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Microdeletion at 4q21.3 is associated with intellectual disability, dysmorphic facies, hypotonia, and short stature

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Abstract

Chromosomal imbalances are a major cause of intellectual disability (ID) and multiple congenital anomalies. We have clinically and molecularly characterized two patients with chromosome translocations and ID. Using whole genome array CGH analysis, we identified a microdeletion involving 4q21.3, unrelated to the translocations in both patients. We confirmed the 4q21.3 microdeletions using fluorescence *in situ* hybridization and quantitative genomic PCR. The corresponding deletion boundaries in the patients were further mapped and compared to previously reported 4q21 deletions and the associated clinical features. We determined a common region of deletion overlap that appears unique to ID, short stature, hypotonia, and dysmorphic facial features.

Keywords

Intellectual disability; submicroscopic microdeletion; translocation; hypotonia; short stature; arrayCGH

INTRODUCTION

Intellectual disability (ID) is a common form of cognitive impairment affecting one to three percent of the worldwide population. Chromosomal imbalances in the form of numerical aneuploidies, microdeletions, microduplications and subtelomere rearrangements are currently a major cause of ID and multiple congenital anomalies. Many of these imbalances are caused by submicroscopic deletions or duplications not detected by conventional cytogenetic methods. Recently, array CGH analysis in patients with ID has led to the identification of submicroscopic deletions and duplications [Hochstenbach et al., 2009; McMullan et al., 2009; Sagoo et al., 2009; Bernardini et al., 2010]. However, assigning pathogenicity to deletions and duplications in patients with ID has been difficult considering

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that a significant number of copy number variants (CNVs) exist in the normal population [Shaikh et al., 2009; Fu et al., 2010; Database of Genomic Variants].

Interstitial deletions of the long arm of chromosome 4 have been reported in a number of individuals with variable clinical phenotypes [Mitchell et al., 1981; Beall et al., 1988; Abuelo et al., 1988; Fagan and Gill, 1989; Nowaczyk et al., 1997; Harada et al., 2002; Velinov et al., 2005; Jacquemont et al., 2006; Dobyns et al., 2008]. Patients with deletions involving 4q21 and 4q22 show several characteristic features such as facial dysmorphism, hypotonia, and small hands with short fingers associated with mild-severe growth and neurodevelopmental delays including ID. In a recent report, analyses of nine patients with overlapping 4q21 deletions of variable sizes (3.2Mb to 15.1Mb) further defined a critical region in 4q21.3 associated with marked growth restriction, ID, hypotonia, distinctive facial features and absent or severely delayed speech [Bonnet et al., 2010]. Additionally, two patients with 4q21.21-22.1 deletions were reported to have polymicrogyria [Dobyns et al., 2008].

Here we identified a microdeletion at 4q21.3 in two patients with ID and chromosome translocations. We mapped the 4q21.3 microdeletion in both patients and compared the extent of the deletion and the associated clinical features of the two patients with previously reported patients with 4q21 deletions. We report on a critical region at 4q21.3 that appears unique for ID, facial dysmorphism, hypotonia and short stature.

MATERIALS AND METHODS

CLINICAL

Patient 1—(CMS11752, Fig. 1A) is a 7 1/3-year-old Honduran girl who was initially evaluated at 6 2/3 years for long-standing neurodevelopmental delays. She is the first and only child born to healthy nonconsanguineous parents following a normal 42 week gestation, labor, and vaginal delivery. Birth weight was 3.0 kilograms (20th centile), and head circumference was 37 centimeters (95th centile). She exhibited hypotonia with a decreased cry and feeding problems secondary to a poor suck. No seizures were reported. The family history was negative for neurodevelopmental disabilities or other systemic illnesses.

She did not crawl until 3 1/2 years, pulled to a stand at 6 years, and currently walks with assistance. She uses both hands, can scribble with a crayon, and can finger feed. She uses a few isolated single words (“mama”, “water”) but no phrases; she exhibits good non-verbal communication. She is attempting toilet training and is described as a happy child with good behavior.

Growth measurements when last examined at 7 1/3 years revealed a head circumference of 51 centimeters (50th centile), weight of 13.6 kilograms (<5th centile, 50th centile for 34 months), and height of 98 centimeters (<5th centile, 50th centile for 42 months). She has craniofacial dysmorphism with frontal bossing, hypertelorism with downslanted palpebral fissures, short philtrum with tented upper lip, malar hypoplasia with an upturned nose/nostrils, and ears with underfolded helices (Fig. 1A). Mild thoracic kyphosis with pectus excavatum was present along with small thumbs, abnormally short second toes (Fig. 1A), and flat feet. The cardiac, pulmonary, abdominal, genitalia, and skin examinations were normal. Neurological exam revealed severe ID, pleasant behavior, decreased muscle bulk, decreased strength, moderate generalized hypotonia with an early “toddler” gait while holding on, and 3+ deep tendon reflexes in all limbs with no pathological reflexes. There were no cranial nerve, cerebellar, or sensory deficits.

