Partial monosomy 8p and trisomy 16q in two children with developmental delay detected by array comparative genomic hybridization

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Abstract. Two cases of liveborn unrelated children with developmental delay and overlapping unbalanced translocations der(8)t(8;16)(p23.2;q23.3) and der (8)t(8;16)(p23.1;q23.1), leading to partial monosomy 8p and partial trisomy 16q, are reported in the present study. The first patient was a 10-year-old boy with mild developmental delay and minor congenital anomalies (borderline microcephaly, clinodactyly, hypertelorism, epicanthus, mild systolic murmur and kidney reflux). The second patient was a 3 year-old girl with developmental delay, gross motor milestone delay and dysmorphic features. Array-comparative genomic hybridization analysis revealed that partial chromosome 8p monosomy extended from 8p23.2 to 8pter (4.8 Mb) in Patient 1 and from 8p23.1 to 8pter (9.5 Mb) in Patient 2, and partial chromosome 16 trisomy extended from 16q23.3 to 16qter (5.6 Mb) in Patient 1 and from 16q23.1 to 16qter (11.7 Mb) in Patient 2. The mechanism of appearance of the rearrangement in association with the genes involved and the architecture of the region is discussed.

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Introduction

Monosomy 8p is a rare chromosomal disorder characterized by deletion of a part of the eighth chromosome. The incidence of the 8p23.1 deletion was estimated at 1:18,542 in amniotic fluid samples and 1:5,072 in postnatal samples (1). Since the first report of an 8p23.1 deletion by Fagan and Morris (2), >50 cases have been reported (3). The majority of the cases are not studied with high resolution molecular techniques or characterized at the molecular level (4). Interstitial deletions of the sub-band 8p23.1 have primarily been associated with facial and other phenotypic abnormalities, whereas terminal deletions are associated with heart defects (3,5). Notably, distal deletion of 8p23.2-pter has additionally been observed in apparently healthy individuals (1).

In the majority of cases, monosomy 8p appears to result from *de novo* errors in early embryonic development that occur for unknown reasons. Associated symptoms and findings differ between cases (6). However, in most cases clinical manifestations including growth deficiency, mental retardation, post-natal growth retardation, developmental delay and speech problems are observed. Furthermore, patients present with common signs of body and craniofacial dysmophisms, in addition to behavioral difficulties (1,3,5,6). Facial dysmorphisms, which are more remarkable in early years, include microcephaly, malformed or low set ears, arched eyebrows, depressed nasal bridge, epicanthus, strabismus, hypermetropia and/or myopia, serrated teeth, short neck and retrognathia. In addition, vertebral abnormalities are frequently observed (7-11).

It has additionally been reported that children with this chromosomal disorder present with behavioral difficulties, including

aggressiveness and attention deficit disorder, and problems associated with cardiovascular and central nervous system (5,9,12). Furthermore, genito-urinary anomalies, in particular cryptorcidism and hypospadias, are observed in boys (6).

In contrast to 8p deletion syndrome, partial trisomies of the terminal 16qter are rare (1). A total of nine cases of partial distal chromosome 16 trisomy have been reported but only a few were studied with high resolution molecular techniques. Only one patient presented a pure partial trisomy 16q24.1q24.3, whereas all the others corresponded to unbalanced translocations where 16q24 was rearranged with other chromosome regions (7,8,10,11,13-15). For two of the patients, there was no detailed phenotypic information (10,15). A number of clinical characteristic features were common in all patients (low birth weight, growth retardation, intellectual disability, muscular hypotonia, small palpebral fissures, long philtrum, low set/dysplastic ears and osteochondroma), so it is difficult to characterize with precision the 16q24 trisomy phenotype or to establish a genotype-phenotype correlation (11) (Table I).

In the present study, two cases of liveborn unrelated children with an unbalanced 8;16 translocation resulting in partial monosomy of chromosome 8 and partial trisomy of chromosome 16 were reported. The effect on the phenotype of monosomy 8 seems to be more prominent than that of trisomy 16. However, this phenotype may result from the rearranged architecture of the region, the structure and function of the genes and regions involved, and their interactions.

Patients and methods

Ethical approval. The present study was approved by the Ethics Committee of the P. & A. Kyriakou Children's Hospital (Athens, Greece) and was performed with respect to the ethical standards of the Declaration of Helsinki, as revised in 2008. Written, informed consent was obtained from the patient's families.

Patient 1. Patient 1 was a 10- and a half-year-old boy, and the second child of healthy, unrelated parents. The first child of the family is a 16-year-old healthy boy. Patient 1 was referred for developmental assessment for speech and language delay. The patient was born following an uncomplicated full term pregnancy with birth weight 3.350 kg, height 51 cm and head circumference (HC) 35 cm. The perinatal history was non-significant. At the age of 8 months the patient was diagnosed with a urinary tract infection and an X-ray investigation revealed urinary reflux (V degree), and a kidney dimercaptosuccinic acid scan revealed 20% decreased left kidney function. The developmental milestones of the patient were slightly delayed as he sat independently at the age of 9 months and walked unaided at the age of 18 months.

On developmental examination at 3 years old the patient was a sociable child, with mild dysmorphic facial and body features including microcephaly, hypertelorism, epicanthus, and clinodactyly. The patient demonstrated good ability for symbolic play and his comprehension ability was limited to one concept per sentence. His speech was limited to 3-4 simple words. His overall developmental level was equivalent to 18 months. According to the Bailey's Scales of Infant Development 2nd edition (16), his mental score was 51 and motor score was 95. Heart auscultation revealed a mild systolic murmur. On neurological examination, the patient was revealed to be slightly hypertonic with borderline microcephaly (HC=48 cm; 3%).

