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Copy-number variant analysis of classic heterotaxy highlights the importance of body patterning pathways

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Abstract

Classic heterotaxy consists of congenital heart defects with abnormally positioned thoracic and abdominal organs. We aimed to uncover novel, genomic copy-number variants (CNVs) in classic heterotaxy cases. A microarray containing 2.5 million single-nucleotide polymorphisms (SNPs) was used to genotype 69 infants (cases) with classic heterotaxy identified from California live births from 1998 to 2009. CNVs were identified using the PennCNV software. We identified 56

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rare CNVs encompassing genes in the NODAL (*NIPBL, TBX6*), BMP (*PPP4C*), and WNT (*FZD3*) signaling pathways, not previously linked to classic heterotaxy. We also identified a CNV involving *FGF12*, a gene previously noted in a classic heterotaxy case. CNVs involving *RBFOX1* and near *MIR302F* were detected in multiple cases. Our findings illustrate the importance of body patterning pathways for cardiac development and left/right axes determination. *FGF12, RBFOX1*, and *MIR302F* could be important in human heterotaxy, because they were noted in multiple cases. Further investigation into genes involved in the NODAL, BMP, and WNT body patterning pathways and into the dosage effects of *FGF12, RBFOX1*, and *MIR302F* is warranted.

Introduction

Classic heterotaxy is a complex congenital disorder characterized by an abnormal arrangement of the internal organs across the left/right axis with at least one major heart defect. Heterotaxy is associated with primary ciliary dyskinesia (PCD), a rare lung disorder caused by mutations in *DNAI1* and *DNAH5* (Kennedy et al. 2007). The range of heterotaxy-associated phenotypes has been previously described in detail (Zhu et al. 2006). Classic heterotaxy is estimated to occur in 1 in 10,000 live births (Lin et al. 2014). Heterotaxy accounts for roughly 3 % of all congenital heart disease (CHD) cases (Zhu et al. 2006). There are conflicting results with regard to whether the risk is increased for Asians (Rigler et al. 2015), African Americans (Lin et al. 2014), or Hispanics (Rigler et al. 2015). Recurrence in families and a 2:1 male-to-female prevalence in some studies (Ferencz et al. 1997; Lin et al. 2014) suggest a genetic component.

Heterotaxy is associated with primary ciliary dyskinesia (PCD), a rare disorder of the respiratory tract, caused by more than 30 genes (Kurkowiak et al. 2015; Lobo et al. 2015), including *DNAI1* and *DNAH5* (Kennedy et al. 2007). Several genes have been linked to human laterality disorders (Kennedy et al. 2007; Sutherland and Ware 2009; Zhu et al. 2006), including mutations in the transforming growth factor-β (TGF-β) family involved in the NODAL signaling pathway (Shen 2007). Other genes have been identified through their association with PCD and influence on ciliary structure and function. However, known genes account for only approximately 10–20 % of sporadic cases of heterotaxy (Fakhro et al. 2011; Sutherland and Ware 2009). Animal studies have identified numerous laterality genes indicating the likelihood of additional human laterality genes (Zhu et al. 2006). Furthermore, the role of copy-number variants (CNVs) in heterotaxy has received little attention (Fakhro et al. 2011; Rigler et al. 2015). The goal of the present study was to determine whether additional CNVs are involved in classic heterotaxy.

Materials and methods

Cases

Heterotaxy cases were identified via the California Birth Defects Monitoring Program's (CBDMP's) population-based, active ascertainment birth defects registry. The methods have been described in detail previously (Croen et al. 1991). In brief, trained staff collected diagnostic and demographic information. Each diagnosis was assigned a code from the

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CBDMP Six Digit Code. A study performed on the CBDMP registry determined that the completeness of ascertainment exceeded 93 % (Schulman and Hahn 1993).

Heterotaxy cases were identified from all live births (N = 761,860) from 1998 to 2009 with maternal residence in a San Joaquin Valley County, excluding births at military facilities. All the cases with heterotaxy [British Paediatric Association (BPA) codes 759.300–759.390, 746.805] and at least one other heart defect (BPA 745.000–747.499) were identified. We hypothesized that using a restrictive definition of heterotaxy would enable us to identify recurring and potentially causal CNVs without confounding by cases with other genetic abnormalities or syndromes. Situs inversus totalis is not associated with CHD and, therefore, was excluded. Heterotaxy cases with associated aneuploidy, additional genetic syndrome or another major, non-heterotaxy associated malformation, e.g., diaphragmatic hernia, oral cleft, and multiple dysmorphic features were excluded from study. We also excluded any cases of PCD because it has already been linked to over 30 genes; genetic causes have been identified for approximately two thirds of cases (Kurkowiak et al. 2015), and it is uncertain that the remaining cases have a genetic cause. We wanted to explore conditions that remained unexplained. A total of 132 cases of heterotaxy were reviewed, 82 cases met the inclusion criteria, and 69 could be matched to newborn dried blood spots (DBS) for the DNA analysis.

To ensure that the identified CNVs were not present in the unaffected population, we investigated frequencies of the identified CNVs validated in the cases in an unaffected group of infants. We randomly selected 165 infants without birth defects who derived from the same population (county and year of birth) as the cases, obtained their bloodspots, and performed qPCR copy-number assays using at least one probe per CNV region.

Vital records data were obtained and analyzed using Fisher's exact test or t test. Prior to genotyping and analysis, cases were given a random identification number and all personally identifying information was removed. The California Department of Public Health (IRB 13-03-1164), the New York State Department of Health (IRB 07-007), and the NIH Office of Human Subjects Research (OHSRP 11631) reviewed and approved this study.

CNV detection, selection, and validation

The 69 cases, 10 controls, and one HapMap sample were batched and genotyped along with one sample and a trio in duplicate taken from an unrelated project. The methods have been described previously (Rigler et al. 2015). In brief, samples were genotyped using the Illumina HumanOmni2.5-8_v1-1_B bead arrays and the Infinium LCG assay protocol. The mean sample call rate ± SD (range) was 99.73 ± 0.005 (95.50–99.91). The mean log R ratio deviation was 0.116 ± 0.024 (0.088–0.201). Single nucleotide polymorphism genotype reproducibility was 100 % for the unaffected controls and 99.99 % for the trio. A total of 2,284,686 autosomal markers were included in the CNV analysis. CNVs were called and annotated using the Illumina's cnvPartition algorithm (version 3.2.0) and PennCNV (version 2011/05/03). For additional information, see the Supplementary Materials and Methods.

