Cryptogenic Epileptic Syndromes Related to SCN1A

Twelve Novel Mutations Identified

Claudio Zucca, MD; Francesca Redaelli, PhD; Roberta Epifanio, MD; Nicoletta Zanotta, MD; Antonino Romeo, MD; Monica Lodi, MD; Pierangelo Veggiotti, MD; Giovanni Airoldi, MD; Cinzia Baschirotto, BS; Loreto Martorell, PhD; Renato Borgatti, MD; Nereo Bresolin, MD; Maria Teresa Bassi, PhD

Background: Sodium channel alpha 1 subunit gene, SCN1A, is the gene encoding the neuronal voltage-gated sodium channel α1 subunit (Na_1.1) and is mutated in different forms of epilepsy. Mutations in this gene were observed in more than 70% of patients with severe myoclonic epilepsy of infancy (SMEI) and were also found in different types of infantile epileptic encephalopathy.

Objective: To search for disease-causing mutations in SCN1A in patients with cryptogenic epileptic syndromes (ie, syndromes with an unknown cause).

Design: Clinical characterization and molecular genetic analysis of a cohort of patients.

Setting: University hospitals, rehabilitation centers, and molecular biology laboratories.

Patients: Sixty unrelated patients with cryptogenic epileptic syndromes.

Main Outcome Measures: Samples of DNA were analyzed for mutations and for large heterozygous deletions encompassing the SCN1A gene. A search for microdeletions in the SCN1A gene was also performed in the subset of patients with SMEI/SMEI-borderland who had negative results at the point mutation screening.

Results: No large deletions at the SCN1A locus were found in any of the patients analyzed. In contrast, 13 different point mutations were identified in 12 patients: 10 with SMEI, 1 with generalized epilepsy with febrile seizures plus, and 1 with cryptogenic focal epilepsy. An additional search for SCN1A intragenic microdeletions in the remaining patients with SMEI/SMEI-borderland and no point mutations was also negative.

Conclusions: These results confirm the role of the SCN1A gene in different types of epilepsy, including cryptogenic epileptic syndromes. However, large deletions encompassing SCN1A were not common disease-causing rearrangements in this group of epilepsies.
It was recently found that intragenic and whole SCN1A gene deletions also occur in a variable percentage (8%-27%) of patients with SMEI without point mutations in SCN1A.16-21 The overlapping yet heterogeneous clinical features of the epilepsy syndromes associated with SCN1A mutations recently led a group of researchers to search for mutations in this gene in a large sample of unselected patients with epileptic encephalopathies (including SMEI) with onset primarily during the first year of life.4 In that study, they identified mutations in patients with cryptogenic generalized epilepsy (CGE) and cryptogenic focal epilepsy (CFE) and in a subgroup classified as having severe infantile multifocal epilepsy.

Based on these results, we decided to screen the SCN1A gene for mutations and whole gene deletions in 60 patients with cryptogenic epileptic syndromes with onset in the first 2 years of life to evaluate the prevalence of SCN1A abnormalities in this heterogeneous group of patients. All the patients with SMEI and SMEB who had negative results at the point mutation screening were also analyzed for the presence of intragenic heterogeneous deletions in the SCN1A gene. The results of these screenings are presented and discussed.

METHODS

PATIENTS

Sixty patients (59 from Italy and 1 from Spain), selected based on the criteria detailed herein, were referred for molecular analysis to the Laboratory of Molecular Biology at the E. Medea Scientific Institute, Lecco, Italy. These patients were first seen with an epileptic syndrome of unknown cause, which started in the first 2 years of life; this diagnosis was based on normal magnetic resonance imaging findings and negative results of metabolic and cytogenetic investigations.

