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# Parental mosaicism detection and preimplantation genetic testing in families with multiple transmissions of de novo mutations

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► Additional supplemental material is published online only. To view, please visit the journal online (<http://dx.doi.org/10.1136/jmg-2022-108920>).

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Received 6 September 2022  
Accepted 14 January 2023



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**To cite:** Xu N, Shi W, Cao X, et al. *J Med Genet* Epub ahead of print: [please include Day Month Year]. doi:10.1136/jmg-2022-108920

## ABSTRACT

**Background** De novo mutations (DNMs) are linked with many severe early-onset disorders ranging from rare congenital malformation to intellectual disability. Conventionally, DNMs are considered to have an estimated recurrence rate of 1%. Recently, studies have revealed a higher prevalence of parental mosaicism, leading to a greater recurrence risk, resulting in a second child harbouring the same DNM as a previous child. **Methods** In this study, we included 10 families with DNMs leading to adverse pregnancy outcomes. DNA was extracted from tissue samples, including parental peripheral blood, parental saliva and paternal sperm. High-throughput sequencing was used to screen for parental mosaicism with a depth of more than 5000× on average and a variant allele fraction (VAF) detection limit of 0.5%.

**Results** The presence of mosaicism was detected in sperms in two families, with VAFs of 2.8% and 2.5%, respectively. Both families have a history of multiple adverse pregnancies and DNMs shared by siblings. Preimplantation genetic testing (PGT) and prenatal diagnosis were performed in one family, thereby preventing the reoccurrence of DNMs.

**Conclusion** This study is the first to report the successful implementation of PGT for monogenic/single gene defects in the parental mosaicism family. Our study suggests that mosaic detection of paternal sperm is warranted in families with recurrent DNMs leading to adverse pregnancy outcomes, and PGT can effectively block the transmission of the pathogenic mutation.

## INTRODUCTION

De novo mutations (DNMs) are defined as genetic variants present in offspring but not detectable in either parent.<sup>1</sup> DNMs are the most severe kind of uncommon genetic variation: since they have been exposed to less intense natural selection, they are more detrimental on average than inherited variations.<sup>2</sup> DNMs have now been identified as the cause of a large percentage of severe early-onset diseases, ranging from rare congenital malformation syndromes to more common neurodevelopmental disorders, such as severe intellectual disability, epileptic encephalopathy, autism spectrum disorder (ASD) and schizophrenia.<sup>3–7</sup> These illnesses account for a significant share of all patients seen in neurology and clinical genetics departments worldwide. Clinical exome sequencing combined with prenatal

## WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ De novo mutations (DNMs) are one of the most severe types of rare genetic variation. According to conventional knowledge, the likelihood of recurrent DNMs for another pregnancy among parents of a DNM-affected child is modest, typically 1%. With the breakthrough of genome sequencing tools, it has become clear that apparently healthy parents have a higher prevalence of mosaicism for DNMs than previously assumed. There is, however, a paucity of research detecting parental mosaicism in families with multiple transmissions of DNMs.

## WHAT THIS STUDY ADDS

⇒ We show that paternal sperm mosaicism is present in a subset of families with DNMs and causes the multiple transmissions of DNMs. Next generation sequencing (NGS)-based targeted preimplantation genetic testing and prenatal diagnosis are able to effectively block the transmission of the disease-causing mutation caused by parental mosaicism.

## HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The study guides future work of genetic counselling, risk assessment and fertility options for parents of children with genetic disorders caused by DNMs. Our study demonstrates that for families with recurrent DNMs, mosaicism detection of paternal sperm is necessary.

diagnosis has revealed that 48%–63% of pathogenic or likely pathogenic variations linked with fetal structural abnormalities appear to be DNMs, with autosomal-dominant illnesses being the most common.<sup>8–10</sup> According to conventional knowledge, the likelihood of recurrent DNMs for another pregnancy among parents of a DNM-affected child is modest, typically 1%.<sup>11</sup> On the other hand, mosaicism is frequently disregarded as a source of harmful variation in uncommon monogenic disorders. With breakthroughs in genome sequencing tools, it has become clear that apparently healthy parents have a greater prevalence of somatic/germline mosaicism for DNMs than previously assumed. Recent studies of deep whole-genome sequencing results have shown that somatic mosaicism is found

in approximately 10% of parents who bear children with DNMs associated with ASD, with levels of variant allele fraction (VAF) ranging from 1% to 40% in the peripheral blood.<sup>12,13</sup> More crucially, pathogenic variants due to parental mosaicism may be passed down to subsequent offspring, resulting in unanticipated recurrences of the same DNMs in the affected children.<sup>14</sup> The incidence of recurrent DNMs among siblings increases to 24% with >1% VAF in parental blood cells and 50% with >6% VAF in parental blood cells.<sup>15</sup>

The birth of children with highly penetrant genetic disorders for which drug therapies remain limited poses considerable psychological, social and economic challenges. Thus, many couples wish to avoid the recurrence of the same disease in a second child. Genetic counselling, risk assessment and fertility options for parents of children with genetic disorders caused by DNM present clear challenges.<sup>16</sup> It is especially important to identify parental mosaicism if the same DNM causes two or more unfavourable pregnancies.

