*Cardiology in the Young* (2015), **25**, 1155–1172
doi:10.1017/S1047951114001838



*Original Article*

# Array comparative genomic hybridisation testing in CHD

Hannah B. Hightower,[1] Nathaniel H. Robin,[2] Fady M. Mikhail,[2] Namasivayam Ambalavanan[1]

[1]*Department of Pediatrics;* [2]*Department of Genetics, University of Alabama at Birmingham, Birmingham, Alabama, United States of America*

**Abstract** *Background:* CHD is the leading cause of mortality due to birth defects. Array comparative genomic hybridisation (aCGH) detects submicroscopic copy number changes and may improve identification of the genetic basis of CHD. *Methods:* This is a retrospective analysis of 1252 patients from a regional referral centre who had undergone aCGH. Of the patients, 173 had CHD. A whole-genome custom-designed oligonucleotide array with >44,000 probes was used to detect copy number changes. *Results:* Of the 1252 patients, 335 (26.76%) had abnormal aCGH results. Of the 173 patients with CHD, 50 (28.9%) had abnormal aCGH results versus 284 (26.3%) of 1079 non-cardiac patients. There were six patients with CHD who had well-described syndromes such as Wolf–Hirschhorn, trisomy 13, DiGeorge, and Williams. Of the patients with CHD, those with left-sided heart disease had the highest proportion (14/31; 45.13%) of abnormal aCGH results, followed by those with conotruncal heart disease (10/29; 34.48%), endocardial cushion defects (13/50; 26%), complex/other heart disease (12/52; 23.08%), and patent ductus arteriosus (1/11; 9.09%). *Conclusions:* Patients with CHD are at a substantial risk of having microdeletions and microduplications. The incidence of abnormalities on aCGH analysis is higher than identified with karyotype, and identification of copy number changes may help identify the genetic basis of the specific heart defects. However, aCGH may not have a significant diagnostic yield in those with isolated CHD. Further research using larger data sets may help identify candidate genes associated with CHD.

Keywords: CHD; array comparative genomic hybridisation (aCGH); copy number variants

Received: 18 June 2014; Accepted: 1 September 2014; First published online: 8 October 2014

CHD ARE THE MOST COMMON MAJOR BIRTH DEFECTS with an estimated incidence of 10/1000 live births.[1–3] Despite great advances in medical care for these newborns, considerable morbidity and mortality continue to be associated with CHD as it is the leading cause of infant death and illness associated with birth defects.[4] Of the total number of cases of CHD, 20% are due to aneuploidy and other chromosomal syndromes, with the remaining 80% classified as "sporadic" CHD.[5] Most patients with CHD do not have affected family members and the low recurrence rate suggests polygenic inheritance, but there may also be a high rate of de novo mutations.[6] Other than single-gene analysis, chromosomal microarray testing has been shown to have a high diagnostic yield by detecting the genetic basis of disease due to pathogenic genomic copy number variants. Copy number variants are duplications and/or deletions that cause a change in the gene dosage. If they are found in >1% of the general population, they are considered polymorphisms; however, if present in <1% of individuals, they are more likely to be disease causing.[7]

Array comparative genomic hybridisation (aCGH) is a DNA microarray-based technology that detects submicroscopic copy number variants in the genome in the kilobase range making it a more sensitive

Correspondence to: N. Ambalavanan, MD, Women and Infants Center, University of Alabama at Birmingham, 176F Suite 9380, 619 South 19th Street, Birmingham, AL 35249-7335, United States of America. Tel: +205 934 4680; Fax: +205 934 3100; E-mail: nambalavanan@peds.uab.edu

modality when compared with traditional karyotype analysis, which has a resolution of only 5–10 Mb.[7] The increased resolution over conventional karyotyping is therefore at least fivefold and is the major advantage of this molecular technique.[8] aCGH is widely used in clinical practice and recent studies have demonstrated its ability to detect pathogenic copy number variants in 10–15% of patients with developmental delay, intellectual disability, and multiple congenital anomalies.[9] In addition, Reddy et al[10] showed an increase of 41.9% in diagnosis of clinically important deletions and duplications by aCGH in stillbirths.

Previous studies have indicated a high rate of chromosomal abnormalities occuring in children with CHD. Bachman et al[11] examined 45 CHD patients by karyotype analysis and aCGH and identified an additional 22.2% of patients with copy number variants, suggesting aCGH should be used as a first-tier test for neonates with CHD. Warburton et al[12] analysed 223 families with at least one child affected by conotruncal or hypoplastic left heart disease and found a substantially higher rate of de novo copy number variants in probands with CHD than in control families (9 versus 2%). Further work by Carey et al compared long-term outcomes in patients with single ventricles with and without copy number variants. Children with CHD had 10% more rare copy number variants who in turn had worse neurocognitive and growth outcomes at 14 months of age.[13]

Even with increasing evidence of improved detection of chromosomal imbalances with aCGH, many questions remain unanswered. Of the individuals evaluated by aCGH, are those with CHD at higher risk of abnormal results? And do specific copy number variants correspond to specific heart defects? The purpose of this study was to identify aCGH abnormalities in patients with CHD and compare them with a population without CHD. We also wished to determine whether there were common copy number variants in CHD, which may suggest involvement of novel genes in heart disease or development.

## Methods

This was a retrospective cohort study of all patients who underwent aCGH between January, 2009 and March, 2011 at the University of Alabama at Birmingham. The Institutional Review Board at The University of Alabama at Birmingham approved the study. The University of Alabama at Birmingham is a regional referral centre and the only paediatric cardiac surgery centre in the state of Alabama. There was an estimated 60,000 live births per year in Alabama during the time of the study of which ~2.3/1000 required some sort of cardiac intervention in the 1st year. Therefore, it is estimated that each year 138 children in the state of Alabama would require cardiac intervention within the 1st year of life. We collected aCGH results from a database maintained by the University of Alabama at Birmingham Department of Genetics and clinical diagnostic information regarding their heart disease and other conditions from electronic medical records. Informed consent was waived as no personal identifying information was collected.

All individuals who underwent an aCGH for any reason during the time of the study were evaluated. Individuals with CHD were compared with individuals without heart disease. The majority of aCGH analyses were performed on patients who also had neurodevelopmental problems and/or congenital anomalies. These additional diagnoses are listed in Table 1 along with cardiac diagnosis and results of the aCGH. The diagnosis of CHD was confirmed when it was made by a cardiologist with echocardiographic confirmation. As this is a retrospective study, the diagnoses were obtained from medical records and were made by referring physicians during clinical visits. Patients were only excluded if test results or medical information was not available. Patients with CHD were placed into one of five categories based on the nature of structural anomalies, including left-sided heart disease – aortic valve anomalies, hypoplastic left heart syndrome, and coarctation of the aorta; endocardial – atrial septal defect, ventricular septal defect, and atrioventricular canal defect; conotruncal – tetralogy of Fallot, transposition of the great arteries, double outlet right ventricle, interrupted aortic arch, and truncus arteriosus; patent ductus arteriosus; and complex/other heart disease – pulmonic stenosis/atresia, total anomalous pulmonary venous return, and more complex defects such as complex atrioventricular canal and transposition of the great arteries, and complex double outlet right ventricle and coarctation of the aorta.[12]

High-resolution whole-genome aCGH analysis was performed using the $4 \times 44$k Agilent oligo-array (Agilent Technologies, Santa Clara, California, United States of America). This is a custom-designed array that is based on the International Standards for Cytogenomic Arrays consortium design. DNA was extracted from the patients' peripheral blood using the Qiagen blood mini kit (Qiagen, Valencia, California, United States of America). DNA labelling, slide hybridisation, washing, and scanning were performed following the manufacturer's protocol. The arrays were scanned using the Agilent high-resolution microarray scanner (Agilent Technologies). The scanned arrays were analysed using the "Feature

https://doi.org/10.1017/S1047951114001838 Published online by Cambridge University Press

Extraction v9.5" and "DNA Analytics v4.0" software (Agilent Technologies). All genomic breakpoints were mapped using the UCSC genome browser using human genome build 36 (NCBI36/hg18). Interpretation of abnormal aCGH results was carried out according to the American College of Medical Genetics standards and guidelines for interpretation and reporting of postnatal constitutional copy number variants. Copy number variants were classified into benign, variant of uncertain clinical significance, likely benign, variant of uncertain significance, likely pathogenic, and pathogenic.[14]

## Results

A total of 1309 patients underwent aCGH analyses between January, 2009 and March, 2011. A total of 57 patients were excluded from the study because of inadequate records or inability to match to medical records. Of the remaining 1252 patients who were analysed, there were 173 patients who had a diagnosis of CHD. The diagnosis of CHD ranged from simple defects such as a ventricular septal defect or valvular stenosis to commonly described defects such as tetralogy of Fallot or transposition of the great vessels. Some patients had more complex and mixed structural CHD.

Many patients with CHD were diagnosed with known syndromes. Of the patients, eight had well-described cytogenetic syndromes: three patients with Williams syndrome, two patients with CHARGE syndrome, one patient with DiGeorge syndrome, one patient with trisomy 13, and one patient with Wolf–Hirschhorn syndrome. In addition, several patients were ultimately diagnosed with other syndromes or single-gene disorders such as campomelic dysplasia, Smith–Lemli–Opitz, Sotos, Noonan, Alagille, and Beckwith–Wiedemann syndromes. Of the 173 patients with identified CHD, 151 had other clinical problems including developmental delays, dysmorphisms, or congenital malformations, as described in Table 1. Therefore, only 22 patients could be considered as having isolated CHD. Of those 22 patients, only three had abnormal array results and they were all duplications: one patient with supravalvular aortic stenosis had a duplication at 5p14.1; a male infant with aortic hypoplasia, ventricular septal defect, and an anomalous right subclavian artery had a duplication at Xp27.1; and an infant with d-transposition, ventricular septal defect, and coarctation of the aorta had a duplication at 6q11.1.

