About TVX5 gene - 2021

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The BrS and LQTS present as congenital or acquired disorders with diagnostic ECGs (ST-segment elevation and prolonged QT interval, respectively) and increased risk for SCD.
Our understanding of the 2 disease forms (congenital vs. acquired) differs. A female patient on quinidine for AF who develops VF is diagnosed with "acquired LQTS" and is discharged with no therapy other than instructions to avoid QT-prolonging medications.
In contrast, an asymptomatic male patient who develops a Brugada ECG on provocative teste is diagnosed with "asymptomatic BrS" and could be referred for an electrophysiological evaluation that could result in CDI implantation.
The typical patient undergoing defibrillator implantation for BrS is asymptomatic but has a Brugada ECG provoked by a drug. (**Ofer Havakuk 1, Sami Viskin 2 A Tale of 2 Diseases: The History of Long-QT Syndrome and Brugada Syndrome J Am Coll Cardiol. 2016 Jan 5;67(1):100-8. doi: 10.1016/j.jacc.2015.10.020**.)

T-BOX TRANSCRIPTION FACTOR 5; TBX5 HGNC Approved Gene Symbol: TBX5**.** Cytogenetic location: 12q24.21
Using exon trap analysis of genomic clones from an interval on chromosome 12q2 containing the locus for Holt-Oram syndrome (HOS; 142900) Terret et al. (**Terrett, J. A., Newbury-Ecob, R., Cross, G. S., Fenton, I., Raeburn, J. A., Young, I. D., Brook, J. D. Holt-Oram syndrome is a genetically heterogeneous disease with one locus mapping to human chromosome 12q. Nature Genet. 6: 401-404, 1994.**), a developmental disorder affecting the heart and limbs, **Li et al. (Li, Q. Y., Newbury-Ecob, R. A., Terrett, J. A., Wilson, D. I., Curtis, A. R. J., Yi, C. H., Gebuhr, T., Bullen, P. J., Robson, S. C., Strachan, T., Bonnet, D., Lyonnet, S., Young, I. D., Raeburn, J. A., Buckler, A. J., Law, D. J., Brook, J. D. Holt-Oram syndrome is caused by mutations in TBX5, a member of the Brachyury (T) gene family. Nature Genet. 15: 21-29, 1997)** identified 2 developmentally expressed genes of the Brachyury (T) family.

These genes share a common DNA-binding motif (T-box) and were designated TBX3 (601621) and TBX5, in line with their mouse homologs.
Basson et al (**Basson, C. T., Bachinsky, D. R., Lin, R. C., Levi, T., Elkins, J. A., Soults, J., Grayzel, D., Kroumpouzou, E., Traill, T. A., Leblanc-Straceski, J., Renault, B., Kucherlapati, R., Seidman, J. G., Seidman, C. E. Mutations in human TBX5 cause limb and cardiac malformation in Holt-Oram syndrome. Nature Genet. 15: 30-35, 1997**.) refined the mapping of the HOS locus to 12q24.1 by fluorescence in situ hybridization using a cosmid containing D12S129, which was tightly linked to HOS.
From the critical region they likewise isolated a gene with a high degree of homology to mouse Tbx5 and identified several mutations in TBX5 in affected members of HOS families.
Members of the T-box gene family act as transcription factors and the conserved T-box domain serves as a DNA binding domain.
In addition to heart and forelimb, murine Tbx5 transcripts are expressed in genital papilla, lung, pharynx, and thorax body wall, all tissues that are not affected in HOS patients. Basson et al. (1997) commented that this observation may suggest a more restricted pattern of expression of human TBX5; alternatively, the consequences of TBX5 haploinsufficiency on organ morphogenesis may differ between tissues.

