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Short Report

Interstitial deletions of chromosome 6q: genotype–phenotype correlation utilizing array CGH

Klein OD, Cotter PD, Moore MW, Zanko A, Gilats M, Epstein CJ, Conte F, Rauen KA. Interstitial deletions of chromosome 6q: genotype–phenotype correlation utilizing array CGH.
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Interstitial deletions of the long arm of chromosome 6 are relatively rare, with fewer than 100 cases reported. Phenotypic variation is in large part due to differences in size and location of the segmental aneuploidy. We report three new patients with interstitial deletions of chromosome 6q defined at the molecular level by array comparative genomic hybridization (array CGH). In two of three cases, the molecular breakpoints differed from those indicated by conventional karyotyping, demonstrating the enhanced resolution of array CGH. Two patients had minimal deletions of 6 and 8.8 Mb involving 6q16.2→q21, and the third patient had a deletion of 11.3 Mb spanning 6q15→q21. All three had developmental delay, craniofacial dysmorphology, and functional eye disorders, suggesting that genes affecting brain and craniofacial development are located in 6q16.2→q21, the deleted region common to all three patients. Furthermore, gene(s) for discordant phenotypic features, such as central diabetes insipidus, may reside at 6q15, the monosomic region unique to patient 3. All three cases described here showed loss of paternal alleles within the deleted segment, providing further evidence of the predominantly paternal origin for 6q deletions and rearrangements.

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Key words: array CGH – array comparative genomic hybridization – chromosome 6q – genotype-phenotype correlation – interstitial deletion – Prader-Willi syndrome – *SIM1* gene

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Deletions of the long arm of chromosome 6 are relatively rare, with fewer than 100 cases reported. Phenotypic variation is due to differences in size and location of the segmental aneuploidy. Three phenotypic groups associated with 6q deletions were proposed based on conventional karyotypes: del(6)(q11→q16) patients have a high incidence of upslanting palpebral fissures, and thin lips with occasional microcephaly, micrognathia, cardiac anomalies, and umbilical or inguinal hernias; del(6)(q15→q25) patients have hypertelorism, intrauterine growth retardation, abnormal respiration, and upper limb malformations; and del(6)(q25→qter) cases have retinal abnormalities, cleft palate, and genital hypoplasia (1). The vast majority of patients with 6q deletions have mental retardation, ear anomalies, hypotonia, and postnatal growth retardation (1). Additionally, several cases with interstitial deletions of 6q have shown some features of the Prader-Willi syndrome (PWS), including hypotonia, obesity, and developmental delay, perhaps because of deletion of the *SIM1* gene at 6q16.2 (2, 3).

In this report, we present three new cases with interstitial deletions of 6q. Aside from one recently published case of a deletion of band 6q16 (4), all of the previous cytogenetic studies on 6q

deletion patients have been performed using conventional techniques, which have limited resolution. We present a case series of interstitial 6q deletions analyzed by array comparative genomic hybridization (array CGH), a method that allows for high-resolution analysis of chromosomal aneuploidy and, therefore, enables improved genotype–phenotype correlation.

Subjects and methods

Patient 1

The proband was the second child born to non-consanguineous 29-year-old parents. The birth weight was 2.8 kg (5th–10th percentile), length was 50.8 cm (50th percentile), and head circumference was 32.5 cm (third percentile). Evaluation at 4 months showed alternating esotropia and hypermetropia and by 7 months there were fine and gross motor delays. At 10 months, the patient's weight and head circumference were at the fifth percentile and his length at the 10th percentile. Phenotypic features included bilateral epicanthal folds, low-set and laterally protruding ears, a simple right helix, short, upturned nose, umbilical hernia, and central hypotonia. At 1 year, his head circumference had fallen more than two standard deviations below the fifth percentile.

The proband did not walk until 26 months, and at 5 years did not speak. Magnetic resonance imaging (MRI) at 4 years showed delayed myelination, and an electroencephalogram at 5 years demonstrated background slowing but no epileptiform activity. At the age of 11, his height was 144 cm (50th percentile), his weight was 46 kg (75th–90th percentile), and he had basic speech. Examination showed a myopathic expression, bitemporal narrowing, brachycephaly, hypotelorism with supraorbital fullness, downslanting palpebral fissures, small, low-set ears, malar hypoplasia, and tented lips (Fig. 1), as well as fifth finger clinodactyly and feet with shortened fourth rays, two-three toe syndactyly, and a wide gap between the first and second toes. The patient had gynecomastia and keratosis pilaris on his upper arms (Table 1).

