BRIEF REPORT

Human Mutation

OFFICIAL JOURNAL
HGVS
HUMAN GENOME VARIATION SOCIETY
www.hgvs.org

Genetic Abnormalities in *FOXP1* Are Associated with Congenital Heart Defects

Sheng-Wei Chang,[1] Mona Mislankar,[1] Chaitali Misra,[1] Nianyuan Huang,[1] Daniel G. DaJusta,[4] Steven M. Harrison,[4] Kim L. McBride,[1,2] Linda A. Baker,[4,5] and Vidu Garg[1,2,3]*

[1]*Center for Cardiovascular and Pulmonary Research and The Heart Center, Nationwide Children's Hospital, Columbus, Ohio;* [2]*Department of Pediatrics, The Ohio State University, Columbus, Ohio;* [3]*Department of Molecular Genetics, The Ohio State University, Columbus, Ohio;* [4]*Department of Urology, University of Texas Southwestern Medical Center, Dallas, Texas;* [5]*McDermott Center for Human Growth and Development/Center for Human Genetics, University of Texas Southwestern Medical Center, Dallas, Texas*

Communicated by Reed E. Pyeritz

Received 6 December 2012; accepted revised manuscript 3 June 2013.
Published online 13 June 2013 in Wiley Online Library (www.wiley.com/humanmutation). DOI: 10.1002/humu.22366

ABSTRACT: **The etiology for the majority of congenital heart defects (CHD) is unknown. We identified a patient with unbalanced atrioventricular septal defect (AVSD) and hypoplastic left ventricle who harbored an ~0.3 Mb monoallelic deletion on chromosome 3p14.1. The deletion encompassed the first four exons of *FOXP1*, a gene critical for normal heart development that represses cardiomyocyte proliferation and expression of Nkx2.5. To determine whether *FOXP1* mutations are found in patients with CHD, we sequenced *FOXP1* in 82 patients with AVSD or hypoplastic left heart syndrome. We discovered two patients who harbored a heterozygous c.1702C>T variant in *FOXP1* that predicted a potentially deleterious substitution of a highly conserved proline (p.Pro568Ser). This variant was not found in 287 controls but is present in dbSNP at a 0.2% frequency. The orthologous murine Foxp1 p.Pro596Ser mutant protein displayed deficits in luciferase reporter assays and resulted in increased proliferation and Nkx2.5 expression in cardiomyoblasts. Our data suggest that haploinsufficiency of *FOXP1* is associated with human CHD.**

Hum Mutat 34:1226–1230, 2013.

KEY WORDS: *FOXP1*; cardiomyocyte; hypoplastic left heart syndrome; atrioventricular septal defect

Congenital heart disease (CHD) is the most common type of birth defect with a birth prevalence of six to eight per 1,000 [Hoffman and Kaplan, 2002]. CHD results in significant morbidity and mortality during childhood, and even with the recent advances in medical and surgical management, it remains a leading cause of infant death worldwide. CHD occurs when the normal process of cardiac morphogenesis is disrupted. The etiology of CHD remains largely unknown and is proposed to be multifactorial with genetic and environmental factors playing critical roles [Kathiresan and Srivastava, 2012].

Evidence supporting genetic contributors comes from reports of familial cases for nearly each subset of CHD and epidemiologic studies, which have demonstrated an increased recurrence risk [Ferencz et al., 1989]. The earliest evidence of specific genetic factors is the link between CHD that occurs in the setting of syndromes associated with chromosomal abnormalities such as Trisomy 21 and 22q11 deletion. Genetic etiologies for nonsyndromic CHD have mostly been discovered by studying large kindreds with multiple affected family members using positional cloning approaches [McBride and Garg, 2011]. The identification of CHD-causing genes has been aided by our increasing molecular knowledge of the developmental pathways that govern normal heart development [Garg, 2006]. Even with this increased knowledge, the etiology for the majority of nonsyndromic CHD remains unclear.

Recent studies have demonstrated the utility of microarray-based approaches to uncover subtle chromosome abnormalities in children with multiple birth defects [Richards and Garg, 2010; Richards et al., 2008]. Chromosomal microarray or array comparative genome hybridization is a relatively new technology that has gained clinical utility to investigate genetic etiologies for complex forms of CHD, especially when it is associated with other birth defects [Payne et al., 2012; Richards and Garg, 2010]. Use of this technology has been important for identifying chromosomal abnormalities in children with CHD, and even for discovering new genetic syndromes associated with chromosomal abnormalities. In some instances, the identified chromosomal abnormalities are small, allowing for the identification of the CHD-causing gene.

