protective factor against the risk of developing Dravet syndrome (table 2). None of the patients who experienced their first seizure after 12 months of age developed Dravet syndrome.

**Association of mutations with Dravet syndrome.** We found 123 different mutations, 65 of which were novel, in 200 individuals (table e-1). Fifty-four mutations (42.2% of 128 individuals for whom

heritability was tested) were de novo and 74 (57.8%) were inherited, 6 of which were from a parent with somatic mosaicism. Mutations were distributed throughout the gene (figure 2), and except for familial cases, only 11 were observed in more than one individual.

We evaluated the effect of missense mutations using different bioinformatic tools (appendix e-1) based on functional prediction scores and conservation scores. We deemed as damaging all substitutions predicted to be deleterious by at least 2 conservation and 2 prediction algorithms and all the mutations resulting in loss of function (nonsense, frameshift, splicing, and genomic rearrangements). To estimate the allelic frequency of the mutations, we interrogated public frequency databases (appendix e-1). We identified 71 different missense mutations, of which 62 were not present in databases, 6 were reported in the Exome Aggregation Consortium to have a frequency of <0.0001%, and 3 (p.Thr1250Met, p.Arg542Gln, and p.Arg604His) with a frequency

**Table 2** Multilevel logistic regression with robust estimator for standard error

	OR	95% CI	p Value
<b>Mutation type</b>			
Missense mutations outside the pore region	1.000		
Missense mutations in the pore region	0.580	0.080-4.215	0.590
Splicing	0.300	0.051-1.759	0.182
All truncating	1.542	0.326-7.294	0.585
Nonsense	0.686	0.108-4.376	0.691
Fs/rearrangements	8.567 <sup>a</sup>	1.828-40.140	0.006
<b>Family history</b>			
No	1.000		
Yes	0.492	0.144-1.689	0.260
Age at last follow-up	0.997	0.990-1.004	0.437
Age at seizure onset	0.647 <sup>a</sup>	0.541-0.774	0.000
<b>Fever at first seizure</b>			
No	1.000		
Yes	3.016	0.703-12.933	0.137
<b>Seizure types</b>			
Clonic-tonic/clonic	1.000		
Focal	7.174	0.698-73.747	0.097
Myoclonic	4.465	0.199-99.936	0.345
Absence	—		
<b>Seizure duration, min</b>			
0-5	1.000		
5-30	2.052	0.490-8.589	0.325
>30	3.330	0.692-16.017	0.133

Abbreviations: CI = confidence interval; Fs = frameshift mutations; OR = odds ratio. Age at seizure onset was analyzed as a continuous variable. Seizure duration was divided into 3 classes: 0 to 5, 5 to 30, and >30 minutes.

<sup>a</sup>Significant values according to CIs and p values.

of  $\geq 0.0001\%$  (table e-1). We grouped mutations into 5 classes: missense, missense falling into the pore-forming region, splicing, nonsense, and frameshift (including rearrangements of entire exons). Multilevel logistic regression revealed that frameshift mutations and rearrangements confer a significantly higher risk of developing Dravet syndrome (OR = 8.567, 95% CI 1.828–40.140,  $p = 0.006$ , table 2) with respect to missense mutations.

**Diagnostic test.** To compare the diagnostic accuracy of all parameters, we estimated sensitivity, specificity, ROC area, DOR, and positive and negative predictive values (table 3). We performed this analysis on the 119 patients for whom information on all 8 phenotypic and genotypic items was available (table 1). Patients carrying variants of uncertain significance (p.Thr1250Met, p.Arg542Gln, and p.Arg604His) were excluded from this analysis. ROC analysis showed age at seizure onset to accurately recognize