Gene Function
Tbx5 is involved with the development of the four chambers in the heart, the electrical conducting system, and the septum separating the right and left sides of the heart.
(**Boogerd CJ, Evans SM (February 2016). "TBX5 and NuRD Divide the Heart". Developmental Cell. 36 (3): 242–4. doi: 10.1016/j.devcel.2016.01.015. PMC 5542051. PMID 26859347.**)
The Tbx5 gene is a transcription factor that codes for the protein called T-box 5. Along with playing roles in the development of the heart, septum, and electrical system of the heart, it also activates genes that are involved in the development of the upper limbs, the arms and hands.
This gene is involved in patterning major aspects of the heart; however, it is also involved in the muscle connective tissue for muscle and tendon patterning. A study showed that deletion of Tbx5 in forelimbs causes disruption in the muscle and tendon patterning without affecting the skeletons development. (**Hasson, Peleg; DeLaurier, April; Bennett, Michael; Grigorieva, Elena; Naiche, L. A.; Papaioannou, Virginia E.; Mohun, Timothy J.; Logan, Malcolm P. O. (19 January 2010). "Tbx4**

and Tbx5 Acting in Connective Tissue Are Required for Limb Muscle and Tendon Patterning". Developmental Cell. 18 (1): 148–156. doi: 10.1016/j.devcel.2009.11.013..)

Tbx5 expression is in the cells of the lateral plate mesoderm which form the forelimb bud and the cascade of limb initiation.

In its absence no forelimb bud forms. Diseases and defects associated with this gene are Holt-Oram syndrome, both have to do with limb defects and several other abnormalities. The cardiac defects include defects in the septum dividing the left and right sides of the heart, conduction system abnormalities, and other problems. The exact mechanism that Tbx5 activates gene expression is still being discovered and is actively being understood.

Hiroi et al. (**Hiroi, Y., Kudoh, S., Monzen, K., Ikeda, Y., Yazaki, Y., Nagai, R., Komuro, I. Tbx5 associates with Nkx2-5 and synergistically promotes cardiomyocyte differentiation. Nature Genet. 28: 276-280, 2001**) found that TBX5 associates with NKX2-5 (600584) and synergistically promotes cardiomyocyte differentiation.

Both directly bind to the promoter of the gene encoding cardiac-specific natriuretic peptide precursor type A (NPPA; 108780) in tandem, and the 2 transcription factors show synergistic activation. P19CL6 cells efficiently differentiate into beating cardiomyocytes expressing cardiac-specific genes after treatment with 1% dimethyl sulfoxide (DMSO).

Hiroi et al. found that P19CL6 cell lines overexpressing wildtype Tbx5 started to beat earlier and expressed cardiac-specific genes more abundantly than did parental P19CL6 cells, whereas cell lines expressing the G80R mutation (601620.0004), which causes substantial cardiac defects with minor skeletal abnormalities in HOS, did not differentiate into beating cardiomyocytes.

Contrariwise, the R237Q mutation (601620.0003), which causes upper limb malformations without cardiac abnormalities, activated the Nppa promoter to an extent similar to that of wildtype TBX5.

Garg et al. (**Garg, V., Kathiriya, I. S., Barnes, R., Schluterman, M. K., King, I. N., Butler, C. A., Rothrock, C. R., Eapen, R. S., Hirayama-Yamada, K., Joo, K., Matsuoka, R., Cohen, J. C., Srivastava, D. GATA4 mutations cause human congenital heart defects and reveal an interaction with TBX5. Nature 424: 443-447, 2003**.) demonstrated that GATA4 (600576) interacts with TBX5 and showed that a missense mutation in GATA4, G296S (600576.0001), abrogated this interaction. Conversely, interaction of GATA4 and TBX5 was disrupted by specific human TBX5 missense mutations that cause similar cardiac septal defects.

