Brain MRI Findings in Severe Myoclonic Epilepsy in Infancy and Genotype–Phenotype Correlations

*Pasquale Striano, †Maria Margherita Mancardi, *Roberta Biancheri, ‡Francesca Madia, §Elena Gennaro, ‡Roberta Paravidino, §Francesca Beccaria, §§Giuseppe Capovilla, #Bernardo Dalla Bernardina, #Francesca Darra, ¶Maurizio Elia, **Lucio Giordano, ††Giuseppe Gobbi, ††Tiziana Granata, †††Francesca Ragona, §§§Renzo Guerrini, §§§Carla Marini, ¶¶Davide Mei, ##Francesca Longaretti, ¶¶Antonino Romeo, †Laura Siri, ***Nicola Specchio, ***Federico Vigevano, †††Salvatore Striano, ††††Fabio Tortora, ††††Andrea Rossi, *Carlo Minetti, ###Charlotte Dravet, †Roberto Gaggero, and *Federico Zara

*Muscular and Neurodegenerative Disease Unit, Institute “G. Gaslini,” University of Genova, Italy; †Epilepsy Unit, Department of Child Neuropsychiatry, “Institute G. Gaslini,” Genova, Italy; ‡Laboratory of Genetics, E.O. Ospedali Galliera, Genova, Italy; §Department of Child Neuropsychiatry, Ospedale “C. Poma,” Mantova, Italy; ¶Department of Child Neuropsychiatry, Policlinico G.B. Rossi, Verona, Italy; ¶¶Department of Neurology, Oasi Institute for Research on Mental Retardation and Brain Aging, Troina, Italy; **Department of Child and Adolescent Neuropsychiatry, Spedali Civili, Brescia, Italy; ††Unit of Child Neuropsychiatry, Ospedale Maggiore “C.A Pizzardi,” Bologna, Italy; †††Division of Child Neurology, Istituto Nazionale Neurologico “C. Besta,” Milano, Italy; §§Department of Child Neurology and Psychiatry, University of Pisa and IRCCS Fondazione Stalla Maris, Calambrone, Pisa, Italy; ‡‡Division of Child Neuropsychiatry, Fondazione Istituto Neurologico “C. Mondino,” University of Pavia, Italy; ¶¶¶Center for Child Epilepsy, A. O. “Fatebenefratelli e Oftalmico,” Milano, Italy; ***Neurology Division, Bambino Gesu Children’s Hospital, Roma, Italy; ††††Epilepsy Center, Federico II University, Napoli, Italy; ‡‡‡Neuroradiology, Federico II University, Napoli, Italy; §§§Neuroradiology, Institute “G. Gaslini,” University of Genova, Italy; †††††Centre Saint-Paul-Hopital Henri Gastaut, Marseille, France

Summary: Introduction: To determine the occurrence of neuro-radiological abnormalities and to perform genotype–phenotype correlations in severe myoclonic epilepsy of infancy (SMEI, Dravet syndrome).

Patients and Methods: Alpha-subunit type A of voltage-gated sodium channel (SCN1A) mutational screening was performed by denaturing high-performance liquid chromatography (DHPLC) and multiplex ligation probe amplification (MLPA). MRI inclusion criteria were: last examination obtained after the age of 4 years on 1.5-T systems; hippocampal cuts acquired perpendicular to the long axis of the hippocampus; qualitative assessment was performed on T1-weighted, T2-weighted, proton density, and 1–3 mm thick coronal FLAIR images.

Results: We collected 58 SMEI patients in whom last MRI was performed at or later than 4 years of age. SCN1A mutations occurred in 35 (60%) cases. Thirteen (22.4%) out of 58 patients showed abnormal MRIs. Eight patients showed cortical brain atrophy of which 3 associated to ventricles abnormalities, 1 to cerebellar atrophy, 1 to white matter hyperintensity; 3 patients had ventricles enlargement only; 1 patient showed hippocampal sclerosis (HS); 1 had focal cortical dysplasia. Genotype–phenotype analysis indicated that abnormal MRIs occurred more frequently in patients without SCN1A mutations (9/23; 39.1%) compared to those carrying SCN1A mutations (4/35; 11.4%) (p = 0.02).

Conclusion: Different brain abnormalities may occur in SMEI. Only one case with HS was observed; thus, our study does not support the association between prolonged febrile seizures and HS in SMEI. Abnormal MRIs were significantly more frequent in patients without SCN1A mutations. Prospective MRI studies will assess the etiological role of the changes observed in these patients. Key Words: Severe myoclonic epilepsy of infancy—Dravet syndrome—MRI—SCN1A—Genotype–phenotype correlations.

