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Case Report

Genetic effects of a 13q31.1 microdeletion detected by noninvasive prenatal testing (NIPT)

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Abstract: Microdeletions of chromosome 13q31.1 are relatively rare. These types of deletions may cause different genetic effects on genotypes and/or phenotypes. There are several ways to detect microdeletions; noninvasive prenatal testing (NIPT) is the newest detection method. In this study, we aimed to investigate the genetic effects of a 13q31.1 microdeletion detected by NIPT and to reconfirm the feasibility of this procedure in predicting sub-chromosomal copy number variations (CNVs). The 13q31.1 microdeletion, which has previously been described as a disease-associated fragment, was detected by NIPT in a pregnant woman. To validate the finding and to explain the origin of this sub-chromosomal CNV, we collected fetal amniotic fluid and parental blood samples and tested the samples using array-based comparative genomic hybridization (aCGH). Karyotype analysis was performed on all of the samples to rule out balanced or mosaic anomalies. The aCGH results confirmed the NIPT findings. We detected the same type of microdeletion in the fetus and the mother via aCGH. The mother had a normal phenotype; therefore, in a post-test genetic counseling session, we predicted a normal phenotype for the fetus. After delivery, the normal phenotype of the newborn confirmed our prediction. Based on the present study, this 13q31.1 microdeletion may be considered as a chromosomal polymorphism. This study also reconfirmed the feasibility of obtaining a molecular karyotype of a fetus via NIPT.

Keywords: Microdeletion/microduplication, NIPT, Acgh, prenatal diagnosis, SLITRK1, SLITRK6, 13q31.1

Introduction

Previous research has shown that copy number variations (CNVs) play important roles in certain human phenotypic variations or diseases [1, 2]. Some syndromes, such as Williams-Beuren syndrome, Angelman/Prader-Willi syndrome, Charcot-Marie-Tooth syndrome, and others, are caused by CNVs or segmental duplications/deletions [3, 4]. Similar to single-nucleotide polymorphisms (SNPs), most CNVs exist as genetic polymorphisms, while only a few of them are pathogenic variations [1-3, 5]. The emergence of array-based comparative genomic hybridization (aCGH) and SNP microarrays (SNP arrays) has greatly accelerated the discovery of CNVs in the human genome. These chromosomal microarray techniques, with their high resolutions, are increasingly used in prenatal diagnosis throughout the world [6-9].

In a traditional prenatal diagnosis program, when genetic aberrations are suspected in a fetus' genome, invasive procedures, such as chorionic villus sampling (CVS), amniocentesis, or percutaneous umbilical cord blood sampling (PUBS)/cordocentesis, are used to provide fetal samples for genetic detection by well-established genetic analysis techniques, such as G-band karyotyping, fluorescence in situ hybridization (FISH), quantitative fluorescence PCR (QF-PCR), or chromosomal microarray analysis (CMA, including aCGH and SNP arrays) [8, 10-12]. These programs did not change until 1997, when the discovery of free fetal DNA (ffDNA) in maternal plasma promoted the development of noninvasive prenatal diagnosis [13]. This noninvasive method was significantly advanced with the development of massively parallel sequencing (MPS), which is also referred to as next-generation sequencing

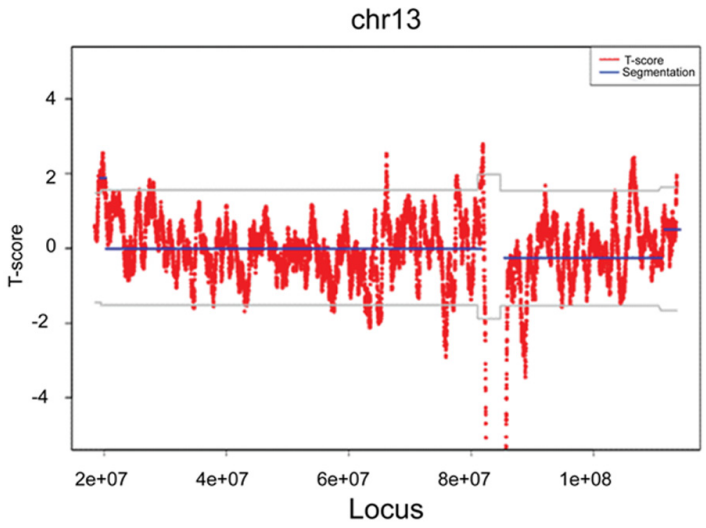

Figure 1. NIPT study of the maternal plasma, illustrating a suspicious 3.71-Mb deletion in the long arm of chromosome 13 (chr13: 81667889-85377286).