Garg et al. (2003) concluded that their results implicate GATA4 as a genetic cause of human cardiac septal defects, perhaps through its interaction with TBX5. Murakami et al. **(Masao Murakami 1, Junji Tominaga, Ryosuke Makita, Yasunobu Uchijima, Yukiko Kurihara, Osamu Nakagawa, Tomoichiro Asano, Hiroki Kurihara Biochem Biophys Res Commun. 2006 Jan 13;339(2):533-9. doi: 10.1016/j.bbrc. 2005.10.214. Epub 2005 Nov 15.Transcriptional activity of Pax3 is co-activated by TAZ**) found that TAZ (WWTR1; 300394) was a potent TBX5 transactivator. TAZ associated with TBX5 and stimulated TBX5-dependent promoters by interacting with the histone acetyltransferases p300 (EP300; 602700) and PCAF (602303). YAP (606608), a TAZ-related protein, also stimulated TBX5-dependent transcription.
TBX5 with HOS-associated truncation mutations could not be stimulated by TAZ, but TBX5 with HOS-associated point mutations was unimpaired in its ability to respond to TAZ.
By microdissection of the mouse ventricular conduction system, followed by serial analysis of gene expression (SAGE) of the left bundle branch. Moskowitz et al. **(Moskowitz, I. P. G., Kim, J. B., Moore, M. L., Wolf, C. M., Peterson, M. A., Shendure, J., Nobrega, M. A., Yokota, Y., Berul, C., Izumo, S., Seidman, J. G., Seidman, C. E. A molecular pathway including Id2, Tbx5, and Nkx2-5 required for cardiac conduction system development. Cell 129: 1365-1376, 2007)** identified Id2 (600386) as a conduction system-specific transcript.
Analysis of the Id2 promoter showed that conduction system-specific expression of Id2 was dependent on Nkx2.5 and Tbx5. They concluded that a molecular pathway including Id2, Nkx2.5, and Tbx5 coordinates specification of ventricular myocytes into the ventricular conduction system lineage.
Takeuchi et al. defined the minimal requirements for transdifferentiation of mouse mesoderm to cardiac myocytes.
They showed that 2 cardiac transcription factors, Gata4 and Tbx5, and a cardiac-specific subunit of BAF chromatin-remodeling complexes, Baf60c (SMARCD3;601737), can direct ectopic differentiation of mouse mesoderm into beating cardiomyocytes, including the normally noncardiogenic posterior mesoderm and the extraembryonic mesoderm of the amnion.
Gata4 and Baf60c initiated ectopic cardiac gene expression. Addition of Tbx5 allowed differentiation into contracting cardiomyocytes and repression of noncardiac mesodermal genes. Baf60c was essential for the ectopic cardiogenic activity of Gata4 and Tbx5, partly by permitting

binding of Gata4 to cardiac genes, indicating a novel instructive role for BAF complexes in tissue-specific regulation. ((**Takeuchi, J. K., Bruneau, B. G. Directed transdifferentiation of mouse mesoderm to heart tissue by defined factors. Nature 459: 708-711, 2009.**) concluded that the combined function of these factors establishes a robust mechanism for controlling cellular differentiation, and may allow reprogramming of new cardiomyocytes for regenerative purposes.

Gros and Tabin showed that mesenchymal limb progenitors arise through localized epithelial-to-mesenchymal transition (EMT) of the coelomic epithelium specifically within the presumptive limb fields.

This EMT is regulated at least in part by TBX5 and FGF1, 2 genes known to control limb initiation.

These authors showed that limb buds initiate earlier than had been thought, as a result of localized EMT rather than differential proliferation rates**.) Gros, J., Tabin, C. J. Vertebrate limb bud formation is initiated by localized epithelial-to-mesenchymal transition. Science 343: 1253-1256, 2014**.)

Nadadur et al. (,(**Nadadur, R. D., Broman, M. T., Boukens, B., Mazurek, S. R., Yang, X., van den Boogaard, M., Bekeny, J., Gadek, M., Ward, T., Zhang, M., Qiao, Y., Martin, J. F., Seidman, C. E., Seidman, J., Christoffels, V., Efimov, I. R., McNally, E. M., Weber, C. R., Moskowitz, I. P. Pitx2 modulates a Tbx5-dependent gene regulatory network to maintain atrial rhythm. Sci. Transl. Med. 8: 354ra115, 2016.))** identified a cis regulatory element containing a functional T-box-binding site in the promoter of PITX2 (601542) that was bound by TBX5.

The major T allele of a common SNP, rs1906595, disrupted the central nucleotide of the T-box-binding motif, whereas the minor G allele of the SNP completed the canonical T-box-binding element.

The major allele of rs1906595 completely abolished cis regulatory element activity in response to TBX5 in transfected HEK293 cells and in HL-1 mouse atrial cardiomyocytes.

Shox2 (602504) is essential for the formation of the sinoatrial valves and for the development of the pacemaking system of the heart. Puskaric et al. (2010) (**Puskaric, S., Schmitteckert, S., Mori, A. D., Glaser, A., Schneider, K. U., Bruneau, B. G., Blaschke, R. J., Steinbeisser, H., Rappold, G. Shox2 mediates Tbx5 activity by regulating Bmp4 in the pacemaker region of the developing heart. Hum. Molec. Genet. 19: 4625-4633, 2010**) analyzed putative targets of Shox2 and identified Bmp4 (112262) as a direct target.

