

Chromosome 1q21.1 Contiguous Gene Deletion Is Associated With Congenital Heart Disease

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Abstract—Congenital heart disease (CHD), comprising structural or functional abnormalities present at birth, is the most common birth defect in humans. Reduced expression of connexin40 (Cx40) has been found in association with atrial fibrillation, and deletion of Cx40 in a mouse model causes various structural heart abnormalities in 18% of heterozygotes. We screened 505 unrelated CHD cases for deletions or duplications of the Cx40 gene (*GJA5*) by real-time quantitative PCR, in order to determine whether altered copy number of this gene may be associated with a cardiac phenotype in humans. Dosage of Cx40 flanking genes (*ACPL1* and Cx50 gene, *GJA8*) was determined by real-time PCR for all apparent positive cases. In total, 3 cases were found to carry deletions on chromosome 1q21.1 spanning *ACPL1*, Cx40, and Cx50 genes. Absence of heterozygosity was observed in all 3 index cases over a 1.5- to 3-Mb region. Samples from the parents of two cases were obtained, and microsatellites across 1q21.1 were genotyped. One of the apparently unaffected parents was found to carry this deletion. All 3 index cases presented with obstruction of the aortic arch as the common structural cardiac malformation, and had no consistent dysmorphic features. Genotyping of 520 unrelated normal controls for this deletion was negative. We hypothesize that this 1q21.1 multigene deletion is associated with a range of cardiac defects, with anomalies of the aortic arch being a particular feature. (*Circ Res.* 2004;94:1429-1435.)

Key Words: congenital heart disease ■ contiguous deletion syndrome ■ connexin40

Congenital heart defects (CHD) are the most common anomalies at birth in humans, with moderate and severe forms observed in about 6 per 1000 live births.¹ They comprise a heterogeneous group of defects including cardiac malformations, cardiomyopathies, and cardiac arrhythmias with genetic and/or environmental causes.² In the majority of CHD cases, the causes are unknown; however, technical advances in genetics are leading to the identification of a greater proportion of chromosomal microdeletions, and single gene mutations in CHD.^{3,4} Genes that are known to play a role in normal heart function are increasingly being investigated for mutations that may be associated with alterations in heart development.

The cardiac gap junction protein, connexin40 (Cx40), is primarily restricted in expression to the conduction system and atria.⁵ Reduced expression of Cx40 has been found in association with impaired conduction of dilatory signals along arterioles, mitral valvular disease, and chronic atrial fibrillation.^{6,7} Previous attempts at identifying point mutations in the gene encoding Cx40 (gap junction protein α -5, *GJA5*) in cases of familial atrial fibrillation syndrome have proven unsuccessful.⁸ However, a recent systematic study of

the anatomy and histology of Cx40-deleted mice has revealed a variety of complex congenital cardiac malformations in these animals.⁹ We examined the possibility that dosage of Cx40 could contribute to some forms of CHD in humans. We screened 505 CHD cases for alterations in Cx40 gene copy number and found three cases that were deleted (haploinsufficient) for this gene.¹⁰ We then found that in all three cases the deletion extended beyond the Cx40 gene, encompassing at least six additional genes. We propose that this 1q21.1 deletion, which includes the Cx40 gene, is a novel genetic cause of congenital heart disease.

Materials and Methods

Participants

Peripheral blood samples were drawn from a total of 505 unrelated index cases with congenital heart defects of varying severity, detected before 17 years of age. These cases were referred to a molecular genetic diagnostic facility to rule-out a tentative diagnosis of either DiGeorge/velocardiofacial syndrome (439 cases) or Williams syndrome (66 cases). Blood samples were obtained from a wide variety of clinical sites with no predefined referral criteria. DNA was extracted using either standard phenol-chloroform-isoamyl alcohol methods or the QIAmp DNA Blood Mini Kit (Qiagen) and solubi-

Original received January 13, 2004; revision received April 19, 2004; accepted April 20, 2004.