(NGS). The establishment of noninvasive prenatal testing (NIPT) rests on the discovery of ffDNA, and its core technology is MPS. NIPT can predict some common fetal aneuploidies, such as trisomies 13, 18, and 21 (T13, T18, and T21) and sex chromosome abnormalities. NIPT not only provides an accurate way to screen these chromosomal aberrations but also reduces the need for invasive procedures to detect these fetal chromosomal aneuploidies [14-17]. NIPT can also predict sub-chromosomal CNVs in the fetus and offers a promising method for noninvasive molecular genetic prenatal diagnosis [18-20].

Microdeletions of chromosome 13q31.1 are relatively rare. SLITRK1 and SLITRK6 are both located in the region of 13q31.1 and are SLITRK gene family members. In a previous study, the SLITRK1 gene was associated with Gilles de la Tourette syndrome (GTS) and Trichotillomania, which typically manifest as neuropsychological disorders related to alterations in dopamine metabolism and neurotransmission involving frontal-subcortical neuronal circuits [21-23]. The SLITRK6 gene is associated with autosomal-recessive congenital myopia and prelingual sensorineural hearing loss [24]. The main aim of our study was to explore the genetic effects of the 13q31.1 microdeletion detected by NIPT and aCGH. This microdeletion may be a newfound polymorphism of the human chromosome. Additionally, we also reconfirmed the feasibility of NIPT for the prenatal detection of microdeletions/microduplications.

Materials and methods

Participants and samples

The study participant was at 17 weeks' gestation. In the second trimester plasma screening, she was notified that the fetus had an increased risk of having T21 syndrome. To avoid invasive prenatal diagnosis procedures, an NIPT was offered to further screen for common fetal chromosomal abnormalities. The NIPT results revealed a microdeletion in the mixture of the fetal and maternal genomes, which indicated that the microdeletion could come from both the fetus and the mother. To confirm the NIPT detection and to determine its origin, amniocentesis was used to obtain a fetal amniotic fluid sample at 19 weeks' gestation, and parental blood samples were also collected. The study was approved by the Research Ethics Committee of Shandong Provincial Hospital Affiliated with Shandong University. Written informed consent was obtained from all of the participants or guardians that participated in this research.

Noninvasive prenatal testing

Five milliliters of maternal peripheral blood was collected into a blood collection tube containing ethylenediaminetetraacetic acid dipotassium salt (EDTA-K2), and the maternal plasma was separated and transferred into a new tube after centrifuging the sample at 1600 g for 10 min. The supernatant was then transferred into a sterile tube and centrifuged at 14,000 g for another 10 min. The plasma fraction was aliquoted and stored at -80°C for future processing. All subsequent standard procedures, including the isolation of cell-free DNA, library construction, and sequencing, were performed in the clinical laboratory of BGI-Shenzhen, China. The details regarding NIPT methods have been described previously [25]. We used bioinformatic methods combined with a locally weighted polynomial regression to eliminate GC-bias and a binary hypothesis to obtain a higher accuracy for aneuploidy detection. The

