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When is a child with status epilepticus likely to have Dravet syndrome?

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Summary
Purpose: To identify clinical risk factors for Dravet syndrome (DS) in a population of children with status epilepticus (SE).
Material and methods: Children aged between 1 month and 16 years with at least one episode of SE were referred from 6 pediatric neurology centers in Switzerland. SE was defined as a clinical seizure lasting for more than 30 min without recovery of normal consciousness. The diagnosis of DS was considered likely in previously healthy patients with seizures of multiple types starting before 1 year and developmental delay on follow-up. The presence of a *SCN1A* mutation was considered confirmatory for the diagnosis. Data such as gender, age at SE, SE clinical presentation and recurrence, additional seizure types and epilepsy diagnosis were collected.

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SCN1A analyses were performed in all patients, initially with High Resolution Melting Curve Analysis (HRMCA) and then by direct sequencing on selected samples with an abnormal HRMCA. Clinical and genetic findings were compared between children with DS and those with another diagnosis, and statistical methods were applied for significance analysis.

Results: 71 children with SE were included. Ten children had DS, and 61 had another diagnosis. *SCN1A* mutations were found in 12 of the 71 patients (16.9%; ten with DS, and two with seizures in a Generalized Epilepsy with Febrile Seizures + (GEFS+) context). The median age at first SE was 8 months in patients with DS, and 41 months in those with another epilepsy syndrome ($p < 0.001$). Nine of the 10 DS patients had their initial SE before 18 months. Among the 26 patients aged 18 months or less at initial SE, the risk of DS was significantly increased for patients with two or more episodes (56.3%), as compared with those who had only one episode (0.0%) ($p = 0.005$).

Conclusion: In a population of children with SE, patients most likely to have DS are those who present their initial SE episode before 18 months, and who present with recurrent SE episodes.

Introduction

Differentiating seizures at early stages of Dravet syndrome (DS) from infantile-onset seizures that will remain isolated, or seizures observed in the context of a self-limited epilepsy, is often difficult, and the diagnosis of DS may be significantly delayed (Hattori et al., 2008). Early recognition of DS is nevertheless fundamental. First, it may help to initiate the most beneficial treatments, such as the combination of valproic acid, clobazam and stiripentol, likely to be efficient in a certain number of patients (Chiron et al., 2000). Second, it may help avoiding drugs frequently chosen as first-line treatment options in the very young with focal seizures, but contraindicated in DS because of their potential for seizure exacerbation, such as carbamazepine, phenobarbital, phenytoin or lamotrigine (Dravet et al., 2005). In addition, unnecessary and costly investigations may be avoided (Brunklaus et al., 2013). Finally, important prognostic and counseling indications may be given to families, especially since the demonstration that germinal mosaicism for DS may occur (Depienne et al., 2006, 2009; Suls et al., 2010). Seventy to 80% of children with DS carry *SCN1A* mutations (Hirose et al., 2013), and gene testing is recommended in patients whose clinical presentation is consistent with DS (Hirose et al., 2013). However, despite important recent developments, genetic analyses remain costly and may not always be available on a regular basis. It is therefore important to identify specific clinical features that may allow a better selection of patients in which *SCN1A* testing should be tested in priority. The features considered suggestive for DS and which should prompt gene testing, as published in the recent recommendations for *SCN1A* testing in patients with epilepsy, include recurrent, febrile or afebrile prolonged hemiclonic seizures or generalized status epilepticus (SE) appearing in a developmentally normal infant (Hirose et al., 2013). Our objective was to identify additional clinical features suggestive of DS in a population of children with SE, in order to further delineate the group of those for whom gene testing may be most useful. We therefore collected a group of children with SE and retrospectively classified their epilepsy syndrome, based on clinical data. We then screened all patients for *SCN1A* mutations, first to confirm DS in those for which this diagnosis was suspected clinically and, second, to see if mutations were found in patients with other epilepsy syndromes. Finally, we tried to identify early clinical features that would allow predicting an evolution to DS in this cohort of patients.