Shox2 interacted directly with the Bmp4 promoter and activated transcription.
Ectopic expression of Shox2 in Xenopus embryos stimulated transcription of Bmp4, and silencing of Shox2 in cardiomyocytes led to a reduction in the expression of Bmp4.
Using Tbx5 del/+ mice, a model for Holt-Oram syndrome (142900), and Shox2 -/- mice, These authors showed that the T-box transcription factor Tbx5 was a regulator of Shox2 expression in the inflow tract, and that Bmp4 was regulated by Shox2 in this compartment of the embryonic heart.
In addition,
Tbx5 acted cooperatively with Nkx2-5 (600584) to regulate the expression of Shox2 and Bmp4. Puskaric et al. (2010) concluded that their work established a functional link between Tbx5, Shox2, and Bmp4 in the pacemaker region of the developing heart.

Gene Structure

Yi et al. (**Yi, C.-H., Russ, A., Brook, J. D. Virtual cloning and physical mapping of a human T-box gene, TBX4. Genomics 67: 92-95, 2000**.) Yi et al determined that the TBX5 gene contains 9 exons and spans more than 47 kb.
Osterwalder et al. (**Osterwalder, M., Barozzi, I., Tissieres, V., Fukuda-Yuzawa, Y., Mannion, B. J., Afzal, S. Y., Lee, E. A., Zhu, Y., Plajzer-Frick, I., Pickle, C. S., Kato, M., Garvin, T. H., Pham, Q. T., Harrington, A. N., Akiyama, J. A., Afzal, V., Lopez-Rios, J., Dickel, D. E., Visel, A., Pennacchio, L. A. Enhancer redundancy provides phenotypic robustness in mammalian development. Nature 554: 239-243, 2018.)** showed that the pervasive presence of multiple enhancers with similar activities near the same gene confers phenotypic robustness to loss-of-function mutations in individual enhancers.
They used genome editing to create 23 mouse deletion lines and intercrosses, including both single and combinatorial enhancer deletions at 7 distinct loci required for limb development including Gli3 (165240), Shox2, Tbx3 (601621), Tbx5, and Lhx5 (605992). Unexpectedly, none of the 10 deletions of individual enhancers caused noticeable changes in limb morphology.
By contrast, the removal of pairs of limb enhancers near the same gene resulted in discernible phenotypes, indicating that enhancers function redundantly in establishing normal morphology.
In a genetic background sensitized by reduced baseline expression of the target gene, even single enhancer deletions caused limb abnormalities,

suggesting that functional redundancy is conferred by additive effects of enhancers on gene expression levels.
A genomewide analysis integrating epigenomic and transcriptomic data from 29 developmental mouse tissues revealed that mammalian genes are very commonly associated with multiple enhancers that have similar spatiotemporal activity.
Systematic exploration of 3 representative developmental structures (limb, brain, and heart) uncovered more than 1,000 cases in which 5 or more enhancers with redundant activity patterns were found near the same gene. Osterwalder et al. (2018) concluded that their data indicated that enhancer redundancy is a remarkably widespread feature of mammalian genomes that provides an effective regulatory buffer to prevent deleterious phenotypic consequences upon the loss of individual enhancers.

Mapping

Basson et al. (1997) positionally cloned the TBX5 gene from the HOS critical region on 12q24.1. Yi et al. (2000) estimated that the TBX3 gene(601621) and the TBX5 gene are about 350 kb apart on chromosome 12q23-q24.