Of the 1252 total patients included in the study, 335 (26.76%) had abnormal aCGH results. Of the 173 patients with identified CHD, 50 (28.9%) had abnormal aCGH results. Of the 50 cardiac patients with abnormal aCGH, 34 had also been examined by traditional karyotype analysis, and 12 of the 34 karyotypes were abnormal including six translocations, one ring chromosome, two deletions, one duplication, one marker chromosome, and one trisomy (Table 1). Although it is well documented in the previous literature that aCGH is more sensitive than traditional karyotype in identifying copy number variants, our cohort did not have enough karyotypes performed to have statistical evidence to support this. Of the 1079 patients without a diagnosis of CHD, 284 (26.3%) had abnormal aCGH results, not statistically different from those with CHD.

The cardiac patients were analysed by category with the highest percentage of abnormal aCGH results being in the left heart disease group with 14/31 (45.16%), and lower percentages in other categories ($p = 0.03$ by $\chi^2$ compared with all other categories combined; Table 2).

To determine whether any of the aCGH abnormalities spanned regions known to be involved in cardiac development, we evaluated chromosomal regions that span genes known to be involved in cardiac development or disease. There were three patients in this study who were noted to have copy number variants involving two of these genes. *TBX5* (12q24.2) encodes a protein called T-box 5 that plays an important role during embryonic development. T-box 5 is a transcription factor that activates genes involved in the normal development of the upper limbs and the heart, specifically the formation of the ventricular septum and the electrical system.[15] This area was duplicated in a patient with a right ventricular dominant atrioventricular canal, a small left ventricle and hypoplastic transverse aorta, and transposed great vessels with anterior main pulmonary artery. She also had 11 rib pairs with the fusion of T5–T6 and dysplastic vertebrae. There were two patients who had abnormalities at 8p23.1 that span the *GATA4* gene. *GATA4* is a zinc finger transcription factor thought to regulate genes involved in embryogenesis and in myocardial differentiation and function. Mutations in this gene have been associated with cardiac septal defects.[16] A patient had an 8p23.1 duplication that spans *GATA4* with multiple aCGH abnormalities: arr 8p23.3p23.1(181,530-6,867,773)x3, 8p23.1(10,856,762-12,448,433)x3, and 18q23(73,323,973-76,111,164)x1, with the phenotype of pulmonic stenosis, a hypoplastic right ventricle, and aortic stenosis. Another patient with a partial atrioventricular canal and left superior vena cava had an 8p23.1 deletion: arr 8p23.1(11,623,512-11,653,694)x1 that does not include *GATA4*. She also had a congenital diaphragmatic hernia, frontal bossing, high nasal bridge, and mild hypotonia.

Other genes implicated in cardiac disease were reviewed. *ZIC3* is a gene that encodes a putative zinc

https://doi.org/10.1017/S1047951114001838 Published online by Cambridge University Press

Table 1. Cardiac patients who had abnormal array results with their specific abnormality and CHD.

| aCGH result | Cardiac diagnosis | Other diagnosis | Karyotype |
|---|---|---|---|
| arr 7q11.23(72,404,049-73,771,409)x1 | Aortic stenosis | Williams syndrome | n/a |
| arr 15q14(31,745,910-37,000,416)x1 | Tetralogy of Fallot | Cleft palate, developmental delay | n/a |
| arr 4q34.1q35.2(176,125,327-190,706,472)x1, 8q23.3q24.3(117,138,625146,250,965)x3 | ASD | Small omphalocele, anteriorly placed anus, cleft palate | 46,XX,der(4)t(4;8)(q34.1;q23.3) |
| arr 22q11.21(17,041,524-20,139,150)x3 | ASD, VSD | Coloboma, amblyopia, left microophthalmia | n/a |
| arr 22q11.21(17,299,742-19,835,558)x1 | Tetralogy of Fallot | DiGeorge syndrome | n/a |
| arr 5p14.2(23,539,185-24,587,824)x4 | VSD | Intestinal duplication cyst | 46,XX |
| arr 9pterqter(204,167-140,193,874)x2~3 | ASD, VSD | Dysmorphic features | 46,XX |
| arr 7q11.23(72,404,049-73,771,409)x1 | Supravalvular aortic stenosis | Williams syndrome | n/a |
| arr 16p11.2(29,581,255-30,098,210)x1 | Tetralogy of Fallot, pulmonary atresia with MAPCAS | Absent left kidney, bilateral optic nerve coloboma, abdominal wall defect, dysplastic rib cage, vertebral anomalies, seizures | 46,XX |
| arr 15q11.2(20,249,686-20,851,879)x3 | Unbalanced AV canal | Abdominal hernia, posterior cleft palate, vertebral anomalies | 46,XY |
| arr 15q11.2(20,249,686-20,610,655)x1 | VSD | Right microtia, left sensorineural hearing loss | n/a |
| arr 7q11.23(72,404,049-73,771,409)x1, 15q1.2(20,249,68620,851,879)x3 | Pulmonic stenosis | Williams syndrome, tracheal stenosis | 46,XY |
| arr Xq13.3q21.1(75,940,969-76,731,764)x3, 5q35.2q35.3(175,491,749-177,355,507)x1 | Large secundum ASD, Wolff–Parkinson–White syndrome | Developmental delay, coarse facial features, large head size (Sotos syndrome) | 46,XX |
| arr Xq23(113,589,767-114,623,576)x3, 15q11.2(20,316,792-20,769,096)x1 | VSD, coarctation of the aorta | Short stature, retrognathia, rib anomalies | 46,XX |
| arr 5p14.1(28,088,325-28,783,564)x3 | Supravalvular aortic stenosis | None | n/a |
| arr 12q24.13q24.33(112,690,731–132,278,200)x3, 14q32.31q32.33(101,324,423-106,34,665)x1 | Right ventricular dominant AV canal with small left ventricle and hypoplastic transverse aorta, transposed great vessels with anterior MPA | 11 rib pairs with fusion of T5–T6, dysplastic vertebrae | 46,XX,der(14)t(12;14)(q24.13;q32.31) |
| arr Xp22.33/Yp11.32(1,474,170-1,661,107)x1 | Tetralogy of Fallot | Cryptorchism, mild ptosis | 46,XY |
| arr 3p26.2(4,001,138-4,844,315)x3 | Coarctation of aorta | Two-vessel cord, absent left kidney, left facial and ear tags | 46,XY |
| arr 16p12.1(21,744,793-22,315,573)x1 | Tetralogy of Fallot | Developmental delay, seizure disorder, mandibular crowding | n/a |
| arr Xp22.33/Yp11.32p11.31(312,889-1,739,016)x3 | VSD, PFO, bilateral SVC | Tracheoesophageal fistula, oesophageal atresia, butterfly vertebra T9, hemivertebrae L3–L4, right multicystic kidney, undescended testicles, tethered cord | 46,XY |
| arr 20p13p12.1(27,988-17,314,365)x3, 21q22.2q22.3(39,981,232-46,914,886)x1 | Large perimembranous VSD and ASD | Frontonasal encephalocele, hemivertebrae, anomalous optic discs, mild ptosis | 46,XY,der(21)t(20;21)(p12.1;q22.2)mat |
| arr 1p36.33p36.21(769,390-15,648,728)x1, 4p16.3p14(41,213-36,935,854)x3 | Tetralogy of Fallot | Malrotation, seizures, ventriculomegaly, central hypogonadism, bilateral foot deformities, cleft lip and palate, hip dimples, hiatal hernia, bilateral blepharophimosis, coloboma or posterior iris | 46,XY,der(1)t(1;4)(p36.2;p14) |
| arr 12p13.33(881,753-1,686,512)x3 | Hypoplastic left heart syndrome | Asperger syndrome, bipolar syndrome, seizures | n/a |
| arr 9p24.3(193,993-1,655,435)x3 | Tetralogy of Fallot with pulmonary atresia | Conductive hearing loss, scoliosis, hemivertebrae (CHARGE) | n/a |