Material and methods

The research protocol was approved by the ethics committees of all participating centers, and informed consent was obtained from the parents or legal representatives of all children.

Patient data

SE was defined as a clinical seizure lasting more than 30 min according to direct witnesses. The clinical diagnosis of DS was made on the basis of a retrospective analysis of clinical data in children whose follow-up was long enough to clarify the epilepsy and neurological phenotype. It was considered likely in initially healthy patients with seizures of multiple types starting before 1 year and developmental delay on follow-up. The presence of a *SCN1A* mutation was considered confirmatory for the diagnosis in those suspected clinically. Children, whose familial history revealed the presence of febrile seizures in at least one additional member, were considered as being part of the Generalized Epilepsy with febrile Seizures + (GEFS+) spectrum.

Patients were eligible if they had presented at least one episode of SE between 1 month and 16 years, whatever the acute cause, and related or not to any chronic cerebral pathology. Refusal of the patient or legal representatives to participate, insufficient data on the duration of seizures despite conversation with a direct witness, premature birth with corrected age of less than 1 month at the time of SE, and electrical SE (*i.e.* without any overt clinical manifestations) were exclusion criteria.

Patients with SE were identified by physicians from Pediatric Neurology Units of six major Swiss Children's Hospitals (Geneva, Lausanne, Basel, Bellinzona, Neuchâtel, and St Gallen), either after retrospective analysis of their database of patients with seizures, or when patients presented at the emergency department with an initial SE episode. The recruitment occurred between 2009 and 2012. Clinical information including personal and family history, age at first

seizure, seizure types, age at first SE, provoking factors, SE recurrence and total number of episodes, interval between first and second SE episode, and likely epilepsy syndrome diagnosis was collected for all included patients.

Genetic analyses

Blood samples of all included patients were collected and DNA was analyzed for *SCN1A* mutations at the Genetic Medicine Service, University Hospitals, Geneva, Switzerland. Genomic DNA was extracted from EDTA blood by a salting out procedure (Gentra PureGene, QIAGEN, Hombrechtikon, Switzerland). To detect partial or complete deletions/duplications on *SCN1A* gene, multiplex ligation-dependent probe amplification (MLPA) was performed using Salsa MLPA P137 *SCN1A* reagent (MHRC-Holland, Amsterdam, The Netherlands). Quantification analysis was carried out using GeneMarker software v1.6 after separation of polymerase chain reaction (PCR) products from patients (in duplicates) against controls by capillary electrophoresis on an ABI 3100 (or 3500) DNA Analyzer (Applied Biosystems, Foster City, USA).

A first selection of patients possibly harboring a *SCN1A* mutation was carried out by HRMCA performed on a Light-Cycler 480 (Roche Applied Science, Mannheim, Germany). *SCN1A* was divided in 40 fragments (primers and conditions available upon request).

DNA from patients was tested in parallel with DNA from control subjects. Normalized and temperature-shifted difference plots were compared between samples. Fifty-one patients in which *SCN1A* mutations were previously identified in another laboratory (Dr C. Depienne, Paris) were also tested with HRMCA, of which 49 were positive (sensitivity: 96%).

Fragments showing abnormal HRMCA profiles were further amplified (30 couples of primers, conditions available upon request). Polymerase chain reaction (PCR) fragments were purified with Microclean (Web Scientific, Crewe, United Kingdom). Direct cycle sequencing of the purified products was performed on both strands sequenced with the Big Dye Terminator Cycle Sequencing Kit v3.1 (Applied Biosystems), cleaned-up (Agencourt CleanSEQ, Dye-terminator removal, Beckman Coulter, Beverly, USA) on a Beckman Biomek NX Span robot, and further analyzed on capillary electrophoresis with the ABI 3100 (or 3500) DNA analyzer using primers designed to cover all exons and their flanking sequences. Sequences were further analyzed with the GeneSearch v3.6 software (PhenoSystems, Lillois, Belgium).

