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Incomplete Trisomy Rescue Reveals the Mechanism Underlying Discordance Between Noninvasive Prenatal Screening and Prenatal Diagnosis

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ABSTRACT

Background: Uniparental disomy (UPD) is a specific type of chromosomal variation in which both chromosomes of a homologous pair are inherited from the same parent. It is responsible for a wide range of disorders. Monosomy rescue and trisomy rescue are the two main hypotheses of UPD generation.

Methods: An older parturient woman with a positive noninvasive prenatal screening (NIPS) test but a negative prenatal diagnosis was referred to the hospital. Trio whole exome sequencing (trio-WES) and ddPCR were further performed.

Results: Utilizing Trio-WES analysis, our research identified a maternal segmental UPD on chromosome 16, characterized by isodisomic genomic segments at the ends of the chromosome arms and heterodisomic genomic segments near the centromere. Moreover, several nuanced signs pointing to the paternal chromosome 16 were discovered, suggesting a low level of trisomy 16 mosaicism. A homozygous missense mutation (c.1499C>T; p.Ala500Val) was also detected in the fetal *TBC1D24* gene, passed down from the heterozygous carrier mother. Furthermore, ddPCR analysis verified a 3% mosaic level of trisomy 16.

Conclusion: We have quantitatively verified for the first time a combination of trisomy 16 mosaicism and maternal segmental UPD 16 due to incomplete trisomy rescue, illuminating the cause of the mismatch between positive NIPS and negative prenatal diagnoses.

1 | Introduction

Noninvasive prenatal screening (NIPS) is one of the most sensitive and specific screening tests for the common trisomies 13, 18, and 21 by massively parallel sequencing of fetal cell-free DNA fragments in maternal plasma, which are primarily derived from placental trophoblast cells (Zhang et al. 2022). With increasing capacity, NIPS can identify other atypical autosomal

Abbreviations: AABR, automatic auditory brainstem response; CNVs, copy number variations; ddPCR, droplet digital PCR; EEG, electroencephalography; hUPD, uniparental heterodisomy; IUGR, intrauterine growth retardation; iUPD, uniparental isodisomy; MRI, magnetic resonance imaging; MS-MLPA, methylation-specific multiplex ligation-dependent probe amplification; NICU, neonatal intensive care unit; NIPS, noninvasive prenatal screening; OAE, oto-acoustic Emission; OMIM, online mendelian inheritance in man; SNP-array, single nucleotide polymorphism array; SNVs, single nucleotide variations; STR, short tandem repeat; UPD, uniparental disomy; UPD(16)mat, maternal uniparental disomy of chromosome 16; UPDmat, maternal uniparental disomy; UPDpat, paternal uniparental disomy; WES, whole exome sequencing.

Yanan Wang and Yong Zhou contributed equally to this work.

aneuploidies and significant copy number variations (CNVs). As most trisomies are lethal, only cases of fetal mosaicism resulting from trisomy rescue can survive. Invasive prenatal diagnosis is conducted by detecting fetal decidual cells in the amniotic fluid, which can reflect the actual condition of fetal chromosomes. However, in rare cases, placental and fetal chromosomal inconsistencies can occur, which are a principal cause of false-positive and false-negative NIPS results. As such, trisomy rescue can be a source of discordant NIPS results, as well as the formation of uniparental disomy (UPD).

UPD, a rare genetic disorder, occurs when both chromosomes of a homologous pair are inherited from a single parent. A pair of duplicates may stem from either the mother (UPDmat) or the father (UPDpat). A recent study based on four million individuals from the general population estimates a whole-chromosome UPD incidence of 1 in 2000 (Nakka et al. 2019). Based on the mechanism of their production, UPDs can be categorized into four types (Scuffins et al. 2021): uniparental heterodisomy (hUPD), inherited from a single parent's two homologous chromosomes; and uniparental isodisomy (iUPD), where a single parent's two homolog copies lead to chromosomal homozygosity. On the other hand, mixed UPD includes segments of both uniparental heterodisomy and isodisomy. Additionally, segmental UPD signifies single-parent disomy impacting a chromosome segment, while the rest exhibits dual-parent inheritance. Unlike numerical or structural chromosomal aberrations, UPD does not change the number or structure of chromosomes. However, it is associated with a wide range of disorders mainly due to the unbalanced expression of imprinted genes (Mulchandani et al. 2016) or the homozygosity of autosomal recessive mutations (Boluda-Navarro et al. 2021). Consequently, detecting UPD will significantly enhance the diagnostic effectiveness of genetic examinations. This will also prove beneficial in the genetic counseling of patients. Short tandem repeat (STR) markers have historically identified UPD (Liu et al. 2020). Within medical settings, identifying UPD instances is possible through single nucleotide polymorphism array (SNP-array) analysis (Kearney et al. 2011). Furthermore, employing methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) is a standard method for examining UPD, as advised by the ACMG (Del Gaudio et al. 2020).

