Novel Homozygous Nonsense Mutation Associated with Bardet-Biedl Syndrome in Fetus with Congenital Renal Malformation

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Research Article

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Abstract

Background: Bardet-Biedl syndrome (BBS) is a rare autosomal recessive genetic disorder with clinical and genetic heterogeneity. BBS is more commonly reported in adults and children than in fetuses.

Method: Here, a retrospective study of 210 fetuses with congenital renal malformation was performed. These fetuses were performed invasive prenatal diagnosis. Chromosome karyotype analysis, whole exome sequencing (WES), and a single nucleotide polymorphism array (SNP-array) were used.

Results: We found the intrauterine phenotype of a fetus with enlarged kidneys, enhanced echo, and oligohydramnios, and the molecular characterizations of the fetus with BBS. The results of chromosome karyotype analysis and SNP-array on the fetus were normal. WES, however, revealed homozygous mutation of c.1177C>T (p.Arg393*) on exon 12 of the *BBS1* gene, and heterozygous variation of c.2704G>A (p.Asp902Asn) on exon 22 of the *CC2D2A* gene. According to ACMG guidelines, c.1177C> T was identified as a pathogenic mutation and c.2704G>A was identified as an uncertain significance. Sanger sequencing showed that there was heterozygous mutation of c.1177C>T and heterozygous variation of c.2704G>A in the parents of the fetus.

Conclusion: WES identified a novel homozygous nonsense mutation c.1177C>T in the *BBS1* gene of a Chinese fetus with congenital renal malformation. The finding provides more insight into *BBS1* mutations in Asian populations in general, and provides a basis for genetic counseling.

Background

Bardet-Biedl syndrome (BBS) (MIM 209900) is a rare autosomal recessive genetic disorder. The prevalence of BBS in European and North American populations is about 1/160,000-1/140,000 [1], and the incidence in Asian populations is even lower, at about 1 in 18 million [2]. Its main characteristics are intellectual disability, retinopathy pigmentosa, polydactyly (toe), obesity, gonadal hypoplasia, renal dysplasia, and short stature [3, 4]. Secondary clinical manifestations include developintellectual disability, motor and neurological dysfunction, speech disorders, and behavioral abnormalities, as well as eye cataracts, strabismus, and astigmatism.

So far, a total of 21 genes that cause BBS phenotypes have been identified [5, 6]. Different BBS-related genes lead to different morbidities. For example, reports showed that BBS related to BBS1 [7], BBS2 [8], BBS6 [9], BBS9 [10], BBS10 [11], and BBS12 [12] gene mutations accounted for 23.3%, 8.1%, 5.8%, 6.0%, 20%, and 5%, respectively [13]. The mutation type frequencies of different BBS genes were different in different ethnic groups. *BBS1* was found to have a high mutation frequency in European populations, leading to the occurrence of BBS, while *BBS7* gene mutation was more commonly found in the Chinese population. Although current studies have found that mutations in 21 BBS genes can result in BBS phenotypes, only 80% of patients have mutations located in these genes, and about 20% of BBS instances are unrelated to them. This suggests that there are still discoveries to be made about some

BBS-related genes, necessitating further study. Several challenges still exist regarding the genetic diagnosis and treatment of this disease.

BBS is a relatively rare condition. Because it heavily damages multiple systems and organs, it results in a very high disability rate. At present, our understanding of the pathogenic molecular mechanism of BBS is still not complete, and there are no special treatments targeting this condition [14]. Therefore, avoiding consanguineous marriage and using effective prenatal screening are still important preventive measures for BBS [15, 16].

To our knowledge, no instances of BBS associated with *BBS1* variants have been reported in the Chinese population. We retrospectively analyzed 210 fetuses with congenital renal malformation, and found a fetus with congenital renal malformation diagnosed with *BBS1* mutation in an Asian population and analyzed their pedigrees to explore the relationship between intrauterine phenotypes and fetal genotypes. This was done with the ultimate goal of improving our diagnostic and monitoring methods, as well as our understanding of the disease overall.

