RESEARCH ARTICLE

AMERICAN JOURNAL OF medical genetics PART A

Analysis of Invdupdel(8p) Rearrangement: Clinical, Cytogenetic and Molecular Characterization

Fe Amalia García-Santiago,[1,5]* Víctor Martínez-Glez,[2,5] Fernando Santos,[3,5] Sixto García-Miñaur,[3,5] Elena Mansilla,[1,5] Antonio González Meneses,[6] Jordi Rosell,[5,7] Ángeles Pérez Granero,[7] Elena Vallespín,[2,5] Luis Fernández,[2,5] Blanca Sierra,[7] María Oliver-Bonet,[8] María Palomares,[2,5] María Luisa de Torres,[1,5] María Ángeles Mori,[2,5] Julián Nevado,[2,5] Karen E. Heath,[4,5] Alicia Delicado,[1,5] and Pablo Lapunzina[3,5]

[1]Cytogenetics Unit, Institute of Medical and Molecular Genetics (INGEMM), IdiPAZ, Hospital Universitario La Paz, Madrid, Spain

[2]Functional and Structural Genomics Unit, Institute of Medical and Molecular Genetics (INGEMM), IdiPAZ, Hospital Universitario La Paz, Madrid, Spain

[3]Clinical Gentics Unit, Institute of Medical and Molecular Genetics (INGEMM), IdiPAZ, Hospital Universitario La Paz, Madrid, Spain

[4]Molecular Endocrinology Unit, Institute of Medical and Molecular Genetics (INGEMM), IdiPAZ, Hospital Universitario La Paz, Madrid, Spain

[5]CIBERER, Centro de Investigación Biomédica en Red de Enfermedades Raras, ISCIII, Madrid, Spain

[6]Dysmorphology Unit, Hospital Virgen del Rocío, Sevilla, Spain

[7]Genetics Unit, Hospital Son Espases, Palma de Mallorca, Spain

[8]Research Unit, Hospital Son Espases, Palma de Mallorca, Spain

Manuscript Received: 16 June 2014; Manuscript Accepted: 22 October 2014

Inverted duplication 8p associated with deletion of the short arms of chromosome 8 (invdupdel[8p]) is a relatively uncommon complex chromosomal rearrangement, with an estimated incidence of 1 in 10,000–30,000 live borns. The chromosomal rearrangement consists of a deletion of the telomeric region (8p23-pter) and an inverted duplication of the 8p11.2–p22 region. Clinical manifestations of this disorder include severe to moderate intellectual disability and characteristic facial features. In most cases, there are also CNS associated malformations and congenital heart defects. In this work, we present the cytogenetic and molecular characterization of seven children with invdupdel(8p) rearrangements. Subsequently, we have carried out genotype–phenotype correlations in these seven patients. The majority of our patients carry a similar deletion but different size of duplications; the latter probably explaining the phenotypic variability among them. We recommend that complete clinical evaluation and detailed chromosomal microarray studies should be undertaken, enabling appropriate genetic counseling. 2014 Wiley Periodicals, Inc.

Key words: invdupdel(8p); inversion; duplication; deletion; chromosome 8; genomic rearrangement; FISH; chromosomal microarray

How to Cite this Article:
García-Santiago FA, Martínez-Glez V, Santos F, García-Miñaur S, Mansilla E, Meneses AG, Rosell J, Granero ÁP, Vallespín E, Fernández L, Sierra B, Oliver-Bonet M, Palomares M, de Torres ML, Mori MÁ, Nevado J, Heath K, Delicado A, Lapunzina P. 2014. Analysis of invdupdel(8p) rearrangement: Clinical, cytogenetic and molecular characterization. Am J Med Genet Part A. 9999:1–8.

INTRODUCTION

Interstitial inverted duplication 8p associated with 8pter deletion (invdupdel[8p]) is a complex and relatively uncommon chromosomal rearrangement, with an estimated incidence in the general population of 1 in 10,000–30,000 liveborn infants. Clinical manifestations of this genetic alteration include mild to severe intellectual disability, characteristic facial features, CNS malformations such as hypoplasia/agenesis of the corpus callosum (80%), skeletal

*Correspondence to:
Fe A. García Santiago, BSc, PhD, Instituto de Genética Médica y Molecular (INGEMM), IdiPAZ; Hospital Universitario La Paz, Paseo de la Castellana 261, Madrid, 28046, Spain.
E-mail: feamalia.garcia@salud.madrid.org
Article first published online in Wiley Online Library (wileyonlinelibrary.com): 00 Month 2014
DOI 10.1002/ajmg.a.36879

abnormalities such as scoliosis/kyphosis (60%), hypotonia (66%), and congenital heart defects (25%) [Guo et al., 1995; de Die-Smulders et al., 1995; Devriendt et al., 1999].