Owing to its formation process, UPD is found in nearly every chromosome. In the case of the majority of chromosomes, UPDs have minimal impact on individuals (Del Gaudio et al. 2020). Presently, the majority of documented cases of UPD on chromosome 16 (UPD(16)) are identified as maternal uniparental heterodisomies (UPD(16)mat). The clinical features of UPD(16) are attributable to trisomy 16 mosaicism and homozygosity for autosomal recessive mutations. Furthermore, the unbalanced expression of imprinted genes might also be a reason (Inoue et al. 2019; Kalousek et al. 1993). However, some previous studies did not support UPD(16) as a definitive imprinting disorder (Benn 2021; Scheuvens et al. 2017). More importantly, there is a close connection between trisomy 16 and UPD(16), with both often existing simultaneously or independently in the placenta and fetus. Scheuvens et al. found no significant differences in phenotypic features (Scheuvens et al. 2017). It is recognized that the clinical phenotype of cases of trisomy 16 and UPD(16) lacks specificity, with reports spanning a spectrum from a normal phenotype to intrauterine growth retardation (IUGR) and severe malformations (Kotzot and Utermann 2005). Seven genes have been identified as carriers of autosomal recessive disorders linked to UPD on chromosome 16 (Catarzi et al. 2012; Inoue et al. 2019).

In this study, we identified a homozygous missense alteration in the *TBC1D24* gene (OMIM *613577) in an affected baby diagnosed with UPD(16)mat, which could indicate low-level mosaic aneuploidies (~3%) resulting from incomplete trisomy rescue. Moreover, we suggest the mechanism underlying the discordance between a positive NIPS result and a negative prenatal diagnosis.

2 | Subject and Methods

2.1 | Ethical Compliance

Genetic examinations were conducted after the expectant mother and her spouse signed written informed consent. This research received its ethical sanction under the identifier LYFY-YCCZ-2023006.

2.2 | Clinical Presentation

A 37-year-old pregnant woman (G5P2) was referred to our hospital for NIPS in the 17th gestational week due to advanced maternal age. The NIPS results for trisomy 13, 18, and 21 were expected, but they suggested an increased number of chromosome 16 (z score = 8.9). Following a favorable result from the NIPS, the amniotic fluid specimen was subjected to analysis using G-banding karyotypes. Nine of the 39 metaphase cells counted were analyzed, uncovering a standard karyotype (46, XY). Concurrently, the mother's amniotic fluid and both parents' peripheral blood samples underwent low-depth whole-genome sequencing (CNV-seq) to identify any genomic microdeletions or microduplications. Unexpectedly, the outcomes of the CNV-seq turned out to be negative. Subsequently, the expectant mother chose to proceed with her pregnancy. Prenatal ultrasound examinations conducted at 22 and 25 weeks of pregnancy revealed potential intrauterine growth limitations in the fetus, such as diminished head and abdominal circumferences and reduced lengths of the humerus and femur (Figure 1). For a deeper investigation into the presence of pathogenic variants in the fetus, it was advised to perform trio whole-exome sequencing (Trio-WES). Before the WES results were available, the woman delivered a meager birth weight (approximately 1100 g), and the male infant had birth asphyxia, grunting, and hypotonia at 31 weeks of gestation. One minute after birth, the recorded Apgar score was 7, and the baby was then transferred to the neonatal intensive care unit (NICU) for emergency care. Owing to challenges in feeding, the baby received intravenous nutritional support. Two months later, the baby was discharged from the NICU.

