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*Published in:*
JOURNAL OF MEDICAL GENETICS

*DOI:*
10.1136/jmedgenet-2018-105672



*Publication date:*
2019

ORIGINAL ARTICLE

# Assessment of parental mosaicism in *SCN1A*-related epilepsy by single-molecule molecular inversion probes and next-generation sequencing

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► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/jmedgenet-2018-105672).

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Received 8 August 2018
Revised 24 September 2018
Accepted 30 September 2018
Published Online First 27 October 2018



**To cite:** de Lange IM, Koudijs MJ, van 't Slot R, *et al*. *J Med Genet* 2019;**56**:75–80.

## ABSTRACT

**Background** Dravet syndrome is a severe genetic encephalopathy, caused by pathogenic variants in *SCN1A*. Low-grade parental mosaicism occurs in a substantial proportion of families (7%–13%) and has important implications for recurrence risks. However, parental mosaicism can remain undetected by methods regularly used in diagnostics. In this study, we use single-molecule molecular inversion probes (smMIP), a technique with high sensitivity for detecting low-grade mosaic variants and high cost-effectiveness, to investigate the incidence of parental mosaicism of *SCN1A* variants in a cohort of 90 families and assess the feasibility of this technique.

**Methods** Deep sequencing of *SCN1A* was performed using smMIPs. False positive rates for each of the proband's pathogenic variants were determined in 145 unrelated samples. If parents showed corresponding variant alleles at a significantly higher rate than the established noise ratio, mosaicism was confirmed by droplet digital PCR (ddPCR).

**Results** Sequence coverage of at least 100× at the location of the corresponding pathogenic variant was reached for 80 parent couples. The variant ratio was significantly higher than the established noise ratio in eight parent couples, of which four (5%) were regarded as true mosaics, based on ddPCR results. The false positive rate of smMIP analysis without ddPCR was therefore 50%. Three of these variants had previously been considered de novo in the proband by Sanger sequencing.

**Conclusion** smMIP technology combined withnext generation sequencing (NGS) performs better than Sanger sequencing in the detection of parental mosaicism. Because parental mosaicism has important implications for genetic counselling and recurrence risks, we stress the importance of implementing high-sensitivity NGS-based assays in standard diagnostics.

## INTRODUCTION

Dravet syndrome (MIM: 607208) is a severe genetic encephalopathy, characterised by intractable epileptic seizures and a delayed psychomotor development, resulting in mild to severe intellectual disability (ID) in most patients. Walking difficulties and behavioural problems are common comorbidities.[1–4] Pathogenic variants in *SCN1A*, which codes for the α-subunit of the neuronal sodium channel Nav1.1, are found in 70%–100% of patients with Dravet syndrome. *SCN1A* variants can, however, also cause milder phenotypes, such as genetic epilepsy febrile seizures plus (GEFS+) syndrome or febrile seizures only.[5–7]

The varying disease severity of phenotypes caused by *SCN1A* pathogenic variants are partly due to differences in mutation types and amino acid changes, which can cause different grades of channel dysfunction.[8] However, an important part of the disease variability is still unexplained, and multiple modifying factors have been suggested.[9–13] We have recently shown that mosaicism in patients is an important modifier of *SCN1A*-related disease severity.[12] Mosaicism arises when a variant occurs postzygotically, leading to genetically distinct cell populations. Patients that carry a mosaic pathogenic variant might be less severely affected, since unaffected cells are also present.[8 12 14–18] Variant carriers can even be free of symptoms when very low percentages of mosaicism are present, caused by mutations occurring relatively late in embryonic development. They can, however, still transmit the variant to their children when it is present in gonadal tissue. Unfortunately, low-grade parental mosaicism can be missed in molecular diagnostics, as regularly used Sanger sequencing fails to detect low percentages of a variant allele,[19] which may also be the case when NGS is used with limited coverage. Moreover, variants are not always present in DNA isolated from blood. A number of patients with a presumed de novo *SCN1A* variant will therefore actually have a parent with low-grade mosaicism: this percentage has been estimated to be 7%–13%[17 20 21] and is illustrated by multiple case reports of families with healthy or mildly affected parents and multiple severely affected children.[22–28]

Detecting parental mosaicism has important implications for genetic counselling: recurrence risks rise when father or mother carries the pathogenic variant allele too, which might affect decisions regarding family planning and prenatal testing when they are aware of this. Other techniques are needed to reliably test for parental mosaicism. Droplet digital PCR (ddPCR), although proven to

be an extremely sensitive method for detecting low-grade mosaicism,[21 29] has the disadvantage of needing specifically designed probes for each assessed variant, which is not feasible in regular diagnostics. Another assay that can be used is single-molecule molecular inversion probes (smMIP) capture and sequencing, a deep NGS technique with high sensitivity for detecting low-frequency variants and high cost-effectiveness.[30–32] In this study, we use smMIPs to investigate the incidence of low-grade parental mosaicism of *SCN1A* variants in a cohort of 90 families and assess the feasibility of this technique.