Echocardiography revealed a small ventricular septal defect without hemodynamic alterations. Metabolic screening revealed a mild elevation of glutamate in blood amino acids and small proteinuria involving lysine, arginin and cystin. His bone age was increased (equivalent to 6-year-old boy). Thyroid function, brain magnetic resonance imaging scans, visual and audiological examinations, urine amino acids and blood lactic acid levels were healthy.

The patient attended mainstream kindergarten and received early intervention services twice a week based on a Portage Scheme. His development was followed up at regular intervals in the Developmental Unit and was monitored according to his needs.

At the age of 3 years and 9 months, his cognitive and language skills were equivalent to the level of a 20-month-old, with severe behavioral difficulties characterized by frequent temper tantrums. At the age of 4 years, 3 months, the cognitive abilities of the patient increased to the level of a 30-month-old while his language skills remained at a 26-month level. His behavior had improved but he remained a difficult child, presenting with hyperactivity, aggressiveness and impulsiveness.

He was additionally observed at the age of 5 years and 2 months. He had made significant developmental progress and his cognitive skills were equivalent to a 4 year and 6 month level, with language skills equivalent to a 2 year and 9 month level. According to Griffiths Scales (17), his performance developmental subquotient (DQ) was 87 and his language DQ was 51. His weight was 19 kg (50th centile), his height was 107 cm (20th centile) and his HC was 49.5 cm (3rd centile). The dysmorphia of his facial features remained mild and passed unnoticed. He was well integrated in mainstream kindergarten and his parents were planning to place him in a mainstream school with extra educational help.

The patient was re-evaluated at the age of 7 years. He was well integrated into the 1st grade of mainstream primary school with special educational provision. His behavior had significantly improved and he was sociable and co-operative. His cognitive abilities were increased, with a developmental level of 5 years and 8 months with a general DQ of 86. His weight was 24 kg (25th centile), his height was 119 cm (25th centile) and his HC was 49.5 cm (below 3rd centile). Dysmorphia of his body and facial features remained mild. On neurological examination, he was revealed to be slightly hypertonic. His thyroid functions and detailed endocrinological examination (GH, IGF1, prolactin, LH, FSH, 17-OH prog., cortisol, insulin) proved normal. Previous echo-triplex results additionally proved normal.

The patient was last observed at the age of 10 years and 6 months. He was attending the 3rd grade of the same mainstream primary school with special educational support. He remained sociable with severe attention deficit disorder, impulsivity and lack of self-confidence. His cognitive deficits were more evident in reading and mathematics. His developmental level was equivalent to that of a healthy 6-year-old, with mild phonological and morphological language problems. His general DQ was 78. The dysmorphia of his body and

Table I. Clinical characteristics associated with 8p23 and 16q24 regions in the literature.

Clinical characteristics	8p23.1 →pter	8p23.2 →pter	16q24.1 →qter	16q24.1 →qter	Patient 1	Patient 2	Other studies	Total
Prematurity					-	+		
Post-natal growth retardation	+	-	-	+	-	-	7-12	7/15
Low birth weight	-	-	-	+	-	-	7,8,11	3/3
Developmental delay	+	-	-	-	+	+	5,9,12	15/20
Mental retardation	+	+	-	+	-	-	5,6,9,10	14/20
Behavioral/ neurodevelopmental (hyperactivity, aggressiveness, no self-confidence, attention deficit disorder, anxious)	+	-	-	-	+	+	5,9,12	25/58
Dysmorphic craniofacial features								
Microcephaly	+	+	-	-	+	-	5,6,9,12	13/21
Hypertelorism					+	+		
Epicanthus	-	-	-	+	+	+	7,12,14	4/5
Broad forehead					-	+		
Arched eyebrows	+	+	-	-	-	+	6	1/1
Diffuse depigmentation of retina					-	+		
Alternating esotropia					-	+		
Long philtrum	-	-	-	+	-	+	10,11,14	3/5
Thin face					-	+		
Thin lips	+	-	-	+	-	+	9,14	5/9
Small mouth					-	+		
Retrognathia	+	-	-	-	-	+	9	8/8
Depressed nasal bridge	+	+	-	-	-	+	6,9	2/9
Dysplastics/low set ears	+	-	-	+	-	+	5,7,8,9,11,12	12/23
Major malformations								
Clinodactyly					+	-		
Laryngeal stridor/laryngomalacia	+	+	-	-	-	+	6	1/1
Cardiovascular system problems	+	+	-	-	+	-	6;16	3/3
Abdominal distension					-	+		
Necrotizing enterocolitis					-	+		
Genito-urinary anomalies	+	+	-	-	+	-	5,6,9	9/28
Central nervous system								
Speech problems	+	-	-	-	+	-	5,9,12	5/20
Dystonic posturing					-	+		
Myelination delay					-	+		

facial features (microcephaly, hypertelorism, epicanthus and clinodactyly) was more evident. On neurological examination, he remained slightly hypertonic with brisk reflexes but without focal neurological signs. His weight was 34 kg (25th centile), his height was 140 cm (25th centile) and his HC was 49.5 cm (<3rd centile).

Patient 2. Patient 2 was a girl was born to non-consanguineous healthy parents at 36 weeks of gestational age, following a normal pregnancy and an uncomplicated delivery. Prenatal karyotype was performed due to advanced maternal age, and it was normal. The family history was unremarkable and there was no previous history of infertility or spontaneous abortion prior to this pregnancy. The birth weight was 2,400 g (25th centile), height 48 cm (75th-90th centile), and HC 30.5 cm (2nd-10th centile). Apgar scores were 9 and 10 at 1 and 5 min, respectively.

