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Chromosomal abnormalities among children born with conotruncal cardiac defects

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Abstract

BACKGROUND—Conotruncal heart defects comprise 25%-30% of non-syndromic congenital heart defects. This study describes the frequency of chromosome abnormalities and microdeletion 22q11 associated with conotruncal heart malformations.

METHODS—From a population base of 974,579 infants/fetuses delivered, 622 Californian infants/fetuses were ascertained with a defect of aortico-pulmonary septation. Infants whose primary cardiac defect was tetralogy of Fallot (n=296) or D-transposition of the great vessels (n=189) were screened for microdeletions of 22q11.

RESULTS—Fourteen (2.3%) of the 622 infants/fetuses had chromosomal abnormalities. Thirty infants, 10% of those whose primary defect was tetralogy of Fallot, had chromosome 22q11 microdeletions. Right aortic arch, abnormal branching patterns of the major arteries arising from the thoracic aorta, and pulmonary artery abnormalities were observed more frequently in these children.

CONCLUSIONS—We found an unusual number of infants with an extra sex chromosome and a conotruncal defect. Infants with tetralogy of Fallot due to 22q11 microdeletion showed more associated vascular anomalies than infants with tetralogy but no 22q11 microdeletion. Although these associated vascular anomalies provide clues as to which infants with tetralogy of Fallot are more likely to carry the microdeletion, the overall risk of 10% among all infants with tetralogy of Fallot warrants chromosome analysis and FISH testing routinely.

Keywords

congenital heart defect; conotruncal defect; tetralogy of Fallot; transposition of the great vessels; 22q11 microdeletion

INTRODUCTION

About 0.4-0.6% of newborn infants are delivered with a moderate or severe congenital heart defect (Hoffman et al., 2002; Pierpont et al., 2007). These congenital heart defects are etiologically heterogeneous, and genetic and environmental causes have been proposed for many specific defects (Pierpont et al., 2007; Jenkins et al., 2007; Nora 1968). Conotruncal heart defects, which result from abnormalities of the process of aortico-pulmonary septation, make up about 25%-30% of non-syndromic congenital heart defects (Debrus et al., 1996).

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Conotruncal malformations include tetralogy of Fallot, double outlet right ventricle, persistent truncus arteriosus, and transposition of the great vessels. Recurrence risk data indicate that non-syndromic conotruncal defects have some genetic basis. For tetralogy of Fallot, the recurrence risk in siblings is about 3%, if there is no other affected first degree relative (Nora et al., 1970). Pierpont and coworkers (1988) found that in families with truncus arteriosus and interruption of the aortic arch (type B), the recurrence rate of congenital heart defects was 6.6% and 2.1%, respectively.

Single gene disorders have been linked with certain congenital heart defects. For example, Alagille syndrome (mutations in *JAG1*) is associated with tetralogy of Fallot, pulmonary valve stenosis, and pulmonary hypoplasia, while atrial septal defects and atrioventricular conduction problems are more characteristic of Holt-Oram syndrome (mutations in *TBX5*) (Pierpont et al., 2007). Some studies have reported chromosomal abnormalities among 8-13% of neonates with congenital heart malformations, but few studies have reported on chromosomal abnormalities and specific types of conotruncal heart defects (Ferencz et al., 1989; Voigt et al., 2002; Iserin et al., 1998; Goldmuntz et al., 1993 and 1998). For this analysis, we examined a population-based series of cases that were ascertained as part of a case-control study to describe chromosomal abnormalities occurring among infants/fetuses with conotruncal cardiac malformations.

MATERIALS AND METHODS

The research protocols used for this study were approved by the California Committee for the Protection of Human Subjects and Research Center Oakland Institutional Review Boards.

Study Population

For these analyses, we used clinical data derived from a population-based case-control study, the California Study of Birth Defect Causes. We identified cases and controls from a base population of 974,579 live-born, stillborn, and electively terminated fetuses delivered of mothers who resided in Los Angeles, San Francisco, or Santa Clara counties and who delivered between July 1999 and June 2004. Case infants (diagnosed within 1 year after birth) or fetuses were ascertained by birth defects registry staff, who reviewed hospital logs and medical records at every birth and pediatric referral hospital in the three counties to identify those with defects of aortico-pulmonary septation [tetralogy of Fallot and pulmonary valve atresia with ventricular septal defect (VSD), d-transposition of the great vessels (D-TGV), double outlet right ventricle (DORV), and truncus arteriosus communis]. Anatomic and physiologic features of each case were confirmed by review of all echocardiographic, cardiac catheterization, surgical, or autopsy reports. Reports of chromosomal karyotypes and FISH analyses were obtained by reviewing prenatal diagnostic reports, in-patient medical records, and logs of cytogenetic labs.