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DOI: 10.1161/01.RES.0000130528.72330.5c

TABLE 1. Summary of the Genes Known to be Deleted in the 1q21.1 Region

Gene	Protein	Function	Expression
<i>PRKAB2</i>	Protein kinase, AMP-activated, β 2 regulatory subunit	Reacts to high AMP/ATP ratio to downregulate biosynthetic pathway ³¹	Skeletal and cardiac muscle
<i>FMO5</i>	Flavin containing monooxygenase 5	Catalyzes oxidation of nucleophilic N, S, and P in drugs and pesticides ³²	Spleen, liver, brain, breast, kidney
<i>CHD1L</i>	Chromodomain helicase DNA binding protein 1-like	Transcriptional regulation, chromatin remodeling, and DNA repair ^{33,34}	Wide
<i>BCL9</i>	B-cell CLL/lymphoma 9	Overexpression associated with B-cell CLL ³⁵	Ubiquitous
<i>ACPL1</i>	Acid phosphatase-like protein 1	Associated with interstitial cells of Cajal function ^{14,36}	Small intestine, colon, pancreas, leukocytes
<i>GJA5</i>	Connexin40	Atrial ventricular conduction, haploinsufficiency is associated with structural heart defects ^{7,9}	Heart conduction system and atria ⁵
<i>GJA8</i>	Connexin50	Maintenance of human lens transparency, missense mutations cause pulverulent zonular cataract ³⁷	Lens epithelium and fibre cells, retina, brain, cornea ¹⁵

lized in TE (pH 8.0). University of Alberta Health Research Ethics Board approval was obtained for clinical evaluation of del 1q21.1 positive cases, and for contact of adult family members for further clinical and molecular analysis. Peripheral blood samples from the parents of two index cases were subsequently obtained.

Control DNA was obtained from the blood of 520 anonymous unrelated individuals without any known congenital heart defects.

Real-Time PCR

All samples were initially screened for deletions within the DiGeorge critical region (DGCR) and Williams syndrome critical region (WSCR) using a multiplexed array of 3 quantitative reactions for each disorder.^{11,12} A TaqMan Cx40 gene Pre-Developed Assay Reagent (PDAR; Applied Biosystems) was multiplexed as a 2-copy control in all reactions. Positive (DGCR and WSCR deletion), wild-type, and “no template” controls were included in each analysis. All real-time PCR amplifications were performed on an ABI Prism 7900HT Sequence Detection System (Applied Biosystems) using a 384-well plate. Each of the three WSCR genes and three DGCR genes were assayed in a separate reaction and each assay was performed in triplicate (ie, a total of 18 assays per sample). Reaction mixtures, thermal cycling conditions, and data analyses followed standard protocols.¹³ Any samples that had reproducible threshold cycle number (Ct) values consistent with a duplication for both the DGCR and the WSCR, or a deletion of CX40 (ie, a cycle threshold of 0.5 cycles less than the wild-type) were reanalyzed for copy number of the Cx40 gene in addition to flanking genes (*GJA8* and *ACPL1*; Table 1). Primers and major-groove-binding probe sets were designed for the detection of *ACPL1* exon 1 and *GJA8* exon 2 (Table 2), using Primer Express software (Applied Biosystems).

Semiquantitative PCR

Samples that had a one cycle increase in Cx40 gene Ct value by real-time PCR analysis were amplified using a different primer pair located outside the open reading frame of this gene (Table 2). Each reaction used standard reagent amounts and cycling conditions according to PLATINUM *Taq* manufacturer’s (Invitrogen) recommendations, except that amplification was limited to 27 cycles. PCR products were run on a 1.0% agarose gel, then ethidium bromide-stained and visualized using an ImageMaster VDS gel documentation system (Amersham Scientific).

Microsatellite Analysis

Samples were haplotyped using primers for 2 previously characterized microsatellites within the 1q21.1 critical region (D1S2344 and D1S2612; Table 2). Genomic sequence data were used to identify an additional array of short tandem repeat (STR) loci within the 1q21.1 critical region (CTS03, CTS04, CTS05, CTS07, CTS08, and CTS16; Table 2). Each reaction used standard reagent amounts (with fluorescently-tagged forward primers) and cycling conditions according to PLATINUM *Taq* manufacturer’s (Invitrogen) recommendations, at varying annealing temperatures (Table 2). PCR products were pooled and sized on an ABI PRISM 310 capillary electrophoresis system (Applied Biosystems) and alleles were identified using GeneScan and Genotyper software. Quantitative data were compared with microsatellite data for all real-time PCR positive samples.