2.3 | Noninvasive Prenatal Screening (NIPS)

Peripheral blood was collected from the pregnant woman in the 17th week of gestation. Then, plasma was separated, and

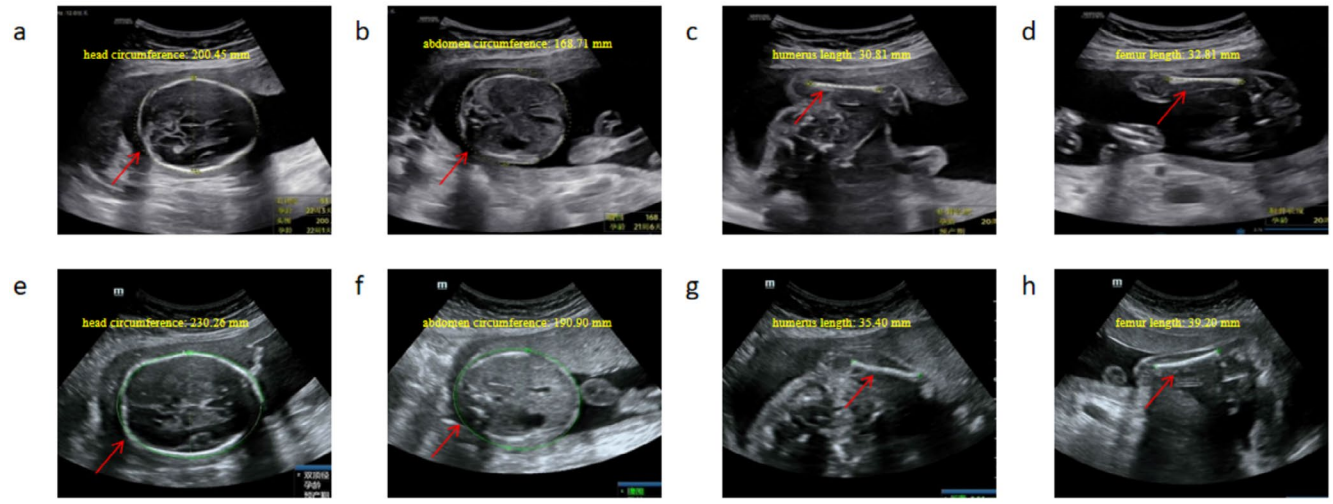

FIGURE 1 | Fetal ultrasound images. (a–d) Head circumference, abdomen circumference, humerus length, and femur length at 22 weeks of gestation, respectively; (e–h) head circumference, abdomen circumference, humerus length, and femur length at 25 weeks of gestation.

cell-free DNA was extracted. The DNA was used for library construction and sequenced on the Illumina Hiseq2000 platform. The GC-corrected normalized chromosome representation value was calculated for each chromosome, and the z-score ($z \geq 3$ as positive cut-off) was used to determine whether a sample was aneuploid.

2.4 | Karyotyping and CNV-Seq

The amniotic fluid sample was collected from the pregnant woman, and G-band karyotype analysis was performed at a 450-band resolution according to the standard protocol. A minimum of 30 metaphases were counted. Subsequently, genomic DNA was extracted from the amniotic fluid sample, and copy number variation was assessed by low-coverage massively parallel CNV sequencing. The procedures of CNV-seq include library construction, quality control, purification, sequencing, and bioinformatic analysis, as previously described (Zhang et al. 2023). Finally, the pathogenicity of CNVs was interpreted using Online Mendelian Inheritance in Man (OMIM) and ClinGen.

2.5 | Whole Exome Sequencing (WES)

To identify disease-causing variants carried by the proband, such as single nucleotide variations (SNVs), small insertions and deletions, or variants occurring at a splice site, WES was performed on amniotic fluid samples from the pregnant woman and peripheral blood samples from the parents. Researchers discovered a homozygous missense alteration in the *TBC1D24* gene (NM_001199107.2:c.1499C>T; p.Ala500Val). NC_000016.9 was used as the reference sequence for the coding regions of the *TBC1D24* gene. The workflow of WES and the annotation and classification of variations have been reported in our prior study (Wang et al. 2024). Sanger sequencing was then applied to confirm the variations identified in the proband.

2.6 | Droplet Digital PCR (ddPCR)

The WES analysis indicated a maternally derived UPD 16 combined with a low level of trisomy mosaicism. To determine the proportion of trisomy 16 in the proband, two paternally derived variants absent in the mother were confirmed by ddPCR in both amniotic fluid and blood of the premature neonate. Probe and primer information for the two variants are shown in Table S1. The ddPCR analysis was performed using a Bio-Rad QX200 system with automated droplet generation following the manufacturer's instructions.