Methods

Study participants

A retrospective study of 210 fetuses with congenital renal malformation in the Fujian Provincial Maternal and Child Health Hospital from November 2016 to February 2021 was performed. These fetuses were performed invasive prenatal diagnosis. Amniocentesis, chorion villus sampling or umbilical cord blood was performed according to the pregnant woman's gestational age.

Chromosome karyotype analysis

Transabdominal amniocentesis was performed under the guidance of ultrasound, and 40 ml of amniotic fluid was extracted. A volume of 20 ml was cultured *in vitro* under aseptic conditions, and another 20 ml was used for DNA extraction. The cultured amniotic fluid cells were harvested, fixed, and prepared for karyotyping and G banding. Chromosomal abnormalities were described according to the International System of Human Cytogenetics Nomenclature (2016).

Single nucleotide polymorphism array (SNP-array)

The experiment was conducted in strict accordance with standard operating procedures provided by Affymetrix, including DNA extraction, preparation, digestion and ligation, as well as amplification, purification, and fragmentation. DNA labeling, hybridization, washing, staining, and scanning were conducted according to those same procedures as well. The data were analyzed using a kit supported by CHAS 2.0 software. Then, the SNP array structure was analyzed, in combination with the relevant

databases, to determine the nature of the copy number variation (CNV) found. These reference databases included DGV (http://dgv.tcag.ca/dgv/app/home), DECIPHER (http://decipher.sanger.ac.uk/), OMIM (http://www.omim.org), ISCA (http://www.iscaconsortium.org), and CAGdb (http://www.cagdb.org/), among others. CNVs can be divided into five categories [17, 18]: pathogenic, possibly pathogenic, of uncertain clinical significance (VUS), possibly benign, and benign. For the VUS, it is recommended to conduct SNP-array testing in on fetal cells isolated from maternal peripheral blood to further clarify the nature of CNV, in combination with pedigree analysis.

Whole exome sequencing (WES)

The fetal DNA was interrupted, and a library was prepared. Then, the exon of the target gene, and DNA in the adjacent shear region, were captured and enriched via Roche KAPA HyperExome chip. Finally, the mutation was detected using the MGISEQ-2000 sequencing platform. The quality control index of sequencing data was as follows: the average sequencing depth of the target region was ≥ 180X, and loci with average depths of > 20X in the target region accounted for over 95% of total loci. Sequenced fragments were compared with the UCSC hg19 human reference genome to remove duplicates. INDEL and genotype detection were performed using GATK. ExomeDepth was used for copy number variation detection at the exon level, and genes were named according to the Human Genome Organization Gene Nomenclature Committee (HGNC). Variants were named according to Human Genome Variation Society (HGVS) nomenclature. The reference database and prediction software versions were below: Clinvar (2020-03-16), ESP6500 (V2), and 1000 Genomes (Phase3), as well as GnomAD (r2.0.1), ExAC (r0.3.1), and BPGD*(V3.1). SecondaryFinding_Var*(v1.1_202.3), dbscSNV (1.1), and SpliceAI (1.3) were used too, alongside dbNSFP (2.9.1), SIft, MutationTaste, and Polyphen2. The pathogenic properties of the variants were classified according to American Society of Medical Genetics and Genomics (ACMG) and American Society of Molecular Pathology (AMP) sequence variation interpretation guidelines [19-22] . The Clingen Working Group on the Interpretation of Sequence Variations and the Society for Clinical Genome Sciences (ACGS) were consulted to refine our interpretation of the guidelines.

Sanger sequencing to validate pedigree

Briefly, total 5 mL of peripheral blood samples from both parents of the fetus was collected, and EDTA was used to prevent coagulation. DNA was extracted using a DNA extraction kit (Tiangen Biochemical Technology [Beijing] Co., Ltd.), and the operation was conducted according to the attached instructions. Suspected pathogenic loci, found by WES, were amplified by PCR. After purification and quantification, the products were sequenced using a ABI 3130 Genetic Analyzer, and the obtained sequences were compared with human wild-type sequences.