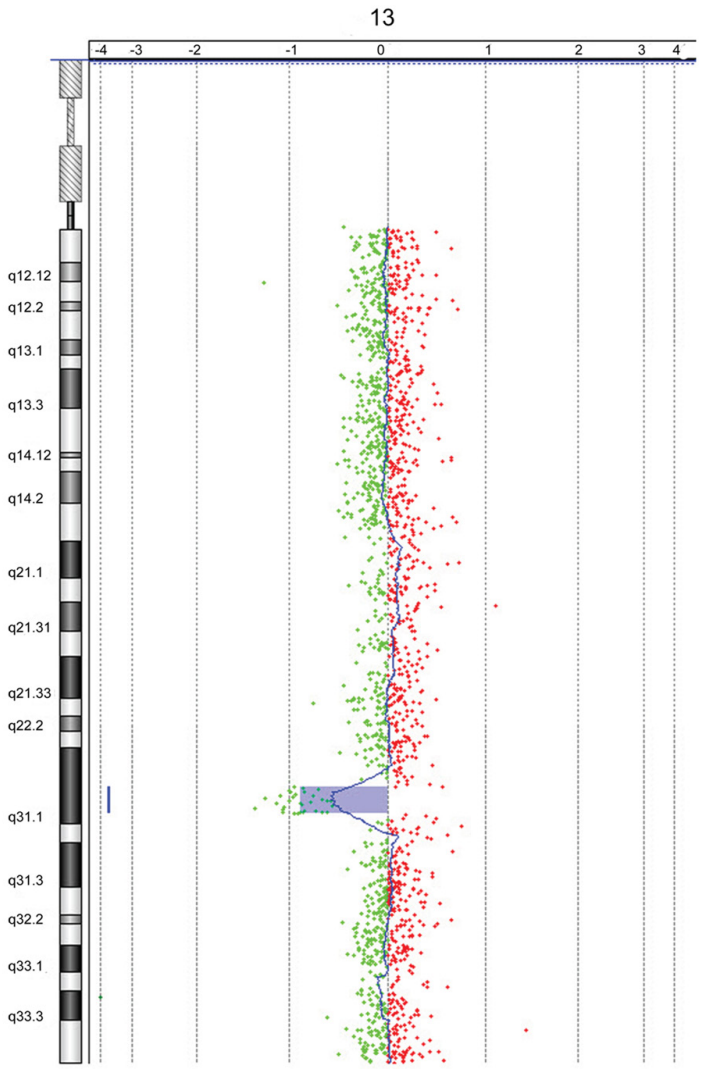

Figure 2. aCGH analysis of fetal uncultured amniocytes, showing a 3.05-Mb deletion at chromosome band 13q31.1 [arr 13q31.1 (83, 494, 767-86, 543, 280) ×1].

risk assessments for T21, T18, and T13 were performed using this test. We also designed a pipeline for Fetal Copy-number Analysis through Maternal Plasma Sequencing (FCAPS) to detect microdeletions and microduplications. Referring clinicians were notified if any of these additional abnormalities were suspected [25, 26].

Array-based comparative genomic hybridization

In this study, aCGH was used to confirm the existence of the genomic rearrangement that was detected by NIPT to further understand its origin. Total genomic DNA was extracted from 10 ml of fetal amniotic fluid or 2 ml of uncultured venous blood samples from the parents with a commercially available Amniotic Fluid Genomic DNA Extraction Kit and a Blood Genomic DNA Extraction kit, respectively (both from BioChain Institute Inc., Newark, CA), according to the manufacturer's instructions. As previously described, for each aCGH experiment, 400 ng of purified DNA and normal sex-matched DNA (BioChain Institute, Inc.) were digested with 10 U Alu I and 10 U Rsa I (Promega, Madison, WI, USA) and differentially labeled with cyanine-5 (cy5) and cyanine-3 (Cy3) fluorescent dyes using a Genomic DNA Enzymatic Labeling Kit (Agilent, Santa Clara, CA). aCGH analysis was performed using 8×60 K commercial arrays (Agilent). This platform contains 60-mer oligonucleotide probes spanning the entire human genome with an overall mean probe spacing of 50 kb. Previous studies showed that 95.5% of confirmed pathogenic copy number changes are > 500 kb, while 3.95% are 300 to 500 kb, and a single case (0.56%) is < 300 kb. In our study, the threshold value filter was set as a continuous change in Probe 6, and the test of CNV resolution was > 300 kb [27, 28]. After hybridization, the arrays were scanned with a dual-laser scanner (Agilent), and the images were extracted and analyzed using Feature Extraction software (Agilent) and Workbench genomics software, respectively [28].

Karyotyping

The amniotic fluid and the blood samples collected in this study were processed in parallel with cell culture and conventional karyotyping (G-banding) to exclude mosaic or balanced chromosome abnormalities.