This study included 10 families with adverse pregnancies caused by DNMs, and parental mosaicisms were screened by high-throughput sequencing. Fertility interventions were offered to families with gonadal mosaicism using preimplantation genetic testing (PGT) and prenatal diagnosis.

## MATERIALS AND METHODS

### Ethics statement

The study was authorised by the International Peace Maternity and Child Health Hospital and the Obstetrics and Gynecology Hospital of Fudan University, and all participants provided informed consent in accordance with ethical norms.

### Subjects

Ten families with adverse pregnancies caused by DNMs participated in this study. All participants were recruited from April 2020 to March 2021 at the Outpatient Department of the Reproductive Center at International Peace Maternity and Child Health Hospital and the Obstetrics and Gynecology Hospital of Fudan University.

### DNA extraction and mutation detection

Standard procedures were used to extract genomic DNA from peripheral blood samples from the 10 families using the MagNA Pure LC DNA Isolation Kit (Roche Diagnostics, GmbH, Mannheim, Germany), including the probands in each pedigree. Whole-exome sequencing (WES) library construction and sequencing were performed using the Illumina platform according to the manufacturer's protocols. Over 180 000 exons and 10 bp flanking regions from 22 000 genes are covered by the detection. Exome sequencing was performed on the HiSeq2000 sequencing platform (Illumina). The Agilent SureSelectXT All Exon Kit 51Mb was used to accomplish whole-exome capture and massively parallel sequencing. Sequenced reads were gathered, filtered for quality and aligned to the human reference genome (UCSC Genome Browser hg19) using the Burrows-Wheeler Aligner. Single-nucleotide variants and small insertions or deletions (indels) were identified using the Genome Analysis Toolkit and annotated with the ANNOVAR software. The guidelines of the American College of Medical Genetics and Genomics were used for variant interpretation and categorisation.<sup>17</sup> The dbNSFP database was used to obtain functional predictions for the identified variations.<sup>18</sup> Blank controls were processed under identical circumstances to demonstrate the absence of contamination. Each mutation we found will be

confirmed by bidirectional Sanger sequencing. Target regions of mutations were amplified using specified primers (online supplemental table S1) and sequenced on an Applied Biosystems 3500Dx sequencer.

### Screening for mosaicism

The mother's DNA (saliva and peripheral blood) and father's DNA (saliva, peripheral blood and sperm) were extracted using the QIAamp DNA micro kit (Qiagen). Genomic DNA was amplified and sequenced for the library with primers for the variants of interest. High-throughput sequencing of sections within the detection range in the genomic DNA of the studied patients was performed on the MGI2000 sequencing platform to base call the sequenced fragments. The target VAF for each sample was estimated after alignment to the human reference genome (GRCh38) and the elimination of duplicate reads. The DNA from proband was used as a positive control, and NA12878 DNA (Coriell Institute) was used as a negative control. Negative control and a progressive dilution of positive control (from 0.01% to 10%) were used to measure background noise and the limit of detection. Targeted regions were examined at a depth of more than 5000× on average, with a VAF detection limit of 0.5%.

### In vitro fertilisation

COH was conducted with hCG. GnRH antagonists are a multiple-dose flexible regimen for the prevention of ovarian hyperstimulation syndrome. Intracytoplasmic sperm injection (ICSI) was performed on metaphase II oocytes, and the resultant embryos were cultured to the blastocyst stage for biopsy. The biopsied blastocysts were cryopreserved for further embryo transfer cycles using the vitrification procedure with individual tubes containing single blastocysts.

### Identity testing and haplotype analysis

According to the instructions, short tandem repeats were used for identity testing and detecting probable maternal contamination with an identification detection kit (R1004T; GENESKY, Shanghai, China). Whole-genome amplification of each embryo biopsy sample was performed using the general sample processing kit for gene sequencing (Yikon Genomics, China), following the manufacturer's instructions. Following the recommendations issued by the European Society for Human Reproduction, for design, we chose sites with a heterozygosity rate of 0.2–0.8 and only two base types within 2 Mb upstream and downstream of the mutant gene.<sup>19</sup> Approximately a total of 187 single nucleotide polymorphisms (SNPs) were designed for haploid linkage analysis.

### Prenatal diagnosis

Pregnancy was verified by a blood hCG level of 25 U/L 14 days after transplantation and determined by ultrasound of the fetal sac with heart rhythm in the uterine cavity 30–40 days after transplantation. Amniocentesis was used to perform prenatal diagnosis in the second trimester. Twenty microliters of amniotic fluid were collected under ultrasound guidance. The genotypes of the fetuses were validated by Sanger sequencing after genomic DNA was extracted as described above.