Each patient has been followed up for a variable period (2-28 years) since the onset of the epileptic seizures. Clinical data were obtained from all the patients, and particular attention was given to family history of seizure disorders; seizure onset, type, frequency, and precipitating factors; drug response; and evolution of electroclinical data. Several polygraphic-polysomnographic electroencephalograms were recorded. The patients enrolled in this study could, thus, be classified into 6 different subgroups according to the International League Against Epilepsy classification of epileptic syndromes22 and the International League Against Epilepsy proposed diagnostic scheme for epilepsies23: SMEI (n=14), SMEB (n=6), GEFS+ (n=12), West syndrome (n=7), CFE and multifocal epilepsies (n=8), and CGE (n=13). The term SMEB was used for cases of SMEI without several key features of SMEI.24 All the patients with West syndrome enrolled in this study were negative for mutations in the genes known for this disease (aristaless-related homeobox [MIM 300382, GenBank NM_139058] and cyclin-dependent kinase-like 5 [MIM 300203, GenBank NM_003159]). The CGE subgroup comprises 4 cases of Lennox-Gastaut syndrome. The ethics committee of the E. Medea Scientific Institute approved the study. Informed consent was obtained from the parents of children and from adults of normal intellect.

MOLECULAR 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) using flanking intronic primers and standard PCR conditions. The PCR fragments were sequenced using a kit (BigDye Terminator Sequencing Kit; Applied Biosystems, Foster City, California) and were run on a genetic analyzer (ABI 3130 XL; Applied Biosystems). The SCN1A mutation nomenclature is based on RefSeq AB093548.1 (considering the A of the ATG as nt 1) according to the recommendations of the Human Genome Variation Society. The appropriate PCR fragment from parents’ DNA (where available) was sequenced in all cases in which an SCN1A mutation was detected to distinguish between de novo and familial variants. All the reported nucleotide changes were checked in a panel of 250 control subjects. Quantitative PCR was performed using probes designed on intron 3 and exons 8 and 26 of the SCN1A gene. The reactions were run on a detection system (ABI 7900HT Sequence Detection System; Applied Biosystems). Multiplex ligation-dependent probe amplification analysis was performed using a kit (SALSA MLPA P137 Kit; MRC-Holland, Amsterdam, the Netherlands). Possible changes in exonic splicing enhancers were assessed using the exonic splicing enhancer finder algorithm (accessible at http://rulai.cshl.edu/tools/ESE).

RESULTS

Of the 60 patients analyzed, 12 were found to carry mutations in SCN1A: 10 with SMEI, 1 with GEFS+ and 1 with CFE. No mutations were found in the remaining epilepsy subgroups. The clinical features of patients with mutations are summarized in Table 1 and described in the following subsections, grouped by the type of epilepsy syndrome.

SEVERE MYOCLONIC EPILEPSY OF INFANCY

All patients with mutations were born after an uneventful pregnancy and delivery. Epilepsy started at 3 to 8 months of life in all patients with febrile (n=8) or afebrile (n=2) generalized (n=5) or hemiclonic (n=5) seizures changing sides. Prolonged seizures or status epilepticus was reported in all the patients except 3. Seven patients started experiencing segmental or massive myoclonus jerks after 12 months of age; 4 developed partial seizures and 5 experienced atypical absence seizures; 1 patient had tonic seizures. At the neurologic examination, 4 patients developed ataxia. Five patients had severe mental retardation, while 4 showed only mild cognitive deficits. Each patient was treated with more than 3 antiepileptic drugs without achieving complete seizure control.

CRYPTOGENIC FOCAL EPILEPSY

One patient had a family history of unspecified epilepsy in a maternal uncle, although both parents were healthy. The delivery was complicated, and the patient has had mild psychomotor retardation since the first year of life, with lateralized (right) neurologic signs and a left ptosis observed at age 10 months. Epilepsy started at age 3 months with generalized tonic-clonic seizures. Hemiclonic seizures involving only the left side subsequently occurred, sometimes followed by febrile and afebrile convulsive status epilepticus. This patient is now severely
mentally retarded. Electroencephalograms revealed slowed background activity and bursts of epileptiform abnormalities bilaterally over the frontal areas.