Two days following birth, the patient presented with abdominal distension and bloody stools. An X-ray revealed the presence of air outside the intestines in the abdominal cavity. Necrotizing enterocolitis with perforation was diagnosed and surgical removal of the caecum was performed, and the ileo-cecal valve was perforated. However, three months following surgery, she presented with intestinal obstruction caused by narrowing of the previously diseased bowel, requiring further surgical intervention. In addition, the neonatal period was complicated by laryngeal stridor due to laryngomalacia. Some dysmorphic features and dystonic posturing were noticed in

Table II. Genotypic information of Patient 1 at the chromosome 8 STS markers obtained by quantitative polymerase chain reaction and gene dosage assay.

STS name	Gene	Position (bp)	Deletion	Primer	Sequence ('5-3')	size
STS-N21307	LOC286161	427685-427914	Yes	F	CAGGTTGGCAAGTGAAATAC	230
				R	GCAGTAGTGGCATGAAGC	
SHGC-149177	DLGAP2	952948-953243	Yes	F	GCCTCCTGGGATAAAAATCCTTT	296
				R	GGTTTGCTCTCCTGATTTAGGGT	
SHGC-149177	CLN8	1728163-1728478	Yes	F	AAGAGCAAGAGGAGCAGGAAAAC	316
				R	GTGAAACATGTGAATCATCAGCC	
SHGC-105022	CSMD1	4126904-4127196	Yes[a]	F	TTTTATTTTGGATCAGGCAACCT	293
				R	TGTGCTTTGAACCACACTCCTAA	
RH119760	CSMD1	4950952-4951296	Yes	F	TATCCAGTCTCTGCATTTGATGG	345
				R	AGAATCCCAAAGGAGTTACCGAA	
A004X20	MCPH1	6302850-6303049	Yes	F	TAAGTTTTCCTTCTCTTCTGTAG	216
				R	AAGGACATGATGATGATT	
SHGC-77726	MCPH1	6478893-6479173	Yes[a]	F	GAAGTAAACTGCAACAGTTCGCC	281
				R	TCTTCTTTCCGCTGTAGGGC	
RH120376	TDH	11224233-11224519	No	F	AAAATCCACGCTTTGACCTAACA	287
				R	TGGTAAGGGAATGAGTGTGTTCA	
RH11694	GATA4	11617203-11617417	No	F	TGCACATTGCTGTTTCTGCC	234
				R	GTTTGTGGGTTAGGGAGGGT	

[a]One in three replications provided conflicting results. STS, sequence tagged site.

early infancy. The patient acquired head control at the age of 6 months, trunk control at the age of 9 months, and autonomous deambulation at the age of 12 months. The patient started to speak at two years of age, but then stopped any further development of verbal language and developed a preference for gestural communication. Verbal comprehension was good.

Extensive studies for metabolic diseases (including blood and urine amino acids, urine organic acids, blood lactate, pyruvate and ammonia) gave normal results. Electroencephalogram, audiometric examination, cardiological evaluation including echocardiogram, X-rays of the thorax and renal ultrasound returned normal results. Ophthalmologic assessment (at 3 months of age) revealed diffuse depigmentation of the retina. Brain magnetic resonance (at 6 months of age) revealed myelination delay. At 7 months of age, the patient's height was 62 cm (10th centile), weight was 5.035 g (<3rd centile) and HC was 39.5 cm (<2nd centile). Morphological evaluation evidenced a thin face, broad forehead, low-set and posteriorly rotated ears, bilateral pits above the tragus, arched eyebrows, hypertelorism, epicanthus inversus, depressed nasal bridge, long philtrum, thin lips, small mouth with down-turned corners, and retrognathia. Neurological examination revealed developmental delay, with gross motor milestones limited to uncompleted head control. Dystonic axial posturing and fluctuating muscular tone of the four limbs was present. Alternating esotropia was additionally observed.

Cytogenetic and fluorescence in situ hybridization (FISH) analyses. Chromosome analysis was performed from 2-2.5 ml cultured blood lymphocytes using Giemsa banding and high resolution banding techniques obtained following cell culture synchronization and thymidine incorporation. FISH studies were performed using a set of probes specific for 8p (TelVysion 8p SpectrumGreen D8S504) and 16q (TelVysion 16q SpectrumOrange 16qTEL013) subtelomeres according to the manufacturer's protocol (Vysis; Abbott Molecular, Des Plaines, Illinois, USA) (18). The slides were washed and counterstained with 4',6-diamidino-2-phenylindole, and cells were examined under a Zeiss Axioplan II, Imager.M1/Imager.Z1 fluorescence microscope equipped with a triple-bandpass filter (Zeiss GmbH, Jena, Germany). Digital images were captured and stored with Isis software version 3.4.0 (MetaSystems, Altlussheim, Germany).

Array comparative genomic hybridization (aCGH), polymerase chain reaction (PCR) and microsatellite analysis. High molecular weight genomic DNA was extracted from the patient's blood lymphocytes using aQiamp DNA Blood Midi kit (Qiagen, Inc., Valencia, CA, USA). aCGH analysis was performed with DNA from cultured amniocytes in order to characterize the extent of the deletion in Patient 1 and to justify the clinical findings in Patient 2. Molecular karyotyping was performed via oligonucleotide aCGH platforms using an 100 kb resolution array kit 44K (Agilent Technologies, Inc., Santa Clara, CA, USA). Gene dosage for 9 sequence tagged sites (STSs) from chromosome 8 was performed by PCR using the LightCycler FastStart DNA Master SYBR Green 1 Kit (Roche Diagnostics, Monza Italy), according to the manufacturer's instructions, on a Roche LightCycler 1.5 instrument (Roche Diagnostics). Primer sequences for the telomeric STSs amplified, including the genesceroid-lipofuscinosis, neuronal 8 (*CLN8*), CUB and Sushi multiple domains 1 (*CSMD1*), microcephalin 1 primary autosomal recessive 1 (*MCPH1*) and GATA binding protein 4 (*GATA4*), are listed in Table II. Altogether,