In this study, we identified a 23-day-old patient with complex CHD consisting of unbalanced atrioventricular septal defect (AVSD; right ventricle dominant), hypoplastic left ventricle and aortic arch, left atrioventricular (AV) valve (mitral valve) stenosis, bilateral superior vena cavae, and transposed great vessels along with bilateral cryptorchidism with germ cell hypoplasia (Supp. Fig. S1A). The child died at 8 months of age due to multiple complications related to a long postoperative course that followed cardiac surgery. A clinical chromosomal microarray was performed (Oligo V8.1, Baylor College of Medicine Medical Genetics Laboratories) and revealed a monoallelic microdeletion on chromosome 3p14, spanning a minimum of 0.261 Mb (chr3: 71,455,422–71,716,260) to a maximum of 0.325 Mb (chr3: 71,443,671–71,768,367). Fluorescence in situ

Additional Supporting Information may be found in the online version of this article.

*Correspondence to: Vidu Garg, Center for Cardiovascular and Pulmonary Research and The Heart Center, Nationwide Children's Hospital, Room #WB4221, Columbus, OH 43205. E-mail: vidu.garg@nationwidechildrens.org

Contract grant sponsors: American Pediatric Society/Society of Pediatric Research Student Research Program; Children's Heart Foundation and the Research Institute at Nationwide Children's Hospital.

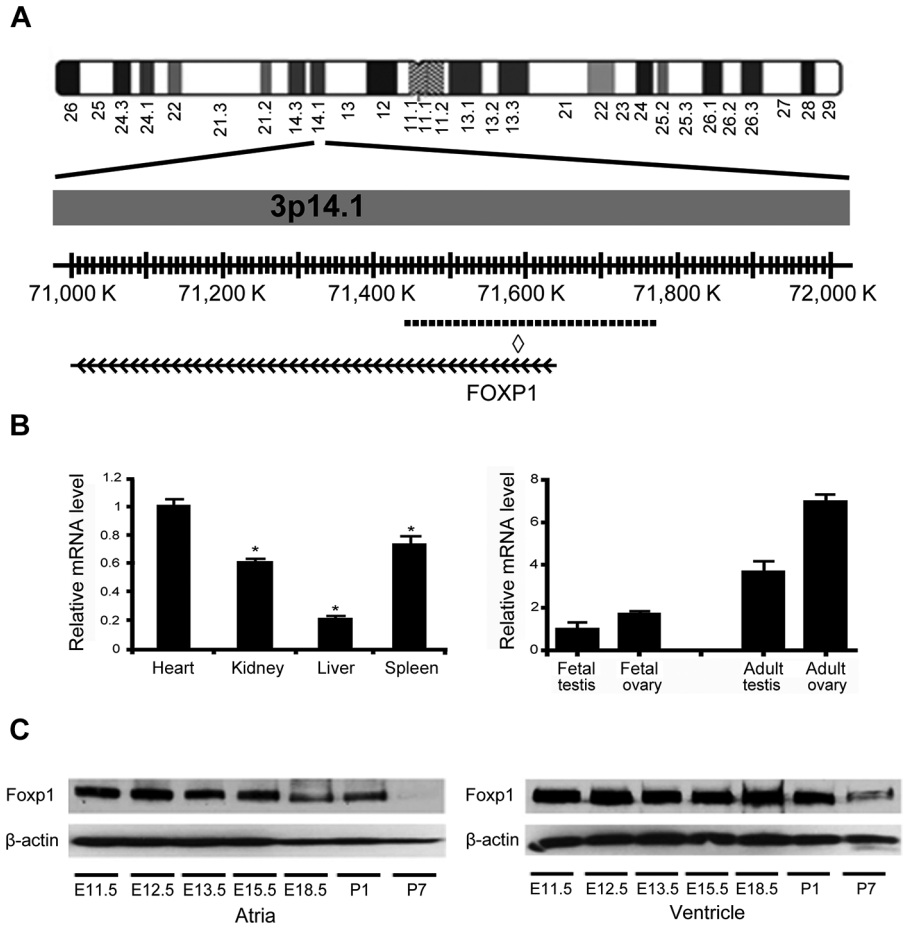
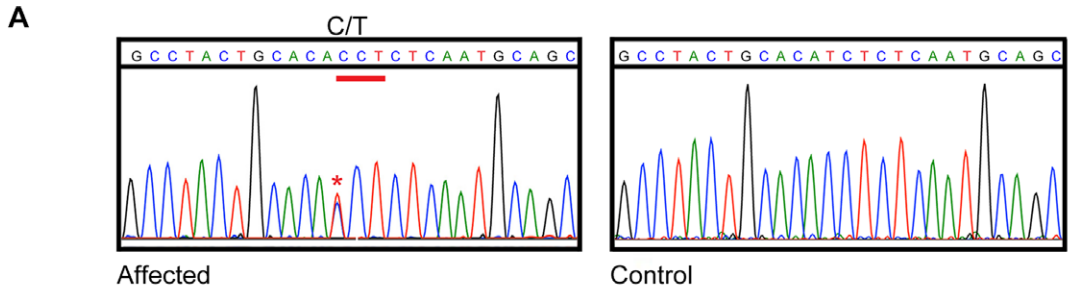


