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Clinical and Genomic Characterization of Distal Duplications and Deletions of Chromosome 4q: Study of Two Cases and Review of the Literature

Michael R. Rossi^{1,2}, Miriam S. DiMaio¹, Bixia Xiang^{1,3}, Kangmo Lu¹, Hande Kaymakcalan¹, Margretta Seashore¹, Maurice J. Mahoney¹, and Peining Li^{1,*}

¹Department of Genetics, Yale School of Medicine, New Haven, Connecticut

²Department of Human Genetics, Emory University School of Medicine, Atlanta, Georgia.

³Department of Human Genetics, Miller School of Medicine, University of Miami, Miami, FL, USA.

Abstract

Variable clinical presentations of patients with chromosomally detected deletions in the distal long arm (q) of chromosome 4 have been reported. The lack of molecular characterization of the deletion sizes and deleted genes hinders further genotype-phenotype correlation. Using a validated oligonucleotide array comparative genomic hybridization (oaCGH) analysis, we examined two patient with apparent chromosomal deletions in the distal 4q region. In the first, oaCGH identified a 2.441 megabase (Mb) duplication and a 12.651 Mb deletion at 4q34.1 in a pregnant female who transmitted this aberration to her son. This mother has only learning disabilities while her son had both renal and cardiac anomalies in the newborn period. Unrecognized paternal genetic factors may contribute to the variable expression. The second patient is a 17-year-old female with a history of Pierre Robin sequence, cardiac abnormalities and learning disabilities. She was diagnosed prenatally with a de novo 4q deletion, and oaCGH defined a 16.435 Mb deletion of 4q34.1 to 4q35.2. Phenotypic comparison and subtractive genomic mapping between these two cases suggested a 4 Mb region possibly harboring a candidate gene for Pierre Robin sequence. Our cases and review of reported cases with genomic findings indicated the presence of familial variants with variable expressivity as well as de novo or inherited pathogenic simple deletion, duplication and complex deletion and duplication in the distal 4q region.

Keywords

array comparative genomic hybridization; 4q distal duplications and deletions; Pierre Robin Sequence

INTRODUCTION

The first report of a deletion of the long arm (q) of chromosome 4 predates modern G-banding techniques [Ockey et al., 1967]. With an estimated incidence of 1/100,000, there are over 100 patients with interstitial and terminal 4q deletions in the literature; a collectively termed 4q-syndrome is usually associated with a constellation of clinical findings including developmental delay (DD), mental retardation (MR), Pierre Robin sequence (PRS), and anomalies of digital, craniofacial, skeletal and extremities, cardiovascular and other systems

*Correspondence to: Peining Li, PhD, Laboratory of Molecular Cytogenetics, Department of Genetics, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06520-8005, Tel: 203-785-6317; Fax: 203-785-7342; peining.li@yale.edu.

[Strehle and Bantock, 2003]. As expected, the severity of the phenotype is correlated with the size of the deletion and ranges from individuals with only minor physical findings involving band 4q34 to more severe abnormalities noted in individuals with deletions involving 4q31 [Townes et al., 1979; Fryns et al., 1981; Mitchell et al., 1981; Lin et al., 1988; Caliebe et al., 1997; Robertson et al., 1998; Tsai et al., 1999; Keeling et al., 2001; Strehle and Bantock, 2003]. However, the lack of genomic delineation for almost all of these cytogenetically detected cases has hindered further characterization of genotype-phenotype correlations for these heterogeneous chromosomal abnormalities.

In this report, we applied genome-wide oligonucleotide array comparative genomic hybridization (oaCGH) to two cases with prenatally-detected distal deletions of chromosome 4q. The first case study involves a mother and her newborn baby, both of whom carry a novel submicroscopic duplication and a large deletion of a distal 4q. The second describes a follow-up study of a young woman who carries a deletion initiated at 4q34.1. Comparing the clinical features and genomic findings of these patients and patients reported in the literature demonstrated clinical and genomic heterogeneity associated with distal 4q deletions and duplications, and further dissected possible critical regions for DD/MR, multiple congenital anomalies (MCA), PRS, congenital heart defects (CHD), and limb or digital anomalies (LA/DA).

MATERIALS AND METHODS

Chromosome G-banding and FISH Analysis

Chromosome analysis was performed on G-band metaphases prepared from chorionic villus samples (CVS) and cultured peripheral blood lymphocytes according to the laboratory's standard protocols. Twenty metaphases were analyzed for each individual sample. Fluorescence in situ hybridization (FISH) was performed on metaphase slides using the ToTelVysion™ Mixture 4 (Vysis/Abbott, Abbott Park, Illinois) probe cocktail containing 4p, 4q and 21q subtelomeric probes. Hybridization and wash conditions followed the manufacturer's protocol, and at least five metaphase spreads were assessed for the presence of the subtelomeric region of chromosome 4q.

