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Chromosomal microarray detects genetic risks of neurodevelopmental disorders in newborns with congenital heart disease

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Original Article

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Abstract

Objective: To compare the genetic testing results of neonates with CHD by chromosomal microarray to karyotyping and fluorescence in situ hybridisation analysis. *Methods:* This was a single-centre retrospective comparative study of patients with CHD and available genetic testing results admitted to the cardiac ICU between January, 2004 and December, 2017. Patients from 2004 to 2010 were tested by karyotyping and fluorescence in situ hybridisation analysis, while patients from 2012 to 2017 were analysed by chromosomal microarray. *Results:* Eight-hundred and forty-nine neonates with CHD underwent genetic testing, 482 by karyotyping and fluorescence in situ hybridization, and 367 by chromosomal microarray. In the karyotyping and fluorescence in situ hybridisation analysis group, 86/482 (17.8%) had genetic abnormalities detected, while in the chromosomal microarray group, 135/367 (36.8%) had genetic abnormalities detected ($p < 0.00001$). Of patients with abnormal chromosomal microarray results, 41/135 (30.4%) had genetic abnormality associated with neurodevelopmental disorders that were exclusively identified by chromosomal microarray. Conotruncal abnormalities were the most common diagnosis in both groups, with karyotyping and fluorescence in situ hybridisation analysis detecting genetic abnormalities in 26/160 (16.3%) patients and chromosomal microarray detecting abnormalities in 41/135 (30.4%) patients ($p = 0.004$). In patients with d-transposition of the great arteries, 0/68 (0%) were found to have genetic abnormalities by karyotyping and fluorescence in situ hybridisation compared to 7/54 (13.0%) by chromosomal microarray. *Conclusions:* Chromosomal microarray identified patients with CHD at genetic risk of neurodevelopmental disorders, allowing earlier intervention with multidisciplinary care and more accurate pre-surgical prognostic counselling.

The aetiology of CHD is multifactorial and involves genetics, embryology, and environmental exposures.[1] Some forms of CHD are known to have a specific genetic cause[2,3] and most, if not all, patients with moderate-to-severe CHD will undergo some form of genetic testing. As genetic technologies have evolved, so has the detection of genetic variants associated with CHD. Karyotyping was one of the first genetic testing modalities used clinically, it revealed chromosomal aberrations in 8–13% of neonates with CHD.[4] This era was followed by combined testing with karyotyping *and* fluorescence in situ hybridisation analysis. The combination was able to diagnose aneuploidies and more subtle genetic abnormalities such as deletions and duplications.[5] Our previous institutional review identified chromosomal abnormalities in 17.8% of neonates with structural heart disease by karyotyping and fluorescence in situ hybridisation analysis.[6] Chromosomal microarray analysis is a valuable clinical tool for the identification of submicroscopic chromosomal aberrations that cannot be detected by conventional cytogenetic methods.[7]

In this study, we sought to compare the diagnostic yield of two different eras of genetic testing at our institution: chromosomal microarray, conducted between 2012 and 2017 and karyotyping and fluorescence in situ hybridisation analysis, conducted between 2004 and 2010 as previously reported.[6] In both eras, patients with CHD routinely underwent genetic testing upon admission to the CICU, with neonates admitted between 2004 and 2010 undergoing karyotyping and fluorescence in situ hybridisation analysis and neonates admitted between 2012 and 2017 undergoing chromosomal microarray testing.

Materials and methods

The Institutional Review Board at Nicklaus Children's Hospital approved this retrospective comparative study. Karyotyping and fluorescence in situ hybridisation analysis data on 482 neonates less than 30 days of age with CHD admitted to the cardiac intensive care unit at Nicklaus Children's Hospital between 2004 and 2010 were reported in our previous study.[6]

Inclusion criteria for chromosomal microarray analysis were all patients less than 30 days of age with CHD admitted to the CICU between 2012 and 2017. Demographic data including gestational age, birth weight, gender, and race were collected. The presence of dysmorphic features was confirmed by physical examination by consulting board-certified geneticists. Cardiac lesions were evaluated and diagnosed by transthoracic echocardiography. Genetic testing via chromosomal microarray is currently standard of care for all neonates admitted with CHD to the CICU at Nicklaus Children's Hospital.