DNA samples of the parents of positive patients were tested for potential transmission of the deletion or mutation, according to the methods described earlier (MLPA analysis or direct sequencing).

Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/), MutPred (http://mutpred.mutdb.org/) and SIFT (http://sift.jcvi.org/) programs were used to predict functional effects of amino acid substitutions identified by direct sequencing. Values close to 1 are highly suggestive of a variant of pathological significance for the first two programs; for SIFT, variants are classified as ''damaging'' or ''tolerated'' substitutions.
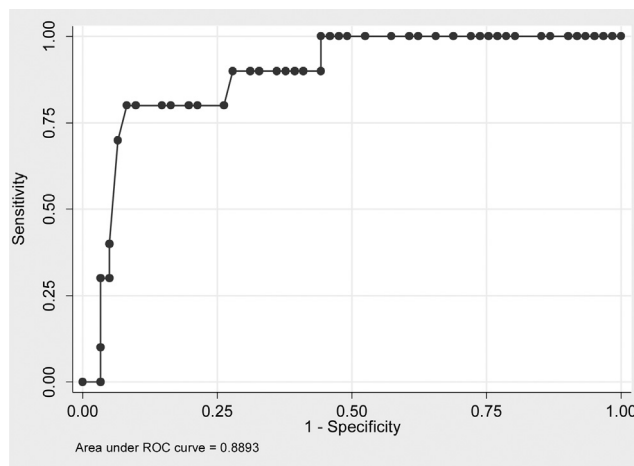

Figure 1 DS likelihood as a function of age at initial SE episode.

Statistical analyses

Patient characteristics were described by frequencies and percentage, or by median, minimum and maximum values. The 95% confidence intervals around proportions were obtained by the exact method of Clopper-Pearson. Comparison between patients with particular syndromes, like DS, and the other patients with SE were performed with Fisher's exact test or Wilcoxon's test. The performances of the age at first status epilepticus to detect DS were assessed by a non parametric ROC curve. The area under the curve is given with the 95% confidence interval. All statistical analyses were performed with S-plus 8.0 for Windows (Insightful Corp., Seattle, WA, USA) and STATA 11.0 (StataCorp. 2009. *Stata Statistical Software: Release 11*. College Station, TX: StataCorp LP). The significance level was set at 0.05.

Results

Clinical features

During the recruitment period, 71 patients fulfilled our inclusion criteria and were referred for genetic testing. Among them, 10 (14.1%) were eventually diagnosed with DS, and 61 had another diagnosis: 5 (7.0%) had seizures in a GEFS+ context, 22 (31.0%) had another epileptic syndrome, and 34 (47.9%) had no identified epilepsy syndrome (Supplemental data Table 3). The characteristics of all children are presented in Table 1.

The median age at first SE in those with DS was 8 months (range, initial SE: 4—32 months), significantly lower than in those with another epilepsy diagnosis (median: 41 months, range: 3—172 months, Supplemental data Table 4) (*p* < 0.001, area under the ROC curve 0.899 (95% CI: 0.79—0.989) (Fig. 1). We set an age limit for initial SE at 18 months to perform further subgroup analyses because this cut-off provided good sensitivity and specificity (90.0% (95% CI: 55.5—99.7) and 72.1% (95% CI: 59.2—82.9), respectively) in the diagnosis of DS. In the 45 patients aged 18 months or more at the time of first SE, only one (2%) had DS. Among those aged 18 months or less (*N* = 26), 9 (35%) patients had

Table 1 Status epilepticus characteristics as a function of epilepsy syndrome diagnosis: patients with clinical Dravet syndrome are compared with those with another clinical diagnosis.