Figure 1. Deletion of *FOXP1* on chromosome 3 and Foxp1 expression in human and mouse. **A:** Schematic of chromosome 3. Lined arrow shows location of FOXP1 while dashed line indicates location of *FOXP1* microdeletion in region of chromosome 3p14.1. ◊, miR-1284 locus. **B:** FOXP1 mRNA is expressed at higher levels in the human fetal heart as compared with other fetal tissues by qRT-PCR. FOXP1 mRNA is expressed at higher levels in the human fetal and adult ovary than the fetal and adult testis by qRT-PCR. Primer sequences are shown in Supp. Table S2. **C:** Expression of Foxp1 protein in murine atria and ventricles at various embryonic and postnatal timepoints. β-actin is shown as loading control. *, $P < 0.05$.

hybridization analysis also confirmed the deletion and revealed the same loss in the unaffected mother (Supp. Fig. S1B). This deletion has only been found in this patient and his mother in the database of Baylor College of Medicine Medical Genetics Laboratories and is considered to be a very rare variant. Based upon the NCBI36/hg18 database, the deletion encompassed only the first four exons of the *FOXP1* gene (MIM #605515) and hsa-miR-1284, a not well-studied miR that is not expressed in the developing heart (Fig. 1A and data not shown).

Foxp1 is a member of the Forkhead box family of transcription factors and has a demonstrated role in murine cardiac development [Wang et al., 2004]. To determine if *FOXP1* is expressed in the developing human heart, we performed quantitative real-time (qRT)-PCR using fetal cDNA. We found that FOXP1 was expressed at higher levels in the fetal human heart than in other human fetal tissues (Fig. 1B). Due to the bilateral cryptorchidism with germ cell hypoplasia, we performed qRT-PCR using fetal and adult testes and ovaries cDNA, finding that FOXP1 is expressed in the developing gonads with higher expression in the mature gonads (Fig. 1B). In the mouse embryo, a similar pattern of a high level of cardiac expression as compared with other organs was found (Supp. Fig. S2). To further define the cardiac expression of Foxp1, we determined the expression of Foxp1 protein in murine atria and ventricles during different embryonic and postnatal timepoints. Western blotting was performed using wild-type mouse hearts from embryonic day (E) 11.5 to postnatal day (P) 7. Foxp1 protein expression levels were higher during embryogenesis in both the atria and ventricles as compared with postnatal day 7 (Fig. 1C).