Chromosomal microarray testing was performed using the Affymetrix CytoScan HD Array, which features 2.6 million genetic markers including ~750,000 single-nucleotide polymorphisms and 1.9 million non-polymorphic probes for the detection of copy number variations, loss of heterozygosity, uniparental isodisomy, regions indicated by descent, and measurements of low-level mosaicism and heterogeneity.

All patients with genetic results not reported as "normal chromosomal microarray" were categorised as an anomalous chromosomal microarray result. Anomalous chromosomal microarray results included aneuploidies, deletions, duplications, loss of heterozygosity, and variants of uncertain significance. Chromosomal microarray results were also subdivided into four subcategories: abnormal results with a known association with CHD; abnormal results with no known association with CHD; variants of uncertain significance; and benign familial variants. Abnormal results with known associations with CHD consisted of abnormal genetic findings with well-known associations with cardiac lesions such as deletions involving *TBX1*, *GATA4*, *CREBBP*, *ELN*.[8] Abnormal results with no known association with CHD consisted of abnormal, but well-described genetic variants without documented associations with CHD. Variants of uncertain significance represent a chromosomal anomaly (i.e. deletion or duplication) for which there are insufficient data, thus, the impact on health and disease risk is uncertain.[7] Benign familial variants are a chromosomal anomaly with no association with a specific condition.[9]

Descriptive data are presented as mean ± standard deviation for normally distributed continuous data or median (25–75% interquartile range) otherwise and as n (%) for categorical data. Chi-square tests were used to compare differences in proportions between the categorical outcome groups, and the Mann–Whitney U-test or Student's t-test were used to test for differences between groups for continuous variable, depending on the distribution. All statistical tests were two-tailed and a p-value <0.05 was considered significant. Analyses were performed with SPSS version 23.0 (IBM Statistics, Chicago, IL, USA).

Results

Eight-hundred and forty patients underwent genetic testing in 2 different eras of genetic testing at our institution; there were no statistically significant differences in gestational age or gender in patients tested between 2012 and 2017 and between 2004 and 2010 (Table 1).

Three-hundred and sixty-seven neonates admitted to the CICU at Nicklaus Children's Hospital between 2012 and 2017 met the inclusion criteria (Table 2). 135/367 (36.7%)patients had anomalous chromosomal microarray analysis results. Of the anomalous results, deletions were the most common finding, present in 52/135 (38.5%), followed by gains/duplications (41/135; 30.4%), aneuploidies (17/135; 13%), loss of heterozygosity (14/135; 10%), multiple anomalous results (9/135; 6.7%), and XX/XY mosaicism (Table 2). According to our sub-classification, abnormal results with a known genetic association with CHD were present in 43/135 (31.8%) of the abnormal results; abnormal results with no known association with CHD comprised 29/135 (21.5%) of abnormal results; variants of uncertain significance were present in 61/135 (45.2%); and benign familial variants were present in 2/135 (1.5%) patients (Table 2).

Of all patients with abnormal chromosomal microarray analysis results, 76/135 (56.3%) of patients had genetic abnormalities known to be associated with risk for neurodevelopmental disorders. In 46/76 (60.5%) of patients with neurodevelopmental disorders, the genetic abnormality would not have been identified by karyotyping and fluorescence in situ hybridisation analysis testing alone. Karyotyping and fluorescence in situ hybridisation analysis can identify aneuploidies and 22q11 deletions, so patients with trisomy 13, 18, and 21 and 22q11 deletion syndrome were excluded from the analysis of neurodevelopmental disorders. Forty-six genetic abnormalities were detected by chromosomal microarray analysis that is known to be associated with neurodevelopmental disorders such as autosomal-dominant intellectual disability, Jacobsen syndrome, and various seizure disorders. A complete list of the identified genetic abnormalities and their associated neurodevelopmental disorders and cardiac lesions are shown in Table 3.