In most case series, D-transposition of the great vessels and tetralogy of Fallot together represent about 75% of all defects of aortico-pulmonary septation. Because these two defects were the focus of the maternal interview component of our investigation, we classified each cardiac defect case (n=622) into mutually exclusive groups that reflect pathogenesis of the underlying anatomical cardiac abnormality (see Table 1). There was also one case of a conjoined twin who had D-TGV, but we excluded this twin pair from the investigation. Thus, of the 622 conotruncal defect cases that were ascertained and reviewed, 485 (78%) had either tetralogy of Fallot (n=296) or D-TGV (n=189) as their primary underlying cardiac defect (see Table 1).

Genotyping Methods

A significant percentage of certain conotruncal defects is thought to be attributable to microdeletion of chromosome 22q11. Less than half of the subjects in this investigation had a chromosomal karyotype performed; however, fewer had fluorescent in situ hybridization (FISH) studies (usually with probes D22S75 or TUPLE 1) performed to detect 22q11 microdeletions. We used the quantitative real-time PCR (RT-PCR) approach described by Kariyazono and coworkers (2001) to determine copy number of the 22q11 region for each infant with a primary diagnosis of tetralogy of Fallot or D-TGV who was not clinically tested by FISH for a 22q11 microdeletion. Genomic DNA was used to amplify the ubiquitin fusion degradation gene (UFD1L) within the 22q11 critical region. We designed a TaqMan® probe within exon 12 of UFD1L, using primers and TaqMan® probe sequences as listed in Table 2. Our source DNA was residual dried blood spots from newborn screening blood specimens (Guthrie paper) matched to each case. The DNA was extracted using a modification of the salting out method (Iovannisci 2000) and was resuspended in 10ul TE buffer. We used the RT-PCR technique described by Kariyazono and colleagues (2001), with the following modifications: the volume of each reaction was 10ul and contained 0.4uM fluorogenic probe, 0.8uM for each primer, 1x TaqMan® Universal PCR Mastermix (Applied Biosystems #4324018), 1x TaqMan® RNaseP Control Reagents (VIC) (Applied Biosystems #4316844), and 1-5ng of genomic DNA. Each sample was analyzed in triplicate. Thermal cycling consisted of a hold at 95°C for 10 minutes, and then 45 cycles of a two-step PCR consisting of 95°C for 15 seconds and 60°C for 1 minute. Quantitative analysis was performed using an Applied Biosystems PRISM 7900 Sequence Detection System. To determine copy number, we used the method described in Applied Biosystems "Guide to Performing Relative Quantitation of Gene Expression Using Real-Time Quantitative PCR" (http://www.appliedbiosystems.com/support/apptech/#rt_pcr). A positive control DNA sample with a 22q11.2 microdeletion was obtained from the Coriell Institute for Medical Research (catalog ID-NA13325; DiGeorge syndrome), and was co-amplified parallel to the study samples.

As expected, most subjects showed copy number around 2.0 (no microdeletion), several subjects revealed copy number of 1.0-1.4 for two or three of the RT-PCR assays. Bacterial artificial chromosome array comparative genomic hybridization (BAC array-CGH) was used to confirm which of these cases had 22q11 microdeletions. Array-CGH slides containing >32,000 BAC clones covering >99% of the human genome sequence were generated, as described (Osoegawa et al., 2008). Test DNA from the 8 cases who showed copy number of <1.5 was labeled with Cy3 and reference DNA sample was labeled with Cy5; both were then hybridized to the array-CGH slides. After washing, images were captured and processed. Normalized hybridization results were presented graphically by plotting chromosome positions (X-axis) against $\log_2$ (test/reference) values (Y-axis) (see Figure 1). Deletion/duplication loci were first automatically scored using CGHplotter and GLAD (gain and loss analysis of DNA), and then manually inspected as described (Osoegawa et al., 2008).