Routine high-resolution chromosome analysis in the patient showed a 46,XX,t(4;16)(q21.3;q24) karyotype. Both parents were seen and are clinically normal and had normal karyotypes (mother: 46,XX and father: 46,XY), indicating the *de novo* nature of the chromosome translocation in the patient.

The patient had normal subtelomeres by MLPA, creatine kinase, plasma amino acids, and transferrin isoelectric pattern. The EEG and brain CT scan were normal.

Patient 2—(CMS3307, Fig. 1B) is a 44-year-old female with profound ID, scoliosis, brachydactyly, proportionate short stature, and small hands and feet with short toes. She is one of seven children born to her mother. Pregnancy was complicated by toxemia in the third trimester and by an unspecified kidney problem. She was small for her term gestational age with a birth weight of 2.3 kilograms. Her mother initially noticed a “weak cry” and began noticing delayed neurodevelopmental milestones by 6 months of age. She was nonverbal and did not sit independently until 4 years of age.

Physical examination at the age of 37 years revealed her head circumference to be 55 cm (60th centile). Her adult height had been recorded in the past at 140.33 centimeters (<5th centile) and her weight 42.55 kilograms (<5th centile). She was wheelchair-bound. She was still nonverbal, quite pleasant, and exhibited some episodic laughter. She appeared to have mild dysmorphic facial features with a broad nose and full lips. Her teeth were widely spaced with thickened alveolar ridges (Fig. 1B). Her palpebral fissures were slightly down-slanted, and she had a short philtrum. Her ears were mildly posteriorly rotated. Her neck was short. She had small hands and very small feet with very short toes and small nail beds (Fig. 1B).

She had normal EEG and thyroid studies. Routine high-resolution chromosome analysis in the patient showed an apparently balanced translocation t(6;10)(q22.1q23.2). Both parents were reportedly clinically normal. The mother had a normal karyotype (46,XX). The father's blood sample was not available.

Molecular Cytogenetics

Cytogenetic analysis was performed essentially as described previously [Bhalla et al 2008; Griggs et al., 2009]. In brief, genomic contigs, clones, markers, mapping, and sequence information were obtained from the NCBI databases (<http://www.ncbi.nlm.nih.gov/genome/guide/human>). BAC and PAC clones were obtained from commercial resources and DNA was isolated using Qiagen Mini-Prep columns, labeled by incorporation of digoxigenin-11-dUTP or biotin-16-dUTP by nick translation using DNA polymerase. For *in situ* hybridization, metaphase chromosome spreads were obtained from lymphoblastoid cells using conventional methods and fluorescence *in situ* hybridization (FISH) was performed. Commercially available chromosome-specific labeled centromeric alphoid probes were used to identify specific chromosomes.

Whole Genome Array CGH and Quantitative PCR

Genomic DNA was purified using the Zymo DNA Clean & Concentrator™ kit (ZymoResearch, Orange, California). DNA concentration and purity were determined with a ND-1000 Spectrophotometer (NanoDrop Technologies, Delaware). DNA was labeled, hybridized to the Affymetrix GeneChip® Human Mapping 250K Nsp Array, and washed according to the manufacturer's commercial protocol (Affymetrix). Data quality assessment was generated using the DM (Dynamic Modeling) calling algorithm within the Affymetrix GTYPE software package. Intensity information and copy number status for each probe set was extracted using Affymetrix Genotyping Console™ Version 2.0 software.

Genomic quantitative PCR was performed using iQ SYBR Green Supermix (Bio-Rad) on an iCycler iQ real-time PCR detection system (Bio-Rad) as described in Griggs et al. [2009].

Multiplex PCR for Detection of Relative Dosage

Three compatible reference primer sets, with Cy5 –labeled forward primers and unlabeled reverse primers, in the X-linked dystrophin (DMD) gene (Chamberlain et al., 1988) and a Cy5 labeled forward primer and unlabeled reverse primer for the experimental genes were used in the PCR mix at a final concentration of 0.25 μ M each, with 250 μ M dNTPs, 0.75 M betaine, 1X GoTaq buffer, and 2.5 units GoTaq DNA polymerase (Promega). Ten nanograms of DNA were used for amplification following the PCR conditions: an initial denaturation step at 94°C for 300 s and 25 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 45 s, extension at 72°C for 120 s and a final extension step at 72°C for 300 s. Each sample was analyzed by an automated capillary electrophoresis instrument (Beckman CEQ) that separates and roughly quantifies the amplicons relative to each other and generates peak area measures (relative fluorescence units X mm) for each of the amplicons in a sample. The data were compiled in an Excel spreadsheet. The reference peak area values were averaged and the peak area for the experimental amplicon was divided by the average and expressed as a ratio. To express the data as copy number, the calculated ratio for each sample was multiplied by 2. The product was then divided by the gender-matched control ratio for the amplicon.

