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Specific Association of Missense Mutations in *CRELD1* with Cardiac Atrioventricular Septal Defects in Heterotaxy Syndrome

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Keywords

congenital heart defects; atrioventricular septal defect; genetic etiology; heterotaxy syndrome; complex genetic disease; laterality defects

Heterotaxy refers to an abnormal developmental condition characterized by randomized arrangement of the thoracic and/or abdominal visceral organs, including the heart, lungs, liver, stomach, and spleen. The organs are either located randomly with respect to the left-right (L-R) axis or to one another. This genetic syndrome is characterized by multiple congenital malformations and complex cardiovascular malformations, which are often reported to be the major cause of morbidity and mortality in this population. Heterotaxy syndrome most often occurs as a sporadic condition, although familial cases with autosomal dominant, autosomal recessive and X-linked inheritance are known [Belmont et al., 2004]. Family studies have been successful in identifying several causative or contributing genes, but it is clear that other modifiers play a role in the phenotypic outcome as there is both incomplete penetrance and variable expression in families with known causative mutations.

Heterotaxy syndrome is associated with approximately 3% of all congenital heart defects (CHD), which have an overall prevalence of about 1:10,000 live births [Lin and Pierpont, 2000]. In fact, heterotaxy syndrome is most often diagnosed in the newborn when there is cyanotic congenital heart disease [Sutherland and Ware, 2009]. Atrioventricular septal defects (AVSD) are a common form of heart defect associated with heterotaxy, but are frequently part of more complex congenital heart malformations in those individuals. The incidence of CHD in heterotaxy is high, with estimates ranging from 50-100% of affected individuals having some type of heart defect [Applegate et al., 1999; Lin et al., 2000].

CRELD1 was the first single gene to be associated with AVSD when it was identified as the causative gene for the *AVSD2* locus [Robinson et al., 2003]. Missense mutations in *CRELD1* occur in approximately 5-10% of individuals with AVSD, including non-syndromic AVSD [Posch et al., 2008; Robinson et al., 2003; Zatyka et al., 2005] and AVSD in individuals Down syndrome [Guo et al., 2010; Kusuma et al., 2011; Maslen et al., 2006]. The highly specific genetic association between *CRELD1*-missense mutations and AVSD suggests that mutations in *CRELD1* significantly increase the risk of developing an AVSD.

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CRELD1 encodes a cell surface protein that is similar to NOTCH proteins in structure with a single pass transmembrane domain, tandemly repeated EGF domains (including two calcium binding EGF domains). However, there is a unique CRELD domain, previously referred to as a WE domain [Rupp et al., 2002], in place of the DSL binding domains of the NOTCH proteins. This highly conserved domain is likely a site of ligand binding.

We had previously identified a potentially pathogenic *CRELD1* mutation in an individual with heterotaxy-related AVSD [Robinson et al., 2003]. That missense variant, c.320G>A, predicts an amino acid substitution p.Arg107His in the CRELD domain, and has a high probability of being deleterious to the protein (PolyPhen score 0.999). In this current study we sought to determine if *CRELD1* mutations are associated with other manifestations of heterotaxy or coincide only with AVSD. Study subjects were identified and DNA samples obtained under a research protocol approved by the Baylor College of Medicine Ethics Committee. DNA was provided for analysis as de-identified samples. DNA analyses were performed under a research protocol approved by the Oregon Health & Science University Ethics Committee. The *CRELD1* gene, including introns, intron-exon boundaries, and regulatory regions, was resequenced by standard Sanger technology through the Oregon Clinical Translational Research Institute core laboratory for 126 individuals with heterotaxy with and without heart defects. The positions of variants in the cDNA sequence were based on NCBI reference sequence NM_001077415.2. The phenotypic details for the study cohort are shown in Table I. Consistent with previous estimates, 70% of this cohort had CHD including a high percentage of complex heart malformations. In addition to the defects listed in Table I, other cardiovascular malformations included right sided cardiac apex, mesocardia, hypoplastic left heart, left atrial isomerism, double aortic arch, patent ductus arteriosus, double outlet right ventricle, hypoplastic left ventricle, and interrupted inferior vena cava.

Resequencing of *CRELD1* identified heterozygous missense variants in three individuals. No other potentially damaging variants were identified. The three rare variants were absent iñ100 race/ethnic group matched controls, and were not present in the 1,000 Genomes Database. Only one of the missense changes was found in patients with an AVSD. We used the web-based algorithms PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2) and MutPred (http://mutpred.mutdb.org/) to predict the possible impact of the amino acid substitutions on the structure and function of CRELD1. Both prediction programs give probability scores for likelihood that a missense variant will be damaging to the protein. In addition, MutPred provides hypotheses regarding the nature of any effect on protein structure or function. All coding variants found in this study are listed in Table II, including the PolyPhen-2 and MutPred scores. All variants were heterozygous changes.

