

CASE REPORT

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Clinical diagnostic exome evaluation for an infant with a lethal disorder: genetic diagnosis of TARP syndrome and expansion of the phenotype in a patient with a newly reported *RBM10* alteration

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Abstract

Background: Diagnostic Exome Sequencing (DES) has been shown to be an effective tool for diagnosis individuals with suspected genetic conditions.

Case Presentation: We report a male infant born with multiple anomalies including bilateral dysplastic kidneys, cleft palate, bilateral talipes, and bilateral absence of thumbs and first toes. Prenatal testing including chromosome analysis and microarray did not identify a cause for the multiple congenital anomalies. Postnatal diagnostic exome studies (DES) were utilized to find a molecular diagnosis for the patient.

Exome sequencing of the proband, mother, and father showed a previously unreported maternally inherited RNA binding motif protein 10 (*RBM10*) c.1352_1353delAG (p.E451Vfs*66) alteration. Mutations in *RBM10* are associated with TARP syndrome, an X-linked recessive disorder originally described with cardinal features of talipes equinovarus, atrial septal defect, Robin sequence, and persistent left superior vena cava.

Conclusion: DES established a molecular genetic diagnosis of TARP syndrome for a neonatal patient with a poor prognosis in whom traditional testing methods were uninformative and allowed for efficient diagnosis and future reproductive options for the parents. Other reported cases of TARP syndrome demonstrate significant variability in clinical phenotype. The reported features in this infant including multiple hemivertebrae, imperforate anus, aplasia of thumbs and first toes have not been reported in previous patients, thus expanding the clinical phenotype for this rare disorder.

Keywords: *RBM10* protein, Human, Exome, Clinical diagnostic sequencing, TARP syndrome, Oligodactyly

Background

Since 2011, Diagnostic Exome Sequencing (DES) has proven to be cost effective and beneficial in providing a broad spectrum of previously undiagnosed patients with molecular genetic diagnoses while broadening the phenotype of known genetic diseases. The American College of Medical Genetics and Genomics recommends proper utilization of DES in the clinical assessment of individuals with a suspected genetic condition in which prior genetic

testing fails to lead to a diagnosis [1]. The application of DES has allowed many undiagnosed patients who have endured an extensive diagnostic odyssey to receive a definitive genetic diagnosis [2–11].

Fetal and neonatal DES is being used more frequently in cases of infant demise, with or without congenital anomalies [4, 12]. Several studies have shown the clinical utility of DES for prenatal and neonatal patients due to increasing ease of DES and knowledge of the human genome [13–15].

Herein, we report a previously undiagnosed neonatal patient with a poor prognosis, who was identified to have a

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hemizygous *RBM10* alteration detected by DES establishing a molecular genetic diagnosis of TARP syndrome.

Case presentation

Clinical description

The male proband was born after a prenatal course complicated by anhydramnios and multiple fetal anomalies. Prenatal ultrasound identified bilateral renal aplasia, bilateral talipes, intrauterine growth restriction, and oligohydramnios that progressed to anhydramnios. Amniocentesis was performed during the pregnancy with normal karyotype and microarray. He was delivered at 35 weeks with a birth weight of 1.320 kg (<2%), length 40 cm (<3%) and head circumference 27 cm (<3%) to non-consanguineous parents of Mexican descent. Additional anomalies noted after birth included multiple hemivertebrae, imperforate anus, bilateral undescended testes, aplasia of thumbs and first toes, cleft palate, pulmonary hypoplasia, brachycephaly, and low-set ears (Fig. 1). A renal ultrasound demonstrated atrophic, dysplastic kidneys with scattered parenchymal cysts suggestive of cystic renal dysplasia. No other renal anomalies, such as hydronephrosis were seen. Multiple cardiac anomalies were identified at autopsy including atrial septal defect, unicuspid aortic valve, bicuspid pulmonic valve, and coarctation of the aorta. A head ultrasound showed an unusual head shape with flattening of the posterior calvarium, potentially related to brachycephaly with no other anomalies noted. An MRI was not performed. The patient's family history is unremarkable.

The infant's prognosis was poor due to severe pulmonary hypoplasia. Whole exome testing was sent shortly after birth with a trio of the infant and parental samples. The infant died at day two of life from respiratory failure.

