CLINICAL REPORT

AMERICAN JOURNAL OF medical genetics PART A

Chromosome 4q Deletion Syndrome: Narrowing the Cardiovascular Critical Region to 4q32.2–q34.3

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Received 4 March 2011; Accepted 2 November 2011

The 4q deletion syndrome is a rare chromosome deletion syndrome with a wide range of clinical phenotypes. There is limited clinical phenotype and molecular correlation for congenital heart defects (CHDs) reported so far for this region primarily because many cases are large deletions, often terminal, and because high-resolution array has not been reported in the evaluation of this group of patients. CHDs are reported in about 60% of patients with 4q deletion syndrome, occurring in the presence or absence of *dHAND* deletion, implying the existence of additional genes in 4q whose dosage influences cardiac development. We report an 8-month-old patient with a large mid-muscular to outlet ventricular septal defect (VSD), moderate-sized secundum-type atrial septal defect (ASD), thickened, dysplastic pulmonary valve with mild stenosis and moderate pulmonic regurgitation, and patent ductus arteriosus (PDA). Illumina CytoSNP array analysis disclosed a de novo, heterozygous, interstitial deletion of 11.6 Mb of genomic material from the long arm of chromosome 4, at 4q32.3–q34.3 (Chr4:167236114–178816031; hg18). The deleted region affects 37 RefSeq genes (hg18), including two provisional microRNA stemloops. Three genes in this region, namely *TLL1* (Tolloid-like-1), *HPGD* (15-hydroxyprostaglandin dehydrogenase), and *HAND2* (Heart and neural crest derivatives-expressed protein 2), are known to be involved in cardiac morphogenesis. This report narrows the critical region responsible for CHDs seen in 4q deletion syndrome. © 2012 Wiley Periodicals, Inc.

Key words: 4q deletion syndrome; ventricular septal defect (VSD); atrial septal defect (ASD)

How to Cite this Article:
Xu W, Ahmad A, Dagenais S, Iyer RK, Innis JW. 2012. Chromosome 4q deletion syndrome: Narrowing the cardiovascular critical region to 4q32.2–q34.3.
Am J Med Genet Part A 158A:635–640.

INTRODUCTION

Since its first description [Ockey et al., 1967], the clinical phenotype of 4q deletion syndrome has been delineated by several groups [Mitchell et al., 1981; Yu et al., 1981; Lin et al., 1988; Strehle and Bantock, 2003], with an incidence estimated at 1/100,000 and overall mortality of 28% [Strehle and Bantock, 2003]. The most common anomalies observed in patients include mental deficiency (92%); postnatal onset of growth deficiency (83%); craniofacial anomalies including broad nasal bridge (94%), rotated ears (56%), cleft palate (94%), and micrognathia (94%); congenital heart defects (CHDs) (50%) including ventricular septal defect (VSD), patent ductus arteriosus (PDA), peripheral pulmonic stenosis, aortic stenosis, atrial septal defect (ASD), tetralogy of Fallot, aortic coarctation, tricuspid atresia; and digital anomalies (especially of the fifth finger) (50–88%). Gastrointestinal and renal anomalies are less common [Mitchell et al., 1981; Yu et al., 1981; Lin et al., 1988; Strehle and Bantock, 2003].

The severity of malformations varies widely and depends on the chromosomal position and deletion size. Loss of 4q31–q34 region is responsible for most of the clinical phenotype [Giuffrè et al., 2004], and Keeling et al. [2001] proposed that the 4q33 region is the critical region for 4q deletion syndrome. However, no reports have used contemporary high-resolution array CGH that would promote assembly of an accurate genotype/phenotype correlation for the CHDs.

We report an 8-month-old male patient with CHDs, growth delay, and minor skeletal anomalies with a loss of 11.6 Mb of genomic material from the long arm of chromosome 4, at 4q32.3–q34.3 (Chr4: 167236114–178816031; hg18). This region contains 37 RefSeq genes including several with potential heart and/or vascular relevance: *TLL1* (Tolloid-like-1), *HPGD* (15-hydroxy-

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Published online 2 February 2012 in Wiley Online Library (wileyonlinelibrary.com).
DOI 10.1002/ajmg.a.34425

prostaglandin dehydrogenase), and *HAND2* (Heart and neural crest derivatives-expressed protein 2). Together with other reports, our description narrows the critical region responsible for the CHDs in 4q deletion syndrome to Chr4:167236114–178816031.

