Novel homozygous nonsense mutation associated with Bardet–Biedl syndrome in fetuses with congenital renal malformation

Meiyi Cai, SMa, Min Lin, BAa, Na Lin, SMa, Liangpu Xu, BAg, Hailong Huang, PhDa,*

Abstract

Background: The Bardet–Biedl syndrome (BBS) is a rare autosomal recessive disorder, characterized by clinical and genetic heterogeneity. BBS is more commonly reported in adults and children than in fetuses. Here, a retrospective study on 210 fetuses with congenital renal malformation was conducted.

Methods: The fetuses were diagnosed using invasive prenatal tests, including chromosome karyotype analysis, whole exome sequencing (WES), and single-nucleotide polymorphism array. We found the intrauterine phenotype of a fetus presenting enlarged kidneys, enhanced echo, and oligohydramnios; therefore, the fetus was characterized to have BBS.

Results: Chromosome karyotype analysis presented normal results. Analysis using an Affymetrix CytoScan 750K array revealed 2 homozygous regions. However, WES revealed a homozygous mutation of c.1177C>T (p.Arg393*) on exon 12 of BBS1 and a heterozygous variation of c.2704G>A (p.Asp902Asn) on exon 22 of C2D2A. The American College of Medical Genetics and Genomics guidelines identified c.1177C>T and c.2704G>A as a pathogenic mutation and of uncertain significance, respectively. Sanger sequencing identified homozygous mutation, that is, c.1177C>T and heterozygous variation, that is, c.2704G>A in the parents of the fetus.

Conclusions: WES identified a novel homozygous nonsense mutation c.1177C>T in BBS1 of a Chinese fetus with congenital renal malformation. This finding provides insight into the BBS1 mutations in Asian populations in general and shows the necessity of genetic counseling.

Abbreviations: BBS = Bardet–Biedl syndrome, CNV = copy number variation, SNP = single-nucleotide polymorphism, VUS = uncertain clinical significance, WES = whole exome sequencing.

Keywords: Bardet–Biedl syndrome, congenital renal malformation, rare autosomal recessive genetic disorder, whole exome sequencing.

1. Introduction

Bardet–Biedl syndrome (BBS; MIM 209900) is a rare autosomal recessive disorder. The prevalence of BBS in the European and North American populations is very low, that is, approximately 1/160,000 to 1/140,000,[5,6] and that in Asian populations is even lower, that is, approximately 1 in 18 million.[7] BBS is characterized by intellectual disability, retinopathy pigmentosa, polydactyly (toes), obesity, gonadal hypoplasia, renal dysplasia, and short stature.[8] Secondary clinical manifestations include developmental disability, motor and neurological dysfunction, speech disorders, and behavioral abnormalities, as well as eye cataracts, strabismus, and astigmatism.

A total of 21 genes associated with BBS phenotypes have been identified so far,[5-6] and different BBS-related genes result in different morbidities. For example, BBS related to BBS1,[7] BBS2,[8] BBS6,[9] BBS9,[10] BBS10,[11] and BBS12[12] mutations accounted for 23.3%, 8.1%, 5.8%, 6.0%, 20%, and 5% of the cases, respectively.[13] The mutation frequencies of the BBS genes differ among ethnic groups. Mutation frequency of BBS1 is high in European populations, thereby leading to the occurrence of BBS, while BBS7 mutation is more commonly found in the Chinese population. Although mutations in 21 BBS genes that can result in the BBS phenotypes have been identified, only 80% of the patients show mutations located in these genes, and the remaining 20% of BBS instances are...
unrelated to these genes. Therefore, further identification of other BBS-related genes is necessary. Several challenges still exist regarding the genetic diagnosis and treatment of this disease.

BBS is a relatively rare condition and has a very high tendency of causing disabilities as it heavily damages multiple systems and organs. At present, our understanding of the pathogenic molecular mechanism of BBS is incomplete, and no special treatments targeting this condition have been designed. Therefore, avoiding consanguineous marriage and using effective prenatal screening are important preventive measures to lower the occurrence of BBS.

