A new molecular mechanism for severe myoclonic epilepsy of infancy: Exonic deletions in SCN1A
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Severe myoclonic epilepsy of infancy (SMEI) [MIM 607208] is a devastating encephalopathy of childhood in which 80% of cases are associated with de novo mutations in the sodium channel SCN1A gene. The gene encoding the α1-subunit of the sodium channel SCN1A [MIM 182389] is established as the most clinically relevant of all the known epilepsy genes, with more than 100 different pathogenic mutations characterized. Mutations are dominant and result in a range of clinical severity from the usually mild phenotypes of the familial syndrome generalised epilepsy with febrile seizures plus [MIM 604233] to the most severe phenotypic expression in SMEI. Most SMEI-associated mutations are de novo; however, at least 20% of a large number of well-defined clinical cases do not have detectable coding mutations in any known genes, including SCN1A.

We explored the hypothesis that whole exon deletions or duplications (undetectable by standard mutation screening in the presence of a normal chromosomal homologue) could account for these residual SMEI cases. Such microchromosomal aberrations are commonly associated with certain genetic diseases, e.g., Duchenne and Becker muscular dystrophies, and mental impairment associated with subtelomeric rearrangement. Multiplex ligation-dependent probe amplification (MLPA) is a new diagnostic tool that efficiently detects genomic deletions and duplications of pathogenic significance.

Methods. From a cohort of 64 clinically diagnosed SMEI patients screened by highly sensitive denaturing high-performance liquid chromatography (Transgenomic WAVE), all but 13 (20%) had the expected pathogenic molecularly diagnosed coding and splicing mutations of SCN1A of the nature previously characterized and catalogued. All 64 patients in the cohort had classic features of SMEI, with onset of convulsive seizures, usually precipitated by fever, in the first year of life. These were followed by the development of other seizure types including myoclonic and partial seizures; the EEG showed generalized spike-wave activity. Development was normal in the first year, and then it slowed. There were no consistent phenotypic differences between those with and those without detectable mutations. The Austin Health Human Research Ethics Committee approved this study. Informed consent was obtained for all subjects.

From the 13 cases with no detectable SCN1A mutation, the DNA was re-extracted using a Qiagen QIAquick PCR purification kit. We performed MLPA using the custom-made SALSA MLPA P137 SCN1A kit (MRC-Holland, Amsterdam, The Netherlands). This generates one unique probe for each of 25 of the 26 SCN1A coding exons and a set of control sites outside this gene. Application of the probe set simultaneously detects any number of adjacent exon deletions or duplications of SCN1A, with probes mismatched to the related SCN2A and SCN3A exon sequences to ensure SCN1A exon specificity.

MLPA analysis was carried out using the manufacturer’s instructions. PCR products were separated and quantified by capillary electrophoresis on an ABI 3100 Avant DNA analyzer (Applied Biosystem), using GeneMapper analysis software (version 3.7). The area under the curve of each peak, as calculated by GeneMapper and each representing a known exon, was transferred to an Excel file. The area of each peak was then divided by the total area of all peaks in each case. Then the ratio for each peak in all samples was averaged. The individual peak ratios are then normalized by dividing them by the average ratio for that peak. A normal result from this calculation is 1.0, a deletion 0.5, and duplication 1.5. We set thresholds of <0.75 for deletions and >1.25 for duplications.

Results. Quantitative MLPA analysis applied to those 13 children with no detectable coding mutations detected two children (15%; 2/13) with unequivocal SCN1A exonic deletions.

Neither child had any distinguishing clinical features compared to the remainder of the SMEI cohort. One had a deletion of the contiguous SCN1A exons 21-26. In this case, the repeatable haploid loss of any one exon is inter-
nally validated by concurrent haploid loss of the other five contiguous exons.

The second child had a repeatable detectable deletion only of exon 21 sequence. In the absence of confirmatory deletions of adjacent exons, exon 21 was sequenced to exclude rare mismatch between the exon-specific probe and its target DNA sequence, thereby excluding interference to primer binding and thus validating the presence of this deletion.

To determine the size and breakpoints of the deletion that excised the exon 21 sequence, we carried out a set of long-range PCR amplifications (Platinum Taq DNA Polymerase High Fidelity from Invitrogen) using nested primers designed at regular intervals from the sequence within the flanking introns. These reactions amplified fragments of predicted size from the intact wild-type chromosome, and we looked for the presence of a smaller fragment from the deleted homologue. Sequence analysis of such a fragment spanning the deleted region revealed that the deletion was 6,499 bp in size. The deletion, IVS20 + 1195_1195 del TATT, removes 5,781 bp of intron 20 adjacent to exon 21, all of exon 21, and 346 bp of intron 21 adjacent to exon 21. DNA repair was accompanied by an insertion of 4 bp (TATT) where the DNA breakpoints rejoined.

Parental testing confirmed a de novo origin of the deletion in both patients, consistent with the origin of most sporadic cases of SMEI in those who have the conventional coding and splice site mutations. Neither patient had any consistent phenotypic differences from those cases with other types of mutation or with remaining cases of clinically typical SMEI in which no mutations of any type have yet been detected.

Discussion. Both deletions are predicted to be pathogenic. Exon 21 affects the DIIIS5-S6 loop of the SCN1A protein. The exon 21-26 deletion truncates the SCN1A protein at the same position. A similar STOP truncation (R1407X) in the same region has previously been reported twice as the pathogenic basis for SMEI.

MLPA should be considered an important second-tier testing strategy for children with SMEI if initial sequencing of SCN1A does not detect coding or splice site mutations. These studies need to be extended to other cohorts to determine a more accurate estimate of deletion frequency, given that our initial estimate was based only on 13 sequence-negative patients satisfying all the clinical diagnostic criteria for SMEI.

The diagnosis of SMEI remains clinical; finding an appropriate molecular abnormality provides strong laboratory support for the diagnosis. Diagnosis aids selection of antiepileptic medication as specific drugs are effective and others may exacerbate seizures in this syndrome. Failure to detect SCN1A mutations should not change the choice of drug therapy based on the initial clinical diagnosis because molecular testing can support but not exclude the clinical diagnosis. Further, making a molecular diagnosis clarifies the etiology, leads to avoidance of potentially invasive tests when a deletion is found, and allows definitive genetic counseling and molecular testing for the families of affected children.

Note added in proof. Suls L, Claeys KG, Goossens D, et al. (Hum Mutat 2006 [Epub ahead of print]) reported three cases of microchromosomal deletions in patients with features of SMEI which included deletions for one copy of the entire SCN1A gene. Thus, deletions involving one copy of SCN1A can include as little as a single coding exon within SCN1A as our report using MLPA demonstrates, or include copies of as many as 21 additional contiguous genes as demonstrated using the approach of Suls et al.

References

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