Results

Clinical phenotype

Among 210 fetuses with congenital renal malformation, the intrauterine phenotype of a fetus showed that both kidneys were enlarged and echo was enhanced, resulting in what was suspected to be infantile polycystic kidney disease. At the same time, the amniotic fluid index was 2.9 cm, which was slightly low (Figure 1).

Chromosome karyotype analysis

Prenatal cytogenetic analysis of amniotic fluid revealed a normal karyotype: 46, XY (Figure 2).

SNP-array

Analysis via use of the Affymetrix CytoScan 750K Array was normal.

WES

WES revealed homozygous variation of c.1177C>T (NM_024649.4, p.Arg393*) in exon 12 of the *BBS1* gene of the fetus (Figure 3). The homozygous variation of c.1177C>T leads to premature termination of protein coding at amino acid position 393, which results in truncated protein production. This ultimately affects normal protein function. According to the ACMG guidelines, c.1177C>T was identified as a pathogenic mutation, with PVS1, PM2, and PM3 as criteria.

These criteria are defined by several standards. PVS1 occurs when the pathogenic mechanism of a disease is loss of function (LOF) and the mutation is detected as nonfunctional mutation. This mutation can occur as a nonsense mutation, frameshift mutation, start codon mutation, or as the deletion of one or more exons. PM2 occurs when variations are not found in the normal control population in the ESP, 1000, and EXAC databases. Finally, PM3 occurs when recessive genetic diseases and the pathogenic variant is detected in the trans position.

WES also revealed heterozygous variation of c.2704G>A (NM_00108052.2.2, p.Asp902Asn) in exon 22 of the fetus's *CC2D2A* gene (Figure 4). According to the ACMG guidelines, c.2704G>A was a significant unknown mutation (PM2). PM2: Variations not found in normal control population in ESP database, 1000 database, and EXAC database.

The results of Sanger sequencing for validation of pedigree

Sanger sequencing showed that there were heterozygous mutations at the same gene positions in DNA samples from the fetus's parents. The parents' *BBS1* genes also exhibited heterozygous variation of exon

12 c.1177C>T (NM_024649.4, p.Arg393 *) (Figure 3). At the same time, the parents also displayed heterozygous variation of the *CC2D2A* gene, on exon 22 c.2704G>A (NM_00108052.2.2, p.Asp902Asn) (Figure 4).

Pregnancy outcome

The pregnancy was terminated at 25 weeks of gestation, and the parents of the fetus did not consent to a post-induction autopsy.

Discussion

In this study, an intrauterine ultrasound was conducted to determine the phenotype of a fetus with bilateral-kidney enlargement, enhanced echo, polycystic kidney, and an amniotic fluid index of 2.9 cm (a low level at 24^{+2} weeks of gestation). We first used traditional karyotype analysis and an SNP-array to conduct genetic testing on the fetus, but no abnormalities were detected. Further genetic testing of the fetus was then performed using WES. This revealed homozygous variation of c.1177C>T (NM_024649.4, p.Arg393*) in exon 12 of the fetus's *BBS1* gene. Sanger sequencing also showed that there were heterozygous mutations in the same positions on genes in the parents of the fetus. This is consistent with the autosomal recessive inheritance of BBS.

BBS1 (OMIM:209901) is located on chromosome 11q13 and is also known as *BBS2L2*. At present, 94 pathogenic variants of *BBS1* have been reported by the HGMD. Mutation of the *BBS1* gene is the most common cause of BBS, and is responsible for 25% of all BBS incidences. The exact type of mutation varies among ethnic groups, with the most common *BBS1* variant (p.M390R) accounting for about 80% of all *BBS1* mutations in the European population [23, 24]. Mykytyn et al. [25] conducted genetic screening on 129 patients with BBS and found that 30% of them possessed at least one *M390R* mutation. BBS proteins encoded by different BBS genes are known to function throughout the formation of the BBS complex, including the BBSome, which consists of seven BBS proteins (BBS1, BBS2, BBS4, BBS5, BBS7, BBS8, and BBS9) [26–29]. Mutation of the *BBS1* gene results in abnormal function of the BBSome, which, in turn, affects the function of microcilia and various systems in the body [30]. The homozygous variation of c.1177C>T (NM_024649.4, p.arg393*) in exon 12 of the *BBS1* gene has not been reported in the Chinese population.