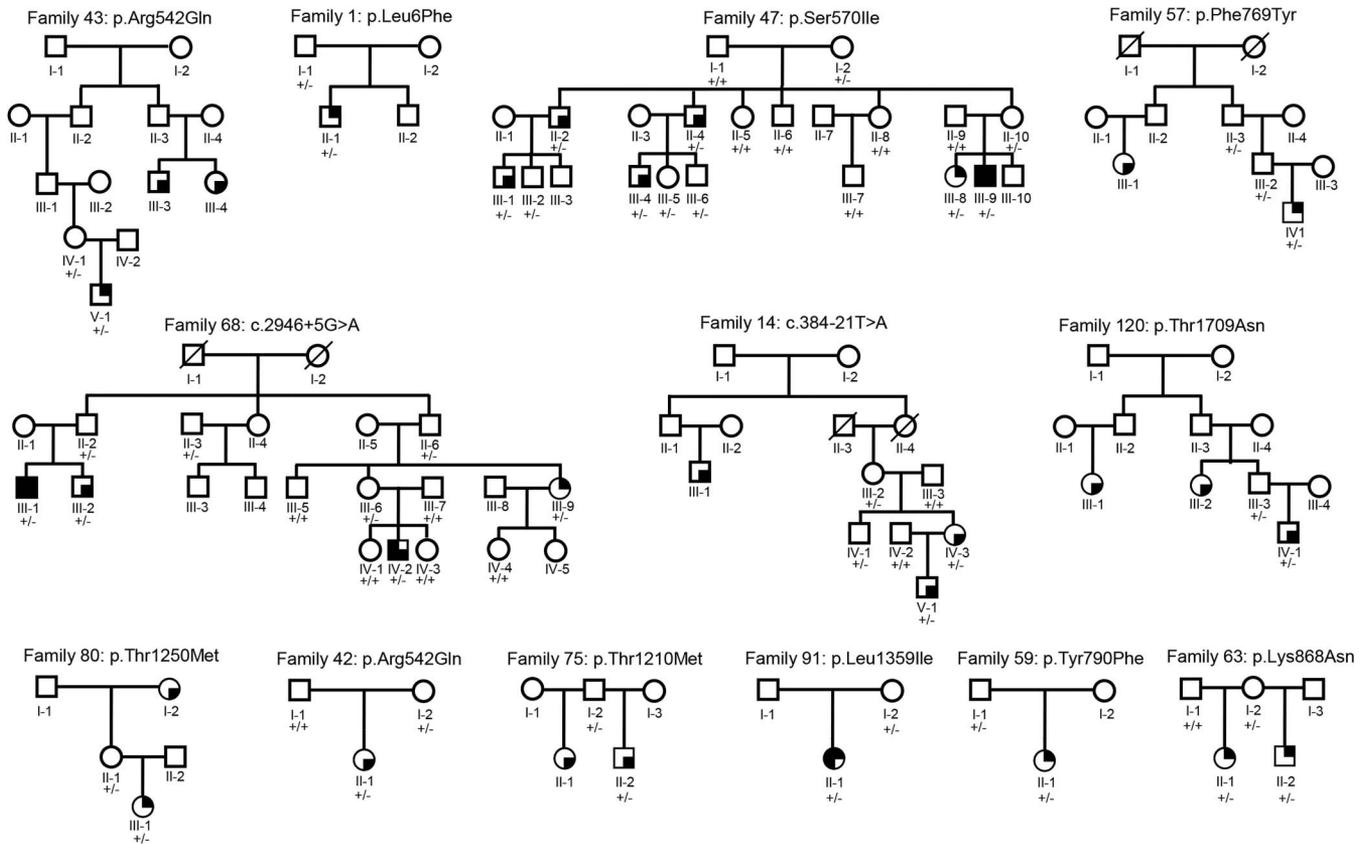
patients with Dravet syndrome. On the basis of this parameter, we identified 3 subgroups within which the probability of developing Dravet syndrome was significantly different: 85% in the 0- to 6-month seizure-onset group, 51% in the 6- to 12-month group, and none after the 12th month of age. The optimal diagnostic cutoff was 6 months of age (sensitivity = 83.3%, 95% CI 72.7–91.1; specificity = 76.60%, 95% CI 62.0–87.7, table 3).

On the basis of the results shown in table 3, we explored a combination of parameters that, however, did not yield higher significance than the test based on age at first seizure.

**DISCUSSION** Previous studies have examined the spectrum of *SCN1A* mutations associated with Dravet syndrome<sup>8,12</sup> and suggested clinical criteria for *SCN1A* screening based on early seizure characteristics.<sup>13</sup> These studies, performed on patient populations whose clinical characteristics were highly suggestive of Dravet syndrome, have contributed to delineate its genotypic and phenotypic spectra. The approaches used, however, could not address the opposite perspective of defining the risk of divergent outcomes in a population of mutation-positive patients whose clinical presentation is relatively similar at seizure onset. Considering that *SCN1A* screening is widely performed soon after early prolonged/repeated FS appear, at a stage when either benign or ominous outcomes are still possible, we attempted to identify reliable early clinical and mutational outcome predictors that can help clinicians to deal with a frequently encountered dilemma.