**GENERALIZED EPILEPSY WITH FEBRILE SEIZURES PLUS**

The family history for this patient was unavailable. The patient’s parents agreed to donate blood for genetic analysis but refused to be clinically evaluated. The patient was born after an uneventful pregnancy and delivery. Psychomotor development was normal. Epilepsy started in the first year of life with febrile generalized tonic-clonic seizures. Generalized afebrile tonic-clonic seizures appeared at age 3 years. He has never experienced prolonged seizures or status epilepticus. The patient is now 6 years old, and the seizures are well controlled with sodium valproate therapy. His intelligence and neurologic examination results are normal.

**MOLECULAR ANALYSIS**

Mutation screening of *SCN1A* performed by direct sequencing of all exons led to the identification of 13 mutations in 12 patients (Table 2). Two truncating mutations in exon 1 (p.Y63X) and exon 22 (p.W1434X) and 8 missense mutations distributed in exon 2 (p.R118S), exon 8 (p.D366E and p.R377Q), exon 18 (p.L1207P), exon 21 (p.V1335M and p.V1358S), exon 23 (p.Y1462C), and exon 26 (p.R1928G) were found. Mutations p.L1207P and p.R1928G were present in the same patient (Table 2). Frameshift mutations due to a single base deletion in exon 19 (c.3774delA) or a 4-base pair deletion in exon 26 (c.5536_5539delAAAC) were also identified in 2 patients. The 2 deletions cause premature stop codon 63 and 10 base pairs downstream (p.L1296fs and p.K1846fs), respectively. A single splice mutation affecting the donor splice site of exon 8 (c.1170 + 1 G→A) was identified in 1 patient. No cell line from the patient was available to check the mutation effect on gene transcription; however, the base change most likely leads to either exon skipping or use of a cryptic splice donor site. All the mutations identified are novel except for c.5536_5539delAAAC, which has already been reported.13,14,21 None of the novel mutations were present in a panel of 250 controls. The mutations are scattered throughout the Na.1.1 subunit protein, with the S5–S6 segments (the pore-forming regions) of 2 domains, DI and DIII, being the more frequent targets (Table 2). Most of the mutations found, which could be tested for segregation within the families (8 of 9), turned out to be de novo. In addition to the point mutation screening, we wanted to exclude the presence of large heterozygous deletions encompassing the entire *SCN1A* gene in all point mutation-negative patients. Quantitative PCR was, thus, performed using probes specific for intron 3 and exons 8 and 26 of the *SCN1A* gene. No large heterozygous *SCN1A* deletions could be found in any of the patients tested. The *SCN1A* intragenic microdeletions were also excluded by using the multiplex ligation-dependent probe amplification technique only in patients with SMEI/SMEB who had negative results at point mutation screening.

### Table 1. Clinical and Electroclinical Features of Patients With SCN1A Mutations

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<td>15</td>
<td>12</td>
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<td>5</td>
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<td>SMEI</td>
<td>SMEI</td>
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<td>+</td>
<td>+</td>
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<td>3</td>
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<td>6</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>–</td>
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<td>SI</td>
<td>SI</td>
<td>SI</td>
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<td>SI</td>
<td>N</td>
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<td><strong>Photoparoxysmal response</strong></td>
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<td>+</td>
<td>–</td>
<td>–</td>
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<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>NA</td>
</tr>
</tbody>
</table>

Abbreviations: ABS, absence seizures; Bab, Babinski; CFE, cryptogenic focal epilepsy; EEG, electroencephalographic; GEFS+, generalized epilepsy with febrile seizures plus; GTC, generalized tonic-clonic; H, hemiclonic; M, moderate; MI, mild; MR, mental retardation; MRI, magnetic resonance imaging; N, normal; NA, not available; NAD, nonabnormal data; ref, refractory; resp, responsive; S, severe; SI, slowly; SMEI, severe myoclonic epilepsy of infancy; +, present; –, absent.