Statistical Analysis

A comparison was made between the del 1q21.1 mutation frequency in the CHD group and the non-CHD control group. This was calculated as a binomial variable. The probability of success “ π ” was

TABLE 2. Primers and Probes Used for Real-Time PCR Analysis of *ACPL1* and *GJA8*, Primers Used for Semiquantitative PCR Analysis of *GJA5*, and Primers Used for Microsatellites (STR) Spanning the 1q21.1 Region

Gene/STR	Forward Primer	Reverse Primer	Annealing Temp °C	Probe
<i>ACPL1</i>	AGGCCGATGGCCAGTGT	CGAGCCCCGTGTGCGAA	60	TCGACCGCAGCCTG
<i>GJA8</i>	CTGCCAAGCCTTTCAA	TGGTAGCCCCGGGACAA	60	AGGAGAAGATCAGCACAGG
<i>GJA5</i>	CCAGACCTTACAGAACATCCTT	AGTGAGAAAGCATCAGTTCAG	60	
<i>D1S2344</i>	TCATGGGACTCTCCATCA	AAATACTCAGGAAATGGCCTA	70→65	
<i>D1S2612</i>	GCTGTTCTTAGGGCTTTTCC	AACTTGGGCTTCTCTGCTTC	70→65	
<i>CTS03</i>	CAGAATAACTACTCCATTGTGCATGT	CATATTAATTCACAAGCCAAGATA	60	
<i>CTS04</i>	GGCAGGATGGTGCTCTTT	GACCAGCCTGGACACCA	70→65	
<i>CTS05</i>	CAATATCAGGCCACTGCACT	GGATAATCTAATGGTGGCATTCT	70→65	
<i>CTS07</i>	CCATTATCTTGTGTGCTACAAGTTT	CTCAATTTGTAGATGACATGATCCTA	67→62	
<i>CTS08</i>	AGAAATTCCTGCCACAACGTGA	GGTAGTGAGGTATACTGGTGTACAA	67→62	
<i>CTS16</i>	GTCAATGTTCTTGGTATATCTACAGA	TCTAAGCTCAATTTTCAAAAAGCA	62→57	