		Patient number (%) (n = 71)	GEFS+, Undefined or other epilepsy diagnosis (n = 61)	Dravet syndrome (n = 10)	p
SCN1A, n (%)	Normal	59 (83.1%)	59 (96.7%)	0 (0.0%)	<0.001
	Abnormal	12 (16.9%)	2 (3.3%)	10 (100.0%)	
Febrile SE, n (%)	No	36 (50.7%)	32 (52.5%)	4 (40.0%)	0.51
	Yes	35 (49.3%)	29 (47.5%)	6 (60.0%)	
SE episodes, n (%)	One	37 (52.1%)	36 (59.0%)	1 (10.0%)	0.006
	Two	13 (18.3%)	9 (14.8%)	4 (40.0%)	
	Multiple	21 (29.6%)	16 (26.2%)	5 (50.0%)	
Focality, 1st SE, n (%)	Hemiclonic, focal clonic, focal myoclonic	15 (21.1%)	12 (19.7%)	3 (30.0%)	0.43
	Other	56 (78.9%)	49 (80.3%)	7 (70.0%)	
Focality, all SE, n (%)	Hemiclonic, focal clonic, focal myoclonic	16 (22.5%)	13 (21.3%)	3 (30.0%)	0.68
	Other	55 (77.5%)	48 (78.7%)	7 (70.0%)	
Age at epilepsy onset, years	Median [Min—Max]	2 [0.1;14]	3 [0.1;14]	0.5 [0.3;0.9]	<0.001
Age at 1st SE, months	Median [Min—Max]	30 [3;172]	41 [3;172]	8 [4;32]	<0.001
Age at 2nd SE, months	Median [Min—Max]	46 [5;182]	57 [11;182]	11 [5;69]	0.002
Interval 2nd/1st SE, months	Median [Min—Max]	3.5 [0.2;61]	5 [0.2;35]	2 [0.2;61]	0.44

DS. In this subgroup, the risk of DS was significantly greater for those with two or more SE episodes, as compared with those with one episode only (56.3% (95% CI: 29.9—80.21) *vs* 0.0% (95% CI: 0.0—30.8), $p = 0.005$).

In addition, nine of the 10 patients (90%) with DS had two or more episodes of SE at the time of inclusion in the study, as compared with 25/61 (41%) of those with another epilepsy diagnosis ($p = 0.005$).

A rule was created to evaluate the risk of DS, based on age at 1st SE of 18 months or less, and SE recurrence, as represented in Fig. 2. Its sensitivity and specificity, when both parameters were considered together, were 90.0% (95%CI: 55.5—99.7) and 88.5% (95%CI: 77.8—95.3), respectively. The positive and negative predictive values for DS were 56.3% (95% CI: 29.9—80.2) and 98.2% (95% CI: 90.3—100.0), respectively.

Patients with DS had their second episode of SE at a median interval of 2 months. This interval between SE episodes was not significantly different from patients with other diagnoses.

Supplementary Table 3 related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.eplepsyres.2014.02.019.

Neither the presence of fever, nor specific seizure semiology (such as hemiclonic or focal clonic seizures during any of the SE episodes) was significantly different in children with DS than in those with another diagnosis.

Nine (90%) of the ten patients with DS were boys, as compared with 31 (50.8%) of the 61 with another epilepsy diagnosis ($p = 0.04$).

Genetic analyses

SCN1A variants were observed in 12 (16.9%) of our 71 patients (all of the 10 patients with clinical DS, and 2 children with seizures in a GEFS+ context) (Table 2). In our DS patients, 2 had heterozygous complete deletion of *SCN1A* (patients 3 and 7), one had a partial heterozygous deletion (patient 4, EX8-EX15del in heterozygosity), one had a frameshift mutation (patient 8, deletion of 2 nucleotides), one had a splice site mutation (patient 10), one had a nonsense mutation (patient 5), and 3 had missense mutations (patients 2, 6, 11), mostly with severe predicted consequences on protein function. One patient with DS (patient 10) was found to have an intronic mutation with Next Generation Sequencing (Dr A. Suls, Antwerpen) after our initial analyses came back negative. Six mutations were *de novo*. In 3 children (patients 5, 7, and 8), either one or both parents were not available for testing to determine the pattern of inheritance. In another patient (patient 9), a boy with encephalopathy and multiple SE episodes, the variant T1174S was inherited from his mother. His family history was however negative for epilepsy. The pathogenicity of this variant is somewhat controversial and is described in