Most *BBS1* variants include missense, deletion/insertion, and splicing mutations, and have been reported to produce typical BBS phenotypes [31–34]. According to recent studies, 90% of BBS patients have retinal degeneration in their clinical phenotypes of BBS patients [35], 90% have abnormal renal development and function [36], and 72-92% are obese [37]. Additionally, 63-81% have polydactyly/deformity [38], and more than half have intellectual disability and/or gonadal dysplasia [39]. Fetuses in this study had a nonsense variant of *BBS1* with biallelic loss of function. Renal abnormalities in the sonographic phenotype of the fetus in our own study are consistent with previously reported clinical abnormalities in the renal

development of those with *BBS1* mutations. The parents of the fetus did not consent to post-induction autopsy, so it is not clear whether the fetus had other clinical manifestations associated with *BBS1* mutations.

WES has the advantage of being able to rapidly and efficiently detect all potentially pathogenic mutations at once [40]. However, its huge data output also brings great challenges when it comes to bioinformatics analysis and clinical interpretation [41]. In this study, WES also revealed heterozygous variation of c.2704G>A (NM_00108052.2.2, p.Asp902Asn) in exon 22 of the fetus's *CC2D2A* gene. This gene is primarily involved in the development of COACH syndrome (OMIM:216360), Joubert syndrome 9 (OMIM:612285), and Meckel syndrome 6 (OMIM:612284).

COACH syndrome is an autosomal, recessive, inherited disorder [42], with intellectual disability, ataxia (due to cerebellar hypoplasia), and liver fibrosis as typical clinical features. Joubert syndrome is also an autosomal, recessive, inherited disease [43], which manifests clinically through cerebellar ataxia, ocular movement dysfunction, vermis hypoplasia, and thickening of the upper cerebellar foot. Meckel syndrome, another autosomal recessive inherited disease [4444], is a fatal multiple congenital anomaly disorder with clinical features that include brain malformation, polycystic kidney malformation, polydactyl deformity, cleft lip and palate, cardiac abnormality, central nervous system malformation, liver fibrosis and bone dysplasia. Heterozygous variation of c.2704G>A (NM_00108052.2.2, p.Asp902Asn) in exon 22 of the *CC2D2A* gene was also found in the parents of the fetus. According to ACMG guidelines, c.2704G>A was identified as a significant unknown mutation. Further study is needed to determine if the heterozygous variation of c.2704G>A is related to congenital renal dysplasia, along with more relevant future case reports.

Conclusion

In conclusion, we identified the novel nonsense variant c.1177C>T (p.Arg393 *) in the *BBS1* gene of a Chinese family. As far as we know, this variant of c.1177C>T, which is considered to be a pathogenic homozygous variant, is the first to be reported in the *BBS1* gene in an Asian population. The genetic etiology of the fetus was determined by analyzing the pathogenicity of the c.1177C>T variant in combination with the fetal intrauterine phenotype. It is also necessary to carry out prenatal genetic diagnosis in subsequent pregnancies by the parents of the fetus, as both carry pathological variants of *BBS1*.

Abbreviations

BBS: Bardet-Biedl syndrome; WES: whole exome sequencing; SNP-array: a single nucleotide polymorphism array; CNV: copy number variation; LOF: loss of function.

Declarations

Ethics approval and consent to participate

The studies were approved by the ethics committee at the Fujian Provincial Maternal and Child Health Hospital. All patients consented to participate and signed written-informed consents. All methods were performed in accordance with the relevant guidelines and regulations.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and/or analyzed during the current study are available from the corresponding author.

Conflict of Interest

The authors declare that they have no competing interest.

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Authors' contributions

Liangpu Xu: Designed the study. Meiying Cai: Wrote the manuscript. Hailong Huang revised the article. Xianguo Fu and Min Lin performed the statistical analyses. Na Lin interpreted the data.