3 | Results

3.1 | NIPS, Karyotyping and CNV-Seq Analysis

The NIPS result at 17 weeks of gestation suggested an increased number of chromosome 16 (Z score = 8.9), indicating a high risk of trisomy 16. Prenatal diagnosis was then carried out by amniocentesis to identify the causes. However, karyotyping and CNV-seq analysis based on amniocentesis yielded negative results (Figure S1).

3.2 | WES Analysis Identified an UPD of Chromosome 16

The Trio-WES method was also employed to pinpoint the disease-inducing variants in the problem. Figure 2A illustrates that the proband's DNA-based polymorphic markers indicate a UPD on chromosome 16, which includes two distinct maternal chromosomes. Moreover, the maternal UPD(16) identified isodisomic genetic segments at the end of the chromosome arms and heterodisomic genomic segments near the centromere (Figure 2B). Several nuanced signs pointing to the paternal chromosome 16 confirmed the mosaic trisomy 16 (Figure 2A). SNP markers originating from the father are distributed across chromosome 16, signifying a total trisomy of 16 (Figure 2C).

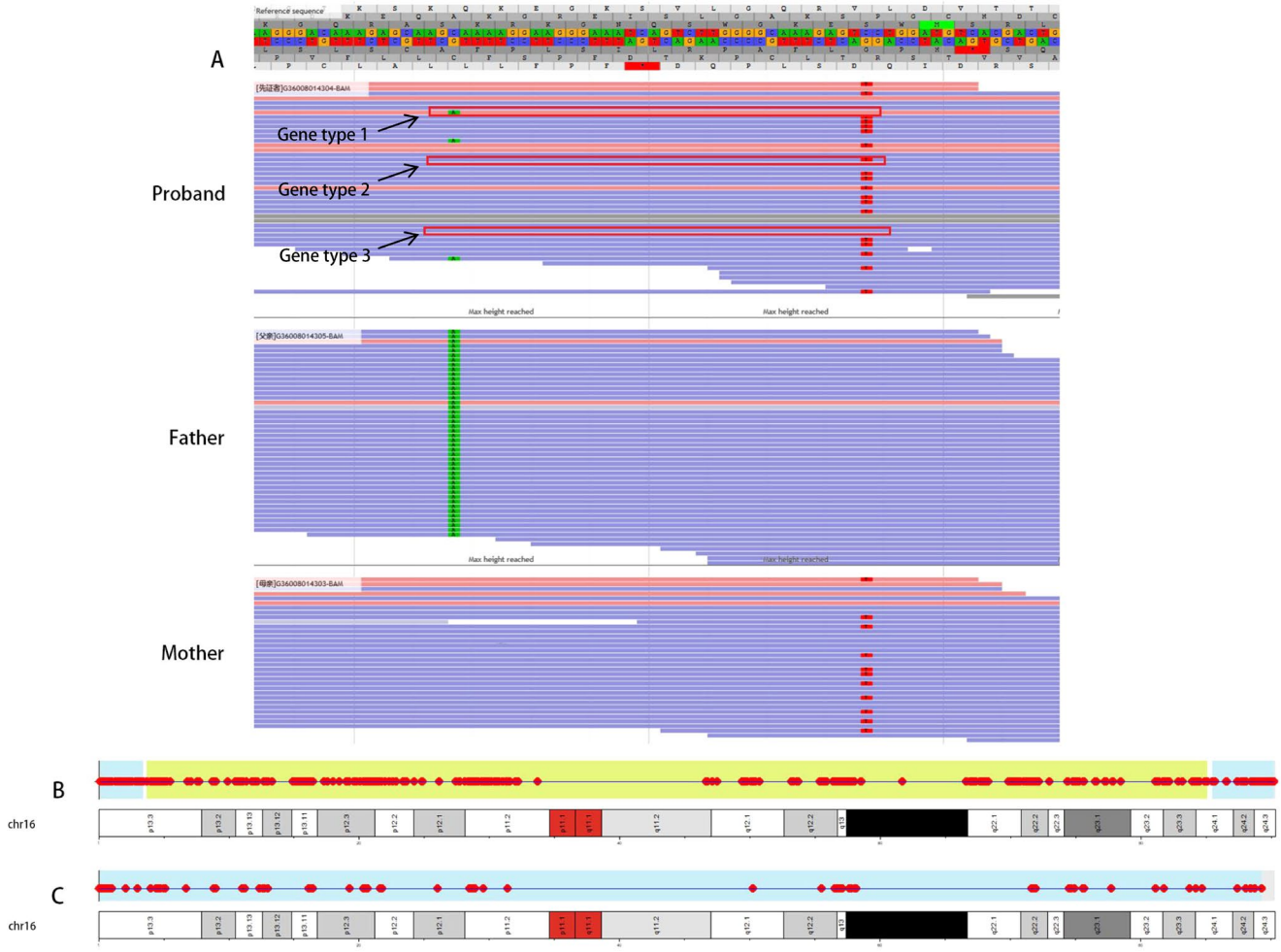

FIGURE 2 | Visualization file of BAM for the proband and parents. (A) shows three genotypes harboring in the proband. (B) Distribution diagram of the SNP in the maternal chromosome 16. (C) Distribution diagram of the SNP in the paternal chromosome 16. Red dots in (B) and (C) represent SNP loci; The yellow area in B represents maternal heterodisomic genomic segments in the prenatal sample. The blue area in B represents maternal isodisomic genomic segments in the prenatal sample. The blue area in C represents hemizygous paternal segments. Genotype 1 (chr16-84,271,158-A & chr16-84,271,193-C) derived from the father; Genotype 2 (chr16-84,271,158-C & chr16-84,271,193-T) derived from the mother; Genotype 3 (chr16-84,271,158-C & chr16-84,271,193-C) derived from the mother.

