

Clinical Report

Holt–Oram Syndrome Associated With Anomalies of the Feet

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Holt–Oram syndrome (HOS) (OMIM 142900) is characterized by upper-extremity malformations involving the radial, thenar, or carpal bones and a personal and/or family history of congenital heart defects (CHDs). It is inherited in an autosomal dominant manner. The *TBX5* gene located on chromosome 12 (12q24.1) is the only gene currently known to be associated with HOS and is associated with variable phenotypes. We report on the clinical and molecular

characterization of a HOS family with three affected individuals and a novel mutation (Lys88ter). We discuss genotype–phenotype correlations, the presence of foot anomalies in one affected individual, and the role of atypical features in HOS differential diagnosis. © 2008 Wiley-Liss, Inc.

Key words: Holt–Oram syndrome; *TBX5*; foot anomalies

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INTRODUCTION

With a prevalence of 1/100,000, Holt–Oram syndrome (HOS) (OMIM 142900) is the most common of the “heart-hand syndromes,” in which skeletal malformations are associated with cardiac structural and/or conduction defects. As the prototypical heart-hand disorder, HOS is characterized by upper-extremity malformations involving the radial, thenar, or carpal bones and a personal and/or family history of congenital heart defects (CHDs) inherited in an autosomal dominant manner. The upper-limb malformations are fully penetrant, but have variable expressivity. Congenital heart defects occur in approximately 75% of affected individuals [Basson et al., 1994; Sletten and Pierpont, 1996]. Mutations in the *TBX5* gene (12q24) cause HOS [Li et al., 1997]. *TBX5* germline mutations are specific for HOS, occurring in 22–42% of clinically diagnosed cases of HOS [Brassington et al., 2003; Heinritz et al., 2005; McDermott et al., 2005; Borozdin et al., 2006; McDermott and Basson, 2006], increasing to 74% when more strict clinical criteria are used [McDermott et al., 2005].

We report on the clinical and molecular characterization of a family with three individuals affected by HOS. We discuss the genotype–phenotype correlations in light of the presence of foot anomalies in one affected individual, the significance of atypical features, and the decision to perform molecular testing in heart-hand syndromes.

CLINICAL REPORT AND MOLECULAR ANALYSIS

The family pedigree is illustrated in Figure 1.

Patient II-1 (Proband) had an unremarkable prenatal history and normal development. She had surgical closure of an atrial septal defect (ASD) at the age of 22 years. Preoperative echocardiography showed a secundum-type ASD, and mitral valve

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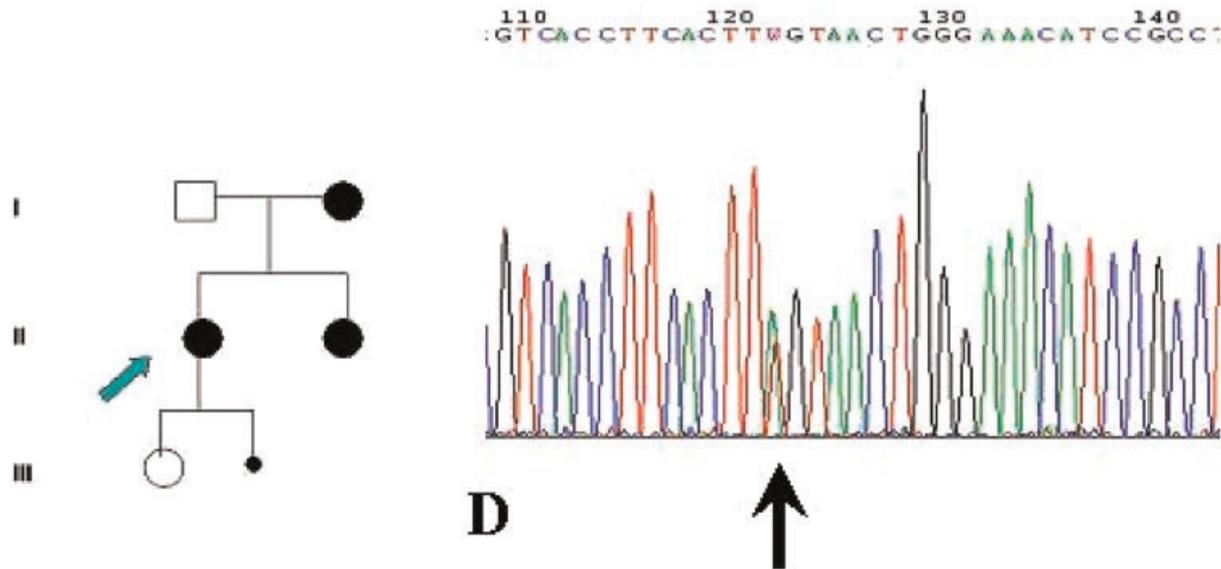