RESULTS

Forty-two percent of the 622 conotruncal defect cases of Table 1 had routine chromosomal karyotypes performed and documented in their medical records. Fourteen (5%) of them had chromosomal abnormalities (see Table 3). Among the 14 are three infants with tetralogy of Fallot who had an extra sex chromosome. A fourth infant with a sex chromosome abnormality (mosaic 45,X/46,XX/47,XXX) had a complex cardiac malformation including L-transposition of the great vessels, double-inlet left ventricle, and atrioventricular canal defect. Three infants had *de novo* terminal deletions (Table 3). One tetralogy of Fallot case had a *de novo* terminal deletion of 6p, while two others had abnormal #5 chromosomes; both

had terminal 5p deletions with additional material of uncertain origin translocation to distal 5p. Two other tetralogy of Fallot cases were associated with extra marker chromosomes of unknown origin. One infant with double outlet right ventricle and tetralogy of Fallot had an interstitial deletion of 7q. Additionally, two infants with tetralogy of Fallot had *de novo* apparently balanced translocations, with breakpoints as noted in Table 3. Among the 189 infants whose primary cardiac defect was D-TGV, 34% had routine karyotypes and each was normal. Only one chromosome abnormality was found among an infant with DORV, an insertion of unknown origin to the long arm of chromosome 16.

Among the 622 conotruncal defect cases, we identified only four who had single gene disorders. Individuals with Alagille syndrome, Treacher Collins syndrome, and Cornelia de Lange syndrome had tetralogy of Fallot, while one infant with L-TGV and double-inlet left ventricle was diagnosed with Holt-Oram syndrome.

As Table 1 shows, we ascertained 296 infants/fetuses with tetralogy of Fallot and 189 with D-transposition of the great vessels as their primary cardiac defects. Thus, using these case definitions, the birth prevalence of tetralogy of Fallot is 3 per 10,000 total births while D-TGV is 2 per 10,000 total births. Of the 296 infants/fetuses with tetralogy of Fallot, only 87 (29%) were clinically tested for 22q11 microdeletion using FISH, and 28 showed the microdeletion. Forty (21%) of the 189 infants/fetuses with D-TGV were also tested, but in contrast, no 22q11 microdeletion was detected. We carried out RT-PCR for exon 12 of the UFD1L gene for 303 of 347 subjects (199 tetralogy of Fallot and 148 D-TGV) who had no record of clinical FISH testing. Nine of 303 infants showed low copy number results (<1.5) that suggested a 22q11 microdeletion. To confirm the microdeletion, we performed BAC array-CGH assays. Two individuals with tetralogy of Fallot were confirmed with a 22q11 microdeletion. Figure 1 shows a graphic representation of the chromosome 22q array-CGH results of one of these infants with tetralogy of Fallot. Thus, among 296 tetralogy of Fallot subjects, thirty (10%) had a microdeletion at 22q11. No infant with D-TGV (n=189) was found to have a 22q11 microdeletion.

Because our study population represents one of the only population-based samples of infants with 22q11 microdeletions and tetralogy of Fallot, we compared the frequency of some associated cardiovascular anomalies among these 30 tetralogy of Fallot cases with 22q11 microdeletion versus the 266 without. Table 4 shows the frequencies of associated cardiovascular anomalies among infants/fetuses with tetralogy of Fallot (with and without 22q11 microdeletion) or D-TGV. We found that right aortic arch, abnormal branching patterns of the major arteries arising from the thoracic aorta (right and left subclavian artery anomalies, innominate artery anomaly, anomalous single artery arising from aortic arch), and pulmonary artery abnormalities were more common among tetralogy of Fallot than D-TGV cases. Moreover, each of these three associated cardiovascular abnormalities was more common among tetralogy of Fallot cases with chromosome 22q11 microdeletions than those without. Among the associated cardiovascular abnormalities, no infant with D-TGV had an anomalous left subclavian artery, whereas four infants with tetralogy of Fallot (two with deletion 22q11 and two without) had anomalous origins of their left subclavian artery. Although carotid and subclavian artery anomalies have been described with 22q11 microdeletions, we found two other individuals born with unusual branching patterns from the aortic arch, but had normal chromosomal testing and 22q11 FISH results. One infant had tetralogy of Fallot and a single vessel arising from the aortic arch that then branched into 3 arteries, giving rise to both carotid arteries and an innominate artery. Another infant, with D-TGV, had 2 carotid arteries that branched from a single artery arising from the aortic arch. In contrast to the above observations related to anomalous major arterial vessels, great vein anomalies were seen more commonly in patients with tetralogy of Fallot and normal chromosomes. A great vein anomaly (e.g. left-sided superior vena cava and/or inferior vena

cava) was reported among 13% of tetralogy of Fallot cases with normal chromosomes, compared to a single infant (3%) among those with 22q11 microdeletions (see Table 4). Coronary artery anomalies were much more likely to occur with D-TGV than tetralogy of Fallot (29% vs. 6%).