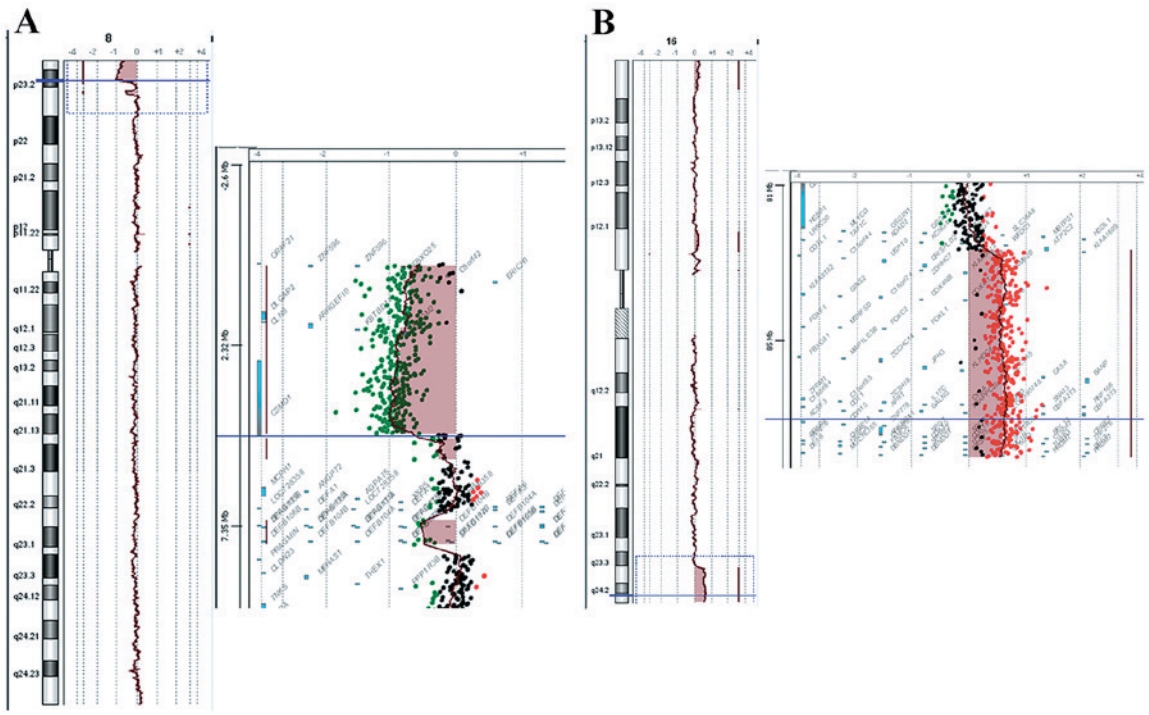

Figure 1. Array comparative genomic hybridization results for Patient 1. (A) *De novo* 4,8 Mb deletion in the short arm of chromosome 8 located at the 8p23.1 to 8pter. (B) *De novo* 5,6 Mb duplication in the long arm of chromosome 16 located at the 16q24.1 to 16qter.

the analyzed region covered ~11 Mb of DNA of the telomeric 8p region. PCR was performed using the following program: 95˚C for 10 min, followed by 40 cycles of 95˚C for 10 sec, 55˚C for 10 sec, and 72˚C for 25 sec. Copy-number/genome of each STS was evaluated by a relative quantification method using the software RelQuant (Roche Diagnostics). A 156 bp fragment of the human beta-globin gene (HBB) was used as reference DNA for normalization and amplified in separate capillaries simultaneously to the STS targets. Primer sequences for HBB were as follows: forward 5'-CAGCTCACTCAGTGT GGCAAAG-3' and reverse 5'-AGGTTCTTTGAGTCCTT TGGGG-3'. Relative standard curves were produced using 5 control DNA samples to correct for differences in efficiency of amplification between STS target and reference DNA. For each locus the test was replicated three times.

Bioinformatic analyses. Sequence features of 8p and 16q regions were analysed in the University of California Santa Cruz (UCSC) Genome Browser (19) using data from the International Standards for Cytogenomic Arrays Consortium (ISCA; www.iscaconsortium.org/) database (20,21) and the corresponding data track for UCSC genes. The Basic Local Alignment Search Tool (BLAST) algorithm (http://blast.ncbi.nlm.nih.gov/Blast.cgi) was used for analysis (22). The results were used to study the nucleotide sequence similarity between the breakpoint regions.

Results

Patient 1. The conventional karyotype of Patient 1 revealed 'additional' material in the short arm of chromosome 8 (46, XY, 8p+). aCGH analysis revealed the chromosomal origin of the additional material and the exact position of the breakpoints, namely a deletion of 8p and a duplication of 16q. The 8p deletion was a 4,8 Mb deletion of the distal short arm of chromosome 8 with the proximal breakpoints between 4,814,649 bp (last deleted oligo) and 4,833,351 bp (first normal oligo), with the last oligonucleotide present in the array at 8p position 161,472 kb being deleted (Fig. 1A). The deleted region of the *CSMD1* gene began at the first intron. The 16q duplication was a 5.6 Mb duplication of the long arm of chromosome 16 with the proximal breakpoint between 84,468,454 bp (normal) and 84,511,640 bp (duplicated), and the last oligonucleotide present in the array at 16q position 88,690,571 bp being duplicated (Fig. 1B). The size of the breakpoint intervals were 18,702 bp for 8p and 43,186 bp for 16q. The analysis revealed an unbalanced translocation, and the aCGH karyotype was 46,XY,der(8)t(8;16)(p23.2;q23.3) dn.arr[hg18]8p23.3p23.2(151,472-4814649)x1,16q23.3q24.3 (84,511,640-88,690,571)x3.

