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Microdeletions and Microduplications in Patients with Congenital Heart Disease and Multiple Congenital Anomalies

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Abstract

Objective—Multiple genetic syndromes are caused by recurrent chromosomal microdeletions or microduplications. The increasing use of high-resolution microarrays in clinical analysis has allowed the identification of previously undetectable submicroscopic copy number variants (CNVs) associated with genetic disorders. We hypothesized that patients with congenital heart disease and additional dysmorphic features or other anomalies would be likely to harbor previously undetected CNVs, which might identify new disease loci or disease-related genes for various cardiac defects.

Design—Copy number analysis with single nucleotide polymorphism-based, oligonucleotide microarrays was performed on 58 patients with congenital heart disease and other dysmorphic features and/or other anomalies. The observed CNVs were validated using independent techniques and validated CNVs were further analyzed using computational algorithms and comparison with available control CNV datasets in order to assess their pathogenic potential.

Results—Potentially pathogenic CNVs were detected in twelve of 58 patients (20.7%), ranging in size from 240 Kb to 9.6 Mb. These CNVs contained between 1 and 55 genes, including *NRP1, NTRK3, MESP1, ADAM19*, and *HAND1*, all of which are known to participate in cardiac development.

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Conflict of interest: None.

Conclusions—Genome-wide analysis in patients with congenital heart disease and additional phenotypes has identified potentially pathogenic CNVs affecting genes involved in cardiac development. The identified variant loci and the genes within them warrant further evaluation in similarly syndromic and nonsyndromic cardiac cohorts.

Keywords

Microdeletion; Microduplication; Copy Number Variant; Congenital Heart Disease

Introduction

Congenital heart defects (CHDs) are the most common major birth defect with a reported prevalence of 4–8 per 1000 live births.[1–3] Though the majority of heart malformations occur in isolation, approximately 25% occur in conjunction with other congenital defects and are often part of a specific malformation pattern or genetic syndrome.[4] Large chromosomal alterations, submicroscopic deletions, and single gene defects have been identified in genetic syndromes characterized in part by CHD (reviewed in Pierpont et al.[5]). Molecular definition of these genetic variants has increased the understanding of the developmental basis of the associated cardiac defects. These analyses have also at times provided insight into the genetic contribution to the much larger group of nonsyndromic patients with CHDs.

Recent investigations have demonstrated a high frequency of copy number variants (CNVs) in the human genome.[6–9] In conjunction with single nucleotide polymorphisms, CNVs are likely to contribute to genetic heterogeneity and phenotypic variability.[10] There is increasing evidence that rare CNVs identified in disease cohorts as compared with a control population may define new candidate disease genes in disorders such as schizophrenia and autism.[11–15] We hypothesized that patients with cardiac defects and additional congenital malformations were likely to have CNVs that could in turn identify new candidate disease-related loci and genes for CHDs. In addition, as microarray technology and analyses are adopted for clinical use, the identification of novel CNVs allows for comparison with a growing list of such alterations in this patient population for both research and clinical purposes. To that end, patients with CHDs and additional congenital anomalies were evaluated for unique structural variants using available single nucleotide polymorphism (SNP)-based microarray technology and analytical methods.

Materials and Methods

Study Cohort

Study patients were recruited from the Cardiac Center at The Children's Hospital of Philadelphia (CHOP) from 1992 to 2007 to study the genetic basis of congenital heart disease. All patients consented to participate in a protocol approved by the Institutional Review Board for the Protection of Human Subjects at the CHOP. Upon consent, all available medical records including cardiac records and specialty consultations were reviewed for cardiac anatomy, dysmorphic features, and additional congenital anomalies. Previous genetic testing was recorded. A three-generation pedigree was obtained for the majority of subjects by a genetic counselor. Parental samples were obtained and processed

for DNA extraction whenever possible. Patients with a known genetic syndrome (such as Turner or Alagille syndromes) or known chromosomal alteration (such as trisomy 21 or 22q11.2 deletion) were excluded from this study.