The invdupdel(8p) consists of a deletion distal to the 8p23 region followed by an intermediate intact segment, and a proximal inverted duplication of various extensions. Rearrangements are mediated mainly by two olfactory receptor gene clusters or defensin repeat (ORDRs) at the breakpoints; the polymorphic 8p23 inversion between these clusters increases the susceptibility on 8p to rearrangements [Giglio et al., 2001; Shimokawa et al., 2004; Zuffardi et al., 2006]. The inversion allele is frequently found in one of the parents, and has an estimated allele frequency of 27% in the normal Japanese population [Sugawara et al., 2003].

To date, only three patients with invdupdel(8p) have been cytogenetically and molecularly characterized. In this work, we present seven patients with invdupdel(8p), the cytogenetic and molecular characterization of six of them and its genotype–phenotype correlations.

PATIENTS AND METHODS

Patients

All clinical patients' clinical data is listed in Table I. Briefly, Patient 1 is a girl and first child of healthy unrelated parents. Neonatal examination was normal. Speech development was delayed and she subsequently presented with attention deficit hyperactivity disorder (Fig. 1A; Table I). Patient 2, a boy, was the second child of healthy nonconsanguineous parents. He had prolonged cholestatic jaundice during the neonatal period that resolved around 4–6 months of age. Motor delay (milestones) was slightly delayed. He could sit without support at 9 months but had not yet achieved an independent gait at 17 months (Fig 1B; Table I). Patient 3 was the first girl of a nonconsanguineous couple. Birth weight was 3,550 g (75th centile). She presented with global developmental delayed with head support at 8 months and could sit around 12 months (Fig. 1C; Table I). Patient 4 is a male with severe intellectual disability and many other physical findings (Fig. 1D; Table I). Patient 5 is a 7-year-old girl, with no data on previous clinical history. She was noted to have brachycephaly, asymmetric and triangular face, broad forehead, short palpebral fissures, low-set, posteriorly rotated ears, wide mouth, and clinodactyly of both fifth fingers (Table I). Patient 6 is a 6-year-old male with dysmorphic features, with no data on previous clinical history (Table I). Patient 7 was a female newborn. She was the first girl of nonconsanguineous parents. Her birth was uneventful and at term. Birth weight was 3,110 g (50th centile). She died at 6 weeks of age secondary to a cardiomyopathy leading to multiorganic failure. No apparent external malformations were noted although the patient was not formally examined by a clinical geneticist (Table I).

G-Banded chromosome analysis. Metaphase chromosome preparations were obtained from cultures of peripheral blood lymphocytes. Slides were GTG banded using standard methods.

Fluorescence in situ hybridization (FISH) analysis. FISH using BAC DNA probes was performed on metaphase chromosomes of the patients and their parents. BAC clones and DNA templates were prepared following the CytoChip QC protocol (Genycell Biotech, Granada, Spain) and labeled using the BlueFish Probes Labeling Protocol (BlueGnome, Cambridge, UK). Images were collected and merged using Ikaros Karyotyping and Isis Imaging Systems (MetaSystems, Germany).

BAC clones RP11–399J23 (GenBank: AC068353) and RP11–589N15 (GenBank: AC025857.5) (8p23.1) were used as probes for two-color FISH analysis on paternal chromosomes to confirm the polymorphic inv(8) (p23) as previously described [Sugawara et al., 2003]. BAC clones RP11–366G13 (GenBank: AC091165.15) (8p12) and RP11–255E13 (GenBank: AC091162.9) (8p22) were used to confirm the inversion of the duplicated segment on Patients 2–6. BAC clones RP11–524J7 (chr8:47806399–47995237) red or RP11–65O11 (GenBank AC127507) green (8q11.1/8p11.1) were used to confirm the origin of the small de novo supernumerary marker chromosome in Patient 1. The inversion of the duplicated segment in this patient was evaluated using BAC clone RP11–399J23 (red). Subtelomere FISH analysis were performed in all patients. Chromosome 8 painting was performed using Kreatech probe spectrum orange and FISH with subtelomeric chromosome 8p probes with Kreatech probe spectrum green.

