Prenatal detection of TAR syndrome in a fetus with compound inheritance of an *RBM8A* SNP and a 334-kb deletion: A case report

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Abstract. Thrombocytopenia-absent radius syndrome (TAR) is a rare genetic disorder that is characterized by the absence of the radius bone in each forearm and a markedly reduced platelet count that results in life-threatening bleeding episodes (thrombocytopenia). Tar syndrome has been associated with a deletion of a segment of 1q21.1 cytoband. The 1q21.1 deletion syndrome phenotype includes Tar and other features such as mental retardation, autism and microcephaly. This study describes a case of a prenatally diagnosed fetus with compound inheritance of a small (334 kb) deletion, as detected by array-comparative genomic hybridization, and a 5' untranslated region (UTR) low-frequency allele (rs139428292) in gene RBM8A as detected by Sanger sequencing. The study describes the first case of prenatal analysis of TAR syndrome in a fetus with compound inheritance of a 334-kb deletion in the 1q21.1 region and a low-frequency 5' UTR single nucleotide polymorphism, and provides confirmation of the causal nature of the RBM8A gene in the diagnosis of TAR syndrome.

Introduction

Thrombocytopenia-absent radius (TAR) syndrome (MIM 274000) is a rare condition (0.5:100,000 in Spain) (1), characterized by absence of the radii with the presence of thumbs and thrombocytopenia (2). Numerous studies have identified the presence of a minimally deleted 200-kb region at chromosome band 1q21.1 in patients with TAR, but it is not sufficient to cause the phenotype (3,4). A study identified two rare single nucleotide polymorphisms (SNPs) in the regulatory region of the RBM8A gene that are involved in TAR syndrome through the reduction of the expression of the RBM8A-encoded Y14 protein (4). The first allele (rs139428292 G>A), which is located in the 5' untranslated region (UTR) region of the gene, was demonstrated to have a minor allele frequency (MAF) of 3.05%, and the second allele (rs201779890 G>C), located in the first intron of the gene, exhibited a MAF of 0.42%, in 7,504 healthy individuals from Cambridge BioResource (Cambridge, UK) (4,5).

Prenatal detection of the disease may be possible for pregnancies known to be at risk and for pregnancies in which radial anomalies are identified on routine ultrasound examination, which has traditionally been used to identify TAR syndrome, using a variety of molecular genetics methods.

The present study describes the clinical, molecular and molecular cytogenetic studies of a prenatally diagnosed fetus with TAR syndrome with compound inheritance of a small (334 kb) deletion, as detected by array-comparative genomic hybridization (CGH), and of a 5'UTR low frequency allele (rs139428292) in gene RBM8A, as detected by Sanger sequencing.

Case report

Case presentation. This case report is presented with the consent of the patient's family. A 29-year-old female, gravida 3, para 1, with a 3.5-year-old healthy child, presented in the first trimester of pregnancy. Previous family history revealed a pregnancy with prenatal ultrasound findings consistent with TAR syndrome at 12 weeks. Increased nuchal translucency (NT; 4.9 mm), omphalocele with intestinal contents and a single umbilical artery were observed. The hands were in ulnar flexion and there was bilateral absence of one of the long bones of the

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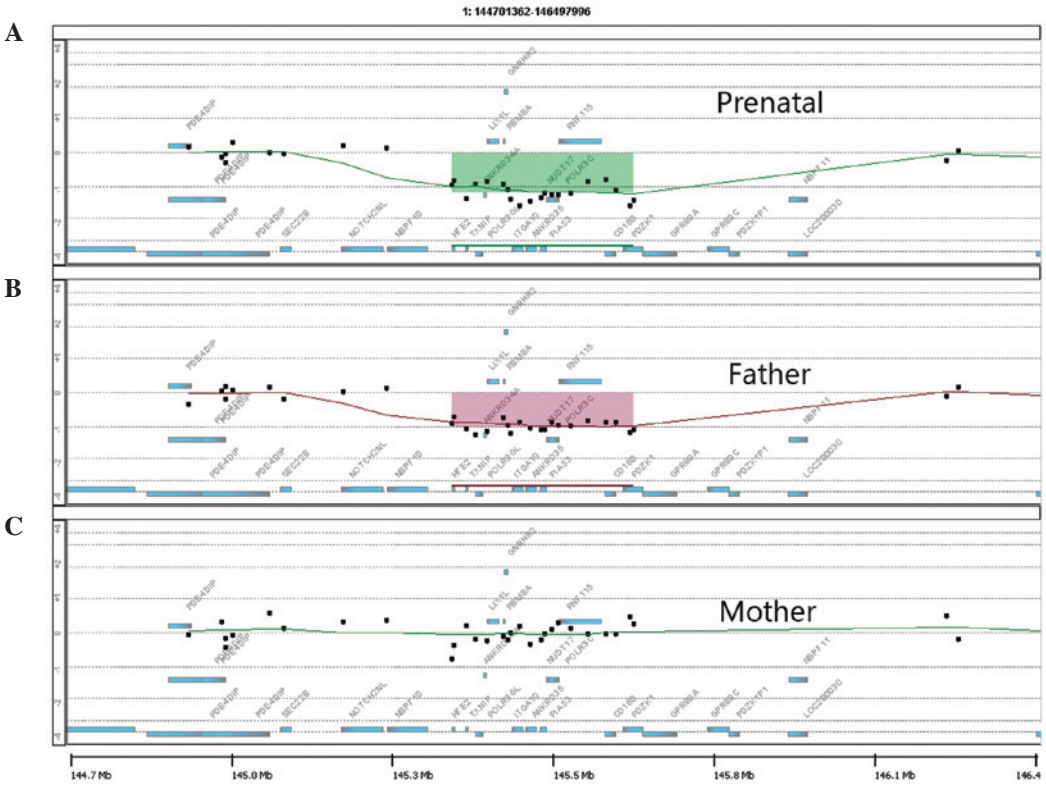