Dysmorphic features were defined as physical examination findings that were deemed abnormal rather than a variant of normal. Dysmorphic features were identified by physical examination and documented by the same group of geneticists in the two eras of testing. In the patients that underwent chromosomal microarray analysis testing, dysmorphic features were documented in 84/367 (22.9%) patients. The most commonly described dysmorphic features were malformations of the ears, mouth, eyes, and limbs. Of the patients with dysmorphic features, 50/84 (59.5%) had abnormal chromosomal microarray analysis results ($p < 0.00001$) (Table 1). In our previous study, dysmorphic features were present in (68/86) 79.1% of patients with abnormal genetic results.[6] Of note, our previous study classified sacral dimple, cleft, and/or tuft of hair as dysmorphic features, whereas the current study did not.

Multiple cardiac lesions were identified both with and without genetic associations. Comparing our results to our previous study using karyotyping and fluorescence in situ hybridisation analysis,[6] chromosomal microarray analysis detected a higher number of genetic abnormalities (36.7% of patients) than karyotyping or fluorescence in situ hybridisation analysis (17.8% of patients) (Table 4). Conotruncal abnormalities were the most common cardiac diagnosis in our cohort and comprised 41/135 (30.4%) of patients with abnormal chromosomal microarray analysis results. Surprisingly, 7/54 (13.0%) patients with d-transposition of the great arteries were found to have abnormal chromosomal microarray analysis results, while karyotyping and fluorescence in situ hybridisation analysis detected no genetic abnormalities in this population.

Discussion

With rapid advances in genomic technologies, various forms of genetic testing are now offered to CHD patients and their families. However, there is little consensus on the optimal test, the specific clinical indication for testing, the interpretation of test results, and their prognostic yield. At our institution, since 2004, every neonate admitted with CHD has undergone some form of genetic testing. Between 2004 and 2010, all neonates admitted to the cardiac ICU

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Table 1. Comparison of demographics and cardiac lesions between the karyotype and FISH testing group versus the CMA group and between normal and abnormal CMA results

	Karyotype and FISH (n = 482)	Chromosomal microarray (n = 367)	p-value	Normal CMA (n = 232)	Abnormal CMA (n = 135)	p-value
Gender			**0.890**			**0.21**
Female	198 (41)	149 (41)		88 (59%)	61 (40.1%)	
Male	284 (59)	218 (59)		144 (66.1%)	74 (34%)	
Race/Ethnicity			**<0.00001**			**0.62**
White	170 (35)	74 (20.2)		43 (22%)	31 (8.4%)	
Black	90 (19)	41 (11.1)		24 (10.3%)	17 (13%)	
Hispanic	204 (42)	208 (57)		136 (59%)	72 (53%)	
Other	18 (3.7)	44 (12)		29 (13%)	15 (11%)	
Dysmorphic findings			**0.0001**			**<0.00001**
Present	169 (35)	84 (22.9)		34 (14.7)	50 (37)	
Absent	313 (65)	283 (77.1)		198 (85.3)	85 (63)	
Gestational age			**0.0502**			**0.4**
37–42 weeks	403 (83.6)	319 (86.9)		199 (85.7)	120 (88.9)	
34–36 weeks	47 (9.8)	32 (8.7)		21 (9)	11 (8.1)	
32–34 weeks	24 (55.0)	9 (2.5)		5 (2.2)	4 (3)	
30–31 weeks	7 (1.4)	2 (0.5)		2 (0.9)	0	
<30 weeks	1 (0.2)	5 (1.4)		5 (2.2)	0	
Cardiac group						
Heterotaxy	12 (2.5)	9 (2.4)	**0.97**	6 (2.6)	3 (2.2)	**0.83**
Conotruncal defects	160 (33.2)	135 (36.8)	**0.28**	94 (40.5)	41 (30.4)	**0.052**
Right side obstructive	51 (10.6)	24 (6.5)	**0.04**	19 (8.2)	5 (3.7)	**0.093**
Left side obstructive	108 (22.4)	61 (16.6)	**0.04**	36 (15.5)	25 (18.5)	**0.46**
Single ventricle	89 (18.5)	63 (17.2)	**0.62**	30 (12.9)	33 (24.4)	**0.005**
Septal defects	29 (6.0)	26 (7.1)	**0.53**	15 (6.5)	11 (8.1)	**0.54**
Other	33 (6.8)	49 (13.4)	**0.001**	32 (13.8)	17 (12.6)	**0.74**
Median birth weight (kg) (n = 361)	N/A	N/A		3.069	2.946	**0.978**

Values expressed as n (%).
CMA = chromosomal microarray; FISH = fluorescence in situ hybridisation analysis.