Microarray studies. Three different chromosomal microarray (CMA) platforms were used to delineate the genomic alterations in six patients.

Firstly, an Agilent-based, in-house-designed 8 × 60 K genome-wide oligonucleotide custom array format (KaryoArray® v3.0; [Rodriguez-Revenga et al., 2013; Vallespin et al., 2013] which covers more than 380 clinically relevant genomic imbalance regions) (Agilent Technologies. Santa Clara, CA). This CMA had an average probe density of 43 kb. Array experiments were performed as recommended by the manufacturer (Agilent Technologies). The arrays were then scanned by the Agilent Microarray Scanner and analyzed with the Feature Extraction software (v9.1, Agilent Technologies) and the analysis and visualization of Karyoarray® data was performed using Agilent Genomic WorkBench 6.5.0.18. The Aberration Detection Method 2 (ADM-2) quality weighted interval score algorithm identifies aberrant intervals in samples that have consistently high or low log ratios based on their statistical score.

Secondly, a 12-plex oligonucleotide-based CGH array of 135,000 probes covering the whole genome (NCBI36/hg18) was applied to some patients. Images and data were analyzed with NimbleScan 2.6 software (Roche NimbleGen, Inc., Madison, WI 53719), and NimbleGen Systems DEVA 1.2.1 software using default parameters.

Finally, Illumina Human610-Quad BeadChip SNP arrays were utilized in two patients, according to the manufacturer's specifications (Illumina, San Diego, CA). Images and data were analyzed with the Beadstudio 3.2 software (Illumina). GenCall scores <0.15 at any locus were considered "no calls." The metric used was the log R ratio, which is the log (base 2) ratio of the observed normalized R value for a SNP divided by the expected normalized R-value.

Microsatellite marker analysis. To confirm the parent-of-origin and types of rearrangements on invdupdel(8p), 16 chromosome 8 microsatellites were analyzed in the proband and parents (primers and conditions available upon request). DNA was extracted from peripheral blood leukocytes using the Blood Core B Kit (Qiagen, Valencia, CA).

TABLE I. Clinical and Genomic Findings in Seven Patients From This Report and Five Individuals From Literature With Invdupdel(8p)