Table 1. *Continued*

| aCGH result | Cardiac diagnosis | Other diagnosis | Karyotype |
|---|---|---|---|
| arr 1q21.1(144,854,346-146,375,859)x1 | Unbalanced AV canal and pulmonic stenosis | Abdominal situs inversus, asplenia, congenital hiatal hernia, microgastria (heterotaxy syndrome) | 46,XX |
| arr 13q34(114,076,863-114,110,891)x1 | ASD | Developmental delay | 46,XY,r(13)(p13q34) |
| arr 2q22.1q22.2(142,003,513-142,612,513)x1, 5q23.2(121,714,400-124,265,622)x1, 18q22.2q23(65,740,013-74,825,495)x1 | Truncus arteriosus and left aortic arch | Two-vessel cord | 46,XX,del(18)(q22.2q23) |
| arr 8p23.3p23.1(181,530-6,867,773)x3, 8p23.1 (10,856,762-12,448,433)x3, 18q23 (73,323,973-76,111,164)x1 | Pulmonic stenosis, hypoplastic right ventricle, aortic stenosis | Hydrops, bilateral hydronephrosis | 46,XY,der(18)t(8;18) (p23.1;q23) |
| arr 2q11.1q12.1(94,964,824-102,140,648)x3 | VSD | Unilateral left cleft lip, asymmetric gluteal cleft | mos 47,XY,+mar[25]/46, XY[5] |
| arr Xp21.1(36,974,995-37,184,519)x0 | Unbalanced AV canal with dominant double outlet right ventricle | Imperforate anus, duodenal atresia | 46,XY |
| arr Xp27.1(139,411,928-139,714,069)x2 | Critical aortic hypoplasia, large VSD, retroesophageal anomalous right subclavian artery | None | 46,XY |
| arr 16p13.3(36,566-306,525)x1, 17q25.3 (73,165,786-78,637,983)x3 | PDA | Dysmorphic facies | n/a |
| arr 17q25.1(69,359,232-70,118,688)x3 | Ventricular inversion and small VSD | Sinus inversus and malrotation | 46,XX |
| arr 15q13.3(29,885,762-30,297,218)x3 | bicuspid aortic valve | Small size, bilateral inguinal hernias, unilateral undescended testicle, penile torsion | 46,XY |
| arr 1q23.1q23.3(156,864,063-162,368,957)x1 | Tetralogy of Fallot with pulmonary atresia | Scoliosis, developmental delay, cognitive delay, dysmorphic features | n/a |
| arr 13q31.2(86,548,624-86,905,954)x1 | Coarctation of Aorta, mitral valve stenosis, aortic stenosis, Shone's syndrome (hypoplastic left heart syndrome) | Gastroschisis, small optic nerves, agenesis of sorpus callosum, septo-optic dysplasia | n/a |
| arr 15q11.2(20,249,686-20,851,879)x3 | Pulmonary atresia with intact ventricular septum | Dysmorphic septum | 46,XX |
| arr 14q24.2q23.33(71,527,659-106,334,665)x3 | bicuspid aortic valve | Small size, dysmorphic | 46,XX,dup(14) (q24.2q32.33) |
| arr 2p14(64,436,584-64,926,957)x3 | ASD, VSD | Neutropenia, bilateral radial-ulnar fusion, Klippel–Feil syndrome, syringomelia | 46,XY |
| arr 6q11.1(62,029,851-62,935,353)x3 | D-transposition of the great arteries, VSD, coarctation of aorta | None | 46,XY |
| arr 4p16.1(9,314,567-10,060,689)x3 | Coartation of the aorta | Hydrocephalus | 46,XX |
| arr 15q26.2q26.3(93,810,852–100,201,137)x1 | Coarctation of aorta, small VSD | Developmental delay, small size, mild clinodactyly | 46,XX,del(15)(q26.2) |
| arr 4p16.3p16.2(62,24703,306,755)x1 | VSD | Wolf–Hirschhorn syndrome (developmental delay, small size, VSD, prominent forehead and glabella) | 46,XX |
| arr 13q12.11q34(19,309,335-114,110,891)x3 | Tricuspid atresia, pulmonary atresia, small right ventricle, enlarged right atria and left ventricle, large VSD | Trisomy 13: bilateral hydronephrosis, bifid thumbs, crossed polydactyly | 47,XY,+13 |
| arr 8p23.1(11,623,512–11,653,694)x1 | Partial AV canal, left SVC | Congenital diaphragmatic hernia, frontal bossing, high nasal bridge, mild hypotonia | 46,XY |

Table 1. *Continued*

| aCGH result | Cardiac diagnosis | Other diagnosis | Karyotype |
|---|---|---|---|
| arr 17q21.31(39,493,007-39,718,923)x1 | mild Ebsteins and malformation of tricuspid valve | Developmental delay, dysmorphic features, mental retardation, short stature, microcephaly, bilateral club feet, hypotonia, supernumery teeth, bilateral cryptorchism, inguinal hernias | n/a |
| arr 6q24.3(147,930,940-148,631,887)x3 | Bicuspid aortic valve with stenosis | Myopia, dysmorphic features | n/a |
| arr 22q11.2(17,041,524-19,891,6700)x3 | Coarctation of aorta | Developmental delay, poor social skills, autism | 46,XY |
| arr 15q11.2(20,316,792–20,851,879)x3 | Transposition of great arteries, VSD | Laryngomalacia, required Gtube | 46,XY,t(4;21)(p12;q11.2) |
| arr 7q21.13(88,152,547-89,638,023)x3 | Dilated aortic root, small VSD | Myopia, learning problems | n/a |

aCGH = array comparative genomic hybridisation; ASD = atrial septal defect; AV = atrioventricular; MAPCAS = major aortopulmonary collaterals; MPA = main pulmonary artery; PDA = patent ductus arteriosus; PFO = patent foramen ovale; SVC = superior vena cava; VSD = ventricular septal defect

finger transcription factor whose cytogenetic location is at Xq26.2 and has been implicated in heterotaxy and isolated CHD.[17] NK Homeobox 5 (*NKX2-5*) located at 5p35.1 is essential in cardiac development, and mutations cause various congenital heart malformations.[18] *GATA6* (cytogenetic location 18q11.2) encodes for a transcription factor, which is broadly expressed in the developing heart and is critical for normal cardiac morphogenesis.[19] *LEFTY* located at 1q42.12 is implicated in left/right axis malformation and has been demonstrated in mouse models.[20] *CRELD1* is located at 3p25.3 and has been implicated in atrioventricular septal defects.[21] *NODAL* is also involved in left- to right-axis malformation and heterotaxy.[22] *TBX20* located at 7p14.2 is involved in morphology of the heart and has been implicated in atrial septal defects and tetralogy of Fallot.[23] No cardiac patients in this study had array abnormalities that correspond to these cytogenetic loci.

Patients with abnormal aCGH results but no CHD are listed in Table 3. Common microdeletions and microduplications such as 22q11 and 15q11 were observed in this population as well. However, the non-cardiac group had many results involving the sex chromosomes X and Y. There was no significant overlap in abnormal results between the cardiac and non-cardiac groups. A non-cardiac patient with preaxial polysyndactyly of the great toes, frontal bossing, and developmental delay had a deletion in the cytogenetic location of *TBX20*.

## Discussion

Our study demonstrates the utility of aCGH in CHD in a reasonably large single-centre cohort. In our cohort, although patients with CHD had no more copy number variants than patients without CHD, patients with left-sided heart disease had an increased rate of microdeletions and microduplications. We also identified specific aCGH anomalies associated with CHD in this cohort that may enable future mechanistic studies of abnormal cardiac development.

aCGH is capable of identifying submicroscopic chromosomal abnormalities that may be missed by traditional karyotype. It has been increasingly used to detect abnormalities in individuals with developmental delay, intellectual disability, autism spectrum disorders, and/or multiple congenital anomalies.[24] The G-banded karyotype is the classic test for cytogenetic abnormalities, but it may miss imbalances in the 5–10 Mb range. It is also limited because of interpersonal and interlaboratory variation in detection rates. aCGH is more sensitive at identifying pathologic copy number variants owing to the higher resolution. The increased resolution allows for increased detection of disease-causing genes. In a

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Table 2. Diagnostic yield of aCGH analysis in patients with various categories of CHD.

| | Left heart disease | Conotruncal heart disease | Endocardial heart disease | Other/complex heart disease | Patent ductus arteriosus |
|---|---|---|---|---|---|
| Abnormal aCGH result | 14 | 10 | 13 | 12 | 1 |
| Normal aCGH result | 17 | 19 | 37 | 40 | 10 |
| Total | 31 | 29 | 50 | 52 | 11 |
| % Abnormal | 45.16 | 34.48 | 26 | 23.08 | 9.09 |

aCGH = array comparative genomic hybridisation

sample of 532 stillbirths, aCGH provided better detection of genetic abnormalities (8.3% by aCGH versus 5.8% by karyotype; $p = 0.007$).[10]

aCGH may permit the identification of abnormalities in genes known to be involved in cardiac development, and help in identifying possible novel genes involved in CHDs. In our study, of the 50 patients with CHD and abnormal aCGH results, three had abnormalities involving genes known to be involved in cardiac development (*TBX5* and *GATA4*). Several novel genes that may possibly be involved in CHD were identified. A patient with tetralogy of Fallot, developmental delay, seizure disorder, and mandibular crowding had the following array abnormality: arr 16p12.1(21,744,793-22,315,573)x1. When examining the deleted region with the USCS Browser (http://www.genome.ucsc.edu/cgi-bin/hgGateway), multiple genes, such as *0T0A, UQCRC2, VWA3A, EEF2K,* and *P0LR3E*, were identified in the deleted area, but none of them have found to be associated with CHD.[25]

Another patient with a partial atrioventricular canal, left superior vena cava, congenital diaphragmatic hernia, frontal bossing, high nasal bridge, and mild hypotonia had the following deletion: arr 8p23.1 (11,623,512-11,653,694)x1. This location corresponds to the *NEIL2* gene (*Homo sapiens* nei endonuclease VIII-like 2), which belongs to a class of DNA glycosylases homologous to the bacterial Fpg/Nei family (MIM 608933). These glycosylases initiate the first step in base excision repair by cleaving bases damaged by reactive oxygen species and introducing a DNA strand break via the associated lyase reaction. This gene has been found to be important in predisposition to certain cancers.[26] There were five copy number variants at this locus, four deletions and one duplication, also described by Soemedi et al.[27]

Soemedi et al[27] described an association between recurrent 15q11.2 deletions and those with CHD, but no genes in the region have been previously associated with CHD. Our population with CHD had only one patient with 15q11 deletion, but three with a duplication of the locus. Greenway et al[28] described recurrent copy number variants in patients with tetralogy of Fallot, including copy number variants at 1q21.1, 3p25.1, 7p21.3, and 22q11.2. In our study, no patients were identified with copy number variants involving 3p25.1 or 7p21.3. There was one patient with a deletion of 1q21.1 that had an unbalanced atrioventricular canal and heterotaxy. There were three patients with copy number variants involving 22q11.2, of which two were duplications and one was a deletion. The patient with a deletion had non-syndromic tetralogy of Fallot. The one with duplication had an atrial septal defect, ventricular septal defect, left micropthalmia, and colobomas. The other had coarctation of the aorta along with developmental delay and a diagnosis of autism.