Molecular Genetics *Genotype/Phenotype Correlations*

To understand better the role of TBX5 in forelimb and heart development, Basson et al. (1999) studied the clinical features of Holt-Oram syndrome caused by 10 different TBX5 mutations.
Defects predicted to create null alleles caused substantial abnormalities in both limb and heart. In contrast, missense mutations produced distinct phenotypes: gly80-to-arg (601620.0004) caused significant cardiac malformations but only minor skeletal abnormalities, whereas arg237-to-gln (601620.0003) and arg237-to-trp (601620.0005) caused extensive upper limb malformations but less significant cardiac abnormalities. Amino acids altered by missense mutations were located on the 3-dimensional structure of a related T-box transcription factor, Xbra (of X. laevis), bound to DNA.
Residue 80 is highly conserved within T-box sequences that interact with the major groove of target DNA; residue 237 is located in the T-box domain that selectively binds to the minor groove of DNA. These structural data, taken together with the predominant cardiac or skeletal phenotype produced by each missense mutation, suggested that organ-specific gene activation by TBX5 is predicated on biophysical interactions with different target DNA sequences.

In a study of 55 probands with Holt-Oram syndrome, Brassington et al. (**Brassington, A.-M. E., Sung, S. S., Toydemir, R. M., Le, T., Roeder, A. D., Rutherford, A. E., Whitby, F. G., Jorde, L. B., Bamshad, M. J. Expressivity of Holt-Oram syndrome is not predicted by TBX5 genotype. Am. J. Hum. Genet. 73: 74-85, 2003.**) found 17 mutations, including 6 missense mutations, in TBX5 and 2 mutations in the SALL4 gene (607343), which is the site of mutations causing some cases of Duane-radial ray syndrome (607323).
Their results suggested that neither the type of mutation in TBX5 nor the location of the mutation in the T box is predictive of the expressivity of malformations in individuals with HOS.
Bamshad (**Bamshad, M. J. Personal Communication. Salt Lake City, Utah 6/26/2003.**) stated that 2 cases in which SALL4 mutations were found in the report of Brassington et al. (2003) had been referred to him and his coworkers and were not personally examined by them.
He said that in 1 case, after a mutation in SALL4 was found, the primary care physician reexamined the patient and noted the presence of ophthalmoplegia, making the diagnosis of Duane-radial ray syndrome. Furthermore, Bamshad (2003) had not considered kidney defects typical of HOS and the affected mother of the patient who was later diagnosed with Duane-radial ray syndrome had pelvic kidneys.
Kohlhase (**Kohlhase, J. Personal Communication. Goettingen, Germany 7/16/2003.**) suggested that the SALL4 mutation in the second 'HOS' case of Brassington et al. (2003) was actually a rare polymorphism, as it did not affect a functional domain, was not conserved between mouse and man, and the unaffected parent also carried the mutation.

Animal Model

In the mouse, 4 of the T-box genes, i.e., the T locus (601397), Tbx1 (602054), Tbx6 (602427), and Tbr1, are dispersed throughout the genome. Li et al. (1997) noted that the other family members, Tbx2 (600747) to Tbx5, exist as 2 clusters, having evolved from a common ancestor by 2 duplication events. Tbx2 and Tbx4 (601719) map together on mouse chromosome 11 (TBX2 is on 17q in the human), and Tbx3 and Tbx5 map on mouse chromosome 5 and human chromosome 12, respectively.
However, it is Tbx2 and Tbx3 that form a cognate pair, likewise Tbx4 and Tbx5, with each pair showing related limb-associated expression.
Pitx1 (602149) and Tbx4 encode transcription factors that are expressed throughout the developing hindlimb, but not in forelimb buds. **Logan and**