Patient 2

The proband was the first male child born to non-consanguineous parents. The mother was 37 years old and the father was 30 years old. The birth weight was 3.1 kg (25th percentile); birth head circumference and length were not available. The patient had dysmorphic features,

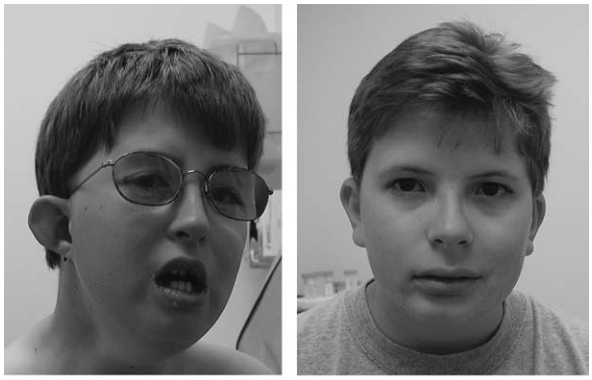

Fig. 1. Photographs of patients 1 and 3. (Patient 2 declined photography.)

including low-set ears with over-folded helices. At 3 months, the patient had normal height and weight at the 50th percentile and head circumference at the 25th percentile. Craniofacial dysmorphia consisted of brachycephaly, a broad forehead, bulbous nasal tip, and mild retrognathia. The ears were low-set with a flat superior helix and simple helices. There was a hemangioma over the posterior fontanel. At 4 months of life, he had poor head control and decreased tone. The patient had strabismus and nystagmus requiring surgical repair (Table 1), and he also had retinitis pigmentosa, as did an otherwise normal younger brother.

At 6.5 years of age, weight was 17.7 kg (10th percentile) and height was 1.1 m (<5th percentile). In addition to the dysmorphia mentioned above, the patient had bitemporal narrowing, epicanthal folds, downslanting palpebral fissures, and a thin upper lip. Hands and feet were normal. There was no evidence of scoliosis, and no brain, chest, or abdominal imaging had been performed. The patient had mild developmental delay.

Patient 3

The proband was the second male child born to healthy non-consanguineous parents. Birth weight was 3.950 kg (95th percentile) and length was 53 cm (75th–90th percentile). In the neonatal period, the patient was irritable and had sucking difficulties, and hypotonia was noted at approximately 4 months of age and he later developed global delay in psychomotor skills and language. MRIs done at ages 15 months, 9 and 12 years revealed no relevant findings.

Physical examination at age 13 showed a head circumference >95%, hypertelorism, epicanthic folds, broad nasal bridge, low-set ears, and mild retro- and micrognathia (Fig. 1). He had fifth

Table 1. Summary of clinical and cytogenetic characteristics of three cases of interstitial 6q deletions