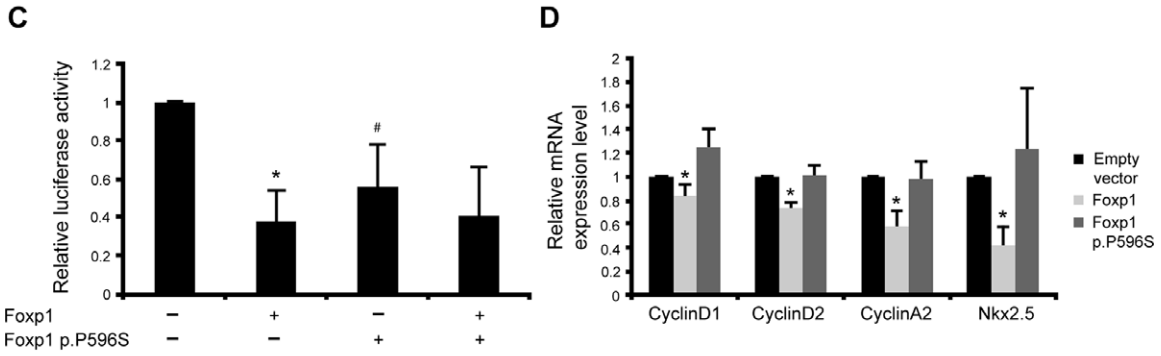
The discovery of a rare chromosomal microdeletion that harbored a cardiac transcription factor implicated in heart development in a patient with complex CHD consisting of an unbalanced AVSD and hypoplastic left ventricle suggested that *FOXP1* may be contributing to the etiology of similar types of CHD. Therefore, we screened patients with AVSD or hypoplastic left heart syndrome (HLHS) for mutations in FOXP1 (primer sequences are available in Supp. Table S1). We sequenced the coding region of *FOXP1* in DNA samples from 82 patients, 10 with AVSD and 72 with HLHS. We discovered two unrelated patients who harbored a heterozygous C to T mutation at nucleotide position 1702, c.1702C>T (with +1 correspond to the A of ATG (start codon) in the GenBank accession number NM_032682.5). This variant predicts the nonsynonymous amino acid change, p.Pro568Ser, and has been submitted to the NCBI Clin Var database (http://www.ncbi.nlm.nih.gov/clinvar/) (Fig. 2A). One African-American patient had HLHS (with mitral valve and aortic valve atresia), whereas the other subject of Hispanic ethnicity had an unbalanced AVSD, pulmonary atresia, and single ventricle in the



B

Nucleotide change	Amino acid change	Cardiac phenotype	Allele frequency (%) in patients	Allele frequency (%) in control	Allele frequency (%) in dbSNP
c.1702C>T	p.Pro568Ser	1) HLHS with MA, AA 2) Unbalanced AVSD with PA and single ventricle (Heterotaxy)	1.2 (2/164)	0 (0/574)	0.2076 (27/13006)

HLHS, hypoplastic left heart syndrome; MA, mitral atresia; AA, aortic atresia; AVSD, atrioventricular septal defect; PA; pulmonary atresia