## RESULTS

### Mutation detection

In the current study, a total of 10 families with DNMs were included. DNMs detected in probands of 10 families are shown

**Table 1** De novo mutations detected in probands of 10 families

Family	Pathogenic gene	Transcript ID	DNA_variant	Amino acid changes	Classification of variants†
1	<i>SMARCA2</i>	NM_003070.5	c.553C>G	p.(Gln185Glu)	Likely pathogenic
2	<i>SF3B4</i>	NM_005850.4	c.29delA	p.(Asn101Ilefs*30)	Pathogenic
3	<i>PKD1</i>	NM_001009944.2	c.7863+1G>A	p.(?)	Likely pathogenic
4	<i>RERE</i>	NM_012102.3	c.32_33delAA	p.(Lys11fs)	Pathogenic
5	<i>PHEX</i>	NM_000444.6	c.58C>T	p.(Arg20X)	Pathogenic
6	<i>SYNGAP1</i>	NM_006772.2	c.2295-2A>G	p.(?)	Pathogenic
7	<i>TP63</i>	NM_003722.5	c.1010G>A	p.(Arg337Gln)	Likely pathogenic
8	<i>KIF11</i>	NM_004523.4	c.139C>T	p.(Arg47Ter)	Pathogenic
9	<i>GNAS</i>	NM_000516.4	c.493C>T	p.(Arg165Cys)	Likely pathogenic
10	<i>ACTA1</i>	NM_001100.3	c.1120C>A	p.(Arg374Ser)	Likely pathogenic

\*Termination of protein translation.

†The interpretation and classification of variants were based on ACMG guidelines.<sup>17</sup> ACMG, American College of Medical Genetics and Genomics.

in table 1 and online supplemental figure S1. Among 10 families, 5 of them possessed the pathogenic variants and 5 the likely pathogenic variants.

### History of 10 families

Clinical data of 10 families (including the presence of DNMs in other siblings, history of adverse pregnancy and clinical phenotype of the proband) are summarised in table 2. Family 1 and Family 2 have a history of multiple adverse pregnancies and DNMs shared by siblings. Detailed pedigree diagrams of the 10 families are shown in figure 1.

#### Family 1

The proband of Family 1 (figure 1A, II-1) is a boy who had intellectual disability and frequent, uncontrollable epilepsy since he was 2 years old. The genetic testing revealed a heterozygous pathogenic *SMARCA2* mutation in the affected boy (c.553C>G). The proband's mother (figures 1A1–2) had an ongoing pregnancy and prenatal diagnosis was offered to the family for the fetus after the *SMARCA2* variant was identified in the proband. By amniocentesis, it was confirmed that the same variant c.553C>G was present in the fetus (figure 1A, II-4).

#### Family 2

The mother of Family 2 (figure 1B, I-2), 38 years of age, with a history of adverse pregnancy (one induced labour with multiple lymphangiomas at 28 weeks gestation and two miscarriages

due to fetal arrest in the first trimester), conceived after PGT for aneuploidies. At 23+5 weeks of gestation, the ultrasound indicated a fetus with left varus foot, left upper limb dysplasia, possible small mandible possible and growth restriction. WES of labour induction revealed de novo *SF3B4* mutations (c.29delA) in the fetus (figure 1B, II-4).

#### Family 3

The proband of Family 3 (figure 1C, II-1) was a middle-aged man who came to our hospital due to years of nulliparity. Multiple semen examinations showed the volumes were less than 2 mL, and all were immotile spermatozoa. The sperm survival rate was 1% and the percentage of normal morphology was 1.9%. Ultrasonography suggested the presence of multiple anechoic areas of varying size in the renal parenchyma. Trio-whole exome sequencing (trio-WES) revealed the presence of NM\_001009944.2 (*PKD1*): c.7863+1G>A de novo heterozygous mutations in the proband (figure 1C, II-1).

#### Family 4

The proband of Family 4 (figure 1D, II-1) was a boy weighing 2.900 kg (13th centile) and was 50.0 cm (40th centile) in length at birth. He suffered from hypotonia in the neonatal period and was finally diagnosed with global developmental delay and severe intellectual disability when he was 2. He also had left eye exotropia. WES revealed the presence of *RERE*: c.32-33delAA heterozygous mutations in the proband (figure 1D, II-1).

#### Family 5

The proband of Family 5 (figure 1E, II-2) was a middle-aged woman with short stature, severe genu varus and low blood phosphorus. Other family members (mother, father and brother) of the proband have normal phenotypes. WES revealed the presence of NM\_000444.6 (*PHEX*): c.58C>T (p. Arg20X) heterozygous mutation in the proband (figure 1E, II-2).