Table 2. Breakdown of abnormal chromosomal microarrays and their associations with CHD

Abnormal results by association with CHD	n = 135
Abnormal results with known association with CHD	43 (31.9%)
Abnormal results with no known association with CHD	29 (21.5%)
Variant of unknown significance	61 (45.2%)
Benign familial variant	2 (1.5%)
Type of abnormal chromosomal microarray results	**n = 135**
Aneuploidies	17 (12.6%)
Deletions	52 (38.5%)
Duplications/gains	41 (30.4%)
Loss of heterozygosity/homozygosity	15 (11.1%)
Multiple abnormal results	9 (6.7%)
Others	1 (0.7%)

Values expressed as n (%).

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Table 3. All genetic defects exclusively identified by chromosomal microarray associated with neurodevelopmental abnormalities

Abnormal CMA: autosome/sex chromosome aneuploidy or 22q11.2 deletion	30 (39.5%)
Abnormal CMA: genetic abnormality with neurodevelopmental abnormality	46 (60.5%)
Abnormal CMA with associated neurodevelopmental abnormality	76 (56.3%)

Genetic abnormalities identified by CMA associated with neurodevelopmental abnormalities

Cardiac diagnosis	Abnormal CMA genetic abnormality	Genetic abnormality	Neurodevelopmental abnormality
Aortic stenosis, unicommisural valve	Loss 8p23.3	*GATA4*	8p23.1 syndrome: intellectual disability
Aortic stenosis, unicommisural valve	Homozygosity identified on several chromosomes	Multiple genes	Joubert syndrome, limb-girdle muscular dystrophy
Atypical cleft mitral valve	Duplication 1q41r and deletion 6p25.3p25.1	Trisomy 1q41-qter syndrome; pathogenic: 6 MB terminal deletion of 6 MB 6p25.1-pter	Developmental delay, dysmorphic features, seizures, brain malformations
Cardiac haemangioma	Gain 15q13.3	*CHRNA7*	Intellectual disability, behavioural problems, neuropsychiatric disease, autism, hypotonia
Coarctation, bicuspid aortic valve	7q11.23 loss, 15q13.3 gain	*GTF21, NCF1, GTF2IRD2, CHRNA7*	Williams–Beuren syndrome, *CHRNA7* gene: intellectual disability, behavioural problems, neuropsychiatric disease, autism, hypotonia
Coarctation, bicuspid aortic valve	Loss 3q25.1	*AADAC*	Tourette syndrome
Coarctation, VSD	Loss 9p24.3	*KANK1*	
Coarctation, VSD	Loss 15q11.2	*TUBGCP5, CYFIP1, NIPA1, NIPA2*	Developmental delay, seizures, autism
Coarctation	Loss 6p25.3p24.3	Deletion 6p syndrome	Central nervous malformations, hearing loss, CHD, developmental delay
Coarctation	Loss 7q31.1	*IMMP2L*	Autism spectrum disorders and neurodevelopmental disorders are may be considered as a candidate gene for Tourette syndrome
Coarctation	Loss 15q15.3	*STRC, CATSPER2, CKMT1A*	Male sensorineural hearing loss and infertility (deafness-infertility syndrome)
Coronary artery fistula	Deletion on chromosome 17p12	*PMP22*	Hereditary neuropathy
DILV	Loss 16p13.3	*RBFOX1*	Global developmental delay, autism spectrum disorder, epilepsy
DORV	Loss 9p24.3	*KANK1*	Spastic quadriplegic cerebral palsy
DORV	Gain 17q25.1	*RPL38, TTYH2, DNA12*	Autism spectrum disorder
DORV	Gain 1q42.21q44, Loss 9q34.3	Gain: 93 OMIM genes, Loss: 65 OMIM genes	Global developmental delay, hypotonia, seizures
DORV	Gain 15q11.2	*NIPA1*	Spastic paraplegia, poor motor coordination, limited expressive language, autism, ADHD/ADD, OCD
DORV	Gain 15q13.3	*CHRNA7*	Adult-onset schizophrenia, intellectual disability, autism spectrum disorder, behavioural problems, hypotonia
D-TGA	Gain 8p22	*ASAH1, NAT1, NAT2, PSD3*	Hypotonia
D-TGA	Loss 4q35.1	*PDLIM3, SORBS2, TLR3*	Developmental delay
D-TGA	10p12.1q11.22-17.5 MB HMZ	*RAB19, MTAP, RET*	*RAB19*: Warberg micro syndrome, MTAP: spastic ataxia, RET: central hypoventilation syndrome
Dysplastic PV	Loss 16p13.3	Includes *CREBBP* or *CBP*	Rubinstein–Taybi syndrome
Heterotaxy	Homozygosity on 7q34q36.1	*CLCN1, PRSS1, TPK1*	Genes in the homozygous regions – myotonia congenita, thiamine metabolism syndrome 5-episodic encephalopathy type

