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Array comparative genomic hybridization as a clinical diagnostic tool in syndromic and nonsyndromic congenital heart disease

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BACKGROUND: Congenital heart diseases (CHDs) are often associated with other congenital anomalies, dysmorphic features, and developmental delay, and only a few cases of chromosomal abnormalities are detected by conventional cytogenetic techniques. The microarray comparative genomic hybridization (CGH) analysis allows the identification of submicroscopic genomic rearrangements.

METHODS: During the past 3 y, 55 of 330 patients referred for array CGH had CHD of unknown etiology plus at least one additional indication of abnormal chromosomal phenotype. High-resolution 1×244K or 4×180K Agilent arrays were used in this study (average resolution 7–13 kb).

RESULTS: Copy-number variations were detected in 37 of 55 patients, and in 29 of 37 patients there were genes that have been associated with CHD. All 37 patients had at least one additional phenotypic abnormality: 30 of 37 had one or more other congenital anomalies, 23 of 37 had dysmorphic features, 16 of 37 had intellectual disability, 13 of 37 had abnormal magnetic resonance imaging, 10 of 37 had hypotonia, and 7 of 37 had seizures. In 9 of 55 patients, unexpected genomic rearrangements in relation to their phenotype were identified.

CONCLUSION: In patients with CHD and at least one additional indication of abnormal chromosomal phenotype, array CGH analysis could detect possible submicroscopic chromosomal abnormalities and provide proper genetic counseling.

Congenital heart disease (CHD), with a frequency of 19 to 75 in 1,000 births, is a major cause of childhood morbidity and mortality worldwide (1). Recent advances in medical and surgical management have resulted in 85% of affected children surviving to adulthood (2). Classic studies, including the Baltimore–Washington Infant Study, have shown that CHD is multifactorial/complex in nature because of both genetic predisposition and environmental factors (3).

Chromosomal abnormalities are a common cause of CHD, but conventional cytogenetic techniques (G-banded karyotype) are able to detect imbalances 5–10 Mb in size. Submicroscopic aberrations can be detected using more recent genetic diagnostic methodologies such as high-resolution comparative genomic hybridization (CGH) technology, which can also identify copies of DNA, called copy-number variations (CNVs). A CNV is defined as a chromosomal region of at least 1 kb in size that differs in copy number compared with the reference human genome. The CNV can be a benign variation (without clinical significance), or it could have unclear clinical significance or be pathogenic. For clinical relevance, the CNV must be categorized as benign, of unclear significance, or pathogenic, thus contributing to the patient's phenotype. To further interpret the nature of the CNV, the qualifier copy-number loss (deletion) or copy-number gain (duplication) should also be included. Microdeletions or microduplications, which are clinically significant genomic rearrangements, have been identified in patients with intellectual disability/developmental delay and/or multiple congenital anomalies, with otherwise normal conventional karyotype (4). The main advantage of array CGH is its ability to detect any quantitative changes in DNA; it is 10–10,000-fold more detailed than conventional karyotype, depending on the size of the target and the coverage and density of probes in the array (5). This study describes the application of array CGH to detect submicroscopic genomic rearrangement in children with CHD and syndromic features.

RESULTS

Of the 55 patients with CHD who were examined by array CGH, large genomic rearrangements (CNVs) were detected in 37 patients (67%) (20 males/17 females). In 23 patients, more than 1 CNV was detected, and in total, 77 deletions and 22 duplications were identified, ranging in size from 0.08 to 19.01 Mb (**Table 1**). The types of heart defect in each of the 37 patients with a pathogenic CNV detected are shown in **Table 1**. The assignment of CNVs pathogenicity was based on the gene content according to the University of California Santa Cruz Genome Browser database (http://genome.ucsc.edu/) human genome build 18 or 19 (6). In addition, these patients presented

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Table 1. Submicroscopic rearrangements (CNVs) in our patients with congenital heart diseases