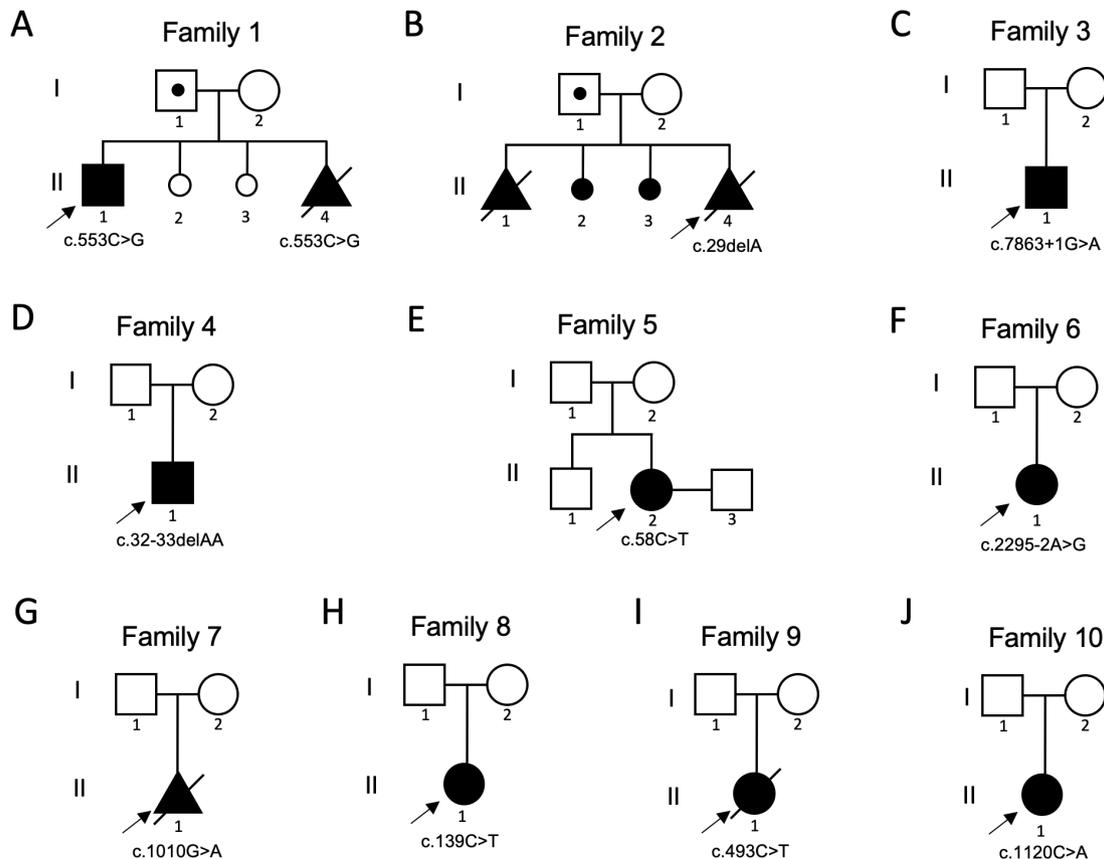
#### Family 6

The proband of Family 6 (figure 1F, II-1) was a boy with language delayed, stereotyped behaviour, aggressive behaviour, lack of eye contact and social interaction and was diagnosed of ASD when he was 8. Convulsive seizures began at the age of 2 years 10 months, manifested by myoclonus and atonia and occurred at a frequency varying from 10 seizures per day. WES revealed the presence of NM\_006772.2 (*SYNGAP1*): c.2295-2A>G heterozygous mutation in the proband (figure 1F, II-1).

**Table 2** Clinical characteristics of 10 families

Family	DNMs shared by siblings	History of adverse pregnancy*	Clinical phenotype of probands
1	Yes	2	Intellectual disability, recurrent epilepsy
2	Yes	4	Fetus with left varus foot, growth restriction
3	No	1	Polycystic kidney disease
4	No	1	Left eye exotropia, growth retardation
5	No	1	Hypophosphatemic rickets
6	No	1	Language delay
7	No	1	Split-hand/split-foot malformation
8	No	1	Microcephaly
9	No	1	Pseudohypoparathyroidism
10	No	1	Myasthenia, dysphagia

\*Criteria included unexplained spontaneous abortions, embryonic arrest and stillbirths or a live baby affected with the monogenic disorder.



**Figure 1** Pedigrees of 10 families. Squares indicate males. Circles indicate females. The black arrows indicate the probands. The filled black symbols indicate the affected patients. Square with black dot in the centre represents mosaic father. Triangles represent fetuses of unknown sex. The small solid circles represent abortions. A slash through the symbol means deceased.

#### Family 7

The proband of Family 7 (figure 1G, II-1) was a fetus whose mother's labour was induced at 23 weeks gestation because of a prenatal diagnosis of split-hand and foot malformation by ultrasound. WES revealed the presence of NM\_003722.5 (*TP63*): c.1010G>A heterozygous mutation in the fetus (figure 1G, II-1).

#### Family 8

The proband of Family 8 (figure 1H, II-1) was a girl who presented with microcephaly, lymphedema and chorioretinal dysplasia since birth. The mother and father of the proband have normal phenotypes. Trio-WES revealed the presence of NM\_004523.4 (*KIF11*): c.139C>T (p. Arg47Ter) de novo heterozygous mutations in the proband (figure 1H, II-1).