DISCUSSION

Fourteen (2.3%) infants/fetuses were established with a chromosome aneuploidy. Four of them had a sex chromosome abnormality. These four occurred among 120 females and 146 males who had karyotypes performed. XXX, XXY, and XYY sex chromosome abnormalities each occurs with a frequency of approximately 1 per 1000 livebirths of the appropriate sex (Gardner and Sutherland, 1996). Associations between a sex chromosome abnormality and congenital cardiac defects have been described for Klinefelter (47,XXY) and Turner (45,X) syndromes. Patent ductus arteriosus is the most common cardiac anomaly in Klinefelter syndrome (Visootsak and Graham, 2006). In contrast, aortic valve abnormalities and coarctation of the aorta are commonly found among girls with 45,X Turner syndrome (Mazzanti and Cacciari, 1998). However, no investigations of infants born with an extra X or Y chromosome has identified an increased risk of conotruncal cardiac defects. Thus, we observed an apparent excess of infants with a conotruncal defect who had an extra sex chromosome. As this is the first such association, additional data on chromosomal investigations is warranted.

We found the frequency of chromosome 22q11 microdeletion in children with congenital conotruncal heart malformations to be approximately 10%. This figure is consistent with the frequencies found in other studies (McElhinney et al., 2003; Lammer et al., 1996; Webber et al., 1996). It has been well-established that chromosome 22q11 abnormalities are associated with DiGeorge (DGS), velo-cardio-facial (VCFS), and conotruncal anomaly face syndromes (CTAFS), with frequencies reported to be 83% in DGS, 68% in VCFS, and 100% in CTAFS (Takahashi et al., 1995). But association between a 22q11 microdeletion to non-syndromic conotruncal cardiac defects has not been studied on a large scale. Most of the existing studies have a small cohort of subjects or only represent a case series in that particular institution (Goldmuntz et al., 1998; Iserin et al., 1998; Amati et al., 1995). Our research is the first population-based study of this magnitude to examine the frequency of chromosome aberrations in children with conotruncal heart defects.

Additionally, like other studies (Lammer et al., 1996; Momma et al., 1996 and 1997; Johnson et al., 1995), we found that chromosome 22q11 microdeletions are prevalent in a significant subset of individuals with a conotruncal cardiac defect and another cardiac anomaly. In our cohort of 485 tetralogy of Fallot and D-TGV cases, we found significant association with right aortic arch, pulmonary artery, or major artery branching pattern anomaly. In the 68 infants/fetuses found with a right aortic arch, 24% possessed a 22q11 microdeletion. Of the 98 infants with pulmonary artery hypoplasia, 11% had a microdeletion in the chromosome 22q11 area; and 23% of the 13 patients with atretic main pulmonary arteries also had the deletion. We identified 3 (30%) of the 10 patients with abnormal branching patterns of the aorta with the same microdeletion. Other cardiac defects reported to be associated with conotruncal defects and chromosome 22q11 microdeletions include major aortopulmonary collaterals (Momma et al., 1997), pulmonary valve atresia (Momma et al., 1996; Johnson et al., 1995), and interruption of the aortic arch, especially type B (Lammer et al., 1996; Lewin et al., 1997; McElhinney et al., 2001). Based on these observations, we propose the clinical diagnosis of conotruncal cardiac defects with these associated anomalies can be used as a screening tool for detection of chromosome 22q11 microdeletion.

Furthermore, our findings are in agreement with other studies showing that children with TGV or DORV rarely have a 22q11 microdeletion (Pierpont et al., 2007). Notably, in our cohort of tetralogy of Fallot with normal chromosomes, there was also a significant fraction of subjects (13%) with anomalous great veins (see Table 4). This is well above the reported prevalence of a left-sided superior vena cava in the general population, which is estimated to be about 0.3%; and in those with congenital heart disease, which ranges from 2.8% to 4.3% (Biffi et al. 2001).

In the other cases of chromosomal deletions, a glance at the deleted genes provides some interesting insight. For example, located on the short arm of chromosome 6 is *JARID2*, an ortholog to the mouse *jumonji* (*jmj*) gene. *Jmj* in mice encodes a protein that is important for normal heart development; homozygous *jmj* mutant mice all die at birth. Double-outlet right ventricle was seen in all but two embryos; a prominent ventricular septal defect was seen in all *jmj*-null homozygotes (Lee et al., 2000). In other *jmj*-null mice, defects of the bulbus cordis are reported (Takeuchi et al., 1999). Another gene, *Cav-1*, is on the long arm of chromosome 7. In knockout mice, there is myocardial hypertrophy, associated pulmonary hypertension, and impaired calcium signaling (Murata et al., 2007; Patel et al., 2007; Park et al., 2003). Fifty percent of *cav-1* null mice in one study had reduced life span after 2 years and died suddenly probably from severe right heart failure or from an acute arrhythmia (Park et al., 2003). Perhaps these genes are also important in heart development in humans; thus, chromosomal aberrations as found in infants of our population might result in some of the ascertained congenital heart defects.