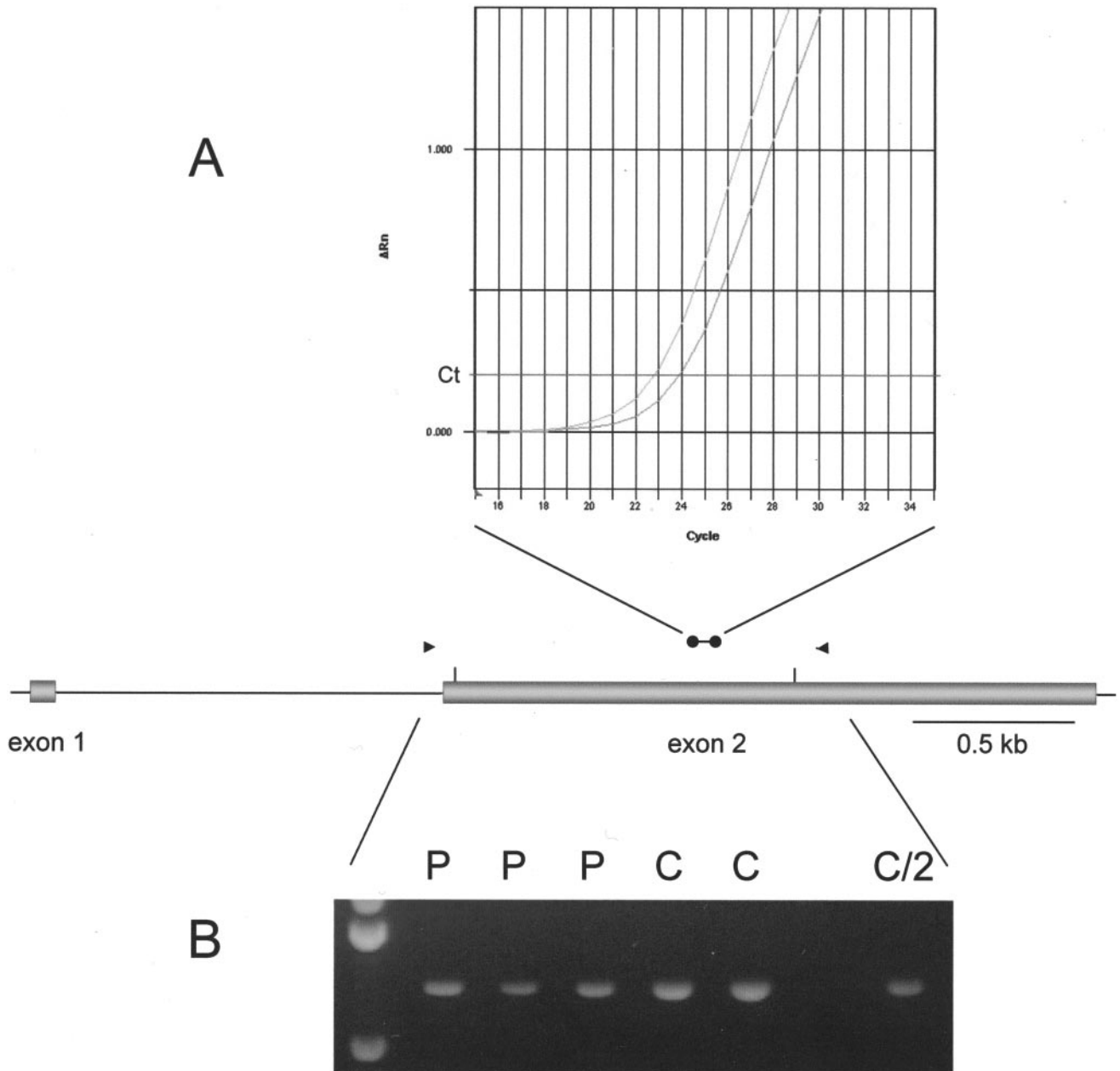


Figure 1. Schematic of GJA5 (Cx40 gene) with (A) real-time PCR data showing 1 cycle lag in amplification rate of 70-bp portion at cycle threshold (Ct) of exon 2 in patient sample relative to normal control, amplified region is illustrated on schematic as solid circles with connecting line, and (B) ethidium bromide stained agarose gel image showing semiquantitative PCR of entire open reading frame (ORF), using primer sites illustrated as arrowheads, from 3 patient samples (P) and 2 normal control samples (C) relative to 1/2 template concentration of normal control (C/2). Cx40 translation start and stop sites (ORF boundaries) are depicted by vertical lines above exon 2.

defined as the number of 1q21.1 deleted cases within the CHD group. Then S , the total number of successes in n trials, was the number of del 1q21.1 cases out of the total number of unrelated control samples tested.

Results

Real-Time and Semi-Quantitative PCR Analysis

Of 505 congenital heart defect cases tested, 16/505 (3.2%) were positive for a deletion within the DiGeorge syndrome critical region (DGCR), and 8/505 (1.6%) were positive for a deletion within the Williams syndrome critical region (WSCR). Of the remaining 481 cases, 3 had Cx40 gene-

normalized results consistent with either a duplication of both the DGCR and the WSCR, or a deletion of the Cx40 gene. Analysis of copy number, revealed a one cycle increase in Cx40 gene at cycle threshold for these samples (Ct; Figure 1), indicating that a deletion of the Cx40 gene was present. Sequencing across the Cx40 gene open reading frame did not reveal any single nucleotide polymorphisms that could be refractory to amplification or probe hybridization (results not shown). A cycle-limited PCR amplification of Cx40 gene exon 2 using a different primer pair, also resulted in less product than normal controls (Figure 1). Of 520 unrelated control samples screened by real-time PCR for the presence

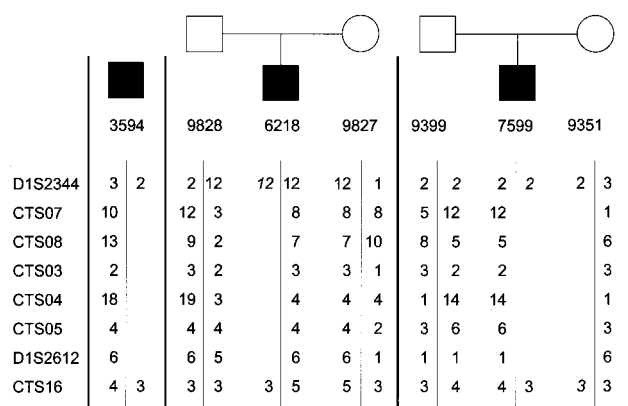


Figure 2. Summary of genotypes obtained for microsatellites spanning the 1q21.1 region. Unrelated index cases are shaded black. Genotypes for the parents of samples 6218 and 7599 are included. Alleles in italics are inferred, based on genotypes of other family members.