To gather a sample population that was representative of the spectrum of *SCN1A*-associated phenotypes, as a first step, we collected clinical details on 200 individuals with damaging *SCN1A* mutations, including healthy relatives of mutation carriers. Of the 182 patients with seizures, 53.6% had Dravet syndrome and 46.4% had milder conditions, including GEFS+ (27%), FS (16.2%), and focal seizures (3.2%). Eighteen remaining relatives of probands (9% of the whole sample) were mutation carriers who never experienced seizures. We then analyzed 7 clinical parameters usually available at seizure onset—sex, family history of epilepsy, age at/fever at/type of/duration of first seizure, and abnormalities at first EEG—and found 5 of them to exhibit a significantly different distribution in the Dravet and non-Dravet groups. While the Dravet group had a higher frequency of onset within the sixth month ( $p < 0.001$ ), of focal seizures ( $p = 0.003$ ), and of seizures lasting >5 minutes ( $p = 0.001$ ), family history ( $p < 0.001$ ) and evidence of fever at seizure onset ( $p = 0.019$ ) were more frequent in the non-Dravet group.

**Figure 2** Pedigrees of families exhibiting incomplete penetrance of *SCN1A* mutations



● = Dravet syndrome; ○ = FS; □ = generalized/genetic epilepsy with febrile seizures plus; ■ = focal epilepsy; +/- = heterozygous *SCN1A* mutation; +/- = absence of the *SCN1A* mutation.

Multilevel logistic regression showed that age at seizure onset was the more relevant parameter associated with Dravet syndrome (OR = 0.647, 95% CI 0.541–0.774,  $p < 0.001$ ). The diagnostic ROC analysis identified age at seizure onset as the main and statistically relevant indicator, with the optimal diagnostic cutoff for age being 6 months (sensitivity = 83.33%; specificity = 76.60%). The risk for Dravet syndrome declined progressively according to the age group at seizure onset, being 85% in the 0- to

6-month group, 51% in the 6- to 12-month group, and 0% after the 12th month.

We observed 123 different *SCN1A* mutations. As previously reported,<sup>14,15</sup> truncating mutations were more represented in patients with Dravet than in those without Dravet syndrome (40% vs 13%). We also observed 13 truncating mutations associated with mild phenotypes and 37 missense mutations outside the pore-forming region in Dravet syndrome, thus confirming the variable phenotypic consequences of

**Table 3** Diagnostic accuracy of analyzed parameters

	Sensitivity, %	Specificity, %	ROC area	DOR	PPV, %	NPV, %
<b>Age at seizure onset, mo</b>						
0-6	83.33	76.60	0.7996	16.4	84.50	75
0-12	97.22	51.06	0.7414	36.5	75.30	92.30
<b>No family history</b>	63.89	70.21	0.6705	4.17	76.70	55.90
<b>First seizure duration (&gt;5 min)</b>	61.11	72.34	0.6673	4.11	77.20	54.80
<b>Mutation (truncating)</b>	48.61	80.85	0.6473	3.99	79.50	50.70
<b>Focal seizures</b>	19.44	95.74	0.5759	5.43	87.50	43.70
<b>Afebrile seizures</b>	27.78	82.98	0.5538	1.88	71.40	42.90

Abbreviations: DOR = diagnostic odds ratio; NPV = negative predictive value; PPV = positive predictive value; ROC = receiver operating characteristic. Truncating refers to nonsense, frameshift mutations, and rearrangements.

*SCN1A* mutations.<sup>1</sup> Although genotype-phenotype studies have pointed out that less severe phenotypes are more common with missense than truncating mutations<sup>16–18</sup> and that missense mutations in the pore-forming region tend to be associated with more severe phenotypes,<sup>19</sup> correlations between specific mutations and specific phenotypes are weak.<sup>20</sup> A meta-analysis of 155 missense *SCN1A* mutations indicated that the physicochemical properties of amino acid changes influence the epilepsy phenotype and could be used to predict the phenotype associated with each mutation.<sup>21</sup> Similarly, an attempt to correlate the Grantham score of missense mutations, a measure of physicochemical differences between amino acids, with phenotypic outcome demonstrated that the type of amino acidic substitution does not independently predict different phenotypes within the spectrum of *SCN1A*-related epilepsies.<sup>15</sup> Our estimation through ROC analysis of the value of different mutation types to predict the risk of developing Dravet syndrome sets the best cutoff grouping truncating mutations vs splicing and missense mutations but could not predict with high confidence Dravet syndrome vs milder phenotypes (sensitivity = 48.61%, specificity = 80.85%). Therefore, localization and type of *SCN1A* mutations are, on their own, less accurate predictors of outcome than assessment based on age at seizure onset. Combining mutational data with clinical parameters did not significantly improve the discrimination performance of the test.