To our knowledge, no instances of BBS associated with the BBS1 variants have been reported in the Chinese population. We retrospectively analyzed 210 fetuses with congenital renal malformation, and among these we diagnosed 1 fetus with BBS1 mutation in the Chinese population. We further analyzed their pedigrees to explore the relationship between intrauterine phenotypes and fetal genotypes to improve the diagnostic and monitoring methods, as well as our understanding of the disease.

2. Methods

2.1. Ethical approval and consent to participate

The studies were approved by the ethics committee at the Fujian Provincial Maternal and Child Health Hospital (no. 2014042). All patients signed written-informed consents to participate in this study.

2.2. Study participants

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

2.3. Chromosome karyotype analysis

Transabdominal amniocentesis was performed using ultrasound, and 40 mL of amniotic fluid was extracted. Of the extracted amniotic fluid, 20 mL was cultured in vitro under aseptic conditions, and the remaining 20 mL was used for DNA extraction. The cultured cells from the amniotic fluid were harvested, fixed, and prepared for karyotyping and G banding. Chromosomal abnormalities were described according to the International System of Human Cytogenetics Nomenclature (2016). Forty karyotypes were counted in each case, and 5 were analyzed karyotypes; the count and analysis of karyotypes were increased in case of any abnormality.

2.4. Single-nucleotide polymorphism array

Experiments were conducted in strict accordance with the standard operating procedures provided by Affymetrix. The data were analyzed using CHAS 2.0 software. The single-nucleotide polymorphism (SNP) array structure was analyzed in combination with the relevant databases to determine the nature of the obtained copy number variation (CNV). The reference databases included DGV, DECIPHER, ISCA (http://www.iscaconsortium.org), and CAGdb. CNVs can be divided into 5 categories, i.e., pathogenic, possibly pathogenic, of uncertain clinical significance (VUS), possibly benign, and benign. For the VUS category, it is recommended to conduct SNP analysis in the fetal cells isolated from maternal peripheral blood in combination with pedigree analysis to further clarify the nature of CNV.

2.5. Whole exome sequencing

A library was prepared from the fetal DNA. Then, the exons of the target genes and DNA in the adjacent shear region were captured and enriched using a Roche KAPA HyperExome chip. Finally, mutations were 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 >20x in the target region accounted for over 95% of the 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 to detect CNV 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 following reference databases and prediction software versions were used: Clinvar (2020-03-16), ESP6500 (V2), 1000 Genomes (phase 3), GnomAD (r2.0.1), ExAC (r0.3.1), BPGD* (V3.1), SecondaryFinding_Var*(v1.1_202.3), dbscSNV (1.1), SpliceAI (1.3), dbNSFP (2.9.1), Sift, MutationTaste, and Polyphen2. The pathogenic properties of the variants were classified in accordance with the sequence variation interpretation guidelines recommended by the American Society of Medical Genetics and Genomics (ACMG) and the American Society of Molecular Pathology.

The Clingen Working Group on the Interpretation of Sequence Variations and the Society for Clinical Genome Sciences were consulted to refine our interpretation of the guidelines.

2.6. Sanger sequencing to validate pedigree analysis

Peripheral blood samples (5mL) from both parents of the fetus were collected, and ethylene diamine tetraacetic acid was used to prevent coagulation. DNA was extracted using a DNA extraction kit (Tiangen Biochemical Technology Co., Ltd, Beijing, China) according to the manufacturer’s instructions. Suspected pathogenic loci found by whole exome sequencing (WES) were amplified using polymerase chain reaction. After purification and quantification, the products were sequenced using an ABI 3130 Genetic Analyzer, and the obtained sequences were compared with human wild-type sequences.

3. Results

3.1. Clinical phenotype

Among the 210 fetuses with congenital renal malformation, the intrauterine analysis of 1 fetus exhibited enlarged kidneys and enhanced echo; this resulted in the diagnosis of suspected to be infantile polycystic kidney disease. The amniotic fluid index was slightly low (2.9 cm; Fig. 1A–C).