The utility of routine aCGH analysis in all patients with CHD is currently unclear. Richards and Garg performed aCGH on 40 individuals, 20 of whom had CHD and other anomalies or developmental delay (syndromic CHD), and the others had isolated CHD. The risk of having disease-causing copy number variants was 45% in syndromic CHD, compared with none with isolated CHD.[7] Similarly, Richards et al recruited 20 children with CHD and additional birth defects and compared them with 20 children with isolated CHD. They detected a 25% rate of copy number variants in the population with additional defects – mostly in those with neurologic defects – but none in the population with isolated CHD. This study advocates for screening of copy number variants in children with CHD and a neurologic abnormality.[29] In addition, Erdogan et al[6] described copy number variants in 105 individuals with isolated CHD. They only detected 18 rare copy number variants and the majority were duplications. Therefore, although rare copy number variants may play a role in isolated CHD, aCGH may not be clinically indicated.[6,7]

In contrast, Thienpont et al examined 60 patients with CHD and either a second major malformation and/or mental handicap who could not be diagnosed genetically with clinical exam and karyotyping. Of the patients, 30% carried imbalances that are not described in normal individuals.[30] Breckpot expanded this study to an additional 90 patients and found that 31% had potentially significant imbalances.[8]

https://doi.org/10.1017/S1047951114001838 Published online by Cambridge University Press

Table 3. Non-cardiac patients who had abnormal array results.

| aCGH result | FISH/karyotype/other | Diagnosis |
|---|---|---|
| arr 17p13.1(7,256,581-7,651,389)x3 | 46,XY | FTT |
| arr 22q11.1qter(15,957,762-49,525,271)x2~3 mosaic in 10% of cells | 46,XY 22q13(ARSAx3)[20]/22q11.2 (Tuple1x2),22q13(ARSAx2)[180] | Dysmorphic, short stature, malocclusion, left Duane anomaly |
| arr 12q24.11q24.33(108,854,486-132,278,200)x3 | 46,XY,der(15)t(12;15)(q24.11;p13) de novo, MECP2: neg, Angelman methylation: normal | Pyloric stenosis, tethered cord, GT/fundo, non-verbal |
| arr 15q11.2q13.1(21,258,345-26,194,049)x1 | + methylation for Angelman syndrome | Angelman syndrome: developmental delay, symmetric small (<5%) |
| arr 10q11.21q21.3(45,525,496-69,488,100)x3 | 46,XY,dup(10)(q21.3q11.2) | Developmental delay, microcephaly |
| arr 18q21.32q22.3(56,096,296-68,859,944)x3,18q22.3q23 (68,910,174-76,111,164)x1 mat | 46,XY,der(18)inv dup(18)(q22.3q21.32) del(18)(q22.3) mat | Umbilical hernia, R undescended testes, CLCP, left aural atresia |
| arr 15q13.3(29,759,738-30,297,359)x1 | | Hypotonia, developmental delay |
| arr Xp22.31(6,498,521-8,057,652)x2, 21q22.3(45,448,874-45,715,920) x3 | | Macrocephaly, dysmorphic |
| arr 1-22(39,986 oligos)x2,X(2,745 oligos)x1,Y(367 olgios)x0 | 45,X | Turner's syndrome |
| arr 16p13.11p12.3(14,817,506-18,539,625)x3 | | Abnormal gluteal cleft, tethered cord, abnormal bladder |
| arr 5q34(160,081,094-160,661,741)x3 | | Mild dysmorphic features |
| arr 15q11.2q13.2(20,249,686-28,865,266)x3~4 | 47,XY,+mar | Hypospadius, developmental delay |
| arr 16p13.11(14,956,052-16,102,229)x1 | | Abnormal body odour, seizures, enuresis, developmental delay |
| arr 10q26.3(130,828,396-135,284,309)x1 | 46,XX,del(10)(q26.3) | Developmental delay, decreased tone, triangular faces |
| arr 16q24.1(83,052,026-84,005,631)x1 | | Microcephaly, short stature |
| arr 22q11.2(17,299,742-19,835,558)x1 | | Conductive hearing loss, learning problems, small ears with thickened helices, and broad nasal bridge (VCFS syndrome) |
| arr Xq27.1q27.2(140,081,946-140,385,424)X0, 16p13.13(11,454,370-11,700,205)x3 | | Macrocephaly, language delay |
| arr 12q24.21q24.22(114,370,263-116,551,886)x3 | | FTT, developmental delay, short stature, gaze paralysis, nystagmus, right hydronephrosis, scoliosis, short digits, partial absenct of corpus callosum |
| arr 4q26q35.2(119,832,405-191,133,809)x3 | 46,XY,der(11)t(4;11)(q26;p15.5) | Dysmorphic, undescended testes |
| arr 1q31.1(74,460,676-75,514,794)x3 | 46,XY,t(7;11)(q11.23;q23.3) | None |
| arr 5q23.1(116,096,090-118,353,346)x3, 10q26.13(125,000,397-126,634,880)x3 | | Developmental delay, seizures |
| arr 15q11.2(20,249,686-20,769,096)x1 | | Sagittal craniosynostosis, developmental delay, dysmorphism |
| arr 1q44(244,616,624-245,059,490)x3 | | Velopharyngeal insufficiency, language delay |
| arr 17q31.31(38,594,317-39,298,117)x3 | | Midline CLCP, callosal agenesis, basal encephalocele, ventriculomegaly, R optic coloboma |
| arr 1p36.33p36.32(769,390-4,301,751)x1 | | Dysmorphic features |
| arr 7q35(146,082,080-146,294,374)x1 | | Velopharyngeal insufficiency, developmental delay, central hypothyroidism, migraines |
| arr 16p13.2(6,567,938-6,861,113)x1 | | FTT, microcephaly |
| arr 17q11.2(22,654,693-26,691,794)x1 | NF gene deletion confirmed by MPLA | CP, NF1 |
| arr Xp22.31(6,498,521-8,057,652)x2 | | Hypotonia, mild ventriculomegaly |
| arr 1q21.1(144,854,346-146,375,859)x1 | | Low-set, posteriorly rotated ears, CP |
| arr 1p36.33p36.13(769,390-18,108,676)x1 | | 29 weeks, agenesis of corpus callosum, ventriculomegaly, microophthalmia, hypertelorism, microphallus, rockerbottom feet, single umbilical artery |

**Table 3.** *Continued*

| aCGH result | FISH/karyotype/other | Diagnosis |
|---|---|---|
| arr 7p21.3(8,210,155-8,456,743)x3 | | Seizures, feeding problems |
| arr 16q22.1(65,382,157-67,166,581)x1 | | Hypertonia, dysmorphic, FTT, microcephaly, developmental delay, bilateral hearing loss |
| arr 9p24.3p24.1(210,053-4,960,293)x1 | | Hypotonia, hypospadius, trigger thumbs. Developmental delay |
| arr 2q13(110,219,566-110,322,167)x1, 16q12.1(45,818,341-46,076,657)x1 | | Type IXb glycogen storage disease |
| arr 9q34.3(139,588,642-140,114,744)x1 | | Hypotonia, developmental delay, dysmorphic features |
| arr 15q13.1q13.2(27,040,409-28,153,557)x1 | | Velopharyngeal insufficiency, hypertelorism |
| arr 16p13.11(14,506-16,218,712)x3 | | Tall stature, seizures, developmental delay |
| arr 16p13.11(14,876,156-16,432,990)x3, 16p12.1(21,744,793-22,315,573)x3 | | Developmental delay, ADHD, OCD |
| arr 16p11.2(29,581,255-30,098,210)x3 | | Bilateral sensorineural hearing loss |
| arr 16p11.2(29,581,255-30,098,210)x1 | | Dysmorphic features |
| arr 15q26.3(96,955,900-97,240,516)x3 | | Developmental delay, seizures, bilateral hippocampal atrophy |
| arr 5q14.3(88,228,848-88,592,452)x1 | | Seizures, macrocephaly, developmental delay |
| arr 15q11.2q13.3(20,249,686-30,686,991)x3~4 | 47,XX,+idic(15)(q13) | Hypotonia, dysmorphic, developmental delay |
| arr 15q11.2(20,316,792-20,851,879)x3 | | Autism, short stature |
| arr 22q11.22q11.23(21,341,813-21,973,364)x3 | | Bulbous nose, micrognathia, bilateral sensorineural hearing loss, developmental delay |
| arr 7p14.2p12.3(36,287,252-48,408,979)x1 | 46,XY,del(7)(p12.3p14.2) | Pre-axial polysyndactyly of great toes, developmental delay, frontal bossing, (Gregg syndrome?) |
| arr 22q11.21(17,041,524-19,835,558)x1 | | CP, VCFS |
| arr Xp22.31(8,408,059-8,757,658)x2 | | Hemoptysis, Kallman syndrome |
| arr 17p12p11.2(15,741,871-20,464,746)x1 | | Smith–Magenis syndrome (cerebellar and cord hypoplasia, FTT) |
| arr 7q31.1(112,431,891-112,873,844)x1, 15q13.1q13.2(27,040,409-28,153,557)x3 | | Cleft palate |
| arr 13q31.3(90,863,461-91,065,511)x1, 18q12.1(26,575,242-28,242,634)x1 | | MR |
| arr 15q11.2(20,249,686-20,851,879)x3 | | None |
| arr 16p11.2(29,581,255-30,098,210)x1 | | Relative macrocephaly, developmental delay |
| arr 21q22.3(44,104,494-46,914,886)x1 | | Submucous CP, hypodontia, abnormal sweating |
| arr 10q26.13q26.3(123,502,981-135,167,000)x1 | 46,XX,del(10)(q26.13) | Esotropia, arthrogryposis, developmental delay |
| arr Xp22.2(11,668,991-13,222,165)x1 | | 27 WGA preemie, macroglossia, hepatomegaly, short stature, developmental delay |
| arr 8p23.1(8,644,932-9,135,108)x3, 20q11.1q11.1(28,265,913-29,611,848)x3 | | Myotubular myopathy |
| arr 3p23(31,780,528-32,095,156)x1, 15q13.3(29,885,762-30,297,359)x3 | | Autism, café au lait spots |
| arr 4q34.2(177,519,174)x3 22q12.3(32,323,633-32,494,828)x1 | | Muscular dystrophy |
| arr 22q11.21q11.21(20,138,750-21,235,209)x1 | | Septo-optic dysplasia, panhypopituitarism, digital abnormalities, MR |
| arr 13q12.3(28,038,932-28,860,210)x3 | 46,XX,t(12;16)(q24.1;q12.1) | None |
| arr 2q11.2(96,130,087-97,380,146)x1 | | Bilateral sensorineural hearing loss, behaviour problems |
| arr 15q11.2(20,249,686-20,769,096)x1 | | FTT, developmental delay |
| arr 9p24.3(193,933-305,169)x1, 11p11.2(45,938,662-46,327,805)x3 | | Developmental delay |