Patient	Phenotype	CNVs: chromosomal region and size (Mb)[a]	Inheritance	Known syndrome	Type of heart defect	Significant candidate genes relating to CHD[b]
1	CHD, PF, MCA	Del 1p36.33 (0.717 Mb), Del 3q27.1 (0.423 Mb), Del 4q21.22–q22.1 (5.6 Mb), Del 16p13.3 (2.97 Mb)	*De novo*	1p36.33 Microdeletion syndrome	Atrial septal defect	*DVL1*, *CHRD*
2	CHD, MCA, MR	Dup 1q41 (0.37 Mb), Del Xp11.3 (0.042 Mb), Del Xq21.31 (0.118 Mb)	*De novo*		Ventricular septal defect	*DISP1*
3	CHD, MR, MCA, E	Del 16p13.13 (0.304 Mb)			Dysplastic mitral valve	
4	CHD, MR, MCA, PF	Del 11q23.3–q25 (14.7 Mb)	*De novo*	Jacobsen syndrome	Pulmonary valve stenosis	*ETS1*, *KCNJ5*, *SCN3B*
5	CHD, PF, MCA, HY	Del 22q13.31–q13.33 (5.8 Mb)			Aortic regurgitation	*WNT7B*, *SCO2*
6	CHD, PF, MR, MCA	Del 2q37.3 (0.162 Mb), Del 10p13 (1.03 Mb)			Atrial septal defect	
7	CHD, MR, MCA	Del 7q11.21 (0.413 Mb)		Williams syndrome	Atrial septal defect, aortic dilatation	*ELN*
8	CHD, MCA	Del 2q35 (0.145 Mb), Del 7q21.11 (0.021 Mb), Del 8p22 (0.74 Mb), Del 15q22.31 (0.77 Mb), Del 20q13.2 (0.031 Mb), Del 22q11.23 (0.25 Mb), Del Xp22.12 (0.053 Mb)	pat, pat and mat, pat, *de novo*, pat, pat, *de novo*	CHARGE	Aortic regurgitation	*IGLL3*
9	CHD, PF, MCA	Del 1p36.33 (0.084 Mb), Del 4p16.3 (0.037 Mb), Del 8q11.21 (0.04 Mb), Del 20q13.3 (0.098 Mb), Del 22q11.21–q11.22 (1.164 Mb)		1p36.33 Microdeletion syndrome	Right aortic arch regurgitation	*DVL1*, *GATA5*, *HIC2*, *TOP3B*
10	CHD, PF, MCA, MR, HY, E	Del 1p36.33–p36.32 (2.778 Mb), Dup 21q2–q21.3 (0.58 Mb)		1p36.33 Microdeletion syndrome	Ventricular septal defect, pulmonary hypertension	*DVL1*
11	CHD, MCA	Del 1p34.2 (0.016 Mb), Dup 3p25.2 (0.061 Mb), Del 3q27.2 (0.008 Mb), Del 15q22.2 (0.014 Mb), Dup Xp22.32 (0.1 Mb), Dup Xq12 (0.026 Mb)			Atrial septal defect	
12	CHD, PF, MR, MCA	Dup 9p24.3–p22.1 (19.09 Mb), Del 13q33.1–q34 (13.6 Mb)			Atrial septal defect, double chamber right ventricle, congestive cardiac failure	*COL4A1*
13	CHD, PF, MR, MCA, HY	Del 22q11.21 (2.5 Mb)		DiGeorge syndrome	Atrial septal defect	*TBX1*, *DGCR8*
14	CHD, PF, MR, MCA, HY	Del 2q13 (0.118 Mb), Dup 15q11.2q13.1 (7.47 Mb)			Atrial septal defect	
15	CHD, MR, MCA	Dup 20p12.2 (0.219 Mb), Dup 21q21.3 (0.095 Mb)			Ventricular septal defect	*ADAMTS1*, *ADAMTS5*
16	CHD, PF, MR, MCA, E	Del 22q11.21 (2.793 Mb)		DiGeorge syndrome	Atrial septal defect, ventricular septal defect	*TBX1*, *DGCR8*
17	CHD, PF, MCA, HY	Dup 3q13.32 (0.074 Mb), Del 10q 26.3 (0.043 Mb), Dup 16p12.1 (0.023 Mb)			Ventricular septal defect	
18	CHD, MCA, MR	Del 16p11.2 (0.53 Mb)			Aortic regurgitation	*KCTD13*, *TBX6*