Tabin (Logan, M., Tabin, C. J. Role of Pitx1 upstream of Tbx4 in specification of hindlimb identity. Science 283: 1736-1739, 1999.) injected a retroviral vector carrying Pitx1 into the wing field of chicken embryos.
Misexpression of Pitx1 in the chick wing bud induced distal expression of Tbx4, as well as HoxC10 and HoxC11, which are normally restricted to hindlimb expression domains.
Wing buds in which Pitx1 was misexpressed developed into limbs with some morphologic characteristics of hindlimbs: the flexure was altered to that normally observed in legs, the digits were more toe-like in the relative size and shape, and the muscle pattern was transformed to that of a leg. Expression of Tbx5, normally expressed only in the forelimb, was not altered by Pitx1 misexpression.
Koshiba et al. (**Koshiba-Takeuchi, K., Takeuchi, J. K., Matsumoto, K., Momose, T., Uno, K., Hoepker, V., Ogura, K., Takahashi, N., Nakamura, H., Yasuda, K., Ogura, T. Tbx5 and the retinotectum projection. Science 287: 134-137, 2000.**) studied Tbx5 gene expression in developing chick eye.
Expression was first detected in a broad area at stage 11 and then became restricted to the dorsal half of the eye in a graded fashion, with the strongest signal in the dorsal-most end.
Misexpression of the Tbx5 gene in the ventral side of the eye induced dorsalization of the ventral side and altered projections of retinal ganglion cell axons. Thus, in the chick, Tbx5 is involved in eye morphogenesis and is a topographic determinant of the visual projections between retina and tectum.
Sowden et al. (**Sowden, J. C., Holt, J. K. L., Meins, M., Smith, H. K., Bhattacharya, S. S. Expression of Drosophila omb-related T-box genes in the developing human and mouse neural retina. Invest. Ophthal. Vis. Sci. 42: 3095-3102, 2001**) examined the role of Drosophila 'optomotor blind' (omb)-related T-box genes in the development of human and mouse retina.
Murine Tbx2, Tbx3, and Tbx5 and human TBX2 cDNAs were isolated from retina cDNA libraries by hybridization to the Drosophila omb gene. Human and mouse TBX2, TBX3, and TBX5 were expressed asymmetrically across the embryonic neural retina, with highest levels of mRNA within dorsal and peripheral retina.
The dorsoventral gradient of TBX2 expression disappeared before the ganglion cell layer (GCL) formed. Its expression became restricted to the

inner neuroblastic retina and later to the GCL and inner nuclear layer (INL).
The dorsal expression domains of TBX5 and TBX3 were maintained during formation of the GCL. As the retina matured, TBX3 expression was restricted to the INL, and TBX5 was expressed within the GCL. The authors concluded that the expression patterns of TBX2, TBX3, and TBX5 within the developing retina support the idea that the encoded transcription factors play a role in providing positional information important for topographic mapping in differentiation of distinct cell types across the laminar axis of the retina.
Bruneau et al, (**Bruneau, B. G., Nemer, G., Schmitt, J. P., Charron, F., Robitaille, L., Caron, S., Conner, D. A., Gessler, M., Nemer, M., Seidman, C. E., Seidman, J. G. A murine model of Holt-Oram syndrome defines roles of the T-box transcription factor Tbx5 in cardiogenesis and disease. Cell 106: 709-721, 2001**.) generated heterozygous Tbx5 -/+ mice to study the mechanisms by which TBX5 haploinsufficiency causes cardiac and forelimb abnormalities in Holt-Oram syndrome.
Tbx5 deficiency in homozygous mice (Tbx5 -/-) decreased expression of multiple genes and caused severe hypoplasia of posterior domains in the developing heart. Tbx5 haploinsufficiency also markedly decreased atrial natriuretic factor (Anf, or Nppa) and connexin-40 (Cx40;12013) transcription, implicating these as Tbx5 target genes and providing a mechanism by which 50% reduction of T-box transcription factors causes disease.
Direct and cooperative transactivation of the Anf and Cx40 promoters by Tbx5 and the homeodomain transcription factor Nkx2-5 was also demonstrated. These studies provided a potential explanation for Holt-Oram syndrome conduction system defects, suggested mechanisms for intrafamilial phenotypic variability, and accounted for related cardiac malformations caused by other transcription factor mutations.
T protein (6601397) is vital for the formation and differentiation of posterior mesoderm and for axial development in all vertebrates. A mutation in this gene underlies the mouse Brachyury phenotype, a lethal phenotype manifested by abnormal notochord, absent somites, and reduced allantois. Ghosh et al (**Ghosh, T. K., Packham, E. A., Bonser, A. J., Robinson, T. E., Cross, S. J., Brook, J. D. Characterization of the TBX5 binding site and analysis of mutations that cause Holt-Oram syndrome. Hum. Molec. Genet. 10: 1983-1994, 2001. [PubMed: 11555635**) identified[APR1] an 8-bp core sequence that is part of the

Brachyury consensus-binding site. TBX5 bound to the full palindromic Brachyury binding site and to the half-palindrome, whereas Brachyury did not bind to the TBX5 site. Amino acids 1-237 of TBX5 were required for DNA binding.