Microarray Experiments

Genomic DNA was extracted from whole blood or lymphoblastoid cell lines using standard techniques. Patient DNA samples used in the study were derived either from whole blood or from lymphoblastoid cell lines. However, all parental DNA samples were derived directly from blood. The microarray experiments were performed using the Affymetrix GeneChip 100 K Affymetrix arrays, which is comprised of the 50 K XbaI and 50 K HindIII arrays (Affymetrix, Santa Clara, CA, USA). For each of the two arrays, 250 ng of genomic DNA from each subject was processed and labeled using reagents and protocols supplied by the manufacturer. After hybridization, the microarrays were processed in the Affymetrix GeneChip Fluidics Station 450 and resultant image files (.CEL file) were analyzed with the Affymetrix GeneChip DNA Analysis Software package. The mean SNP call rate of the XbaI arrays was >97% and the median call rate (MCR) was >95%. The SNP call rate and MCR were lower overall for the HindIII experiments. Data derived from the HindIII experiments were entirely excluded if the SNP call rate and/or MCR fell below 90% and were only used to confirm rather than discover CNVs identified first from the XbaI experimental dataset.

Copy Number Analysis

The data obtained from the Affymetrix 100 K arrays were further analyzed to detect copy number alterations using three different approaches and algorithms. Copy number analysis was first performed using the Affymetrix Chromosome Copy Number Analysis Tool (CNAT).[16] The likelihood of each CNAT prediction was determined by assessing the calculated copy number, log 10 (*P* value) and presence or extent of loss of heterozygosity, as previously described.[17] In the second approach, the CNAT copy number output was further analyzed by using the segmentation algorithm circular binary segmentation (CBS).[18] CBS is a statistical method that allows the detection of pattern changes that may correspond to copy number differences. In the third approach, the 100 K arrays were analyzed using the CNAT for Gene Chip (CNAG v1.1).[19] Although CNAT and CNAG analyzed the data from the XbaI and HindIII chips separately, combined high-quality data from both chips (when available) were used for the CBS analysis, and the resulting output and plots were inspected for copy number variations. Only those CNVs identified by all three analytical approaches were carried forward to validation. Chromosomal coordinates are based on the hg17/ NCBI 35 build from the University of California Santa Cruz (UCSC) Genome Browser.

The copy number calls obtained from each of the three approaches described above were compared with all available control datasets for copy number variation. Such comparison included the Database of Genomic Variants (DGV) that contains CNVs reported by 49 distinct studies.[20] We also compared the copy number data found in our cohort to 305 samples analyzed using the Affymetrix 50 K and 100 K arrays for other studies to determine if any of the novel CNVs detected in our cohort were seen previously to eliminate array-specific background. This set included healthy controls as well as patients with multiple congenital anomalies. We further compared our data with a copy number variation database

generated from our prior analysis of 2026 healthy controls analyzed using Illumina HumanHap550 K arrays.[21]

Fluorescence In Situ Hybridization

Metaphase spreads and interphase nuclei were prepared from subject-derived lymphoblastoid cell lines and normal control individuals, and were subsequently hybridized with selected probes for fluorescence in situ hybridization (FISH) using standard protocols. Five region-specific bacterial artificial chromosome (BAC) clones were chosen (Table 1). All BAC clones were obtained through CHORI BACPAC Resources (Oakland, CA, USA). BAC DNA was isolated (mini-prep kit; Qiagen, Valencia, CA, USA) and labeled by nick translation (Nick Translation Reagent Kit, Vysis, Inc., Abbott Park, IL, USA) using Spectrum Orange/Green dUTP (Vysis, Inc.). Commercial region-specific probes (Vysis, Inc.) were used as control probes. Probes were confirmed to localize to the appropriate target region on normal metaphase spreads before they were used for FISH of patient samples. Twenty metaphase spreads and twenty interphase nuclei were counted for each patient to confirm the presence of a deletion or duplication, respectively.

Quantitative Polymerase Chain Reaction

Quantitative Polymerase Chain Reaction (qPCR) probes and primers were designed using the Primer Express software (Applied Biosystems, Inc., Foster City, CA, USA) or custom designed by Applied Biosystems based on the submitted gene sequence. A comparative Ct method[22] was used to calculate the relative gene number. β-Actin was employed as an endogenous control. Each sample (case, parents and controls) was assayed in triplicate. Two known control samples were analyzed on each reaction plate for calibration, as required in the comparative threshold cycle (ddCt) method. A no-template control (background) was also included in each assay. qPCR was carried out using an ABI prism 7500 (Applied Biosystems) in a 96-well optical plate in a final reaction volume of 25 uL. Thermal cycling conditions were according to the TaqMan Universal PCR Protocol (Applied Biosystems).