Table 1 Continued on next page

Table 1. Continued

Patient	Phenotype	CNVs: chromosomal region and size (Mb)[a]	Inheritance	Known syndrome	Type of heart defect	Significant candidate genes relating to CHD[b]
19	CHD, PF, MR, MCA	Del Xp21.3 (0.028 Mb)			Atrial septal defect	
20	CHD, PF, MR, E, HY	Del 7q11.23 (1.9 Mb)		Williams syndrome	Pulmonary valve stenosis	*ELN*
21	CHD, PF, MCA, HY	Dup 18p11.32–p11.21 (14.72 Mb), Dup 18q21.32 (0.768 Mb), Del 18q21.32–q23 (19.76 Mb)			Atrial septal defect	*EMILIN2*, *MRCL2*, *MRCL3*, *MYOM1*, *LPIN2*, *LAMA1*, *CIDEA*, *KCNG2*
22	CHD, PF, MCA	Del 22q11.21–11.23 (1.86 Mb)		Distal DiGeorge syndrome	Atrial septal defect	*TOP3B*, *TOP3B2*, *HIC2*
23	CHD, PF, MCA	Del 2p25.3 (1.1 Mb), Dup 9q34.3 (0.798 Mb), Dup 12p11.1 (0.615 Mb), Del 15q15.1 (0.099 Mb), Del 16p13.3 (0.436 Mb), Del 22q13.32 (0.074 Mb), Dup 16p13.3 (0.028 Mb)		9q Subtelomeric microduplication syndrome	Dysplastic mitral valve	*CACNA1B*, *EHMT1*
24	CHD, PF, MR, MCA	Del 3p11.1 (0.088 Mb), Del 4p16.3 (0.112 Mb), Del 8p21.3 (0.064 Mb), Del 10q26.11–q26.3 (14.55 Mb), Del Xp 21.3 (0.156 Mb), Dup Xq27.1 (0.068 Mb), Del Xq28 (1.98 Mb)			Pulmonary valve stenosis	*BAG3*, *NKX1-2*
25	CHD, MR, MCA	Del 5p13.33 (0.139 Mb), Del 5p14.3–p14.2 (0.84 Mb), Del 7p22.1 (1.7 Mb), Del 9p13.1 (0.75 Mb)			Aortic regurgitation	
26	CHD, PF, MCA, HY	Del 16p13.2 (0.093Mb)			Coarctation of the aorta	*RBFOX1*
27	CHD, PF, MR, MCA	Del 6p24.1–p22.3 (6.64 Mb)			Ventricular septal defect, coarctation of the aorta	*EDN1*, *DTNBP1*, *MYLIP*
28	CHD, PF, MR	Del 3p13 (0.083 Mb), Dup 9q34.3 (3.12 Mb), Dup 18q23 (0.834 Mb)		9q Subtelomeric microduplication syndrome	Atrioventricular septal defect	*KCNT1*, *NOTCH1*
29	CHD, MR, MCA	Del 5q35.3 (2.32 Mb), Dup 17q25.1–q25.3 (10.14 Mb), Del Xp21.1 (0.034 Mb)			Pulmonary valve stenosis	*MAML1*, *FLT4*
30	CHD, MR, MCA	Dup 2q35 (0.134 Mb), Dup 6q22.31 (0.958 Mb)			Aortic regurgitation	*PRKAG3*, *WNT6*, *CYP27A1*
31	CHD, PF, MR, MCA	Dup 17q21.31	mat	17q21.31 Microduplication syndrome	Atrial septal defect	
32	CHD, PF, MR, MCA, HY	Del 1p31.2–p22.3 (18.44 Mb)			Atrial septal defect	*NEXN*
33	CHD, PF, MR, MCA	Del 3p14.1 (0.65 Mb), Del 7p22.3 (0.68 Mb), Del 7q34 (0.9 Mb), Del 9q34.3 (0.78 Mb), Del 11p15.5 (0.98 Mb), Del 17q25.3 (0.53 Mb), Del 19p13.3 (0.75 Mb)	*De novo*, mat, mat, mat, mat, ---, mat		Atrial septal defect, coarctation of the aorta	*KCNQ1*
34	CHD, PF, MR, MCA	Del 8q24.3 (4.26 Mb), Dup 9q34.3 (5.41 Mb), Del 10q26.3 (1.72 Mb)		9q Subtelomeric microduplication syndrome	Sinus venosus atrial septal defect	*ADAMTS13*, *NOTCH1*, *EHMT1*