## METHODS

### Participants

A portion of the parents of a previously described cohort of 176 patients clinically affected by *SCN1A*-related seizures[12 13] were tested for low-grade mosaicism. In this cohort, only participants with pathogenic variants (class 5) and likely pathogenic variants (class 4), according to the American College of Medical Genetics and Genomics criteria,[33] in *SCN1A* were included. *SCN1A* variants in index patients had been detected in diagnostic laboratories (University Medical Center Utrecht, Utrecht, the Netherlands; Laboratory for Neurogenetics, Institute Born-Bunge, University Antwerp, Antwerp, Belgium; Radboud University Nijmegen Medical Center, Nijmegen, the Netherlands; and Duncan Guthrie Institute of Medical Genetics, Glasgow, UK) by Sanger sequencing, NGS epilepsy gene panels, whole exome sequencing or by multiplex ligation-dependent probe amplification (MLPA). Only parents for whom DNA was available were considered for inclusion. In most families, parents had been assessed for carriership of the pathogenic *SCN1A* variant by Sanger sequencing in a regular diagnostic setting; families in which parents were shown to be heterozygous or mosaic carriers of their children's pathogenic variants were regarded as participants themselves and excluded from additional DNA testing. Families in which mosaicism was demonstrated in the proband in previous research[12] were also excluded, since this rules out parental mosaicism. Informed consent was obtained from participants, according to the Declaration of Helsinki.

### Molecular analyses

#### Mosaicism screening by smMIPs and NGS

All *SCN1A* exons were captured by smMIPs, as described earlier,[12 31] and sequenced (see online supplementary data 1 for more details) in parental DNA extracted from lymphocytes. The resulting data were analysed using commercial software (SeqNext module of Sequence Pilot; JSI medical systems, Ettenheim, Germany) (see online supplementary data 2 for more details). Reads with the same single-molecule tag were assembled into one consensus read to correct for PCR and sequencing artefacts. In addition, the molecular tag discriminates unique reads from PCR duplicates, allowing the determination of quantitative sequence coverage of reads originating from unique DNA molecules. *SCN1A* pseudogene reads were removed from alignment and analysis.

Parents of patients with deletions and duplications spanning more than one smMIP were excluded from analyses, since those variants cannot be detected by smMIPs.

#### Statistical analysis of smMIP data

We determined whether the patients' pathogenic variants were present in the sequencing data of their respective parents. Only variants that were present in both forward and reverse reads were counted to filter out likely false positive reads. High coverage is needed to detect low-grade mosaicism with a high confidentiality: a unique coverage of 300× is needed to detect 1% of alternative allele reads with a 95% probability level (calculated based on a binomial distribution). Therefore, parents with a coverage <100× (needed to detect 3% of alternative allele reads with a 95% probability level, calculated based on a binomial distribution) and no alternative allele reads at the location of their child's pathogenic variant were excluded from statistical analyses.

We established the false positive rate of the variants detected in the parents to determine whether they were true variants and not sequencing errors leading to false positives. The overall percentage of variant reads of each possible low-grade mosaic variant was determined in 145 unrelated samples. P values were calculated for each parent in whom a possible mosaic variant was present, based on a binomial distribution and the average percentage of variant reads in unrelated controls, to determine whether the percentage of variant reads deviated significantly from the established noise ratio. Variants in parents with significant p values, corrected for multiple testing (below 0.05 divided by the number of tested parents), were considered to be likely true low-grade mosaic variants.

#### Confirmation by ddPCR

All likely true mosaic variants were validated by ddPCR if probes could be designed (see online supplementary data 3 for more details).

## RESULTS

### Participants

DNA was available for both parents in 101 families. The probands of six families carried a duplication or deletion of *SCN1A* that could not be detected by smMIPs and were therefore excluded. High-grade mosaicism was previously established in probands of seven of these families, which were excluded. Ninety complete families remained and were analysed as described. The 26 families for which DNA of both parents was not available contained five mosaic family members, of which four were parents of probands that were found to carry the same mutation as their children in regular diagnostics (Sanger sequencing).

### Molecular analyses

#### Mosaicism screening by smMIPs and NGS

A coverage of at least 100× unique coverage at the location of the known pathogenic variant of their children was reached for 80 complete parent couples. Seventy of these 80 families had been previously assessed for parental carriership by Sanger sequencing in standard diagnostic procedures, and their children's variants had been deemed de novo (figure 1). Parents of the other 10 families had previously declined Sanger sequencing of their own DNA. The average unique read depth in these parents was 1663× (ranging from 112× to 8990×, median: 1203×). No corresponding pathogenic variant alleles were detected in parents with a unique coverage <100×.

Corresponding variant alleles were detected in 29 parents, belonging to 22 different families; in seven families, both parents carried variant alleles. Variants in six parents, of which two belonged to families in which both parents carried variant reads, were regarded as false positives, since their variant reads were never present in both a forward and reverse read and had low-quality scores (14). The remaining 23 parents belonged to 18 different families and carried 16 different variants (two variants were present twice in the cohort of probands).