Multiplex Ligation Dependent Probe Amplification

Multiple ligation-dependent probe amplification (MLPA) was performed using “Salsa MLPA kit P250 DiGeorge” on genomic DNA according to the manufacturer’s protocol (MLPA-HD Kit, MRC-Holland, Amsterdam, The Netherlands). PCR amplification was carried out on an ABI 9700 thermocycler (Applied Biosystems), and electrophoresis was performed using the ABI 3700 DNA analyzer (Applied Biosystems) with Rox 500 size standards. Gene Marker from Soft-Genetics (State College, PA, USA) was used to analyze the data.[23]

For the 12 potentially pathogenic CNVs detected in our study, 8/12 inherited CNVs were also validated (using the above methods) in the transmitting parent using samples derived directly from blood. Of the remaining four CNVs, three patient DNA samples (subject 2560, BG-212, BG-420) were extracted directly from blood and one case (subject 425) was confirmed by clinical testing (subject 425) using a new sample of DNA extracted from blood. Therefore, none of the 12 CNVs represent artifacts from cell line transformation.

Results

Patients presenting to The Cardiac Center at the CHOP for cardiac care at various ages for various reasons were consented to participate in a protocol on the genetic basis of congenital heart disease. Cases were selected for this study if they had CHD in addition to significant dysmorphic features and/or congenital malformations but did not have a specific genetic diagnosis or recognized chromosome alteration using standard techniques. A total of 58 patients met these criteria and were tested for novel CNVs (Table 2). Two additional patients with a previously confirmed 22q11.2 deletion and tetralogy of Fallot were used as positive controls for the microarray experiment and subsequent computational analysis. The cohort was predominantly Caucasian (n = 47), and also included African Americans (n = 6), Asians (n = 2), and mixed races (n = 3).

The cardiac phenotype of the study cohort consisted of a wide spectrum of defects (Table 2). All patients were examined by a cardiologist, and most patients underwent a clinical genetic evaluation; none were diagnosed with a specific clinical genetic syndrome or chromosomal alteration by conventional analyses. In particular, a total of 39 patients had a high-resolution karyotype, of which two had normal variants (inv 9 and 46, XY.ish del [Y] [qter]). Fifty-one of the 58 patients had tested negative for a 22q11.2 deletion by commercially available FISH assays, while the remaining seven were not tested for a 22q11.2 deletion given their cardiac diagnosis and lack of characteristic features. Thirteen subjects were also tested for sub-telomeric deletions and were not found to have any chromosomal alterations. A clinical geneticist examined 43 of the 58 subjects and reviewed a photograph of one additional subject for facial dysmorphia. Fourteen subjects had no record of a formal clinical genetics examination in our institution but were noted to have syndromic features by the physician of record.

Patients were genotyped using SNP arrays, and CNVs (microdeletions and microduplications) were identified by analyses described in Methods. The CNVs detected in each of our patients were compared with databases of CNVs that had previously been detected in healthy control individuals (see Methods). Twelve of the 58 patients (20.7%) were found to have relatively large, rare CNVs that contain recognized genes (Tables 3 and 4). All 12 patients were Caucasian and demonstrated a wide range of clinical features and cardiac defects including conotruncal, septal, endocardial, and left-sided defects (Table 3). Both chromosomal deletions (n = 8) and duplications (n = 4) were identified (Table 4). The identical CNVs were not identified in the DGV or the internal control datasets. One of these CNVs, a 12p11.22–11.23 deletion, overlapped entirely with a duplication of CNV in a single control individual out of 2026 healthy controls; and one control deletion overlapped 59.4% percent with a duplication CNV in 18p11.32 in one patient. Each of the overlapping control CNVs in these cases was rare (<0.05% frequency), and more importantly, each comprised a different type of alteration (i.e., duplication vs. deletion) than those of the respective study patient. Furthermore, the patient's CNV within 18p11.32 (subject 2179) had substantially different endpoints and gene content as compared with the control CNV.

We therefore considered these 12 CNVs to be of further interest. Each of the 12 CNVs was validated by one or more additional experimental methods. Eleven of the twelve CNVs were

confirmed by qPCR, five of which were also confirmed by FISH (Figure 1 and Table 4). The novel deletion of 22q11.2 in v 472 was confirmed by MLPA.[23] This case contributes to the growing number of patients recognized to have variant deletions of the 22q11.2 locus who are not otherwise detected by a commercially available FISH assay.[23–26] When available, parental samples were also evaluated for the CNV detected in their offspring. Two CNVs were confirmed to be de novo, while the question of inheritance could not be evaluated in three patients because of unavailable parental samples. Of the 12 CNVs detected in the patients, eight were found in parents with no reported congenital anomaly, although medical examination was not possible (Table 3). Though siblings were not available for medical examination or genetic testing, none were reported to have congenital anomalies.