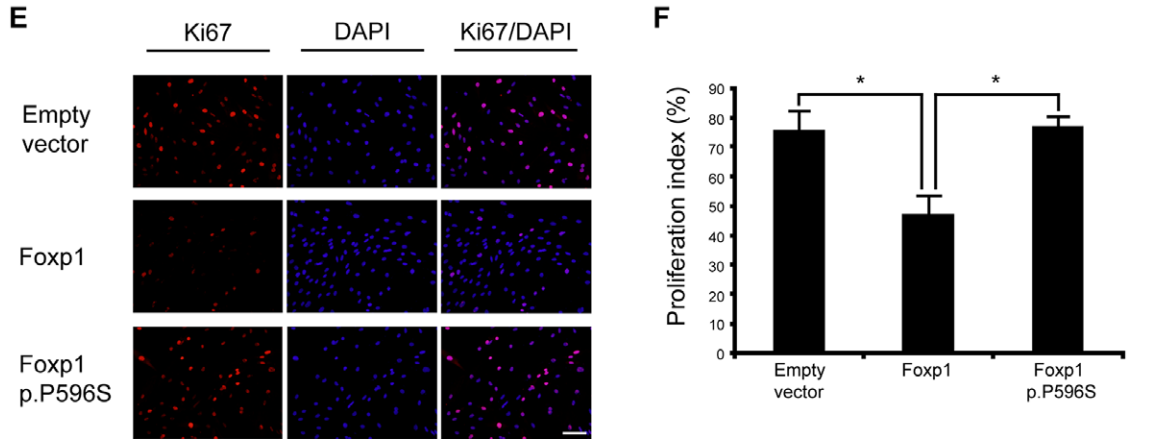


Figure 2. Identification of FOXP1 c.1702C>T variation (p.Pro568Ser) in individuals with CHD and functional analysis of murine Foxp1 P596S protein. **A:** Sequence chromatogram showing heterozygous C to T transition at nucleotide position 1702 in affected subject as compared with control individual. Asterisk (*) indicates the location of c.1702C>T transition as +1 corresponds to the A of the ATG of *FOXP1* (GenBank accession number NM_032682.5). **B:** Summary of nonsynonymous FOXP1 p.Pro568Ser variation identified in children with CHD. **C:** Luciferase reporter assays using the 3-kb mouse Nkx2.5 promoter/enhancer demonstrate that the murine Foxp1 p.P596S mutant protein without wild-type Foxp1 does not repress the Nkx2.5-luciferase reporter as compared with wild-type Foxp1. The addition of Foxp1 p.P596S mutant did not affect the ability of wild-type Foxp1 to repress reporter activation. *, $P < 0.05$ comparison between empty vector and Foxp1; #, $P < 0.05$ comparison between wild-type Foxp1 and Foxp1 p.P596S. Luciferase activity is normalized to β-gal. **D:** Expression of CyclinD1, CyclinD2, CyclinA2, and Nkx2.5 mRNA was increased with transfection of Foxp1 p.P596S plasmid alone as compared with wild-type Foxp1 in H9C2 cardiomyoblasts as measured by qRT-PCR. Primer sequences are shown in Supp Table S2. **E:** Transient transfection of Foxp1 p.P596S mutation without wild-type Foxp1 is unable to repress proliferation of H9C2 cardiomyoblasts as compared with wild-type Foxp1 as measured by immunofluorescent staining for Ki67 and quantified in (**F**). DAPI (4′,6-diamidino-2-phenylindole) is shown in blue. *, $P < 0.05$. Scale bar indicates 100 μm.

setting of heterotaxy syndrome (Fig. 2B). The parental DNAs were not available to determine inheritance. The FOXP1 protein is 677 amino acids in length and contains a homologous DNA-binding forkhead domain and two nuclear localization signals (NLS) [Banham et al., 2001], and the p.Pro568Ser variant was 22 codons from the NLS (Supp. Fig. S3A). The proline residue at codon 568 is highly conserved between species and bioinformatics tools, which predict whether an amino acid substitution affects protein function, suggest deleterious effects with a score of 0.05 and a disease-causing probability value of 0.9985 based upon SIFT (Sorting Intolerant from Tolerant) and MutationTaster, respectively (Supp. Fig. S3B). However, another algorithm, PolyPhen-2, predicted this sequence variant to be benign. This nucleotide change was not found in a diverse control population of 287 individuals (96 Caucasian-American, 95 Hispanic, and 96 African American). This nucleotide variant (rs147674680) has been reported in dbSNP with a frequency of 0.6% in African Americans (27/4406) and an overall frequency of 0.2% (27/13006 alleles). It was present in 1.2% (2/164 alleles) of the affected population (Fig. 2B; Fisher's exact test, $P = 0.05$).

Foxp1 functions as a direct transcriptional repressor of Nkx2.5, a key regulator of cardiomyocyte proliferation during cardiac development [Tu et al., 2009]. As previously reported, Foxp1 is able to repress the activity of a luciferase reporter containing a previously identified –3 kb murine Nkx2.5 promoter [Zhang et al., 2010]. We generated the orthologous mutation in murine *Foxp1*, p.Pro596Ser (Supp. Fig. S3B), and found that the Foxp1 p.P596S mutant protein without wild-type Foxp1 had a decreased ability to repress the Nkx2.5-luciferase reporter when transiently transfected in HEK293 cells. However, it did not show this decreased ability with wild-type Foxp1 cotransfection, demonstrating the lack of dominant negative effect of Foxp1 p.P596S mutation (Fig. 2C). Wild-type Foxp1 and Foxp1 p.P596S mutant express at comparable protein levels in HEK293 cells (Supp. Fig. S3C). This in vitro assay suggested that the p.P596S mutation in Foxp1 had functional deficits.

Murine studies have demonstrated that the loss of *Foxp1* in the developing myocardium results in increased cardiomyocyte proliferation, leading to increased myocardial mass and neonatal lethality [Zhang et al., 2010]. To investigate the effects of Foxp1 p.P596S mutation on cardiomyocyte proliferation, we transfected wild-type Foxp1 and the mutant Foxp1 p.P596S in H9C2 cells, a rat cardiomyoblast cell line, and assessed cell proliferation using the proliferation marker, Ki67. Wild-type and mutant Foxp1 were expressed at comparable levels in H9C2 cells (Supp. Fig. S3C), and as expected, transfection of Foxp1 decreased cell proliferation by ~40% as compared with cells transfected with empty vector. However, the Foxp1 p.P596S mutation without wild-type Foxp1 demonstrated an inability to repress cell proliferation, and levels of proliferation were similar to control (Fig. 2E and 2F). Consistent with this, the mRNA levels of CyclinD1, CyclinD2, and CyclinA2 assessed by qRT-PCR were not repressed in H9C2 cells transfected with Foxp1 p.P596S mutant compared with wild-type Foxp1 (Fig. 2D). Interestingly, we also noted increased expression of Nkx2.5, a key regulator of cardiomyocyte proliferation (Fig. 2D). Similar results were seen with HL-1 cardiomyocytes, a murine atrial cardiomyocyte cell line (data not shown). These in vitro studies suggest that murine Foxp1 p.P596S mutation, which is orthologous to the human FOXP1 p.P568S mutation, potentially has decreased ability to repress cardiomyocyte proliferation via a Nkx2.5-mediated mechanism.