Bioinformatics

To better understand the 13q31.1 microdeletion, we evaluated the deleted region with the information provided by the Online Mendelian

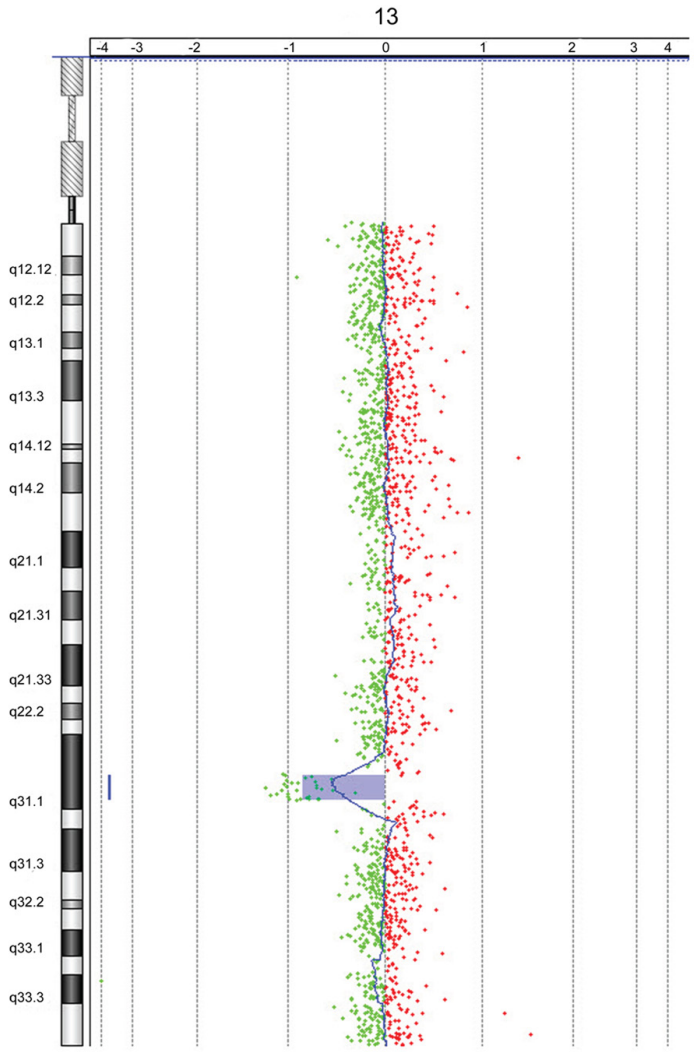

Figure 3. aCGH analysis of uncultured maternal peripheral blood, showing a 2.82-Mb deletion at chromosome band 13q31.1 [arr 13q31.1 (83, 724, 773-86, 543, 280) ×1].

Inheritance in Man database (OMIM, http://omim.org/), the DECIPHER Database (http://decipher.sanger.ac.uk), PubMed (http://www.ncbi.nlm.nih.gov/pubmed/), and the Database of Genomic Variants (DGV, http://www.ncbi.nlm.nih.gov/dbvar/).

Results

Detection of the 13q31.1 microdeletion via NIPT

The NIPT results revealed that the ffDNA was negative for T21, T18, and T13, but an obvious deletion in chromosome 13 was identified. Based on FCAPS analysis of maternal plasma, the t-score of the chromosomal section adjacent to position 80 Mb was significantly lower than the rest of chromosome 13, indicating a suspicious 3.71-Mb deletion in the long arm of chromosome 13 (chr13: 81667889-85377286) (**Figure 1**).

Validation of the NIPT results by aCGH

Whole-genome aCGH analysis on uncultured amniocytes detected a 3.05-Mb deletion at chromosome band 13q31.1 [arr 13q31.1 (83, 494, 767-86, 543, 280) ×1] (**Figure 2**). Whole-genome aCGH analysis on uncultured maternal peripheral blood revealed a 2.82-Mb deletion at chromosome band 13q31.1 [arr 13q31.1 (83, 724, 773-86, 543, 280) ×1] (**Figure 3**). There were no significant aberrations found in the paternal blood examination.

Karyotype analysis results

The fetus and both parents had normal karyotypes. The chromosomal analysis of the fetus showed a 46, XX karyotype. The mother had a 46, XX karyotype and the father had a 46, XY karyotype.