The estimated size of the CNVs varied from 240 Kb to 9.6 MB and contained between five and 295 SNP-based probes on the Affymetrix arrays. The number of genes predicted to map into the 12 CNVs ranged from one to 55 with highly variable predicted functions of the encoded proteins (Table 4, and Discussion).

Overall, 12 CNVs were identified in 58 cardiac patients with multiple congenital anomalies which by virtue of gene content and absence in large control datasets may therefore contribute to disease risk and pathogenesis. As the eight inherited CNVs could represent very rare events, and the heritability of two cases could not be confirmed, the prevalence of novel CNVs range from 3% to 21% in our cohort (two of 58 confirmed de novo events vs. 12 of 58 overall events).

Discussion

Multiple deletion and duplication syndromes have been described both clinically and on a molecular basis.[27] The genetic analysis of these syndromes has provided insight into the etiology of the associated congenital anomalies. For example, the recognition that the majority of patients with DiGeorge syndrome carried a 22q11.2 deletion[28–31] led to the discovery that a large number of cardiac patients with a subset of syndromic findings carried a 22q11.2 deletion.[32] Further molecular investigation demonstrated that *TBX1* plays a critical role in cardiovascular development.[33,34] The 22q11.2 deletion as well as other recurrent rearrangements associated with known genetic syndromes (e.g., Prader Willi, Williams-Beuren, and Smith-Magenis syndromes) likely result from nonallelic homologous recombination between highly homologous segmental duplications (reviewed in Emanuel & Shaikh and Shaw & Lupski[27,35]). The recognized prevalence of segmental duplications and other unstable architecture in the human genome predicts the potential existence of additional deletion and duplication syndromes.[35–37] Array technology provides the opportunity to evaluate disease populations for undiscovered, potentially disease-related microdeletions and microduplications.

This study was undertaken to identify potential disease-related loci in a cardiac population with multiple congenital anomalies. We hypothesized that a subset of cardiac patients with additional anomalies would be the most likely to harbor previously undetected, disease-related CNVs. Since the initiation of this study, the high prevalence, variability, and

complexity of CNVs in the general population have become increasingly apparent.[6–10] Chromosomal regions with numerous overlapping CNVs, both from apparently healthy and diseased individuals, have been described, which complicate distinction of pathogenic from nonpathogenic CNVs for specific disease-associated deletions and duplications. Recently, several studies found an increased burden of rare or de novo CNVs in disease populations with autism or schizophrenia as compared with normal controls, highlighting the potential pathogenicity of rare or unique CNVs.[11–15]

The current investigation identified 12 CNVs in 58 patients that were not identified in several large control cohorts, two of which were particularly large deletions (5q32–34 and 10pl2.1-l 1.21). Of interest, Shahdadpuri and colleagues[38] report a similar deletion of 10p 12.1–11.21 in a single case with pseudoarthrosis of the clavicle, copper beaten skull, multiple small ventricular septal defects, bicuspid aortic valve, and coarctation of the aorta. Though a distinct cardiac phenotype, this case supports the potential pathogenicity of this deletion given that genetic variants are often associated with a range of cardiac defects, thereby demonstrating variable expressivity. Of note, the DiGeorge-like syndrome (DGS2) and hypoparathyroidism, sensorineural deafness and renal dysplasia syndrome (HDR) loci map near but distal to the 10p 12.1–11.21 deletion reported here. Although several single gene disorders map into the 5q32–34 locus, there appear to be no reports of a similar deletion in any patients with congenital anomalies. None of the other 11 CNVs have been reported in cases with CHDs to our knowledge.[39–12]