Table 2 *SCN1A* mutations in the 12 patients with abnormal genetic results. DS = Dravet syndrome, GE = Genetic epilepsy, GEFS+ = Genetic Epilepsy with Febrile Seizures+, SNP = Single Nucleotide Polymorphism, GTCS = generalized tonic-clonic seizures, sz = seizure.

| Patient	Sex	Epilepsy Diagnosis/Context	*Clinical data (age at sz onset/Seizure type/development)*	*SCN1A* abnormality type HGVS nomenclature (traditional nomenclature)	Parental transmission (parent phenotype)	Predicted mutation severity		
						Mutpred	Polyphen 2	SIFT
1	F	GEFS+	24 Months/GTCS/mild school difficulties	c. [1888C > T];[=], p.Arg630Trp(R630 W)	Mother (normal)	0.51	1	Damaging
2	M	DS	4 Months/tonic sz, GTCS, myoclonias, absences/mental retardation	c. [5492T > C];[=], p.Phe1831Ser (F1831S)	*De novo*	0.64	1	Damaging
3	M	DS	6 Months/myoclonias, GTCS/mental retardation	c. [105-?_5511 + ?del];[=] (complete heterozygous deletion)	*De novo*	N/A	N/A	N/A
4	M	DS	5 Months/repeated focal febrile sz/normal non-verbal skills	c. [1086-?_2881 + ?del];[=] (EX8-EX15 heterozygous deletion)	*De novo*	N/A	N/A	N/A
5	M	DS	6 Months/absences, focal dyscognitive, GTCS, tonic sz/mental retardation	c. [1129C > T];[=], p.Arg377Stop (R377X)	*Unknown*	N/A	N/A	N/A
6	M	DS	4 Months/absences, GTCS, myoclonias/mental retardation	c. [747T > G];[=], p.Asp249Glu (D249E)	*de novo*	0.54	1	Damaging
7	M	DS	6 Months/GTCS, myoclonias, absences/mental retardation	c. [105-?_5511 + ?del];[=] (complete heterozygous deletion)	Unknown (mother negative, father not tested)	N/A	N/A	N/A
8	M	DS	10 Months/GTCS/mental retardation	c. [3425−3426 delAA];[=], p.Lys1142Argfs*5	Unknown	N/A	N/A	N/A
9	M	DS	4 Months/myoclonias/mental retardation	c. [3521C > G];[=], p.Thr1174Ser (T1174S)	Mother (normal)	0.3	0	Tolerated
10	F	DS	8 Months/myoclonias/mild school difficulties	c. [965−2 A > C];[=] (splice site variant)	*De novo*	N/A	N/A	N/A
11	M	DS	5 Months/absences, GTCS, myoclonias/mental retardation	c. [1088C > G];[=], p.Thr 363Arg (T363R)	*De novo*	0.84	1	Damaging
12	F	GEFS+	6 Months/GTCS/normal	c. [5438C > T];[=] p.Met1823Thr (M1823 T)	*De novo*	0.473	0.36	Damaging
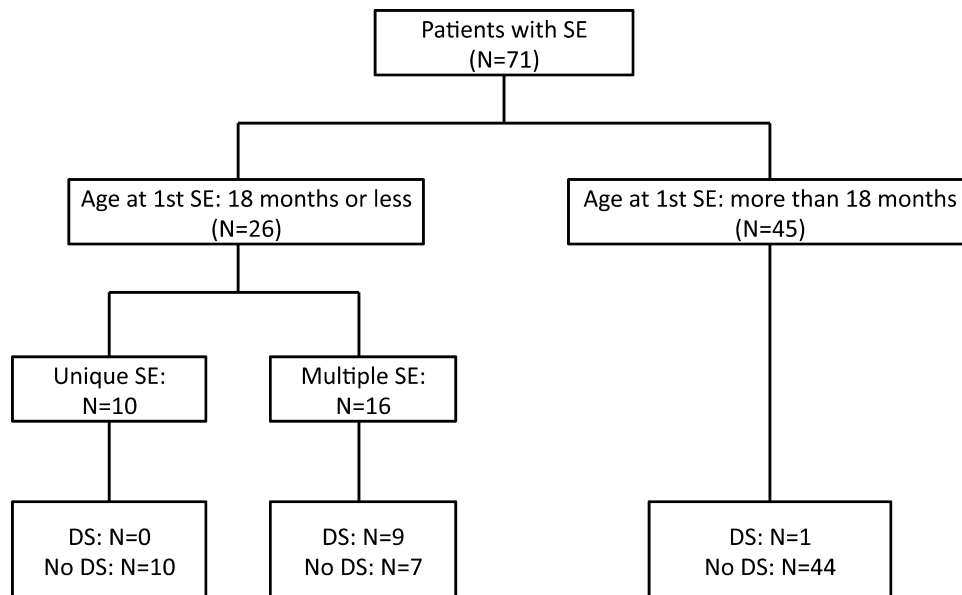
Figure 2 Distribution of Dravet syndrome patients according to age at initial status epilepticus and status epilepticus recurrence.