Copy number variants were excluded if they were shorter than 20 kb, contained fewer than ten SNP probes, overlapped more than 35 % with common CNVs in Hap-Map or CHOP, or

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overlapped more than 50 % with similar-type CNVs in an in-house reference CNV database compromised of unaffected controls and cases of other unrelated birth defects. The remaining CNVs were uploaded to DGV (build37/hg19, DGV release date 2014-10-16, and date accessed 2014-10-28) and analyzed for overlap. A CNV was selected for further analysis if it had less than 50 % overlap with variants present in DGV or if the CNV encompassed a gene in the non-overlapped region. Although the DGV is a very valuable resource, some studies in the database have very small sample sizes and all could potentially include false positives. Furthermore, some methods of CNV detection, such as BAC arrays, are known to overestimate the start and end points of CNVs. For these reasons, we chose to ignore overlap with variants represented by thin lines (denoting low confidence) in the DGV browser and overlap with variants from studies using similar methods. A study that included subjects with cardiovascular defects was also excluded.

Copy number variants with poor B-allele frequency/log R ratio scatterplot data quality (poor likelihood to validate) were excluded from further analysis. The remaining CNVs were considered candidate CNVs for heterotaxy. Of the 58 identified CNVs, 19 were prioritized for validation based on whether they were present in multiple subjects, contained biologically relevant genes, or overlapped CNVs that had been previously reported in heterotaxy cases. Studies were performed using two to three quantitative real-time polymerase chain reaction (qPCR) TaqMan assays (Applied Biosystems, Carlsbad, CA, USA) per region. Validations were performed as previously described (Rigler et al. 2015). For further details, see Supplementary Materials and Methods. One probe was excluded due to discordant results obtained when retesting multiple samples with low confidence calls (Supplemental Table 1). All assays were tested in each of the 69 heterotaxy cases and 10 control subjects. We subsequently screened all validated CNVs against an additional 165 control samples from unaffected California births using at least one assay targeting each area of interest. Therefore, a total of 175 unaffected controls were screened using at least one assay in each candidate CNV region.

Known heterotaxy gene screening

A custom AmpliSeq panel (Life Technologies, Carlsbad, CA) was used to screen for mutations in the following genes: *ACVR2B, NODAL, FOXH1, ZIC3, CFC1, NKX2-3, CRELD1, LEFTY2, SESN1, GDF1, KTU, RPGR, TXNDC3, DNAH11, DNAI2, DNAI1, DNAH5, FOXI2, NKX6-2*, and *GJA1* (Supplemental Table 2). The genes were chosen based on evidence that they were associated with heterotaxy or PCD. The panel was run on an Ion Torrent platform as previously described (Rigler et al. 2015). For further details, see Supplementary Materials and Methods. Due to poor coverage, we chose to exclude CFC1 mutations from the analysis. The selected mutations are summarized in Table 1.

Results

Of the 761,860 live births, 82 met our case definition with a birth prevalence of 1 in 9300 live births. Cases were statistically more likely to be born to Asian mothers (13.4 vs. 6.8 %; $P = 0.0158$), but not statistically more likely to be born to African–American (7.4 vs 4.6 %; $P = 0.2247$) or Hispanic (50.6 vs 58.8 %; $P = 0.1359$) mothers (Table 2). Mothers with less

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than a high school education had an increased risk for having an infant with classic heterotaxy (67.9 vs 35.1 %; $P < 0.0001$). Infants with classic heterotaxy were more likely to be of a lower birth weight (mean of 2973 vs. 3327 g; $P < 0.0001$), but surprisingly, no significant difference was seen for gestational age (mean of 266 vs. 269; $P = 0.2949$). This conflicts with the previous reports (Lin et al. 2014; Rigler et al. 2015). No statistical sex difference was observed between the cases and the controls (59.8 vs. 51.1 % male, respectively; $P = 0.1162$).

PennCNV identified 6085 CNVs in the primary microarray analysis. After the initial round of exclusions outlined in the Methods, 56 candidate CNVs from 38 cases remained. We prioritized 19 of the 56 CNVs for validation and further investigation. Using qPCR, 14 CNVs validated as duplications, three as heterozygous deletions, and two did not validate (false positive CNV call from microarray data). The predicted CNVs ranged in size from 21 to 840 kb. The validated CNVs are described in more detail in Table 3. Candidate CNVs not selected for qPCR validation are listed in Supplemental Table 4. No pattern of cardiac defects was seen in the 38 cases with candidate CNVs (Table 4).

In individual case infants, we identified rare CNVs involved in NODAL, BMP, FGF, and WNT body patterning pathways. These CNVs included an 840 kb duplication at 5p13.2 involving *NIPBL*, a gene that influences *lefty-2* expression in zebrafish (Muto et al. 2011); a 617 kb deletion at 16p11.2 encompassing *PPP4C* and *TBX6*, genes linked to BMP and NODAL signaling (Hadjantonakis et al. 2008; Jia et al. 2012), respectively; a 120 kb duplication at 8p21.1 spanning *FZD3*, a gene that activates the planar cell polarity (PCP) pathway of WNT signaling (Komatsu and Mishina 2013); and a 22 kb duplication at 3q29, overlapping *FGF12*, a gene overlapped by a CNV in a previous heterotaxy cohort our group studied (Rigler et al. 2015).

We also identified a 29–37 kb duplication at 6p12.1 in two cases. This duplication was upstream of *ZNF451*, a zinc finger protein that likely interacts with Smad4, a key player in the BMP and TGF-β-signaling pathways (Feng et al. 2014). Two additional cases carried an approximately 370 kb duplication at 16p13.3, involving *RBFOX1*, a gene encoding an RNA-binding protein expressed in the heart (Kuroyanagi 2009). Another CNV, duplicated in one case, overlapped *MIR302F*, a micro-RNA whose function has not been determined. Two additional deletions in this region were detected during validations.