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Figures

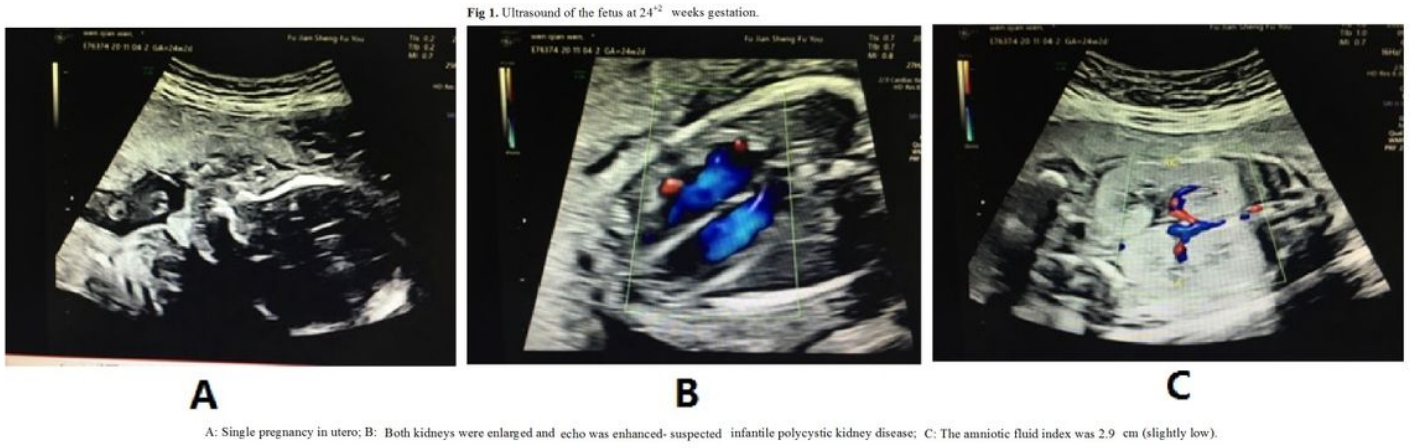

Figure 1

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Figure 2

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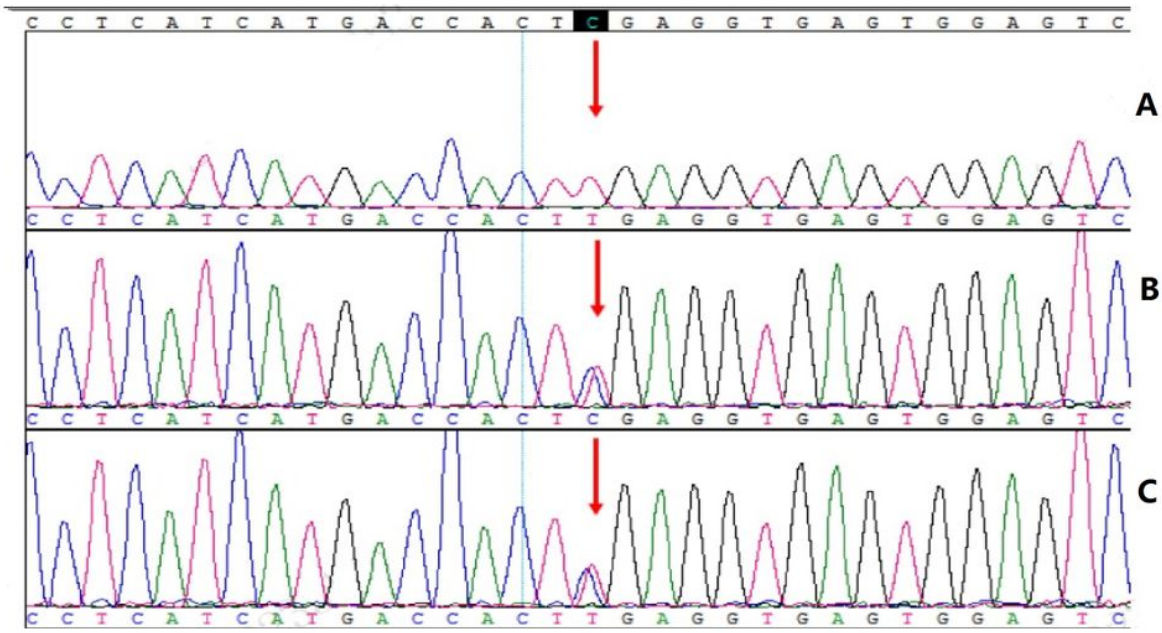
Fig 3. *BBS1* gene sequencing of the fetus and his parents.