A list of Online Mendelian Inheritance in Man (OMIM; https://www.omim.org/) genes deleted and duplicated is presented in Table III. FISH analysis was performed to confirm the aCGH data. Three signals were detected; one on chromosome 8 and two on chromosome 16 of the 16q subtelomeric probe. FISH analysis performed in the parents revealed a normal result, indicating a *de novo* rearrangement.

Patient 2. Prenatal diagnosis due to elevated maternal age revealed a normal karyotype of 46, XX. During the neonatal period and due to dysmorphic features, hypotonia and clinical complications, aCGH analysis was performed. The analysis revealed a deletion of 9.5 Mb of the distal short arm of chromosome 8 with the proximal breakpoints between 95,48,146 bp (last deleted oligo) and 95,62,020 bp (first normal oligo), with the last oligonucleotide present in the array at 8p position

Table III. List of OMIM genes deleted and duplicated in both patients.

Deletion

Patient 1		Patient 2	
Gene	OMIM	Gene	OMIM
FBX025	609098	*FBX025*	609098
DLGAP2	605438	*DLGAP2*	605438
CLN8	607837	*CLN8*	607837
ARHGEF10	608136	*ARHGEF10*	608136
MYOM2	603509	*MYOM2*	603509
CSMD1	608397	*CSMD1*	608397
		MCPH1	607117
		ANGPT2	601922
		AGPAT5	614796
		DEFB1	602056
		DEFA6	600471
		DEFA4	601157
		DEFA1	125220
		DEFA3	604522
		DEFA5	600472
		DEFB103B	606611
		SPAG11B	606560
		FAM90A7P	613044
		FAM90A10P	613047
		DEFB4A	602215
		CLDN23	609203
		MFHAS1	605352
		ERI1	608739
		PPP1R3B	610541
		TNKS	603303

Duplication

Patient 1		Patient 2	
Gene	OMIM	Gene	OMIM
ATP2C2	613082	*WWOX*	605131
COTL1	606748	*MAF*	177075
USP10	609818	*MAFTRR*	616264
CRISPLD2	612434	*DYNLRB2*	607168
ZDHHC7	614604	*CENPN*	611509
KIAA0513	611675	*ATMIN*	614693
FAM92B	617274	*GCSH*	238330
GSE1	616886	*PKD1L2*	607894
GINS2	610609	*BCO1*	605748
EMC8	604886	*GAN*	605379
COX4I1	123864	*CMIP*	610112
IRF8	601565	*PLCG2*	600220
LINC01082	614978	*SDR42E1*	616164
LINC01081	614977	*HSD17B2*	109685
FENDRR	614975	*MPHOSPH6*	605500
FOXF1	601089	*CDH13*	601364

Table III. Continued.

Duplication

Patient 1		Patient 2	
Gene	OMIM	Gene	OMIM
MTHFSD	616820	*HSBP1*	604553
FOXC2	602402	*MLYCD*	606761
FOXL1	603252	*OSGIN1*	607975
FBXO31	609102	*SLC38A8*	615585
MAP1LC3B	609604	*MBTPS1*	603355
JPH3	605268	*DNAAF1*	613190
SLC7A5	600182	*TAF1C*	604905
CA5A	114761	*KCNG4*	607603
BANP	611564	*WFDC1*	605322
ZNF469	612078	*ATP2C2*	613082
ZFPM1	601950	*COTL1*	606748
IL17C	604628	*USP10*	609818
CYBA	608508	*CRISPLD2*	612434
MVD	603236	*ZDHHC7*	614604
SNAI3	612741	*KIAA0513*	611675
RNF166	617178	*FAM92B*	617274
CTU2	617057	*GSE1*	616886
PIEZO1	611184	*GINS2*	610609
CDT1	605525	*EMC8*	604886
APRT	102600	*COX4I1*	123864
GALNS	612222	*IRF8*	601565
TRAPPC2L	610970	*LINC01082*	614978
CBFA2T3	603870	*LINC01081*	614977
ACSF3	614245	*FENDRR*	614975
CDH15	114019	*FOXF1*	601089
ANKRD11	611192	*MTHFSD*	616820
SPG7	602783	*FOXC2*	602402
RPL13	113703	*FOXL1*	603252
CPNE7	605689	*FBXO31*	609102
DPEP1	179780	*MAP1LC3B*	609604
CHMP1A	164010	*JPH3*	605268
SPATA33	615409	*SLC7A5*	600182
CDK10	603464	*CA5A*	114761
ZNF276	608460	*BANP*	611564
FANCA	607139	*ZNF469*	612078
SPIRE2	609217	*ZFPM1*	601950
TCF25	612326	*IL17C*	604628
MC1R	155555	*CYBA*	608508
TUBB3	602661	*MVD*	603236
AFG3L1P	603020	*SNAI3*	612741
GAS8	605178	*RNF166*	617178
GAS8-AS1	605179	*CTU2*	617057
URAHP	615805	*PIEZO1*	611184
PRDM7	609759	*CDT1*	605525
		APRT	102600
		GALNS	612222
		TRAPPC2L	610970
		CBFA2T3	603870
		ACSF3	614245

Table III. Continued.

Duplication			
Patient 1		Patient 2	
Gene	OMIM	Gene	OMIM
		CDH15	114019
		ANKRD11	611192
		SPG7	602783
		RPL13	113703
		CPNE7	605689
		DPEP1	179780
		CHMP1A	164010
		SPATA33	615409
		CDK10	603464
		ZNF276	608460
		FANCA	607139
		SPIRE2	609217
		TCF25	612326
		MC1R	155555
		TUBB3	602661
		AFG3L1P	603020
		GAS8	605178
		GAS8-AS1	605179
		URAHP	615805
		PRDM7	609759

OMIM, Online Mendelian Inheritance in Man.