Genetic abnormalities involving genes critical for normal cardiomyocyte proliferation and gonadal differentiation are becoming increasingly recognized as etiologic contributors to human congenital anomalies [Lourenco et al., 2011; Richards and Garg, 2010]. Here, we have identified three affected unrelated individuals with rare genetic abnormalities of *FOXP1*. The individuals have complex CHD spanning a spectrum of unbalanced AVSD with an associated hypoplastic ventricle to HLHS. The rare heterozygous p.Pro568Ser variant in *FOXP1* was found in 2/82 affected individuals and demonstrated decreased repressive ability in transactivation assays. Consistent with this, the orthologous murine Foxp1 mutant protein (p.P596S) demonstrated the inability to repress cardiomyocyte proliferation and Nkx2.5 expression in rodent cardiomyocyte cell lines. These findings suggest that mutations in *FOXP1* are associated with congenital cardiac defects.

The *FOXP1* microdeletion that we identified in our index case was inherited from his mother who did not manifest complex CHD. Similarly, the *FOXP1* p.Pro568Ser mutation has been reported in public databases at very low frequency. Due to the relatively higher allele frequency for the p.Pro568Ser variant in African Americans (0.6%), we cannot rule out the possibility that it represents an uncommon polymorphism in this population. While our observations with the *FOXP1* microdeletion and p.Pro568Ser mutation could be accounted for by incomplete penetrance or potentially be explained by genetic modifiers, it is becoming recognized that rare deleterious variants may play a role in the etiology of birth defects, especially nonsyndromic CHD. This is consistent with the rare variant hypothesis, in which disease susceptibility is due to the additive effect of genetic variants [McBride and Ware, 2012]. For example, some mutations in *GATA4*, a transcription factor crucial for normal cardiac and testicular development, have been linked to CHD in a highly penetrant Mendelian fashion [Garg et al., 2003], whereas other rare heterozygous point mutations in *GATA4* can also show variable penetrance [Lourenco et al., 2011; Schluterman et al., 2007; Tomita-Mitchell et al., 2007]. Accordingly, our literature review has revealed two additional cases of chromosome 3p deletions involving *FOXP1* in patients with cardiac anomalies or cryptorchidism. One patient has speech delay, contractures, hypertonia, and blepharohimosis, whereas the other has cardiac, genital, and cerebral malformations along with delayed neuro- and psycho-motor development [Pariani et al., 2009; Tutulan-Cunita et al., 2012]. These and additional patients with chromosomal deletions encompassing *FOXP1* have a spectrum of anomalies primarily learning deficits [Horn et al., 2010]. The patient with the *FOXP1* deletion that we describe died at 8 months of age precluding the assessment of developmental outcomes. Microcephaly with mild hydrocephalus and mild hippocampal neuronal depopulation was noted at autopsy, but this may have been related to the prolonged hospitalization.

The Forkhead box (Fox) family of transcriptional repressors is defined by a highly conserved forkhead DNA-binding domain [Zhang et al., 2010]. *FOXP1* is a member of the Forkhead box family, and has been identified as an important regulator of cardiac development, lung alveolar morphogenesis, esophageal myogenesis, and neurogenesis. Loss of *Foxp1* in mice results in lethality by embryonic day (E) 14.5 with severe cardiac anomalies consisting of defects in endocardial cushion formation, ventricular septation, and myocardial maturation, whereas abnormalities in gonadal development have not been reported [Wang et al., 2004]. *Foxp1* is expressed in the myocardium as well as endocardium of the developing heart and has been shown to be important for endocardial cushion development and cardiomyocyte proliferation [Zhang et al., 2010]. In the myocardium, Foxp1 has been shown to negatively regulate cardiomyocyte proliferation by repressing Nkx2.5 expression; however, in the endocardium, Foxp1 promotes cardiomyocyte proliferation through regulation of Fgf signaling [Zhang et al., 2010]. These studies highlight the role of Foxp1 in cardiomyocyte proliferation but the link between cardiomyocyte proliferation and human CHD is not well understood.