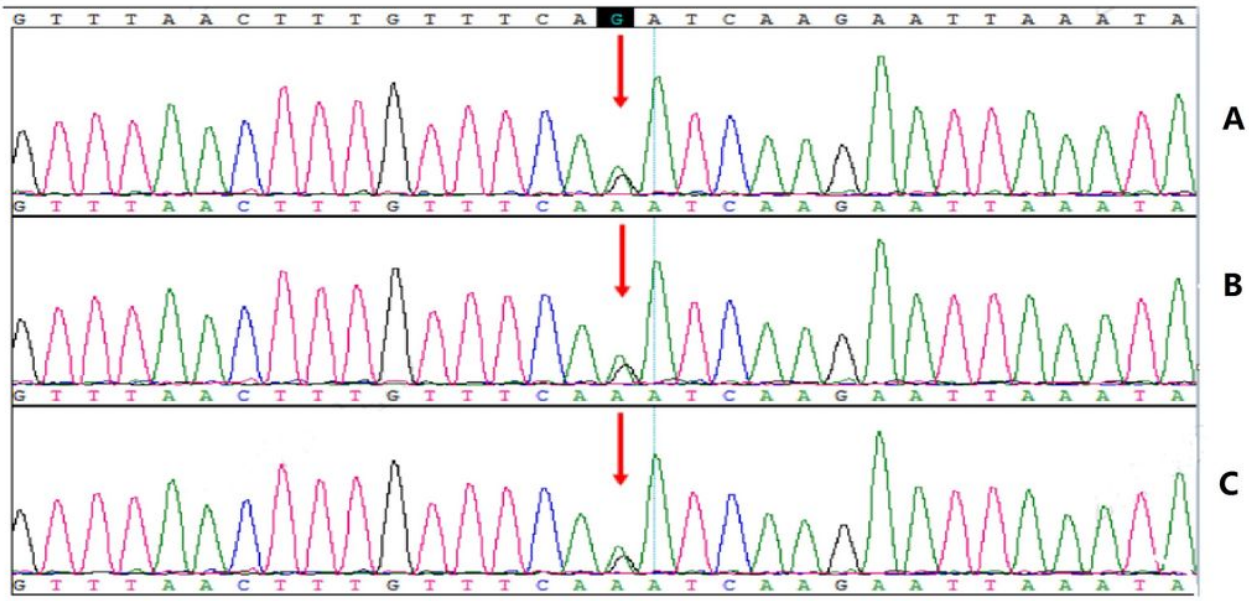


A: homozygous variation of c.1177C>T (NM_024649.4, p.Arg393*) in exon 12 of the fetus's *BBS1* gene; B: heterozygous variation of c.1177C>T (NM_024649.4, p.Arg393*) in exon 12 of the mother's *BBS1* gene; C: heterozygous variation of c.1177C>T (NM_024649.4, p.Arg393*) in exon 12 of the father's *BBS1* gene.

Figure 3

Please See image above for figure legend.

Fig 4. CC2D2A gene sequencing of the fetus and his parents.



A: heterozygous variation of c.2704G>A (NM_ 00108052.2.2, p.Asp902Asn) in exon 22 of the fetus's *CC2D2A* gene; B: heterozygous variation of c.2704G>A (NM_00108052.2.2, p.Asp902Asn) in exon 22 of the mother's *CC2D2A* gene; C: heterozygous variation of c.2704G>A (NM_00108052.2.2, p.Asp902Asn) in exon 22 of the father's *CC2D2A* gene.

Figure 4

Please See image above for figure legend.