*1* Always on the same side (see the “Results” section).
COMMENT

Sixty patients with cryptogenic epileptic syndromes characterized by seizure onset in the first 2 years of life were tested for point mutations and whole gene deletions in SCN1A. No large deletions including the SCN1A gene were found in any patients, indicating that these rearrangements are not frequently associated with cryptogenic epileptic syndromes. However, 13 SCN1A point mutations were detected in 12 patients. Most of these patients have SMEI (10 of 12), with the remaining having GEFS+ and CFE.

SEVERE MYOCLOMIC EPILEPSY OF INFANCY

The large percentage of SMEI in patients with SCN1A mutations was expected based on the reported data. In the present study, 10 (71%) of 14 patients with SCN1A analyzed showed SCN1A mutations, and this is in line with the results obtained in the most recently reported large patient sample screenings. One patient with SMEI carried 2 heterozygous mutations in exons 18 and 26 of SCN1A. Both changes affect evolutionary conserved residues and fall within gene regions representing frequent targets of mutations. However, we cannot exclude that at least 1 of the changes represents a rare variant. These data, together with the results of the deletion screening, indicate that SCN1A mutation detection in patients with SMEI is still lower than 100%. This reinforces the idea that the molecular genetic basis of SMEI is not fully known yet, as was previously hypothesized.

GENERALIZED EPILEPSY WITH FEBRILE SEIZURES PLUS

We observed an SCN1A mutation in 1 GEFS+ patient (patient 9) of the 12 analyzed (8%). This percentage is consistent with the reported frequency of SCN1A mutations in families with GEFS+ (5%-10%). Other genes known to be involved in this type of epilepsy, such as SCN1B, SCN2A, and GABRG2, still need to be screened in this sample.

CRYPTOGENIC FOCAL EPILEPSY

Unlike in the results of a recent study, only 1 of 8 patients with CFE in our study had an SCN1A mutation (12.5% vs 22%). The only mutation found was the c.5536_5539delAAAC deletion, which has been reported so far in 4 other patients with a different clinical diagnosis. The patient described herein is classified as having CFE owing to the presence of focal electroencephalographic abnormalities, hemiclonic seizures occurring always on the same side, and lateralized neurologic deficits. Three patients already described with this mutation had SMEI, and a fourth was defined as having SMEB without generalized spike waves. The identification by different researchers of the same mutation in apparently different clinical phenotypes might be due to either a blurred clinical distinction between these epileptic syndromes or other genetic or environmental factors playing a role in the expression of the epilepsy phenotype. This idea would be consistent with the marked variability widely reported in patients with SMEI and related parents carrying the same mutation and with the hypothesis of a polygenic origin of SMEI, as previously suggested. Functional data on the mutant protein will contribute to addressing this issue.

The negative results obtained in the other subgroups of patients, such as the West and Lennox-Gastaut syndromes subgroups and the CGE subgroup, are only partly unexpected. Indeed, the previous identification of only 1 SCN1A mutant case in the West and Lennox-Gastaut syndromes, together with the present negative results, indicates that SCN1A is a rare cause of disease in these syndromes. These findings also suggest that an SCN1A mutation search may not be necessary in routine diagnostic practice for these forms of epilepsy, but rather that it should be considered for research purposes.

Overall, the negative results at the SCN1A mutation screening in the CGE subgroup, compared with the remarkable percentage (24%) of patients with SCN1A mutations in the CGE subgroup analyzed by Harkin and colleagues, might be explained by the likely clinical heterogeneity between the 2 CGE subgroups tested. The