Table 3. *Continued*

| aCGH result | FISH/karyotype/other | Diagnosis |
|---|---|---|
| arr Xq21.1q21.3(83,587,497-86,980,627)x2 | | Microcephaly, developmental delay |
| arr 15q11.2(20,316,792-20,769,096)x1 | | Developmental delay |
| arr 7q33q36.3(137,146,219-158,811,468)x3 | 46,XX,der(7)dup(7)(qter>q33::pter>qter) | Developmental delay, cerebellar abnormality, dysmorphic features |
| arr Xq28(152,342,492-154,905,065)x2 | Ish der(Y)t(X;Y)(qter;pter) | Developmental delay, microcephaly, seizure disorder |
| arr 6q27(168,425,470-170,763,155)x1, 15q24.2q24.3(73,913,181-75,908,449)x3 | | Speech delay, motor delay |
| arr 6q27(168,425,470-170,763,155)x1, 15q24.2q24.3(73,913,181-75,908,449)x3 | | Developmental delay, speech problems |
| arr 6q27(168,425,470-170,763,155)x1 | | Developmental delay, autism |
| arr 15q11.2(20,316,792-20,628,002)x1 | | MR, L renal aplasia, R renal hyperplasia, renal transplant required |
| arr 15q11.2q13.1(20,851,479-26,365,196)x3 | | Small size, developmental delay, behaviour problems |
| arr 7p21.3(8,210,155-8,456,743)x3 | | None described |
| arr Xpterp11.1(680-58,098,337)x1, Xq11.1qter(61,848,217-154,905,221)x3 | Karyotype: 46,X,i(X)(q10) | Speech delay, Turner sydrome |
| arr 1p36.31(6,594,333-6,899,375)x1 | | Hypernasal speech, developmental delay, MR, ataxic gait |
| arr 7q21.3q22.1(97,777,630-100,025,134)x3 | | Infantile spasms, developmental delay |
| arr Xp22.2(9,756,750-9,912,388)x3, Xp22.2(13,393,866-13,727,371)x3 | | Short stature, low-set ears |
| arr 14q13.3(35,694,263-35,973,724)x1 | | Congenital hypothyroidism, developmental delay |
| arr 14q24.2(72,245,680-72,802,625)x3 | | Rocker bottom feet, poor mineralisation of calvarium, renal failure |
| arr 13.21.1(54,712,988-56,924,587)x1, 15q21.2(50,045,029-50,313,619)x1 | | Right amblyopia, obesity, learning problems |
| arr Xp21.3p21.2(28,683,919-29,753,365)x0, 11q22.3(102,758,873-102,858,472)x1 | | Dysmorphic, developmental delay |
| arr 16p12.1(21,744,793-22,315,573)x1 | | Prune belly syndrome, bladder outlet obstruction |
| arr 1p21.1(103,239,476-103,290,337)x1, 4q35.2(187,875,597-188,241,675)x3, 6p21.32(32,151,341-32,270,649) | | CP, facial dysmorphism (Stickler syndrome-1p21.1 deletion) |
| arr 13q21.33q22.1(70,444,948-73,508,503)x1 | | Developmental delay, short stature, hx of hypotonia |
| arr 15q14(37,490,118-37,890,533)x3 | | Developmental delay |
| arr Xp22.31(6,498,521-8,057,652)x3 | | 32-week triplet, seizures |
| arr 15q13.3(29,818,304-30,423,251)x3 | | Developmental delay, autism |
| arr 1q21.1(144,136,552-146,375,859)x1 | | Learning difficulties, dysmorphic |
| arr 3q26.2(171,319,429-171,680,037)x3 (uncertain significance) | | Macrocephaly, developmental delay, large size (PTEN mutation) |
| arr 1q21.1(144,698,313-146,375,859)x1 | | Bilateral club feet, hip dislocation |
| arr 1q21.1(144,136,552-144,511,100)x3 | | Macrocephaly, hydronephrosis, patent omphalomesenteric duct |
| arr 13q21.1q34(55,598,617-114,110,891)x4 (fibroblasts) | 47,XY,+der(?)(??::13q34>13q21.1::13q21.1>13q34::??)[22]/46,XY[10] | Hypospadius, Dandy–Walker malformation, absence or hypoplasia of cerebellar vermis, hypomelanosis of Ito (mosaic partial trisomy 13) |
| arr 1p36.33p36.32(1,440,704-2,986,776)x1 | | Developmental delay, no speech, FTT, obesity |
| arr 16p13.11(14,817,506-16,432,990)x3, 16p12.1(21,744,793-22,315,573)x3 | | Speech delay, behaviour problems |
| arr 16p13.11(14,817,506-16,432,990)x3, 16p12.1(21,744,793-22,315,573)x3 | | Learning delay, ADD |

**Table 3.** *Continued*

| aCGH result | FISH/karyotype/other | Diagnosis |
|---|---|---|
| arr 13q21.33q31.3(68,148,854-89,165,781)x1 | 46,XX,t(8;20)(q13;p11.23),del(13)(q21.33q31.3) | Mild hypotonia, poor feeder, developmental delay, Gtube |
| arr Xp22.31(6,498,521-8,057,652)x0 | | Ichthyosis, developmental delay (X-linked ichthyosis/steroid sulfatase deficiency) |
| arr 6q16.1q21(97,817,699-105,946,615)x1, 13q34(109,816,315-110,077,527)x3 | 46,XY,t(4;6;12)(p12;q21;q22) | Developmental delay |
| arr 17q25.1(69,359,232-70,118,688)x3 | | Patent parietal foramina |
| arr 18q23(75,575,242-75,954,259)x3 | | Overgrowth |
| arr 2q14.3(122,933,784-123,960,286)x1 | | Dysmorphic |
| arr 10p12.1p11.23(27,418,734-29,276,069)x1 | | Mild MR |
| arr 17p13.3(129,202-494,470)x3 | | Microcephaly, immune def |
| arr 2p12(77,201,049-78,040,191)x1 | | Cleft palate, language delay |
| arr Xp22.33q28(501-154,861,689)x3, 14q21.2(41,269,035-42,160,020)x1 | 47,XXX | Developmental delay, seizures |
| arr 20p11.21(24,996,890-25,414,606)x3 | | Developmental delay, hypotonia |
| arr Xp22.33q28(701-154,861,689)x3 | 47,XXX | Developmental delay, learning problems, microcephaly |
| arr Xp22.31(6,498,521-8,057,652)x3, 16q23.2(79,425,060-79,600,816)x1 | | Cleft palate, speech delay |
| arr 12p11.23(27,197,387-27,651,560)x3 | | Bilateral cleft lip and palate, microcephaly |
| arr 4q21.22q21.3(85,507,877-87,642,276)x1 | | Relative macrocephaly, ptosis, developmental delay |
| arr 17p13.1(7,060,354-7,335,313)x1 | | Hypotonia, microcephaly |
| arr 8p12(29,800,507-30,462,740)x3, 17p13.3(3,490,013-3,505,283)x1 | | Dysmorphic |
| arr 15q11.2(20,316,992-20,636,537)x1 | | Macrocephaly, developmental delay |
| arr 2q37.2(236,134,887-236,353,965)x1 | | Bilateral celft lip, macrocephaly, dysmorphic |
| arr 11q13.2(67,114,580-67,331,119)x3 | | Neurodegenerative d/o NOS |
| arr 12q14.1q14.2(60,757,794-61,699,924)x3, 15q26.3(97,124,964-97,337,543)x1 | | Absent corpus callosum, very mild developmental delay |
| arr 20p12.1(12,704,525-13,278,104)x1 | | Macrocephaly, developmental delay |
| arr 13q31.1q31.1(85,548,824-86,905,854)x1 | | Bilateral progressive sensorineural hearing loss, learning problems |
| arr Xp22.3(7,155,159-7,704,332)x2 | | Macrocephaly, hypotonia, developmental delay |
| arr 16p13.11(14,876,156-16,102,220)x1, 16p11.2(29,500,084-30,240,223)x3 | | Microcephaly, dysmorphic |
| arr 16q24.1(83,649,938-83,958,170)x1 | | Developmental delay, strbismus, hyperteolorism, flat nasal bridge, hypertonia |
| arr 1q21.1(144,698,313-146,375,859)x3 | | Macrocephaly, developmental delay |
| arr 22q11.21(19,355,454-19,574,144)x3 | | Hypotonia, relative macrocephaly, developmental delay |
| arr 4q28.3q31.1(136,497,083-140,089,577)x1, 6q27(168,425,470-170,732,174)x1, 15q24.2q24.3(73,913,181-75,908,449)x3 | | Developmental delay, dysmorphic, hypernasal speech |
| arr Xp22.33p21.3(159,409-29,234,377)x3, 13q34(112,517,673-114,110,891)x1 | 46,XX,der(13)t(X;13)(p21.3;q34) | FTT, cleft palate, developmental delay, seizures, ventilator dependence |
| arr 6p25.1(5,110,220-5,334,346)x1 | | Nystagmus, ocular albinism (GPR143 mutation), developmental delay |
| arr 8p23.1(9,350,950-9,582,080)x1 | | Developmental delay, dysmorphic |
| arr 6q25.3q27(159,461,547-170,732,174)x1 | | Developmental delay, IUGR, tethered spinal cord, microcephaly, FTT |
| arr 15q11.2(20,316,792-20,769,096)x1 | | Developmental delay, dysmorphic (flat midface, small mouth, small ears) |