Patient	Patient 1	Patient 2	Patient 3	Patient 4 (Le Caignec et al.)
Sex	M	M	M	F
IUGR	+	−	−	−
Weight at birth	2.8 kg (5–10%)	3.1 kg (25%)	3.9 kg (90%)	2.5 kg (5%)
Height at birth	50.8 cm (50%)	N.A.	53.3 cm (90%)	49 cm (25–50%)
HC at birth	32.5 cm (3%)	N.A.	N.A.	N.A.
Brachycephaly	+	+	−	−
Microcephaly	+	−	−	−
IPD	Hypoteloric	Normal	Hyperteloric	Hyperteloric
Epicanthal folds	−	+	Mild	N.A.
Downslanting PF	+	+	−	−
Ears abnormal/low	+/+	+/+	−/+	+/−
Nose	Long narrow	Wide mid-section	High bridge	Upturned
Microretrognathia	+	+	+	+
Thin upper lip	−	+	−	−
Lips	Full/tented upper	Thin upper	Full	Everted lower
Bitemporal narrowing	Mild	+	+	−
Fifth finger clinodactyly	+	−	+	N.A.
Foot anomalies	Shortened fourth ray	−	N.A.	N.A.
Toes	Wide gap between 1/2 and 2/3 syndactyly	−	Wide gap between 1/2 and second toe clinodactyly	N.A.
Diabetes insipidus	−	−	+	−
Increased wt:ht ratio/ gynecomastia	+	−	+	N.A.
Wide spaced nipples	+	−	+	N.A.
Renal/GU anomalies	Normal renal US	N.A.	Post-urethral valve	N.A.
Cardiac anomalies	Clinically normal	Clinically normal	Clinically normal	N.A.
Eye/vision anomalies	Esotropia/visual maturation delay	Strabismus	Myopia	Strabismus/ hypermetropia
Psychiatric disorder	−	Increased oral sensory sensitivity	ADHD/OCD	N.A.
Neurologic	−	−	Bowel/bladder control	N.A.
Skin	Keratosis pilaris	Hemangiomas	−	N.A.
Scoliosis	Mild	−	+	N.A.
Urinary bladder atonia	−	−	+	N.A.
Developmental delay	+	+	+	+
MRI results	Delayed myelination	N.A.	Brain normal	Brain normal
Hypotonia	+	+ (neonatal)	+	+
Small stature	+	+	−	−
Conventional karyotype	46,XY,del(6)(q16.2q21)	46,XY,del(6)(q15q16.2)	46,XY,del(6)(q15q16.2)	N.A.
Array deletion	6q16.2→6q21	6q16.2→6q21	6q15→6q21	6q15→6q21
6q Breakpoint: proximal non-deleted flanking clone	92,090,255 bp	92,090,255 bp	89,816,141 bp	91,352,279 bp
6q Breakpoint: distal non-deleted flanking clone	108,554,969 bp	108,282,370 bp	105,560,195 bp	107,058,135 bp
Deletion size range (bp)	8.8–16.4 Mb	6–16.2 Mb	11.3–15.7 Mb	12.9–15.7 Mb

ADHD, attention deficit/hyperactivity disorder; IPD, intrapupillary distance; IUGR, intrauterine growth retardation; N.A., not available; OCD, obsessive–compulsive disorder; PF, palpebral fissure; MRI, magnetic resonance imaging; GU, genitourinary; US, ultrasound.

finger clinodactyly bilaterally, and a wide gap between the first and second toes with second toe clinodactyly. He also had myopia and truncal obesity.

The patient had frequent ear infections in early childhood, bilateral foot contractures, and central diabetes insipidus. He had attention deficit/hyperactivity disorder and mild obsessive–compulsive disorder requiring medication (Table 1).

Cytogenetic and parental origin analyses

Cytogenetic analysis and GTG-banding were repeated on all three patients using standard

techniques on metaphases from peripheral blood lymphocytes.

Genotyping of the probands and parents was performed using chromosome 6 sequence tagged site (STS) markers (Sigma-Proligo, Boulder, CO) (Table 2) within the region of deletion. PCR amplification was performed using standard procedures and amplicons were sized using capillary electrophoresis (CEQ2000XL analyzer; Beckman Coulter, Fullerton, CA).

Array CGH analysis

Array CGH analysis was performed using a microarray consisting of 2464 BAC, PAC, and P1 clones printed in triplicate (HumArray2.0) as previously described (5, 6).

Results

Karyotype analysis demonstrated the following abnormal karyotypes in the three patients: patient 1, 46,XY,del(6)(q16.2q21), patient 2, 46,XY,del(6)(q15q16.2), and patient 3, 46,XY, del(6)(q15q16.2) (Fig. 2). Parental karyotypes were normal. Genotyping of the probands and their parents with chromosome 6 STS markers showed that the deletion in each case was on the paternally derived chromosome 6 (Table 2).