Bioinformatics analysis of the deleted fragment

The deleted 13q31.1 region contains 2 disease-related genes (**Figure 4**): SLITRK1 (Gene/Locus MIM number: 609678), which is associated with GTS (Phenotype MIM number: 137580) and Trichotillomania (Phenotype MIM number: 613229), and SLITRK6 (Gene/Locus MIM number: 609681), which is associated with deafness and myopia (Phenotype MIM number: 221200).

Discussion

The karyotype analysis of cultured metaphase cells is considered to be a “gold standard” in the prenatal diagnosis of chromosomal diseases, but the traditional karyotype has its obvious limitations. Karyotyping can only recognize chromosomal abnormalities of 5 Mb or greater. Furthermore, the turnaround time for cytogenetic analysis is long. FISH appears to compen-

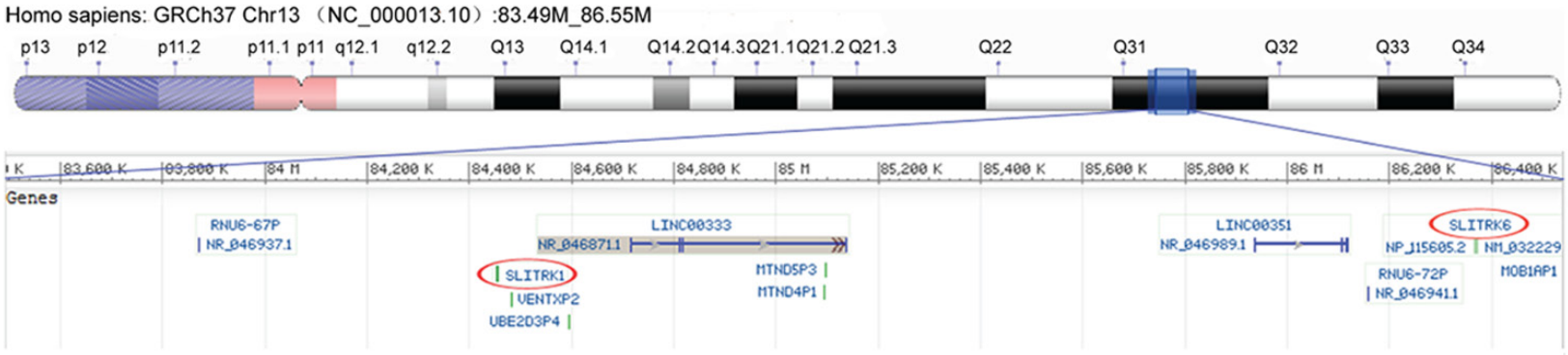

Figure 4. Two disease-causing genes, SLITRK1 and SLITRK6 on chromosome 13q31.1. Search for the fetal microdeletion on chromosome 13:83, 494, 767-86, 543, 280 with the dbVar Genome Browser. Two disease-causing genes, SLITRK1 and SLITRK6, were identified.

sate for this constraint because it has advantages in the detection of targeted fragments or points. However, FISH detects only its intended targets and may provide no information regarding additional abnormalities [12]. CMA provides high-resolution genome-wide screening of the CNVs, and it could be used to detect the gains and losses of genomic DNA fragments even when the fragments are unknown and have discrete genomic loci [8, 10]. Therefore, CMA is widely used in prenatal diagnosis and other clinical diagnoses. CMA needs only a few micrograms of fetal genomic DNA for prenatal diagnosis, but DNA samples cannot be obtained unless an invasive procedure is performed [10, 11]. NIPT was initially used in the prediction of T13, T18, T21 and sex chromosome abnormalities in prenatal diagnosis studies. A recent study reported that NIPT also had an advantage in the prediction of the subchromosomal aberrations. With the development of NIPT, we could obtain a fetal molecular karyotype just from the ffDNA in maternal plasma, and the molecular karyotype could be of equivalent or even greater resolution than the CMA [18-20]. Thus, due to its noninvasive nature and greater accuracy, NIPT has advantages in prenatal diagnosis studies. In the present study, we observed two normal karyotype cases that both carried the same microdeletion detected by NIPT.