#### Family 9

The proband of Family 9 (figure 1I, II-1) was the first child of healthy and non-consanguineous Chinese parents. Pseudohypoparathyroidism was diagnosed at the age of 5 days due to elevated thyroid stimulating hormone levels. Echocardiography revealed the presence of the ventricular septal defect. She had several times generalised seizures. WES revealed the presence of NM\_000516.4 (*GNAS*): c.493C>T heterozygous mutation in the proband (figure 1I, II-1).

#### Family 10

The proband of Family 10 (figure 1J, II-1) was diagnosed with hypotonia after birth and complained of feeding difficulties and inability to breathe spontaneously in the neonatal period. The

physical examination was notable for scoliosis. She uses ventilatory support 24 hours a day with a tracheostomy. WES revealed the presence of NM\_001100.3 (*ACTA1*): c.1120C>A heterozygous mutation in the proband (figure 1J, II-1).

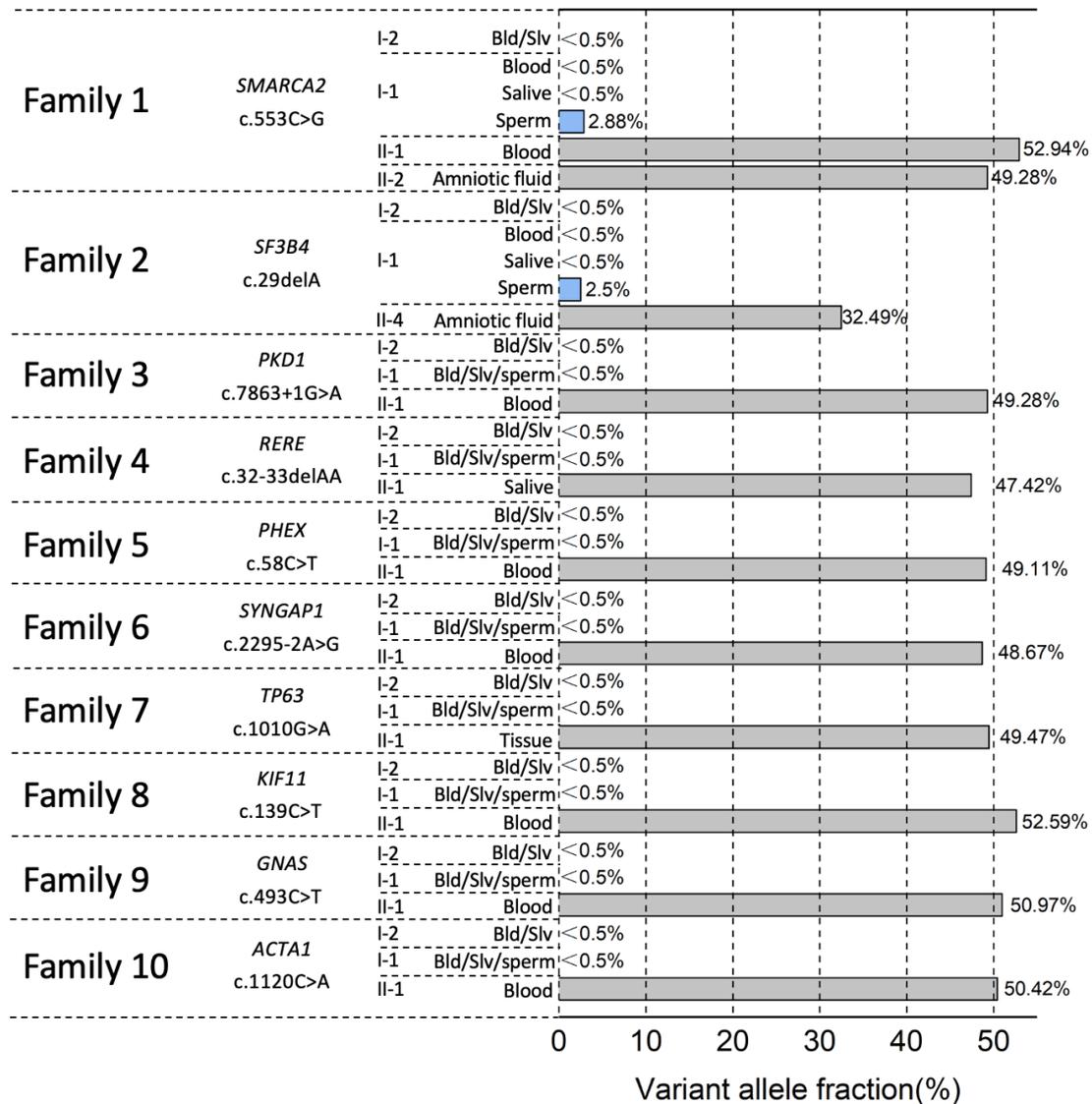
#### Detection of parental mosaicism

Figure 2 shows the results of the mosaicism detection from 10 families. Among 10 families, two parental mosaicisms (Family 1 and Family 2) were detected, and no mosaicism (VAF<0.5%) was detected in the remaining eight families. The VAF of *SMARCA2* was 2.88% in the paternal sperm of Family 1 and was reported as a case report in our previous study.<sup>20</sup> The VAF of *SF3B4* was 2.5% in the paternal sperm of Family 2.