By array CGH analysis (Fig. 3a–c), patient 1 had a single loss in copy number of clones on chromosome 6q that was represented by five BAC clones with a $\log_2$ ratio $= -0.88 \pm 0.03$. The mean ratio is slightly higher than the ideal $\log_2$ ratio of -1 for reasons discussed by Snijders et al. (5). Patient 2 also exhibited single copy loss of clones on chromosome 6q as represented by four BAC clones with a $\log_2$ ratio $= -0.79 \pm 0.11$. Patients 1 and 2 had similar proximal breakpoints that lie between the same two genomic clones annotated to cytogenetic bands 6q15 (RP11-113K7 non-deleted) and 6q16.2 (RP11-14G17 deleted). The distal molecular breakpoints of patients 1 and 2 were both within cytogenetic band 6q21 but between discordant clones (Table 1). Patient 3 had a larger deleted region on 6q as demonstrated by six BAC clones with a $\log_2$ ratio $= -0.72 \pm 0.15$. The proximal breakpoints were between two genomic clones annotated to 6q15 (RP11-52B15 non-deleted and RP11-13I21 deleted), and distal breakpoints between two clones annotated to 6q16.3 (RP11-73D20 deleted) and 6q21 (RP11-47E20 non-deleted). Based on the sequence positions, the minimum and maximum deletion size ranges were as follows: patient 1, 8.8–16.4 Mb; patient 2, 6–16.2 Mb; and patient 3 11.3–15.7 Mb. No other causal copy number alterations were detected. Based on the array analysis, the 6q breakpoints were refined at the molecular level as follows: patient 1 del(6)(q16.2q21), patient 2 del(6)(q16.2q21), and patient 3 del(6)(q15q21) (Fig. 3).

Table 2. Genotype analyses of the families identify the parental origin of the deletion.

	D6S1565 6q16.2	D6S1543 6q16.3	D6S283 6q16.3
Patient 1	272	109	259
Father	268	107,111	259,286
Mother	272	109,111	259,266
Conclusion	Paternal deletion	Paternal deletion	Uninformative
Patient 2	271	109	255
Father	267,269	107,111	255,264
Mother	271	109,111	255
Conclusion	Paternal deletion	Paternal deletion	Uninformative
Patient 3	270	110	258
Father	268	100	254
Mother	270	110	264,258
Conclusion	Paternal deletion	Paternal deletion	Paternal deletion

Discussion

We present three patients with interstitial 6q deletions, the first such case series to be analyzed by array CGH. The probands presented here, and the recently reported case (4), had developmental, ear and eye abnormalities (Table 1), suggesting genes that affect brain and craniofacial development in 6q16.2→q21, the deleted region common to the patients.

All four of the patients with similar deletions by array CGH – the three analyzed here and the recently reported case (4) – had developmental delay. One of our patients had delayed myelination on head MRI and two others had normal MRIs, indicating that haploinsufficiency for genes in the deleted regions causes defects in central nervous system (CNS) function rather than major structural defects in the brain. Candidate genes important for CNS development in the common 6q deleted region include the genes *EphA7* and *GRIK2*. *EphA7* encodes a member of the ephrin family of molecules that have been implicated in mediating developmental events, particularly in the nervous system (7). *GRIK2* encodes a glutamate receptor that, in mouse, plays a role in the induction of long-term potentiation in the hippocampus (8). Other CNS

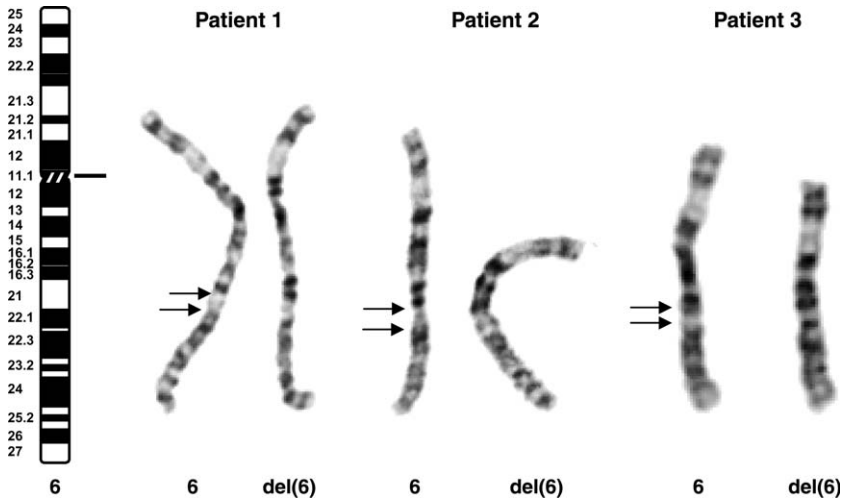

Fig. 2. Partial karyotypes and ideogram of the normal and deleted chromosomes 6 from the probands. The extent of the deletions, in each case, is indicated by the arrows.

findings common to all four patients were eye anomalies of various types, and all four patients were hypotonic.