Table 3. *Continued*

| aCGH result | FISH/karyotype/other | Diagnosis |
|---|---|---|
| arr Xp22.33q28(501-154,861,689)x3 | 47,XXX | Developmental delay, learning problems, premature menopause |
| arr 2p21(44,384,434-45,184,192)x3 | | Macrocephaly |
| arr 2q13(110,219,566-110,322,167)x1, 15q11.2q13.1(21,258,345-26,194,049)x1 | | Angelman syndrome |
| arr 15q13.2q13.3(28,741,818-30,297,359)x1 | | Bathrocephaly, FTT, dysmorphic, developmental delay |
| arr 15q11.2q13.1(21,258,345-26,885,085)x3 | | Macrocephaly, developmental delay |
| arr 16p13.11(15,458,603-16,102,220)x1 | | FTT, dysmorphic, developmental delay |
| arr 2p12p11.1(83,606,532-91,263,529)x3 | 46,XY,dup(2)(p11.1p12) | Asperger's syndrome, growth retardation, ADHD, Chiari 1 malformation, R exotropia, diet-controlled diabetes mellitus, small penis |
| arr 9p24.1(6,188,339-6,574,339)x3 | | Congenital small bowel obstruction |
| arr 16p13.2(6,328,449-6,534,496)x1 | | Autism?, dysmorphic |
| arr Xp22.12(20,645,025-21,401,470)x3 | | Developmental delay, seizures |
| arr 16p13.3(4,480,265-4,635,094)x1 | | Partial aplasia of the corpus callosum, speech delay, dysmorphic, nystagmus |
| arr 11p11.2(46,433,617-46,818,781)x3, 11q13.2(67,114,780-67,330,978)x3 | | Autism, macrocephaly, seizure disorder |
| arr 16p13.12p13.11(14,669,540-16,432,990)x3 | | Obesity, acanthosis nigricans, normal dev |
| arr 3q13.33(120,945,219-122,212,944)x3, 16p11,2(29,581,255-30,098,210)x1 | | Cleft palate, speech delay, dysmorphic |
| arr Xp21.2p21.1(31,047,552-31,896,746)x3 | | Hypermobility, strabismus, congenital bilateral ptosis, speech delay |
| arr 15q13.2q13.3(28,741,818-30,297,359)x1 | | Developmental delay |
| arr 5q23.2q31.2(124,428,862-137,903,960)x3 | 46,XY,dup(5)(q23.2q31.2) | Developmental delay, dysmorphic, hypotonia |
| arr Yq12(57,486,861-57,720,889)x3, 2p21(45,353,739-45,815,227)x3 | 46,XX,der(22)t(Y;22)(q12;p13) | Saethre–Chotzen syndrome, Duane's anomaly, craniosynostosis, congenital absence of the ovaries |
| arr 20q13.33(60,784,651-62,379,259)x1 | 46,XY,r(20)(p13q13.33).ish r(20)(20pter+,20qter-) | Neonatal seizure disorder, dysmorphic, developmental delay, nystagmus |
| arr 22q11.21(17,041,524-19,891,670)x1 | | Velocardiofacial syndrome, developmental delay |
| arr 6q12(65,421,469-65,911,511)x1, 15q13.2q13.3(28,741,818-30,297,359)x1 | | Unilateral CLCP |
| arr 3q28(191,060,445-191,151,486)x1 | | CLCP |
| arr Xp22.1q22.3(102,411,476-104,410,522)x1, 13q34(109,978,836-110,119,527)x3 | | Mitochondrial disorder, static encephalopathy |
| arr 13q21.33(70,871,358-71,265,056)x1 | | Bilateral vocal cord paralysis, hypotonia, gross motor delays |
| arr 10p15.3(2,160,995-2,809,665)x1 | | Seizures, dysmorphic features |
| arr Xp11.4(37,864,291-38,334,337)x0 | | OTC, liver transplant, retinitis pigmentosa |
| arr 16p12.1(21,744,793-22,315,573)x1 | | Juvenile rheumatoid arthritis |
| arr 11p15.5(1,700,280-1,974,184)x3 | | Short stature, small size, developmental delay |
| arr 16p13.13(10,324,839-10,557,062)x1 | | Self-mutilation, MR, FTT |
| arr 3p24.1(28,474,355-28,675,828)x4 | | ADHD, seizures, developmental and speech delays |
| arr 3p23(31,780,528-32,095,156)x1, 4q13.1(59,593,471-60,638,473)x1 | | Microcephaly |
| arr 5q13.1q13.2(68,169,005-71,056,864)x3 | | Autism, self-injurious behaviour, speech delay |
| arr 1q32.1(198,027,109-198,788,199)x3 | | Congenital diaphragmatic hernia |
| arr 10q11.22q11.23(46,404,719-51,451,056)x1 | | Seizures, central hypothyroidism, hypotonia, developmental delay |

**Table 3.** *Continued*

| aCGH result | FISH/karyotype/other | Diagnosis |
|---|---|---|
| arr 1p34.3p34.2(39,251,628-40,052,795)x3 | Connexin 26 (DFNB1) hearing loss | Bilateral sensorineural hearing loss, dysmorphic features |
| arr 7q11.23(72,404,049-73,771,409)x3 | | Developmental delay, PDD NOS, delayed puberty, short stature |
| arr 10q11.22q11.23(46,404,719-51,451,056)x1 | | Speech and motor delays |
| arr 15q13.2q13.3(28,517,605-30,186,356)x3 | | Mild dysmorphisms |
| arr 15q13.3(29,818,104-30,297,359)x3 | | Developmental delay, obesity, macrocephaly |
| arr Xp22.33(525,776-1,152,910)x3 | | Cognitive delay, scoliosis, |
| arr 16p11.2(29,581,255-30,098,210)x1 | | 16p11.2 microdeletion syndrome, obestity, behaviour problems |
| arr Xp11.22(53,419,254-53,431,241)x2, Xq21.1(82,644,205-83,016,297)x2, 19q13.12q13.2(41,237,198-43,804,171)x3 | | Infantile spasms |
| arr 15q11.2(20,249,686-20,769,096)x1, 16p11.2(28,768,832-28,938,701)x1 | | Obesity, developmental delay, MR/PDD, dysmorphic |
| arr 1q21.1(144,854,346-146,375,859)x1, 16p11.2(28,768,832-28,938,701)x1 | | NF1, developmental delay, seizure disorder, right ventricular hypertrophy |
| arr 15q13.2q13.3(28,741,818-30,686,991)x3 | | Developmental delay, small size, FTT, leg length discrepancy, sacral dimple |
| arr 1q21.1(144,136,552-144,511,100)x3 | | Developmental delay, FTT, renal artery stenosis, small size, hypoglycaemia |
| arr 15q13.3(29,818,104-30,297,359)x3 | | Speech delay, self-stimulatory behaviour |
| arr 15q13.3(29,818,104-30,297,359)x3 | | Developmental delay, FTT, seizures, umbilical hernia |
| arr 10q25.1(110,430,505-111,079,597)x1 | | FTT, bilateral congenital glaucoma |
| arr 9q22.32(96,645,584-96,937,867)x1 | | Small teeth, dysmorphic |
| arr 2p25.3(1,014,584-1,474,321)x3 | | Speech delay, hypogammaglobulinaemia, FTT, short stature |
| arr 20p13(429,360-1,070,257)x3 | | Bilateral incomplete cleft palate |
| arr 18p11.31p11.23(6,962,810-8,046,016)x3 | | Bilateral CLCP, dysmorphic |
| arr 1q25.2(176,032,538-176,607,157)x3 | | Seizures |
| arr 5p15.2(12,872,852-14,299,349)x3 | | 2–3 toe syndactyly, enlarged ventricles |
| arr 9q33.1(117,245,933-117,842,139)x3 | | Hypotonia, developmental delay |
| arr 1q44(244,616,624-245,059,490)x3 | | Seizures, sensory integration disorder |
| arr 15q11.2(20,316,792-20,769,096)x1 | | 23 WGA, ARPKD |
| arr 15q11.2(20,316,792-20,697,714)x1 | | Developmental delay, ankylglossia |
| arr Xq26.3q27.1(137,766,149-138,668,327)x2 | | Seizures, myelomeningocele, hydrocephalus, Chiari 2 malformation, neurogenic bladder |
| arr 2q24.1(155,817,812-156,697,629)x3, 17p12(14,052,297-15,382,932)x1 | | Arthrogryposis, myopia, developmental delay, scoliosis |
| arr Xp22.31(6,498,521-8,057,652)x3 | | Small size, developmental delay |
| arr 16q23.1(75,630,868-76,229,279)x3 | | Facial dysmorphism, developmental delay |
| arr Xp22.31(6,498,521-8,057,652)x3, 17q11.2(25,964,992-26,320,616)x1 | | Small size, developmental delay |
| arr 3p22.1(41,364,999-41,735,606)x1 | | CLCP, bilateral 4–5 toe syndactyly, bilateral short 5th metacarpals, left short 2nd finger |
| arr 8p23.2(2,296,133-3,115,040)x3 | | Minor dysmorphic features, autism, seizures |
| arr 13q31.1q31.2(85,548,824-86,905,854)x1 | | Bilateral progressive sensorineural hearing loss |
| arr 16q22.3w23.3(71,134,306-81,682,703)x1 | 46,XX,del(16)(q22.3q23.3) | Bilateral CLCP, broad and high nasal bridge, underdeveloped pinnae, bilateral 5th finger clinodactyly |
| arr Xq21.1(76,814,158-76,923,996)x1 | | Learning problems, partial agenesis of corpus callosum, GH def, FTT |