of a one cycle increase in the Cx40 gene Ct value relative to the DGCR and WSCR, none were detected ($P=0.039$). Real-time PCR assays for genes flanking Cx40 gene (*ACPL1* and Cx50) in the three cases with a lower copy number of Cx40 gene showed a 1.0 Ct increase for both flanking genes (results not shown). These results are consistent with a deletion in these 3 CHD cases that includes *ACPL1*, Cx40, and Cx50 genes (Table 1).

Microsatellite Analysis

DNA from the three index cases, and parents of cases 6218 and 7599, was genotyped with an array of microsatellite markers spanning the 1q21.1 region (Figure 2). Absence of two distinct alleles (heterozygosity) was seen in the index cases spanning markers CTS07 to D1S2612. One case (3594) was heterozygous at the proximal flanking marker D1S2344, and all three cases were heterozygous at the distal flanking marker CTS16. Individual 6218 had maternal alleles spanning the CTS07 to D1S2612 region. Both of this infant's parents were heterozygous through this region, indicating that the deletion was paternal in origin. Paternity of this infant was tested and verified (results not shown). Individual 7599, for which parental samples were also obtained, had paternal

alleles present in the CTS07 to D1S2612 region. This infant's father (9399) was heterozygous at markers CTS07 to CTS16. However, this infant's mother (9351), showed contiguous absence of heterozygosity through the same 1q21.1 region as her son (Figure 2). Real-time PCR analysis was performed on sample 9351 and showed a 1.0 Ct increase for *ACPL1*, Cx40 gene, and Cx50 (results not shown). These results are consistent with a chromosome 1q21.1 deletion that spans a 1.5- to 3-Mb region between markers D1S2344 and CTS16 in 3 CHD-affected cases and one apparently unaffected parent.

Clinical Findings

Medical records of the three 1q21.1 deletion cases were examined to determine whether they could represent a clinically defined subgroup. This included review of operative notes from six surgical procedures, two cardiac catheterizations, and 41 echocardiographic (ECG) examinations. Initial electrocardiography showed normal time intervals in all three patients. In follow-up, 33 ECG recordings and one Holter recording were available. Patient 7599 developed a complete right bundle block and also had a brief period of junctional ectopic tachycardia after surgical closure of the ventricular septal defect, patient 3594 has persistent, left axis deviation of the QRS complex, and patient 6218 has had no unusual ECG features. No other significant dysrhythmias or conduction abnormalities have been detected. The clinical features are presented in Table 3. One patient (3594) had dysmorphic features and minor neurological abnormalities in addition to cardiac defects. After a clinical genetics assessment, this individual was strongly suspected to have fetal alcohol syndrome. However, he is adopted and no contact with his biological parents is available at present. All three patients had trileaflet aortic valves. Two of the patients (3594 and 7599) have required re-operation for recurrent subaortic stenosis. All three patients are unrelated and of relatively diverse ethnic background: Caucasian (3594), Somali (6218), and French Canadian (7599).

The mother of patient 7599 (9351) has been seen in genetics clinic and was found to carry the same 1q21.1 deletion as her son (Figure 2). She is asymptomatic; clinical examination and a cardiac ultrasound are also normal.

TABLE 3. Summary of Clinical Findings for Chromosome 1q21.1-Deleted Index Cases

	3594	6218	7599
Major cardiac lesion	Discrete coarctation of aorta	Interrupted aortic arch-type A	Interrupted aortic arch-type B
Other cardiac lesion	Subaortic membrane	Aortopulmonary window	VSD with muscular subaortic stenosis
Re-operation	Subaortic stenosis	None	Subaortic stenosis
Gender	M	M	M
Birth weight	Normal	Normal	Normal
Birth length	Normal	Normal	Normal
Head circumference	Normal	Normal	Normal
Significant dysphagia	+	-	+
Seizures	+	-	-
Developmental delay	+	-	-
Dysmorphic facies	+	-	-

VSD indicates ventricular septal defect.