Eight of the 12 CNVs were inherited from reportedly unaffected parents. Nonetheless, these inherited CNVs still hold pathogenic potential. First, the parents are normal by report but none underwent physical inspection for mild syndromic features or echocardiograms for subclinical cardiac malformations. A high level of variability in phenotypic expression of CNVs has been observed in other genetic syndromes, where patients display a wide range in the number and severity of features despite equivalent chromosomal alterations. In most cases, the pathogenic CNV is inherited from a reportedly “normal” or mildly affected parent.[43,44] For example, approximately 6–10% of cases of 22ql 1.2 deletion are inherited from a parent who is not recognized to have syndromic features or carry the deletion until after their offspring is diagnosed and the parent subsequently tested.[45] Thus, upon closer inspection some study parents might prove to harbor unsuspected syndromic features. Furthermore, a CNV may be of clinical significance eventhough it is inherited from a seemingly “healthy” parent because of other complex mechanisms, including incomplete penetrance, the effect of imprinted genes or modifier, a point mutation in a recessive gene, parental mosaicism, and position effect.[46–48] Cardiovascular and other birth defects are increasingly recognized to be complex traits where a combination of genetic and environmental factors contributes to the risk of disease. Therefore, it is possible that these CNVs contribute to the risk of disease though phenotypic expression is variable.

The putative disease-related CNVs contain a wide range of genes in both number and predicted function. Several genes are of particular interest given their previously established role in cardiovascular development. In particular, *NRP1* (neuropilin-1), deleted in case 425 with a ventricular septal defect, is a coreceptor for class 3 semaphorins (critical for neuronal development) and for some members of the vascular endothelial growth factor family

(critical for cardiovascular development) (reviewed in Pellet-Many et al.[49]). Targeted disruption of *Nrp1* in the mouse results in abnormal development of the embryonic branchial arches and truncus arteriosus.[50] *NTRK3* (neurotrophin 3) and *MESP1* (mesoderm posterior 1) are both deleted in case 723 with a ventricular septal defect. Multiple cardiovascular anomalies were noted in the mouse following targeted disruption of *Ntrk3* including ventricular septal defects, truncus arteriosus, and characteristic findings of tetralogy of Fallot.[51] *MESP1* is a member of the bHLH transcription factor family and is expressed in early mesoderm at the onset of gastrulation (reviewed in Saga et al.[52]). Disruption of *Mesp1* expression in mice results in early malformations of heart tube formation and looping.[53]

Moreover, *ADAM19* and *HAND1* are both deleted in case BG-212 with an atrioventricular canal defect. *ADAM19*, a metalloprotease-disintegrin, is expressed in the conotruncal and atrioventricular cushions such that mice lacking *Adam19* expression display ventricular septal defects and defective semilunar and atrioventricular valves.[54,55] In the mouse, the basic helix-loop-helix transcription factor *Hand1* is initially expressed in the precardiac mesoderm, but it is later restricted to the anterior and posterior segments of the straight heart tube, which become the conotruncus and left ventricle.[56–58] The role of *Hand1* in cardiac development has been harder to discern given early embryonic lethality in the null mouse,[59] but mice with a conditional *Hand1* null allele display multiple congenital heart malformations including ventricular septal defects, hypoplastic left ventricles, outflow tract anomalies, hyperplastic atrioventricular cushions, and a disorganized muscular septum.[60] A recent report described a loss-of-function mutation in *HAND1* in hypoplastic human ventricles.[61]

Thus, several of the genes altered in our patients have been documented to play critical roles in cardiovascular development and appear to be worthy of further evaluation for deletion, duplication, or mutation in a cardiac cohort. It is equally possible that additional genes in the putative disease-related CNVs have unrecognized roles in cardiovascular development and may also prove to be viable candidate genes. Therefore, these loci may contain one or more disease-related genes that warrant further evaluation in a similarly syndromic and/or nonsyndromic cardiac cohort. In addition, because the current study cohort had a wide range of associated congenital anomalies, subjects with similar noncardiac anomalies could be tested for the same or overlapping CNV.

Finally, this study underscores the growing importance of genome-wide microarray analysis for the detection of unique CNVs in patients with multiple congenital anomalies for both research and clinical purposes. Syndromic patients without a clear etiology or genetic diagnosis are evaluated by microarray analysis for clinical diagnostic purposes with increasing frequency. Two recent studies[39,40] similarly identified de novo and inherited unique CNVs in syndromic cardiac patients, though both studies report distinct CNVs from those identified in this study. Furthermore, Erdogan and colleagues[42] and Greenway and colleagues[41] identified de novo and inherited unique CNVs in nonsyndromic patients with CHDs and tetralogy of Fallot, respectively. Collectively, these studies begin to describe a variety of potentially disease-related chromosomal alterations that may define new deletion or duplication syndromes. These studies also provide a basis of comparison for clinical diagnostic purposes. Identification of similar or overlapping CNVs and/or the identification

of disease-related mutations in gene(s) mapping into these regions in patient samples will further elucidate their role in abnormal phenotypes, including cardiac disease and other congenital abnormalities.