Analysis via ddPCR of the amniotic fluid sample showed the proband possessed 3.61% and 3.27% of the paternal variants *MRPL28*:c.417T>C p.Y139=and *WDR59*:c.1866+17T>C, in that order. The outcome verified a low level of trisomy 16 mosaicism in the subject. Nonetheless, the ddPCR test on the newborn's blood sample showed no significant findings.

3.3 | Identification of Pathogenic Variants by WES

WES detected a homozygous missense mutation (c.1499C>T; p.Ala500Val) in the *TBC1D24* gene within the proband. This mutation is located in the terminal regions of chromosome arms (Figure 3A). The WES data indicated that the genetic variation originated from the healthy mother, a heterozygous carrier, and the wild-type father (Figure 3B). Sanger sequencing was used to confirm the mutation (Figure 3C). This variant was classified as pathogenic according to the ACMG guidelines (PM2_Supporting+PM3_Very strong+PP4). Moreover, the variant is predicted to be "damaging," "probably damaging," and "disease-causing" by SIFT, Polyphen2, and Mutation Taster, respectively.

3.4 | Follow-Up

Follow-up information was available from the parents 3 months after discharge from the hospital. At 3 months and 4 days of age, the baby was admitted to the hospital due to anemia, with a weight of 6.0 kg and a head circumference of 39.2 cm. The male infant exhibited regular facial features and was in good mental health. However, the infant failed the initial neonatal hearing screening (Oto-acoustic Emission, OAE) and the automatic auditory brainstem response (AABR). It is essential to enhance audio-visual stimulation. Structural and functional MRI revealed mild atrophic changes in the bilateral temporal lobes and enlarged subarachnoid spaces (Figure 4), suggesting the possibility of cerebral atrophy.