RESULTS AND DISCUSSION

Mapping of the t(4;16) Chromosome Translocation and Identification of a Deletion at 4q21 in Patient 1

Using FISH, we localized the 16q24 breakpoint in Patient 1 to within the clones RP5-991G20 and RP11-77K12 (data not shown). Clone RP11-328J14 mapped proximal and several clones including clone RP11-105F24, RP11-281J19 mapped distal to the 16q24 breakpoint. Several clones from the 4q21.3 chromosomal band were used as FISH probes. The 4q breakpoint was mapped distal to the clone RP11-341K3 (Fig. 2).

However, FISH analysis with additional clones revealed an apparent deletion at 4q21.3. Clones RP11-338B23, RP11-57B24, RP11-722P15 and RP11-147K21 gave a signal on only the normal chromosome 4 but not on the derivative chromosome 4 or the derivative chromosome 16 (Figs. 2, 3A, 3B and data not shown). The map location of FISH signals suggested that the deletion was likely to be not present at the translocation breakpoint but distal to the breakpoint in 4q21.3 since at least one clone (RP11-341K3) (Fig. 2) mapped distal to the 4q translocation breakpoint and located centromeric to the most proximal deleted clone RP11-338B23 (Fig. 3B). Furthermore, clone RP11-8N8 which is located distal to the 4q breakpoint (Fig. 3C), mapped telomeric to the most distal deleted clone RP11-147K21.

Mapping of the Chromosome t(6;10) Translocation Breakpoints in Patient 2

With Patient 2, we used a set of BAC/PAC clones spaced across chromosomal band 10q23 as FISH probes and mapped the breakpoint at 10q23.3 to a single BAC clone (RP11-94M14). This clone gave a signal on the normal chromosome 10 and the derivative chromosome 6 (data not shown). Clones RP11-411A19, RP11-4L15, RP11-165M8, RP11-399O19, RP11-80H5 and RP11-477D14 mapped proximal and clones RP11-30G13, RP11-103A2, RP11-152G7, RP11-360G10 and RP11-71F8 mapped distal to the 10q23 breakpoint. No gene was found to be disrupted at the 10q23.3 breakpoint.

Similarly using clones from the chromosomal band 6q22, we mapped the chromosome 6q breakpoint between clones RP11-231A18 and RP1-238J17. Three clones (RP11-505O14, RP11-510G24, RP11-447O8), located further telomeric to the most proximal probe (RP11-231A18), were found to be deleted in Patient 2 and gave a signal on the normal chromosome 6 but not on the derivative 6 nor the derivative 10. The identified deletion at the 6q22 breakpoint was found to be similar to a reported CNV (Variation_3633, Variation_7519, DGVS). GTG-banding of the patient's normal and derivative chromosomes 6 and 10 (Fig. 3D) and FISH results (Fig. 3E) are shown for clone RP11-390H10 which mapped proximal and clone RP11-231N13 which mapped distal to the 6q breakpoint, and clone RP11-447O8 which was found to be deleted.

Array CGH Analysis in Patients 1 and 2

We analyzed lymphocyte DNA from Patient 1 and Patient 2 using the Human Mapping 250K Nsp Array. The array CGH analysis in Patient 1 further confirmed presence of the microdeletion identified using FISH analysis (Fig. 2). In Patient 2, we identified a novel microdeletion at 4q21.2-q23 that overlapped with the 4q microdeletion in Patient 1 (Fig. 2). We looked at the log2ratio and corresponding negativeLog10Pvalues and determined the extent of the deletion in each case.

The array CGH data and subsequent *in silico* analyses provided tentative boundaries for each microdeletion and a list of genes deleted in each case (Fig. 2). A copy number loss of approximately 5.1 Mb between positions 81,055,150 – 86,169,140 (Hg18/NCBI Build 36) in Patient 1 and of approximately 5.9 Mb between positions 81,658,220 – 87,569,120 (Hg18/NCBI Build 36) in Patient 2 were determined. Array CGH analysis also confirmed the polymorphic deletion CNV at 6q22, identified by FISH analysis, in Patient 2. Additionally, we also identified a copy number loss of approximately 0.89 Mb on chromosome 7q31.33 between positions 124,960,000 – 125,768,400. This region contains two previously known CNVs and contains no genes.