Finally, we identified three CNVs, two duplications, and one heterozygous deletion, which overlapped with CNVs reported in a previous heterotaxy cohort (Fakhro et al. 2011). In all cases, the CNVs detected were smaller than previously noted, providing a more focused region of interest. We detected a 36 kb duplication at 2q37.3, a 230 kb duplication at 18p11.21, and a 27 kb heterozygous deletion at 8p23.2.

Discussion

Establishing the left axis/right axis is essential for normal organogenesis and provides the basis for correct heart looping (Srivastava and Olson 2000). However, the exact mechanisms that establish this asymmetry and drive heart development and differentiation are still largely

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unknown. The current data on laterality disorders underscore the importance of nodal cilia and leftward nodal flow in the developing embryo for these processes. NODAL, BMP, WNT, and FGF signaling are significant factors in left/right axis determination and cardiac development in multiple animal models (Rochais et al. 2009; Shiraishi and Ichikawa 2012). Our current study identified several rare CNVs in humans, encompassing genes involved in these critical signaling pathways.

The establishment of the left–right body axis in most vertebrates begins with the creation of leftward nodal flow. Nodal flow contributes to the asymmetrical expression of *Nodal* (Komatsu and Mishina 2013). Our analysis identified a duplication at 5p13.2 that overlapped *NIPBL*, a gene linked to NODAL signaling. In zebrafish, knockdown of *nipbla/b* results in heart and laterality defects. *Nipbla/b* mutants exhibit reduced expression of several genes linked to NODAL signaling, such as *lefty-2*, as well as reduced expression of *dnah9*, a gene that encodes a protein necessary for cilia motility in Kupffer's vesicle, a structure analogous to the mouse node (Muto et al. 2011). In humans, *NIPBL* has been linked to Cornelia de Lange syndrome, which is associated with CHD.

A deletion in 16p11.2 in one case encompassed *TBX6*. TBX6 regulates *Delta-like 1 (Dll1)*, a Notch ligand that is upstream of Nodal. Thus, TBX6 may influence both the Notch- and NODAL-signaling pathways. *Tbx6* null mice exhibit abnormal heart looping and are characterized by nodal cilia that are abnormal in both structure and motility (Hadjantonakis et al. 2008). *TBX6* is a member of the T-box family of transcription factors that act as transcriptional repressors or activators and play key roles in development. *TBX1* is associated with the heart defects present in 22q11.2 deletion syndrome (Yagi et al. 2003).

A second gene, *PPP4C*, was found in the same deletion as *TBX6*. *PPP4C* encodes the catalytic subunit of PPP4, a serine/threonine phosphatase member of the PPP family. Ppp4 regulates a variety of cellular functions, including transcriptional and replication activities of chromatin (Cohen et al. 2005). In zebrafish, *ppp4c* knockdown results in increased embryonic dorsalization and decreased ventralization (Jia et al. 2012). Furthermore, Ppp4c has been demonstrated to bind to Smad1/Smad5, regulating the BMP signaling pathway during dorso-ventral patterning (Jia et al. 2012). BMP signaling genes have previously been linked to human heterotaxy (Rigler et al. 2015). In animal studies, Bmp signaling has been shown to influence left/right patterning and Nodal expression (Smith et al. 2011). A similar microdeletion was identified in a pair of identical twins with aortic valve abnormalities (Ghebranious et al. 2007).

We identified a duplication at 6p12.1 upstream of *ZNF451*, in two cases. *ZNF451* is a member of the zinc finger protein family and is a transcriptional cofactor. ZNF451 binds to Smad4, forming a complex with Smad2 and Smad3, key components of the TGF-β signaling pathway (Feng et al. 2014). Both *NODAL* and members of the BMP family belong to the TGF-β superfamily.

WNT signaling also plays a role in embryonic patterning and cardiogenesis. WNT signaling can be grouped into three pathways, each involving specific frizzled receptors. We identified a duplication in 8p21.2 overlapping *FZD3*, a gene that encodes one of the frizzled receptors

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that activates the PCP pathway. The PCP pathway has been implicated in embryonic narrowing and elongation (convergent extension), cell migration, and determination of cell fate (Vladar et al. 2009). In mice, the PCP pathway influences the positioning and orientation of nodal cilia, thereby influencing nodal flow (Komatsu and Mishina 2013).

Interestingly, we detected a duplication in 3q29 overlapping *FGF12*, a gene deleted in an individual in our previous heterotaxy cohort (Rigler et al. 2015). Animal studies have implicated members of the FGF family in several key developmental processes, including cilia development, Nodal-dependent endoderm induction, and specification of the early cardiac mesoderm (Mizoguchi et al. 2006; Neugebauer et al. 2009; Rochais et al. 2009). However, FGF12 belongs to the FGF homologous factor subfamily, which unlike other members of the FGF family, does not bind to FGF receptors. In mice, *fgf12* is expressed in the myocardium of the developing heart (Hartung et al. 1997). Thus, further investigation is required to determine how this variant is related to cardiac and laterality defects.

A duplication in 16p13.3 involving *RBFOX1* was found in two cases. *RBFOX1* is a member of the Fox-1 family of RNA-binding proteins in mammals. RBFOX proteins are involved in alternative splicing, which influences gene expression during embryonic development and differentiation (Kuroyanagi 2009). Knockdown of rbfox1 l and rbfox2 in zebrafish embryos results in cardiac muscle defects (Gallagher et al. 2011). Partial deletions of *RBFOX1* have been previously identified in infants with complex congenital heart defects (Lale et al. 2011; Li et al. 2012), and 16p13.3 has been noted as a candidate locus for heart malformations (Geng et al. 2014). In addition to the partial duplication of *RBFOX1*, Case 10 had a homozygous *DNAH11* mutation predicted to alter splicing (Table 1). *DNAH11* mutations are associated with heterotaxy; however, it is not known if this point mutation is pathogenic.

We also noted a 184 kb duplication in 18q12.1 encompassing *MIR302F*. Partially overlapping deletions were detected in two additional cases during validations. The additional cases were detected as heterozygous delections at probe Hs06501555_cn during qPCR validations; although breakpoints of the deletions cannot be estimated, Hs06501555_cn is 1.4 Kb from *MIR302F*. Little is known about *MIR302F*, but studies using synthetic human *MIR302* (*MIR302a-d*) showed targeted inhibition of *LEFTY1* and *LEFTY2*, two key genes in the NODAL signaling pathway (Rosa et al. 2009). It is possible that *MIR302F* has a similar role to the other members of the *MIR302* family and could influence NODAL signaling genes.