(*Continued*)

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Table 3. (*Continued*)

Cardiac diagnosis	Abnormal CMA genetic abnormality	Genetic abnormality	Neurodevelopmental abnormality
HLHS	Loss 11q24.1q25	*SORL1, KIRREL3*	Jacobsen syndrome: intellectual disabilities, delayed speech, motor disabilities
HLHS	Gain 1q21q1q21.2	*PRKAB2, PDIA3P, FMO5, CHD1L, BCL9, ACP6, GJA5, GJA8, GPR89B, GPR89C, PDZK1P1, NBPF24, NBPF11*	1q21.1 microduplication syndrome: developmental delay
HLHS	Loss 7q31.1	*IMMP2L*	Autism spectrum disorders and neurodevelopmental disorders are may be considered as a candidate gene for Tourette syndrome
HLHS	Duplication 1q42.3	*TBCE*	Progressive encephalopathy, optic atrophy, intellectual disability
HLHS	22q11.21 microdeletion	*PI4KA, SERPIND1, SNAP29, CRKL, LZTR1, THAP7, P2RX6, SLC7A4, BCRP2*	Hypotonia and developmental delay
HLHS	Gain 9p24.3, Loss 15q11.2	*DOCK8, EHMT1, CACNA1B, TUBGCP5, CYFIP1, NIPA1, NIPA2*	Kleefstra syndrome, developmental delay, behavioural problems, autism, epilepsy
HLHS	Gain 9p24.3	*DOCK8, KANK1*	Autosomal-dominant mental retardation
HLHS	Gain 15q13.3	*CHRNA7*	CHRNA7: adult-onset schizophrenia, intellectual disability, autism spectrum disorder, behavioural problems, hypotonia
HLHS	Loss 7q31.1	*IMMP2L*	Autism spectrum disorders, neurodevelopmental disorders, and candidate gene for Tourette syndrome
HLHS	Loss of 6q24.3q25.1	16 OMIM genes	6q24.3q25 deletion syndrome: intellectual disability
HLHS	Gain 6p22.3	*ATXN1*	Autosomal-dominant spinocerebellar ataxia
IAA (Type B)	Xp21.2 loss, 9p24.31q13 gain	149 OMIM genes	Duchenne's muscular dystrophy, global developmental delay
PA/IVS	Loss 15q31.1	*HERC2*	Autosomal recessive mental retardation
PDA	Loss 1p36.33p36.22	1p36 deletion syndrome	Developmental delay, hypotonia, seizures, hearing loss and vision defects
TAPVR (Infradiaphragmatic)	Loss 15q11.2	*TUBGCP5, CYFIP1, NIPA1, NIPA2*	15q11.2 microdeletion: developmental and speech delays, seizures, autism spectrum disorders and neuropsychiatric disorders
TAPVR (Infradiaphragmatic)	Loss 6q24.3q25.1	*TUBGCP5, CYFIP1, NIPA1, NIPA2*	Developmental and speech delays, seizures, autism spectrum disorders and neuropsychiatric disorders
TAPVR (Infradiaphragmatic)	Duplication and triplication on chromosome 22q11.1q11.21	51 OMIM genes	Intellectual disability, learning disability, delayed psychomotor development, and hypotonia.
TAPVR (Supracardiac)	15q11.2-511kb deletion	*TUBGCP5, CYFIP1, NIPA2, NIPA1, WHAMML1, GOLGA8IP*	Delayed development, behaviour problems, idiopathic epilepsy, autism spectrum disorder
TOF	Gain 8p23.1	6OMIM genes	Developmental delay
TOF	Gain Xp22.31	*VCX, PNPLA4*	Cognitive deficits, seizures
Truncus arteriosus (type A2)	Loss 8q21.12q22.2	52 OMIM genes	Hypotonia, global developmental delay, and Dandy–Walker anomaly
VSD	Loss of heterozygosity on 7q11.22q21.12	*CD36, POR, POR, HGF, NCF1*	Deafness
VSD	Loss 6q26	*PARK2*	Autistic spectrum disorder