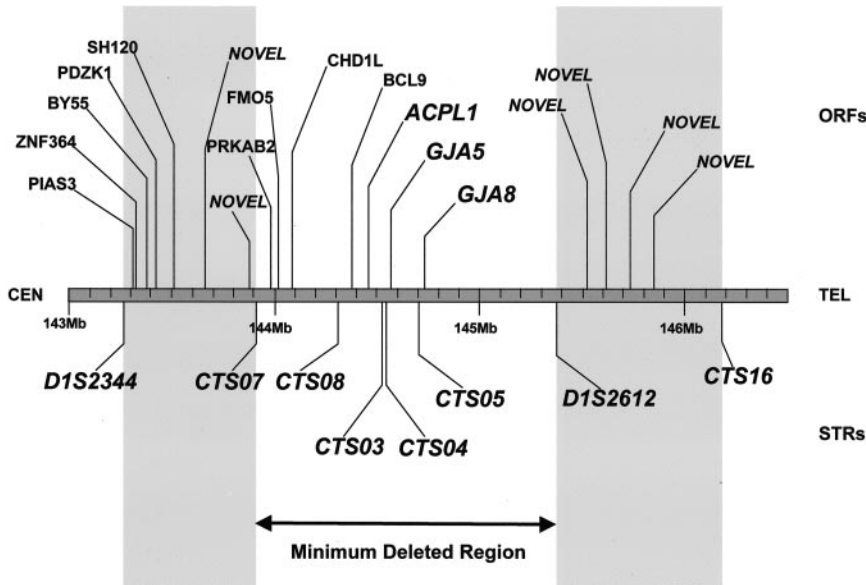


Figure 3. Summary of mapping data for genes (open reading frames; ORFs) and short tandem repeats (STRs) in the chromosome 1q21.1 region. Gene names follow standard nomenclature. Corresponding protein names and functions for genes within the minimum deleted region are listed in Table 1. Predicted genes with no current known function are labeled as "NOVEL." Deletions were confirmed by real-time PCR for genes in larger italicized print (*ACPL1*, *GJA5*, and *GJA8*). Arrow indicates the minimum deleted region of approximately 1.5 Mb, based on real-time and microsatellite genotyping results. Flanking gray regions contain the centromeric and telomeric deletion breakpoints. The maximum possible deletion extends from D1S2344 to CTS16: ≈ 3 Mb.

Discussion

Several microdeletion syndromes are associated with congenital heart defects.³ There are, however, no previous reports of a contiguous gene microdeletion syndrome localized to chromosome 1q21.1. In the present investigation, real-time PCR analysis of the chromosome 1q21.1 region from a total of 505 congenital heart disease cases identified three unrelated cases with a deletion in this region spanning at least three genes. These genes (*ACPL1*, *GJA5*, and *GJA8*) code for acid phosphatase-like protein 1, connexin40 (Cx40), and connexin50 (Cx50) proteins (Table 1).^{10,14,15} In order to determine the full extent of this deletion, we genotyped eight different polymorphic DNA tandem repeats (microsatellites) that map across this region (Figure 2). This microsatellite analysis was performed on two of the three cases known to carry the deletion along with their parents. The other deletion carrier was adopted and was, therefore, analyzed without samples from his biological parents. All three congenital heart defect cases have single microsatellite alleles from marker CTS07 to marker D1S2612. These markers span a 1.5-Mb region within 1q21.1, which includes a total of seven genes (*PRKAB2*, *FMO5*, *CHD1L*, *BCL9*, *ACPL1*, *GJA5*, and *GJA8*; Table 1 and Figure 3). One of the two CHD patients for which parental analysis was possible (6218) had a paternally derived (9828) de novo deletion, because only maternal alleles were observed in this region and both parents are heterozygous (Figure 2). The other CHD patient (7599) inherited this deletion from his mother (9351), because only paternal alleles were observed, and because this individual's mother had single microsatellite alleles through this 1.5-Mb region (Figure 2). The mother's deletion was subsequently confirmed by real-time PCR analysis of her *ACPL1*, *GJA5*, and *GJA8* genes. The maximum extent of this deletion is delineated by microsatellite markers D1S2344 and CTS16 (Figures 2 and 3). Heterozygosity for each of these markers were observed in one of the CHD cases (3594), and the other three cases known to carry the deletion (6218, 7599, and 9351) were either heterozygous or showed inheritance consistent with two copies of the same allele (homozygous) for these markers. D1S2344 and CTS16 are 3 Mb apart. Therefore,