Patient	Coordinates of the chromosomal alterations and size of the rearrangement (CMA/SNP/ Karyotypes)	Intellectual disability	Hypotonia	Typical facial features	CNS abnormalities	Congenital heart defects	Skeletal abnormalities
Patient 1	Del 8p23.1(330,897–6,420,809): 6.09 Mb; Dup 8p12→8p21 (28,529,348–39,899,187): 11.37 Mb;mosaic dup 8p11.218q11 (41,348,847–48,885,448): 7.54 Mb; dup 8q24.3 (143,626,319–146,157,954): 2.53 Mb	Mild delayed Speech development, attention deficit hyperactivity disorder	No	Small pointed chin, wide nasal bridge and a thick vermilion of the lower lip. Cutis marmorata	No	No	Asymmetry of lower limbs: both legs were of the same length but the mid-leg girth of the left one was 1 cm smaller than the right one
Patient 2	Del 8p23.1 (1–6,901,486): 6.90 Mb;Dup 8p12→8p23.1 (12,627,630–36,027,465): 23.40 Mb	Mild delayed	Yes	Prominent forehead, cupped simple ears, smooth philtrum	Fronto-parietal atrophy	No	Bilateral fifth finger clinodactyly
Patient 3	Del 8p23.1 (1–7,233,949): 7.3 Mb;Dup 8p12→8p23.1 (12,554,743–34,577,042): 22.03 Mb	Moderate delayed	Yes	Protruding tongue in the absence of macroglossia	Thinning of the corpus callosum. Enlarged extra-axial spacing	PDA[a]	Brachydactyly. Abnormal palmar creases
Patient 4	Del 8p23.1 (1–6,925,869): 6.94 Mb;Dup 8p11.1→8p23.1 (12,554,743–41,232,360): 28.76 Mb	Severe delayed	Yes	Macrocephaly, narrow and small forehead, facial asymmetry, micrognathia, ptosis, smooth filtrum, macroglossia, spaced teeth, narrow palate, tendency to open mouth and large ears	Corpus callosum agenesis	No	Valgus feet
Patient 5	Del 8p23.1 (1–6,900,000): 6.90 Mb;Dup 8p12→8p23.1 (12,296,000–32,800,000): 20.5 Mb	Severe delayed	Yes	Brachycephaly, broad forehead with bitemporal narrowing, facial asymmetry, short palpebral fissures, straight and narrow nose, low-set, posteriorly rotated ears, and large mouth	Corpus callosum agenesis	VSD[c]	Bilateral fifth finger clinodactyly
Patient 6	Del 8p23.1 (1–6,900,000): 6.90 Mb;Dup 8p11.2→8p23.1 (12,296,000–43,700,000): 31 Mb	Mild/moderate delayed	Yes	Protruding ears, straight nose with bulbous tip. High palate, thick vermilion of lips and micrognathia	NA	NA	NA
Patient 7	Kariotype:46,XX,del(8)(p23.3) invdup(8) p21.1p23.2)	NA	NA	NA	Corpus callosum agenesis.	DORV[b] + VSD[c]	NA
Mehmet et al. (2010)	Del 8p23.1: 6.71 Mb;Dup 8p11.2→8p23.1: 29.26 Mb	Severe delayed	Yes	Broad forehead, hypertelorism, strabismus of the left eye, blue sclera, retrognathia and high palate	Corpus callosum agenesis.	Dextrocardia, PDA[a]	NA
Hand et al. (2010)	Del 8p23.1: 6.8 Mb;Mosaic Del 8p21.2: 1.7Mb; Mosaic Dup p21.2→p23.1:11Mb	Mild/moderate delayed	Yes	Small pointed chin, a wide nasal base. No facial dysmorphisms	No	NA	Bilateral single palmar creases without clinodactyly
Buysse et al. (2009)	Del 8p23.1l: 6.9 Mb; Dup 8p22: 3.4 Mb;Dup 8qter→24.13: 20.9 Mb	Moderate delayed	No	Hypertelorism, intermittent strabismus of the left eyes, heterochromia iridis of the right eye, upslanting palpebral fissures, blue sclerae and slight retrognathia. Low-set, posteriorly rotated Ears	No	Supravalvular pulmonary stenosis.	Bilateral simian crease
Caglayan et al. (2009)	Del 8p23.1: 6.99 Mb;Dup 8p11.2→8p23.1: 31.51 Mb	Severe delayed	Yes	Microcephaly, frontal bossing, Malformed ears, thin vermilion of upper lip, abnormal maxilla or mandible, strabismus, coloboma	Corpus callosum agenesis.	No	No
Vermeesch et al. (2003)	Karyotype: 46,XX,del(8)(p23.3invdup(8) (p21.1p23.2)	Moderate delayed	Yes	Upward slanting palpebral fissures, synophrys, left preauricular tag. Pigmentation anomalies	No	No	Thumbs low set, bilateral clinodactyly

NA: not available. Patient 4 died within the first few days of life due to a multiorgan failure secondary to her underlying heart defect
[a]Persistent ductus arteriosus.
[b]Double outlet right ventricle.
[c]Ventricular septal defects.

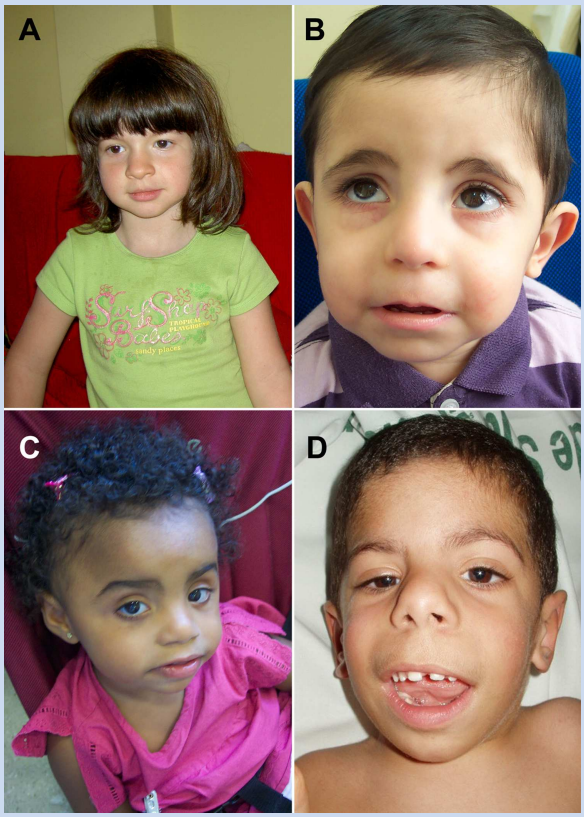

FIG. 1. Facial features of Patients 1–4. Typical facial features of invdupdel(8p) in Patients 1 (A), 2 (B), 3 (C) and 4 (D): Small pointed chin, broad-base nose, prominent forehead, detached, rotated and/or simplified ears, smooth philtrum and facial asymmetry.