DILV = double inlet left ventricle; DORV = double outlet right ventricle; DORV w/HLV = DORV with hypoplastic left ventricle; d-TGA = d-transposition of the great arteries; PV = pulmonary valve; HLHS = hypoplastic left heart syndrome; IAA = interrupted aortic arch; PA/IVS = pulmonary atresia with intact ventricular septum; PDA = patent ductus arteriosus; TAPVR = totally anomalous pulmonary venous return; TOF = tetralogy of Fallot; VSD = ventricular septal defect.

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Table 4. Cardiac lesions and their frequency of genetic abnormalities detected by different testing modalities

Frequency of abnormal result by cardiac lesion	Karyotyping and FISH abnormal, n (%)	Total, n	CMA abnormal, n (%)	Total, n	p-value
All cardiac lesion	86 (17.8)	482	135 (36.8)	367	<0.00001
Heterotaxy	1 (8.3)	12	3 (33.3)	9	0.15
Conotruncal defects	26 (16.3)	160	41 (30.4)	135	0.004
DORV	3 (13.6)	22	12 (54.5)	22	
d-TGA	0 (0)	68	7 (13.0)	54	
l-TGA	0 (0)	2	0 (0)	2	
TOF	9 (40.9)	22	12 (38.7)	31	
TOF absent PV	1 (50)	2	0	0	
TOF/PA	7 (24.1)	29	3 (21.4)	14	
Truncus arteriosus	6 (42.9)	14	6 (54.5)	11	
Hemitruncus	0 (0)	1	1 (100)	1	
Right side obstructive	3 (5.9)	51	5 (20.8)	24	0.050
Pulmonary atresia	0 (0)	15	0	0	
Dysplastic TV	1 (50)	2	0 (0)	2	
Pulmonary stenosis	2 (14.3)	14	2 (18.2)	11	
PA/IVS	0 (0)	9	3 (30)	10	
Hypoplastic RV	0 (0)	11	0 (0)	1	
Left side obstructive	21 (19.4)	108	25 (41.0)	61	0.0025
Mitral stenosis	0 (0)	1	0 (0)	0	
Aortic stenosis	2 (22.2)	9	5 (45.5)	11	
Coarctation	12 (15.2)	79	17 (39.5)	43	
IAA	7 (36.8)	19	3 (60)	5	
Shone's complex	0	0	0 (0)	2	
Single ventricle	11 (12.4)	89	33 (52.4)	63	<0.00001
DILV	0 (0)	2	1 (33.3)	3	
HLHS	8 (11.3)	71	23 (59.0)	39	
TA	1 (14.3)	7	1 (11.1)	9	
DORV w/HLV	1 (14.3)	7	3 (60)	5	
Aortic Atresia	1 (50)	2	0 (0)	1	
Unbalanced AVC	0	0	5 (83.3)	6	
Septal Defects	19 (65.5)	29	11 (42.3)	26	0.084
VSD	2 (40)	5	6 (46.2)	13	
Primum ASD	1 (100)	1	0	0	
ASD	0	0	0 (0)	2	
AVC	16 (69.6)	23	0	0	
Balanced AVC	0	0	5 (45.5)	11	
Other	5 (10.2)	49	11 (33.3)	33	0.0502
Ebstein Anomaly	1 (33.3)	3	1 (20)	5	
APVR	3 (13.0)	23	8 (57.1)	14	
Coronary Anomaly	1 (33.3)	3	0	0	
Coronary Artery Fistula	0	0	1 (100)	1	
Rhabdomyoma	0 (0)	1	0	0	
Tumour/Mass	0	0	1 (50)	2	
Cor Tritriatum	0 (0)	1	0	0	

R=anomalous pulmonary venous return; ASD=atrial septal defect; AVC=atrioventricular canal; DILV=double inlet left ventricle; DORV=double outlet right ventricle; DORV w/HLV=DORV with hypoplastic left ventricle; d-TGA=d-transposition of the great arteries; HLHS=hypoplastic left heart syndrome; PA/IVS=pulmonary atresia with intact ventricular septum; l-TGA=l-transposition of the great arteries; TOF=tetralogy of Fallot; VSD=ventricular septal defect.