Table 1 Continued on next page

Table 1. Continued

Patient	Phenotype	CNVs: chromosomal region and size (Mb)[a]	Inheritance	Known syndrome	Type of heart defect	Significant candidate genes relating to CHD[b]
35	CHD, MCA	Del 10q21.3 (0.108 Mb), Del Xq21.1 (1.83 Mb)			Sinus venosus atrial septal defect	*CTNNA3*
36	CHD, PF, MR	Del 10q21.3 (0.041 Mb)			Sinus venosus atrial septal defect	*CTNNA3*
37	CHD, PF, MCA	Dup 10p12.33 (1.022 Mb), Del 21q22.3 (0.49 Mb), Del 22q12.3 (0.81 Mb)			Aortic regurgitation	*CACNB2*

CHARGE, Coloboma of the eye, Heart defects, Atresia of the choanae, Retardation of growth and/or development, Genital defect, Ear anomalies and/or deafness; CHD, congenital heart disease; CNV, copy-number variation; E, epilepsy; HY, hypotonia; mat, maternal inheritance; MCA, multiple congenital anomalies; MR, mental retardation; pat, paternal inheritance; PF, peculiar facies; UCSC, University of California, Santa Cruz.

[a]Agilent 244K arrays with average resolution of 8.9 kb. [b]UCSC database (http://genome.ucsc.edu/), UCSC Genome Browser, human genome build 18.

with the following: 30 of 37 had one or more other congenital anomalies, 23 of 37 had dysmorphic features, 16 of 37 had intellectual disability, 16 of 37 had other anomalies, 13 of 37 had abnormal magnetic resonance imaging, 10 of 37 had hypotonia, and 7 of 37 had seizures. Array CGH performed on parental DNA for 5 patients, indicated that the genomic rearrangement was of maternal origin in 2 and *de novo* in the remaining 3.

DISCUSSION

In this study, using array CGH, we examined 55 patients with CHD and at least one of the following conditions: other congenital anomalies, dysmorphic features, or developmental delay. CNVs were detected in 37 of 55 patients (67%). In 29 of 37 patients, CHD was attributed to genes identified in specific genomic regions that are considered to be associated with heart disease. In the remaining 8 patients, the CNVs identified did not include known genes associated with heart disease (**Table 1**).

The overall clinical phenotype in 23 of 55 patients resulted from a combination of several CNVs. In 14 patients, we identified known microdeletion and microduplication syndromes: DiGeorge (3), 1p36 microdeletion (3), 9q subtelomeric microduplication (3), Williams syndrome (2), CHARGE (Coloboma of the eye, Heart defects, Atresia of the choanae, Retardation of growth and/or development, Genital defect, Ear anomalies and/or deafness) (1), 17q21–31 microduplication (1), and Jacobsen syndrome (1) (**Figure 1**). In general, it is given that phenotypes of microduplications are milder as compared with those caused by microdeletions in the same chromosomal region; this was confirmed by our cases and previously published results (6).