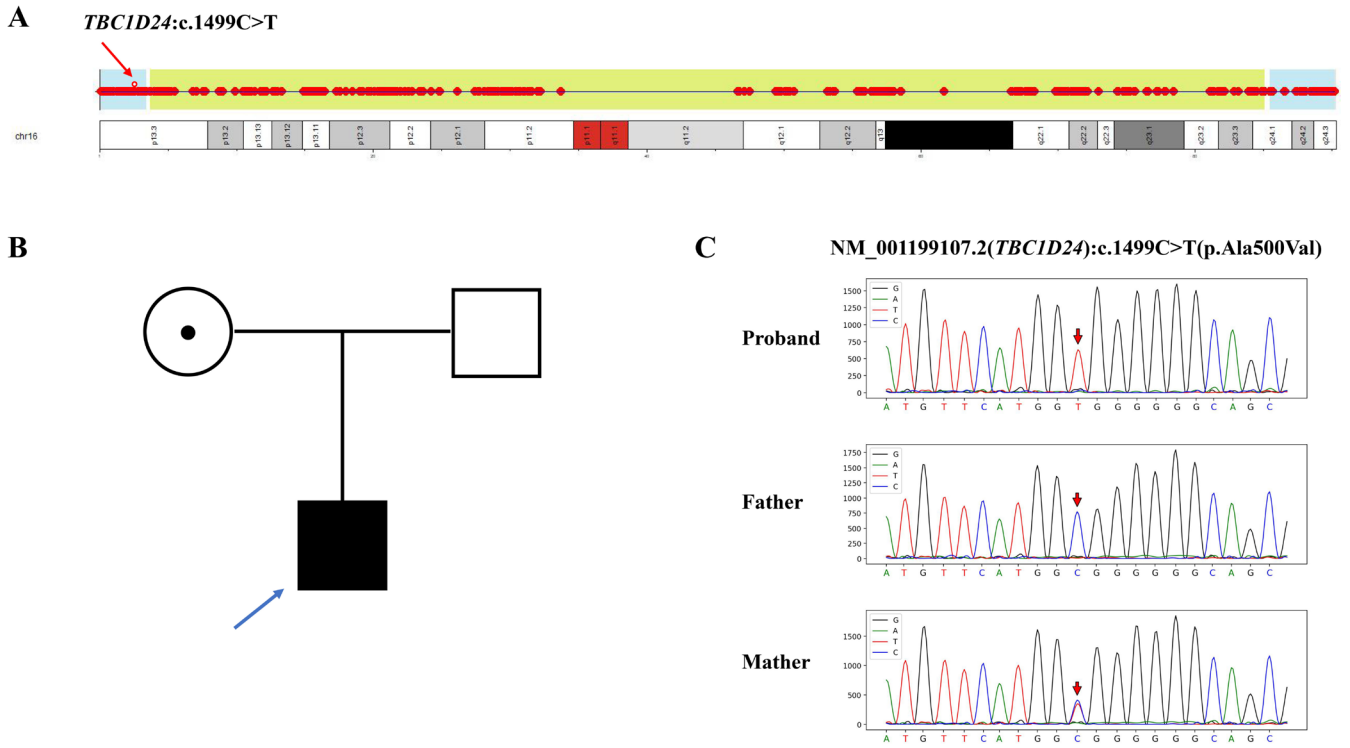

FIGURE 3 | Pedigree of the family and Sanger sequencing validation. (A) Location of identified mutation on chromosome 16. The red arrow indicates the *TBC1D24* (NM_001199107.2:C.1499C>T; p.Ala500Val) variant located in the isodisomic genomic segment of maternal homologs. (B) Family pedigree. The arrow indicates the proband. (C) Sanger sequencing shows a homozygous missense mutation in *TBC1D24* in the proband, while the mother is heterozygous and the father is normal. Amniotic fluid samples from the pregnant woman and peripheral blood from the parents were used for Sanger sequencing validation.