#### PGT and prenatal diagnosis of Family 2

Patients of Family 2 chose PGT for monogenic/single gene defects (PGT-M) to avoid the occurrence of abnormal pregnancy. Despite the low rate of sperm mosaicism, the PGT was performed due to the advanced parental ages and history of recurrent miscarriages.

After the gonadotropin-releasing hormone (GnRH) antagonist protocol, the oocytes of the patients were retrieved and fertilised by ICSI. Embryo biopsy followed by copy number variation (CNV) sequencing showed that embryo 1 and embryo 2 of the mother in Family 2 had normal CNV (figure 3C). However, targeted NGS sequencing of informative SNPs revealed that the normal CNV embryos had acquired the mutation haplotype from their father (figure 3A). Sanger sequencing of DNA from all embryos showed the absence of *SF3B4* variants at c 29



**Figure 2** Detection of parental mosaicism of 10 families. The VAFs in sperm of the fathers in families 1 and 2 were 2.8% and 2.5%, respectively. No mosaicism (VAF<0.5 %) was detected in the remaining eight families. VAF, variant allele fraction.

(figure 3B). Considering that the VAF mosaicism in the sperm was only 2.8% and haplotype linkage analysis can produce false positives in samples carrying the paternal mutation, blastocysts carrying the paternal mutant haplotype may not truly contain the *SF3B4* pathogenic variants. Normal CNV embryos with normal *SF3B4* according to Sanger sequencing results were selected for transplantation.

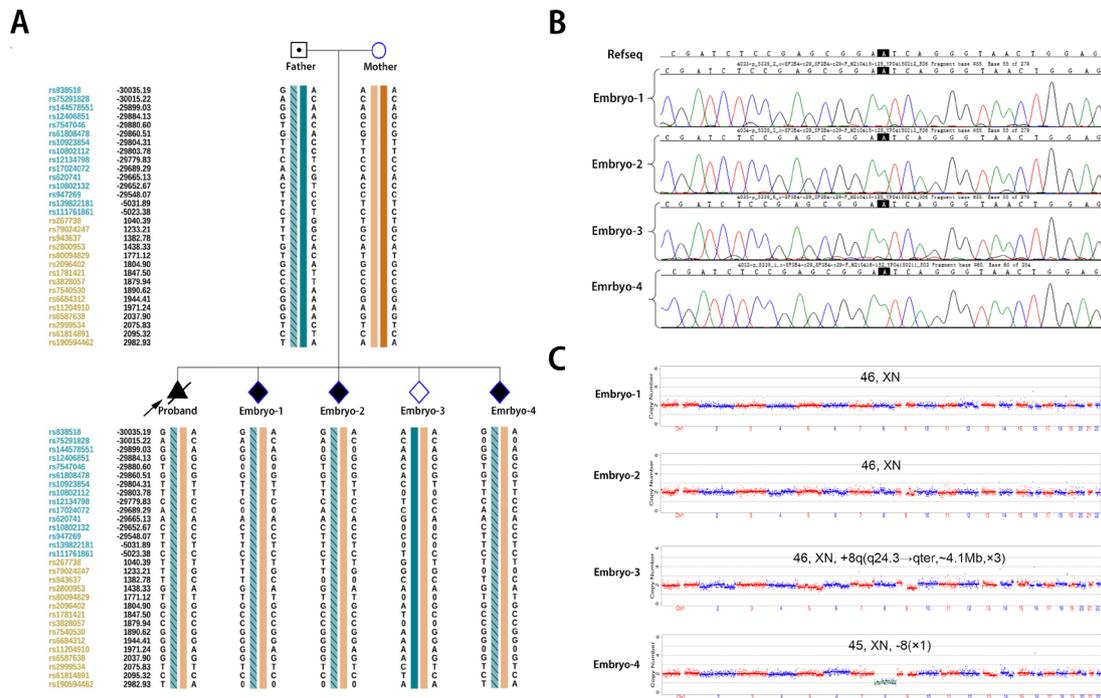
Successful pregnancy was confirmed by human chorionic gonadotropin (hCG) and ultrasound examination. Prenatal diagnosis results showed that the fetus did not carry mutations, which confirmed the accuracy of PGT (online supplemental figure S2). The baby of Family 2 was born at full term and was healthy.