Oligonucleotide array comparative genomic hybridization (oaCGH)

Genomic DNA was extracted from whole blood or cultured chorionic villus samples using the Gentra Puregene kit (Qiagen, Valencia, CA). DNA concentration was measured using a NanoDrop spectrophotometer (ND-1000, Thermo Fisher Scientific Inc., Waltham, MA) and high molecular weight DNA quality was verified by agarose gel electrophoresis. For each sample, 2 µg of genomic DNA was used for oaCGH analysis following the manufacturer's protocol for the Agilent Human Genome CGH microarray 44K kit (Agilent Technologies, Inc, Santa Clara, CA). This laboratory has validated the oaCGH procedure to offer a 99% sensitivity and a 99% specificity with an average analytical resolution of 0.3–0.5 Mb using log₂ ratio from five to seven contiguous probes [Xiang et al, 2008]. All base pair designations for the Agilent 44K array, which are positioned according to the May 2004 Assembly, were converted to the most recent March 2006 Assembly using the UCSC Human Genome browser (<http://genome.ucsc.edu/>).

RESULTS

Clinical findings

Case 1—A 33-year-old pregnant mother (Pedigree II-2, Fig 1-A) with learning disabilities was referred for genetic counseling to discuss optional recessive gene screening. The mother elected to have fragile X syndrome carrier testing and peripheral blood karyotyping. She

was found to have a fragile X syndrome premutation allele of approximately 80 CGG repeats and an abnormal karyotype, 46,XX,del(4)(q34.3), on G-band metaphase analysis. Because of these findings, CVS was collected at 13 weeks gestation. The fetus (III-1) inherited his mother's normal *FMR1* allele with a CGG repeat size in the normal range (data not shown). The karyotype of cultured fibroblasts from the CVS was 46,XY,del(4)(q34.3) and appeared to be identical to the deletion found in the mother; FISH analysis confirmed a subtelomeric deletion at the del(4) chromosome (Fig 1-B). Peripheral blood karyotypes of the mother's parents (I-1 and I-2) and her husband (II-1) were normal.

The mother (II-2) who carries the del(4) chromosome had, with the exception of learning disabilities, an unremarkable medical history. Her two siblings (II-3 and II-4), neither of whom were available for testing, had no history of learning difficulties, but her brother (II-3) had complex congenital heart disease. The father (II-1) was reported to have a history of learning disabilities but otherwise an unremarkable medical history.

Prenatal ultrasound examination identified renal anomalies in the fetus. The pregnancy resulted in the term birth of a boy with Apgar scores of 9. Birth weight was 3490 g (50th centile), length was 53.5 cm (75–90th centile) and head circumference was 36.0 cm (50th centile). Postnatal examination revealed moderate left kidney pelvicaliectasis with normal corticomedullary differentiation, severe right kidney pelvicaliectasis with lack of corticomedullary differentiation and bilateral hydronephrosis with evidence of reflux. Cardiac anomalies were not observed prenatally, but echocardiography in the newborn period revealed an inlet membranous ventricular septal defect (VSD), an apical muscular VSD, a patent foramen ovale and patent ductus arteriosus (PDA). Physical exam in the neonatal period showed an unusual narrow attachment of the lobes of both pinnae to the face, mild to moderate micrognathia, and an inferior iris coloboma of the right eye. Pediatric ophthalmologists examined both eyes and found no other ocular defects, specifically no retinal or choroidal coloboma.

Case 2—This 17-year-old female was assessed at birth for PRS, micrognathia, fluttering nystagmus, and a series of cardiac findings including Ebstein anomaly and atrial septal defect (ASD). Chromosome analysis was performed prenatally, and an abnormal karyotype, 46,XX,del(4)(q34.1), involving a deletion of the distal portion of chromosome 4 was reported. Parental chromosome analyses showed normal results.

Because of respiratory compromise and unstable hemodynamic and cardiovascular status during the first few days of life, she underwent emergency tracheostomy on the third day of life. At age eight years, this patient was re-evaluated for developmental delay. The patient was under the 5th percentile for height and weight, was learning disabled and showed minor dysmorphic features including micrognathia, hypertelorism with outer canthal distance of 9cm (75–97th centile) and inner canthal distance of 3.5cm (>97th centile), short nose with abnormal bridge (Fig. 2). Other findings included clinodactyly of the left and right fifth toes, and an in-toe gait. Most recently, this patient was followed up for learning difficulties compounded by attention deficit hyperactivity disorder. She was in special education classes, and had bouts of anger and depression, suspicious of bipolar disorder. The in-toe gait was persistent, requiring braces. Primary amenorrhea and myopia were newly reported findings.