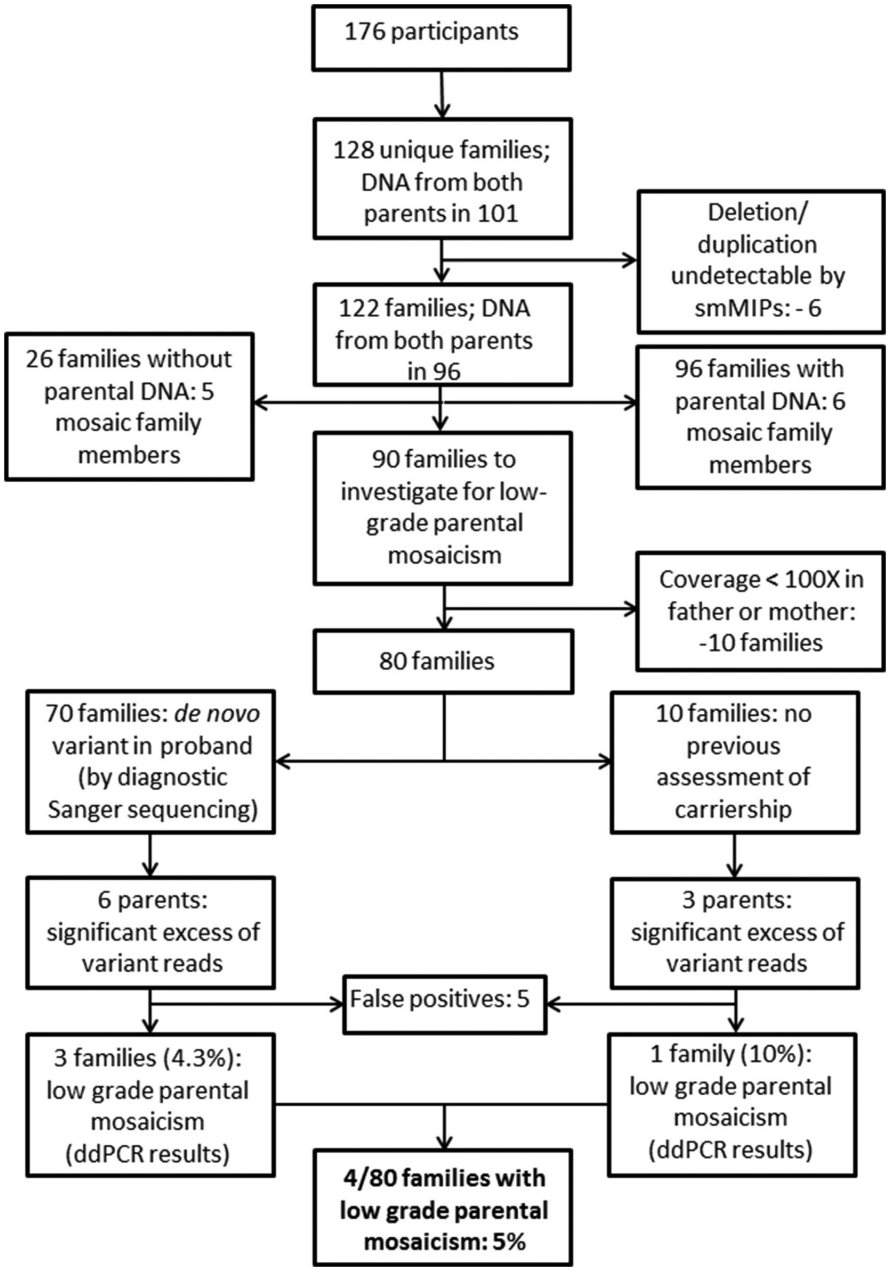


**Figure 1** Flow chart of detected mosaic pathogenic variants in the complete cohort described here and in previous work. ddPCR, droplet digital PCR; smMIPs, single molecule molecular inversion probes.

### Statistical analysis of smMIP data

The percentages of variant reads in unrelated parents (noise ratios) are shown in table 1 for all 16 variants. A percentage of variant reads significantly higher than the established noise ratio was found in nine parents (table 1, bolded p values). Unexpectedly, a significantly high ratio of variant allele was found in both father and mother of one family (variant 1). The father of this parent couple, in which a much lower ratio of variant allele was found than in the mother (0.63% vs 6.47%), was regarded as a false positive, since it is extremely unlikely that both parents are true mosaics for the same variant. The low p value in this father might be explained by the location of the variant in a poly-T sequence, for which less reliable NGS results are seen.

### Confirmation by ddPCR

Mutation specific ddPCR probes could be designed for six of the eight likely mosaic parents. Low-grade mosaicism could be confirmed in three: a significantly higher percentage of droplets positive for the variant allele was observed than in negative controls (table 1). The confirmed mosaic variants belonged to the parents with the lowest p values that we established for the percentages of variant compared with noise ratios. One of the parents (family 1) for whom ddPCR probes could not be designed (due to their variant being in a poly-T sequence) had an even lower p value ($7.5 \times 10^{-88}$), the highest percentage of variant alleles (6.47%), and two affected sons; we therefore regard her as a true mosaic variant carrier as well. The other parent for whom no ddPCR probes could be designed however (variant 7) had a p value in the same range as the variants that could not be confirmed by ddPCR (0.0006) and the lowest percentage of variant alleles (0.17%). This father was therefore regarded as a false positive. In summary, we have detected low-grade parental mosaicism in 4 out of 80 parent couples (5%) (figure 1). In three of these four families, parents had been assessed for carriership

