Published in final edited form as:

Congenit Heart Dis. 2014 ; 9(1): 83–86. doi:10.1111/chd.12097.

The prevalence of 16p12.1 microdeletion in patients with left-sided cardiac lesions

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Abstract

Setting—Left-sided cardiac lesions have a birth prevalence of approximately 1 in 1000 and have been shown to be heritable in pedigree studies. A large microdeletion at 16p12.1 is associated with childhood developmental delay, and initial studies describing this deletion identified left-sided lesions as an enriched phenotype compared with a control population.

Objective—To determine whether patients with left-sided cardiac lesions have an increased frequency of 16p12.1 microdeletions as compared to control populations.

Design—A cohort of 262 probands with left-sided lesions, including 53 with isolated aortic stenosis/bicuspid aortic valve, 83 with coarctation of the aorta with or without aortic stenosis/bicuspid aortic valve, and 126 with hypoplastic left heart syndrome were assessed for copy number variation at 16p12.1. The control cohort included 595 patients with conotruncal defects as a cardiac control and 971 healthy children.

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Conflict of Interest: None

Author Contributions

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Results—We detected one patient in the left-sided lesion cohort with a large duplication partially overlapping the reported 16p12.1 microdeletion, along with one patient each in the conotruncal and control cohorts with a deletion in the same region. None of these patients had dysmorphic features, extracardiac malformations or developmental delay.

Conclusion—In our cohort, structural variation at 16p12.1 was not identified with increased frequency in patients with left-sided lesions as compared to controls.

Keywords

congenital heart disease; left-sided lesions; hypoplastic left heart syndrome; 16p12.1; copy number variation

Introduction

Collectively, left-sided congenital cardiac lesions (LSL), including hypoplastic left heart syndrome (HLHS), aortic valve stenosis (AS) and coarctation of the aorta (CoA), have a birth prevalence of approximately 1 in 1000.(1) Numerous pedigree studies have demonstrated that LSL are heritable, but the inheritance pattern is most consistent with a complex trait and the underlying etiology of most LSL remains elusive.(2–6)

Recent studies by Girirajan *et al.* and Antonacci *et al.* identified a large and consistent region of hemizygous deletion of 521 kb within 16p12.1 that was associated with childhood developmental delay (OR = 7.2).(7,8) Of the 42 patients in the combined discovery and replication cohorts with the microdeletion, 21 patients had available clinical data, and 7 of 21 had congenital heart disease (CHD), including 5 with left-sided lesions. Two patients had hypoplastic left heart syndrome (HLHS), two had HLHS variants (Shone complex with total anomalous pulmonary venous return, and double outlet right ventricle with mitral atresia), and one had a bicuspid aortic valve (BAV) and ventricular septal defect. The number of patients with LSL in the Girirajan cohort is enriched compared with the general population. (7) Interestingly, McBride *et al.* in 2009 also identified 16p12 as a locus with suggestive linkage in a combined cohort of patients with AS, CoA and HLHS.(6)

We therefore hypothesized that a cohort of patients with LSL might have an increased frequency of 16p12.1 microdeletion as compared to controls. To explore this hypothesis, we surveyed a large cohort of subjects with LSL for 16p12.1 structural variation using SNP arrays. LSL subjects were compared to control cohorts with conotruncal cardiac defects and healthy pediatric individuals.

Methods

The study protocol was approved by The Children's Hospital of Philadelphia Institutional Review Board on Human Subjects and patients were recruited after informed consent. A cohort of 262 probands of European ancestry with LSL (isolated AS/BAV, CoA ± AS/BAV or HLHS) was uniformly ascertained in a single center. Patients with a genetic diagnosis or association (e.g., VATER) upon clinical genetic examination were excluded. All patients were genotyped on an Illumina HH550v3 microarray (previously described) and annotated

for copy number variation using structural variation detection software CNV Workshop and PennCNV.(9,10,11) We note that the Illumina HH550v3 microarray was also used to successfully detect the 16p12.1 microdeletion in the original study.(7) Copy number calls required concordance for CNV type on both algorithms with 60% size overlap, with thresholds of >10 SNPs for deletions and >20 SNPs for duplications. Our control cohorts included 595 ethnicity-matched genotypes from probands with conotruncal cardiac defects as a CHD control and 971 ethnicity-matched healthy children recruited from randomized well-child visits throughout The Children's Hospital of Philadelphia Healthcare Network. (11) Controls were genotyped on matched arrays and analyzed using the same CNV algorithms and parameters. CNVs identified in the region of interest were validated by quantitative PCR using a TaqMan® Copy Number Assay (Hs01843299_cn, Chr16:21968850 Build 37 (21964609–219946668), Applied Biosystems, California, USA) following standard protocols.