We further verified the deletions at 4q21.3 using a recently devised multiplex PCR-based relative dosage analysis for detection of copy number variations (see Materials and Methods) and DNA isolated from patient's blood (Fig. 4). We used three amplicons (*RASGEF1B*, *SEC31A*, *ARHGAP24*) and observed deletion of the *RASGEF1B* and *SEC31A* amplicons in both patients. However, the *ARHGAP24* amplicon was only deleted in Patient 2 (Fig. 4 and data not shown). The results were further verified using real time PCR of genomic DNA (data not shown). Furthermore, the deletion in Patient 1 was found to be *de novo* as neither parent carried the 4q21.3 microdeletion (Fig. 4).

The deletion region at 4q contains multiple annotated genes (Fig. 2), any one of which could possibly be responsible for the neurodevelopmental defects and other observed features in these two patients.

Delineation of the 4q21.2 Microdeletion Region Apparently Associated with Intellectual disability, Dysmorphic Facies, Hypotonia and Short Stature

Figure 2 and Table I show the deletion region and clinical features in case 1 and case 2 and five previously reported cases with the 4q21 deletion [Harada et al., 2002; Velinov et al., 2005; Dobyns et al., 2008; Bonnet et al., 2010]. Most previously reported 4q deletions are relatively larger than the 4q21.3 deletions in case 1 and case 2 [Fig. 2 and Harada et al., 2002; Velinov et al., 2005; Dobyns et al., 2008]. The patients with the 4q21.3 deletions shown in Figure 2 have a small region of overlap of approximately 4.0 Mb. All have severe ID or neurodevelopmental delay, no speech or severely delayed speech, facial dysmorphism with prominent forehead, hypertelorism and pointed chin giving a triangular facial

appearance, hypotonia, and short stature (Table I). However, very recently deletion analyses in nine patients with 4q21.1–4q21.3 deletions further revealed a smaller region of overlap of 1.37 Mb [Bonnet et al., 2010]. Thus, our findings further complement the findings in previously published reports and strengthen the notion that the 4q21.3 region is associated with ID, dysmorphic features, hypotonia and short stature. These key characteristic clinical features likely result from deletion of a gene or multiple genes located in the region of overlap (Fig. 2). Some of the notable candidate genes for some of the common clinical features in these patients are *PRKG2*, *RASGEF1B*, *SEC31A*, *CCDC98* and *WDFY13*. *RASGEF1B* appears to be a strong candidate for the neurodevelopmental features in these patients as it is present within the minimal region shared by all 4q21.3 deletions (Fig. 2). The *ARHGAP24* gene appears to be a good candidate gene for brain disorders. However, this gene is located at the telomeric end of the critical deletion and is not deleted in Patient 1. Deletion screening of patients with key clinical features associated with the 4q21.3 deletion will further determine if the 4q21.3 deletion is recurrent in patients with ID and may further refine the deletion region associated with ID. Furthermore, re-sequencing of genes from the minimal deletion region would determine if a recessive mutation is unmasked by the deletion.

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Figure 1. Patient 1 (A) and Patient 2 (B) exhibited normocephaly with dysmorphic facial features including frontal bossing, hypertelorism, downslanted palpebral fissures, and a short philtrum. Patient 1 (A) had short second toes. Patient 2 (B) had small feet with all toes shortened and small nail beds.

Figure 2. Array CGH profiles of chromosome 4 in Patient 1 and Patient 2 and a schematic representation of chromosome 4q21.3 showing the 4q21.3 deletions (indicated by black bars). The log2ratio of about -0.5 indicates the deletion is heterozygous. Physically mapped BAC clones used in FISH analysis of chromosome 4 are indicated by gray bars. A representative gene content of the 4q21.3 deletion and known copy number variants are shown. Arrows indicate direction of transcription. Genes used for quantitative genomic PCR are indicated in bold types. Overview of the deletions in Patient 1 and Patient 2 described in this report and five previously reported deletions involving the 4q21.3 region [JP patient, Harada et al., 2002; Patients LRA04-022a2 and LR07-256, Dobyns et al., 2008; Patients 1 and 8, Bonnet et al., 2010]. Shaded area indicates the smallest region of overlap.

A

B

C

Figure 3.