Figure 1. Array-CGH identifies the 334-kb deletion in the long arm of chromosome 1, located within the 1q21.1 region (chr1:145,413,388-145,747,269) in the (A) fetus, (B) father and (C) mother. CGH, comparative genomic hybridization.

forearm (either the radius or ulna), which was not possible to clearly define at that stage. The adjusted risk for trisomy 21 was 1 in 54, whereas the adjusted risk for trisomies 18 and 13 was 1 in 260. Following genetic counseling, the parents opted for invasive prenatal diagnosis, and chorionic villus sampling (CVS) was performed. Chromosome analysis revealed a normal female karyotype (46, XX). No further genetic analysis was performed. The karyotypes of the parents were normal. Pregnancy was terminated by choice at 13 weeks. There was no pathological examination of the fetus due to the lack of parental consent.

In the presented pregnancy, ultrasound examination at 13 weeks of gestation revealed an increased NT of 8.1 mm. The fetal crown-rump length was 53.1 mm and the fetal heart rate was 171 beats per minute. The upper limbs were shorter than normal, but no other structural defects were identified during the first trimester ultrasound examination. Following genetic counseling, the parents opted for invasive prenatal diagnosis.

Cytogenetics analysis. CVS culture was according to the standard procedures (6). CVS was performed at 13 weeks and fetal DNA was extracted directly using InstaGene matrix resin (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The chorionic villi were cultured and GTG banding (300-400 bands) of chromosomes revealed a normal male karyotype (46, XY). Due to their previous history, the parents decided to further investigate with molecular cytogenetic testing.

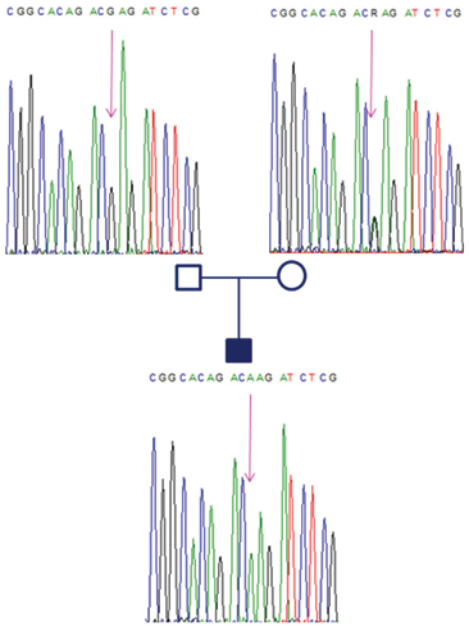

Figure 2. Electropherograms of the nucleotides flanking the SNP rs139428292, located in the 5'UTR of the RBM8A gene of the parents and the fetus presenting clinical signs of TAR syndrome. The arrows indicate the position of the SNP in the electropherograms. SNP, single nucleotide polymorphism; UTR, untranslated region; TAR, thrombocytopenia-absent radius syndrome.

Microarray analysis. Array-CGH analysis was conducted on DNA from CVS using a SurePrint G3 Human GE 8x60k, Oligo Microarray kit that has a backbone resolution of ~200 kb (Agilent Technologies, Santa Clara, CA, USA), as described in a previous study (7). The statistical analysis, using aberration detection method-2 [aberration filter/threshold set at 6 and annotation genomic build NCBI37 (NCBI, Bethesda, MD, USA)], revealed a 334-kb deletion in the long arm of chromosome 1,

located within the 1q21.1 region (chr1:145,413,388-145,747,269) of the fetus (Fig. 1A). The same chromosomal abnormality was identified in the father (Fig. 1B) and no abnormal copy number variations were identified in the mother (Fig. 1C).