Three of the CNVs we found overlap CNVs found in a previous heterotaxy cohort (Fakhro et al. 2011). The first, a duplication in 2q37.3, encompassed *CXXC11*, a member of the receptor transporter protein family. Little is known about this family of proteins. A second duplication, in 18p11.21, overlapped *FAM210A* and *LDLRAD4*. Like *CXXC11*, there is a lack of research on *FAM210A* and *LDLRAD4*. *LDLRAD4* (also known as *C18ORF1*) has been shown to inhibit TGF-β signaling in a dose-dependent manner (Nakano et al. 2014). This case also had a heterozygous nonsynonymous mutation in *NODAL* that was previously reported as pathogenic (Mohapatra et al. 2009). It is possible that both the *NODAL* mutation and the duplication in 18p11.21 are contributing to the individual's phenotype. The third CNV, a deletion in 8p23.2, did not encompass any genes. In addition to Fakhro et al. (2011),

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another CNV investigation identified a duplication in 8p23.2 in an individual with tetralogy of Fallot (Campos et al. 2015). In addition to the deletion in 8p23.2, this case had three exonic heterozygous mutations in *DNAH11* (Table 1), all with unknown significance. It is possible that these point mutations are nonpathogenic or that both these mutations and the deletion of 8p23.2 are contributing to heterotaxy in this individual. In all three cases, our identified CNVs were smaller than those noted by Fakhro et al. (2011), narrowing the critical region. Two duplications that validated in single cases (3p21.31 and 12p13.33) were each found in a control subject. In both cases, 3 and 11, sequence variants were also identified (Table 1). Case 3 had a heterozygous *FOXI2* mutation that was predicted to be pathogenic by both PolyPhen and SIFT. *FOXI2* is a gene deleted in an individual in our previous heterotaxy cohort (Rigler et al. 2015). Case 11 had two nonsense *DNAH5* mutations. Mutations in *DNAH5* are known to cause PCD. It is possible that the duplications in these subjects are nonpathogenic and the gene mutations are the cause of heterotaxy. Alternatively, both the duplications and the gene mutations may contribute to a multifactorial inheritance of heterotaxy.

Multifactorial inheritance of heterotaxy has been suggested (Sutherland and Ware 2009). Heterozygous mutations in PCD genes have been seen in heterotaxy cases with PCD (Nakhleh et al. 2012). Functional validation of a trans-heterozygous interactions provides support of an oligogenic model of heterotaxy (Li et al. 2016). Results of our combined sequence and CNV analysis could support multigenic inheritance of heterotaxy. Fifteen of 38 heterotaxy cases studied carried both a candidate CNV and a sequence variant (Supplemental Table 5). As the majority of the sequence variants we identified were heterozygous, it is possible that both the sequence variants and the candidate CNVs are contributing to heterotaxy.

This is only the second population-based study of classic heterotaxy (Rigler et al. 2015). By excluding a variety of isolated abnormalities, we hoped to uncover CNVs seen in multiple cases that were not associated with a secondary phenotype. We were also able to analyze demographic characteristics associated with heterotaxy, noting an increased prevalence among Asians. Cases were also more likely to be lower birth weight and born to mothers with less than a high school education. Interestingly, cases were no more likely to be born at an earlier gestational age.

This study has several strengths and weaknesses. Because it is a population-based study, we know that we have a representative set of cases. The data available through the CBDMP registry have been collected and coded by well-trained staff and has excellent and unbiased ascertainment of cases. We also validated 17 CNVs and additional point mutations, ensuring that no false positives are present in our final candidate heterotaxy-associated CNV list. Our CNV selection criteria included filtering against the DGV database to assure that the detected CNVs were absent or rare in normal subjects. Furthermore, to rule out the CNVs specific to our population, we screened the CNVs in 175 control subjects from the California population. Due to the nature of the CNV validation method (TaqMan Copy-number assays), we are unable to determine the exact breakpoints of CNVs. However, if a single probe for a given CNV was detected in a control subject, we tested the other probes spanning the region in that control subject to determine minimum predicted overlap. Using one assay per CNV

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region, we are effectively ruling out the presence of CNVs with breakpoints matching those detected in the cases in the 175 control subjects. There were limitations to our case identification, as cases were reported from live births and losses or terminations would be missed. Cases can also be missed or excluded based on non-reporting or incorrect coding. Because cases were ascertained and DNA obtained from a large, population-based newborn cohort, it was not possible to obtain data or DNA from parents to determine whether CNVs were inherited or de novo mutations. This was a CNV/gene finding study that we hope will provide leads for future triad and model organism studies. In addition, determining the clinical relevance of CNVs can be challenging due to the potential inaccuracies in mapping breakpoints and difficulty in estimating CNV frequency.

In conclusion, we identified several CNVs that included genes linked to body patterning not previously found in human heterotaxy cases, including *NIPBL, TBX6, PPP4C, FZD3*, and *ZNF45.* CNVs involving *RBFOX1* and near *MIR302F* were seen in more than one case, indicating potential importance in human heterotaxy. We also replicated associations between heterotaxy and *FGF12* (Rigler et al. 2015) and 2q37.3, 8p23.2, and 18p11.21 (Fakhro et al. 2011) mutations in an independent population. These genes could be investigated further by sequencing or analyzing genes in the related pathways. Our study provides more evidence that mutations in body patterning genes are implicated in classic heterotaxy and, therefore, merit additional investigation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

Campos CM, Zanardo EA, Dutra RL, Kulikowski LD, Kim CA. Investigation of copy number variation in children with conotruncal heart defects. Arq Bras Cardiol. 2015; 104:24–31. DOI: 10.5935/abc.20140169 [PubMed: 25387403]

Cohen PT, Philp A, Vazquez-Martin C. Protein phosphatase 4–from obscurity to vital functions. FEBS Lett. 2005; 579:3278–3286. DOI: 10.1016/j.febslet.2005.04.070 [PubMed: 15913612]