Genomic characterization of 4q duplication and deletions

For Case 1, oaCGH analysis was performed using genomic DNA samples extracted from the fetus's CVS and the parental blood samples. The chromosome 4 abnormality was further delineated as a 2.441 Mb duplication and a 12.651 Mb deletion in the mother and her fetus (Fig 1-C). The aberration was defined cytogenetically as der(4)dup(4)(q34.1q34.3)del(4)

(q34.3) and molecularly as a one copy gain of chr4:175,649,768–178,091,166 involving nine known genes from *HPGD* to *VEGFC*, and a one copy loss of chr4:178,470,008–191,121,285 containing about 40 known refseq genes from *NEIL3* to *FRG1* and one microRNA hsa-mir-1305 (chr4:183,327,440–183,327,525) at 4q35.1 (<http://genome.ucsc.edu/>). The duplication event was initiated within a gap of approximately 0.153 Mb (chr4:175,496,729–175,649,768) between probes on the Agilent 44K array. Likewise, there is a 0.378 Mb probe gap (chr4:178,091,166–178,470,008) between the duplication and deletion events. No other copy number variants (CNVs) were found in the fetus and the parents.

For Case 2, oaCGH analysis detected a 16.435 Mb deletion (chr4:174,685,919–191,121,195, from genes *HAND2* to *FRG1*) extending from 4q34.1 to the subtelomeric region of 4q35.2 (Fig. 3). There is a gap of approximately 135 kb (chr4:174,551,045–174,685,919) between probes on the Agilent 44K array, and the deletion event was initiated at some point within this interval.

The minimal region of overlap among the three individuals described in this report is a 13 Mb region (chr4:178,470,008 – 191,121,285) in Case 1. The database of genomic variants (<http://projects.tcag.ca/variation/>) describes at least 16 genes (*NEIL*, *AGA*, *ODZ3*, *ENPP6*, *IRF2*, *ANKRD37*, *CCDC110*, *FAM149A*, *ZFP42*, *TRIML2*, *TRIML1*, *FRG1*, *TUBB4Q*, *DUX4C*, *FRG2*, and *DUX4*) within this distal 4q region associated with loss of copy number CNVs. Both losses and gains of copy number CNVs were noted from genes *ZFP42* to *DUX4* (189.1 Mb to 191.2 Mb) indicating approximately 2 Mb region of benign CNV at the 4qter. The extended deletion region in Case 2 contains 12 known refseq genes (*HAND2*, *MORF4*, *FBXO8*, *HPGD*, *GLRA3*, *ADAM29*, *GPM6A*, *WDR17*, *SPATA4*, *ASB5*, *SPCS3*, and *VEGFC*), and only the *GPM6A* gene is documented as a CNV. The duplicated region (chr4:175,649,768–178,091,166) in case 1 shared nine known genes (from genes *HPGD* to *VEGFC*) as the extended region of deletion in case 2 (chr4:174,685,919–178,091,166). Collating CNV information within this region may help to refine the disease-causing candidate genes.

DISCUSSION

Approximately 14% of chromosomal 4q terminal or interstitial deletions were inherited from parental carriers of balanced translocation or unbalanced abnormalities [Strehle and Bantock, 2003]. Familial transmission of a chromosomally recognized 4q terminal deletion, del(4)(q34.2), was first reported in a mother and her two sons [Descartes et al., 1996]. Our case 1 is the second reported familial mother-to-son transmission of a G-band 4q deletion. What appeared to be a terminal 4q deletion by routine metaphase analysis is actually a novel compound duplication and deletion by oaCGH, which demonstrates the necessity of oaCGH analysis to delineate the genomic content of chromosomal rearrangements. The phenotypic variability between the mother and child raises questions of what roles haploinsufficient and triple-sensitive genes or other yet-to-be-described genetic factors play in the overall phenotype. The father had learning disabilities which could also have an unidentified genetic component that may likely contribute to his son's phenotype. Subtelomeric FISH analysis of 11,688 cases detected 4qter deletions in eight cases; two of them were inherited from normal paternal carriers and considered to be normal variants and one was transmitted from an abnormal maternal carrier [Ravnan et al., 2006]. For patients with 4q subtelomeric or chromosomal deletions, follow-up parental analysis should be considered.