Table 3. *Continued*

| aCGH result | FISH/karyotype/other | Diagnosis |
|---|---|---|
| arr Xp22.2(10,361,188-10,430,507)x2, 18q12.2(33,360,783-35,431,070)x1 | | Language delay |
| arr 16p11.2(29,500,084-30,240,223)x3 | | Dysmorphic features |
| arr 2q37.3(240,374,606-241,140,951)x3 | | Developmental delay, thin upper lip, small ears |
| arr 14q32.2q32.33(99,567,895-106,109,395)x1 | | Dysmorphic, SGA |
| arr 5p14.3(18,894,105-19,085,088)x1, 17q25.1(69,269,385-70,118,688)x3 | | Learning problems |
| arr 1p13.3(107,244,652-107,532,500)x3, 15q13.1(27,040,409-27,359,856)x3 | Mosaic for marker chromosome | Recurrent pregnancy loss |
| arr 16q11.2(45,058,042-45,466,778)x3 | | Cyclical vomiting |
| arr 3p26.3(68,749-1,277,000)x3 | | Possible movement disorder |
| arr 17q12(31,474,318-33,323,172)x3 | | Marfan syndrome |
| arr 4q28.3(135,162,486-135,399,760)x1, 8q24.2(130,949,130-131,112,163)x4 | | Developmental delay, dysmorphic features, seizures, HIE at 3 weeks of age |
| arr 18q22.1(63,877,985-64,683,664)x3 | | Left-sided congenital diaphragmatic hernia, bilateral hydronephrosis, spinal segmentation anomalies, extra ribs, rib anomalies |
| arr 12q24.31(122,495,058-123,220,177)x1 | | Seizures, behaviour problems, developmental delay |
| arr 22q11.22q11.23(21,341,813-22,225,763)x3 | | Developmental delay, dysmorphic |
| arr Xp22.31(6,498,521-8,057,652)x2 | | Developmental delay, scoliosis, syrinx, Chiari malformation |
| arr 3q22.3(139,779.696-140,188,359)x3 | | FTT, cleft palate, large ant fontanelle, low-set ears, large eyes, small mouth |
| arr 15q11.2(20,316,792-20,769,096)x3 | | Cleft palate, dysmorphic features |
| arr 16q22.1(66,332,837-67,620,577)x1 | | Cleft lip, bilateraly astigmatism, language delay |
| arr 10q23.31q23.32(92,353,332-93,590,641)x1 | | Developmental delay, h/o FTT, dysmorphic, abnormal gait |
| arr 7q11.23(72,404,049-73,771,409)x3 | | Global developmental delay, macrocephaly, hydrocephalus |
| arr 13q22.3(76,188,803-76,342,979)x1 | | Developmental delay, h/o FTT, ADAMsT13 deficiency, congenital nystagmus |
| arr 4q22.3q23(99.083,904-99,242,122)x1 | | BPD, 23-week preemie |
| arr 22q11.1q11.21(15,777,298-16,319,059)x3, 22q11.21q12.1 (17,041,524-26,318,518)x3 | | Dysmorphic: prominence of metopic suture, frontal upsweep,flaring eyebrows, cupped ears |
| arr 3p12.1(85,906,346-86,925,547)x3, 12p13.33p11.21(100, 482-31,996,388)x3 | 46,XY,der(13)t(12;13)(p11.21;p11.2) | IUGR, dysmorphic, polydactyly, inguinal hernia |
| arr 17p12(14,052,297-14,234,332)x1 | | Developmental delay, seizures, hypoplastic corpus callosum |
| arr 16p11.2(28,768,832-28,928,701)x3 | | Short stature, mild dysmorphic features |
| arr 1q21.1(142,512,849-146,375,859)x1, 14q32,12q32,13(92,575,980-93,371,821)x4 | | Microcephaly, developmental delay, hypotonia |
| arr 5p14.3(22,192,144-22,534,486)x1, 13q14.11(41,024,012-41,378,603)x3 | | Incomplete unilateral cleft lip, left clubfoot, small size, cleft soft palate, pointed chin |
| arr 11q14.1(76,932,496-77,109,432)x1, 16p13.11(14,817,506-16,432,990)x3 | | FTT, microcephaly, post-rotated ears, brachycephaly, smooth philtrum |
| arr 1p13.3(107,397,645-107,642,681)x3 | | Hydrocephalus, large appetite |
| arr 15q13.3(29,818,104-30,297,359)x3 | | Congenital toxo, macrocephaly, seizures/infantile spasms, bilateral hydrocele, hypothyroidism |
| arr 15q11.2(20,316,792-20,769,096)x1 | | Bilateral single palmar crease, FTT |
| arr 4q26q28.1(116,713,770-127,208,160)x1 | | Developmental delay, dysmorphic features |

**Table 3.** *Continued*

| aCGH result | FISH/karyotype/other | Diagnosis |
|---|---|---|
| arr 15q11.2(20,249,686-20,851,879)x3 | | Two-vessel cord, CCAM versus diaphragmatic hernia |
| arr 15q11.2(20,316,792-20,769,096)x1 | | FTT, osteopenia, liver disease |
| arr 14q24.2(71,198,215-72,119,362)x3 | | Amyoplasia, arthrygryposis, Pierre–Robin sequence, club foot, hypotonia |
| arr 10q26.3(131,662,081-132,358,553)x3, 22q11.21(17,299,742-19,835,558)x1 | | Velocardiofacial syndrome, 10q26.3 duplication, language delay, dysmorphic features, postcricoid haemangioma |
| arr 2q37.3(241,240,038-242,656,173)x1, 17p13.3(76,063-572,366)x3 | | Seizure disorder, FTT |
| arr 1q21.1(142,512,849-146,812,300)x3 | | Developmental delay, dysmorphic features, hypospadius, tracheomalacia |
| arr 15q13.2q13.3(28,741,818-30,297,359)x1 | | Macrocephaly, frontal bossing, language delay |
| arr 7q11.22(68,156,592-69,288,822)x1 | | Tethered spine |
| arr 9q34.11(129, 742,847-130,304,437)x1 | | Autism, mild epicanthal folds |
| arr Xp22.31(8,408,059-8,680,899)x3 | | Microcephaly, CP |
| arr 5q15(96,890,163-97,202,364)x1 | | Poor feeder required GT, FTT, relative macrocephaly |
| arr 4q28.3(135,162,486-135,399,760)x1 | | Developmental delay, autism, dysmorphic features, macrocephaly |
| arr 2q37.3(240,879,731-242,656,173)x1, 17p13.3(129,202-572,366)x3, 20q13.31q13.33(55,538,683-62,379,259)x3 | | Dysmorphic features, hypotonia, scoliosis, seizures, developmental delay, GT |
| arr Xp22.31(6,498,521-8,057,652)x2 | | Dysmorphic, laryngotracheomalacia |
| arr 2q14.1(116,112,209-117,998,418)x3 | | Cleft lip, esotropia, congenital adrenal hyperplasia |
| arr 15q11.2q13.1(21,258,345-26,194,049)x1 | | Microcephaly, low-set ears, developmental delay, seizures (Angelman syndrome) |
| arr 11q22.1(98,049,510-98,206,565)x1 | | Speech problems, amblyopia, developmental delay, learning problems |
| arr 1p31.3(63,991,532-64,188,412)x1 | | Developmental delay, macrocephaly, frontal bossing, delayed closure of ant fontanelle, flared eyebrows, large ears |
| arr Xq24(118,628,128-118,966,610)x2 | | Dysmorphic features, hypogammaglobulinaemia, developmental delay |
| arr 15q11.2(20,316,792-20,851,879)x3 | | Cleft lip, pre-auricular skin tags, L single palmar crease |
| arr Xp22.33p11.21(17,045-56,412,285)x1, Xp11.21q28(56,505,028-154,861,689)x3 | 46,X,idic(X)(p11.21) | FTT, dysmorphic features, feeding problems, large simple ears, high-arched palate (Turner syndrome variant) |
| arr 6q22.31q22.33(124,321,882-128,861,602)x1 | | Small size, dysmorphic features, developmental delay (langauge) |
| arr 15q13.3(29,885,762-30,297,359)x3 | | NF1, hydronephrosis, behaviour problems |
| arr 13q21.32(66,807,163-66,913,454)x1 | | Hypotonia, developmental delay (esp language) |
| arr 22q11.21q11.23(20,138,750-22,050,371)x1 | | CLCP, developmental delay, dysmorphic features (VCFS) |
| arr Xp21.1(31,593,344-31,734,805)x0 | | Developmental delay, short stature, deletion of dystrophin gene |
| arr 15q13.1q13.2(26,884,685-28,153,557)x3 | | Spina bifida, hydrocephalus, developmental delay, dysmorphic features, hypernasal speech |
| arr Xq28(147,902,403-148,646,685)x3, 9p13.2p13.1(37,441,438-38,805,616)x3 | | Precocious puberty otherwise normal |
| arr 22q11.21(17,299,742-20,139,150)x3 | | Autism, seizures |
| arr 21q21.3(26,186,543-26,361,041)x1 | | Developmental delay, abnormal speech, ankylglossia, smooth philtrum, poor palatal movement |
| arr 22q11.21(18,782,433-20,139,150)x3 | | Hoarse voice, broad forehead, stellate iris, h/o pyloric stenosis, developmental delay |
| arr 3q26.1(162,139,117-164,531,794)x3, 14q12q13.1(31,097,633-33,436,541)x3, 18q22.1q23(62,315,997-76,111,164)x1 | | Nystagmus, FTT, microcephaly, developmental delay, dysmoprhic features (flat midface with wide set eyes, narrow palpebral fissures, wide nasal bridge, thin lips) |