**Table 1** Possible low grade parental mosaic variants

| | Variant | % variant reads in father (coverage) | % variant reads in mother (coverage) | % variant reads in unrelated parents (coverage) | P values* (father; mother) | ddPCR results†: % of variant alleles |
|---|---|---|---|---|---|---|
| 1 | c.1209del p.(Phe403fs) | 0.63 (630×) | 6.47 (804×) | 0.06 (325 481×) | **0.0004; $7.5\times10^{-88}$** | **Probes could not be designed – mosaicism likely.** |
| 2 | c.5348C>T p.(Ala1783Val) | 2.34 (770×) | | 0.07 (123 200×) | **$3.4\times10^{-21}$** | **0.58% (NC: 0.0001%).** |
| 3 | c.5674C>T p.(Arg1892*) | 6.35 (126×) | | 0.06 (114 869×) | **$1.8\times10^{-14}$** | **8% (NC: 0.15%).** |
| 4 | c.5656C>T p.(Arg1886*) | 0.50 (2403×) | | 0.03 (317 306×) | **$3.0\times10^{-11}$** | **0.83% (NC: 0.05%).** |
| 5 | c.5164A>G p.(Thr1722Ala) | | 0.24 (841×) | 0.001 (172 609×) | **$4.7\times10^{-5}$** | No confirmation. |
| 6 | c.4757G>A p.(Gly1586Glu) | | 0.31 (651×) | 0.002 (248 849×) | **0.00012** | No confirmation. |
| 7 | c.3706–1G>A p.(1236splice) | 0.17 (3580×) | 0.10 (3913×) | 0.03 (435 939×) | **0.0006**; 0.021 | Probes could not be designed – mosaicism unlikely. |
| 8 | c.4219C>T p.(Arg1407*) | 0.56 (718×) | | 0.07 (117 985×) | **0.0013** | No confirmation. |
| 9 | c.602+1G>A p.(201splice) | 0.16 (4324×) | 0.08 (4842×) | 0.049 (685 966×) | 0.0043; 0.121 | |
| 10 | c.2836C>T p.(Arg946Cys) | 0.09 (4350×) | 0.07 (2921×)<br>0.34 (1170×) | 0.07 (420 914×) | 0.165; 0.271<br>0.007 | |
| 11 | c.602C>T p.(Ala201Val) | 0.04 (4508×) | | 0.004 (696 006×) | 0.0164 | |
| 12 | c.1738C>T p.(Arg 580*) | 0.22 (912×)<br>0.09 (2113×) | | 0.06 (285 028×) | 0.079<br>0.216 | |
| 13 | c.1178G>A p.(Arg393His) | 0.12 (1669×) | | 0.05 (226 584×) | 0.155 | |
| 14 | c.580G>A p.(Asp194Asn) | 0.04 (5271×) | 0.04 (5466×) | 0.05 (736 234×) | 0.246; 0.239 | |
| 15 | c.3637C>T p.(Arg1213*) | | 0.03 (5947×) | 0.02 (527 215×) | 0.249 | |
| 16 | c.5269G>A p.(Gly1757Arg) | 0.04 (5371×) | | 0.033 (465 391×) | 0.266 | |

*Threshold values for significance: <0.05/23=0.00217. Significant p values are bolded.
ddPCR, droplet digital PCR; NC, negative control.

of their child's variant, which had been deemed de novo by Sanger sequencing.

### Clinical symptoms

Two of the four mosaic parents (50%) had experienced seizures in the past, although limited details were available: the father of family 4 (0.5% variant alleles) had experienced epilepsy as a child for which phenobarbital was prescribed; the father of family 2 (2.43% variant alleles) had epilepsy as a child and was prescribed antiepileptic drugs until 10 years of age. Seizures were reported in 5% of the remaining non-mosaic parents, who experienced mostly typical febrile seizures (6/8), one single seizure (1/8) and one parent had had two seizures as a child and one as an adult, provoked by alcohol and fatigue. Antiepileptic drugs were prescribed in none.

## DISCUSSION

The presence of low-grade parental mosaicism for pathogenic variants has important implications for genetic counselling. A recurrence risk of 1% is often counselled to parents in the case of a presumed de novo genetic disorder[34]; recurrence risks, however, rise when father or mother are shown to carry variant alleles as well. Parents may make different choices regarding prenatal diagnostics when this knowledge is available. In standard clinical practice, Sanger sequencing in parents is often the method of choice to determine whether the pathogenic variant in a proband is de novo or not. However, Sanger sequencing and regular NGS fail to detect low percentages of variant allele,[19] and a normal result might therefore give parents a false sense of reassurance. By using smMIPs and deep sequencing, we here detected low-grade parental mosaicism in 3 out of 70 families (4.3%) with a previously presumed de novo *SCN1A* variant (based on Sanger sequencing in regular diagnostics) and in a total of 4 out of 80 families (5%).