In summary, this study identified 12 potentially pathogenic CNVs in cardiac patients with multiple congenital anomalies that were not seen in extensive control populations, similar to recent reports.[39,40] Comparison between patients may identify recurrent, disease-related chromosomal alterations in future studies. The evaluation of candidate genes, such as those detailed above, for mutations in nonsyndromic cardiac cohorts may identify novel disease-related genes as well. These investigations begin to expand upon our understanding of cardiovascular disease and may identify novel pathophysiologic mechanisms in the future.

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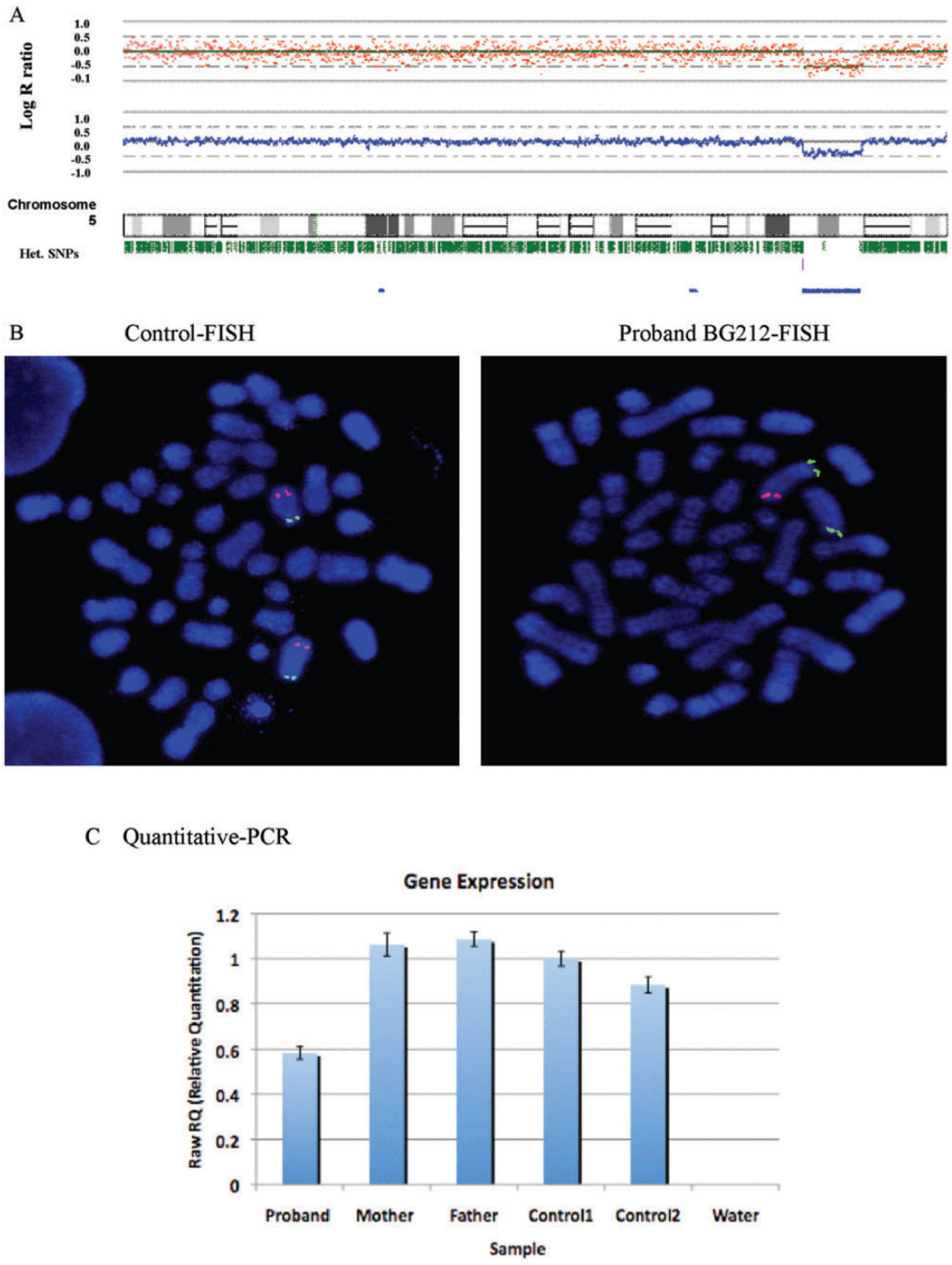

Figure 1.