The NIPT results showed that there was a suspicious 3.71-Mb deletion in the long arm of chromosome 13 (chr13: 81667889-85377286) (**Figure 1**). The subsequent bioinformatic analysis revealed that there were two disease-causing genes known in this fragment. One of the genes was SLITRK1 (Gene/Locus MIM number: 609678), which is associated with GTS (Phenotype MIM number: 137580) and Trichotillomania (Phenotype MIM number: 613229) [21-23]. The other gene was SLITRK6 (Gene/Locus MIM number: 609681), which is associated with autosomal-recessive congenital myopia and prelingual sensorineural hearing loss (Phenotype MIM number: 221200) [24]. To investigate whether this microdeletion originated from the fetus, the mother, or both, we collected fetal amniotic fluid and blood samples from the parents, and aCGH was used to validate this finding and its origin. All of the samples were tested by metaphase karyotype analysis (G-banding) to exclude mosaic or balanced chromosome abnormalities.

The fetal karyotyping and aCGH results confirmed the microdeletion 46, XX. arr 13q31.1 (83, 494, 767-86, 543, 280) ×1 (**Figure 2**), and the simultaneous study of the parents' blood samples revealed that the mother had a similar microdeletion at the same locus, 46, XX. arr 13q31.1 (83, 724, 773-86, 543, 280) ×1 (**Figure 3**). To increase the resolution, 8×60 K commercial arrays (Agilent) were used but could not accurately identify the boundaries of the microdeletion; therefore, based on the sizes and the locations of the lost fragments, we assumed that the mother and the fetus had the same microdeletion. Based on the results of the NIPT and aCGH, we concluded that this microdeletion in the fetus originated from her mother. The mother had normal features and normal behavior, with no myopia or hearing loss. Upon physical examination, she was assessed as having no motor tics, such as head turning, head rotation, laterocollis, eye blinking, lip twisting, chewing, clenching, facial and mouth grimacing, and shoulder shrugs, and no phonic tics, such as throat clearing, coughing, and sniffing. The metaphase karyotype analysis results of the fetus and the parents were all normal. We then proposed that there was a high possibility for the fetus to have the same normal phenotype as the mother. In the post-test genetic counseling session for this couple, we predicted a normal phenotype for the fetus. After delivery, the normal phenotype of the newborn infant confirmed our prediction. The one-year follow-up study showed that the baby had no motor tics, no congenital myopia, and no prelingual sensorineural hearing loss. This study indicated that this microdeletion could be considered as a normal polymorphism at the chromosomal level.

The SLITRK1 gene, which is located at 13q31.1, has been associated with GTS or Trichotillomania [22], but a genome-wide linkage study by the Tourette Syndrome Association International Consortium for Genetics indicated that there was no support for a locus on chromosome 13 in the current study, suggesting that, if SLITRK1 is a susceptibility gene for GTS, it does not have a major effect in the population that was studied [23]. Moreover, not all of the clinical GTSs were associated with the SLITRK1 gene or 13q31.1; microduplications at 15q13.3 and Xq21.31 could also contribute to GTS [29]. In 1989, based on experience with pedigrees of 1,200 Tourette syndrome (TS) families, the

inheritance in TS may be best described as semi-dominant, semi-recessive [30]. For the SLITRK6 gene, previous reports indicated that cases of congenital myopia and prelingual sensorineural hearing loss had autosomal-recessive patterns of inheritance [24]. These studies may help to explain why the phenotypes were normal in the mother and the newborn infant. Based on the results of our present study, we could speculate that this microdeletion is a normal polymorphism on chromosome 13. To obtain more detailed information regarding this microdeletion, further studies are required.

In conclusion, microdeletions at 13q31.1 were identified in a woman and her fetus via NIPT and aCGH. Previous studies demonstrated that this fragment contains 2 disease-related genes. In our one-year follow-up study, the woman and her daughter both had normal phenotypes, which suggests that the loss of this fragment is not harmful and may be considered as a normal polymorphism on chromosome 13. Moreover, NIPT is feasible in the prediction of fetal microdeletions/microduplications. CMA and NIPT could provide large amounts of genomic DNA information. Our study also contributes to data processing of these results in human genome research.

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Disclosure of conflict of interest

None.

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