## DISCUSSION

A total of 10 families with adverse pregnancy outcomes due to DNMs were included in this study. High-throughput sequencing was used to screen for mosaicism in the parental peripheral blood, parental saliva and paternal sperm cells of 10 families, and the mosaicism was detected in the paternal sperm in two families. Despite low VAF (2.88% and 2.5%, respectively), we treated one family with PGT due to the presence of advanced

maternal age, multiple adverse pregnancy outcomes and the strong wishes of the patients. DNMs were effectively avoided in Family 2, resulting in a healthy newborn.

In our study, both families (Family 1 and Family 2) in which mosaicism was detected had DNMs shared by siblings. Multiple clinical studies have shown that couples should be highly suspected of having gonadal mosaic mutations if there is a history of two or more adverse pregnancies caused by the same DNM. Recent studies show that only 3.8% of parents with offspring with DNMs have blood mosaicism; the incidence rises to 57.2% when two or more offspring share the same DNMs.<sup>14,15</sup> The contribution of pathogenic mosaic variants to DNM has been underestimated for several reasons. Despite advances in sequencing technology, detecting mosaicism in human disease has been challenging. Traditional molecular approaches such as Sanger sequencing are incapable of detecting low-level somatic mosaicism with a VAF of less than 10%–20%.<sup>21</sup> With developments in genomic technologies such as NGS, blocker displacement amplification and droplet digital PCR (ddPCR), the capacity to detect modest levels of mosaicism (VAF of 1%) has improved significantly.<sup>22–24</sup> In addition, pathogenic variants may



**Figure 3** Details of biopsy results in embryos of Family 2 who chose PGT-M for IVF. (A) Linkage analysis results. The orange band represents chromosomes from the mother, cyan bands without slash indicate normal chromosomes from the father and cyan bands with slash indicate disease-causing chromosomes from the father. Embryo 3 showed the absence of disease-causing variants. (B) Sanger sequencing results. All embryos showed the absence of SF3B4 variants at c 29. (C) CNV sequencing results. Embryos 1 and 2 had normal CNV. CNV, copy number variant; IVF, in vitro fertilisation; PGT-M, preimplantation genetic testing monogenic/single gene defects.

be tissue-specific or tissue-limited, but most human genetic tests are conducted on DNA extracted from peripheral blood leucocytes. Mosaicism includes germline mosaicism (also known as gonadal mosaicism), somatic mosaicism and gonosomal mosaicism (a combination of germline and somatic mosaicism).<sup>25</sup> In general, only germline mutations have the potential to be transmitted to offspring; thus, the detection of germline mosaicism is particularly important in genetic risk assessment.<sup>16</sup> Only VAF in sperm mosaicism can be detected as limitations prevent detection in maternal germ cells. Massive spermatogonial mitotic proliferation likely underlies the surprising observation that 80% of DNMs in the offspring arise on the paternal haplotype.<sup>26 27</sup> Thus, sperm mosaicism screening can detect the vast majority of at-risk individuals.

Providing accurate recurrence risk estimates and fertility options for families with children with pathological DNMs remains challenging. Deep sequencing of parental blood and tissue for pathogenic DNMs seen in children, according to our findings, should allow meaningful stratification of families into the large majority with recurrence risks of less than 1% and the small minority with recurrence risks of at least an order of magnitude higher. Table 3 summarises factors that impact the risk for DNM recurrence and can contribute to the physician's genetic evaluation.

In this study, two fathers with mosaicisms in sperm were detected, and the VAFs were 2.88% and 2.5% respectively. Now that sperm mosaicisms have been directly quantified, we may determine that recurrence risk arises via discrete and quantifiable effects, depending on the type and VAF of the mutation. Type I mutations are found in terminally or near-terminally postmitotic spermatocytes and sperm cells, and they are thought to account for a large proportion of DNMs. Type II mutations occur in spermatogonial stem cells (SSCs) and accumulate due

to environmental exposures and mitotic mistakes during ageing. Type III mutations arise throughout the male embryogenesis process, seeding many SSCs and contributing to a stable sperm percentage throughout life, which can lead to intrafamilial recurrence. Type I and type II mutations have no or a very low recurrence risk within a family, and because they are random, their occurrence across the population is expected to occur by chance. It is worth noting that type II has a unique increased recurrence risk within families. 'Selfish spermatogonial selection', or a stronger potential for self-renewal resulting from spontaneous mutations in SSCs, leads to clonal growth.<sup>28 29</sup> All known mutations in selfish spermatogonial selection result in the activation of proteins with gain-of-function properties, and most of them have been reported to be associated with various tumour types, involving the tyrosine kinase receptor/RAS/MAPK pathway.<sup>30 31</sup> Given the low amount of mosaicism (2.5 %) as well as the high