Among the many complex forms of CHD, both AVSDs and HLHS are common disorders. AVSDs, which comprise 5%–7% of all CHD, arise from abnormal endocardial cushion development [Hoffman and Kaplan, 2002]. While the majority of AVSD are termed "balanced," with a common AV valve associated with equal-sized ventricles, in a small percentage of AVSD patients, the "unbalanced" location of the common AV valve will lead to the unequal size of the right and left ventricles. HLHS is the most common form of CHD associated with a hypoplastic left ventricle and is defined as when all of the structures on the left side of the heart are severely underdeveloped [Hickey et al., 2012]. The etiology of HLHS is likely related to multiple factors including defects in endocardial and myocardial development [Hickey et al., 2012]. As Foxp1 is expressed in the developing endocardium and myocardium and has been shown to be critical for normal development of the endocardial cushions and myocardium in mouse models, it is plausible that genetic abnormalities in *FOXP1* could contribute to the development of AVSD and HLHS. Further studies are required to determine if *FOXP1* mutations are found in children with other types of CHD.

We found that the murine *Foxp1* p.Pro596Ser mutation demonstrated abnormal activation of the cardiac transcription factor, Nkx2.5. Mutations in *NKX2.5* have been identified as a cause of human CHD, predominantly atrial septal defects and tetralogy of Fallot [McElhinney et al., 2003; Schott et al., 1998]. The majority of *NKX2.5* mutations have been demonstrated to result in loss-of-function primarily due to reduced DNA-binding affinity [Kasahara et al., 2000]. Interestingly, a rare sequence variant in *NKX2.5* that results in a missense mutation (R25C) has been linked to HLHS in multiple studies [Elliott et al., 2003; McElhinney et al., 2003; Stallmeyer et al., 2010]. This R25C mutant protein was shown to increase activation ability unlike other *NKX2.5* mutations [Kasahara et al., 2000]. It is interesting to speculate that abnormal activation of NKX2.5 and its targets may contribute to HLHS.

In conclusion, our study has demonstrated that genetic abnormalities that result in loss of *FOXP1* function are associated with complex forms of CHD in humans. Additional investigation is required to determine the mechanisms by which *FOXP1* mutations contribute to CHD, but we speculate that it may involve abnormal regulation of cardiomyocyte proliferation.

Acknowledgment

The authors wish to thank the participating subjects and families.

References

Banham AH, Beasley N, Campo E, Fernandez PL, Fidler C, Gatter K, Jones M, Mason DY, Prime JE, Trougouboff P, Wood K, Cordell JL. 2001. The FOXP1 winged helix transcription factor is a novel candidate tumor suppressor gene on chromosome 3p. Cancer Res 61:8820–8829.

Claycomb WC, Lanson NA Jr., Stallworth BS, Egeland DB, Delcarpio JB, Bahinski A, Izzo NJ Jr. 1998. HL-1 cells: a cardiac muscle cell line that contracts and retains phenotypic characteristics of the adult cardiomyocyte. Proc Natl Acad Sci USA 95:2979–84.

Elliott DA, Kirk EP, Yeoh T, Chandar S, McKenzie F, Taylor P, Grossfeld P, Fatkin D, Jones O, Hayes P. 2003. Cardiac homeobox gene NKX2–5 mutations and congenital heart disease: associations with atrial septal defect and hypoplastic left heart syndrome. J Am Coll Cardiol 41:2072–2076.

Ferencz C, Boughman JA, Neill CA, Brenner JI, Perry LW. 1989. Congenital cardiovascular malformations: questions on inheritance. Baltimore-Washington Infant Study Group. J Am Coll Cardiol 14:756–763.

Filipeanu CM, Zhou F, Lam ML, Kerut KE, Claycomb WC, Wu G. 2006. Enhancement of the recycling and activation of beta-adrenergic receptor by Rab4 GTPase in cardiac myocytes. J Biol Chem 281:11097–103.

Garg V. 2006. Insights into the genetic basis of congenital heart disease. Cell Mol Life Sci 63:1141–1148.

Garg V, Kathiriya IS, Barnes R, Schluterman MK, King IN, Butler CA, Rothrock CR, Eapen RS, Hirayama-Yamada K, Joo K, Matsuika R, Cohen JC, et al. 2003. GATA4 mutations cause human congenital heart defects and reveal an interaction with TBX5. Nature 424:443–447.

Hickey EJ, Caldarone CA, McCrindle BW. 2012. Left ventricular hypoplasia: a spectrum of disease involving the left ventricular outflow tract, aortic valve, and aorta. J Am Coll Cardiol 59:S43–S54.