Severe myoclonic epilepsy of infancy (SMEI) or Dravet syndrome is an epileptic encephalopathy characterized by early prolonged febrile and afebrile seizures, followed by intractable seizures of different type and secondary psychomotor delay (Guerrini and Dravet, 1997; Dravet et al., 2005). Mutations in the voltage-gated sodium channel.
channel (Nav1.1) alpha-subunit gene (SCN1A) have been reported in 30–70% of SMEI patients (Nabbout et al., 2003; Dravet et al., 2005). Neuroradiological studies are normal in most patients ( Guerrini and Dravet, 1997) but structural abnormalities such as cerebral or cerebellar atrophy of various degree and focal arachnoid cysts have been anecdotally reported ( Dalla Bernardina et al., 1982; Reiner and Renkawek, 1990; Guerrini and Dravet, 1997; Ohki et al., 1997; Dravet et al., 2005). However, only one study systematically investigated structural abnormalities on MRI and found evidence of hippocampal sclerosis ( HS) in 10 out of the 14 children with a clinical diagnosis of SMEI ( Siegler et al., 2005). Among these, six who had been reported to have normal initial MRI developed HS during the course of the disorder ( Siegler et al., 2005). However, no mutational screening of SCN1A was performed.

We reviewed the MRI findings in a large series of patients with SMEI who underwent mutation analysis of SCN1A to determine the occurrence of neuroradiological abnormalities and to perform genotype–phenotype correlations.

PATIENTS AND METHODS

The study includes patients with clinical diagnosis of SMEI ( Commission, 1989) referred for SCN1A testing to the Laboratory of Genetics at Galliera Hospital between January 2001 and January 2006. Clinical data of the patients (including age at seizure onset, seizure types and duration, number and length of episodes of status epilepticus, antiepileptic therapy) were obtained from medical records collected from 12 different epilepsy centers. Status epilepticus was defined as recurrent seizures without complete recovery lasting 30 min or longer. SCN1A point mutations were analyzed by denaturing high-performance liquid chromatography (DHPLC) and sequencing as previously reported ( Nabbout et al., 2003). Patients with SCN1A deletions identified by multiplex ligation probe amplification (MLPA) and fluorescence in situ hybridization (FISH) analysis ( Madia et al., 2006) were excluded from the study.

MRI inclusion criteria and acquisition details are as follows:

1. MRI examinations scanned on 1.5-T systems;
2. last MRI examination performed at or after the age of 4 years;
3. hippocampal cuts acquired perpendicular to the long axis of the hippocampus, to optimize the evaluation of mesial temporal structures; and
4. qualitative assessment of MRI scans performed on T1-weighted images, T2-weighted images, proton density images, and 1–3 mm thick coronal fluid-attenuated inversion recovery (FLAIR) images.

Clinical data and brain MRI examinations were retrospectively reviewed in June 2006. Images were independently examined by two skilled neuroradiologists. Indicators of HS were hippocampal atrophy on T1-weighted images, increased mesial temporal signal intensity alteration on FLAIR or T2-weighted images, or both, usually associated with loss of hippocampal internal structure ( Siegler et al., 2005). The analysis also focused on localization of supratentorial (i.e., white matter abnormal signal, cortical or subcortical atrophy, abnormalities of cortical development) and infratentorial (i.e., brainstem and cerebellum) abnormalities. Marked deep sulci and enlarged subarachnoid spaces were considered as signs of cortical atrophy. Atrophy was considered to have cortical predominance if enlargement was more prominent in the sulci and subarachnoid spaces with respect to the ventricles.

Statistical analysis

Statistical evaluations were assessed using the chi-square test, or the Fisher’s exact test (in case of expected frequencies less than five). The Wilcoxon matched-pairs test was used to determine the magnitude of difference between matched groups.

RESULTS

Patients’ characteristics

We retrospectively identified 58 unrelated patients with clinical diagnosis of SMEI in whom high-resolution MR images were available. The cohort included 32 boys and 26 girls, with an average age at seizure onset of 5.3 months (range: 3–8 months). Thirty-two patients experienced prolonged febrile ( FS) as first disease manifestation. In the remaining 26 subjects, initial seizures were afebrile tonic or clonic or tonic–clonic. During their life, all patients experienced FS, segmental or massive myoclonic jerks and at least one generalized seizure, 28 had absence seizures and 30 had complex partial seizures. Status epilepticus occurred in 40 patients. All children developed from a moderate to severe mental retardation and showed epileptic seizures resistant to 3–7 different antiepileptic drugs (AEDs). Thirty-five (60%) patients showed SCN1A mutations (20 truncating, 12 missense, 1 splice-site, 1 intragenic deletion, spanning exons 2–6) (“mutated group”). The remaining 23 patients did not show SCN1A mutations (“nonmutated group”).