Table 2. SCN1A Mutations

<table>
<thead>
<tr>
<th>Patient No./Phenotype</th>
<th>Amino Acid Change</th>
<th>ESE Domain</th>
<th>Exon/Intron</th>
<th>SCN1A Inheritance</th>
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</thead>
<tbody>
<tr>
<td>2/SMEI c.195T&gt;A</td>
<td>p.Y65X</td>
<td>NA</td>
<td>1</td>
<td>NH2 ter</td>
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<tr>
<td>1/SMEI c.354G&gt;C</td>
<td>p.R118S</td>
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<td>2</td>
<td>NH2 ter</td>
</tr>
<tr>
<td>10/SMEI c.1098T&gt;A</td>
<td>p.D366E</td>
<td>Yes</td>
<td>8</td>
<td>DI-S5-S6</td>
</tr>
<tr>
<td>9/GEFS+ c.1130G&gt;A</td>
<td>p.R377Q</td>
<td>Yes</td>
<td>8</td>
<td>DI-S5-S6</td>
</tr>
<tr>
<td>11/SMEI c.1170+1G&gt;A</td>
<td>NA</td>
<td>IVS8</td>
<td>NA</td>
<td>DI-S5-S6</td>
</tr>
<tr>
<td>3/SMEI c.3774delA</td>
<td>p.L1269fs</td>
<td>NA</td>
<td>19</td>
<td>DIII-S2</td>
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<tr>
<td>5/SMEI c.4030G&gt;A</td>
<td>p.V1335M</td>
<td>21</td>
<td>Yes</td>
<td>DIII-S4-S5</td>
</tr>
<tr>
<td>8/SMEI c.4073G&gt;C</td>
<td>p.W1358S</td>
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<tr>
<td>7/SMEI c.4301G&gt;A</td>
<td>p.W1434X</td>
<td>22</td>
<td>NA</td>
<td>DII-S4-S5</td>
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<tr>
<td>6/SMEI c.4385A&gt;G</td>
<td>p.Y1462C</td>
<td>23</td>
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<td>DII-S6</td>
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<tr>
<td>4/CFE c.5536_5539delAAC</td>
<td>p.K1846fs</td>
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<tr>
<td>12/SMEI c.5782C&gt;G</td>
<td>p.R1928G</td>
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<td>Yes</td>
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<tr>
<td>11/SMEI c.3620T&gt;C</td>
<td>p.L1207P</td>
<td>18</td>
<td>No</td>
<td>DII-DIII linker</td>
</tr>
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</table>

Abbreviations: CFE, cryptogenic focal epilepsy; COOH ter, COOH terminus; ESE, exonic splicing enhancer; GEFS+, generalized epilepsy with febrile seizures plus; NA, not available; ND, not determined; NH2 ter, NH2 terminus; SMEI, severe myoclonic epilepsy of infancy.
same reason may explain the different percentages of SCN1A mutants in the CFE subgroups as well. Such heterogeneity may originate from the different types of epilepsy examined in the 2 studies: the severe epileptic encephalopathies with onset in the first year of life in the first study vs the cryptogenic epileptic syndromes with onset in the first 2 years of life tested herein. Based on this, the present data seem to indicate that the earlier and more severe the clinical and electroencephalographic presentations are, the higher the probability of finding mutations in SCN1A is. However, additional experimental evidence is required to confirm this hypothesis.

SCN1A MUTATIONS

Twelve of the 13 SCN1A mutations identified in this study are novel, thus supporting the concept of mutational heterogeneity that is typical of SCN1A. Most of the mutations found (8 of 9 tested) were de novo, consistent with the data in the literature.4

Seven of the 13 mutations found, including missense, nonsense, and deletions, fall within the S5-S6 segments of the DI and DIII domains of the Na1,1 subunit. Whereas the pathogenic effect of a truncating mutation is clear, the postulated pathogenicity of missense mutations falling within these segments needs functional demonstration. These segments represent the pore-forming region of the channel subunit; therefore, any change affecting the properties of the constituting residues, such as the charge or the steric hindrance (as in mutations p.V1335M and p.Y1462C) or both (as in mutations p.R377Q and p.W1358S), is supposed to variably affect channel activity. In addition, all the identified missense mutations change an evolutionary conserved amino acid residue, and all but 3 (p.W1358S, p.Y1462C, and p.L1207P) determine a putative exonic splicing enhancer sequence change. These sites are well-known to play a role in constitutive and alternative splicing events.20

