Holt-Oram syndrome is an autosomal-dominant condition characterized by congenital cardiac and forelimb anomalies. It is caused by mutations of the \( TBX5 \) gene, a member of the T-box family that encodes a transcription factor. Molecular studies have demonstrated that mutations predicted to create null alleles cause substantial abnormalities in both the limbs and heart, and that missense mutations of \( TBX5 \) can produce distinct phenotypes. One class of missense mutations causes significant cardiac malformations but only minor skeletal abnormalities; others might cause extensive upper limb malformations but less significant cardiac abnormalities. Intrafamilial variations of the malformations strongly suggest that genetic background or modifier genes play an important role in the phenotypic expression of HOS. Efforts to understand the intracellular pathway of \( TBX5 \) would provide a unique window onto the molecular basis of common congenital heart diseases and limb malformations.

**Clinical features**

Holt and Oram first described this syndrome when they reported on a family with atrial septal defects and congenital anomalies of the thumbs [1]. Since then, about 200 clinical papers have been published that further delineate the clinical features of Holt-Oram syndrome (HOS). The prevalence of HOS is 1 of 100,000 live births, and it occurs with wide ethnic and geographic distribution. Its clinical manifestations have proved to be variable [2,3••,4•], but with complete penetrance. All patients with HOS have upper limb anomaly and about 85% to 95% have cardiac malformation. The presence of cardiac malformations, conduction defects and radial ray abnormalities (or both) in an individual, or the presence of radial ray abnormalities with or without cardiac malformations or conduction defects in individuals with a family history of HOS [5••]. The family history should be consistent with autosomal-dominant inheritance.

**Cardiac defects**

Secundum-type atrial septal defect (ASD) and ventricular septal defect (VSD) are the most common heart defects. Other cardiac defects range from asymptomatic conduction disturbances (first-degree heart block) to multiple structural defects. Almost every type of cardiac anomaly has been reported, either singly or as part of a group of multiple defects [6–8]. Sudden death from heart block has been reported. Bruneau et al. summarize the defects in 240 patients [9••]. Among these patients, 58% had ASD, and 28% have VSD. Less common anomalies, such as conduction defect, truncus arteriosus, mitral valve defect, patent ductus arteriosus, and tetralogy of Fallot, occur in 18%, 8%, 4%, 4%, and 3%, respectively.

In an earlier series of studies [3•], heart defects in 189 patients were classified by severity. Among these patients, 66% had single abnormalities, including isolated conduction defects; 16% had “mild” combinations consisting of two or three malformations (eg, ASD, VSD); 11% had “moderate” combinations that required more complicated surgical repair (eg, tetralogy of Fallot and endocardial cushion defect); and 6% had “severe” combinations with life-threatening defects, including hypoplastic left heart, total anomalous pulmonary venous return, and truncus arteriosus.

Diagnosis of heart defects requires electrocardiography and two-dimensional echocardiography with doppler.
Cardiac catheterization may be required to fully define a defect.

**Upper limb anomalies**
Skeletal abnormalities affect the upper limbs exclusively; lower limb abnormalities have not been reported. The abnormalities are always bilateral and often asymmetric, predominantly involving the radial ray. The thumb is the most commonly affected structure and can be triphalangeal, hypoplastic, or completely absent. Abnormalities range from minor (clinodactyly of the fingers, limited supination of the forearms, and sloping shoulders) to severe (reduction deformities, including phocomelia and ectromelia). Clinical recognition of subtle limb anomalies in patients with HOS can require both physical examination and radiographs of the upper extremities.

Poznanski et al. demonstrated that carpal abnormalities are more specific for HOS than are changes in the thumb [10]. Other radiographic abnormalities include posteriorly and laterally protuberant medial epicondyles of the humerus, hypoplastic clavicles, shortened radii, and ulnar hypoplasia (occurring only in patients with radial defects).