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The proband was born at a gestational age of 35 2/7 weeks by cesarean to a healthy 24-year-old gravida 5, para 2–3 mother. A prenatal diagnosis of a CHD was made. His birth weight was 3.16 kg (78th centile), length was 50 cm (85th centile), and head circumference (OFC) was 34 cm (85th centile). The baby was cyanotic at delivery, intubated for poor respiratory effort at birth, and received one dose of surfactant. Initial echocardiogram showed large mid-muscular to outlet VSD with bidirectional shunt, moderate-sized secundum-type ASD, thickened, dysplastic pulmonary valve with mild stenosis, and moderate pulmonic regurgitation. He underwent PDA ligation and VSD repair at age 3 months. His postnatal course was complicated by milk protein intolerance, failure to thrive, and gastroesophageal reflux. There was mild developmental delay at age 7 months, since he was not sitting independently, but was otherwise age-appropriate. On physical exam at 7 months, his weight was 5.9 kg (less than 5th centile), length was 60 cm (less than 5th centile), and OFC was 43.6 cm (23rd centile). He had posteriorly rotated ears with simplified helices, pointed on the right. No other significant dysmorphic facial features were noted (parents declined facial photographs). There was overlap of toes 4–5 on both feet. Nails of fifth fingers were normal bilaterally (Fig. 1). Physical examination was otherwise unremarkable. Renal ultrasound noted bilateral extrarenal pelvis.

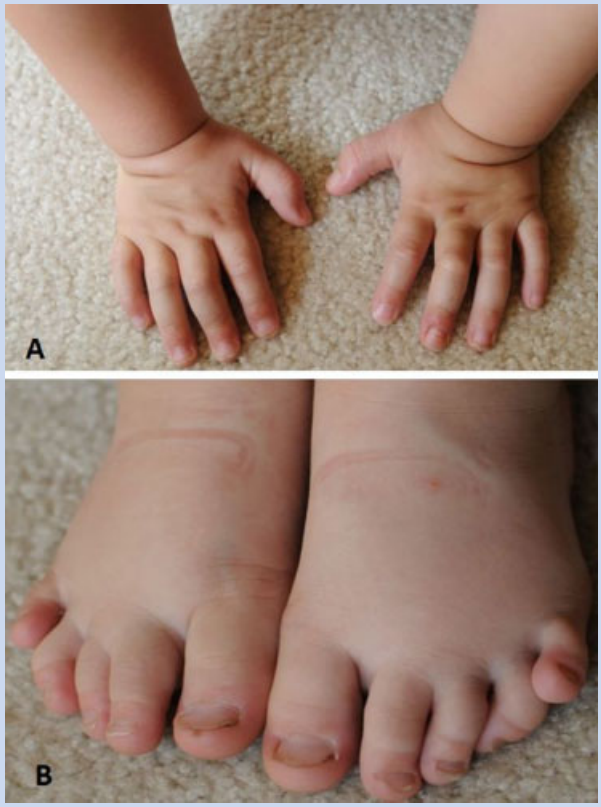

FIG. 1. A: Proband's hands; note normal nails of the fifth fingers bilaterally, illustrating that the deletion does not involve the 4q34 region. B: Proband's feet showing overlapping digits 4 and 5.

METHODS

Postnatal Array CGH Analysis

Array CGH analyses were performed using the custom-designed EMArray Cyto6000 array platform [Baldwin et al., 2008], a sex-mismatched, pooled, normal DNA control for comparison, and standard protocols implemented in the Michigan Medical Genetics Laboratory at the University of Michigan.

Array CGH Data Analysis

The array data were imported into, analyzed and plotted by the Agilent CGH Analytics version 3.5 or DNA analytics version 4.0.81 software (Agilent Technologies, Santa Clara, CA). For reporting of copy number changes detected by CMA, nucleotides are numbered according to Genome Build UCSC hg18 (March 2006) assembly, and results are reported as per the International System for Human Cytogenetic Nomenclature (ISCN) 2009 recommendations.

