Parental mosaicism in epilepsies due to alleged de novo variants

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Summary

Severe early-onset epilepsies are often caused by de novo pathogenic variants. Few studies have reported the frequency of somatic mosaicism in parents of children with severe epileptic encephalopathies. Here, we aim to investigate the frequency of mosaicism in the parents of children with epilepsy caused by alleged de novo variants. We tested parental genomic DNA derived from different tissues for 75 cases using targeted next generation sequencing. Five parents (6.6%) showed mosaicism at minor allele frequencies of 0.8 to 29% for the pathogenic variant detected in their offspring. Parental mosaicism was observed in the following genes: SCN1A, SCN2A, SCN8A and STXBP1. One of the identified parents had epilepsy himself. Our results show that de novo events can occur already in parental tissue and in some cases can be detected in peripheral blood. Consequently, parents affected by low-grade mosaicism are faced with an increased recurrence risk for transmitting the pathogenic variant, compared to the overall recurrence risk for a second affected child estimated at approximately 1%. However, testing for parental somatic mosaicism will help identifying those parents that truly are at higher risk and will significantly improve genetic counseling in the respective families.

Introduction

During the last decade, next-generation sequencing has led to a virtual explosion of gene discovery, raising the number of epilepsy genes to more than 200, explaining 20-25% of all cases with severe early-onset epilepsies that had otherwise no identifiable cause. Most of the cases are caused by de novo pathogenic variants. The overall recurrence
risk for genetic disorders due to alleged de novo variants is estimated to be around 1%\(^2\), due to the risk of parental mosaicism\(^3\). Mosaicism in human genetics refers to a person harboring two or more unique clonal cell populations. In case of parental mosaicism, the respective parent of an affected child carries a cell population without the pathogenic variant, but also a proportion of cells carrying the pathogenic variant. This variant emerged spontaneously in a single cell and will be present in all subsequent cells descending from that particular cell. Mosaic parents are at increased risk of transmitting the disease-associated variant to their offspring with the risk depending on the level of mosaicism within the gonadal tissue. In case of transmission, the offspring will carry the pathogenic variant in all of his or her cells. Only a few studies have attempted to detect the rate of parental mosaicism using next-generation sequencing and identified somatic mosaicism in approximately 8% of parents whose children are affected by severe epileptic encephalopathies\(^4\) including Dravet syndrome\(^5\)–\(^7\). The individual recurrence risk for mosaic parents can hypothetically be as high as 50% and will depend on the level of mosaicism in the reproductive tissue. For males, the rate of gonadal mosaicism can be monitored non-invasively, whereas for females a gonadal biopsy would be necessary, which usually remains unrealistic. Hence, the actual recurrence risk is difficult to estimate relying on blood alone and may not be directly comparable to the rate of mosaicism in peripheral tissues due to the considerable variation described across tissues and body locations\(^8\). This study aims to explore the frequency of low-grade mosaicism in parents of children with epilepsy due to an alleged de novo variant and to highlight the differences between mutant allele frequency and general recurrence risk, critical to allow for proficient genetic counseling.

**Methods**

We collected parental DNA from 93 patients with epilepsy due to an alleged de novo pathogenic variant identified by clinical testing. Most patients suffered from epileptic encephalopathies. After patient recruitment, we identified one of the 93 families to have more than one affected child. Genomic DNA was extracted from blood (93 pairs of parents) and urine using a MagCore\(^\circ\) instrument (RBC Bioscience) according to manufacturer instructions. Oral mucosa was collected using the OCA collect DNA OCR 100 kit (DNA genotek) and genomic DNA was extracted with the prepIT L2P kit (DNA genotek) using manufacturer instructions. Parental DNA samples were screened by deep targeted next generation sequencing. Forty-six families were sequenced using Ion AmpliSeq library technology on an Ion torrent PGM (ThermoFisher) following manufacturer’s instructions, 47 parents by using single-molecule molecular inversion probes (smMIPS)\(^9\). For 26 pairs of parents from the latter group, three tissues, (i) blood, (ii) oral mucosa, and (iii) urothelium were available, representing all three germ layers. To compensate for the generally low quality and quantity of genomic DNA available in urine and oral mucosa, we used a ratio of genomic DNA to smMIPs of up to 1:232. Library preparation was done in sets of 24 samples and 40 pooled smMIPS. Sequencing was performed on an Illumina MiSeq instrument to generate 151 bp paired-end reads using customized sequencing primers. The fastq files were aligned to version GRCh37/hg19 of the human reference genome and analyzed with the software SeqPilot by JSI Medical Systems. To avoid false positive findings at this comparative coverage only variants that were present in both forward and reverse reads were counted\(^7\). For detection of low grade mosaics with smMIPS a minimum coverage of 200 captures has been set according to published literature\(^4\). For AmpliSeq sequencing data a detection minimum of 850 reads has been set. In total 75 trios fulfill these criteria. The analyzed de novo variants in the CACNA1A, CDKL5,
CHD2, GABRA1, GRIN2A, GRIN2B, KCNQ2, PCDH19, SCN1A, SCN2A, SCN8A, SLC2A1 and STXBP1 genes had been previously detected in the blood of the respective children. In case of mosaicism, a second run was performed for validation. The variant-specific sequencing error rate was calculated for each mosaic parent using sequencing data for the respective pathogenic variant from all individuals unrelated to the proband. The vast majority of variants represent missense variants. The study was approved by the local ethical committees and informed consent was obtained from all participating individuals.