3.2. Chromosome karyotype analysis

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

3.3. SNP array

SNP analysis indicated a homozygous region of 41Mb in the q31.1q33 region of chromosome 2 containing 153 OMIM genes and a homozygous region of 28Mb in the q14.3q22.3 region of chromosome 5 containing 51 OMIM genes. No imprinted genes in these regions were identified; however, an increased risk of recessive genetic diseases caused by homozygous mutations was indicated (Fig. 3).
Figure 1. Intrauterine ultrasound phenotype of the fetus. (A) Ultrasound of the fetus at 24 +2 gestational week; single pregnancy in utero. (B) Ultrasound of the fetus at 24 +2 gestational week; both kidneys were enlarged, the echo was enhanced, and infantile polycystic kidney was suspected. (C) Ultrasound of the fetus at 24 +2 gestational week; the amniotic fluid index was 2.9 cm, showing a slightly sparse state.

Figure 2. Karyotype of the fetus from amniotic fluid.
3.4. WES

WES revealed homozygous variation of c.1177C>T (NM_024649.4, p.Arg393*) in exon 12 of BBS1 in the fetus (Fig. 4). This variation leads to the premature termination of protein synthesis at amino acid position 393, resulting in the production of truncated proteins. This ultimately affects protein function. In accordance with the ACMG guidelines, c.1177C>T was identified as a pathogenic mutation, with the PVS1, PM2, and PM3 criteria.

These criteria are defined by several standards. PVS1 occurs when the pathogenic mechanism underlying disease is a loss of function mutation. This mutation can occur as a nonsense, frameshift, or start codon mutation, depending on the deletion of 1 or more exons. PM2 occurs when variations are not found in control population in the ESP, 1000 genome, and EXAC databases. PM3 occurs when recessive genetic diseases and pathogenic variants are detected at the trans position.

WES further revealed a heterozygous variation, that is, c.2704G>A (NM_00108052.2, p.Asp902Asn) in exon 22 of CC2D2A of the fetus (Fig. 5). According to the ACMG guidelines, c.2704G>A is a variant of unknown significance (PM2).

3.5. Sanger sequencing for validation of pedigree

Sanger sequencing identified heterozygous mutations at the same gene positions in the DNA samples of the parents. BBS1 of the parents exhibited heterozygous variation of exon 12 c.1177C>T (NM_024649.4, p.Arg393*) (Fig. 3). The parents also displayed heterozygous variation of CC2D2A on exon 22 c.2704G>A (NM_00108052.2, p.Asp902Asn) (Fig. 4).

3.6. Pregnancy outcome

The pregnancy was terminated at 25 weeks of gestation, and the parents of the fetus did not provide consent for a postinduction autopsy.

4. Discussion

In this study, an intraterine 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 conducted traditional karyotyping and SNP analysis for genetic testing of the fetus. Karyotyping showed no abnormalities. SNP analysis showed no imprinted genes in the 2 homozygous regions, but revealed an increased risk of recessive genetic disease caused by homozygous mutation. WES revealed homozygous variation of c.1177C>T (NM_024649.4, p.Arg393*) in exon 12 of BBS1. Sanger sequencing identified heterozygous mutations in the same positions of genes in the parents of the fetus. These data are consistent with an autosomal recessive inheritance of BBS.

BBS1 (OMIM:209901) is located on chromosome 11q13 and is also known as BBS2L2. Presently, 94 pathogenic variants of BBS1 have been reported by Human Gene Mutation Database. BBS1 mutation is the most common cause of BBS and is responsible for 25% of all BBS incidences. The type of mutation varies among ethnic groups, with the most common BBS1 variant (p.M390R) accounting for approximately 80% of all BBS1 mutations in the European population. Mykytyn et al. conducted genetic screening on 129 patients with BBS and
found that 30% of these patients possessed at least 1 M390R mutation. BBS proteins encoded by different BBS genes are functional throughout the formation of the BBS complex, including BBSome, which consists of 7 BBS proteins (BBS1, BBS2, BBS4, BBS5, BBS7, BBS8, and BBS9). BBS1 mutation results in abnormal function of the BBSome, which in turn affects the function of microcilia and other systems in the body. The homozygous variation of c.1177C>T (NM_024649.4, p.Arg393*) in exon 12 of BBS1 has not been reported in the Chinese population.