The use of smMIPs to detect mosaicism has several advantages. First, the assembly of smMIP reads into one consensus read based on the molecular barcode in each smMIP probe corrects for PCR or sequencing artefacts and therefore reduces sequencing errors. Furthermore, the removal of PCR duplicates from the analyses leads to very accurate percentages of variant alleles, since only one read per unique DNA molecule is taken into account. The smMIP technique with deep sequencing allows us to detect parental mosaicism even when only very small fractions of variant alleles are present. However, this method also has several limitations. No definitive conclusions about mosaicism can be made without confirmation by a second technique, especially when percentages of variant reads are very low. This is illustrated by the three variants for which significant percentages of variant alleles were seen based on smMIP sequencing, of which none could not be validated by ddPCR. Another example of the need for confirmatory tests is the parent couple that both showed a significant percentage of variant reads, of which only one is likely a true mosaic. A solution for this

could be to lower the p value threshold for significance, since the choice of threshold influences the false positive rate. Since all false positive variants had p values in the same range (much higher than the true mosaic variants), this may be a feasible resolution. Besides generating false positive results, mosaic variants may also be missed when using smMIPs, which causes false negative results. While we were able to detect percentages of mosaicism as low as 0.5%, theoretically even lower percentages of mosaicism are possible. Fractions of variants in the noise range (0.001%–0.07%, dependent on variant location) will however not be detected. Even variants with percentages above this noise ratio can be missed when coverage is insufficient at their location; for mosaic variants of 0.5%, a coverage of at least 600× is needed to detect it with a 95% probability level (calculated based on a binomial distribution). In 44 of the non-excluded parents, this coverage level was not reached, and therefore some very low-grade mosaics have possibly remained undetected. Finally, variant alleles will not be detected by sequencing of DNA from blood in the case of purely gonadal mosaicism. Our percentage of families affected by parental mosaicism (5%) is therefore likely an underestimation, which is demonstrated by one family in our cohort with two affected brothers and absence of variant alleles in both parents (905× and 1564×). A previous study[21] has demonstrated paternal mosaicism for *SCN1A* pathogenic variants in sperm that could not be detected in DNA from blood in three fathers. This highlights the limitations of mosaicism detection if only DNA from lymphocytes is investigated.

Studies to investigate the incidence of parental mosaicism of *SCN1A* pathogenic variants have been performed previously in Dravet syndrome families. A recent study detected parental mosaicism in 3 out of 40 families with apparent de novo pathogenic *SCN1A* variants by using smMIPs.[35] No validation studies were performed, which may have led to false positive results; however, as the reported percentages of mosaicism were relatively high (16.7%–30.6%), this risk may be low. Xu *et al*[20] found parental mosaicism in 10% of Dravet syndrome families as well. Yang *et al*[21] reported an even higher incidence: parental mosaicism was detected in 25% of Dravet syndrome families. However, part of these parental mosaics could also be detected by Sanger sequencing, which was an exclusion criterion in our study based on which four families were excluded. The incidences of parental mosaicism in the mentioned studies were 8.6% and 13.3%, respectively, if only mosaics undetectable by conventional methods in clinical practice are taken into account. In our study, the relatedness of families was not confirmed, which makes it possible that additional mosaic parents were missed. However, the much higher incidence of Yang *et al*[21] is most likely due to the use of ddPCR as screening method, which can reach a higher sensitivity than NGS-based methods; variant allele percentages as low as 0.03% were reported, which is in the noise range of our technique. However, the disadvantage of ddPCR as a screening tool is that specifically designed probes are needed for each mutation, which makes it infeasible to implement in standard diagnostics. smMIPs that cover *SCN1A* only have to be designed and ordered once and can then be used for the assessment of all pathogenic variants detectable by NGS, making them very inexpensive: a full *SCN1A* smMIP NGS run for 96 patients could be performed in our lab for approximately €22 per sample (including the use of plastics, reagents, oligos and sequencing, excluding labour costs for laboratory procedures and analyses). In contrast, ddPCR costs €600–€800 per sample for merely ordering custom probes for each patient, and Sanger sequencing could be performed at our diagnostics lab for ~€25–30 per sample. smMIPs might therefore be an attractive cost-effective method to assess parental mosaicism: although it will detect less instances of parental mosaicism than more sensitive methods like ddPCR, it is clear that it outperforms Sanger sequencing; in three families, mosaicism, detected by smMIPs, was missed in regular diagnostics.