151,472 kb being deleted (Fig. 2A). The deleted region of the tankyrase (*TNKS*) gene began at the fifth intron. A duplication of 11.7 Mb of the long arm of chromosome 16 with the proximal breakpoint between 76,961,103 bp (duplicated) and 76,938,723 bp (normal) was observed and the last oligonucleotide present in the array at 16q position 88,690,571 bp was duplicated (Fig. 2B). The size of the breakpoint intervals were 13,874 bp for 8p and 22,380 bp for 16q. The analysis revealed an unbalanced translocation and the aCGH karyotype was 46,XX,der(8)t(8;16)(p23.1;q23.1).arr[hg18]8p23.3p23.1 (151,472-9548146)x1,16q23.1q24.3(76,961,103-88,690,571)x3.

A list of OMIM genes deleted and duplicated is presented in Table III. Microsatellite analysis of the trio revealed that deletion and duplication occurred on maternally-derived chromosomes (Table IV).

Bioinformatic analyses. A possible cause of rearrangements, duplications and deletions is the occurrence of recombination events. To search for a possible breakpoint for recombination, the BLAST algorithm was used to find sequence similarity in the breakpoint regions of the two patients. The breakpoint regions were revealed to contain similar sequences residing in Alu elements of Patient 1 and in L1 elements of Patient 2.

The rearranged regions were viewed in parallel with ISCA consortium data in the UCSC Genome Browser. The 8p region contained multiple pathogenic copy number variations (74 deletions and 31 duplications) described in the ISCA database, while rearrangements in 16q were less frequent (containing 19 deletions and 16 duplications). Manual computations of the ISCA data revealed that 66% of patients with 8p23.3-p23.1 rearrangements (deletions or duplications), and 62% of patients with 16q23.1-q24.3 rearrangements had developmental delay in their pathogenic phenotype (Fig. 3).

Discussion

To the best of our knowledge, this is the first report of a rearrangement involving an 8p deletion and 16q duplication. The two patients presented in this report had subtle facial feature dysmorphia, dysmorphic body features, borderline intelligence and marginal follow up progress, low birth weight and vertebral anomalies, and one presented with cardiovascular abnormalities. The majority of the clinical characteristics of the two patients were associated with those of 8p or 16q chromosome imbalances, but it is difficult to estimate if the clinical phenotype and developmental delay were due to the rearrangement or whether they were the result of 8p monosomy and 16q trisomy separately. It has previously been recognized that deletions in the distal region of chromosome 8p are associated with growth and mental impairment, minor facial dysmorphisms, microcephaly, congenital heart defects and behavioral problems (23). According to all references, 16q trisomy is a rare abnormality due to high rates of mortality and lethality in the prenatal and neonatal period (11,24). Partial 16q trisomy is most often the result of balanced or unbalanced rearrangements, and therefore it is difficult to understand if the commonly observed phenotypic characteristics (dysmorphic facial features, developmental delay, intellectual disability, central nervous system malformations and congenital heart defects) are due to 16q or whether they are the result of changes in genome architecture (24).

More than 2/3 of patients with 8p syndrome have congenital heart defects, suggesting that 8p23.1 maybe critical for heart development (5,25). One of the candidate genes for heart disease is *GATA4* because haploinsufficiency and mutations have been documented in patients and families with atrial septal defects and other cardiac defects associated with 8p23.1 deletion (4,26-29). Chen *et al* (30) studied a four-generation Chinese atrial septal defect family and suggested that a mutation in the *GATA4* gene (c.A899C, p.K300T) may contribute to this congenital heart disease. However, the *GATA4* gene was not deleted in either patient in the present study. The fact that Patient 1 has heart problems suggested either that other genes were responsible for these problems, or that the rearrangement resulted in a structural alteration affecting the function of genes associated with the heart. The *CSMD1* gene (8p23.2) was deleted in Patient 2 but only partially deleted in Patient 1. According to the literature, *CSMD1* loss of function is correlated with head and neck squamous cell carcinoma (31,32), and liver (33,34), lung, breast and skin cancers (31). Deletion of this gene has been reported in a case of craniofacial and body dysmorphisms and mental retardation (35). In Patient 2, two of the deleted genes were *TNKS* and *MCPH1*. These genes are involved in meiosis and mitosis mechanisms. The *TNKS* gene, located at 8p23.1, is involved in sister chromatid cohesion and deletions result in anaphase arrest (36). Páez *et al* (4) identified deletions of *TNKS* gene in patients with mental retardation and

Table IV. Results from microsatellite analysis on Patient 2.

Sample	253-10 Proband	254-10 father	255-10 mother	Origin
D8S201	259.5	255.5/259.5	259.5/267.2	Uninformative
D8S504	200.7	200.7/202.8	198.1/202.7	Maternal
D8S264	138.2	138.1/138.1	126.4/126.4	Maternal
D8S1781	259.4	259.4/263.2	251.1/263.1	Maternal
D8S351	119.2	119/119	105/105	Maternal
D8S1706	228/234.2	228/234.2	228/234.2	Uninformative
D16S3023	79.5/83.5	83.5/83.5	79.5/83.6	Uninformative
D16S413	128/132	130/132	128/132	Uninformative
STS1 (chr16)	210.8*/214.9	214.9/214.9	210.9/210.9	Maternal
STS2 (chr16)	125.2/125.2	125.2/125.2	125.2/125.2	Uninformative
STS3 (chr16)	296.5/296.5	294.6/296.5	296.6	Uninformative
STS4 (chr16)	345.32/352.4/357.8	352.4/359.7	345.4/357.38	Maternal

Polymorphic sequence tagged site markers were selected in the deleted and duplicated regions of chromosomes 8 and 16, respectively.