Results

We performed targeted high-throughput sequencing of parental DNA from blood in 67 families, as well as from blood and somatic tissues including oral mucosa and urothelium to infer mosaicism in another 26 families. Forty-six cases showed a mean read depth of 6600x (858x – 78764x) using Ion AmpliSeq library technology. SmMIPS was used for the remaining 47 cases including the 26 cases for which all three tissues were tested. The uncollapsed mean read depth of blood was 54988x (0x-455756x), 4018x (0x-96606x) for urine epithelium, and 24053x (0x – 209674x) in oral mucosa. After merging the data of smMIPS to unmask PCR-derived duplicates, the collapsed mean read depth of blood was 576x (2x-1974x), 51x (0x-982x) in urothelium and 323x (0x-1807x) in oral mucosa. Five parents (6.6%) showed low-grade mosaicism for the pathogenic variant detected in their child, with minor allele frequencies ranging from 0.8 to 29% and variant specific error rate ranging from 6.9E-04 to 0.0E-00. In four out of these five cases, the mosaicism has been transmitted paternally. Parental mosaicism was observed for pathogenic variants in SCN1A, SCN2A, SCN8A and STXBP1, see Table 1, and all except one mosaic variant were undetectable via Sanger sequencing of parental DNA obtained from blood. One of the mosaic parents had epilepsy himself. He also showed the highest rate of mosaicism (29%), and was the only parent with more than one affected child, carrying the pathogenic variant. This paternal mosaic was retrospectively confirmed by Sanger Sequencing. Clinically, the seizure disorder of the mosaic parent was comparable with his daughters’ SCN8A-related encephalopathy, both in terms of severity and age of onset. Finally, in one case the mosaic was detected solely in the oral mucosa of one parent.

Discussion

Parental segregation testing is routinely performed on blood-derived DNA using Sanger sequencing, which has a limited sensitivity in detecting low-grade mosaicism with minor allele frequencies below 20%\(^1\). Here we performed deep targeted sequencing in parental samples of children with an alleged de novo variant in order to test for low-grade mosaicism\(^2\). We successfully detected low-level mosaicism in 5 of the 75 criteria fulfilling families (6.6%). The rate of mosaicism ranged from 0.8 to 29%. Our detection rate is slightly lower than recently published studies reporting parental mosaicism in 8.3% of families with developmental and epileptic encephalopathy, and in 8.6 - 17% of families with Dravet syndrome due to de novo SCN1A variants, respectively. Some previous studies based on a collection of random cases with de novo variants from various sources. In order to minimize ascertainment bias we did not restrict recruitment to solely the presence of a de novo variant in the genes of interest but also aimed for a recruitment of all consecutive case of de novo variants in the study genes from the respective contributor and included those where samples of both parents were available. By performing smMIPS we used a very sensitive and
cost-efficient method for detecting low-grade variants\textsuperscript{12}. Its advantages are in the molecular tag, which makes it possible to minimize PCR artifacts. Other studies also used alternative methods like digital droplet PCR (ddPCR)\textsuperscript{6}. Some studies even showed that particularly very low-grade mosaics with alternative allele frequencies of below 1% could be detected with ddPCR depending on the level of background noise\textsuperscript{7}. When aiming for such very rare variants, very high sequencing depth would be required for an NGS-based method. Hence, the differences in methodology and sequencing depth may also account for part of the variation in mosaicism detection rates observed across studies\textsuperscript{4–6}.