In summary, our population-based study showed that chromosomal anomalies and 22q11 microdeletions represent a substantial subset of patients with tetralogy of Fallot, but not among infants with D-TGV. Our results illustrate that infants with tetralogy of Fallot due to 22q11 microdeletion have more associated vascular anomalies than infants with tetralogy but no 22q11 microdeletions. Although these associated vascular anomalies provide clues as to which infants with tetralogy of Fallot are more likely to carry a 22q11 microdeletions, the overall risk of about 10% among all infants with tetralogy probably warrants chromosome analysis and FISH in every case.

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Figure 1.

Results of array comparative genomic hybridization were plotted on graphs with chromosome positions on the X-axis and $\log_2(\text{test/reference})$ values on the Y-axis. If the copy number of specific sequences in the test DNA is higher or lower than the reference, the ratio will appear increased or decreased, respectively. In normal circumstances, the ratio between the test (patient's) DNA and the reference DNA will be 1, thus the $\log_2(\text{test/reference})$ value will be 0.

Table 1

Each of the 622 cardiac defect cases was separated into mutually exclusive categories

Primary Cardiac Defect	Number of Infants/Fetuses
L-Transposition of Great Vessels (L-TGV)	60
Endocardial Cushion Defect with TGV or tetralogy of Fallot	27
Double inlet left ventricle with TGV	3
Double outlet right ventricle	44
Truncus Arteriosus Communis	2
D-Transposition of Great Vessels (D-TGV)	190 ^a
Tetralogy of Fallot (includes pulmonary valve atresia with ventricular septal defect)	296
TOTAL	622

^aOne infant was a conjoined twin so we excluded him from further analyses.

Table 2

Primers and TaqMan™ probe sequences used in RT-PCR

Primer	Sequence
UFD1L Forward Primer	5'- AGGCAGATTCGTCGCTTT-3'
UFD1L Reverse Primer	5'- CAGCCAACAGTCCTCACTT-3'
UFD1L probe	5'-FAM-TCTGGAGAAGGACAGTCATTGCCTAAAA-TAMRA-3'

Table 3

Chromosome abnormalities among infants/fetuses with aortico-pulmonary septation defects

Chromosomal Abnormality	Tetralogy of Fallot	L-TGV ^a	DORV ^b
47,XXY	1		
47,XXY/46,XY mosaic	2		
45,X/46,XX/47,XXX mosaic		1	
del 6p24.2	1		
add5p14; ish del (5)(p15.2p15.2)	1		
add5p?15?	1		
46,XY, ins (16;?)(q12.1;?)			1 ^c
46,XY, del(7)(q22q31.2)			1 ^d
46,XY, der(21) t(8;21)(p21.1;p12)mat			1 ^d
46,XX t(10;13)(q24;q14)	1		
46,X? t(1;9)	1		
47,XY +mar mat	1		
48,XX +mar1+mar2. ish mar1(WCP5+), mar2(WCPX+)/ 47,XX+mar1/47,XX+mar2/ 46,XX	1		

^aL-TGV=L-transposition of great vessels^bDORV=double outlet right ventricle^cThis subject was also diagnosed with D-transposition of the great vessels.^dThese individuals also had tetralogy of Fallot.

Table 4

Cardiovascular anomalies associated with tetralogy of Fallot and chromosome 22q11 microdeletion.

Cardiovascular Anomalies	TOF^a + del22q11 (30)	TOF w/o del22q11 (266)	D-TGV^b (189)
Pulmonary Valve Atresia	19 (63%)	70 (26%)	4 (2%)
Pulmonary Artery Atresia	3 (10%)	10 (4%)	0 (0%)
Hypoplastic Pulmonary Artery	11 (37%)	79 (30%)	8 (4%)
Right Aortic Arch	16 (53%)	43 (16%)	9 (5%)
Abnl Branching Pattern of Major Peripheral Arteries ^c	3 (10%)	6 (2%)	1 (0.5%)
Aortopulmonary Collaterals	4 (13%)	11 (4%)	2 (1%)
Coronary Artery Abnormalities	2 (7%)	15 (6%)	54 (28%)
Great Vein Anomalies ^d	1 (3%)	35 (13%)	10 (5%)

^aTOF=tetralogy of Fallot

^bD-TGV=D-transposition of the great vessels

^cIncludes aberrant right or left subclavian artery and innominate artery anomalies.

^dIncludes left-sided superior vena cava and/or inferior vena cava.