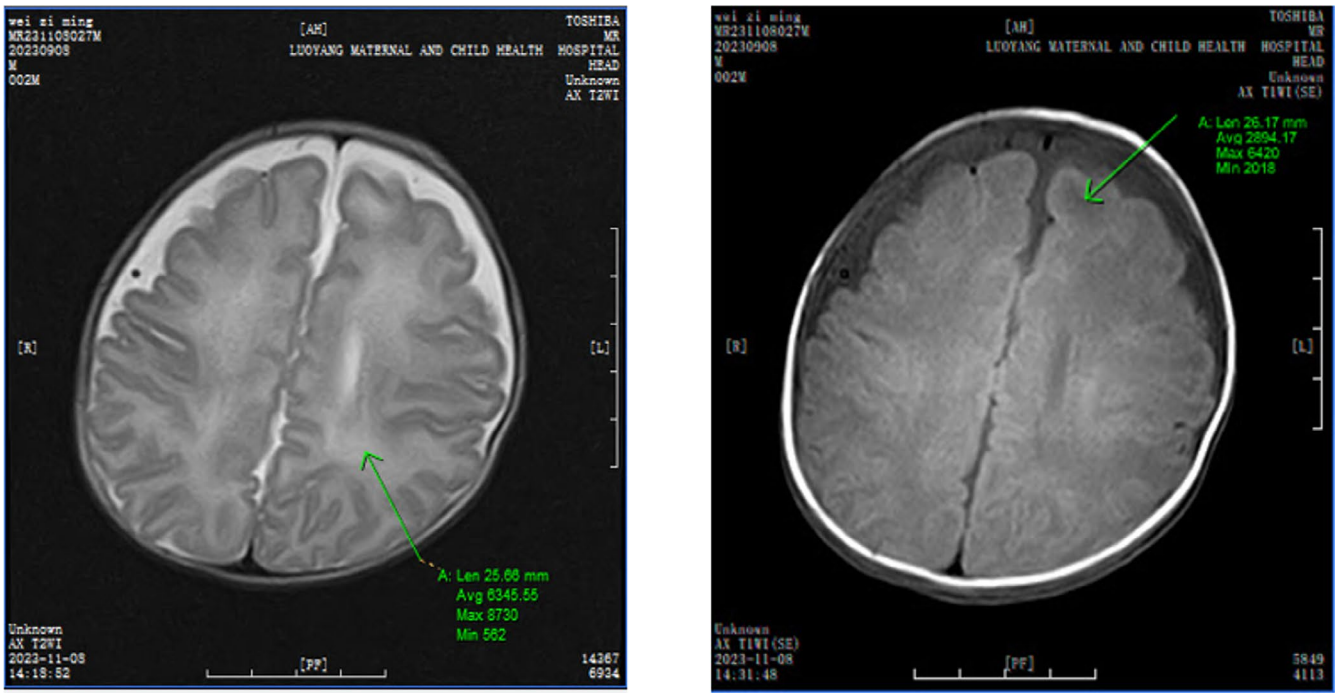

FIGURE 4 | Brain MRI of the proband at three months shows mild cerebellar atrophy.

4 | Discussion

The proband exhibited a UPD on chromosome 16, marked by a combination of partial heterodisomy and isodisomy. Consequently, a homozygous mutation (c.1499C>T; p.Ala500Val) occurred in the *TBC1D24* gene. The prevalent view is that the primary process of UPD formation involves an initial error in meiotic segregation, succeeded by the trisomy

rescue (Kotzot 2004). The progression of maternal age is intricately associated with mistakes in meiotic segregation (Scuffins et al. 2021), potentially playing a role in the development of UPD in a 37-year-old expectant mother, similar to our scenario.

Trisomy rescue is a physiological process that removes excessive chromosomes from cells. Incomplete trisomy rescue occurs when the process is terminated before the target chromosomes are eliminated (Kurtas et al. 2019). Although some cases of incomplete trisomy rescue have been reported (Morán-Barroso et al. 2021; Schlade-Bartusiak et al. 2022), the exact proportion of trisomy is rarely determined.

Karyotyping and CNV-seq analyses are essential prenatal diagnostic techniques in clinical environments, each proficient in identifying chromosomal aneuploid mosaics. Typically, the lower limit of chimerism that can be accurately detected by these two techniques is approximately 5% (Liang et al. 2014; Ma et al. 2021). As a result, cases with low mosaic levels may be underdiagnosed. The research verified that the subject had UPD along with extremely low-level trisomy mosaicism (around 3%) via ddPCR analysis. The disparity could account for the negative outcomes in both karyotyping and CNV-seq analyses of the amniotic fluid sample, in contrast to the NIPS finding, which suggests an increased number of chromosome 16 (z score = 8.9). The positive NIPS result for chromosome 16 may be due to a higher level of trisomy in placental cells, an important component of the free-cell DNA in the maternal plasma (Grati et al. 2020).

Nevertheless, the absence of placental specimens prevented verifying the trisomy count of placental cells. Notably, the trio-WES method has seen a rise in its application for prenatal diagnosis in recent times, capable of identifying various forms of UPD, such as iUPD, hUPD, mixed UPD, and segmental UPD (Scuffins et al. 2021). Consequently, for those more prone to trisomy, according to NIPS, we advise conducting concurrent tests using various techniques, such as CMA, trio-WES, and others, to reduce the risk of underdiagnosis in cases of minor mosaics.

Interestingly, the proportions of *MRPL28*:c.417 T>C p.Y139 = and *WDR59*:c.1866 + 17 T>C were determined by ddPCR analysis. Approximately 3% was detected in the amniotic fluid sample but not in the infant's blood sample. This could be attributed to the potential ability of self-repair and renewal of hematopoietic stem cells (Bello et al. 2018). The errors, such as chromosomal trisomy, would be cleared soon after they occurred. However, the capacity for self-repair and renewal is weaker in somatic cells. Therefore, we speculate that ddPCR analysis using other histocyte types, such as nails, skin, saliva, or hair, may yield positive results.