**Overlapping conditions and differential diagnosis**
Other congenital malformations reported with cardiac malformation and upper limb anomalies, include lung hypoplasia and cardiomyopathy, postaxial or central polydactyly, arachnodactyly, thoracic scoliosis, hemiatrophy of the body, high myopia, Hirschsprung disease, malformations of the urinary system, the Rokitansky-Kuster-Hauser syndrome, cryptorchidism, malformations of renal and cerebral arteries, hypoplastic peripheral upper extremity vasculature, hypoplasia of the left radial artery, pulmonary hypertension, multiple strokes and end-stage renal failure, and malignant tumors [11–21]. These reports probably reflect fortuitous occurrences or represent different conditions. To date, no mutations in TBX5 have been found in individuals with “atypical” phenotypes (Huang et al., unpublished data).

The following autosomal-dominant conditions need to be considered for differential diagnosis:

- **Fanconi anemia syndrome** is characterized by congenital abnormalities. These abnormalities include malformations of the thumbs, forearms, and heart; progressive bone marrow failure with pancytopenia, typically in the first decade; and increased risk for myelodysplasia or acute myelogenous leukemia. The diagnosis of Fanconi anemia syndrome relies on detection of chromosomal breakage or rearrangements in the presence of diepoxybutane or mitomycin C.
- **Thrombocytopenia-absent radius** syndrome: both radii are always absent; the thumbs are always present. By contrast, radial aplasia in HOS is invariably associated with hypoplasia or absence of the thumb [4]. Phocomelia occasionally occurs. The lower limbs can be involved, including club foot and instability of the knee. Thrombocytopenia, present in infancy, generally improves with time. Heart defects can be present.
- **Heart-hand syndrome II (Tabatznik): type D brachydactyly (shortening of the distal phalanx of the thumb with or without shortening of the fourth and fifth metacarpals), sloping shoulders, short upper limbs, bowing of the distal radii, and absence of the styloid process of the ulna with supraventricular tachycardia. Patients can also have mild facial dysmorphism and mild mental retardation [22].**
- **Heart-hand syndrome III: type C brachydactyly (shortening of the middle phalanges) with an accessory wedged-shaped ossicle on the proximal phalanx of the index fingers with sick sinus syndrome [23].**
- **Okihiro syndrome: Duane syndrome (a congenital eye-movement disorder resulting from abnormal development of cranial nerve VI and characterized by absence of abduction of the globe and narrowing of the palpebral fissure on adduction of the globe), upper extremity reduction defects, and cardiac malformation [24].**
- **Long thumb brachydactyly syndrome: elongation of the thumb distal to the proximal interphalangeal joint, often associated with index finger brachydactyly, clinodactyly, narrow shoulders, secondary short clavicles, and pectus excavatum. Occasionally, rhizomelic limb shortening occurs. The cardiac abnormality is often a conductive defect [25].**
- **Vertebral, anal, cardiac, tracheal, esophageal, renal, and limb (VACTERL) anomalies association: radial defects are usually unilateral and accompanied by characteristic other malformations (i.e., imperforate anus, tracheoesophageal [TE] fistula).**

**Genetic counseling and management**
Genetic counseling should be provided to all patients with HOS. Of probands, 60% to 70% have an affected parent, and 30% to 40% have a de novo mutation. Evaluation of both parents is recommended, including physical examination and radiographs of the upper extremities to detect subtle changes of the thumb and carpal bones, and examination of the heart, including electrocardiogram and echocardiogram are recommended.

Risk to siblings depends on the genetic status of the parents. If one of the parents is affected, the siblings of a proband have a 50% risk of inheriting the disease-causing mutation. When the parents are clinically unaffected, the risk to the siblings of a proband appears to be low. Each individual with HOS has a 50% chance of inheriting the disease-causing mutation.
For individuals with conduction defects, regular electrocardiograms are recommended, as conduction defects can get worse with time. Many patients with severe atrioventricular block will need pacemakers. Antiarrhythmic drugs have been used for patients with atrioventricular block, but not for prophylaxis.

**Molecular studies**

The disease gene for HOS, which was linked to the chromosome 12q2 region by studying multiple unrelated families [26•,27•,28•], was identified as TBX5, a member of the T-box gene family [29•,30•]. Mutations in the TBX5 gene were demonstrated in many affected individuals and families [5••,29•,30•,31•,32•,33•]. The coding region of TBX5 cDNA is 1.5 kb with 8 exons. The DNA-binding domain of a TBX5 protein is composed of 180 amino acid residues (amino acid residues 56 to 236) and binds to a 24-nucleotide palindromic DNA duplex in vitro [33•]. It is possible that the protein binds as a dimer interacting with the major and minor grooves of the DNA [34].