Illumina Array

To refine the deletion endpoints, an Illumina Human CytoSNP-12v2.1 BeadChip Kit (Illumina, INC., San Diego, CA) with average probe spacing of 10 kb, was used for the analysis. DNA was quantified with the Quant-iT™ PicoGreen® dsDNA Kit (Invitrogen Corporation, Carlsbad, CA). 200 ng DNA was utilized in an Illumina Infinium® HD Ultra Assay according to the manufacturer's instructions. HumanCytoSNP BeadChips were scanned with an Illumina iScan Reader. Image data were analyzed using the Illumina GenomeStudio (v2010.2) Genotyping Analysis Module (v1.7.4) Software. SNP cluster positions were defined based on the HumanCytoSNP cluster file provided by Illumina, Inc. Genome positions were determined using Build 36.1 (hg18).

Fluorescence In Situ Hybridization (FISH)

Metaphase FISH analysis was performed at Emory Genetics Labs on samples from the proband and the biological parents of the proband, using a custom-labeled Bacterial Artificial Chromosome (BAC) probe, RP11-119J20, located within the region of interest at 4q. A 4q subtelomere-specific probe (Abbott Molecular Inc., Des Plaines, IL) was used as a control.

RESULTS

Array CGH analysis using the EmArray detected a male chromosome profile, with a 11.6 Mb loss of genomic material from the long arm of chromosome 4, at 4q32.3–q34.3. To further define the deletion breakpoints, we used higher density Illumina CytoSNP analysis and this revealed heterozygous loss for

TABLE I. Phenotype Comparison Between Our Case and Published Cases With 4q Deletion Syndrome [Adapted From Strehle and Bantock, 2003]

Characteristic	Percentage	This report
Male/female ratio	0.91	Male
Abnormal parental chromosomes	14% (13/90)	−
Prematurity	14% (12/85)	+, 35 wks GA
Developmental delay	94% (77/82)	+, Mild
Growth failure	60% (56/94)	+, Failure to thrive
Mortality	28% (28/101)	−
Dysmorphic craniofacial features	99% (100/101)	+
Pierre-robin sequence/cleft lip and palate	37% (37/101)	−
Central nervous system	34% (34/101)	No known abn
Ocular system	44% (44/101)	No known abn
Hearing	37% (16/43)	−
Digital anomaly	88% (89/101)	−
Muscular System	45% (45/101)	−
Cardiovascular system	50% (50/101)	+ VSD muscular-outlet; ASD secundum; Pulmonic stenosis
Respiratory tract	32% (32/101)	No known abn
Dentition	18% (18/101)	No known abn
Gastrointestinal tract	40% (40/101)	+, GERD
Hepatobiliary system and pancreas	17% (17/101)	No known abn
Lymphatic system and spleen	8% (8/101)	No known abn
Endocrine system	6% (6/101)	No known abn
Renal and urinary tract	19% (19/101)	+, Extrarenal pelvis
Genitalia	28% (28/101)	−
Skin/hair	43% (43/101)	−

"No known abn" indicates that a comprehensive evaluation of this organ system was not performed, however there are no clinical concerns regarding this system at the last clinical evaluation.
−, no observed clinical problem; +, feature is present.
ASD, atrial septal defect; GA, gestational age; GERD, gastrointestinal reflux disease; VSD, ventricular septal defect.

Chr4:167236114–178816031. The minimal aberrant region contains 37 RefSeq genes (Table I), including *TLL1* (; NM_012464.3), *HPGD* (NM_000860.3) and *HAND2* (also known as *dHAND*; NM_021973.2). To confirm the results as well as to clarify the origin of this microdeletion, we performed FISH analysis on samples from the patient and both parents. FISH utilized BAC probe RP11-119J20 and detected an interstitial deletion in the proband, consistent with the array results (see Fig. 2). A 4q subtelomere-specific probe (Abbott Molecular Inc) was used as a control and it showed a normal hybridization pattern. The 4q deletion is presumed to be *de novo* since it was not detected in either of the biological parents.