Patient 1 was diagnosed with heterotaxy, including dextro-Transposition of the Great Arteries (d-TGA) an atrial septal defect (ASD) and a ventricular septal defect (VSD). Analysis of the subject's DNA showed a heterozygous missense change, c.616G>A, which predicts a substitution of threonine for alanine at amino acid position (p.Ala206Thr). The affected subject is of Hispanic descent. The base substitution was not detected in 93 chromosomes from a Hispanic control population indicating that this is a rare variant in that population. Both PolyPhen and MutPred predict that this rare variant is likely to be benign.

The second alteration was found in a South Asian Indian patient with TGA, coarctation of aorta (CoA), and double inlet left ventricle (DILV). The single base substitution, c.793A>G, was identified at exon 7 of DNA patient. This predicts p.Thr265Ala, changing the position from a polar to a non-polar amino acid. The base substitution was not detected in 102 race-relevant control chromosomes [personal communication, Dr. Subrata K. Dey, West Bengal University of Technology, India], suggesting the variation is unlikely to be a polymorphism.

Both MutPred and Polyphen-2 indicate that this rare variant could possibly have a damaging effect on CRELD1 protein structure, although the probability scores are relatively low compared to most AVSD-associated *CRELD1* mutations. In addition, MutPred did not predict any gain or loss of structural or functional properties of the protein product, which further reduces confidence that this is likely to be a pathogenic alteration.

The third alteration was found in a Hispanic patient with a situs inversus, AVSD, and asplenia, a congenital absence of the spleen. The patient had a family history of two female siblings who died *in utero*. The first sibling was diagnosed with pulmonary artery hypoplasia, dysplastic tricuspid valve, hypoplastic thymus, asplenia; the second sibling died from cystic hygroma, and liver calcification. No samples were available from the siblings for further study. The primary cause of situs inversus in this individual is not known. The mutation found in this patient, c.985C>T, predicts p.Arg329Cys. This highly conserved amino acid residue is located in the second calcium-binding EGF (cb-EGF) domain. This is a recurrent mutation that has been identified in multiple unrelated individuals with non-syndromic AVSD [Robinson et al., 2003], and AVSD with Down syndrome [Guo et al., 2010; Kusuma et al., 2011; Maslen et al., 2006]. It has not been detected in nearly 400 normal controls across these multiple studies, including 100 Hispanic controls. The non-conservative amino acid substitution resulting from this mutation is predicted by both MutPred and PolyPhen-2 to have a high probability of being deleterious, and importantly has been shown to change protein conformation when expressed in cultured cells [Robinson et al., 2003].

This study indicates that deleterious *CRELD1* missense mutations are specifically associated with AVSD and are not correlated with other aspects of the heterotaxy phenotype. Our original study of AVSD in heterotaxy patients was biased as we only studied individuals with an AVSD. There we identified one mutation out of 11 individuals with heterotaxy and a partial AVSD (ostium primum ASD). The finding of one *CRELD1* mutation in only three individuals with AVSD in the study brings this to a total of two mutations in 14 individuals, suggesting that approximately 14% of individuals with heterotaxy-associated AVSD have a *CRELD1* missense mutation. It further supports the premise that deleterious mutations in *CRELD1* contribute to the pathogenesis of AVSD regardless of genetic background, including trisomy 21 and causative mutations for heterotaxy syndrome.

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REFERENCES

Applegate KE, Goske MJ, Pierce G, Murphy D. Situs revisited: Imaging of the heterotaxy syndrome. Radiographics. 1999; 19:837–852. discussion 853-834. [PubMed: 10464794]

Belmont JW, Mohapatra B, Towbin JA, Ware SM. Molecular genetics of heterotaxy syndromes. Curr Opin Cardiol. 2004; 19:216–220. [PubMed: 15096953]

Guo Y, Shen J, Yuan L, Li F, Wang J, Sun K. Novel *CRELD1* gene mutations in patients with atrioventricular septal defect. World J Pediatr. 2010; 6:348–352. [PubMed: 21080147]

Kusuma L, Dinesh SM, Savitha MR, Krishnamurthy B, Narayanappa D, Ramachandra NB. A maiden report on *CRELD1* single-nucleotide polymorphism association in congenital heart disease patients of Mysore, South India. Genet Test Mol Biomarkers. 2011; 15:483–487. [PubMed: 21413875]