A review of cases involving chromosomally detected 4q interstitial and terminal deletions proposed a classification of proximal interstitial, distal and terminal deletions [Strehle and Bantock, 2003]. Distal 4q deletions have been associated with some common features

including DD, MR, PRS, craniofacial dysmorphic features, congenital heart defects of ASD and VSD, digital anomalies (DA) of syndactyly of the 3rd and 4th digits and clinodactyly of 5th digit, and on severe cases limb anomaly (LA) of ulna aplasia and absence of ulnar rays [Keeling et al., 2001; Strehle and Bantock, 2003; Kaalund et al., 2008]. Using minimal deletion regions and subtractive comparison of phenotypes, critical regions of 4q31, 4q33 and 4q34 have been reported for most of the 4q- common characteristics [Robertson et al., 1998], for distal arm development and cleft lip and palate [Keeling et al., 2001] and for digital anomaly [Vogt et al., 2006], respectively. The outlined karyotype-phenotype association was limited by the 3–5 Mb resolution of G-banded chromosomes and should be further characterized for a better genotype-phenotype correlation.

At the time of writing, genomic findings of 12 cases of distal 4q deletions, three cases of distal 4q duplications, one case of a complex intrachromosomal 4q rearrangement, one case with a distal interstitial deletion and one case of a 4q deletion and a 20p duplication were reported; clinical features of six reported cases and our two cases were summarized in Table I [Balikova et al., 2007; Quadrelli et al., 2007; Kitsiou-Tzeli et al., 2008; Shao et al., 2008; Sensi et al., 2008; Kaalund et al., 2008]. Figure 4 shows the heterogeneous genomic abnormalities including familial variants with variable expressivity and pathogenic simple deletions and duplications, as well as complex rearrangements. An approximately 1.3 Mb 4qter deletion transmitted from a normal mother to her two mentally retarded sons was noted [Balikova et al., 2007]. Although detailed clinical information was not available, a recent report of 5,380 cases with DD/MR, and multiple congenital anomalies (MCA) using targeted BAC clone arrays with enhanced coverage of subtelomeric regions detected nine cases of simple 4qter deletion ranging from about 2–5 Mb and three cases of simple 4qter duplication of about 2 Mb [Shao et al., 2008]. Excluding a 2 Mb (189.1–191.2 Mb, from genes *ZFP42* to *DUX*) region with benign CNVs archived in the Database of Genomic Variants (<http://projects.tcag.ca/variation/>), a 3.0 Mb interval (186–189 Mb) of terminal 4qter may contain dosage-sensitive genes. Of the 18 refseq genes in this region, the *HELT* (HES/HEY-like transcription factor), *LRP2BP* (LRP2 binding protein), *SORBS2* (Sorbin and SH3 domain containing 2 isoform 2) and the *FAT* (FAT tumor suppressor 1 precursor) genes may be the candidate genes.

Our Case 2 and three reported simple deletions of 4q32.1 to 4q35.2 showed similar clinical features of DD, MR, craniofacial dysmorphisms, digital anomalies of clinodactyly and congenital heart defects (Table I). Based on the genomic findings, an 11 Mb interval (170–181 Mb) of 4q33q34.3 may harbor the candidate genes for PRS, DA and CHD (Fig. 4). More specifically, the extended 4 Mb deletion region (174–178 Mb) between our two cases could be the critical region for PRS. The *HAND2* gene at 4q34.1, which encodes basic helix-loop-helix transcription factor and functions in the development of the right ventricle and aortic arch arteries and anterior posterior polarization of the limb bud, has been implicated as mediators of congenital heart disease and limb anomalies [Srivastava, 1999; Charite et al., 2000]. Targeted deletion of *HAND2* branchial arch enhancer in mice exhibited a spectrum of craniofacial defects including cleft palate, mandibular hypoplasia and cartilage malformation [Yanagisawa et al., 2003]. Haploinsufficiency of *HAND2* in 4q deletion patients is a likely explanation for cardiac defects, craniofacial dysmorphisms and digital anomalies. However, absence of cardiac features has been noted in several cases with 4q deletions involving the *HAND2* gene [Huang et al., 2002; Vogt et al., 2006; Kaalund et al., 2008], which suggests that other genes or genetic factors could modify the phenotype. In our Case 1 with a duplication and a deletion distal to the *HAND2* gene, the absence of craniofacial and digital anomalies and the presence of cardiac defects suggested there are other candidate genes for heart defects in the 4q34.2–q35.1 region. Interestingly, there is a new microRNA, hsa-mir-1305 at 4q35.1 in this region and its role in gene regulation requires further study. Only the case of 4q terminal deletion and 20p duplication [Kaalund et

al., 2008] presented the characteristic ulnar deficiency, which indicates the requirement of other genetic factors for this severe limb defect.