Identification and confirmation of the chromosomal deletion 5q32-q34 in case BG 212: (A) A copy number output for proband BG212 on chromosome 5 using the Affymetrix 50 K Xba Mapping GeneChip. Red dots represent raw log2 R ratio values for each single nucleotide polymorphism (SNP). Blue line represents copy number inferences based on local mean analysis for 10 consecutive SNPs. Heterozygous SNP calls are shown as green bars below the ideogram. The deletion detected in this proband BG212, based on log2 R ratio, is shown as a blue bar below. (B) To confirm the detected deletion, fluorescence in

situ hybridization (FISH) was performed on a normal control and the proband BG212 using acterial artificial chromosome (BAC) clones RP11-86C20 in the region of 5q32–34. The green signal is a control Vysis probe and the red signal is the test BAC clone RP11-86C20. The control probe (green signal) is seen on both normal control and proband homologues of chromosome 5, whereas the test probe (red signal) is seen on both chromosome 5 homologues of the normal control, but on only one chromosome 5 homolog of the proband, consistent with a deletion in that region. (C) Quantitative polymerase chain reaction (qPCR) was performed on proband and parental genomic DNA samples using probes designed in the region of interest (exon 23 of the *LARP1* gene). The parental samples and the two controls show ddCt ratios of approximately one, consistent with two copies of the *LARP1* gene, while the proband BG212 has a ddCt ratio of about 0.5, consistent with a single copy of the gene. The *x*-axis represents the relative quantification and the *y*-axis is the detector (*LARP1* gene).

Table 1

Test and Control Probes Used for Confirmation of Copy Number Variant by Fluorescence In Situ Hybridization

Subject	Cardiac Dx	Estimated Length (Mb)	Cytoband	VYSIS Probes	BAC Clone
425	Ventricular septal defect	6.9	10p12.1–11.21	PTEN-10q23	RP11-174P15
2561	Tetralogy of Fallot	0.44	20p12.3	ZNF217	RP11-164C17
1624–462	Hypoplastic left heart syndrome	0.29	12p11.22–23	12p13-Tel/AML	RP13-200J3
BG-212	Atrioventricular canal defect	8.8	5q32-34	Telomeric	RP11-86C20
BG-420	Atrial septal defect	2.6	15q22.2–22.31	PML-RARA	RP11-505L16

BAC, bacterial artificial chromosome.

Table 2

Cardiac Phenotype of the Study Cohort (N = 58)

Cardiac Defects	Number of Subjects
Ventricular septal defect*	14
Tetralogy of Fallot	13
Hypoplastic left heart syndrome	11
Coarctation of the aorta	5
Double outlet right ventricle	4
Atrial septal defect (secundum type)	2
Atrioventricular canal defect	2
Other†	7

*Conoventricular or malalignment type ventricular septal defects.

†One each with double inlet left ventricle, d-transposition of the great arteries, interrupted aortic arch type B, right ventricle to aorta with pulmonary valve atresia, pulmonary valve atresia with intact ventricular septum, pulmonary valve stenosis, and tricuspid valve atresia.