qPCR. The array-CGH results were confirmed by copy number profiling using a qPCR method, as described previously (8). Two genes, POLR3GL (chr1:145,456,236-145,470,388) and CD160 (chr1:145,695,798-145,715,614), which map to the ends of the putatively deleted region, were amplified with specific primers using the LightCycler® FastStart DNA Master^{PLUS} SYBR Green I mix (Roche Applied Science, Roche Diagnostics S.p.A., Monza, Italy). The real-time reactions were analyzed on a LightCycler® 1.5 (Roche Diagnostics GmbH, Mannheim, Germany). The concentration of the DNA samples was adjusted by including two reference genes, EIF3L and KDELR3, that are located on chromosome 22. Relative quantification, in respect to a calibration curve used to establish efficiency, was utilized to detect the number of copies of DNA targets per diploid genome. All PCR experiments were replicated three times. qPCR demonstrated the presence of a single copy of the two target genes, POLR3GL (0.96 ± 0.05) and CD160 (0.97 ± 0.07) per diploid genome of the fetus, confirming the array-CGH data. The deletion was also confirmed in the father's DNA, while the DNA of the mother showed the presence of two copies of the genes per diploid genome (data not shown).

Sanger DNA sequencing methods. The sanger method was used to analyze the DNA sequence of the region spanning the 5'UTR and the first intron of the RBM8A gene (chr1:145,507,556-145,513,535) in the fetus and parents. Primers, 5'-ATGGCCACAGAAACACTTCC-3' (forward) and 5'-GGGCGGAATCTCTA-ATCCAC-3' (reverse), were selected to include the two SNPs involved in TAR. The sequencing reactions of the strands were analyzed on an automatic DNA sequencer Applied Biosystems 3500 Genetic Analyzer for sequence typing and fragment analysis (Life Technologies Corporation, Foster City, CA, USA). Genotyping of the sequence demonstrated the presence of the wild-type G allele in the father and revealed a heterozygous (G/A) condition at the 5'UTR polymorphism (rs139428292) in the mother. The father's genotype was hemizygous, as he had the deletion of 334 kb, which included the gene RBM8A. The electropherogram of the fetus only identified the A allele of maternal origin. Thus, the fetus was affected by TAR syndrome, as it had inherited the paternal null allele (microdeletion) and the maternal A allele of the 5'UTR polymorphism (Fig. 2). All subjects of the family showed only the wild-type G allele for the intronic SNP (rs201779890). Following genetic counseling, the parents decided to terminate the pregnancy at 14 weeks of gestation. The couple did not consent for fetal pathology assessment.

In the present case the deletion was ~334 kb at 1q21.1 and included nine hypothetical/unknown protein coding genes according to SWIIS-PROT, TrEMBL and TrEMBL-NEW and their corresponding mRNAs from Genbank (HFE2, TXNIP, RBM8A, GNRHR2, PEX11B, ITGA10, PIAS3, CD160 and PDZK1). The RBM8A gene encodes RNA binding protein Y14, one component of the exon junction complex, which interacts with mRNA and mediates gene expression (9). The Y14 protein is widely expressed and highly conserved among species.

Discussion

In the majority of TAR cases there is a compound inheritance of a microdeletion in 1q.21.1 and of a low-frequency regulatory SNP. Albers *et al* (4) reported that of 55 TAR patients, 51 were known carriers of the 200-kb deletion. In two of the patients, an exonic 4-bp insertion frameshift mutation (605313.0003) and an exonic null heterozygous mutation (605313.0004) were detected, respectively, in the RBM8A gene. In all 53 cases, 1 of 2 low-frequency SNPs were detected in regulatory regions of the RBM8A gene in hetero/hemizygosity. A total of 51 patients carried a deletion (null allele) and one of the two low frequency SNPs, while 2 patients carried a truncation or frameshift null mutation in RBM8A and the low frequency 5' UTR SNP. Consequently, a compound inheritance mechanism of a rare null allele (deletion or mutation-frameshift, null) and one of two low-frequency SNPs in the regulatory regions of RBM8A, causes TAR.

RBM8A has been repeatedly associated with TAR syndrome and functional studies indicate that SNP rs139428292, identified in the present study, results in reduced promoter activity of the gene. A genotype of rs139428292 and a microdeletion/null allele of the RBM8A leads to lower levels of Y14, thus disrupting mRNA processing in several tissues and causing TAR syndrome (4).

The present study describes the first case of prenatal analysis of TAR syndrome in a fetus with compound inheritance of a 334-kb deletion in the 1q21.1 region and of a low-frequency 5'UTR SNP. It also confirms of the causal nature of the RBM8A gene in the diagnosis of TAR syndrome, as recently described by Albers *et al* (4). This study provides additional information for the understanding of TAR syndrome and a diagnostic molecular approach that, according to the existing literature, is able to detect the majority of TAR cases prenatally.

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