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underwent genetic testing by karyotyping or karyotyping and fluorescence in situ hybridisation analysis. When we reported these data in 2016, 86/482 (17.8%) of patients had a chromosomal abnormality.[6] In 2010, the American College of Medical Genetics issued practice guidelines for chromosomal microarray analysis and recommended chromosomal microarray analysis as first-tier testing for postnatal patients with multiple congenital anomalies.[10] In 2012, our institution began to regularly use chromosomal microarray analysis as the screening genetic test in CHD patients. In our cohort, 135/367 (37%) patients had genetic abnormalities detected by chromosomal microarray analysis. As chromosomal microarray analysis offers higher resolution compared to karyotyping and/or fluorescence in situ hybridisation analysis, an increased detection rate of abnormalities is consistent with the current literature.[5,10–12]

Of interest, 76/135 (56.3%) of patients with abnormal chromosomal microarray analysis results had chromosomal abnormality associated with the risk of neurodevelopmental disorders. Of these 76 patients, 30 had one of the following: trisomy 18, 21, or 22, sex chromosomal aneuploidy, or 22q11.2 deletion. All of which can be detected by karyotyping and fluorescence in situ hybridisation analysis. So, 46/76 (60.5%) of patients with risk of neurodevelopmental disorders had genetic abnormalities that were only detectable by chromosomal microarray analysis. Recognising neurodevelopmental problems in patients with CHD is of paramount importance in the neonatal period, since these disorders affect 10% of all patients with CHD and 50% of patients with severe heart disease.[15] Traditionally, consideration of risk factors for CHD-associated neurodevelopmental delay has focused on the role of cardiopulmonary bypass, the byproduct of abnormal physiology, and post-operative complications.[16] It is likely that these factors contribute to some degree of neurodevelopmental delay in our patients, together with genetic abnormalities. Neurodevelopmental risk stratification remains difficult in the neonatal CHD population, but readily identifying patients at high risk due to genetic abnormalities allows early intervention with multidisciplinary care and more accurate pre-surgical prognostic counselling for families. Several studies have shown the benefit of early intervention in these patients and its effect on neurodevelopmental outcomes, such as executive function in at-risk children.[17]

Dysmorphic features were identified and documented by the same group of board-certified geneticists in both studies. In our current cohort, 84/367 patients had dysmorphic features, 50/84 (59.5%) of whom had an abnormal chromosomal microarray analysis result. Two-hundred and eighty-three patients did not exhibit any dysmorphic features, and 85 (30.0%) of these had an abnormal chromosomal microarray analysis result. Of all patients with an abnormal chromosomal microarray analysis result, dysmorphic features were noted in 50/135 (37%) patients. Of note, dysmorphic features were also noted in 34/235 (15%) of patients with a normal chromosomal microarray analysis. Our previous study detected dysmorphic features in 79% of patients with abnormal cytogenetic results and in 25% of patients with normal cytogenetic results,[6] higher than in the current study, which may be due to the inclusion of sacral dimple and cleft as a dysmorphic feature in the previous study. Most dysmorphic patients lacked a specific history or physical examination features to suggest a specific genetic disorder.[10] In some institutions, genetic testing is only considered if the patient exhibits dysmorphic features.[14] However, our data indicate that nearly half of patients with CHD and genetic anomalies do not have any dysmorphic features on physical examination. Thus, using dysmorphic features to risk stratify candidates with CHD for genetic testing may overlook a significant number of patients with genetic abnormalities.