the discussion section. The two non-DS patients with SE and *SCN1A* abnormalities had seizures in a GEFS+ context. One of them (patient 1) was a girl with generalized tonic-clonic seizures mainly observed with fever since the age of 2 years. At seven years, she had attention and expressive language difficulties; her mother had had one febrile seizure in infancy, and her sister had presented two afebrile seizures in childhood (*SCN1A* analysis not performed); her neurological examination, MRI and repeated EEGs were normal. The second patient (patient 12) was a 3 year-old girl with febrile seizures since the age of 6 months. Febrile seizures were also noted in several members of her family, on both parental sides. Her neurological examination and EEG were normal. A mild cerebellar atrophy was suspected on brain MRI at 19 months. Each had a variant of uncertain predicted value with Polyphen and MutPred but classified as Damaging with SIFT (R630 W and M1823T). The first of these abnormalities was inherited from the mother; the other one was *de novo*.

Supplementary Table 4 related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.eplepsyres.2014.02.019.

Discussion

Clinical features that best predict evolution to DS in a cohort of children with SE

In our group of patients with SE, DS was significantly more likely in children with initial SE before 18 months and recurrent SE, than in those with a first SE before 18 months without recurrence, or in those with initial SE after 18 months. In DS patients like in children with other diagnoses, SE recurrence was observed in a median interval of 2 months. Factors reported to be characteristic of seizures in DS patients, such as fever-sensitivity and focal components were not significantly more frequent in SE episodes of our DS patients than in those with another diagnosis, but our small sample size may have caused this lack of statistical power.

Who to test for *SCN1A* mutations?