Methods

Exome library preparation, sequencing, bioinformatics, and data analysis were performed as previously described [4, 16–18]. Briefly, samples were prepared using IDT xGen Exome Research Panel V1.0 and sequenced using paired-end, 150-cycle chemistry on the Illumina HiSeq 2500 (Illumina, San Diego, CA). Approximately 92% of characterized Mendelian disease genes are fully covered (100%) at >20X (Farwell et al. [17]). The sequence data were aligned to the reference human genome (GRCh37) and variant calls were generated using CASAVA and Pindel [19]. Stepwise filtering included the removal of common SNPs, intergenic and 3'/5' UTR variants, non-splice-related intronic variants, and lastly synonymous variants. Variants were then filtered further based on family history and possible inheritance models. Data are annotated with the Ambry Variant

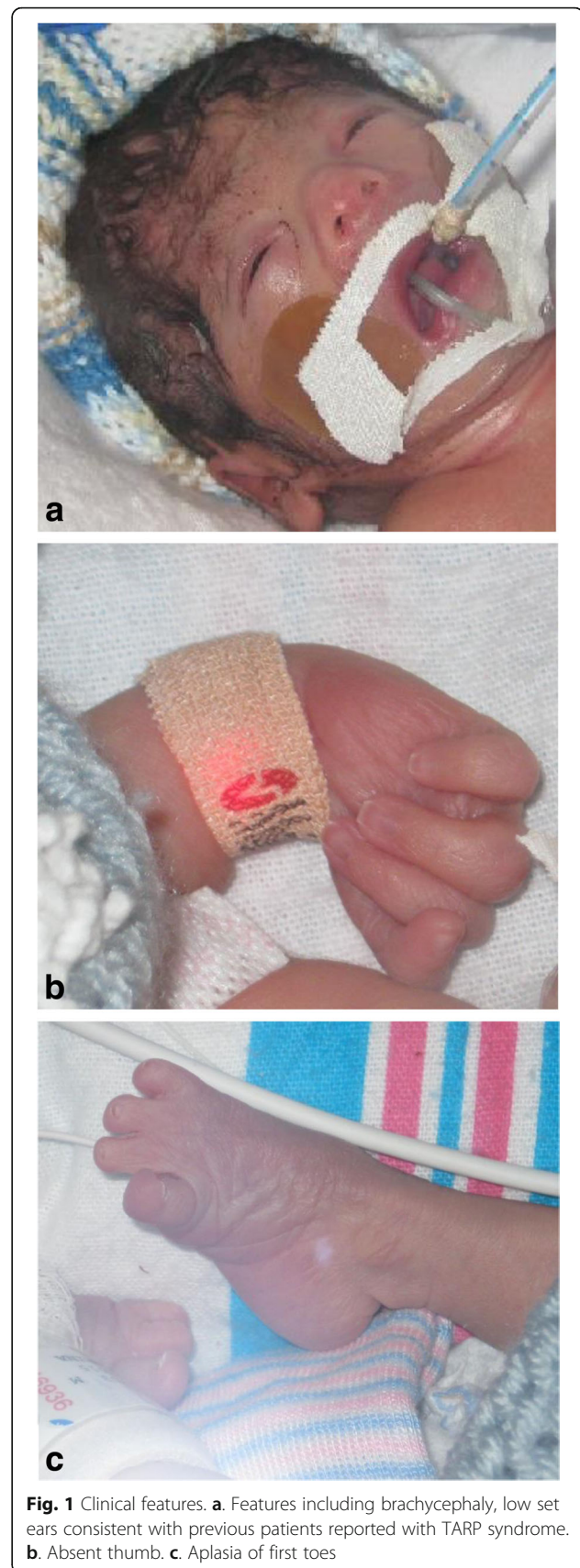


Fig. 1 Clinical features. **a.** Features including brachycephaly, low set ears consistent with previous patients reported with TARP syndrome. **b.** Absent thumb. **c.** Aplasia of first toes

Analyzer tool (AVA) [20]. Identified candidate alterations were confirmed using automated fluorescence dideoxy sequencing.