The only common craniofacial anomalies in the four patients were abnormal or low-set ears and microretrognathia, but there were several other findings that were present in two or more of the patients, such as hypertelorism and bitemporal narrowing. The disparities in the craniofacial and other phenotypes among the four patients could be caused by differences in the deletions between these patients, or by differences in genetic background. Patients with aneuploidy often demonstrate variability in their clinical presentations, and genotype–phenotype correlations can be improved as increasing numbers of patients with overlapping deletions are analyzed (9).

Patients 1 and 3 had increased adiposity, hypotonia, and developmental delay, which have previously been described in several Prader-Willi-like patients with interstitial 6q deletions (2, 3, 10–12). The previously reported patients had, like our patients, obesity, developmental delay, and hypotonia. Also like our patients 1 and 3, those patients did not have the typical craniofacial findings of PWS, such as almond-shaped eyes and a tented upper lip. This unusual phenotype has been proposed to result from deletion of the *SIM1* gene at 6q16.3 (2). The mouse *Sim1* gene ortholog is important in the development of the hypothalamus, which is involved in appetite control (13). Interestingly, patient 2 did not have PWS-like features, which may indicate that this phenotype is not completely penetrant or that there are environmental or genetic modifiers. This point is further supported by the recently reported patient with a 6q15→21 deletion analyzed on a CGH microarray, who also had a deletion in *SIM1* but not a Prader-Willi phenotype (4). This patient had some autistic features, strabismus, hypermetropia, and craniofacial dysmorphology including hypertelorism, everted lower lip, abnormal dentition, and posteriorly rotated ears.

Patient 3 had a more proximal breakpoint than the other two patients, and thus had a unique region of deletion that may contain candidate genes for phenotypes specific to this patient. Patient 3 had central diabetes insipidus, and genes for this condition may reside at 6q15, the monosomic region unique to this individual. He also had psychiatric diagnoses, and interestingly, uniquely among the three cases, he was haploinsufficient for the *rho2* gene at 6q15. This gene encodes a γ-aminobutyric acid receptor which is expressed in the developing brain and is thought to be important for CNS function (14, 15).

It is notable that all three of our patients had paternally inherited deletions. Examination of several large series of patients with *de novo* structural rearrangements has shown that up to 85% of these cases occur as a result of deletions in the paternal germline (16–18). Of the various rearrangements, the most pronounced paternal excess was seen with interstitial deletions (18), and this finding is supported by our data. A recent report showed that in three fetuses with 6q deletions, all had paternal origins (19). The mechanisms underlying this paternal bias are not yet clear but it has been suggested that the excess of paternal errors may reflect an increased occurrence of rearrangements during pre-meiotic divisions in germ cells. There are many more pre-meiotic divisions in the male than in the female germline, and thus sperm, which are constantly generated throughout life, are more exposed to environmental mutagens (17).