DISCUSSION

Chromosome 4q deletion syndrome comprises both interstitial and terminal deletions in the long arm of chromosome 4. Both the reported size and region deleted in 4q deletion syndrome vary greatly among patients, so does the scope of clinical malformations. There are fewer than 200 cases with 4q deletion reported in the literature and ours is the first to use high-resolution array to define the molecular deletion boundaries in relation to the phenotype.

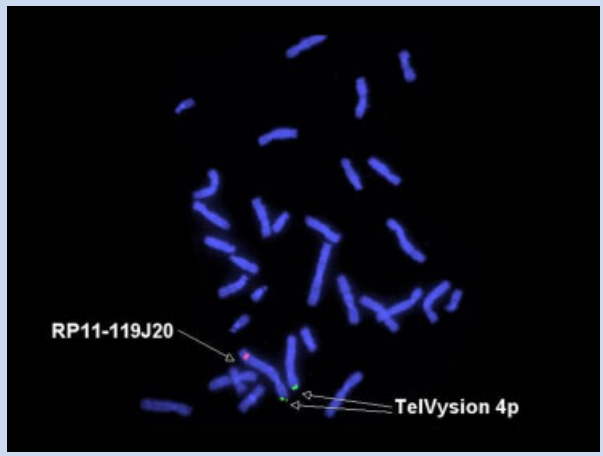

FIG. 2. FISH results of the proband. Bacterial Artificial Chromosome (BAC) probe (RP11-119J20) located within the region of interest (red) detects a 4q32.3–34.3 deletion on one homolog of chromosome 4. A 4q subtelomere-specific probe (Abbott Molecular Inc.) used as a control (green) showed a normal hybridization pattern.

We compared the clinical findings in our patient with the summary of the clinical characteristics in 101 patients with 4q deletion syndrome [Strehle and Bantock, 2003] (Table I). The sole major malformation in our patient included the CHDs consisting of a large mid-muscular to outlet VSD, ASD secundum, thickened, and dysplastic pulmonary valve with mild stenosis. Minor anomalies consisted of posteriorly rotated, simplified helices and overlapping of toes 4–5. However, he had small stature and mild developmental delay.

It has been suggested that the 4q32.3–q34.3 region plays a role in cardiac development. One study of five patients reported three patients with terminal deletions involving the *dHAND* (*HAND2*) gene, and only two of these individuals had CHD. One other patient with a terminal deletion had an ASD, but *dHAND* was not deleted [Huang et al., 2002]. Another study described BAC array comparative genomic hybridization (CGH) evaluation of a patient with mild aortic stenosis and dysplasia of the pulmonary valve showing a *de novo* 4q terminal deletion of 18.9–22.9 Mb [Kitxiou-Tzeli et al., 2008]. Kaalund et al. [2008] also reported a boy with a VSD who had a 21 Mb interstitial deletion of 4q (roughly delineated to position 160,717,000–181,668,000) using a BAC array. However, none of those reports accurately identified the deletion breakpoints so it is impossible to develop clinical correlations with regard to CHDs.

To evaluate the known contribution of genes in the deleted region to clinical phenotype as well as the potential impact of haploinsufficiency to disease or risk, we examined the deleted chromosomal region in our patient for genes and large copy number variants (CNVs). There are 37 documented RefSeq genes (hg18) (Table II) in the deleted region. There are many small CNVs of a few thousand base pairs, but only one reaching 425 kb. CNVs

TABLE II. 37 RefSeq Genes in the 4q32.3-34.3 Deleted Region of the Proband[a]

