A novel mutated sequence in the *T-box* transcription factor-5 (*TBX-5*) gene (c.241A>T) in Holt–Oram syndrome

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Abstract

We report a case of a 31-year-old pregnant woman who was admitted to our perinatology outpatient clinic because of a fetal ventricular septal defect and limb reduction in the upper extremities of fetus revealed by ultrasonographic investigation diagnosed in the 16th week of gestation. First child of the family was diagnosed with Holt–Oram syndrome who had atrial septal defect and upper limb anomalies, whereas the father was documented to have arrhythmia and shortening of upper limbs. The pregnancy was terminated in the 16th week of gestation with the consent of the family. We performed mutation analysis in *T-box transcription factor-5 (TBX5)* gene coding exons, including exon/intron boundaries from peripheral blood or skin fibroblasts. The sequence analysis revealed c.241 adenine (A)>thymine (T) [p. arginine (Arg) 81 Tryptophan (Trp)] alteration in exon-3 of the *TBX5* gene in affected family members and fetus. This is a novel mutation causing Holt–Oram syndrome.

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Introduction

Holt–Oram syndrome (HOS) also called as the heart and hand syndrome or atriodigital dysplasia is an autosomal-dominant, inherited genetic condition that manifests itself as various cardiac malformations and skeletal deformities in the upper extremities (1). Cardiac defects occur in three quarters of the patients with HOS, and they may be structural, such as septal defects or functional, such as arrhythmias (2). Structural cardiac defects are encountered more frequently than functional defects. The most frequent cardiac structural defect is atrial septal defect (ASD) (3). Skeletal defects may be unilateral or bilateral and mostly affects the radial ray of the forearm with its phalanges and the thumb. Hypoplasia or aplasia of the radial, ulnar, or humeral bone, hypoplastic thumb, and absence of the thumb are the most commonly seen characteristic skeletal deformities. The incidence of HOS is 1 in 100,000 live-born babies with a very high penetrance (2). To date, a vast number of mutations have been defined in affected individuals. Approximately 70%–85% of the cases have been attributed to a novel mutation of the *T-box transcription factor-5* (*TBX5*) gene on the long arm of the chromosome 12 (q24.21) (4). *TBX5* is a member of the large T-box transcription factor family, which is present in a wide range of species from worms to humans and known to exert crucial functions in cardiogenesis and skeletal development. In the literature, missense, nonsense, frameshift mutations, splice mutations, and chromosomal rearrangements are defined, which are postulated to cause a disease through *TBX5* haploinsufficiency. Upper extremity anomalies are almost completely penetrant, whereas cardiac anomalies have approximately 75% penetrance (5).

Case Presentation

A 31-year-old Caucasian pregnant woman was referred to our perinatology unit in the 16th week of gestation for advanced fetal investigation for presenting with fetal cardiac and skeletal malformations. No increased risk of chromosomal abnormalities was revealed in the first-trimester screening. An ultrasonographic examination by a senior ultrasonographer revealed a single live fetus compatible with 16 weeks. A ventricular septal defect was located in the membranous septum with a mean diameter of 2.1 mm along with humeral, radial, ulnar aplasia in the left-upper extremity and right radial aplasia seen via ultrasonography. The woman was in a non-consanguineous marriage and had no significant medical or gestational history. Her first child, a 6-year-old girl, had ASD (secundum type, which was recently corrected with an angiographic operation), bilateral radial aplasia, and agenesis

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of thumbs that had been alleviated with two sequential operations known as "index finger pollicization" in the last 2 years. The patient's husband had bradyarrhythmia and needed a pacemaker, and his forearms were short. We learned that his sister also had the same defects. The family was seeking aid to deliver healthy offsprings in the future. Pedigree analysis suggested autosomal dominant inheritance (Figure 1).

The family was informed about fetal anomalies and recurrence risk of future pregnancies. They wanted the pregnancy to be terminated and to receive further genetic evaluation. The postmortem examination of the fetus was in agreement with prenatal ultrasonographic findings, including aplastic radius, aplastic thumb, hypoplastic carpal and metacarpal bones, and radial deviation of the hand on the right-upper extremity; a severely hypoplastic humerus, aplastic radius and ulna, fusion of carpal bones, triphalangeal thumb, and two aplastic fingers on the left side (Figure 2a, b). The ventricular septal defect was confirmed, and no other gross pathology was observed in the postmortem examination. The fetus weighed 150 g. Venous blood sampling was performed in I-2 (the father), I-3 (the mother), and II-1 (the living child) (Figure 1), and dermal tissue sampling in mutation analysis revealed heterozygous c.241A>T alteration in the exon-3 of the *TBX5* gene, causing p.Arg81Trp alteration in the protein. Because we were not able to conduct functional studies to document the effect of this amino acid change in protein, we checked how conserved it is among different species and observed that it was highly conserved (Figure 3). In support of this observation, SIFT (Fred Hutchinson Cancer Research Center, Seattle, WA, USA) (6), and PolyPhen (Department of Genetics, Harvard University, Cambridge, MA, USA) (7) analyses (involving predicting whether an amino acid substitution affects protein function) also indicated that Arg>Trp change at residue 81 would be damaging. A written informed consent was obtained from the patient to present this clinical report. The patient was discharged 2 days after the termination of pregnancy without any problems. We informed the family about the recurrence risk of the disease (50%) and preimplantation genetic diagnosis (PGD) for the determined specific *TBX5* missense mutation prior to future conceptions.