Analysis of the effects of specific substitution mutations that arise in Holt-Oram patients indicated that gly80 to arg (G80R;601620.0004) and arg237 to gln (R237Q;601620.0003) eliminated binding to the target site. Similar target sites are present in the upstream regions of several cardiac-expressed genes, including cardiac alpha-actin (102540), atrial natriuretic factor (108780), cardiac myosin heavy chain alpha (160710), cardiac myosin heavy chain beta (160760), myosin light chain 1A, myosin light chain 1V, and Nkx2.5 (600584).

Cell transfection studies demonstrated that TBX5 activated the transcription of an atrial natriuretic factor reporter construct and this effect was significantly reduced by deletion of the TBX5 binding site.

The authors proposed that the presence of TBX5 binding sites in the upstream regions of these cardiac-expressed genes suggests a role for TBX5 in their regulation.

Ahn et al. (**Ahn, D., Kourakis, M. J., Rohde, L. A., Silver, L. M., Ho, R. K. T-box gene tbx5 is essential for formation of the pectoral limb bud. Nature 417: 754-758, 2002. [PubMed: 12066188**,) demonstrated that in zebrafish, Tbx5 has an early function that precedes the formation of the limb bud itself.

Functional knockdown of zebrafish Tbx5 through the use of an antisense oligonucleotide resulted in a failure to initiate fin bud formation, leading to the complete loss of pectoral fins.

The function of the Tbx5 gene in the development of zebrafish forelimbs seems to involve the directed migration of individual lateral-plate mesodermal cells into the future limb-bud-producing region.

The primary defect seen in the Tbx5-knockdown phenotype is similar to the primary defects described in known T-box gene mutants such as the spadetail mutant of zebrafish and the Brachyury mutant of the mouse (see 601397), which both similarly exhibited an altered migration of mesodermal cells.

Ahn et al. suggested that a common function for many of the T-box genes might therefore be in mediating the proper migration and/or changes in adhesive properties of early embryonic cells.

By gene targeting and transgenic methods, Minguillon et al (**Minguillon, C., Del Buono, J., Logan, M. P. Tbx5 and Tbx4 are not sufficient to determine limb-specific morphologies but have common roles in**

initiating limb outgrowth. Dev. Cell 8: 75-84, 2005.) examined the ability of Tbx4 and Pitx1 to rescue the no-forelimb phenotype of mutant mice with Tbx5 knockout restricted to limbs.
Tbx4 could replace Tbx5 and rescue limb outgrowth, but Pitx1 could not. In contrast to previous chick misexpression studies, Tbx4-rescued limbs had a forelimb-like phenotype, suggesting that Tbx4 alone does not dictate hindlimb morphology and that forelimb characteristics can develop in the absence of Tbx5.
To determine the role of Pitx1 in defining hindlimb characteristics, Minguillon et al. introduced forelimb-targeted Pitx1 into mice expressing endogenous Tbx5 and into mutant mice rescued by Tbx4. In both cases, forelimb-targeted Pitx1 expression caused a partial forelimb-to-hindlimb transformation, indicating that Pitx1 has a role in directing hindlimb morphology.
Human mutations in TBX5, a gene encoding a T-box transcription factor, and SALL4 (607343), a gene encoding a zinc finger transcription factor, cause similar upper limb and heart defects.
Mutations in SALL4 are responsible for the Duane-radial ray syndrome (607323); mutations in TBX5 are responsible for the Holt-Oram syndrome (142900).
Koshiba et al. (**Koshiba-Takeuchi, K., Takeuchi, J. K., Arruda, E. P., Kathiriya, I. S., Mo, R., Hui, C., Srivastava, D., Bruneau, B. G. Cooperative and antagonistic interactions between Sall4 and Tbx5 pattern the mouse limb and heart. Nature Genet. 38: 175-183, 2006**.) showed that Tbx5 regulates Sall4 expression in the developing mouse forelimb and heart; mice heterozygous for a gene trap allele of Sall4 showed limb and heart defects that modeled human disease.
Tbx5 and Sall4 interacted both positively and negatively to finely regulate patterning and morphogenesis of the anterior forelimb and heart.
Thus, a positive and negative feed-forward circuit between Tbx5 and Sall4 ensures precise patterning of embryonic limb and heart and provides a unifying mechanism for heart/hand syndromes.
Zhu et al. (**Zhu, Y., Gramolini, A. O., Walsh, M. A., Zhou, Y.-Q., Slorach, C., Friedberg, M. K., Takeuchi, J. K., Sun, H., Henkelman, R. M., Backx, P. H., Redington, A. N., MacLennan, D. H., Bruneau, B. G. Tbx5-dependent pathway regulating diastolic function in congenital heart disease. Proc. Nat. Acad. Sci. 105: 5519-5524, 2008**.) generated mice with haploinsufficiency of Tbx5 in ventricular myocytes only and observed diastolic dysfunction due to a cell-autonomous defect in myocyte relaxation but no atrial or ventricular septal defects or conduction defects.