Gene	Description	Chromosomal location
TLL1	Metalloprotease	4q32.3
SPOCK3	Proteoglycans	4q32.3
ANXA10	Ca^{2+}-dependent phospholipid-binding protein	4q32.3
DDX60	DEAD (Asp-Glu-Ala-Asp) box polypeptide 60	4q32.3
DDX60L	DEAD box polypeptide 60-like	4q32.3
PALLD	Actin-containing microfilaments	4q32.3
CBR4	Carbonyl reductase 4	4q32.3
SH3RF1	SH3 domain containing ring finger 1	4q32.3–4q33
NEK1	Never in mitosis gene A-related kinase 1	4q33
CLCN3	Chloride channel 3	4q33
C4orf27	Chromosome 4 open reading frame 27	4q33
MFAP3L	Microfibrillar-associated protein 3-like	4q33
AADAT	α-aminoadipate amino transferase	4q33
HSP90AA6P	Heat shock protein 90kDa alpha	4q33
GALNTL6	GalNac Transferase-like 6	4q34.1
GALNT7	GalNac Transferase 7	4q34.1
HMGB2	High mobility group protein 2	4q34.1
SAP30	SIN3-associated polypeptide, 30KD	4q34.1
SCRG1	Scrapie-responsive gene 1	4q34.1
HAND2	Basic helix-loop-helix transcription factor	4q34.1
NBLA00301	Nbla00301 (non-coding RNA)	4q34.1
MORF4	Mortality factor 4	4q34.1
FBXO8	F-box only protein 8	4q34.1
KIAA1712	HBV PreS1-transactivated protein 3	4q34.1
MIR4276	microRNA 4276	4q34.1
HPGD	15-@hydroxyprostaglandin dehydrogenase	4q34.1
GLRA3	Glycine receptor, alpha-3 subunit	4q34.1
ADAM29	A disintegrin and metalloproteinase domain 29	4q34.1
GPM6A	Neuronal membrane glycoprotein M6A	4q34.2
MIR1267	microRNA 1267	4q34.2
WDR17	WD repeat-containing protein 17	4q34.2
SPATA4	Spermatogenesis-associated protein 4	4q34.2
ASB5	Ankyrin repeat and SOCS box containing 5	4q34.2
SPCS3	Signal peptidase complex subunit 3 homolog	4q34.2
VEGFC	Vascular endothelial growth factor C	4q34.3
NEIL3	Endonuclease VIII-like 3	4q34.3
AGA	Aspartylglucosaminidase	4q34.3

[a]Predicted ORFs and predicted microRNA genes are included. Two terminal coding exons of the *TLL1* gene were deleted in the patient.

are discussed below in the context of specific CHD candidate genes. Several genes in this region possess functions that may correlate with aspects of the patient's clinical, in particular cardiac, phenotype. *TLL1*, *HPGD*, and *HAND2* are involved in cardiovascular development and/or pathology as we summarize below.

The *TLL1* gene encodes an astacin-like metalloprotease that shares structural similarity to the morphogenetically important proteases bone morphogenetic protein-1 (BMP1; MIM:112264) and *Drosophila* Tolloid (TLD). Homozygous (*Tll1*−/−) is embryonic lethal in mice from cardiac failure and a constellation of CHDs at midgestation. The major pathological features included incomplete formation of the muscular interventricular septum and an abnormal and novel positioning of the heart and aorta. *TLL1* plays multiple roles in the formation of the interventricular septum and other parts of the heart [Clark et al., 1999]. Heterozygous (*Tll1*+/−) mutant mice appear grossly normal, survive to adulthood, and are fertile [Clark et al., 1999]. We are not aware of a pathological CHD in the *Tll1*+/− mouse. In humans, in 19 unrelated patients with ASD (MIM# 613087) with or without other CHDs, three heterozygous missense mutations were identified in *TLL1* in three patients [Stanczak et al., 2009]. Two mutations (Met182Leu, and Ala238Val) were identified in two patients with ASD only, and another (Leu627Val), was detected in a patient with ASD with an interatrial septum aneurysm [Stanczak et al., 2009]. These data support the hypothesis that heterozygous deletion of *TLL1* may contribute to the development of ASD in our patient. We did not sequence the *TLL1* gene of the non-deleted chromosome. Examination of the *TLL1* gene region in the Database of Genomic Variants (DGV) revealed the presence of five CNVs. One of the CNVs is a 425 kb deletion (Chr4:167057083-167482075) [Sebat et al., 2004]; the other four CNVs are around or less than 1 kb. The 425 kb deletion truncates the *TLL1* gene in the 5′-UTR and was observed in 1 out of 20 control samples [Sebat et al., 2004]. Examination of the EMArray and Illumina CytoSNP data revealed only heterozygous *TLL1* loss in this region.