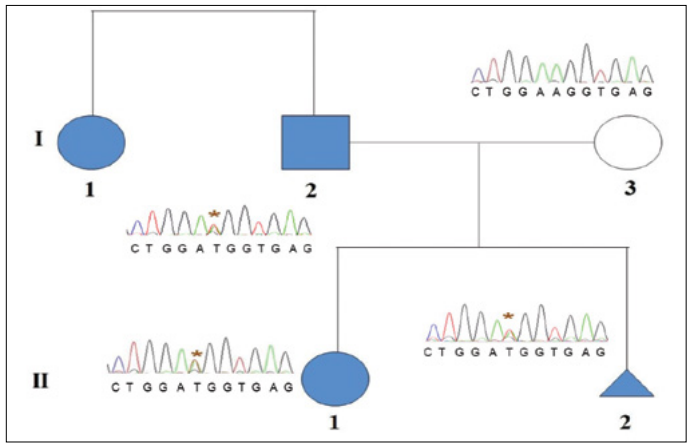

Figure 1. Pedigree is consistent with an autosomal-dominant inheritance pattern. Mutation analysis in affected individuals revealed c.241A>T alteration in exon 3 of *TBX5*

Figure 2. a, b. External view of the fetus (a), X-ray radiogram of the fetus (b)

Discussion

We reported three individuals in the same family with a missense mutation (c.241A>T), causing amino acid change at residue 81 in the T-Box domain. All individuals in the family of the present study had cardiac and limb abnormalities, being less severe in the father and his sister. This observation was in accordance with a previous report in which 19 individuals from the same family had an amino acid change at residue 80, and all of them were found to have cardiac and limb abnormalities (5). These observations provided convincing evidence that alterations around these residues may have more deleterious phenotypic outcomes compared with other residues in *TBX5*.

Individuals with HOS should be properly counseled regarding the disease. As it is clear from the family in the present study, HOS is characterized with full penetrance. We realized that skeletal phenotypes were more severe in fetus and living child than in father, possibly as a result of anticipation. Cardiac and skeletal phenotypes were different between father and two siblings, which is indicative of variable expression in HOS. Molecular genetic analysis is very important to know the type of mutation and which part of the protein is being affected to estimate genotype–phenotype correlation. All of these information will help in discussing the condition with the family in detail during genetic counseling.

This rare genetic syndrome dramatically affects the destiny of families. Sequential cardiac and/or orthopedic operations may be warranted. The family in our case had to travel frequently due to health problems of their first child.

The same kind of mutated sequence may show phenotypic heterogeneity among individuals in a family.

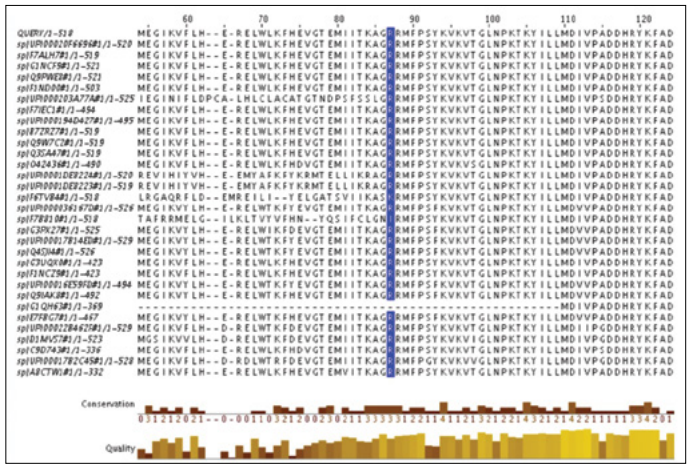

Figure 3. Multiple sequence alignment of TBX5 with its homologs. The altered amino acid is shown to be highly conserved evolutionarily across various species (http://genetics.bwh.harvard.edu)

In this study, the father and his sister had relatively mild cardiac and skeletal defects rather than the affected children. Conduction defects are less frequently seen cardiac problems in HOS (3).