Tbx5-haploinsufficient mice had significantly decreased left ventricular protein levels of SERCA2a (ATP2A2; 108740) and decreased mRNA levels of Tbx5 and Atp2a2.
Similarly, there was a decrease in rate and amplitude of Ca(2+) uptake and Ca(2+) transient prolongation in left ventricular tissue.
The authors demonstrated that Tbx5 activated an Atp2a2 promoter-reporter construct. Doppler analysis of 8 patients with clinically diagnosed Holt-Oram syndrome, 1 of whom was known to carry a mutation in the TBX5 gene, showed diastolic filling abnormalities of variable severity and type. Zhu et al. (2008) concluded that there is a direct genetic pathway regulating cardiac diastolic function, and that patients with structural congenital heart defects may also have underlying anomalies in heart function.
Koshiba et al **(Koshiba-Takeuchi, K., Mori, A. D., Kaynak, B. L., Cebra-Thomas, J., Sukonnik, T., Georges, R. O., Latham, S., Beck, L., Henkelman, R. M., Black, B. L., Olson, E. N., Wade, J., Takeuchi, J. K., Nemer, M., Gilbert, S. F., Bruneau, B. G. Reptilian heart development and the molecular basis of cardiac chamber evolution. Nature 461: 95-98, 2009. Note: Erratum: Nature 461: 550 only, 2009. [PubMed: 19727199**,) examined heart development in the red-eared slider turtle, Trachemys scripta elegans (a chelonian), and the green anole, Anolis carolinensis (a squamate), focusing on gene expression in the developing ventricles.
Both reptiles initially form a ventricular chamber that homogeneously expresses the T-box transcription factor gene Tbx5. In contrast, in birds and mammals, Tbx5 is restricted to the left ventricle precursors. In later stages, Tbx5 expression in the turtle (but not anole) heart is gradually restricted to a distinct left ventricle, forming a left-right gradient.
This suggests that Tbx5 expression was refined during evolution to pattern the ventricles. In support of this hypothesis, These authors showed that loss of Tbx5 in the mouse ventricle results in a single chamber lacking distinct identity, indicating a requirement for Tbx5 in septation. Importantly, misexpression of Tbx5 throughout the developing myocardium to mimic the reptilian expression pattern also results in a single mispatterned ventricular chamber lacking septation.
Thus, they concluded that ventricular septation is established by a steep and correctly positioned Tbx5 gradient, and that their findings provided a molecular mechanism for the evolution of the amniote ventricle and supported the concept that altered expression of developmental regulators is a key mechanism of vertebrate evolution.

Using conditional mouse haploinsufficiency models, Nadadur et alfound that Tbx5 and Ptx2 antagonistically regulated gene expression in atria. Reduced Tbx5 expression profoundly disrupted cardiac channel gene expression and caused action potential abnormalities leading to primary, spontaneous atrial fibrillation.
Concomitant haploinsufficiency for Pitx2 rescued the phenotype. , (**Nadadur, R. D., Broman, M. T., Boukens, B., Mazurek, S. R., Yang, X., van den Boogaard, M., Bekeny, J., Gadek, M., Ward, T., Zhang, M., Qiao, Y., Martin, J. F., Seidman, C. E., Seidman, J., Christoffels, V., Efimov, I. R., McNally, E. M., Weber, C. R., Moskowitz, I. P. Pitx2 modulates a Tbx5-dependent gene regulatory network to maintain atrial rhythm. Sci. Transl. Med. 8: 354ra115, 2016**.)