FIG. 1. Pedigree of the two-generation family with Holt–Oram syndrome (**left**). The open symbols represent unaffected individuals; the arrow indicates the proband. Chromatogram of the identified mutations in the *TBX5* gene (**right**). The arrow indicates the nonsense mutation Lys88Ter (exon 4). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

prolapse with slight regurgitation. On examination at the age of 23 years she had bilateral hypoplasia of the thumbs, more severe on the right side, proximally set thumbs and abnormal forearm pronation and supination (Fig. 2A,B). X rays of the hand showed on the right side extreme hypoplasia of the first metacarpal and severe hypoplasia of the thumb phalanges. On the left side, there was hypoplasia of the thumb phalanges, and hypoplasia of the middle phalanges of the 2nd and 5th fingers (Fig. 3A). The kidneys, gastrointestinal tract, face,

vision and hearing were normal. Laboratory evaluations excluded hematological and/or karyotype abnormalities.

Patient II-2 had normal development. Echocardiography showed minimal mitral regurgitation. On examination at the age of 13 years she had proximally set thumbs bilaterally, hypoplasia and clinodactyly of the 5th fingers bilaterally, abnormal forearm pronation and supination, more marked on the left side (Fig. 2C). X rays of the hands showed hypoplasia of the middle phalanges of the 2nd and 5th fingers,



FIG. 2. **A:** Patient II-1: bilateral hypoplasia of the thumbs, more severe on the right side, proximally set thumb; **(B)** Patient II-1: abnormal forearm pronation and supination; **(C)** Patient II-2: proximally set thumb bilaterally, hypoplasia and clinodactyly of the 5th finger bilaterally; **(D)** Patient I-2: bilaterally absent thumbs, abnormal forearm pronation and supination. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

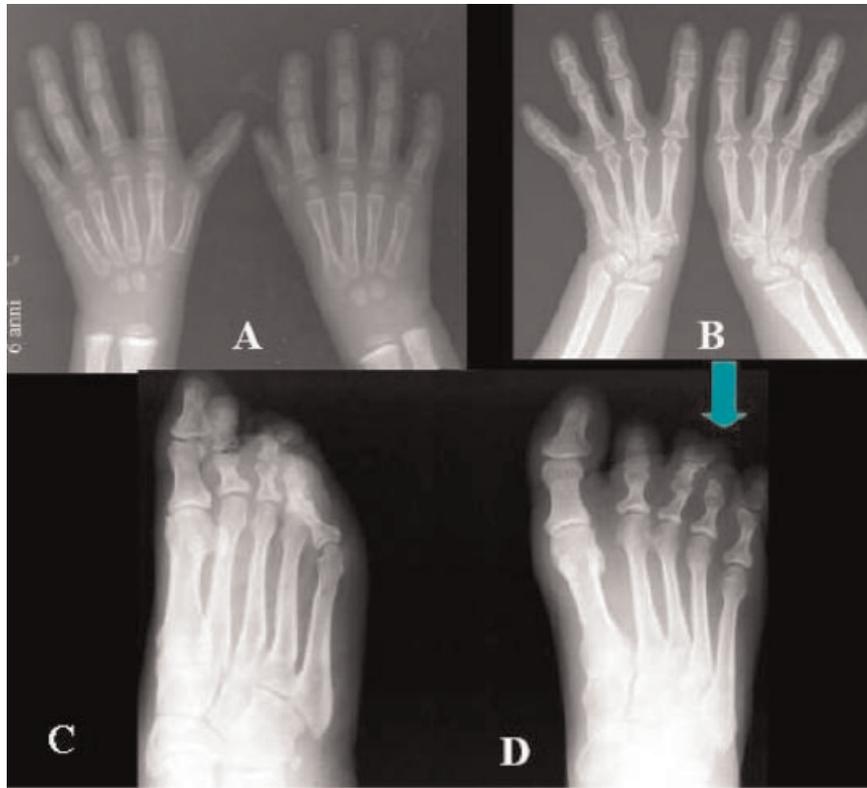


FIG. 3. **A:** Patient II-1. X rays of the hand: on the right side showing extreme hypoplasia of the first metacarpal, and severe hypoplasia of the thumb phalanges; on the left side hypoplasia of the thumb phalanges; hypoplasia of the middle phalanges of the 2nd and 5th finger; **(B)** Patient I-2. X rays of the hand showing bilateral absence of the thumbs, 1st metacarpals, and hypoplasia of the middle phalanges of the 2nd and 5th fingers; **(C,D)** Patient I-2. X rays of the feet showing hypoplasia of the distal and middle 2nd, 3rd, 4th toe phalanges, more marked on the 4th toe, and duplication of the distal phalanges of the 3rd toe (arrow). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

more severe on the right side and abnormal carpal bones with bilateral fusions of scaphoid and trapezium.