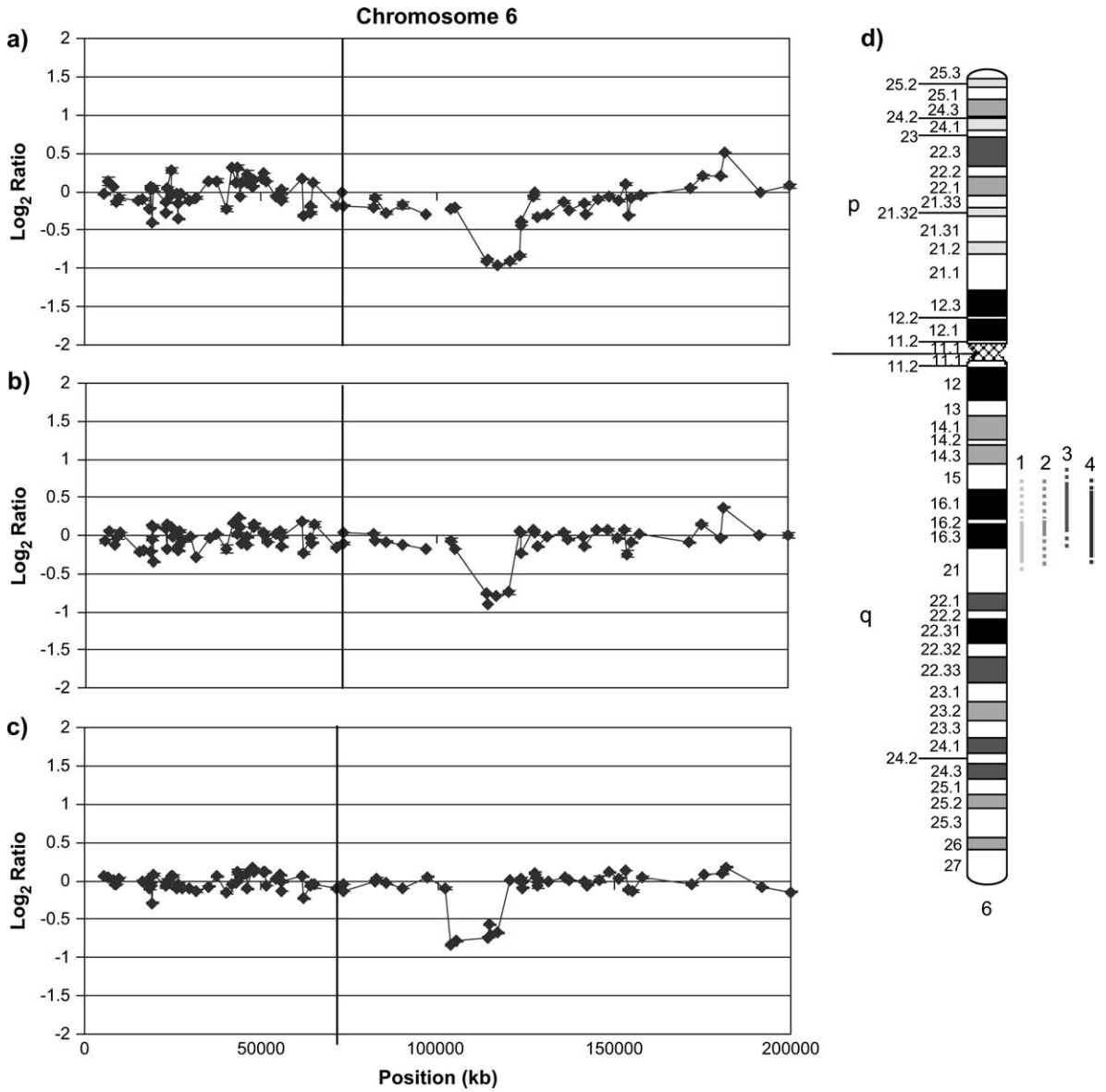

Fig. 3. **(a–c)** Array CGH analysis of patients 1, 2, and 3. Microarray analysis of the three patients demonstrated interstitial deletions of the long arm of one copy of chromosome 6. Patient 1 (panel **a**) had proximal breakpoints between the genomic clones annotated to cytogenetic bands 6q15 (RP11-113K7 non-deleted) and 6q16.2 (RP11-14G17 deleted), and distal breakpoints between two clones annotated to 6q21 (RP11-165E15 deleted and RP11-78P9 non-deleted). Patient 2 (panel **b**) had proximal breakpoints between the genomic clones annotated to cytogenetic bands 6q15 (RP11-113K7 non-deleted) and 6q16.2 (RP11-14G17 deleted), and distal breakpoints between two clones annotated to 6q21 (RP11-47E20 deleted and RP11-165E15 non-deleted). Patient 3 (panel **c**) had proximal breakpoints between two genomic clones annotated to 6q15 (RP11-52B15 non-deleted and RP11-13I21 deleted), and distal breakpoints between two clones annotated to 6q16.3 (RP11-73D20 deleted) and 6q21 (RP11-47E20 non-deleted). **(d)** Ideogram of 6q showing deletions of the three patients described here; patient 4 was previously reported (4). Solid lines represent chromosomal regions known to be deleted (minimal deleted regions). Dashed lines represent regions between deleted and flanking non-deleted BAC clones containing deletion breakpoints. Numbers above bars indicate patient number.

In summary, the improved characterization of segmental aneuploidy by array CGH provides finer mapping of candidate genes for specific abnormalities, and the development of higher-density arrays will enhance mapping further. In two of our three cases, the molecular breakpoints differed from those indicated by conventional karyotyping, demonstrating the enhanced resolution of array CGH.

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