It is interesting that unexpected genomic rearrangements in relation to their phenotype were identified in nine patients. Specifically, two patients showed CNVs involving the genes *KCNQ1* and *CACNB2* (patients 34 and 38), which are associated with long QT and Brugada syndrome, respectively (7,8), whereas in the remaining seven patients (patients 4, 9, 18, 23, 28, 36, and 37) (**Table 1**) the identified CNVs were associated with channelopathies (9). Genetic counseling proves problematic in cases where the CNVs include ion channel genes because it is not certain that they will lead to a late-onset severe cardiac disease.

Most studies of a similar nature rely on determining whether the finding is *de novo* before assigning pathogenicity. Among the limited parental tests performed, in two instances where the CNVs were of maternal origin the maternal phenotype was normal. We acknowledge the paucity of parental testing as a weakness of our study, but because the array CGH test is expensive and is not covered by the Greek National Health Insurance system, the remaining parents refused testing.

The current study indentified the genes relating to CHD in 78% of patients with array CGH. The higher percentage of patients containing CNVs with genes relating to CHD in this study as compared with two previously published studies is probably a result of the different number of patients studied and the higher array CGH resolution (10,11).

Thienpont *et al.* (10) were the first to study array CGH techniques in patients with CHD and other congenital anomalies and normal conventional karyotype. In their study of 60 patients, CNVs were detected in 30% of cases, whereas in 17% some CNVs were located in genomic regions containing genes contributing substantially to the development of the heart, such as *NKX2.5* and *NOTCH1*. However, in most of the genomic aberrations, the genes identified were not associated with CHD.

One year later, Richards *et al.* (11) investigated 40 patients (20 with isolated CHD and 20 with additional congenital anomalies and/or developmental delay) using array CGH. CNVs were detected in 5 of 20 children (25%) with CHD and additional congenital anomalies, whereas no CNVs were found in the remaining 20 children with isolated CHD.

Recently, Sørensen *et al.* (12) examined 402 CHD patients (378 with isolated CHD and 24 with extra cardiac malformations) with a specific multiplex ligation-dependent probe amplification designed for CHD screening. They identified 14 rare CNVs in 13 (3.2%) patients, with >80% of the detected imbalances found in children younger than 5 y. The authors conclude that the multiplex ligation-dependent probe amplification assay should be used as a first-tier screen to detect clinically relevant CNVs for the identification of syndromic patients at an early stage.

The development of new technologies has resulted in a better understanding of genome structure and the role of CNVs. The major importance of our findings in patients with CHD and

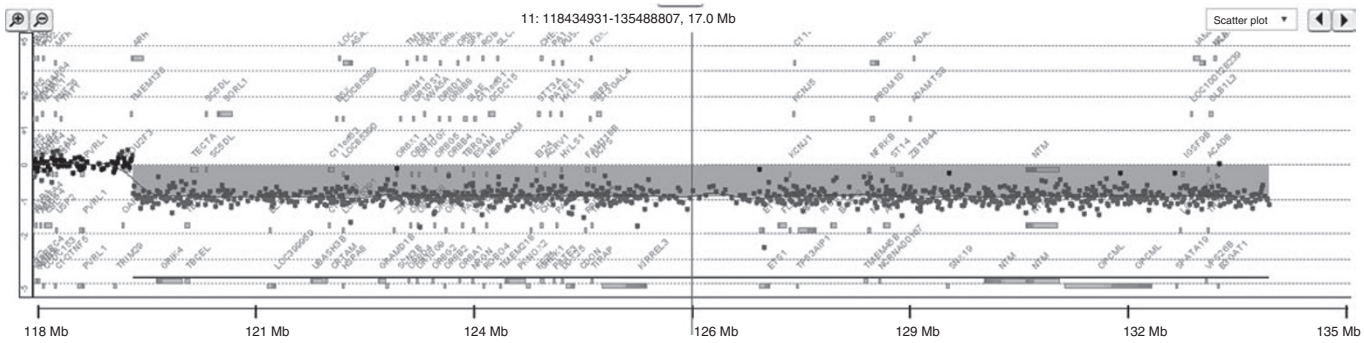

Figure 1. Deletion in the 11q23.3–q25 (14.7 Mb) chromosomal region, Jacobsen syndrome (patient 4).

syndromic features is the association of the CNVs related to CHD, as well as the possible clinical impact of such results for the genetic counseling of families.