**Table 3** Components of genetic counselling for a DNM discovered in a child

Factor	Impact on recurrence risk
Parental gonadal mosaicism	Depending on the type and VAF of the mutation. Mosaicisms in mothers double the probability of recurrence, but the probability is increased more than 50 times in fathers <sup>11</sup>
DNMs shared by siblings	Increases to 23.0% from 1.1% <sup>14</sup>
Mutation origin	Approximately 80% of all DNMs arise in the paternal allele, but mutations of maternal origin determine a higher recurrence risk than paternal mutations <sup>11 46</sup>
Age of mother	Unlikely to change with maternal age <sup>11</sup>
Age of father	Despite an increased risk of a first afflicted offspring, the recurrence risk from paternal mutations diminishes with age <sup>11</sup>
DNM, de novo mutation.	

frequency of recurrence of the paternal pathogenic variant, we anticipate that sperm carrying this variant may have an advantage over normal sperm during conception. The mutated gene *SF3B4* in Family 2, encodes one of the components of the splicing factor 3b (SF3b) complex.<sup>32</sup> The loss or gain of function caused by *SF3B4* mutations is frequently associated with aberrant cell development and plays a role in the pathogenesis of various cancers including hepatocellular carcinoma, ovarian cancer and oesophagus squamous cell carcinoma.<sup>33–36</sup> Whether SF3B4 plays a role in SSC self-renewal, leading to selfish spermatogonial selection, requires further investigation. Specifically, all different types of recurrence risk can be directly assessed by obtaining mosaicism VAF results from sperm sequencing analysis.

Identifying parental mosaicism will improve the technical ability to perform PGT because knowing the parental allele that contains the variant allows PGT techniques to incorporate linked markers.<sup>37</sup> PGT avoids therapeutic termination as an early form of prenatal diagnosis, which has various consequences ranging from severe bleeding, cervical damage and infections to future infertility.<sup>38</sup> In our study, PGT successfully blocked the transmission of the pathogenic mutation in the family in which the father had sperm mosaicism, which provided new ideas for fertility selection in families with mosaic mutations. We strongly recommend that mosaicism detection be conducted for couples in families with multiple adverse pregnancies ( $\geq 2$ ) caused by the same DNM mutation. In the presence of gonadal mosaicism, PGT-M may be implemented for families with a high risk of DNM recurrence, depending on the range of clinical applications and the wishes of the families. The desire to avoid suffering is a common goal across nations, cultures and societies. Some overuse of PGT raises concerns about eugenics and ‘designer babies’ because it may screen for health unrelated traits such as height and intelligence.<sup>39–41</sup> It is important to point out that one of the major substantial requirements for the application of PGT in most countries is to restrict its use only to situations of medical need, prohibiting its use for personal or social reasons.<sup>42–44</sup> The general consensus was that PGT-M is ethically justified when the fetus is at ‘substantial risk’ of ‘severe genetic disease’ and following this principle will avoid excessive genetic testing and the application of PGT.<sup>39–41, 45</sup>

In summary, 10 families with adverse pregnancy outcomes caused by DNMs were included in our study. The fathers were diagnosed with sperm mosaicism in two of these families. Besides, targeted NGS-based PGT and prenatal diagnosis successfully prevent the transmission of the pathogenic mutation in one of the families. Our study suggests that mosaic detection of paternal sperm is warranted in families with recurrent DNMs leading to adverse pregnancy outcomes. In addition, for parental mosaicism, genetic counselling, guided PGT and prenatal diagnosis can effectively pacify patients and improve pregnancy outcomes. This model could be effective in preventing the transmission of severe genetic diseases.

**Acknowledgements** The authors would like to thank the family members for participating in this study.

**Contributors** CX and SC designed the experiments. NX and WS performed the experiments and wrote the manuscript. XC assisted with the experiments. XZ checked the English grammar and polished the English language in the manuscript. LJ and H-FH performed PGT-M test and analysed the biopsy results. All the authors contributed to the article and approved the submitted version. CX is guarantor for this study and accepts full responsibility for the work and/or the conduct of the study, had access to the data, and controlled the decision to publish.

**Funding** This work was supported by the National Key R&D Programme of China (21YFC2701002, 2018YFC1004900), the National Natural Science Foundation of China (Nos. 81971344, 81771638 and 82171677), the Shanghai Municipal

Commission of Science and Technology Programme (No. 21Y21901002, No. 15411966700), Shanghai Municipal Health Commission (202140110, GW-10.1-1-XK07), Shanghai Municipal Commission of Health and family planning (202140110, 20 215Y0216), National Natural Science Foundation of China (82088102), CAMS Innovation Fund for Medical Sciences (2019-I2M-5–064), Collaborative Innovation Program of Shanghai Municipal Health Commission (2020CXJQ01), Clinical Research Plan of SHDC (SHDC2020CR1008A) and Shanghai Frontiers Science Research Base of Reproduction and Development.

**Competing interests** None declared.

**Patient consent for publication** Not applicable.

**Ethics approval** The study was authorised by the International Peace Maternity and Child Health Hospital (GKLW2019-52) and the Obstetrics and Gynecology Hospital of Fudan University (2020-178), and all participants provided informed consent in accordance with ethical norms. Participants gave informed consent to participate in the study before taking part.

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

**Data availability statement** Data are available on reasonable request.

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