MRI findings

From 1 to 5 (mean: 1.9) brain MRI examinations were available for each patient. The last MRI examination was performed at a mean age of 8.8 ± 5.1 years (range: 4–25 years). For 35/58 patients, two or more MRI scans were available with first MRI examination performed at the age of 3.2 ± 2.5 years (range: 4 months to 12 years). Thirteen (22.4%) out of 58 patients showed abnormal MRI findings at the last examination (Fig. 1). For these
patients, the mean age at last MRI was 8.9 years (range: 4–21 years) (Table 1).

Brain abnormalities included

1. **Brain atrophy**: Eight patients showed cortical brain atrophy that was clearly focal (i.e., frontal, occipital) in two.
2. **Ventricle abnormalities**: Six patients showed moderate ventricle enlargement, associated to cortical brain atrophy in three.
3. **Other brain abnormalities**: Left HS, right frontal dysplasia, reduced size of the vermis (associated with frontal atrophy), and supratentorial white matter hyperintensity (associated with brain atrophy) occurred in one case (1.6%) each.

For 8 out of the 13 patients showing MRI abnormalities, a previous imaging study was available (mean age 5.5 years; range 2–7). Brain abnormalities were present in all of these cases and no significant changes were observed during the MRI follow-up.

Moreover, there was no significant difference in the number of episodes of status epilepticus that had occurred within the first 4 years of life between patients showing abnormal MRI (mean: 4.9 ± 7 episodes; range: 0–30) and those with normal imaging (mean: 6.9 ± 6.7 episodes; range: 0–20) (p = 0.06).

**Genotype–phenotype correlations**

Genotype–phenotype correlations are summarized in Table 2. Male/female ratio was 1.7:1 (20/35 males) in the mutated group and 1.7:1 (13/23 males) in the nonmutated group (p = 1.00). Mean age at seizure onset was 5 months (range: 3–8) in the mutated group and 5.5 months (range: 3–8) in the nonmutated group (p = 1.00). The mean age at the time of last MRI was 9.4 ± 5.2 years (range: 4.1 months to 25 years) in the mutated and 8.1 ± 5 years (range: 4–22 years) in the nonmutated group. This difference was not statistically significant (p = 0.09).

Genotype–phenotype analysis indicated that the abnormal MRIs were significantly more frequent in patients without SCN1A mutations (9/23; 39.1%) compared to those carrying SCN1A mutations (4/35; 11.4%) (p = 0.02) (Table 2).

**DISCUSSION**

Our findings confirm that brain abnormalities may occur in SMEI (Reiner and Renkawek, 1990; Dravet et al.,

---

**TABLE 1. Abnormal MRI findings in SMEI patients**

<table>
<thead>
<tr>
<th>Patients ID</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nav1.1 defect</td>
<td>M1333X</td>
<td>L1426R</td>
<td>R393C</td>
<td>R1245Q</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Age at MRI (years)</td>
<td>2</td>
<td>6</td>
<td>4</td>
<td>4.5</td>
<td>21</td>
<td>1</td>
<td>3</td>
<td>13</td>
<td>1</td>
<td>2</td>
<td>5</td>
<td>4</td>
<td>16</td>
</tr>
<tr>
<td>MRI findings</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain atrophy</td>
<td>+ frontal</td>
<td>+ occipital</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ventricles enlargement</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>White matter hyperintensity</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Cerebellar atrophy</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Focal cortical dysplasia</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Hippocampal sclerosis</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

*Plus signs indicate the presence of specific MRI abnormalities; minus signs indicate the absence of such abnormalities. N, not mutated in SCN1A.*

_Epilepsia, Vol. 48, No. 6, 2007_
The duration of epilepsy and age at seizure onset did not correlate with the presence of MRI abnormalities. Moreover, we did not observe a correlation between abnormal MRIs and the frequency of episodes of status epilepticus having occurred within the first 4 years of life.

Genotype–phenotype correlations indicate that abnormal MRIs were significantly more frequent in patients without SCN1A mutations. Four subjects carrying SCN1A mutations showed brain abnormalities at MRI. In one case (pt 4), left HS was documented at the age of 4.5 years and subsequently confirmed at the age of 9 years. In this case, a prolonged left hemiclonic FS at the age of 5 months was the initial manifestation. Subsequently, he experienced repeated (thirty episodes within 4 years of life) prolonged febrile and afebrile alternating hemiconvulsions; thus, it was not possible to search for an association between the side of seizures and HS laterality.

The association between FS and HS has been reported to be strongest for patients with complex or prolonged seizures (VanLandingham et al., 1998) and, recently, HS was reported in 10 out of 14 SMEI patients aged 1.2–16 years (mean: 7.2 years) occurring several months or years after the first seizure (Siegler et al., 2005). In our series, despite most of the patients experienced complex and prolonged FS, only one case with documented HS was observed. Thus, the present data do not support evidence of association between early-prolonged FS and HS, at least in SMEI.