Trisomy 16 is typically considered embryonic lethal unless rescued during early embryogenesis. The fetus in our study was diagnosed with IUGR during prenatal ultrasonography in the 28th week of gestation. This finding is consistent with previous observations in fetuses with low-level trisomy 16 mosaicism who were phenotypically regular at birth (Chen et al. 2021). However, except for IUGR, the infant in our case was born prematurely at 31 weeks gestation with deficient birth weight (~1100 g) and MRI abnormalities at 3 months. Scheuvens and colleagues (Scheuvens et al. 2017) claimed that the clinical symptoms may be caused by trisomy 16 mosaicism and homozygosity of autosomal recessive mutations rather than maternal UPD 16. Given the shallow level of trisomy mosaicism (3%), we are inclined to believe that a homozygous mutation (c.1499C>T; p.Ala500Val) in the *TBC1D24* gene is responsible for the clinical features.

The *TBC1D24* gene encodes a protein with a conserved domain known as the TBC domain. It interacts with small GTPases and regulates endosomal trafficking of synaptic vesicles (Finelli et al. 2019) or axonal outgrowth in human neurons (Aprile et al. 2019). The *TBC1D24* gene has been linked to neurodevelopmental disorders in an autosomal recessive manner (Shao et al. 2022). The c.1499C>T (p.Ala500Val) is one of the most common pathogenic mutations in the *TBC1D24* gene (Zhang et al. 2019). The allele frequency of this variant was 0.0003 in the East Asian population. It was also predicted as 'damaging' or 'disease-causing' by in silico missense prediction. This is the first report of a homozygous missense mutation (c.1499C>T; p.Ala500Val) in the *TBC1D24* gene resulting from segmental uniparental disomy. *TBC1D24*-related disorders exhibit a broad range of symptoms, such as severe developmental delay, epilepsy, and congenital sensorineural hearing loss. In line with these findings, IUGR and hearing loss were also present in our case. In addition, an MRI at the age of 3 months showed cerebral atrophy, but there were no symptoms of epilepsy. Prior reports have shown that individuals carrying the c.1499C>T (p.Ala500Val) variant had epilepsy before the age of 12 months (Lee et al. 2022; Uzunhan and Uyanik 2020). Therefore, electroencephalography (EEG) and magnetic resonance imaging (MRI) are necessary, along with monitoring clinical manifestations in older age and providing timely treatment if epilepsy symptoms are detected.

5 | Conclusions

Collectively, a homozygous mutation (c.1499C>T; p.Ala500Val) in *TBC1D24* due to maternal UPD 16 was identified in a case showing discordance between positive NIPS and negative prenatal diagnosis. Moreover, we confirmed that incomplete trisomy rescue is responsible for UPD formation. Given the pros and cons of methods like CMA, CNA-seq, trio-WES, and chromosomal karyotyping in identifying low-level mosaic cases, where NIPS yields a positive result yet the karyotype remains normal, various methods can be utilized to differentiate genuine mosaicism from erroneous ones precisely. Our study will also help us understand the gene –disease relationship for *TBC1D24*-related disorders.

Author Contributions

Yanan Wang and Yong Zhou conceived and directed the project. Yanan Wang and Yong Zhou wrote the manuscript and performed statistical analysis of data. Yuqiong Chai and Weiwei Zang collected the blood sample and medical information of the patients. Hongchao Wang prepared the samples and performed the WES. Fan Yin analyzed and interpreted the WES data. Qianqian Tan and Zhigang Chen conducted the follow-up study. All authors read and approved the final manuscript.

Acknowledgments

We are very grateful to the family who participated in this investigation.

Ethics Statement

This study was approved by the Ethics Committee in the Luoyang Maternal and Child Health Hospital (Approval No. LYFY-YCCZ-2023006). Written informed consent was signed by the pregnant woman and her husband.

Conflicts of Interest

Yong Zou, Qianqian Tan, and Fan Yin are employed by Puluo (Wuhan) Medical Biotechnology Co. Ltd and Wuhan Kindstar Clinical Diagnostic Institute Co. Ltd. Zhigang Chen is employed by Puluo (Wuhan) Medical Biotechnology Co. Ltd. The remaining authors declare no conflicts of interest.

Data Availability Statement

The raw data of this study is available at https://www.ncbi.nlm.nih.gov/sra/PRJNA1119651.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.