Molecular genetic testing of TBX5 is currently available only on a research basis. Using gene sequencing of the TBX5 coding regions or mutation scanning (single-strand conformation polymorphism [SSCP] followed by sequencing of exons with abnormal band patterns), the mutation detection rate in the TBX5 coding region ranges from 20% to 55% in familial cases and from 15% to 40% in isolated cases with apparently negative family history [31•] (Huang et al., unpublished data). Mutations are distributed throughout the gene, with a few “hot spots” (codon 273) [5••,29•] and nucleotide 824 [30•]. Most mutations lead to premature termination of the protein product. The disease is probably caused by haploinsufficiency of TBX5 [5••,35].

Some tentative genotype-phenotype correlations have been established. Mutations predicted to create null alleles caused substantial abnormalities both in limb and heart [5••]. In one study, patients with frameshift mutations had severe phecomelia, whereas those with missense mutations had normal arms and absent or hypoplastic thumbs [32•]. Missense mutations of TBX5 may have distinct phenotypes. G80R caused significant cardiac malformations but only minor skeletal abnormalities, whereas mutations at amino acid 237 cause severe skeletal abnormalities, but minor cardiac malformations [5••] (Fig. 1). However, intrafamilial variations do exist. Basson et al. [26•] reported two large families with different mutations and showed an interfamilial pattern and intrafamilial variations. It seems that TBX5 mutations serve as gross tune and that other factors (e.g., genetic background) serve as a “finer tuning” for the phenotypic expression of HOS.

To determine the roles of background modifier genes and environment in the phenotypic expression of HOS, we identified a pair of identical twins affected with HOS and compared the clinical features in such genetically identical individuals (monozygotic twins) (Huang et al., unpublished data). Such a comparison provides a tremendous lens onto the role of genetic background and other factors that might contribute to the phenotypic expression of HOS. The twins were first diagnosed based on their hand and heart abnormalities, and their monozygosity was confirmed by genotyping. The same 1-base pair (bp) deletion was detected in both twins. The deletion was predicted to cause a frameshift in the TBX5 coding region, which would be truncated at amino acid residue 263. The defective allele probably encoded an inactive TBX5. Then we analyzed their clinical features. Both twins had similar complex cardiac defects, including secundum ASD, a large membranous VSD, and multiple muscular defects. They also had identical forearm defects: hypoplastic bilateral radial bones, radial club and delayed carpal ossification. However, the hands of the twins were not identical: one twin lacked both thumbs, whereas the other had a remnant of the distal and proximal phalanges of the right thumb and distal phalanx hypoplasia of the left thumb. Thus, although the clinical features of the twins were strikingly similar compared with the wide range of phenotypes observed among individuals bearing the same TBX5 mutations, the discordant features do exist, suggesting that genetic background alone cannot explain discordant features in monozygotic twins. Furthermore, most patients with HOS show asymmetric limb anomalies. Thus far, it seems that all genes identified to be involved in limb development are bilaterally expressed in the developing embryo.

Figure 1. Three-dimensional structure of T-box and the missense mutations found in HOS

Three-dimensional structure of the Xbra T-box bound to a 24 bp DNA target, as a model for the human TBX5 gene. The mutations found in human HOS are I54 (purple), G169 (red), G80 (yellow), and R237 (green). Some missense mutations may interfere with protein-DNA interactions, others may interrupt protein-protein interactions.
 limb. These observations suggest that factors other than the TBX5 mutation itself and genetic background might contribute to this phenotypic variability.

**TBX5 intracellular pathway and prospects**

It is likely that elucidation of the TBX5 intracellular pathway will open a new venue to study the causes of common congenital heart and limb anomalies. TBX5 was found to bind to the T-box binding elements in vitro [33•]. It is possible to identify the candidate genes that might be regulated by TBX5 by searching the human genome database using consensus binding sequences. The genes, particularly those expressed in the developing heart and limbs, would be promising candidates for congenital heart disease and limb malformation. Because TBX5 binding specificity could be determined by a complex of molecules and such cofactors could be heterogeneous, such a nextwork could be complex.