Of the 33 families included in our cohort, all 20 families exhibiting complete penetrance carried truncating mutations, while the remaining 13 exhibiting incomplete penetrance carried either splicing or missense mutations (figure 2). Overall, penetrance was highly mutation-dependent, reaching 100% in families with truncating mutations and 77% in those with segregating missense mutations. Nonpenetrance for *SCN1A* mutations, observed in 9% of our sample, has already been reported in GEFS+<sup>22</sup> and less frequently in Dravet syndrome,<sup>23</sup> but its frequency had not been assessed on large series before. We also observed wide intrafamilial phenotypic variability, with only 5 of 33 families exhibiting the same phenotype in all affected members. High phenotypic variability within the same family<sup>15,23–27</sup> is interpreted as a consequence of epistasis.

From a pathophysiologic perspective, there seems to be an age-at-seizure-onset-dependent response of the brain carrying an *SCN1A* mutation to early epileptogenesis whereby onset within the sixth month almost regularly progresses as an epileptic encephalopathy, while onset after the 12th month never does. It is unlikely that this course was influenced by treatment choices in the population studied because no uniform attitude or protocol exists, with either

immediate treatment of seizures or long-term medication currently being started on the basis of individual preferences concerning drug(s) and timing.

It remains to be clarified whether the worst prognosis related to a younger age at seizure onset can be entirely explained by genetic factors, with earlier onset just being an expression of a lower seizure threshold prompted by the most damaging mutations. Earlier onset of seizure activity can actually by itself induce changes that permanently lower seizure threshold and cause cognitive impairment<sup>28</sup> above and beyond what is caused by the underlying mutation. Studies on animal models have demonstrated that the deleterious consequences of seizures strongly depend on the developmental stage at which they occur: immature neurons having few synapses and more developed neurons that express a multitude of functional synapses endure different consequences.<sup>28,29</sup> Long-lasting effects of seizures may derive from seizure-induced transformation of a naive network to one that has increased seizure susceptibility. In particular, converging evidence has been gathered that in the rat brain, early/prolonged hyperthermic seizures cause permanent changes resulting in long-standing increased excitability.<sup>28</sup> Although it is still unclear whether similar changes occur in the human brain, analogies with the observations that Dravet syndrome develops only when early/prolonged hyperthermic seizures appear in the first year of life, particularly in the first 6 months, are strong.

In pediatric epilepsy practice, young infants with prolonged/repeated fever-related seizures and *SCN1A* mutations pose considerable concerns in terms of their risk of developing Dravet syndrome, a risk that our study sets at  $\approx 50\%$  overall but at 0% in those with seizure onset after the 12th month. It is of primary importance to discern those at higher risk of severe outcomes and to promptly organize management accordingly. Because this study indicates that age at seizure onset is a reliable indicator of outcome, we suggest that seizure onset within the first year of life should prompt appropriate treatment choices, for example, introduction of the stiripentol-clobazam combination<sup>30</sup> before the epileptic encephalopathy is established. Of course this suggestion is valid provided no precious time is lost delaying mutation analysis.<sup>4</sup> Although no controlled evidence exists that more appropriate earlier treatment can limit the progression toward the severe end of the *SCN1A* spectrum, this possibility should be explored through a dedicated trial, which might use the results of this study as a comparator.

#### AUTHOR CONTRIBUTIONS

Study concept and design: V. Cetica, R. Guerrini. Patient collection: R. Guerrini, C. Marini, S. Chiari, A. Ferrari, F. Sicca, N. Specchio,

M. Trivisano, D. Battaglia, I. Contaldo, N. Zamponi, C. Petrelli, T. Granata, F. Ragona, G. Avanzini. Mutation screening and data analysis: V. Cetica, S. Chiari, D. Mei, E. Parrini, D. Pucatti, R. Guerrini. Statistical analysis: L. Grisotto. Drafting of the manuscript: V. Cetica, S. Chiari, R. Guerrini. Critical revision of the manuscript for important intellectual content: R. Guerrini. Obtained funding: R. Guerrini.

## ACKNOWLEDGMENT

The authors thank all the patients and their relatives for participating in this study.

## STUDY FUNDING

This study was funded by the EU 7th Framework Programme (FP7) under the project DESIRE grant N602531 (to R.G.) and RESIDRAS.

## DISCLOSURE

The authors report no disclosures relevant to the manuscript. Go to [Neurology.org](http://Neurology.org) for full disclosures.

Received July 12, 2016. Accepted in final form December 22, 2016.

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