METHODS

Subjects

During a period of 3 y, among the 330 patients evaluated with array CGH, we investigated 55 patients (23 males and 22 females) with array CGH who had CHD according to the available history of complete cardiac evaluation, echocardiogram, and/or cardiac catheterization and operative reports. The types of CHD varied and included the following: 14 subjects with ostium secundum atrial septal defect, 9 with aortic regurgitation, 7 with pulmonic valve stenosis, 5 with a sinus venosus atrial septal defect, 5 with perimembranous ventricular septal defect, 4 with atrioventricular septal defect, 3 with dysplastic mitral valve, 3 with aortic coartaction, 1 with aortic coartaction and perimembranous ventricular septal defect, 1 with aortic coartaction and atrial septal defect, 1 with atrial septal defect and double chamber right ventricle with congestive cardiac failure, 1 with atrial septal defect and aortic dilatation, and 1 with ventricular septal defect and pulmonary hypertension. All subjects had at least one additional indication of chromosomal abnormality: (i) dysmorphic features (26 of 55 patients), (ii) other congenital anomalies (5 urogenital, 15 skeletal, and 17 others), (iii) varying degrees of intellectual disability or learning difficulties (19 of 55 patients), and (iv) neurological disorders (7 seizures, 10 hypotonia, and 15 abnormal magnetic resonance imaging results). Detailed clinical history and examination were provided by experienced clinical geneticists. Clinical data of patients and the results from the array CGH were entered into a database kept at the Department of Medical Genetics. All patients were previously studied by conventional karyotype and/or other diagnostic tests, which were all normal.

The study was approved by the Medical Genetics Review Board of the National Kapodistrian University of Athens, and informed consent for genetic studies was obtained directly from all family members included in the study.

Array CGH Analysis

We used high-resolution commercial Agilent technologies arrays (Santa Clara, CA): the 244K and 4×180K platforms (SurePrint G3 arrays). The 244K platform is composed of >236,000 60-mer oligonucleotide probes for the mapped genes or unique DNA sequences with an average spatial resolution of 7.9–8.9 kb (annotated against National Center for Biotechnology Information build 36, hg18). The 4×180K platform is composed of >170,000 60-mer oligonucleotide probes with average spatial resolution of 13–25 kb (annotated against National Center for Biotechnology Information build 36, hg18, and National Center for Biotechnology Information build 37, hg19). An array CGH test was performed for each patient using the standardized protocol recommended by the manufacturer and as described previously (6). Slides were scanned with an Agilent microarray scanner G2565CA, software version 4.8.4.1. Image data were extracted and converted to text files with Feature Extraction Agilent Technologies version 10.10.1.1. DNA Analytics v.3.5 and/or Agilent Genomic Workbench 6.5 (Santa Clara, CA) were used to plot the $\log_2$ ratio of the signal intensity of each probe. The CNVs were visualized along each chromosome with correspondent National Center for Biotechnology Information annotated gene information for each probe. For the analysis, we used the software built-in aberration detection method (ADM1, threshold 6.7). The cutoff for recording an alteration was ≥4 consecutive probes. For the interpretation of the results, the following databases were used: database of genomic variants (http://projects.tcag.ca/variation/), DECIPHER database (http://www.sanger.ac.uk/PostGenomics/decipher/), and the International Standards for Cytogenomic Arrays (https://www.iscaconsortium.org/) (6).

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