if we assume that the same deletion is common to all four cases, the centromeric deletion breakpoint must lie between D1S2344 and CTS07, and the telomeric deletion breakpoint must lie between D1S2612 and CTS16. This microsatellite data, therefore, defines a common deleted region that is a minimum of 1.5 Mb and a maximum of 3 Mb in size. If the deletion is 3 Mb, it includes five additional genes (Figure 3): protein inhibitor of activated STAT3 (*PIAS3*), zinc finger protein 364 (*ZNF364*), natural killer cell receptor BY55 (*BY55*), PDZ domain-containing-1 protein (*PDZK1*), and putative G protein-coupled receptor SH120 (*SH120*).

Contiguous gene deletion disorders result from haploinsufficiency for several genes and the complete spectrum of phenotypic features can often be difficult to attribute to the loss of any one of these genes in isolation. At least two of the deleted genes in the 1q21.1 region are known to be expressed in cardiac tissue; β_2 AMP-activated protein kinase and Cx40 (Table 1). The combined effect of a reduced quantity of these proteins may be responsible for the specific cardiac defects that were observed. Mutations in the gene encoding the γ_2 subunit of AMP-activated protein kinase (AMPK) have been found to cause familial hypertrophic cardiomyopathy (HCM).^{16,17} No causative point mutations have been detected in the β_2 AMPK subunit gene,¹⁶ and the clinical presentation of HCM does not appear to overlap with the features observed in our 1q21.1 patients (Table 3). However, the possibility remains that haploinsufficiency for the β_2 AMPK subunit may be contributing to the del 1q21.1 phenotype. In a mouse model system, haploinsufficiency for Cx40 alone has been shown to result in a number of cardiac malformations including bifid atrial appendage, ventricular septal defect (VSD), tetralogy of Fallot, and an aortic arch abnormality.⁹ There is considerable overlap between the malformations observed in these Cx40 heterozygous-null (+/-) mice and our del 1q21.1 index cases. It is therefore possible that despite the fact that our CHD cases have a contiguous gene deletion, the Cx40 gene deletion may be the only mutation that is contributing to the CHD phenotype. However, other deleted genes in this 1q21.1 region, such as *CHD1L*, a chromodomain helicase DNA

binding protein 1-like gene that has transcriptional regulatory and chromatin remodeling domains, may be dosage sensitive. The phenotype of the del 1q21.1 disorder may therefore, result from haploinsufficiency for one or more of these proteins.

It is unexpected that a chromosomal microdeletion, resulting in haploinsufficiency for at least seven genes, would be associated with isolated heart defects. Two of the three affected patients did not have any other apparent congenital anomalies. More than 20 other microdeletion syndromes have been identified to date, the vast majority of which are characterized by consistent patterns of physical, behavioral, and mental characteristics.¹⁸ This is presumably due to the combined effects of haploinsufficiency for several genes. It is, therefore, probable that heart-specific genes in the 1q21.1 region are particularly dosage sensitive. This finding has implications for congenital heart disease screening, because there are no other characteristic clinical features of this disorder.

The 505 CHD cases screened for a 1q21.1 deletion in the present study were drawn from patients referred from a variety of clinical sites to rule out a diagnosis of either DiGeorge/velocardiofacial syndrome (439 cases) or William syndrome (66 cases). This study was performed retrospectively from a source of banked DNA samples, and therefore, referral criteria were not defined before submission. This is illustrated by the fact that isolated cardiac defects, coarctation of the aorta, or membranous subaortic stenosis, as seen in two of our three 1q21.1 patients (Table 3), are not typically within the clinical spectrum of either DiGeorge or William syndrome. It is therefore, likely that if we had screened a DNA repository that encompassed a full spectrum of moderate to severe CHD cases, the incidence of del 1q21.1 may have been significantly higher than the 3/481 (0.6%) cases that we observed.

22q11.2 deletion syndrome encompasses DiGeorge syndrome and is commonly associated with a variety of neural crest-derived defects, including conotruncal septation defects.^{19,20} Forty-eight percent of patients with the conotruncal anomaly of subaortic stenosis due to posterior deviation of the infundibular septum with ventricular septal defect and interrupted aortic arch type B have DiGeorge syndrome.²¹ The presence of this particular cardiac anomaly in one of our patients (7599) is therefore suggestive of an association with migration abnormalities of the cardiac neural crest. Patients with congenital heart defects which are suspected to result from neural crest migration abnormalities may warrant screening for del 1q21.1.