Perspective after the initial SE episode

It has been debated whether genetic testing for *SCN1A* abnormalities may help in the differential diagnosis of DS (Hattori et al., 2008; Hirose et al., 2013; Scheffer et al., 2011). Indeed, mutations in *SCN1A* are found in 70—80% of patients with classic DS (Brunklaus et al., 2013; Hirose et al., 2013), which conversely means that up to 20—30% of DS patients have normal *SCN1A* results on ''basic'' gene analyses. Even though recently implemented techniques, such as CGH-array or exome sequencing, may pick up cases previously missed (Hartmann et al., 2012), other genes, such as *PCDH19* (Hynes et al., 2010), *GABRG2* (Harkin et al., 2002), and *SCN1B* (Patino et al., 2009), have been involved in DS. In any case, the genotype-phenotype correlation remains to be established with precision, and there is a need to identify patients at highest clinical risk for DS, to allow proper interpretation of genetic results according to the context (Hattori et al., 2008; Hirose et al., 2013; Scheffer et al., 2011) Our results confirm, as previously suggested (Scheffer, 2011b; Cross, 2012), that waiting for a second SE episode before performing *SCN1A* analyses is a ''reasonable'' approach to investigate children whose initial SE episode is noted before 18 months. This is particularly true for those in which classical criteria of DS are not all fulfilled. Having in mind that the majority of children with DS will present a second SE episode in a short time interval (see Table 1), this proposal may increase the yield of positive findings, and may help avoid an important number of costly procedures if they were rather performed after the initial episode.

Retrospective perspective

Millichap et al. rightly proposed that ''The diagnosis of DS should also be considered in adults with infantile-onset

refractory epilepsy, by reevaluation of childhood history and *SCN1A* testing'' (Millichap et al., 2009). This was illustrated in a subsequent publication on two fathers of children with Dravet syndrome, who had themselves presented with seizures in infancy (Verbeek et al., 2011). *SCN1A* somatic mosaicism was detected in one of them, after the diagnosis had been confirmed in his son (Verbeek et al., 2011). Our study indicates that the age at first SE and SE recurrence may also be used retrospectively, particularly to identify patients at low risk of DS and avoid unnecessary *SCN1A* testing. Among adult patients whose clinical characteristics may retrospectively suggest DS, like developmental delay and refractory seizures since the first year of life, those who have not presented a single SE episode at 18 months or less, as well as those who have not recurred after a first SE before 18 months, are unlikely to have DS. More concretely, our decision rule shows that, in patients with at least one SE episode, those with a single event observed after the age of 18 months have more than 98% chances of having another diagnosis than DS. However, because of the small sample size, the 95% confidence intervals around our estimates are often large. Moreover, we cannot exclude that a certain proportion of patients with DS will not present any evidence for SE episodes. In that perspective, this feature is not supposed to replace the ''traditional'' criteria for DS, but rather to be used as an additional tool to suspect the diagnosis.

Genetic findings and specific epilepsy phenotypes

SCN1A variants were found in a very restricted number of situations in this study: 10 patients had DS and 2 had seizures in a GEFS+ context. The inheritance of *SCN1A* variants was checked whenever possible, and was mostly *de novo* in DS patients, except in one boy who had a missense variant (T1174S) inherited from his asymptomatic mother. This variant was already reported in patients with a wide range of phenotypes, including 3 female members of a family with severe migraines (Gargus and Tournay, 2007), and a girl with DS for whom genetic analyses evidenced two variants, one of which, the T1174S, being transmitted by her asymptomatic mother (Le Gal, personal communication). It has also been reported in one unaffected control (cited in *SCN1A* variant database). With a Polyphen-2 score of zero but a very low allele frequency, the potential pathogenic effect of this variant is still a matter of controversy (Cestele et al., 2013; Frosk et al., 2013).

Zuberi et al. recently reported that truncating mutations correlated with an earlier age of onset of prolonged seizures than missense mutations in 273 patients. This result was not significant, however, if the group was restricted to patients with DS (Scheffer, 2011a; Zuberi et al., 2011). In our study, neither the type, nor the predicted severity of *SCN1A* variants significantly differed between children with DS and those with seizures in a GEFS+ context.

In one of our GEFS+ patients with *SCN1A* abnormalities (patient 12), the variant (M1823T) was *de novo*. To our knowledge, this has only been reported in 4 patients (Scheffer et al., 2011).

Conclusion

In conclusion, if DS is suspected in patients with a current or past history of SE, *SCN1A* testing should be uppermost considered in those with initial SE before 18 months and SE recurrence. If both factors are negative, DS is unlikely.

Conflicts of interest

The authors have no conflicts of interests. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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