In this study, we identified low-grade mosaicism of variants that had remained undetected by Sanger sequencing. Alternatively, mosaicism can be present at higher grades in variants that can be detected by Sanger sequencing. We have recently used smMIPs and NGS to identify high-grade mosaicism in 9% of pathogenic *SCN1A* variants detected in regular diagnostics, of which 9 out of 11 were previously considered heterozygous based on Sanger sequencing results.[12] Similar percentages of high-grade mosaic variants in other genes have been reported previously.[36–38] The grade of mosaicism reflects the timing of mutagenesis: variants occurring during some of the first cell divisions after fertilisation will be present in many cells in multiple tissues, whereas variants that occur later in embryonic development might only be present in a single tissue type.[36] Although the methods of studies differ, which makes comparing exact percentages difficult, relatively similar incidences of high-grade and low-grade mosaic variants are reported, implicating that mutations occur at roughly the same rate during early and slightly later embryonic development. Detecting high-grade mosaicism in a proband is currently the only way to virtually rule out low-grade mosaicism in a parent, since only one member of a family can be mosaic for a specific variant and all techniques can miss very small percentages of (gonadal) mosaicism. Testing probands for high-grade mosaicism therefore adds value to testing parents for low-grade mosaicism in the assessment of recurrence risks, especially because this occurs in a substantial amount of patients (9%).[12] We suggest that analysing both parents and the proband with smMIPs and NGS is currently the most cost-effective way of assessing parental mosaicism in *SCN1A*-related epilepsy after a pathogenic variant is found in regular diagnostics. Since either low-grade or high-grade mosaicism could be detected in a total of 12% of families carrying pathogenic *SCN1A* variants in this cohort (11 probands[12] and 4 parents in 122 families), a substantial amount of families will likely benefit from improved counselling this way.

The percentages of pathogenic variant allele reads detected in parents ranged between 0.5 and 6.5. Two of the four mosaic parents had experienced seizures or epilepsy in the past, in contrast to 5% of non-mosaic parents. Furthermore, more severe epilepsy phenotypes were observed. Unlike in a previous report,[21] the percentage of mosaicism was not necessarily related to seizure symptoms: the two parents with the highest percentages of mosaicism (6.47% and 6.35%) reported no seizures. A reason for this may be that levels of mosaicism in blood do not necessarily correspond to those in brain. Furthermore, different genetic backgrounds are likely to modify the effect of the mosaic pathogenic *SCN1A* variant on the disease outcomes. Symptoms have previously been reported to arise between 12.5% and 25% of pathogenic *SCN1A* variant alleles.[8 12 39] Our results and earlier studies[20 21] suggest that symptoms might already arise below 1%, although this risk of seizures is much lower and we cannot be sure that the seizures have been caused by the *SCN1A* variant.

In conclusion, this study confirms that parental mosaicism of *SCN1A* pathogenic variants is a common phenomenon. Using molecular barcoding to obtain unique sequence reads per DNA fragment analysed, by combining smMIP technology and deep sequencing, can detect parental mosaicism in 4.3% of families with mutations that were previously considered de novo by standard diagnostics. Our reported total incidence of 5% is likely an underestimation, since ultra-low percentages of variant allele and gonadal mosaicism cannot be detected by the used methods. However, our methods perform better than Sanger sequencing used in regular diagnostics, and implementing smMIPs could be a cost-effective way to improve

the accuracy of counselling recurrence risks, which has important implications for families.

**Contributors** IMdL, ECC, MJK, MJAvK, IJN, RFE, SMCS, NVAMK, EHB and BPCK contributed to drafting/revising the manuscript for content. IMdL, EHB and BPCK contributed to the study concept or design. IMdL, MJK, RvS, ACMS, FM, ECC, IJN, RFE, SMCS, EHB and BPCK contributed to acquisition of data. IMdL performed the statistical analysis. EHB and BPCK performed study supervision or coordination.

**Funding** This study was supported by the 'Stichting Vrienden WKZ' (project 1614054) on behalf of Stichting Panta Rhei, and the Dutch Epilepsy Foundation (project 2017-01).

**Competing interests** None declared.

**Patient consent** Not required.

**Ethics approval** The study was approved by the Ethical Committee of the University Medical Center Utrecht. 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 guideline.