Results

In total, 262 Caucasian genotyped probands with LSL were assessed for structural variation at the 16p12.1 locus, including 53 with isolated AS/BAV, 83 with CoA ± AS/BAV, and 126 with HLHS. Of these, 168 (64.1%) were male. Samples were genotyped on a whole genome SNP array. Independent validation of selected CNVs of interest was subsequently conducted using locus-specific TaqMan® assays.

In surveying the LSL cohort for 16p12.1 structural variants, a single subject was found to contain a CNV overlapping the reported consensus region (Table 1 and Fig 1). This patient (1624-091A) presented with bicuspid aortic valve, coarctation of the aorta, hypoplastic aortic arch and muscular ventricular septal defect. The CNV from this patient was a large, maternally inherited duplication spanning 48 SNPs (725 kb) that overlapped the reported deletion region by 60%. Within the conotruncal cohort, one patient with tetralogy of Fallot (284A) was found to contain a large, maternally inherited 45 SNP deletion (460 kb) that was entirely contained both within the reported deletion and the LSL patient duplication. A similar deletion was also identified in one control patient. Notably, each of the three 16p12.1 CNVs detected shared one identical breakpoint and differed only slightly in the number of SNPs they contained, indicating the possibility that they shared a common mechanism of origin. Neither the LSL nor conotruncal patient, nor the control to our knowledge, had dysmorphic features, extracardiac malformations, or developmental delay.

The LSL patient (1624-091A) had one additional CNV identified elsewhere in the genome that was present in normal controls. Likewise, the conotruncal patient (284A) had two additional CNVs that were also represented in healthy controls. Neither the LSL nor conotruncal patient had additional unique or rare CNVs that were not present in controls.

Discussion

We hypothesized that the rare 16p12.1 microdeletion reported by Girirajan *et al.* might be identified with greater frequency in a cohort of patients ascertained by virtue of a left-sided congenital heart defect as compared to controls, given the cardiac phenotype reported by

Girirajan *et al.* in cases carrying the 16p12.1 deletion. In our cohort of 262 patients with LSL, one patient had a large maternally inherited duplication at this locus, but we did not detect any deletions at this locus. However, one patient from a conotruncal cohort with tetralogy of Fallot (TOF) and one control patient had smaller deletions (460 and 475 kb) beginning at the same locus as the duplication detected in the LSL patient. The patient with TOF shows no evidence of developmental delay or intellectual disability, and to our knowledge, neither does the control patient. Our findings also concur with those suggested by Cooper *et al.*, although the cardiac phenotype in Cooper *et al.* was not detailed, and the two cohorts were ascertained for different, though often comorbid, phenotypes.⁽¹²⁾ In the Girirajan study, one of the two control patients had a smaller deletion (102.9 kb shorter) that did not contain the *CDR2* gene, which the authors hypothesized might be contributing to the disease phenotype of developmental delay; this gene is contained by the presently reported deletion in the conotruncal and control patients.

It is possible that 16p12.1 remains a very rare cause of LSL, undetectable by our cohort size; however this remains the largest cohort of unrelated LSL probands reported to date, and success in identifying rare mutations and variants contributing to LSL have been reported in fewer probands (13). This along with the findings in our control patients suggests that 16p12.1 deletions are at best very uncommon in a population ascertained on the basis of this cardiac phenotype.

Acknowledgments

Funding for this work is provided by: R01 HL74094 (EG), P50HL062177 (EG), P50 HL74731 (EG). Additionally, the project described was supported by the National Center for Research Resources, Grant UL1RR024134, and is now at the National Center for Advancing Translational Sciences, Grant UL1TR000003. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

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FIGURE 1. Structural variation at 16p12.1

The pathogenic 16p12.1 microdeletion is shown, as well as two previously reported copy number polymorphisms and a non-pathogenic microdeletion (1). The patients with structural variation identified at the locus of interest are depicted.

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TABLE 1

Structural variation at 16p12.1

Cohort	Proband	Number of SNPs	Chr 16 Start	Chr 16 End	Copy Number	Inheritance
LSL	1624-091A	48	21,856,623	22,581,356	Gain	Maternal
Conotruncal	284A	45	21,856,623	22,316,964	Loss	Maternal
Control	X6946312536	47	21,856,623	22,331,199	Loss	Unknown