Results

Exome sequencing of the family trio (proband, mother, and father) resulted in a mean fold-coverage of captured regions of 135.04x per sample, with >98% covered with at least 10x coverage, and an average mean quality score of 34.56. (Additional file 1: Table S1). Family history inheritance model filtering based on autosomal and X-linked dominant and recessive and Y-linked inheritance models of the proband, mother, and father revealed 24 unique alterations in 17 candidate genes. Manual review of each alteration to rule out sequencing artifacts and polymorphisms along with medical interpretation to rule out genes lacking clinical overlap with the patient's evaluated phenotype (Additional file 2: Table S2) resulted in 1 gene (1 unique alteration) being considered as a candidate (Additional file 3: Table S3). A hemizygous alteration with potential clinical relevance, maternally inherited (*RBM10*) c.1352_1353delAG (p.E451Vfs*66) (Chr X:47040717-47040718), was confirmed by using automated fluorescence dideoxy sequencing (Additional file 4: Figure S1). To our knowledge, the c.1352_1353delAG alteration has not been previously reported in dbSNP, the NHLBI Exome Sequencing Project (ESP) [21], the ExAC database [22] or the 1000 Genomes database. [10].

The full length protein spans 930 residues and consists of six identified domains (Fig. 2). This alteration results in loss of over half of the native protein sequence, including the octamer repeat (OCRE) [23], C2H2-Zinc, and G-patch domains. These domains have been shown, by truncation and domain deletion in the closely related protein RBM5, to be important for spliceosome assembly and recruitment to pre-mRNA [24]. In particular, it has been shown that the OCRE domain binds to the C-terminal tail of snRNP core proteins [25]. Therefore, this alteration is predicted to cause loss of function either through nonsense-mediated decay or loss of functionally important protein domains and is interpreted as a pathogenic mutation.

Discussion and conclusions

Whole exome sequencing and bioinformatics analysis of a male neonate of Mexican ancestry with multiple

congenital anomalies was performed prior to his death at 2 days of age. Testing identified a maternally inherited hemizygous *RBM10* mutation. While rapid exome testing would not have benefitted this particular case, it is possible that rapid testing could aid additional patients with *RBM10* alterations in prognosis and knowledge to expand the clinical phenotype.

While previous testing had failed to provide a diagnosis for the proband, exome sequencing was able to determine a cause post-mortem based on the features evident before his death. This unique case highlights the power of whole exome sequencing as a diagnostic tool even after the patient is deceased. Post-mortem discovery of the cause of this patient's disease may help the parents with future reproductive decision making. The family has the option of preimplantation genetic diagnosis, early prenatal diagnosis in a future pregnancy, adoption, or to avoid future pregnancies. In addition, carrier testing is available for at-risk maternal relatives including three maternal aunts to our proband. When an infant is born with multiple anomalies, identifying a cause is essential for the family to determine recurrence risk in a future pregnancy.

The *RBM10* gene is located on chromosome Xp11.23 and encodes the RNA binding motif protein 10 (OMIM 300080), originally called RNA binding protein S1-1, which participates in alternative splicing of apoptosis-related genes [26, 27]. Hemizygous alterations in *RBM10* cause the X-linked recessive disorder TARP (Talipes equinovarus, Atrial septal defect, Robin sequence, and Persistent left superior vena cava) syndrome. TARP syndrome is a disorder originally described by Gorlin et al. as lethal in males associated with talipes equinovarus, congenital heart defects, and Robin sequence (micrognathia, glossoptosis, and cleft palate) [28]. Additional findings reported in patients with pathogenic variants in *RBM10* include dysmorphic features (hypertelorism, ear abnormalities, wide mouth with downturned corners, upturned nose, supraorbital ridges), airway abnormalities, high arched palate, cryptorchidism, oligohydramnios, intrauterine growth restriction, failure to thrive, rocker bottom feet, hypotonia, cranial nervous system abnormalities, postaxial pes polydactyly, cutaneous syndactyly, sensorineural hearing loss, developmental delay, and seizures [26, 28, 29].