**Provenance and peer review** Not commissioned; externally peer reviewed.

## REFERENCES

1 Dravet C. Les epilepsies graves de l'enfant. *La Vie Médicale* 1978;8:543–8.
2 Brunklaus A, Ellis R, Reavey E, Forbes GH, Zuberi SM. Prognostic, clinical and demographic features in SCN1A mutation-positive Dravet syndrome. *Brain* 2012;135:2329–36.
3 Lagae L, Brambilla I, Mingorance A, Gibson E, Battersby A. Quality of life and comorbidities associated with Dravet syndrome severity: a multinational cohort survey. *Dev Med Child Neurol* 2018;60:63–72.
4 Rilstone JJ, Coelho FM, Minassian BA, Andrade DM. Dravet syndrome: seizure control and gait in adults with different SCN1A mutations. *Epilepsia* 2012;53:1421–8.
5 Claes L, Ceulemans B, Audenaert D, Smets K, Löfgren A, Del-Favero J, Ala-Mello S, Basel-Vanagaite L, Plecko B, Raskin S, Thiry P, Wolf NI, Van Broeckhoven C, De Jonghe P. De novo SCN1A mutations are a major cause of severe myoclonic epilepsy of infancy. *Hum Mutat* 2003;21:615–21.
6 Mulley JC, Scheffer IE, Petrou S, Dibbens LM, Berkovic SF, Harkin LA. SCN1A mutations and epilepsy. *Hum Mutat* 2005;25:535–42.
7 Escayg A, MacDonald BT, Meisler MH, Baulac S, Huberfeld G, An-Gourfinkel I, Brice A, LeGuern E, Moulard B, Chaigne D, Buresi C, Malafosse A. Mutations of SCN1A, encoding a neuronal sodium channel, in two families with GEFS+2. *Nat Genet* 2000;24:343–5.
8 Meng H, Xu HQ, Yu L, Lin GW, He N, Su T, Shi YW, Li B, Wang J, Liu XR, Tang B, Long YS, Yi YH, Liao WP. The SCN1A mutation database: updating information and analysis of the relationships among genotype, functional alteration, and phenotype. *Hum Mutat* 2015;36:573–80.
9 Ohmori I, Ouchida M, Kobayashi K, Jitsumori Y, Mori A, Michiue H, Nishiki T, Ohtsuka Y, Matsui H. CACNA1A variants may modify the epileptic phenotype of Dravet syndrome. *Neurobiol Dis* 2013;50:209–17.
10 Singh NA, Pappas C, Dahle EJ, Claes LR, Pruess TH, De Jonghe P, Thompson J, Dixon M, Gurnett C, Peiffer A, White HS, Filloux F, Leppert MF. A role of SCN9A in human epilepsies, as a cause of febrile seizures and as a potential modifier of Dravet syndrome. *PLoS Genet* 2009;5:e1000649–12.
11 Long YS, Zhao QH, Su T, Cai YL, Zeng Y, Shi YW, Yi YH, Chang HH, Liao WP. Identification of the promoter region and the 5′-untranslated exons of the human voltage-gated sodium channel Nav1.1 gene (SCN1A) and enhancement of gene expression by the 5′-untranslated exons. *J Neurosci Res* 2008;86:3375–81.
12 de Lange IM, Koudijs MJ, van 't Slot R, Gunning B, Sonsma ACM, van Gemert L, Mulder F, Carbo EC, van Kempen MJA, Verbeek NE, Nijman IJ, Ernst RF, Savelberg SMC, Knoers N, Brilstra EH, Koeleman BPC. Mosaicism of de novo pathogenic *SCN1A* variants in epilepsy is a frequent phenomenon that correlates with variable phenotypes. *Epilepsia* 2018;59:690–703.
13 de Lange IM, Gunning B, Sonsma ACM, van Gemert L, van Kempen M, Verbeek NE, Nicolai J, Knoers N, Koeleman BPC, Brilstra EH. Influence of contraindicated medication use on cognitive outcome in Dravet syndrome and age at first afebrile seizure as a clinical predictor in SCN1A-related seizure phenotypes. *Epilepsia* 2018;59:1154–65.
14 Gottlieb B, Beitel LK, Trifiro MA. Somatic mosaicism and variable expressivity. *Trends Genet* 2001;17:79–82.
15 Youssoufian H, Pyeritz RE. Mechanisms and consequences of somatic mosaicism in humans. *Nat Rev Genet* 2002;3:748–58.
16 Verbeek NE, van Kempen M, Gunning WB, Renier WO, Westland B, Lindhout D, Brilstra EH. Adults with a history of possible Dravet syndrome: an illustration of the importance of analysis of the SCN1A gene. *Epilepsia* 2011;52:e23–e25.
17 Depienne C, Trouillard O, Gourfinkel-An I, Saint-Martin C, Bouteiller D, Graber D, Barthez-Carpentier MA, Gautier A, Villeneuve N, Dravet C, Livet MO, Rivier-Ringenbach C, Adam C, Dupont S, Baulac S, Héron D, Nabbout R, Leguern E. Mechanisms for variable expressivity of inherited SCN1A mutations causing Dravet syndrome. *J Med Genet* 2010;47:404–10.
18 Campbell IM, Shaw CA, Stankiewicz P, Lupski JR. Erratum to: Somatic Mosaicism: Implications for Disease and Transmission Genetics. *Trends Genet* 2016;32:138–92.
19 Rohlin A, Wernersson J, Engwall Y, Wiklund L, Björk J, Nordling M. Parallel sequencing used in detection of mosaic mutations: comparison with four diagnostic DNA screening techniques. *Hum Mutat* 2009;30:1012–20.
20 Xu X, Yang X, Wu Q, Liu A, Yang X, Ye AY, Huang AY, Li J, Wang M, Yu Z, Wang S, Zhang Z, Wu X, Wei L, Zhang Y. Amplicon Resequencing Identified Parental Mosaicism for Approximately 10% of "de novo" SCN1A Mutations in Children with Dravet Syndrome. *Hum Mutat* 2015;36:861–72.