Most BBS1 variants include missense, deletion/insertion, and splicing mutations and produce typical BBS phenotypes. Recent studies indicate that 90% of the BBS patients exhibit retinal degeneration, 90% have abnormal renal development and function, and 72% to 92% are obese. Additionally, 63% to 81% of the patients have polydactyly/deformity.
and more than half of the patients exhibit intellectual disability and/or gonadal dysplasia. The fetus in this study exhibited a nonsense BBS1 variant with biallelic loss of function mutation. Renal abnormalities in the sonography results of the fetus are consistent with previously reported clinical abnormalities in the renal development in patients with BBS1 mutations. The parents of the fetus did not provide consent for postinduction autopsy; therefore, whether the fetus had other clinical manifestations associated with BBS1 mutations could not be explored.

WES can rapidly and efficiently detect all potentially pathogenic mutations at once. However, the associated huge data output poses a great challenge for bioinformatic analysis and clinical interpretation. In this study, WES revealed a heterozygous variation, that is, c.2704G>A (NM_00108052.2, p.Asp902Asn) in exon 22 of CC2D2A in the fetus. This gene is primarily involved in the development of the COACH syndrome (OMIM:216360), Joubert syndrome 9 (OMIM:612285), and Meckel syndrome 6 (OMIM:612284).

The COACH syndrome is an autosomal recessive inherited disorder, which exhibits intellectual disability, ataxia (owing to cerebellar hypoplasia), and liver fibrosis as the typical clinical features. Joubert syndrome is an autosomal recessive inherited disease, which manifests clinically as cerebellar ataxia, ocular movement dysfunction, vermian hypoplasia, and thickening of the upper cerebellar foot. Meckel syndrome, another autosomal recessive inherited disease, is a fatal disorder associated with multiple congenital anomalies and characterized by clinical features, including brain malformation, polycystic kidney malformation, polydactyly deformity, cleft lip and palate, cardiac abnormality, malformation of the central nervous system, liver fibrosis, and bone dysplasia. Heterozygous variation, that is, c.2704G>A (NM_00108052.2, p.Asp902Asn) in exon 22 of CC2D2A was identified in the parents of the fetus. Further studies are necessary to determine the relationship of this variation with congenital renal dysplasia.

5. Conclusion
In conclusion, we identified a novel nonsense variant c.1177C>T (p.Arg393*) in the BBS1 gene of a Chinese family. To the best of our knowledge, this pathogenic homozygous variant in BBS1 is the first to be reported in the Chinese population. Importantly, it is necessary to carry out prenatal genetic diagnosis in subsequent pregnancies by the parents of the fetus, as both carry pathological variants of BBS1.

Acknowledgments
We thank all patients for their participation.

Author contributions
Conceptualization: LX and NL.
Writing – original draft: MC.
Writing – review & editing: HH revised the article.
Data curation and formal analysis: ML.