Hoffman JI, Kaplan S. 2002. The incidence of congenital heart disease. J Am Coll Cardiol 39:1890–1900.

Horn D, Kapeller J, Rivera-Brugues N, Moog U, Lorenz-Depiereux B, Eck S, Hempel M, Wagenstaller J, Gawthrope A, Monaco AP, Bonin M, Riess O, et al. 2010. Identification of FOXP1 deletions in three unrelated patients with mental retardation and significant speech and language deficits. Hum Mutat 31:E1851–1860.

Kasahara H, Lee B, Schott JJ, Benson DW, Seidman JG, Seidman CE, Izumo S. 2000. Loss of function and inhibitory effects of human CSX/NKX2.5 homeoprotein mutations associated with congenital heart disease. J Clin Invest 106:299–308.

Kathiresan S, Srivastava D. 2012. Genetics of human cardiovascular disease. Cell 148:1242–1257.

Lourenco D, Brauner R, Rybczynska M, Nihoul-Fekete C, McElreavey K, Bashamboo A. 2011. Loss-of-function mutation in GATA4 causes anomalies of human testicular development. Proc Natl Acad Sci U S A 108:1597–1602.

McBride KL, Garg V. 2011. Heredity of bicuspid aortic valve: is family screening indicated? Heart 97:1193–1195.

McBride KL, Ware SM. 2012. Modifying Mendel: approaches for identification of susceptibility alleles for human cardiovascular malformations. Circ Cardiovasc Genet 5:274–276.

McElhinney DB, Geiger E, Blinder J, Benson DW, Goldmuntz E. 2003. NKX2.5 mutations in patients with congenital heart disease. J Am Coll Cardiol 42:1650–1655.

Pariani MJ, Spencer A, Graham JM Jr., Rimoin DL. 2009. A 785kb deletion of 3p14.1p13, including the FOXP1 gene, associated with speech delay, contractures, hypertonia and blepharophimosis. Eur J Med Genet 52:123–127.

Payne AR, Chang SW, Koenig SN, Zinn AR, Garg V. 2012. Submicroscopic chromosomal copy number variations identified in children with hypoplastic left heart syndrome. Pediatr Cardiol 33:757–763.

Richards AA, Garg V. 2010. Genetics of congenital heart disease. Curr Cardiol Rev 6:91–97.

Richards AA, Santos LJ, Nichols HA, Crider BP, Elder FF, Hauser NS, Zinn AR, Garg V. 2008. Cryptic chromosomal abnormalities identified in children with congenital heart disease. Pediatr Res 64:358–363.

Schluterman MK, Krysiak AE, Kathiriya IS, Abate N, Chandalia M, Srivastava D, Garg V. 2007. Screening and biochemical analysis of GATA4 sequence variations identified in patients with congenital heart disease. Am J Med Genet A 143A:817–823.

Schott JJ, Benson DW, Basson CT, Pease W, Silberbach GM, Moak JP, Maron BJ, Seidman CE, Seidman JG. 1998. Congenital heart disease caused by mutations in the transcription factor NKX2–5. Science 281:108–111.

Stallmeyer B, Fenge H, Nowak-Gottl U, Schulze-Bahr E. 2010. Mutational spectrum in the cardiac transcription factor gene NKX2.5 (CSX) associated with congenital heart disease. Clin Genet 78:533–540.

Tomita-Mitchell A, Maslen CL, Morris CD, Garg V, Goldmuntz E. 2007. GATA4 sequence variants in patients with congenital heart disease. J Med Genet 44:779–783.

Tu CT, Yang TC, Tsai HJ. 2009. Nkx2.7 and Nkx2.5 function redundantly and are required for cardiac morphogenesis of zebrafish embryos. PLoS One 4:e4249.

Tutulan-Cunita AC, Papuc SM, Arghir A, Rotzer KM, Deshpande C, Lungeanu A, Budisteanu M. 2012. 3p interstitial deletion: novel case report and review. J Child Neurol 27:1062–1066.

Wang B, Weidenfeld J, Lu MM, Maika S, Kuziel WA, Morrisey EE, Tucker PW. 2004. Foxp1 regulates cardiac outflow tract, endocardial cushion morphogenesis and myocyte proliferation and maturation. Development 131:4477–4487.

Zhang Y, Li S, Yuan L, Tian Y, Weidenfeld J, Yang J, Liu F, Chokas AL, Morrisey EE. 2010. Foxp1 coordinates cardiomyocyte proliferation through both cell-autonomous and nonautonomous mechanisms. Genes Dev 24:1746–1757.