Croen LA, Shaw GM, Jensvold NG, Harris JA. Birth defects monitoring in California: a resource for epidemiological research. Paediatr Perinat Epidemiol. 1991; 5:423–427. DOI: 10.1111/j.1365-3016.1991.tb00728.x [PubMed: 1754501]

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Fakhro KA, Choi M, Ware SM, Belmont JW, Towbin JA, Lifton RP, Khokha MK, Brueckner M. Rare copy number variations in congenital heart disease patients identify unique genes in left-right patterning. Proc Natl Acad Sci. 2011; 108:2915–2920. DOI: 10.1073/pnas.1019645108 [PubMed: 21282601]

Feng Y, Wu H, Xu Y, Zhang Z, Liu T, Lin X, Feng XH. Zinc finger protein 451 is a novel Smad corepressor in transforming growth factor-beta signaling. J Biol Chem. 2014; 289:2072–2083. DOI: 10.1074/jbc.M113.526905 [PubMed: 24324267]

Ferencz, C.; Loffredo, CA.; Correa-Villaseñor, A.; Wilson, PD. The Baltimore-Washington infant study 1981–1989, perspectives in pediatric cardiology. Vol. V. Futura Publishing; Armonk: 1997. Genetic and environmental risk factors of major cardiovascular malformations.

Gallagher TL, Arribere JA, Geurts PA, Exner CR, McDonald KL, Dill KK, Marr HL, Adkar SS, Garnett AT, Amacher SL, Conboy JG. Rbfox-regulated alternative splicing is critical for zebrafish cardiac and skeletal muscle functions. Dev Biol. 2011; 359:251–261. DOI: 10.1016/j.ydbio. 2011.08.025 [PubMed: 21925157]

Geng J, Picker J, Zheng Z, Zhang X, Wang J, Hisama F, Brown DW, Mullen MP, Harris D, Stoler J, Seman A, Miller DT, Fu Q, Roberts AE, Shen Y. Chromosome microarray testing for patients with congenital heart defects reveals novel disease causing loci and high diagnostic yield. BMC Genom. 2014; 15:1127.doi: 10.1186/1471-2164-15-1127

Ghebranious N, Giampietro PF, Wesbrook FP, Rezkalla SH. A novel microdeletion at 16p11.2 harbors candidate genes for aortic valve development, seizure disorder, and mild mental retardation. Am J Med Genet A. 2007; 143A:1462–1471. DOI: 10.1002/ajmg.a.31837 [PubMed: 17568417]

Hadjantonakis AK, Pisano E, Papaioannou VE. Tbx6 regulates left/right patterning in mouse embryos through effects on nodal cilia and perinodal signaling. PLoS One. 2008; 3:e2511.doi: 10.1371/journal.pone.0002511 [PubMed: 18575602]

Hartung H, Feldman B, Lovec H, Coulier F, Birnbaum D, Goldfarb M. Murine FGF-12 and FGF-13: expression in embryonic nervous system, connective tissue and heart. Mech Dev. 1997; 64:31–39. DOI: 10.1016/S0925-4773(97)00042-7 [PubMed: 9232594]

Jia S, Dai F, Wu D, Lin X, Xing C, Xue Y, Wang Y, Xiao M, Wu W, Feng XH, Meng A. Protein phosphatase 4 cooperates with Smads to promote BMP signaling in dorsoventral patterning of zebrafish embryos. Dev Cell. 2012; 22:1065–1078. DOI: 10.1016/j.devcel.2012.03.001 [PubMed: 22595677]

Kennedy MP, Omran H, Leigh MW, Dell S, Morgan L, Molina PL, Robinson BV, Minnix SL, Olbrich H, Severin T, Ahrens P, Lange L, Morillas HN, Noone PG, Zariwala MA, Knowles MR. Congenital heart disease and other heterotaxic defects in a large cohort of patients with primary ciliary dyskinesia. Circulation. 2007; 115:2814–2821. DOI: 10.1161/circulationaha.106.649038 [PubMed: 17515466]

Komatsu Y, Mishina Y. Establishment of left-right asymmetry in vertebrate development: the node in mouse embryos. Cell Mol Life Sci. 2013; 70:4659–4666. DOI: 10.1007/s00018-013-1399-9 [PubMed: 23771646]

Kurkowiak M, Zi tkiewicz E, Witt M. Recent advances in primary ciliary dyskinesia genetics. J Med Genet. 2015; 52(1):1–9. DOI: 10.1136/jmedgenet-2014-102755 [PubMed: 25351953]

Kuroyanagi H. Fox-1 family of RNA-binding proteins. Cell Mol Life Sci. 2009; 66:3895–3907. DOI: 10.1007/s00018-009-0120-5 [PubMed: 19688295]

Lale S, Yu S, Ahmed A. Complex congenital heart defects in association with maternal diabetes and partial deletion of the A2BP1 gene. Fetal Pediatr Pathol. 2011; 30:161–166. DOI: 10.3109/15513815.2010.547555 [PubMed: 21355681]

Li D, Tekin M, Buch M, Fan YS. Co-existence of other copy number variations with 22q11.2 deletion or duplication: a modifier for variable phenotypes of the syndrome? Mol Cytogenet. 2012; 5:18.doi: 10.1186/1755-8166-5-18 [PubMed: 22487416]

Li Y, Yagi H, Onuoha EO, Damerla RR, Francis R, Furutani Y, Tariq M, King SM, Hendricks G, Cui C, Saydmohammed M, Lee DM, Zahid M, Sami I, Leatherbury L, Pazour GJ, Ware SM, Nakanishi T, Goldmuntz E, Tsang M, Lo CW. DNAH6 and its interactions with PCD genes in heterotaxy and primary ciliary dyskinesia. PLoS Genet. 2016; 12(2)doi: 10.1371/journal.pgen. 1005821

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Lin AE, Krikov S, Riehle-Colarusso T, Frias JL, Belmont J, Anderka M, Geva T, Getz KD, Botto LD. Laterality defects in the national birth defects prevention study (1998–2007): birth prevalence and descriptive epidemiology. Am J Med Genet A. 2014; 164A:2581–2591. DOI: 10.1002/ajmg.a.36695 [PubMed: 25099286]