References
[1] Zaghloul NA, Katsanis N. Mechanistic insights into Bardet-Biedl syndrome, a model ciliopathy. J Clin Investig. 2009;119:428–37.
[2] Makito H, Wataru S, Kenji I, et al. The first nationwide survey and genetic analyses of Bardet-Biedl syndrome in Japan. PLoS One. 2015;10:e016317.
[3] Georges B. On congenital obesity syndrome with polydactyly and retinitis pigmentosa (a contribution to the study of clinical forms of hypophyseal obesity) 1920. Obes Res. 1995;3:387–99.
[4] Biedl AA. Pair of siblings with adipose-genital dystrophy. Obes Res. 2012;3:404–404.
[5] Heon E, Kim G, Qin S, et al. Mutations in C8ORF37 cause Bardet Biedl syndrome (BBS21). Hum Mol Genet. 2016;25:2283–94.
[6] Schaefer E, Stoetzel C, Scheidecker S, et al. Identification of a novel mutation confirms the implication of IFT172 (BBS20) in Bardet-Biedl syndrome. J Hum Genet. 2016;61:447–50.
[7] Leppert M, Baird L, Anderson KL, et al. Bardet-Biedl syndrome is linked to DNA markers on chromosome 1q and is genetically heterogeneous. Nat Genet. 1994;7:108–12.
[8] Kwitek-Black AE, Ca Rmi R, Duyk GM, et al. Linkage of Bardet-Biedl syndrome to chromosome 16q and evidence for non-allelic genetic heterogeneity. Nat Genet. 1993;3:592–6.
[9] Katsanis N, Beales PL, Woods MO, et al. Mutations in MKKS cause obesity, retinal dysplasty and renal malformations associated with Bardet-Biedl syndrome. Nat Genet. 2000;26:687–70.
[10] Yong HK, Joo KS, Seong MW, et al. Retinitis pigmentosa associated with Bardet-Biedl syndrome with BBS9 gene mutation in a Korean patient. Korean J Ophthalmol. 2020;34:94–5.
[11] Scerbo M, Costa F, Obringer C, et al. The study of a total and two hypothalamic-specific BBS10 knockout models highlights the importance of systemic inactivation in the obese phenotype in Bardet Biedl Syndrome. Cilia. 2015;4(Suppl 1):P5.
[12] Nihkhah E, Safarlalizadeh R, Mohammadlaj I, et al. Identification of novel compound heterozygous mutation in BBS12 gene in an Iranian family with Bardet-Biedl syndrome using targeted next generation sequencing. Cell J. 2018;20:284–9.
[13] Priya S, Nampoothiri S, Sen P, et al. Bardet–Biedl syndrome: genetics, molecular pathophysiology, and disease management. Indian J Ophthalmol. 2016;64:620–7.
[14] Williams CL, Uyttinger CR, Green WW, et al. Gene therapeutic reversal of peripheral olfactory impairment in Bardet-Biedl syndrome. Mol Ther. 2017;25:904.
[15] Larroca GT. Prenatal diagnosis of Bardet-Biedl syndrome in a case of hyperechogenic kidneys: clinical use of DNA sequencing. Clin Case Rep. 2017;5:449–53.
[16] Mary L, Chennen K, Stoetzel C, et al. Bardet-Biedl syndrome—antenatal presentation of 45 fetuses with biallelic pathogenic variants in known BBS genes. Clin Genet. 2019;95:384–97.
[17] South ST, Lee C, Lamb AN, et al. ACMG Standards and Guidelines for constitutional cytogenomic microarray analysis, including postnatal and prenatal applications: revision 2013. Genet Med. 2013;15:901–9.
[18] Kearney HM, Thorland EC, Brown KK, et al.; Working Group of the American College of Medical Genetics Laboratory Quality Assurance Committee. American College of Medical Genetics standards and guidelines for interpretation and reporting of postnatal constitutional copy number variants. Genet Med. 2011;13:680–5.
[19] Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17:405–24.
[20] Biesecker LG, Harrison SM. The ACMG/AMP reusable source criteria for the interpretation of sequence variants. Genet Med. 2018;20:1687–8.
[21] Gelb BD, Càvè H, Dillon MW, et al. ClinGen’s RAsOpeth Expert Panel consensus methods for variant interpretation. Genet Med. 2018;20:1334–45.
[22] Zastrow DB, Baudet H, Shen W, et al. Unique aspects of sequence variant interpretation for inborn errors of metabolism (IEM): the ClinGen IEM Working Group and the Phenylalanine Hydroxylase Gene. Hum Mutat. 2018;39:1569–80.