Lin AE, Pierpont ME. Heart development and the genetic aspects of cardiovascular malformations. Am J Med Genet. 2000; 97:235–237. [PubMed: 11376434]

Lin AE, Ticho BS, Houde K, Westgate MN, Holmes LB. Heterotaxy: Associated conditions and hospital-based prevalence in newborns. Genet Med. 2000; 2:157–172. [PubMed: 11256661]

Maslen CL, Babcock D, Robinson SW, Bean LJ, Dooley KJ, Willour VL, Sherman SL. *CRELD1* mutations contribute to the occurrence of cardiac atrioventricular septal defects in Down syndrome. Am J Med Genet A. 2006; 140:2501–2505. [PubMed: 17036335]

Posch MG, Perrot A, Schmitt K, Mittelhaus S, Esenwein EM, Stiller B, Geier C, Dietz R, Gessner R, Ozcelik C, Berger F. Mutations in *GATA4*, *NKX2.5*, *CRELD1*, and *BMP4* are infrequently found in patients with congenital cardiac septal defects. Am J Med Genet A. 2008; 146A:251–253. [PubMed: 18076106]

Robinson SW, Morris CD, Goldmuntz E, Reller MD, Jones MA, Steiner RD, Maslen CL. Missense mutations in *CRELD1* are associated with cardiac atrioventricular septal defects. Am J Hum Genet. 2003; 72:1047–1052. [PubMed: 12632326]

Rupp PA, Fouad GT, Egelston CA, Reifsteck CA, Olson SB, Knosp WM, Glanville RW, Thornburg KL, Robinson SW, Maslen CL. Identification, genomic organization and mRNA expression of *CRELD1*, the founding member of a unique family of matricellular proteins. Gene. 2002; 293:47–57. [PubMed: 12137942]

Sutherland MJ, Ware SM. Disorders of left-right asymmetry: Heterotaxy and situs inversus. Am J Med Genet C Semin Med Genet. 2009; 151C:307–317. [PubMed: 19876930]

Zatyka M, Priestley M, Ladusans EJ, Fryer AE, Mason J, Latif F, Maher ER. Analysis of *CRELD1* as a candidate 3p25 atrioventicular septal defect locus (*AVSD2*). Clin Genet. 2005; 67:526–528. [PubMed: 15857420]

Table I

Cardiovascular defects in the study cohort

Abnormality	Number of patients
ASD,VSD,AVSD with or without other heart defects	8 (3 with AVSD)
D/L-loop TGA	40
D/L-loop TGA with CoA	8
D/L-loop TGA and other heart defects	9
Situs inversus with or without dextrocardia	4
Dextrocardia with other heart defects	7
Heterotaxy only	38
MGA and other heart defects	4
Other types of heart defects	8

ASD, atrial septal defects; VSD, ventral septal defects; AVSD, atrioventricular septal defect; TGA, transposition of the great arteries; CoA, coarctation of the aorta; MGA, malposition of the great arteries.

Table II

Coding variants identified in the study cohort

cDNA Position (db SNP rs#)	Amino Acid Change	Exon and Protein Domains	Functional Predictions	Frequency
c.616G>A (NA)	p.Ala206Thr	Exon 5 (EGF domain)	Benign (PP=0.001; MP=0.374)	1/126
c.793A>G (NA)	p.Thr265Ala	Exon 7 (cbEGF domain)	Possibly damaging (PP=0.466; MP=0.561)	1/126
c.910C>T (rs79223485)	p.Leu304Leu	Exon 8 (cb-EGF domain)	Benign	1/126
c.945G>A (rs76764016)	p.Pro315Pro	Exon 9 (cb-EGF domain)	Benign	3/126
c.985C>T (rs145036576)	p.Arg329Cys	Exon 9 (cb-EGF domain)	**Damaging** (PP=1.00; MP=0.689)	1/126
c.1104G>A (NA)	p.Gln368Gln	Exon 10 (Transmembrane domain)	Benign	1/126

cDNA positions are based on NCBI reference sequence NM_001077415.2.

The National Center for Biotechnology Information Single Nucleotide Polymorphism (dbSNP) database ‘rs’ number designations are noted when available (http://www.ncbi.nlm.nih.gov/projects/SNP/); NA, not available PP = PolyPhen-2 probability score; MP=MutPred general probability score; PP and MP scores >0.50 are considered to be potentially damaging; cbEGF domain = calcium binding EGF domain;

Frequency is number of individuals with the variant over the total number of individuals resequenced in this study.