Table 3. *Continued*

| aCGH result | FISH/karyotype/other | Diagnosis |
|---|---|---|
| arr 22q11.22q11.23(21,341,813-23,289,968)x3 | | Midface hypoplasia, right single palmar crease, mild scoliosis, short 5th fingers, narrow chest (possible Marfans) |
| arr 15q11.2(20,316,792-20,697,714)x3 | | Haemihypertrophy, left inguinla hernia (possible Beckwith–Wiedemann syndrome) |
| arr 3q13.11q13.12(107,791,632-109,336,248)x1 | | Asperger's syndrome, sleep apnoea, retrognathia |
| arr 14q32.12q32.13(92,575,980-93,371,821)x4, 17q12(31,929,968-33,323,172)x3 | | Developmental delay, epicanthal folds, flat nasal bridge |
| arr Xp11.21p11.1(56,573,387-57,027,020)x2 | | Global developmental delay, dysmorphic features |
| arr 5p13.2(36,654,246-37,275,165)x3 | | Developmental delay, hypotonia |
| arr 15q13.3(29,885,762-30,297,359)x3 | | Social delayed, bipolar disorder, food hoarding, hypogonadism, migraines, obesity |
| arr 17q25.1(69,359,232-70,118,688)x3 | | Encephalocele, right optic disc dysplasia, frontal bossing |
| arr 17q25.1q25.3(71,027,089-73,951,793)x2~4, 17q25.1q25.3 (70,668,288-78,637,842)x2~3, 20p13(18,580-170,443)x1~2 | | Hypotonia, global delay, epilepsy |
| arr 17p12(14,052,297-15,382,932)x1 | | Sibling with abnormal array otherwise normal |
| arr 2q24.1(155,817,812-156,697,629)x3 | | Sibling with abnormal array otherwise normal |
| arr 2q24.1(155,817,812-156,697,629)x3, 6p21.1p12.3(45,128,180-45,236,701)x1, 15q11.2(20,249,686-20,812,096)x3 | | Sibling with abnormal array otherwise normal |
| arr 6p21.1p12.3(45,128,180-45,236,701)x1 | | Sibling with abnormal array otherwise normal |
| arr 1q21.2(144,136,552-144,511,100)x1 | | Pompe's disease |
| arr 3q25.33(160,384,901-160,762,878)x3, 17p13.3(1,770,737-2,111,971)x1, 20q13.2(49,565,184-49,765,895)x4 | | Spina bifida, microcephaly, imperforate anus, developmental delay |
| arr 1q21.2(144,698,313-146,375,859)x1, 17p12(14,052,297-15,382,932)x3 | | Charcot–Marie–Tooth, 1q21.2 microdeletion syndrome |
| arr 5q14.3q21.1(91,259,156-100,780,043)x1 | 46,XY,del(5)(q14.3q21.1) | Hypotonia, optic nerve hypoplasia |
| arr 1p32.2p31.1(58,505,806-79,830,067)x3, 1q42.2(230,155,506-231,388,873)x1 | | FTT, frontal bossing, right ptosis, hypertelorism, mild micrognathia, bilateral 5th finger clinodactyly, developmental delay |
| arr 9q34.3(139,389,802-140,193,718)x1 | | Kleefstra syndrome (9q34.3 microdeletion syndrome), developmental delay, dysmorphic features, arachnoid cyst |
| arr 12q14.3(64,025,085-64,638,946)x1 | | IUGR, small umbilical cord, oligohydramnios, retrognathia |
| arr 22q11.22q11.23(21,341,813-23,395,717)x3 | | Microcephaly, learning problems, dysmorphic features |

aCGH = array comparative genomic hybridisation; ADD = attention deficit disorder; ADHD = attention deficit hyperactivity disorder; ARPKD = autosomal recessive polycystic kidney disease; BPD = bronchopulmonary dysplasia; CCAM = congenital cystic adenomatoid malformation; CLCP = cleft lip and cleft palate; CP = cleft palate; FISH = fluorescence in situ hybridisation; FTT = failure to thrive; GH = growth hormone; HIE = hypoxic-ischemic encephalopathy; IUGR = intrauterine growth restriction; MPLA = multiplex ligation-dependent probe amplification; MR = mental retardation; OCD = obsessive-compulsive disorder; OTC = ornithine transcarbamylase; PDD = pervasive developmental disorder; SGA = small for gestational age; VCFS = velocardiofacial syndrome; WGA = whole-genome amplification

In our study, only 22 patients could be described as having isolated CHD, and of those only three had an abnormality detected by aCGH. Similar to the study conducted by Ergogan, all three patients had duplications.[6]

Even with greater detection of variants, many are of unclear significance and thus require additional investigation and provide limited clinical information.[11] Hitz et al compared families with CHD with controls. They identified 73 unique copy number variants in 54 individuals in the left-sided CHD cohort, suggesting that unique copy number variants contribute significantly to left-sided CHD.[31] Our finding that patients with left-sided heart disease had a higher rate of aCGH abnormalities is consistent with this study, and suggests that aCGH may have a higher diagnostic yield in patients with left-sided CHD, and perhaps of little benefit in isolated CHD patients.

Our study is unique in the large number of congenital heart patients who were evaluated, a total of 173 individuals. This number is, however, relatively small considering the wide range of anomalies that can be observed, both in the clinical phenotype and in the aCGH array results. A multi-centre analysis of aCGH data may help identify microdeletions and microduplications containing important genes in cardiac development. A limitation is that there were no real controls because none of the patients were "normal". All patients, either with or without CHD, had a clinical indication for an aCGH. In addition, there were no familial samples as this was a retrospective study. In addition, there was considerable selection bias. Only patients who underwent an aCGH were included. Therefore, patients who may have been diagnosed by other means, such as fluorescence in situ hybridisation, were not included. This may have removed patients with deletions of 22q11 locus from the study. This is important because 22q11 is recognised as being a frequent genetic cause of CHD. There are also potential systemic biases. All patients with cardiac disease who are admitted to the University of Alabama at Birmingham regional neonatal intensive care unit have an aCGH ordered routinely. However, patients transferred in from other hospitals would be missed by the study if they had an aCGH sent to another institution. Moreover, if infants were diagnosed with CHD after the newborn period, they may not have had an aCGH ordered unless a geneticist saw them as an outpatient.

Even with greater detection of genetic abnormalities in CHD, 70% of the patients remain without any identifiable genetic abnormalities. These may be the result of an undiscovered single-gene defect or combination of multiple gene defects, or the result of epigenetic alterations due to environmental effects. Next-generation sequencing may be an alternative modality for individuals with CHD and/or other anomalies. One study tested 250 probands, of which 62 carried 86 mutant alleles that satisfied criteria for a molecular diagnosis giving an overall rate of positive molecular diagnosis of 25%. This higher diagnostic yield supports the use of whole-exome sequencing as a diagnostic test. Questions about cost-effectiveness, accuracy, yield, and integration into clinical care, however, need to be addressed in future studies.[32]

## Conclusion

aCGH analysis may have a role as a first-tier test for individuals with left-sided CHD, or CHD with neurodevelopmental problems and/or multiple congenital anomalies. However, our study shows that it does not have a significant diagnostic yield in patients with isolated CHD. A prospective study of aCGH in patients with CHD that is not confounded by selection bias may clarify the role of copy number variants in patients with isolated CHD. In addition, other modalities such as whole-exome sequencing may have value in identification of candidate genes associated with CHD.

## Acknowledgements

None.

## Financial Support

This research received no specific grant from any funding agency, commercial, or not-for-profit sectors.

## Conflicts of Interest

None.

## Ethical Standards

The authors assert that all procedures contributing to this work comply with the ethical standards of National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research and with the Helsinki Declaration of 1975, as revised in 2008, and has been approved by the institutional review board at the University of Alabama at Birmingham.

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