Table 3

Clinical Features of the Patients with a Novel Copy Number Variant

Subject	Cardiac Anatomy	Locus	Other Anomalies	Age at Evaluation
425	pmVSD	del10p12.1-11.21	Right hemifacial microsomia, posterior embryotoxon, bilateral epicanthal folds, abnormal left inner ear, microtia of right ear, high narrow arched palate, tented lip, butterfly vertebrae	6 years
472	cVSD/CA	del22q11.21	High arched palate, protruberant ears, single kidney, blind vaginal pouch	9 years
723	pmVSD	del15q26.1	Cleft palate, flat facies, decreased ear cartilage, small nose, long fingers, dysplastic ribs, abnormal vertebral bodies, mild scoliosis	Newborn
2179	cVSD	dup18p11.32	Macrocephalic, epicanthal folds, overfolded helix, anteverted nares, high arched palate, hydronephrosis, global developmental delay	2 years
2236	cVSD	dup5q21.1-21.2	Cleft lip, small squared-off ears, sacral dimple, hypospadias, inguinal hernia, right clinodactyly	9 days
2560	cVSD	del9p23	Cleft palate, facial asymmetry, premature graying of the hair, synophrys, mild hirsutism, mild bilateral camptodactyly of fingers, labial hypoplasia, anterior anus, short stature, kyphoscoliosis, small feet with high arch on left side, right-sided pes planus	20 years
714	TOF/PA	dup3q28	Left hydroureter, bilateral hydronephrosis	6 months
2561	TOF/AVC	dup20p12.3	High arched palate, bilateral sensorineural hearing loss, detached left retina, plagiocephaly, mild rhizomelic shortening of upper limbs	11 months
BG-212	AVC	del5q32–34	Cleft palate (soft), bilateral squared and overfolded helices, extra nuchal skin, long fingers, autism	1 year
BG-420	ASD	del15q22.2-22.31	Plagiocephaly, wide-spaced nipples, hypotonia, developmental delay	2 years
1624–197	HLHS	del9p21.2-21.3	Thick helices, scooped nasal bridge, increased nuchal skin, 4–5 syndactyly on left hand	1 year
1624-462	HLHS	del12p11.22-23	Cupped ears, horseshoe kidney, absent left fallopian tube, fused ribs on left, sacral dimple	Newborn

pmVSD, posterior malalignment type ventricular septal defect; cVSD, conoventricular ventricular septal defect; CA, coarctation of the aorta; TOF, tetralogy of Fallot; PA, pulmonary valve atresia; AVC, atrioventricular canal defects; ASD, secundum atrial septal defect; HLHS, hypoplastic left heart syndrome.

Table 4

Novel Copy Number Variants in Study Patients

Subject	CHD	CNV Type	Locus	Coordinates*	Estimated Length (Mb)	Number of SNPs	Number of Genes	Validation	Mother	Father
425	VSD	Deletion	10p12.1-11.21	28 728 683–35 905 297	7.1	164	21	FISH/qPCR	Not del	Not del
472	VSD	Deletion	22q11.21	18 351 387–20 306 802	1.96	5	27	MLPA	Deleted	NA
723	VSD	Deletion	15q26.1	86 439 405–90 753 814	4.3	28	46	qPCR	Not del	Deleted
2179	VSD	Duplication	18p11.32	284 494–918 189	0.64	12	8	qPCR	Duplicated	Not dup
2236	VSD	Duplication	5q21.1-21.2	100 934 417–103 557 609	2.6	120	7	qPCR	Not dup	Duplicated
2560	VSD	Deletion	9p23	9 163 303–9 606 645	0.44	20	1	qPCR	Not del	NA
714	TOF	Duplication	3q28	191 513 690–192 004 582	0.5	19	4	qPCR	Duplicated	Not dup
2561	TOF	Duplication	20p12.3	5 086 589–5 335 788	0.24	8	2	FISH/qPCR	Not dup	Duplicated
BG-212	AVC	Deletion	5q32-34	149 669 664–159 265 067	9.6	295	55	FISH/qPCR	Not del	Not del
BG-420	ASD	Deletion	15q22.2-22.31	61 207 054–63 775 690	2.6	17	38	FISH/qPCR	Not del	NA
1624-197	HLHS	Deletion	9p21.2-21.3	25 498 693–25 849 708	0.35	14	1	qPCR	Deleted	Not del
1624-462	HLHS	Deletion	12p 11.22-23	27 325 018–27 620 210	0.30	17	3	FISH/qPCR	NA	Deleted

*Chromosomal coordinates are based on the hg17/NCBI 35 build from the UCSC Genome Browser.

Number of genes represents the number of RefSeq genes overlapping each region; CHD, congenital heart defects, see details of cardiac anatomy in Table 2; NA, sample not available, Not del, not deleted; Not dup, not duplicated; CNV, copy number variant; SNP, single nucleotide polymorphism; VSD, ventricular septal defect; FISH, fluorescence in situ hybridization; qPCR, quantitative polymerase chain reaction; MLPA, multiple ligation-dependent probe amplification; TOF, tetralogy of Fallot; AVC, atrioventricular canal defects; ASD, secundum atrial septal defect; HLHS, hypoplastic left heart syndrome.