In summary, our cases and recent findings demonstrated the presence of de novo and familial simple deletions and duplications, and complex intra-/interchromosome rearrangements in the distal 4q region. Genomic characterization of additional cases with 4q distal deletions and other modifying genetic and epigenetic factors should help to clarify which genes and pathways are responsible for the spectrum of 4q- syndrome phenotypes.

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Fig. 1.

A: Pedigree of the three-generation family with 4q distal duplication and deletion, fill box indicates affected subjects and arrow to the proband. **B:** Left panel shows chromosomes 4 of the proband (II-2) and her son (III-1). The deletion was designated to the distal portion of 4q34.3 and the duplication of 4q34.1q34.3 was not evident at a resolution of 550 bands. Right panel shows metaphase-FISH confirming the del(4q) in the proband using subtelomeric 4pter (green), 4qter (red) and 21qter (yellow) probes. **C:** Chromosome 4 oaCGH data for the parents (II-1 and II-2) and their child (III-1). The proband and her child carry an identical duplication/deletion of chromosome 4. Base pair designations of the copy number gain (chr4:175,649,768–178,091,166) and copy number loss (chr4:178,470,008–

191,121,285) are according to the March 2006 (hg18) human reference sequence (<http://genome.ucsc.edu/>).

Fig. 2.
Front and side view of the patient from case 2 at 8 years old.

Fig. 3.

The oaCGH analysis on Case 2 shows loss of one copy of the distal portion of chromosome 4 (chr4:174,685,919–191,121,195). A 4q deletion, del(4)(q34.1), is shown in the inset.

Fig. 4. Genomic findings of pathogenic deletions, duplications and complex rearrangements in the distal 4qter region. Critical regions associated with DD/MR/MCA and PRS/DA/CHD and possible candidate genes were shown. (DD: developmental delay, MR: mental retardation, MCA: multiple congenital anomalies, PRS: Pierre Robin sequence, DA: digital anomalies, CHD: congenital heart defects, number within parenthesis denoted number of cases in the report).

Table 1

Summary of clinical features of cases with 4q distal deletions and duplications

Case Ref	Cases with simple deletions					Cases with deletion/duplication			
	Balikova	Case 2	Kitsiou-Tzeli	Quadrelli	Kaalund-2	Case 1	Sensi	Kaalund-1	
Chr. Breakpoints	4q35.2	4q34.1	4q33	4q33	4q32.1q34.3	4q34.1/q34.3	4q32.1~q35.2	4q33	
Deletion size (Mb)	1.3	16.5	18.9–22.9	25.7	21	12.6	~9 (3 dels)	20	
Start seq. (Mb)	~189	174.6	~170	165.5	160.7	178.5	157, 171, 190	171.2	
End seq. (Mb)	~191	191.1	~191	191.2	181.7	191.1	163, 174, 191	191.2	
Duplication size (Mb)						2.5	~3	4.5 (20p)	
Start seq. (Mb)						175.6	182		
End seq. (Mb)						178.1	185		
Origin	mat	de novo	de novo	de novo	de novo	mat	de novo	de novo	
Sex	M	F	M	F	M	M	M	F	
Gestation (weeks)	Term	Term	38	38	40	Term	40	40	
Age	7y	17y	3y	4y	3y	1y	3y	9y	
DD	–	+	+	+	+	+	+	+	
MR	mild	+	+	+	mild	(LD)		+	
Craniofacial dysm.	–	+	+	+	+	+	+	+	
PRS	–	+	+	+	+	–	–	+	
Skeletal/Limb defects	–	–	–	–	+	–	–	+	
Digital anomalies	–	+	+	+	+	–	+	+	
Clinodactyly	–	+	+	+	+	–	+	+	
Cardiac anomalies	–	+	+	+	+	+	+	+	
VSD/ASD/PDA	–	+	+	+	+	+	+	+	
Renal Disorders	–	–	–	–	–	+	–	–	
Hearing loss	–	–	–	–	–	–	+	–	

Ref, reference; +, presence; – absence; DD, developmental delay; MR, mental retardation; dysm., dysmorphism; PRS, Pierre Robin Sequence; ASD, atrial septal defect; VSD, ventricular septal defect; PDA, patent ductus arteriosus; LD, learning disabilities in carrier mother

* seq. sequence mapped to chromosome 4 in Mb location of UCSC human genome browser