Lobo J, Zariwala MA, Noone PG. Primary ciliary dyskinesia. Semin Respir Crit Care Med. 2015; 36(2):169–179. DOI: 10.1055/s-0035-1546748 [PubMed: 25826585]

Mizoguchi T, Izawa T, Kuroiwa A, Kikuchi Y. Fgf signaling negatively regulates Nodal-dependent endoderm induction in zebrafish. Dev Biol. 2006; 300:612–622. DOI: 10.1016/j.ydbio.2006.08.073 [PubMed: 17026981]

Mohapatra B, Casey B, Li H, Ho-Dawson T, Smith L, Fernbach SD, Molinari L, Niesh SR, Jefferies JL, Craigen WJ, Towbin JA, Belmont JW, Ware SM. Identification and functional characterization of NODAL rare variants in heterotaxy and isolated cardiovascular malformations. Hum Mol Genet. 2009; 18:861–871. DOI: 10.1093/hmg/ddn411 [PubMed: 19064609]

Muto A, Calof AL, Lander AD, Schilling TF. Multifactorial origins of heart and gut defects in nipbl-deficient zebrafish, a model of Cornelia de Lange Syndrome. PLoS Biol. 2011; 9:e1001181.doi: 10.1371/journal.pbio.1001181 [PubMed: 22039349]

Nakano N, Maeyama K, Sakata N, Itoh F, Akatsu R, Nakata M, Katsu Y, Ikeno S, Togawa Y, Vo Nguyen TT, Watanabe Y, Kato M, Itoh S. C18 ORF1, a novel negative regulator of transforming growth factor-beta signaling. J Biol Chem. 2014; 289:12680–12692. DOI: 10.1074/jbc.M114.558981 [PubMed: 24627487]

Nakhleh N, Francis R, Giese RA, Tian X, Li Y, Zariwala MA, Yagi H, Khalifa O, Kureshi S, Chatterjee B, Sabol SL, Swisher M, Connelly PS, Daniels MP, Srinivasan A, Kuehl K, Kravitz N, Burns K, Sami I, Omran H, Barmada M, Olivier K, Chawla KK, Leigh M, Jonas R, Knowles M, Leatherbury L, Lo CW. High prevalence of respiratory ciliary dysfunction in congenital heart disease patients with heterotaxy. Circulation. 2012; 125(18):2232–2242. DOI: 10.1161/CIRCULATIONAHA.111.079780 [PubMed: 22499950]

Neugebauer JM, Amack JD, Peterson AG, Bisgrove BW, Yost HJ. FGF signalling during embryo development regulates cilia length in diverse epithelia. Nature. 2009; 458:651–654. DOI: 10.1038/nature07753 [PubMed: 19242413]

Rigler SL, Kay DM, Sicko RJ, Fan R, Liu A, Caggana M, Browne ML, Druschel CM, Romitti PA, Brody LC, Mills JL. Novel copy-number variants in a population-based investigation of classic heterotaxy. Genet Med. 2015; 17:348–357. DOI: 10.1038/gim.2014.112 [PubMed: 25232849]

Rochais F, Mesbah K, Kelly RG. Signaling pathways controlling second heart field development. Circ Res. 2009; 104:933–942. DOI: 10.1161/circresaha.109.194464 [PubMed: 19390062]

Rosa A, Spagnoli FM, Brivanlou AH. The miR-430/427/302 family controls mesendodermal fate specification via species-specific target selection. Dev Cell. 2009; 16:517–527. DOI: 10.1016/j.devcel.2009.02.007 [PubMed: 19386261]

Schulman J, Hahn JA. Quality control of birth defect registry data: a case study. Public Health Rep. 1993; 108:91–98. [PubMed: 8434104]

Shen MM. Nodal signaling: developmental roles and regulation. Development. 2007; 134:1023–1034. DOI: 10.1242/dev.000166 [PubMed: 17287255]

Shiraishi I, Ichikawa H. Human heterotaxy syndrome—from molecular genetics to clinical features, management, and prognosis. Circ J. 2012; 76:2066–2075. DOI: 10.1253/circj.CJ-12-0957 [PubMed: 22864291]

Smith KA, Noel E, Thurlings I, Rehmann H, Chocron S, Bakkers J. Bmp and nodal independently regulate lefty1 expression to maintain unilateral nodal activity during left-right axis specification in zebrafish. PLoS Genet. 2011; 7:e1002289.doi: 10.1371/journal.pgen.1002289 [PubMed: 21980297]

Srivastava D, Olson EN. A genetic blueprint for cardiac development. Nature. 2000; 407:221–226. DOI: 10.1038/35025190 [PubMed: 11001064]

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. DOI: 10.1002/ajmg.c.30228 [PubMed: 19876930]

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Vladar EK, Antic D, Axelrod JD. Planar cell polarity signaling: the developing cell's compass. Cold Spring Harb Perspect Biol. 2009; 1:a002964.doi: 10.1101/cshperspect.a002964 [PubMed: 20066108]

Yagi H, Furutani Y, Hamada H, Sasaki T, Asakawa S, Minoshima S, Ichida F, Joo K, Kimura M, Imamura S, Kamatani N, Momma K, Takao A, Nakazawa M, Shimizu N, Matsuoka R. Role of TBX1 in human del22q11.2 syndrome. Lancet. 2003; 362:1366–1373. DOI: 10.1016/S0140-6736(03)14632-6 [PubMed: 14585638]

Zhu L, Belmont JW, Ware SM. Genetics of human heterotaxias. Eur J Hum Genet. 2006; 14:17–25. DOI: 10.1038/sj.ejhg.5201506 [PubMed: 16251896]