Conotruncal abnormalities were the most common diagnosis in both our previous study and the current chromosomal microarray analysis study. In our previous study, conotruncal defects were present in 33.2% (160/482) of patients and accounted for 30.2% (26/86) of all abnormal genetic results. In the current study, conotruncal defects were present in 36.8% (135/367) of patients and comprised 30.4% (41/135) of patients with abnormal chromosomal microarray analysis results. Of patients with d-transposition of the great arteries, 7/54 (12.9%) had abnormal chromosomal microarray analysis results, while no genetic abnormalities were detected in patients with d-transposition of the great arteries by karyotyping and fluorescence in situ hybridisation analysis.[6] D-transposition of the great arteries is not generally associated with genetic anomalies and has been reported to have a very low recurrence risk,[13] so our result is surprising. Of our patients with d-transposition of the great arteries and abnormal genetic results, one patient had an abnormal result with known association with CHD, two patients had an abnormal result with no known association with CHD, and four patients had variants of uncertain significance. The patient with an abnormal result with known association with CHD had a gain of 622 kb at 8p22, a region containing four Online Mendelian Inheritance in Man genes: *ASAH1, NAT1, NAT2*, and *PSD3*. Duplications of this region have been associated with coarctation of the aorta and generalised neonatal hypotonia.[8] Of the two patients with abnormal results with no known association with CHD, one had a loss of 10p12.1q11.22 and the other had a 47, XYY karyotype.[8] Four patients had variants of uncertain significance: a gain of 246 kb at 15q15.3, loss of 336 kb at 13q21.31, loss of 252 kb at 22q21.1, and a 251 kb loss at 7q34.[8] Despite the current literature reporting genetic abnormalities as rare in patients with d-transposition of the great arteries, 13% of patients with d-transposition of the great arteries and abnormal genetic test results. Of the seven patients with d-transposition of the great arteries and anomalous chromosomal microarray, one patient was at risk of developing a neurodevelopmental disorder.

As expected, we detected a much higher rate of genetic abnormalities by chromosomal microarray analysis compared to the previous era of testing with karyotyping and fluorescence in situ hybridisation analysis at our institution. Despite a large number of results being variants of uncertain significance, we detected several clinically impactful genetic abnormalities. With the current growth in cardiac genetics clinical research, variants of uncertain significance may yet become clinically significant.[18–20] CHD patients who do not display dysmorphic features on clinical examination should undergo genetic evaluation and testing, as 15% of our patients with abnormal chromosomal microarray analysis results had no dysmorphic features identifiable by board-certified geneticists. We identified 60% more patients with potential neurodevelopmental abnormalities by chromosomal microarray analysis compared to karyotyping and fluorescence in situ hybridisation analysis. The ability to recognise patients who are of innately high neurological risk allows us to provide appropriate family counselling, anticipatory guidance, and early interventions. Chromosomal microarray analysis is a valuable routine tool to supplement the lifelong care of patients with CHD.

Genetic testing continues to rapidly advance, as next-generation sequencing of multiple targets or whole-genome sequencing becomes readily available and affordable. Sequencing tests detect variants within single genes and chromosomal microarray detects copy number variants. While a few studies have reported targeted

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use of next-generation sequencing of multiple genes and whole-genome sequencing with high diagnostic yield in CHD,[22,23] further studies are required for routine clinical application in paediatric CHD.

Limitations of this study include the retrospective study design, the comparison of different genetic testing modalities in two different groups rather than the same group, and a lack of long-term follow-up in patients with an identified risk of neurodevelopmental disorders. Nevertheless, despite the two groups examined undergoing different genetic testing modalities, there were no statistically significant differences between the two populations in terms of gender, gestational age, and the majority of cardiac lesion subgroups (Table 1). Birth weight could not be compared, as raw data were not available from the previous study. There was a significant difference in race, possibly attributable to the introduction of electronic medical records in 2012, as race was exclusively documented for each patient in the paper records but the more recent electronic medical records contain two fields for race or ethnicity, with ethnicity often the only documentation and race then auto-populated as "other". The strengths of this study include the large sample size of patients who underwent two distinct genetic testing modalities with available results and clinical correlation.

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Ethical standards. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national guidelines on human experimentation (please name) and with the Helsinki Declaration of 1975, as revised in 2008, and has been approved by Institutional Review Board at Nicklaus Children's Hospital.

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