However, parents without detectable mosaicism in blood could still have an increased recurrence risk, as the variant might be present in other tissues. To address this aspect, we generated sequence data from additional somatic tissues, representative for all three germ layers, i.e. blood for mesoderm, oral mucosa for ectoderm and urothelial cells in urine from endoderm, in 26 families. In one case, a very low-level parental mosaic of 0.9% was detected in oral mucosa, only. However, the limited amount of both, oral mucosa and urine from this parent hampered a confirmation in either a second tissue (urine), or an independent sequencing run. Therefore we are cautious to declare this as a definite case of mosaicism. However, the variant-specific error of 0.0E00 would support its validity.

The associated mutant allele fractions detected in DNA from blood varied from 0.8 to 29%, which is comparable with previously published data from blood, saliva or sperm (0.03% to 39.04%)\textsuperscript{4–6}. The individual recurrence risk in the mosaic parents is hypothetically up to 50%, however the precise recurrence risk is almost impossible to predict.

Moreover, low-grade mosaics have been observed to often occur in the paternal lineage\textsuperscript{13}. Also, the extent of mosaicism in sperm may be higher than in blood, despite their common germ layer origin\textsuperscript{6}. However, exceptional cases of very low-grade mosaics being detected only in sperm have been reported as well\textsuperscript{6}. In summary, we would like to emphasize the different predictive values of (i) mutant allele fractions in an individual with a mosaic \textit{de novo} variant, and (ii) the prevalence of such mosaic carriers in the general population. The latter, i.e. parental mosaicism\textsuperscript{3} is known to be the major factor contributing to the overall recurrence risk for \textit{de novo} genetic disorders of approximately 1%\textsuperscript{2} and remains a valid population risk reference point. Testing for low-grade mosaicism in parents will enable identification of those few parents with a truly elevated recurrence risk, who will be the main contributors to the above-mentioned 1%. Thus, parents with a negative testing result for low-grade mosaicism will in general have a much lower estimated recurrence risk than the 1% of the general population, whereas a positive result will presumably lead to much higher rates. The information from parental testing for low-grade mosaicism will therefore markedly improve genetic counseling of the respective families.

In conclusion, the present study highlights that families of children with \textit{de novo} variants should be informed about the risk of parental mosaicism and the potential possibility of prenatal or preimplantation genetic testing for future pregnancies. Furthermore, it is recommended to perform deep targeted sequencing in parents of children with an alleged \textit{de novo} variant to better evaluate the parental mosaicism status and to identify parents with an elevated recurrence risk.

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Disclosure

None of the authors has any potential conflict of interest to disclose. 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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References


Attachment

Table 1 Parental Mosaicism in 5.4% of tested parents of children with epileptic encephalopathies caused by a putative de novo variant.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Gene</th>
<th>cDNA position</th>
<th>Protein position</th>
<th>Tissue</th>
<th>Total reads</th>
<th>Variant reads</th>
<th>Mosaicism</th>
<th>Transmission</th>
<th>Error rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SCN1A</td>
<td>c.3962dupT</td>
<td>p.(Arg1322Glu/fs*10)</td>
<td>blood</td>
<td>15389</td>
<td>1472</td>
<td>9.6%</td>
<td>mother</td>
<td>3.8E-04</td>
</tr>
<tr>
<td>2</td>
<td>SCN2A</td>
<td>c.2588C&gt;T</td>
<td>p.(Ser863Phe)</td>
<td>blood</td>
<td>2871</td>
<td>223</td>
<td>7.8%</td>
<td>father</td>
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<tr>
<td>3</td>
<td>SCN8A</td>
<td>c.5615G&gt;A</td>
<td>p.(Arg1872Gln)</td>
<td>blood</td>
<td>8876</td>
<td>2573</td>
<td>29.0%</td>
<td>father</td>
<td>6.9E-04</td>
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<tr>
<td>4</td>
<td>STXBP1</td>
<td>c.1387G&gt;T</td>
<td>p.(Glu463Ter)</td>
<td>blood</td>
<td>12147</td>
<td>93</td>
<td>0.8%</td>
<td>father</td>
<td>1.7E-05</td>
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<tr>
<td>5*</td>
<td>SCN2A</td>
<td>c.2660T&gt;C</td>
<td>p.(Val887Ala)</td>
<td>blood, urothelium, oral mucosa</td>
<td>23595(392)<em>, 1734(41)</em>, 24616(214)*</td>
<td>22(0)<em>, 3(0)</em>, 160(2)*</td>
<td>0.6% (0.9%)*</td>
<td>father</td>
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*tested with smMIPS – collapsed coverage in parentheses
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<tr>
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<td></td>
<td></td>
<td></td>
<td>urothelium</td>
<td>1734(41)*</td>
<td>3(0)*</td>
<td>(0.9%)*</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>oral mucosa</td>
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