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Table 1

Potentially pathogenic sequence variants in known heterotaxy genes

Gene	Transcript[a]	DNA change[a]	Protein change[a]	Max MAF[b]	DbSNP rs ID[a]	SIFT (S) and PolyPhen (P) Predictions[g]	Subject ID(s)
FOXI2	NM_207426	c.G622A	p.G208R	N/A	N/A	S-0.04 (deleterious) P-0.979 (probably damaging)	3
LEFTY2	NM_003240	c.C330T	p.N110N	0.0200	rs181651384	N/A	4
DNAH11	NM_001277115	c.G10783A	p.E3595K	0.0001	N/A	S-0 (deleterious) P-0.967 (probably damaging)	8
DNAH11	NM_001277115	c.C11266T	p.R3756C	0.0002	N/A	S-0 (deleterious) P-0.95 (probably damaging)	8
DNAH11	NM_001277115	c.G12175A	p.A4059T	0.0110	rs142372794	S-0 (deleterious) P-0.998 (probably damaging)	8
DNAH11	NM_001277115	c.11203-1G>C	N/A	N/A	N/A	N/A	10[c]
DNAH5	NM_001369	c.C9346T	p.R3116X	N/A	N/A	N/A	11[d]
DNAH5	NM_001369	c.C5281T	p.R1761X	0.0002	N/A	N/A	11
DNAH11	NM_001277115	c.T5359C	p.L1787L	0.0250	rs75932225	N/A	16
DNAH11	NM_001277115	c.A6726G	p.L2242L	0.0140	rs78152348	N/A	16
DNAH11	NM_001277115	c.C10274A	p.T3425K	0.0002	rs367800491	S-0 (deleterious) P-0.885 (possibly damaging)	16
NODAL	NM_018055	c.G778A	p.G260R	0.0035	rs121909283	S-0 (deleterious) P-1 (probably damaging)	16, 21
DNAH5	NM_001369	c.T5224C	p.L1742L	0.0370	rs35963491	N/A	5, 20
DNAH5	NM_001369	c.C9778T	p.Q3260X	N/A	N/A	N/A	20
DNAH5	NM_001369	c.C5281G	p.R1761G	0.0140	rs148891849	S-0.01 (deleterious) P-0.569 (possibly damaging)	20
DNAH5	NM_001369	c.A8268C	p.S2756S	0.0046	N/A	N/A	25
DNAH5	NM_001369	c.G6382A	p.V2128I	0.0006	N/A	S-No score P-0.312 (benign)	25
RPGR	NM_001034853	c.G3351C	p.E1117D	0.0030	rs199691696	S-0.04 (deleterious low confidence) P-0 (unknown)	28[e]
DNAH11	NM_001277115	c.G4124A	p.R1375H	0.0160	rs151018293	S-0.03 (deleterious) P-0.11 (benign)	33
DNAH11	NM_001277115	c.T8266C	p.C2756R	0.0330	rs74667361	S-0.21 (tolerated) P-0.003 (benign)	33
DNAH11	NM_001277115	c.C8362G	p.H2788D	0.0190	rs147478795	S-0 (deleterious) P-0.994 (probably damaging)	33

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Gene	Transcript[a]	DNA change[a]	Protein change[a]	Max MAF[b]	DbSNP rs ID[a]	SIFT (S) and PolyPhen (P) Predictions[g]	Subject ID(s)
CRELD1	NM_015513	c.A1249G	p.I417V	0.0054	rs115361983	S-0.02 (deleterious low confidence) P-0.891 (possibly damaging)	35
SESN1	NM_001199933	c.952_954del	p.E318del	N/A	N/A	N/A	387[f]

Variants shown were detected using the AmpliSeq panel and validated by Sanger sequencing

All variants are heterozygous unless otherwise noted. Two variants called by Torrent Variant Caller did not validate by Sanger sequencing. A *FOXH1* insertion was ruled out via Sanger sequencing. An insertion in *NKX6-2* (c.140dupG, p.G47fs in Case 12) could not be attempted, because the region could not be PCR-amplified despite several designs; this variant is in a region with high GC content (>80 %), and visualization of the sequence reads (AmpliSeq BAM files) in Integrative Genomics Viewer suggests that the variant is also likely to be a false positive artifact

[a]Annotations from ANNOVAR, where available

[b]MAF represents the maximum minor allele frequency from any population included in 1000G, ESP6500, ExAC, and CG46 studies (represented as ‘popfreq_all_20150413’ in ANNOVAR)

[c]Homozygous variant

[d]Torrent Variant Caller called variant as homozygous. Sanger sequencing indicated that the variant was heterozygous

[e]Hemizygous variant

[f]Torrent Variant Caller called heterozygous variant c.953_955del, p.318_319del, and rs201384588. From Sanger trace files, Indelligent (v.1.2) called a heterozygous variant c.952_954del, and p.318del. Manual inspection of the Sanger trace files confirm c.952_954del. Regardless, both variants have the same consequence, which is an in-frame deletion of amino acid 318

[g]SIFT and PolyPhen scores/predictions from Variant Effect Predictor (Ensembl release 85) queried 8/22/2016

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Table 2

Select demographic characteristics of heterotaxy cases and the California reference population

Characteristic[a]	Heterotaxy cases (*n* = 82)	CA live births (*n* = 761,860)	*P* value
Maternal age, years (%)			0.2198[b]
<20	9 (10.98)	109766 (14.41)	
20–34	68 (82.93)	570,167 (74.85)	
35	5 (6.10)	81,803 (10.74)	
Maternal race/ethnicity, *n* (%)			0.0045[b]
Non-Hispanic white	20 (24.69)	219,005 (28.90)	
African American	6 (7.41)	34,744 (4.59)	0.2247[c]
Hispanic	41 (50.62)	445,308 (58.77)	0.1359[d]
Asian	11 (13.58)	51,680 (6.82)	0.0158[e]
Other	3 (3.70)	6936 (1.20)	
Maternal education, years (%)			<0.0001[b]
<12	55 (67.90)	262,503 (35.14)	
12	14 (17.28)	242,396 (32.45)	
>12	12 (14.81)	242,189 (32.42)	
Parity, *n* (%)			0.1376[b]
Nulliparous	22 (26.83)	263,532 (34.63)	
Multiparous	60 (73.17)	497,421 (65.37)	
Infant sex, *n* (%)			0.1162[b]
Male	49 (59.76)	389,168 (51.08)	
Female	33 (40.24)	372,670 (48.92)	
Infant gestational age (mean days ± SD)	266.07 ± 23.94	269.09 ± 16.19	0.2949[f]
Infant birth weight (mean grams ± SD)	2972.9 ± 743.7	3326.81 ± 568.74	<0.0001[f]