(A) GTG- banding of the normal and derivative chromosomes 4 and 16 from Patient 1. (B, C) Metaphase spread from Patient 1 with the t(4;16) translocation showing FISH signals obtained with clones RP11-383B23 (green) and RP11-8N8 (green), with a chromosome 4 centromere-specific alphoid sequence probe (red). Probe RP11-383B23 produced a green signal only on one chromosome 4 but not the other, indicating deletion of this probe in Patient 1. Probe RP11-8N8 mapped distal to the translocation breakpoint and gave signals on the normal chromosome 4 and the derivative chromosome 16. A red signal was present on both the normal and the derivative chromosomes 4. (D) GTG- banding of the normal and derivative chromosomes 6 and 10 from Patient 2. (E) FISH analyses in Patient 2 with the t(6;10) translocation. Probe RP11-390H10 (green) is proximal to the 6q22 translocation

breakpoint. The probe is present on the normal chromosome 6 and the derivative chromosome 6. Probe RP11-447O8 (green) is deleted and showed a signal only on the normal chromosome 6. Probe RP11-231N13 mapped distal to the 6q22 breakpoint and gave signals on the normal chromosome 6 and the derivative chromosome 10. Probe signals are indicated by thick arrows

Figure 4.

Copy number determination for *SEC31A* and *ARHGAP24* using the multiplex PCR-based dosage analysis for detection of copy number variation. DNA samples from a normal female (CMS13914), a normal male (CMS3351a), Patient 2, Patient 1 and her parents (CMS11751 and CMS12130) were studied. Samples were analyzed by automated capillary electrophoresis (Beckman CEQ) and the peak value data was processed and the copy number was calculated as described in Materials and Methods.

Table 1

Clinical features of patients with 4q21.3 deletion.

Patient	Case 1 (This study)	Case 2 (This study)	JP patient [Harada et al., 2002]	LR04-022a2 [Dobyns et al., 2008]	LR07-256 [Dobyns et al., 2008]	Patient 1 [Bonnet et al., 2010]	Patient 8 [Bonnet et al., 2010]
Deletion	del(4)(q21.2;q21.3)	del(4)(q21.2;q21.3)	del(4)(q21.1;q22.2)	del(4)(q21.2;q22.1)	del(4)(q21.2;q23)	del(4)(q21.1;q21.22)	del(4)(q21.2;q21.23)
Age & sex	6-yr-old female	37-year-old female	6-year-old female	4.5-year-old male	8-months-old male	14-month female	14-year-old female
Neuro-developmental	Severe ID	Profound ID	Severe dev delay	Severe dev delay, ID	Severe dev delay	Severe dev delay	Severe dev delay, ID
	Speech delay with few isolated words	Non verbal					Few words at 5 yr
	Hypotonia	Spasticity	Muscle hypotonia	Mild spasticity	Hypotonia	Hypotonia	Hypotonia
				Probable ataxia			
	No seizures	No data	No data	Seizures	No data	No data	No data
Skeletal	Short stature	Short stature	Short stature	No data	No data	Normal length	Short stature
	Small thumbs, short 2nd toes	Small hands and feet, Brachydactyly, short toes				Small hands and feet, Brachydactyly	Small hands and feet, Brachymetatarsy
	Thoracic kyphosis with pectus excavatum	Contractures and scoliosis				Short neck Short limbs	
Craniofacial	Facial dysmorphism	Mild facial dysmorphism	Facial dysmorphism	Facial dysmorphism	Facial dysmorphism	Facial dysmorphism	Facial dysmorphism
	Frontal bossing	Mild frontal bossing		Prominent forehead		Broad frontal bossing	Broad frontal bossing
	Hypertelorism		Hypertelorism			Hypertelorism	Hypertelorism
	Short philtrum, tented upper lip	Short philtrum	Long philtrum, small mouth	Flat philtrum		Long philtrum, thin lips, downturned mouth	Short philtrum
	Underfolded helices with attached lobes	Ears posteriorly rotated		Low-set posteriorly rotated ears		Small ear canals	
			Pointed chin	Pointed chin			
			Triangular facial appearance	Triangular facial appearance			
	No data	Widely spaced teeth	Lower teeth defect	No data	No data	No data	No data
	Normocephaly	Normocephaly	Dolicocephaly	Normocephaly VP shunt	Normocephaly	Normocephaly	Normocephaly
Hearing	No data	No data	Bilateral deafness	No data	Conductive hearing loss	Congenital deafness	No data
Cranial imaging	Normal brain CT	No data	No data	Polymicrogyria and cerebellar vermis hypoplasia on MRI	Polymicrogyria and cerebellar vermis hypoplasia on MRI	Hypoplasia frontal cortex and cerebellar vermis on MRI	Cerebral hypoplasia with dilated ventricles on CT