21 Yang X, Liu A, Xu X, Yang X, Zeng Q, Ay Y, Yu Z, Wang S, Huang AY, Wu X, Wu Q, Wei L, Zhang Y. Genomic mosaicism in paternal sperm and multiple parental tissues in a Dravet syndrome cohort. *Sci Rep* 2017;7:1–13.
22 Depienne C, Arzimanoglou A, Trouillard O, Fedirko E, Baulac S, Saint-Martin C, Ruberg M, Dravet C, Nabbout R, Baulac M, Gourfinkel-An I, LeGuern E. Parental mosaicism can cause recurrent transmission of SCN1A mutations associated with severe myoclonic epilepsy of infancy. *Hum Mutat* 2006;27:389.
23 Gennaro E, Santorelli FM, Bertini E, Buti D, Gaggero R, Gobbi G, Lini M, Granata T, Freri E, Parmeggiani A, Striano P, Veggiotti P, Cardinali S, Bricarelli FD, Minetti C, Zara F. Somatic and germline mosaicisms in severe myoclonic epilepsy of infancy. *Biochem Biophys Res Commun* 2006;341:489–93.
24 Guala A, Peruzzi C, Gennaro E, Pennese L, Danesino C. Maternal germinal mosaicism for SCN1A in sibs with a mild form of Dravet syndrome. *Am J Med Genet A* 2015;167A:1165–7.
25 Marini C, Mei D, Helen Cross J, Guerrini R. Mosaic SCN1A mutation in familial severe myoclonic epilepsy of infancy. *Epilepsia* 2006;47:1737–40.
26 Morimoto M, Mazaki E, Nishimura A, Chiyonobu T, Sawai Y, Murakami A, Nakamura K, Inoue I, Ogiwara I, Sugimoto T, Yamakawa K. SCN1A mutation mosaicism in a family with severe myoclonic epilepsy in infancy. *Epilepsia* 2006;47:1732–6.
27 Selmer KK, Eriksson AS, Brandal K, Egeland T, Tallaksen C, Undlien DE. Parental SCN1A mutation mosaicism in familial Dravet syndrome. *Clin Genet* 2009;76:398–403.
28 Sharkia R, Hengel H, Schöls L, Athamna M, Bauer P, Mahajnah M. Parental mosaicism in another case of Dravet syndrome caused by a novel SCN1A deletion: a case report. *J Med Case Rep* 2016;10:67.
29 Kiss MM, Ortoleva-Donnelly L, Beer NR, Warner J, Bailey CG, Colston BW, Rothberg JM, Link DR, Leamon JH. High-throughput quantitative polymerase chain reaction in picoliter droplets. *Anal Chem* 2008;80:8975–81.
30 O'Roak BJ, Vives L, Fu W, Egertson JD, Stanaway IB, Phelps IG, Carvill G, Kumar A, Lee C, Ankenman K, Munson J, Hiatt JB, Turner EH, Levy R, O'Day DR, Krumm N, Coe BP, Martin BK, Borenstein E, Nickerson DA, Mefford HC, Doherty D, Akey JM, Bernier R, Eichler EE, Shendure J. Multiplex targeted sequencing identifies recurrently mutated genes in autism spectrum disorders. *Science* 2012;338:1619–22.
31 Eijkelenboom A, Kamping EJ, Kastner-van Raaij AW, Hendriks-Cornelissen SJ, Neveling K, Kuiper RP, Hoischen A, Nelen MR, Ligtenberg MJ, Tops BB. Reliable Next-Generation Sequencing of Formalin-Fixed, Paraffin-Embedded Tissue Using Single Molecule Tags. *J Mol Diagn* 2016;18:851–63.
32 Hiatt JB, Pritchard CC, Salipante SJ, O'Roak BJ, Shendure J. Single molecule molecular inversion probes for targeted, high-accuracy detection of low-frequency variation. *Genome Res* 2013;23:843–54.
33 Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, Voelkerding K, Rehm HL. ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 2015;17:405–23.
34 Campbell IM, Stewart JR, James RA, Lupski JR, Stankiewicz P, Olofsson P, Shaw CA. Parent of origin, mosaicism, and recurrence risk: probabilistic modeling explains the broken symmetry of transmission genetics. *Am J Hum Genet* 2014;95:345–59.
35 Myers CT, Hollingsworth G, Muir AM, Schneider AL, Thuesmunn Z, Knupp A, King C, Lacroix A, Mehaffey MG, Berkovic SF, Carvill GL, Sadleir LG, Scheffer IE, Mefford HC. Parental mosaicism in "de novo" epileptic encephalopathies. *N Engl J Med* 2018;387:1646–8.
36 Acuna-Hidalgo R, Bo T, Kwint MP, van de Vorst M, Pinelli M, Veltman JA, Hoischen A, Vissers LE, Gilissen C. Post-zygotic point mutations are an underrecognized source of de novo genomic variation. *Am J Hum Genet* 2015;97:67–74.
37 Ballif BC, Rorem EA, Sundin K, Lincicum M, Gaskin S, Coppinger J, Kashork CD, Shaffer LG, Bejjani BA. Detection of low-level mosaicism by array CGH in routine diagnostic specimens. *Am J Med Genet A* 2006;140:2757–67.
38 Stosser MB, Lindy AS, Butler E, Retterer K, Piccirillo-Stosser CM, Richard G, McKnight DA. High frequency of mosaic pathogenic variants in genes causing epilepsy-related neurodevelopmental disorders. *Genet Med* 2018;20:403–10.
39 Shi YW, Yu MJ, Long YS, Qin B, He N, Meng H, Liu XR, Deng WY, Gao MM, Yi YH, Li BM, Liao WP. Mosaic SCN1A mutations in familial partial epilepsy with antecedent febrile seizures. *Genes Brain Behav* 2012;11:170–6.