Patient I-2, the mother of the other patients, had normal development. Echocardiography showed atrial septal hypermobility, but no shunt. When examined at the age of 42 years, there was bilateral absence of the thumbs (Fig. 2D), abnormal forearm pronation and supination, as well as limited rotation of the right shoulder. The feet were characterized by a bifid appearance of the distal phalanges of the 3rd toes bilaterally, and absence of the distal phalanges of both 4th toes. Radiographs of the hands showed absence of the thumbs and first metacarpals bilaterally, hypoplasia of the middle phalanges of the 2nd and 5th fingers (Fig. 3B). Radiographs of the foot showed hypoplasia of the distal and middle 2nd, 3rd, and 4th toe phalanges, more marked on the 4th toe; duplication of the distal phalanges of the 3rd toe (Fig. 3C,D).

Foot anomalies were not present in the other two affected patients, her daughters, or in the apparently unaffected relatives (I-1, III-1). These individuals declined photographs and radiographs of the feet.

Molecular Genetic Analysis

Mutational analysis in all three affected patients showed a nonsense novel mutation, Lys88ter, in the *TBX5* gene (exon 4) (Fig. 1).

DISCUSSION

Holt-Oram syndrome can be diagnosed clinically based on the presence of upper-limb malformations, and a personal and/or family history of CHDs or cardiac conduction abnormalities. In all affected patients of our family there was hypoplasia of the middle phalanges of the 2nd and 5th fingers (brachydactyly type A4) which may be useful in diagnosis (Figs. 2 and 3). The foot anomalies that we observed in one patient appear to be unique (Fig. 3C,D).

TBX5 mutations have been identified in both familial and sporadic cases of HOS [Brassington et al., 2003] with more than 30 reported mutations (missense, nonsense, frameshift), and at least two recurrent mutations. As in our family, most of the *TBX5* mutations are null alleles which cause the HOS phenotypes through haploinsufficiency [Packham

and Brook, 2003]. The Lys88ter mutation that we found in our family is a novel mutation. Neither the kind of mutation nor its site seems to be predictive of the phenotypic expressivity [Brassington et al., 2003]. Although some genotype-phenotype correlations have been hypothesized for *TBX5* missense mutations [Basson et al., 1999; Yang et al., 2000], other studies have failed to confirm this [Brassington et al., 2003; Heinritz et al., 2005; McDermott et al., 2005]. The number of independent cases of HOS with the *TBX5* mutation described in literature is too small to establish or exclude such a correlation on a statistical basis.

Our report does not contribute to this issue. However, as a single patient with foot anomalies in an otherwise typical HOS family, we call attention to the potential role of the atypical features. Apart from upper limb malformations and CHDs with or without conduction defects, other features include renal, lower limb, craniofacial anomalies, deafness, axillary and tracheal anomalies, vertebral anomalies, abdominal situs inversus [Brassington et al., 2003; Lehner et al., 2003; McDermott et al., 2005]; the majority of such features have been reported in nongenotyped HOS patients.

Brassington et al. [2003] reported a *TBX5* mutation in one patient with typical HOS features and cervical vertebral fusions. Similarly, defects of the anterior vertebral bodies were the only "atypical" features observed in 1 of 14 HOS patients with the *TBX5* mutation described by McDermott et al. [2005], raising the issue of the usefulness of genetic testing in presence of skeletal malformations. Our case supports the view that skeletal abnormalities in *TBX5* positive HOS patients do not seem to be confined to the upper extremities, but can also involve the spine and the lower limbs.

It is difficult to explain these observations in the context of the mechanistic basis of phenotypic changes resulting from *TBX5* haploinsufficiency. Several studies suggest that genetic modifiers determine the severity of CHDs [Basson et al., 1999; Huang, 2002; Brassington et al., 2003]. Recently, using a mouse allelic series, Mori et al. [2006] have demonstrated a high sensitivity of the developing heart to *Tbx5* dosage, providing some interesting clues to the transcriptional and cellular mechanisms involved. In contrast, little is known about *TBX5* mechanisms in HOS skeletal malformations. *Tbx5* expression is predominant in the upper (fore)limbs of all vertebrates, including humans, mice, chickens, and fish, and is critical for growth processes in the developing limb. It was not expressed in the hind limb, which could explain the lack of lower limb defects in HOS [Margulies et al., 2001]. However, although related to cardiac morphogenesis, the study by Mori et al. [2006] clearly indicates the complex pattern of downstream genes whose expression can be influenced by *Tbx5* dosage. These genes are

frequently expressed also in the limbs (i.e., *Dkk3*, *Fbx032*, *Hand1*) and could be involved in skeletal malformations. Moreover, the presence of vertebral anomalies in two HOS cases with *TBX5* mutations is difficult to explain if there is no significant expression of *Tbx5* in the vertebrae (B.G. Brunneau, personal communication). Therefore, even if the presence of additional spine and lower limbs malformations could be coincidental, the possible role of *Tbx5* downstream target genes and/or genetic modifiers (stochastic or environmental factors) cannot be excluded.

In conclusion, our case report documents a new skeletal feature in one individual with HOS which may provide some insight into the phenotypic changes in HOS cases proven by the *TBX5* mutation. Documentation of additional cases will be needed to determine whether the presence or absence of lower limb anomalies can be used as a diagnostic criterion.

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