In their recent study of Cx40 haploinsufficient mice, Gu et al⁹ found conotruncal malformations to be very common. They also described a significant anomaly of the aortic arch. A previous study of combined Cx40 and connexin43 (Cx43)-deficient mice also found numerous abnormalities of cardiac morphogenesis and concluded that the contribution to morphogenesis of the heart was isotype specific.²² Cx43 is highly expressed in neural crest cells,²³ but there is no reported expression of Cx40 in neural crest cells.⁹ However, the mechanisms by which these gap junction proteins effect migration of the cardiac neural crest appear to be related. This apparent association would benefit from a more detailed analysis of how Cx40 affects development of the neural crest-derived outflow tract.

The etiology of del 1q21.1 heart defects could be related to Cx40-dependent blood flow abnormalities, which may be associated with previously characterized disorders. Cx40 has been shown to play a significant role in the propagation of endothelium-dependent vasodilations in a mouse model system, presumably by altering cell communication in arterioles.⁶ Chronic atrial fibrillation and mitral valvular disease (MVD) have also been associated with alterations in expression levels of Cx40.⁷ Mitral valve, vascular, and conduction abnormalities, in addition to coarctation of the aorta and subaortic stenosis, are part of the spectrum of heart defects seen in Shone's syndrome, a relatively rare disorder that is characterized primarily by left heart obstruction.^{24,25} Similarly, Turner syndrome, which results from haploinsufficiency for several sex-linked genes that normally escape X inactivation, is associated with cardiovascular abnormalities, typically including bicuspid aortic valves and coarctation of the aorta.^{26–28} There is some overlap between Shone's syndrome, the cardiovascular features of Turner syndrome, and the defects in our patients; however, none of our patients have MVD or bicuspid aortic valves (Table 3). Conduction defects were found in two of the three del 1q21.1 patients and a discrete coarctation was observed in only one these cases. It is therefore, currently difficult to determine whether del 1q21.1 falls within the spectrum of Shone's syndrome or overlaps with Turner syndrome cardiogenic etiology.

Clearly del 1q21.1 syndrome shows variable penetrance, because the mother of an affected child carries the same deletion, and is asymptomatic. In this instance, the deletion was stably transmitted from mother to child. The extended family history of this affected child includes a maternal first-cousin (his maternal aunt's son) who was reported to have a congenital heart murmur. Although his condition apparently resolved before adolescence, further investigation of this extended family is underway. In the other patient for which we could genotype the parents, the deletion occurred de novo and originated with the father. For most microdeletion syndromes, the frequency of de novo deletions is much higher than inherited deletions, and so we would have expected that the chance of identifying a deleted parent was very low.²⁹ There may also be a sex-related bias to our observation of an asymptomatic deletion-carrier mother, because coarctation of the aorta shows a striking male predominance among individuals with congenital heart defects.³⁰ All of our affected patients are male, suggesting the variable penetrance of del 1q21.1 syndrome may follow this sex-related pattern. The lack of complete penetrance is also consistent with mouse model studies, where cardiac malformations were observed in 18% of Cx40 heterozygous-null (+/–) animals, although genders were not reported.⁹

In summary, we have identified a novel microdeletion syndrome that is associated with congenital heart defects. The 1.5- to 3-Mb deletion of chromosome 1q21.1 encompasses at least seven genes. We have observed this mutation in 0.6% of congenital heart defect cases referred for molecular genetic testing. The clinical profile of these cases is somewhat variable, but obstruction of the aortic arch is a common feature. Classification of these heart abnormalities on the basis of anatomic or functional characteristics may be ob-

sured by clinical heterogeneity and would benefit greatly from the identification of more cases.

Acknowledgments

This work was supported by a grant CLB01/003745 from the Edmonton Community Lottery Board. We thank R. Wevrck for critical reading and helpful comments in the preparation of this manuscript. We also thank the individuals who provided blood samples for testing.

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JOURNAL OF THE AMERICAN HEART ASSOCIATION



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Circ Res. 2004;94:1429-1435; originally published online April 29, 2004;
doi: 10.1161/01.RES.0000130528.72330.5c

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:

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