[23] Mykytyn K, Nishimura DY, Searby C, et al. Identification of the gene (BBS1) most commonly involved in Bardet-Biedl syndrome, a complex human obesity syndrome. Nat Genet. 2002;31:435–8.
[24] Beales PL, Badano JL, Ross AJ, et al. Genetic interaction of BBS1 mutations with alleles at other BBS loci can result in non-Mendelian Bardet-Biedl syndrome. Am J Hum Genet. 2003;72:1187–99.
[25] Mykytyn K, Nishimura DY, Searby CC, et al. Evaluation of complex inheritance involving the most common Bardet-Biedl syndrome locus (BBS1). Am J Hum Genet. 2003;72:429–37.
[26] Stoetzel C, Laurier V, Davis EE, et al. BBS10 encodes a vertebrate-specific chaperonin-like protein and is a major BBS locus. Nat Genet. 2006;38:521–4.
[27] Maria S, Sheila CS, Diana V. Bardet-Biedl syndrome as a chaperonopathy: dissecting the major role of chaperonin-like BBS proteins (BBS6-BBS10-BBS12). Front Mol Biosci. 2017;4:33.
[28] Billingsley G, Bin J, Fiegen KJ, et al. Mutations in chaperonin-like BBS genes are a major contributor to disease development in a multiethnic Bardet-Biedl syndrome patient population. J Med Genet. 2010;47:453–63.
[29] Hodges ME, Scheumann N, Wickstead B, et al. Reconstructing the evolutionary history of the centriole from protein components. J Cell Sci. 2010;123:1407–13.
[30] Hua J, White SR, Shida T, et al. The conserved Bardet-Biedl syndrome proteins assemble a coat that traffics membrane proteins to cilia. Cell. 2010;141:1208–19.
[31] Estrada-Cuzcano A, Koenekoop RK, Senechal A, et al. BBS1 mutations in a wide spectrum of phenotypes ranging from nonsyndromic retinitis pigmentosa to Bardet-Biedl syndrome. Arch Ophthalmol. 2012;130:1425–32.
[32] Deluca AP, Weed MC, Haas CM, et al. Apparent usher syndrome caused by the combination of BBS1-associated retinitis pigmentosa and SLC26A4-associated deafness. JAMA Ophthalmol. 2015;133:967–8.
[33] Bravo-Gil N, Méndez-Vidal C, Romero-Pérez L, et al. Improving the management of inherited retinal dystrophies by targeted sequencing of a population-specific gene panel. Sci Rep. 2016;6:23910.
[34] Carss KJ, Arno G, Erwood M, et al. Comprehensive rare variant analysis via whole-genome sequencing to determine the molecular pathology of inherited retinal disease. Am J Hum Genet. 2017;100:55–90.
[35] Beales PL, Elcioglu N, Woolf AS, et al. New criteria for improved diagnosis of Bardet-Biedl syndrome: results of a population survey. J Med Genet. 1999;36:437–46.
[36] Forsythe E, Beales PL. Bardet-Biedl syndrome. Eur J Hum Genet. 2013;21:8–13.
[37] Iannello S, Bosco P, Cavaleri A, et al. A review of the literature of Bardet–Biedl disease and report of three cases associated with metabolic syndrome and diagnosed after the age of fifty. Obes Rev. 2010;3:123–35.
[38] Tayeh MK, Hsan-Jan Y, Beck JS, et al. Genetic interaction between Bardet-Biedl syndrome genes and implications for limb patterning. Hum Mol Genet. 2008;17:1956–67.
[39] Uralp S, Demircan M, Getin S, et al. Bardet-Biedl syndrome associated with vaginal atresia: a case report. Turk J Pediatr. 2003;45:273–5.
[40] Wright CF, Mccrae JF, Clayton S, et al. Making new genetic diagnoses with old data: iterative reanalysis and reporting from genome-wide data in 1,133 families with developmental disorders. Genet Med. 2018;20:1216–23.
[41] Wright CF, FiczPatrick DR, Firth HV. Paediatric genomics: diagnosing rare disease in children. Nat Rev Genet. 2018;19:253–68.
[42] Doherty D, Parisi MA, Finn LS, et al. Mutations in 3 genes (MKS3, CC2D2A and RPGRIP1L) cause COACH syndrome (Joubert syndrome with congenital hepatic fibrosis). J Med Genet. 2010;47:8–21.
[43] Devi A, Naushad SM, Lingappa L. Clinical and molecular diagnosis of Joubert syndrome and related disorders. Pediatr Neurol. 2020;106:43–9.
[44] Radhakrishnan P, Nayak SS, Shukla A, et al. Meckel syndrome: clinical and mutation profile in six fetuses. Clin Genet. 2019;96:560–5.