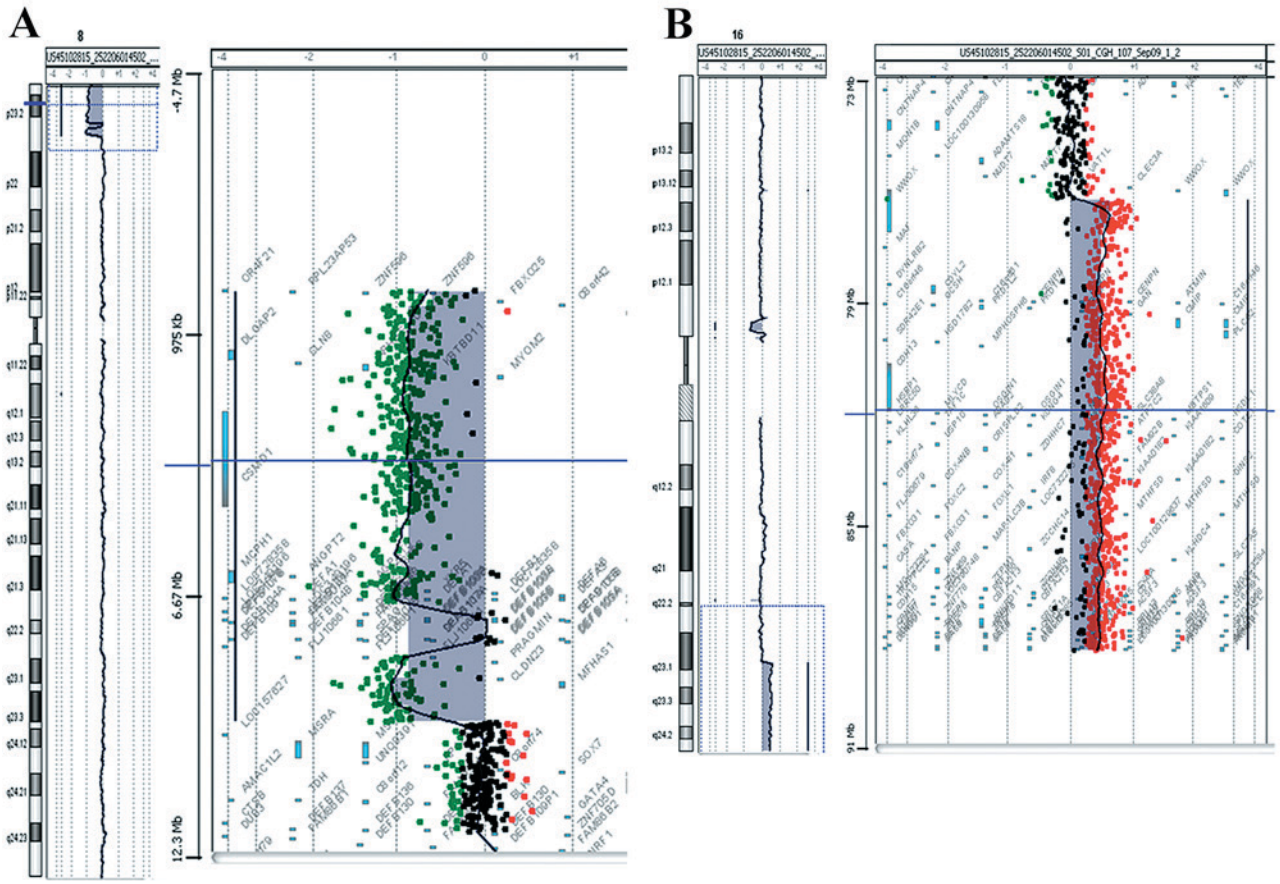

Figure 2. Array comparative genomic hybridization results for Patient 2. (A) *De novo* 9,5 Mb deletion in the short arm of chromosome 8 located at the 8p23 to 8pter. (B) *De novo* 11,7 Mb duplication in the long arm of chromosome 16 located at the 16q23.1 to 16qter.

behavioral problems. TNKS protein positively regulates the Wnt/β-catenin signaling pathway (37). This pathway is critical for healthy embryonic development and cellular differentiation (38). Furthermore, *TNKS* is a candidate gene for Cornelia de Lange Syndrome (CdLS) (3,36), a syndrome characterized by distinctive facial features including well-defined curved and confluent eyebrows, long eyelashes, anteverted nares, micrognathia and downturned corners of the mouth with a thin upper lip. Patient 2 resembled the CdLS facial phenotype, and she is expected to have psychomotor retardation, language acquisition difficulties and behavioral disorders in the autistic spectrum, typical aspects of CdLS. The *MCPH1*