[a] Demographic variables are not available for all cases and controls

[b] *P* value determined using χ^2 analysis

[c] *P* value assigned independently to African American versus all other races

[d] *P* value assigned independently to Hispanic versus all other races

[e] *P* value assigned independently to Asian versus all other races

[f] *P* value determined using *t* test

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Table 3

Validated rare CNVs present in individuals with classic heterotaxy

Locus	Genomic coordinates[a]	Size (bp)	Type	Case ID	Gene(s)/transcript(s)
1q31.1	186,260,615–186,295,524	34,910	Dupl	1	*PRG4; RNU6-1240P; TPR*
2q37.3	242,803,051–242,838,636	35,586	Dupl	2	*AC131097.3; AC131097.4;* ***CXXC11***
3p21.31[b]	49,792,023–49,853,073	61,051	Dupl	3	*CDHR4; FAM212A; IP6K1; MIR5193; UBA7*
3q29	192,320,321–192,342,219	21,899	Dupl	4	***FGF12***
5p13.2	36,965,433–37,805,942[c]	840,510	Dupl	5	*C5orf42;* ***NIPBL****; NUP155; RN7SL37P; RNU6-1190P; RNU6-484P; RNU7-75P; WDR70; AC008869.1*
6p12.1	56,917,520–56,954,518	36,999	Dupl	6	*KIAA1586*
6p12.1	56,918,647–56,947,527	28,881	Dupl	7	*KIAA1586*
8p23.2	5,314,092–5,341,562	27,471	Het Del	8	*None*
8p21.1	28,235,553–28,399,631[c]	164,079	Dupl	9	*FBXO16;* ***FZD3****; MIR4288; RNA5SP259; RP11-181B11.2; ZNF395*
10q21.3	70,091,923–70,452,471[c]	360,549	Dupl	10	*DNA2; HNRNPH3; PBLD; RNA5SP319; RUFY2; SLC25A16; TET1; Y_RNA*
12p13.33[b]	357,319–399,485	42,167	Dupl	11	*KDM5A; RP11-283I3.4; RP11-283I3.6; SLC6A13*
16p13.3	6,752,680–7,122,374[c]	369,695	Dupl	12	***RBFOX1****; RNU7-99P; RP11-420N3.2; RNU6-457P; RNU6-328P*
16p13.3	6,748,624–7,122,374[c]	373,751	Dupl	10	***RBFOX1****; RNU6-457P; RNU6-328P*
16p11.2[d]	29,581,764–30,199,579	617,816	Het Del	13	*AC009133.12; AC009133.14; AC009133.15; AC009133.17; AC009133.20; AC009133.21; ALDOA; ASPHD1; C16orf54; C16orf92; CDIPT; CDIPT-AS1; CORO1A; CTD-2574D22.2; CTD-2574D22.4; DOC2A; FAM57B; GDPD3; HIRIP3; INO80E; KCTD13; KIF22; MAPK3; MAZ; MIR3680-2; MVP; PAGR1;* ***PPP4C****; PRRT2; QPRT; RN7SKP127; RP11-455F5.3; RP11-455F5.4; RP11-455F5.5; SEZ6L2; SPN; TAOK2;* ***TBX6****; TMEM219; YPEL3; ZG16*
16q23.3	83,792,314–83,813,294	20,981	Het Del	14	*CDH13; RP11-298D21.1*
17p13.1	7,265,681–7,296,233	30,553	Dupl	15	*C17orf61-PLSCR3; TMEM256-PLSCR3; TNK1*
18p11.21	13,491,489–13,721,509[c]	230,021	Dupl	16	*AP001010.1;* ***FAM210A; LDLRAD4****; MIR4526; RN7SL362P; RP11-53B2.3; RP11-53B2.4; RP11-53B2.5; RP11-53B2.6*
18q12.1[e]	27,873,601–28,057,691	184,091	Dupl	17	***MIR302F***
20q13.2	52,088,801–52,112,562	23,762	Dupl	18	*AL354993.1; RP4-678D15.1; RP5-823G15.5; TSHZ2*

Bolded genes are discussed in text

Dupl duplication, *Het Del* heterozygous deletion

[a]Genomic coordinates were predicted using PennCNV and correspond to genome build hg19

[b]One control was found to be duplicated in this region (the case identified by microarray also carried a duplication, which was confirmed by qPCR)

[c]Genomic coordinates were predicted using cnvPartition and correspond to genome build hg19

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[d]One control was found to be duplicated in this region (the case identified by microarray carried a deletion in this region, which was confirmed by qPCR)

[e]Two additional cases with partially overlapping deletions were detected during validations

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Table 4

Distribution of associated cardiac defects categorized by the cases with and without copy-number variants of note

Additional heart defects (BPA 4 level)	Infants with CNV findings (*N* = 38)		Infants without CNV findings (*N* = 44)	
	No.[a]	**%[b]**	**No.[a]**	**%[b]**
Common truncus	<5		<5	
Transposition of great vessels	22	9.0	19	7.8
Tetralogy of Fallot	<5		<5	
Common ventricle	10	4.1	12	4.9
Ventricular septal defect	16	6.5	9	3.7
Ostium secundum atrial septal defect	16	6.5	23	9.4
Endocardial cushion defects	28	11.4	30	12.2
Cor biloculare	<5		<5	
Anomalous pulmonary valve	19	7.8	19	7.8
Tricuspid atresia/stenosis/insufficiency	<5		<5	
Congenital stenosis–aortic valve	<5		<5	
Insufficiency/bicuspid/other/unspecified aortic valve	7	2.9	10	4.1
Mitral atresia, stenosis, hypoplasia	6	2.5	<5	
Congenital mitral insufficiency	<5		<5	
Hypoplastic left heart syndrome	5	2.0	<5	
Other specified heart anomalies	28	11.4	35	14.3
Unspecified anomalies of heart	<5		<5	
Coarctation of aorta	7	2.9	<5	
Other anomalies of aorta	19	7.8	21	8.6
Anomalies of pulmonary artery	12	4.9	13	5.3
Anomalies of great veins	35	14.3	32	13.1

To protect confidentiality, exact numbers were not reported for defects seen in less than five cases

[a]Count is by number of defects. Cases may be counted more than once

[b]Percentage of defects in each category