By analyzing Tbx5 knockout mice, Bruneau et al. have shown that several genes are regulated by Tbx5, including atrial natriuretic factor (ANF) and connexin 40 (Cx40) [36••]. Availability of such an animal model allows us to analyze the gene expression pattern using a DNA chip and, therefore, to identify the TBX5 targets that might play important roles in heart development and even be involved in human congenital heart diseases.

TBX5 as a transcription factor was found to interact with NKx2.5 and to synergetically regulate other genes [36••,37•]. However, TBX5 may have other partners, some of them tissue-specific and others spatially regulated. Understanding its protein-protein interactions in a developing heart can also lead to the identification of candidate genes involved in common congenital heart diseases.

The availability of a TBX5 promoter DNA sequence would facilitate isolation of the gene(s) regulating TBX5 expression. We believe these efforts will lead to the identification of the molecular cascades of TBX5 and could provide a unique window onto the causes of more common congenital heart diseases and limb malformations.

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**References and recommended reading**

Papers of particular interest, published within the annual period of review, have been highlighted as: • Of special interest
  • Of outstanding interest


The authors examine TBX5 expression pattern in the developing mouse and chick heart and find that TBX5 expression pattern correlates well with cardiac defects reported in more than 200 patients with HOS in the literature.


24 Okhinen MM, Takai T, Nakano KK, et al.: Duane syndrome and congenital


The disease locus was linked to human chromosome 12q24.1 in two large families, which showed that an intrafamilial pattern of abnormalities does exist. Family A has 19 affected individuals. Clinical evaluation showed that many of them had different cardiac defects. Some individuals had multiple defects, whereas others show vascular hypoplasia without defects in the heart itself. The skeletal anomalies were also variable. In contrast to family A, family B showed severe skeletal abnormalities but milder cardiac malformations. These results strongly suggest that genetic background or modifier genes play an important role in the phenotypic expression of HOS.


29 Basson CT, Bachinsky DR, Lin RC, et al.: Mutations in human TBX5 (corrected) cause limb and cardiac malformation in Holt-Oram syndrome. Nat Genet 1997, 15:30–35. This article demonstrates that mutations in the human Tbx5 gene cause HOS. Tbx5 was cloned from the disease locus on human chromosome 12q24.1. A nonsense mutation in Tbx5 was identified in affected members of one family, and a missense mutation was identified in affected members of another.

30 Li QY, Newbury-Ecob RA, Terrett JA, et al.: Holt-Oram syndrome is caused by mutations in TBX5, a member of the Brachury (T) family gene. Nat Genet 1997, 15:21–29. This article demonstrates that mutations of TBX5 are a cause of HOS. Six mutations were identified, three in HOS families and three in sporadic HOS cases.


33 Ghosh TK, Packham EA, Bonser AJ, et al.: Characterization of the Tbx5 binding site and analysis of mutations that cause Holt-Oram syndrome. Hum Mol Genet 2001, 10:1983–1994. Using an in vitro oligo binding site selection assay, the authors demonstrate that Tbx5 binds to an 8 bp core sequence that is part of the Brachyury consensus-binding site and that Tbx5 also binds to the full palindromic Brachyury binding site and to the half-palindrome. Missense mutations that arise in patients with HOS indicate that G80R and R237Q eliminate binding to the target site.


37 Hiroi Y, Kudoh S, Monzen K, et al.: Tbx5 associates with Nkx2–5 and synergistically promotes cardiomyocyte differentiation. Nat Genet 2001, 28:276–280. Using the yeast two-hybrid system with Nkx2–5 as the "bait," Tbx5 and Nkx2–5 were found to form a complex. The Tbx5/NKX2.5 complex bound to the promoter of the gene for atrial natriuretic factor, and both transcription factors showed synergistic activation. A G80R mutation of Tbx5 did not activate the atrial natriuretic factor or show synergistic activation, whereas R237Q, which causes upper-limb malformations without cardiac abnormalities, activated the Nppa promoter to an extent like that of wild-type Tbx5.