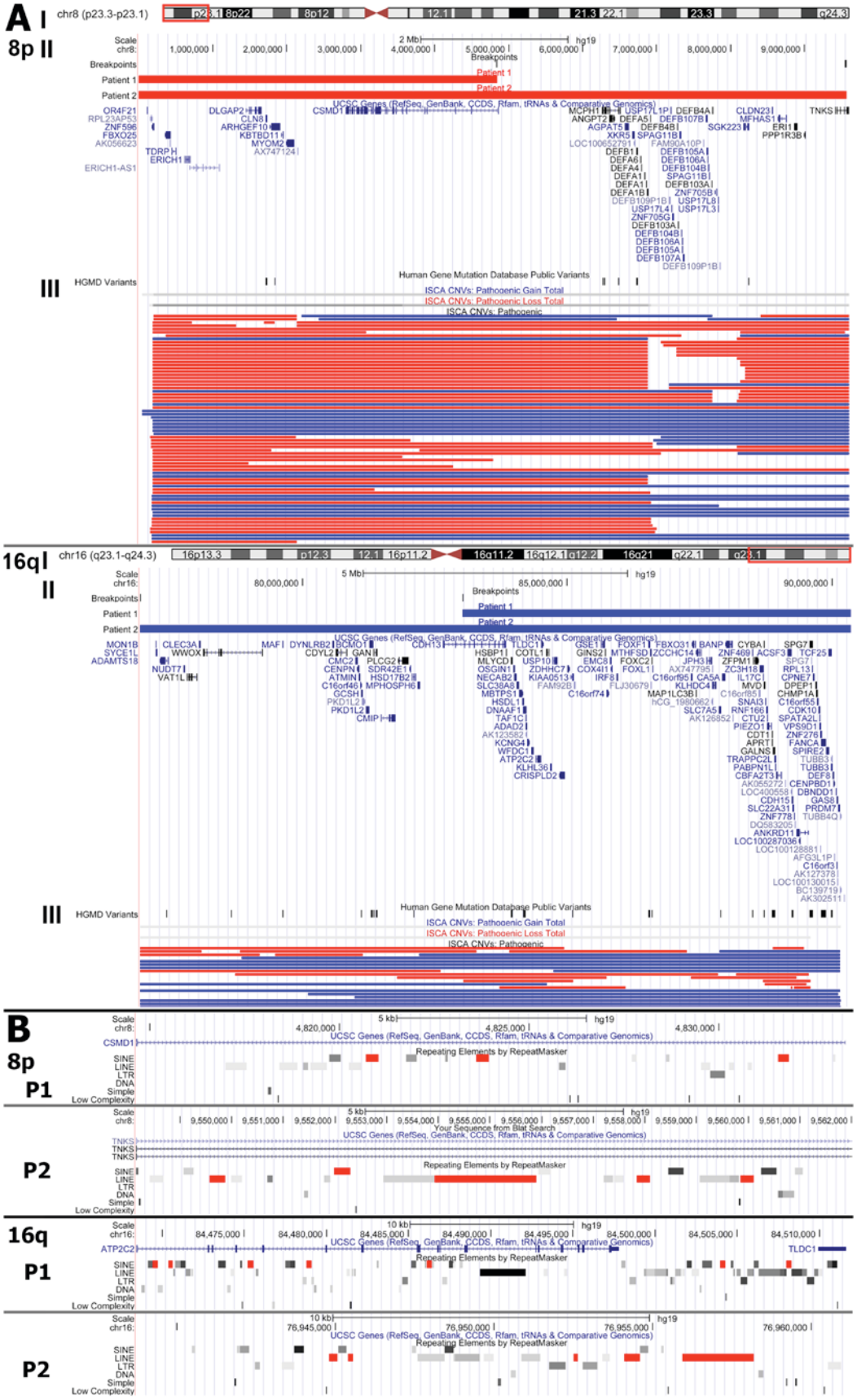

Figure 3. (A) Sequence features of the 8p and 16q translocated regions. The features are presented in parallel tracks. I) Chromosome ideogram representing the translocated regions in the red square. II) Chromosome positions of deleted (in red) and duplicated (in blue) regions are shown in each patient and respective breakpoints and gene positions are highlighted. III) Pathogenic copy number variants in these regions, as published by the ISCA Consortium. Blue lines represent duplications and red lines deletions. (B) Sequence features of the respective 8p and 16q breakpoints for both patients. Presented in parallel tracks: the chromosome scale and chromosome region annotation, genes present in this chromosome region and repetitive genetic elements as annotated by the RepeatMasker tool (www.repeatmasker.org). Red color repeats denote sequences with high similarity, as revealed by BLAST alignment of the respective breakpoints for each patient. P1, patient 1; P2, patient 2.

gene, additionally located in 8p23.1, is involved in preventing cells from prematurely entering mitosis, and truncated mutations have been associated with premature chromosome condensation and were observed in patients with microcephaly, growth impairment and mental retardation (36,39). Another gene located in 8p23.1 is RP1 like 1 (*RP1L1*). Its expression is restricted to the postnatal retina, potentially being involved in retinal development (40). The *RP1L1* gene may not be haploinsufficient in Patient 2, who was diagnosed with diffuse depigmentation of the retina. This gene maybe under the control of translocated regulatory elements, being in the proximity of the breakpoint, and may have resulted in this retinal disorder.

In total, >30 cases with distal 8p deletion have been described in the literature, and 9 with 16q24 duplication, but only a few have been characterized with high resolution molecular techniques (1,11). The 8p region is more often reported to be involved in rearrangements than 16q. Giglio *et al* (41) demonstrated that the olfactory receptor (OR) gene clusters are the substrate for the formation of intrachromosomal rearrangements involving chromosome 8p. Different rearrangements, most of them recurring, are associated with the distal 8p region. Among them there are inv dup(8p), del(8p22) and small marker chromosomes der(8)(p23-pter) (41). Furthermore, seven individuals with balanced and unbalanced translocations between 4p16 and 8p23 demonstrated that the breakpoints fell within the 4p and 8p OR-gene clusters (42).

BLAST alignment in the 8p and 16q regions revealed high similarity regions with several Alu elements in Patient 1 and two similar long interspersed nuclear element 1 elements in Patient 2. Retroelements are known to facilitate recombination events (43). Consequently, a potential mechanism of their appearance maybe unequal cross over between repetitive DNA regions with high sequence similarity (44). The deleted and duplicated regions are regions often correlated with developmental delay, according to the ISCA Consortium (20).

Novel diagnostic methods with great potential have facilitated the study and interpretation of the consequences of chromosome aberrations and revealed that the pathogenicity may be due to complex molecular mechanisms (45,46). A number of the genes identified as deleted or duplicated in these cases may have resulted in developmental delay, but developmental delay may also be the result of rearrangements and changes of important parts of gene structure functional elements, truncated or fusion genes. A multidisciplinary effort aiming to study all cases with 8p;16q rearrangements with combined and accurate methods and tools (cytogenetic, molecular cytogenetic, NGS mapping) along with their clinical phenotypes may elucidate the involvement of the rearrangement, genes involved, participation of control elements and/or interactions with polymorphic regions, and potentially a clear phenotype-genotype correlation.

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