The origin of the other focal abnormalities (i.e., frontal and occipital brain atrophy, right frontal cortical dysplasia) in three children with SCN1A mutations remains unknown. However, in these cases, no focal neurological signs or EEG abnormalities were observed in keeping with the site of the atrophy. Thus, the coexistence of different localized brain lesions in four SCN1A mutant patients could be incidental. However, besides SCN1A mutations, we cannot exclude that the structural brain lesions may have a role in the epileptic encephalopathy in these cases.

Electrophysiological studies in cellular and animal models indicate that epileptogenic mutations in ion channels impair the membrane properties of neurons, thus increasing the probability that neurons will burst in response to normal inputs (George, 2004). Moreover, specific brain abnormalities are not commonly observed in human epileptic inherited channelopathies, including generalized epilepsy with febrile seizures plus (GEFS+) in which SCN1A mutations may be found (Singh et al., 2001; Mulley et al., 2003). The lower incidence of abnormal MRIs in SMEI patients with SCN1A mutations confirms that the hyperexcitability underlying epilepsy in this syndrome has functional rather than structural origin.

Although no specific MRI changes could be identified among the mutation-negative patients, diffuse brain atrophy occurred only in this group. Cross-sectional and longitudinal MRI studies in epileptic patients have demonstrated focal or diffuse brain atrophy, although it is still debated whether its severity is correlated with the duration of epilepsy, seizure frequency, or lifetime seizure number (Kalviainen and Salmenpera, 2002; Liu et al., 2003; Cendes, 2005; Raspall-Chaure et al., 2006). However, diffuse brain atrophy has been already reported in other SMEI patients (Dravet et al., 2005). Putative underlying mechanisms for brain atrophy are probably diverse and complex and include wallerian degeneration, apoptotic cell death, inflammation, and excitotoxicity (Liu et al., 2003). Brain atrophy could be in some cases related to chronic treatment with AEDs. The majority of our patients had been or was on valproate therapy, which has been associated to pseudoatrophy of the brain (Guerrini et al., 1998). The proportion of patients using valproate is approximatively similar for patients with SCN1A mutations and those without SCN1A mutations. Thus, it is unlikely that the use valproate affected our results.

In our series, brain abnormalities did not show a progressive course and were significantly clustered in patients without SCN1A mutations. These structural abnormalities could be involved in the mechanisms of epileptogenicity in our cases; alternatively, they might be expression of abnormal inherited or acquired—circuitries possibly involved in the etiology of epilepsy in these cases.

The presence of patients fitting clinical criteria of SMEI and lacking SCN1A mutations suggests that different genetic or acquired factors other than SCN1A may lead to the SMEI phenotype. Thus, it is likely that SMEI includes different conditions. So far, SMEI patients without SCN1A mutations cannot be differentiated from those showing SCN1A mutations on clinical ground. In the present study we revised MRI data of a large cohort of patients to address this clinical issue and we found a correlation between MRI abnormalities and lack of SCN1A mutations.

### Table 2. Genotype–phenotype correlations in 58 SMEI patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mutated group n = 35</th>
<th>Nonmutated group n = 23</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female ratio</td>
<td>1.7:1 (20/34)</td>
<td>1.7:1 (13/24)</td>
<td>1.00</td>
</tr>
<tr>
<td>Mean seizure onset (years)</td>
<td>5 months (range: 3–8)</td>
<td>5.5 months (range: 3–8)</td>
<td>1.00</td>
</tr>
<tr>
<td>Mean age at last MRI (years)</td>
<td>9.4 ± 5.2 years (4.1 months to 25 years)</td>
<td>8.1 ± 5 years (4–22 years)</td>
<td>0.09</td>
</tr>
<tr>
<td>Abnormal MRI findings</td>
<td>4/35 (11.4%)</td>
<td>9/23 (39.1%)</td>
<td>0.02*</td>
</tr>
</tbody>
</table>

* Statistically significant.
However, nonspecific MRI abnormalities were identified and additional clinical clues should be identified to dissect this complex phenotype.

A limitation of the present study is the qualitative assessment of MRI images. Furthermore, we cannot exclude that very subtle malformations of cortical development could have been missed, although imaging sequences included a high-resolution tridimensional volume acquisition in most cases. Additional research and prospective, quantitative MRI studies are required to further address this issue and ultimately confirm our findings.

Acknowledgments: This work was supported by the Italian Ministry of Health (no. 132/03 to Federico Zara and Carla Marini) and the Italian League against Epilepsy. The authors wish to thank A. Bianchi for his contribution.

REFERENCES