In conclusion, this study represents the second systematic study of SCN1A mutations in cryptogenic epilepsies and confirms the findings of the first study conducted on a larger sample of patients, although with different results for some types of epilepsy. The results of these 2 studies are supported and completed by the previous findings of SCN1A mutations in a condition previously considered to be symptomatic, such as the alleged vaccine encephalopathies (mutations found in 11 of 14 patients) or in adult cases with refractory epilepsy and normal magnetic resonance imaging findings with onset in infancy (10 of 14 patients).5

These data altogether extend the spectrum of the clinical phenotypes associated with SCN1A mutations to include SMEI and other epileptic encephalopathies and different types of cryptogenic epilepsies with clear diagnostic implications. All these studies are actually single pieces of an apparently big puzzle representing the wide and heterogeneous spectrum of pathologic manifestations associated with Na1,1 subunit dysfunctions.

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Author Affiliations: Clinical Neurophysiology Unit (Drs Zucca, Epifanio, and Zanotta), Laboratory of Molecular Biology (Drs Redaelli, Airoldi, Panzeri, Bresolin, and Bassi and Ms Baschirotto), and Department of Neurorehabilitation (Drs Romaniello and Borgatti). E. Medea Scientific Institute, Lecco, Italy; Epilepsy Center, Department of Child Neuropsychiatry and Neurophysiology, Fatebenefratelli e Ospedaliero Hospital, Milan, Italy (Drs Romeo and Lodi); Department of Child Neurology and Psychiatry, IRCCS C. Mondino Foundation, University of Pavia, Pavia, Italy (Dr Veggio); Clinical Neurophysiology Unit, E. Medea Scientific Institute, Conegliano, Italy (Drs De Polo and Bonanni); Child Neuropsychiatry Unit, Azienda Ospedaliera G. Salvini, Garbagnate Milanese, Milan (Dr Cardinali); Molecular Genetics Section, Sant Joan de Déu Hospital, Barcelona, Spain (Dr Martorell); and Dino Ferrari Center, Fondazione IRCCS Ospedale Maggiore Policlinico, Mangiagalli e Regina Elena, Department of Neurological Sciences, University of Milan, Milan (Dr Bresolin).

Correspondence: Maria Teresa Bassi, PhD, Laboratory of Molecular Biology, E. Medea Scientific Institute, Via D. L. Monza 20, 23842 Bosio Parini, Lecco, Italy (mariateresa.bassi@bp.lnf.it).

Author Contributions: Dr Bassi had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Zucca and Bassi. Acquisition of data: Zucca, Redaelli, Epifanio, Zanotta, Romeo, Lodi, Veggio, Airoldi, Panzeri, Romaniello, De Polo, Bonanni, Cardinali, Baschirotto, Martorell, Borgatti, Bresolin, and Bassi. Analysis and interpretation of data: Zucca, Redaelli, Epifanio, Zanotta, Romeo, Lodi, Veggio, Airoldi, Panzeri, Romaniello, De Polo, Bonanni, Cardinali, Baschirotto, Martorell, Borgatti, Bresolin, and Bassi. Drafting of the manuscript: Zucca, Redaelli, Epifanio, Zanotta, Romeo, Lodi, Veggio, Airoldi, Panzeri, Romaniello, De Polo, Bonanni, Cardinali, Baschirotto, Martorell, Borgatti, Bresolin, and Bassi. Critical revision of the manuscript for important intellectual content: Zanotta, Veggio, Airoldi, Romaniello, Bonanni, Cardinali, and Martorell. Obtained funding: Zucca, Romeo, Lodi, Veggio, De Polo, Bonanni, Borgatti, and Bassi. Administrative, technical, and material support: Baschirotto. Study supervision: Zucca, Redaelli, Epifanio, Zanotta, Romeo, Lodi, Veggio, Airoldi, Panzeri, Romaniello, De Polo, Cardinali, Baschirotto, Martorell, Borgatti, Bresolin, and Bassi. Financial Disclosure: None reported.

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