HPGD, (also called *PGDH*) is the main enzyme of prostaglandin degradation. By catalyzing the conversion of the 15-hydroxyl group of prostaglandins into a keto group, this ubiquitous enzyme strongly reduces the biologic activity of these molecules. *Pgdh* −/− mice die between 12 and 48 hr of postnatal life because of PDA leading to congestive heart failure [Coggins et al., 2002]. Treatment with indomethacin rescued the mice. Coggins et al. [2002] concluded that alterations in prostaglandin E_2 (PGE_2) metabolism by PGDH during the perinatal period are essential for permanent closure of the ductus arteriosus. *PGDH* mutations are also reported to be associated with autosomal recessive primary hypertrophic osteoarthropathy (PHO; MIM:259100). Four of 13 PGDH homozygous deficient (c.175_176delCT/p. L59VfsX8, c.232_241delinsCA/p.V78QfsX11, c. 418G > C/p.A140P) patients had a persistent PDA that may have resulted from increased PGE_2 [Uppal et al., 2008]. Though Coggins et al. concluded that alterations in PGE_2 metabolism by PGDH during the perinatal period is essential for the permanent closure of the ductus arteriosus, most patients did not have PDA, suggesting that loss of PGDH activity is not sufficient to cause failure of ductal closure in humans. Supporting this idea, both dominant and recessive inheritance of PHO have been reported, suggesting a complicated role for *PGDH* in the course of disease [Uppal et al., 2008]. In our patient, heterozygous deletion of the *PGDH* gene could contribute to the occurrence of PDA. We did not sequence the *PGDH* gene of the non-deleted chromosome. Examination of the *PGDH* gene region in DGV revealed no CNVs.

The *HAND2* (MIM # 602407) gene encodes a basic helix–loop–helix transcription factor that, along with the closely related HAND1 protein, plays an essential role in cardiac morphogenesis. *Hand2* is particularly important for the formation of the right ventricle and the aortic arch arteries [Srivastava et al., 1997]. *Hand2* is expressed in the developing vascular mesenchyme and its derivative, vascular smooth muscle cells. $Hand2^{-/-}$ mice die at embryonic day 10.5 from heart failure [Srivastava et al., 1997], and have severe defects of embryonic and yolk sac vascular development by embryonic day 9.5 [Yamagishi et al., 2000]. In a group of 131 patients diagnosed with CHDs of the right ventricle, outflow tract, aortic artery, or cardiac cushion, Shen et al. [2010] found seven *HAND2* mutations in 12 patients: Three missense mutations (Pro11Arg, Ser36Asn, and Val83Leu), one synonymous mutation (His14His) and three mutations in the 5′ and 3′ untranslated regions (241A > G, 604C > T, and 3237T > A). In patients with terminal 4q deletions and CHDs, loss of *HAND2* is associated with pulmonary valve stenosis or partial anomalous pulmonary venous return, however, some patients do not have CHDs when *HAND2* is deleted, and others have CHDs when *HAND2* is not deleted [Huang et al., 2002]. Therefore, other genes in the region or elsewhere are likely involved in producing CHDs in these patients. We did not sequence the *HAND2* gene of the non-deleted chromosome. Examination of the *HAND2* gene region in the DGV revealed no CNVs.

Using high resolution array studies we have narrowed the critical region for CHDs in 4q deletion syndrome to an 11.6 Mb interval. The contribution of other genes in the deleted region to CHDs is unknown, and it is certainly possible that other genes proximal or distal to this deletion are also critical for heart development. However, based on the clinical phenotype of our patient and knowledge of gene function from human and animal models, it is attractive to speculate that in our patient *TLL1* loss of function correlates with ASD; that *PGDH* loss of function is implicated in the PDA; and that *HAND2* loss of function was critical in the CHDs of the right side of the heart. Our patient's CHDs could also result from interactions among the *TLL1*, *PGDH*, *HAND2* and other genes in this deleted region or downstream targets. Ongoing identification of patients with smaller deletions in this region of chromosome 4 will help to further narrow the critical region for CHDs in 4q deletion syndrome.

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