Our patient has the overlapping features of intrauterine growth restriction, complications of oligohydramnios,

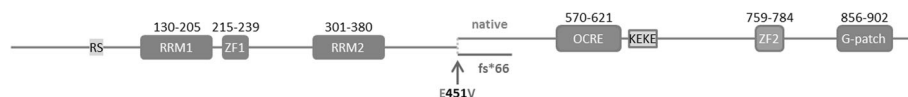


Fig. 2 Domain structure diagram of *RBM10* showing locations of domains (grey rectangles) and sequence biased regions (grey squares). Position of alteration shown with teal arrow, native sequence lost due to this alteration outlined in red, and the alternative 66 amino acid C-terminal sequence gained is depicted as a teal line. No structure or function was identified for this gained sequence

small size, cleft palate, pulmonary hypoplasia, a wide anterior fontanelle, cryptorchidism renal anomaly seen in other patients with TARP syndrome. His other features including multiple hemivertebrae, imperforate anus, aplasia of thumbs and first toes have not been reported in previous patients. While these additional features are present in other additional genetic conditions, due to the age of death, additional clinical diagnoses could not be made and a second pathogenic alteration was not found to explain these features.

The majority of cases reported have resulted in pre- or post-natal male lethality with the exception of two reported patients, one alive at 20 months and one alive at age 3 years and 7 months [26, 29]. Gripp et al. postulated that neonatal lethality was due to cardiac conduction defects, a finding not seen in our patient. To date, there have been no symptoms reported in carrier females and pathogenic variants reported in *RBM10* have been limited to loss of function alleles [26]. Therefore knowledge of the maternally inherited alteration may increase anxiety to due the recurrence risk, but can provide reassurance as additional health surveillance of the mother would not be necessary.

Previous reported cases of TARP syndrome demonstrate significant variability in clinical phenotype. Here, we report an additional patient with a pathogenic *RBM10* alteration demonstrating expansion of the clinical phenotype for this rare disorder. Diagnosis by DES is indispensable as the known phenotype of TARP expands and as limited time is often available to perform genetic testing in affected infants.

Additional files

Additional file 1: Table S1. Run Metrics for family trio. Read depth values exclude the following: reads of low quality, reads that do not align uniquely to the exome, and PCR duplicates. (DOCX 22 kb)

Additional file 2: Table S2. Variant Filtering Based on Inheritance Model & Interpretation (DOCX 20 kb)

Additional file 3: Table S3. List of candidate genes/alterations resulting from inheritance model filtering and the manual removal of NGS artifacts and polymorphisms*. (DOCX 18 kb)

Additional file 4: Figure S1. Pedigree and Co-segregation. Familial pedigree and electropherograms of the c.1352_1353delAG (p.E451Vfs*66) alteration in the proband and additional family members. Shaded shapes indicate affected individuals. Asterisk (*) indicates whole exome sequencing performed. (DOCX 210 kb)

Abbreviations

AVA: Ambry variant analyzer; dbSNP: Database of single nucleotide polymorphism; DES: Diagnostic exome sequencing; ESP: Exome sequencing project; OMIM: Online mendelian inheritance in man; *RBM10*: RNA binding motif protein 10; TARP syndrome: Talipes equinovarus, atrial septal defect, robin sequence, and persistent left superior vena cava syndrome

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Availability of data and materials

The alteration was deposited into ClinVar with accession number SCV000576421 (<http://www.ncbi.nlm.nih.gov/clinvar/>).

Authors' contributions

AH, SC and CJ carried out the clinical evaluation and provided the clinical genetics consultation to the patient and critically revised the final manuscript. ZP drafted the manuscript. IP provided structural analysis and assisted in writing manuscript. EP and ST critically revised the manuscript. All authors read and approved the final manuscript.

Competing interests

Zöe Powis, Igor Petrik, Erika Palmaer, and Sha Tang are employed by and receive a salary from Ambry Genetics. Exome sequencing is among the commercially available tests. Alexa Hart, Sara Cherny and Carolyn Jones have nothing to declare.

Consent for publication

Written informed consent was received from the family for publication of the clinical information and photographs.

Ethics approval and consent to participate

This study is exempt from the Office for Human Research Protections Regulations for the Protection of Human Subjects (45CFR46.101.4). Informed consent from the parents was obtained prior to testing. DNA from the peripheral blood of the patient and both parents was sent to Ambry Genetics (Aliso Viejo, CA) for DES. Furthermore, the clinical information presented herein was collected during the routine clinical care of a patient.

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