Skeletal abnormalities are frequently more severe in upper-left limbs than in upper-right limbs, and an abnormal carpal bone is present in almost all of the affected cases. Furthermore, we observed these specifications in our case, as defined in the literature (2). Skeletal deformities as well as cardiac malformations were heterogeneous among individuals. The literature review provided no explanation for the predominant involvement of the left side of upper limbs, which therefore remains unclear.

The clinical implications of *TBX5* missense mutations, which are less frequently seen, are not clearly known, but it was denoted that these mutations seem to result in lesser severe cases than other types of mutations, predominantly cardiac or skeletal deformities (5, 8).

In a recent review by Al-Qattan and Abou Al-Shaar, the clinical heterogeneity of HOS was well established. They highlighted that there were three different mutation groups in *TBX5* gene: the first one was a single base change, e.g., missense mutations, causing a specific amino acid deficiency with regard to change in the associated nucleotide; second mutation caused extended protein changes; and third was the intragenic duplication of a region (8). A missense mutation altering an amino acid near the amino-terminal end of the T-box causes more considerable cardiac malformations, while a different missense mutation near the carboxyl end causes predominantly more severe upper limb deformities (frequently left-sided deformity) (5). Extended protein mutations are more inclined to cause severe bilateral skeletal malformations and more severe cardiac anomalies. Intragenic duplications have been reported to entail more severe cardiac anomalies rather than severe skeletal anomalies (8). In a recent case report by Kimura et al. (9), duplication screening was offered for families that had unidentified genetic structure. In the case of the family who presented at our clinic, the novel missense mutation (c.241A>T) seemed to cause both cardiac and skeletal malformations and showed a variable expressivity.

PGD is an advanced technique of molecular biology and genetics. It has been applied with high accuracy in HOS and used on affected families. Its combination with embryo cryopreservation and in vitro fertilization has been reported to be effective for the affected families to have several healthy children (10).

In conclusion, molecular genetic analysis is helpful in the management of inherited conditions such as the situation of the family in our study. Genetic counseling is additionally crucial for affected families. In practice, it merits great importance to inquire genetic conditions in family history, particularly when one of the parents raises suspicion of any genetic syndrome (short upper limb and arrhythmia in the father of the present family). This would help couples plan their pregnancies in advance and reduce emotional and economical damage overall. The severity of cardiac and concurrently skeletal defects should be discussed with the family when a decision remains unclear regarding the termination of pregnancy. PGD is an advanced technique and provides an opportunity to have a healthy baby with a normal genetic sequence. c.241A>T is a novel mutation found in a patient diagnosed with HOS according to our comprehensive literature search.

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Informed Consent: *Written informed consent was obtained from patient who participated in this case.*

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References

1 Holt M, Oram S. Familial heart disease with skeletal malformations. Br Heart J 1960; 22: 236-42. [CrossRef]
2. Basson CT, Cowley GS, Solomon SD, Weissman B, Poznanski AK, Traill TA, et al. The clinical and genetic spectrum of the Holt-Oram syndrome (heart-hand syndrome). N Engl J Med 1994; 330: 885-91. [CrossRef]
3. Sletten LJ, Pierpont ME. Variation in severity of cardiac disease in Holt-Oram syndrome. Am J Med Genet 1996; 65: 128-32. [CrossRef]
4. Bruneau BG, Nemer G, Schmitt JP, Charron F, Robitaille L, Caron S, et al. A murine model of Holt-Oram syndrome defines roles of the T-box transcription factor Tbx5 in cardiogenesis and disease. Cell 2001; 106: 709-21. [CrossRef]
5. Basson CT, Huang T, Lin RC, Bachinsky DR, Weremowicz S, Vaglios A, et al. Different TBX5 interactions in heart and limb defined by Holt-Oram syndrome mutations. Proc Natl Acad Sci USA 1999; 96: 2919-24. [CrossRef]
6. Available from: http://sift.jcvi.org/sift
7. Available from: http://genetics.bwh.harvard.edu/pph2
8. Al-Qattan MM, Abou Al-Shaar H. Molecular basis of the clinical features of Holt-Oram syndrome resulting from missense and extended protein mutations of the TBX5 gene as well as TBX5 intragenic duplications. Gene 2015; 560: 129-36. [CrossRef]
9. Kimura M, Kikuchi A, Ichinoi N, Kure S. Novel TBX5 duplication in a Japanese family with Holt-Oram syndrome. Pediatr Cardiol 2015; 36: 244-7. [CrossRef]
10. McDermott DA, He J, Song YS, Kligman I, Basson CT. Update: PGD and Holt-Oram syndrome. Am J Med Genet A 2005; 136: 223. [CrossRef]