RESULTS

G-Banded and FISH Chromosome Analysis

Chromosome analysis revealed extra chromosomal material in 8p in all seven patients (Table I). The small de novo supernumerary marker observed in Patient 1 was also derived from chromosome 8 pericentromeric region as demonstrated by FISH using BAC clones RP11–524J7 red/RP11–65O11 green (8q11.1/8p11.1) (Fig. 2A). The presence of the inverted duplication was confirmed by using BAC clone RP11–366G13.

FISH performed on patients 2 to 6 showed an inverted duplication with BAC clones RP11–366G13 and RP11–255E13 (Fig. 2B). BAC clones RP11–399J23 (8p23) and RP11–589N15 (8p23) showed that the mothers of Patients 2–6 were heterozygous for inv(8)(p23) (Fig. 2C). FISH studies of Patient 1 s parents were normal in this region.

Subtelomeric FISH studies showed an 8p deletion in Patients 1–6 (Fig. 2D), and a subtelomeric signal in both arms for the 8q probe in Patient 1 (Fig. 2E). Chromosome painting in Patients 1–6, revealed that the extra material was all from chromosome 8 (Fig. 2F).

Microarray Studies

Whole genome CMA and/or SNP genotyping was performed in six patients. Array studies in Patients 1–6 showed the presence of a telomeric 8p23-pter deletion followed by a normal region and then a duplication, all variable in size. No other genome wide CMA or SNP genotyping alteration was found in Patients 2–6. Patient 1 also shows a 8q24.3duplication (Fig. 3).

Microsatellite Analysis

Analysis of 13 chromosome 8p microsatellite markers showed that the deletions observed in Patients 2–6 had arisen in the maternally derived chromosomes. Microsatellite analysis of 3 markers of chromosome 8q performed in the parents of Patient 1, demonstrated the paternal origin of the derivative chromosome 8 as well as the supernumerary marker chromosome.

DISCUSSION

Chromosomal rearrangements in the short arm of chromosome 8 are relatively frequent, mainly due to the presence of two olfactory receptors gene clusters or defensin repeats (ORDRs), *REPD* in distal 8p23.1 (REPeat Distal) and *REPP* (REPeat Proximal) in proximal 8p23.1, flanking a region of about 5 Mb on 8p23.1 [Giglio et al., 2001]. In this study, we describe seven children with invdupdel(8p). In cases 1–6 we performed cytogenetic and molecular studies in probands and their parents whilst only cytogenetic studies were performed for patient 7.

Array studies in Patients 1–6 showed the presence of a telomeric 8p23-pter deletion followed by a normal region and then an inverted duplication, all variable in size. To date, only three patients with invdupdel(8p) have been cytogenetically and molecularly characterized [Buysse et al., 2009; Hand et al., 2010; Mehmet et al., 2010] and a summary of these data are shown in Table I.

The breakpoint of the terminal deletion in all cases, except for patients 1 and 3, corresponds to the ORDRs on 8p23.1 region. In Patient 1, the deletion is smaller, with the breakpoint outside of the ORDRs, extending to hg18, 8:6,420,809, and in Patient 3 the deletion is larger, extending to hg18; 8:7,223,949, but the breakpoint is also located in a defensin cluster (defensin b108 pseudogene 2).

The normal segment between the deleted and duplicated regions is similar in all cases except for Patient 1, in whom the normal region among the deletion and the duplication is larger. In cases 2–6, the terminal breakpoints of the duplication correspond to the same ORDR cluster, whilst the proximal breakpoints are variable, leading to duplications ranging from 20 to 31 Mb in size.

In five cases (Patients 2–6) the derivative chromosome 8 was of maternal origin and we have shown that the mothers are carriers of the predisposing inversion polymorphism, as described by Sugawara et al. [2003]. The high frequency of heterozygotes in the normal population (27% among Japanese population) suggest that inv(8) (p23) itself may not have any significant pathological effects on carriers [Shimokawa et al., 2004].

The invdupdel(8p) chromosome results from an ectopic recombination event within the 8p OR gene clusters [Vermeesch et al., 2003]. Floridia et al. [1996] and Giglio et al. [2001] hypothesize that, at maternal meiosis I, there was abnormal pairing of chromosomes

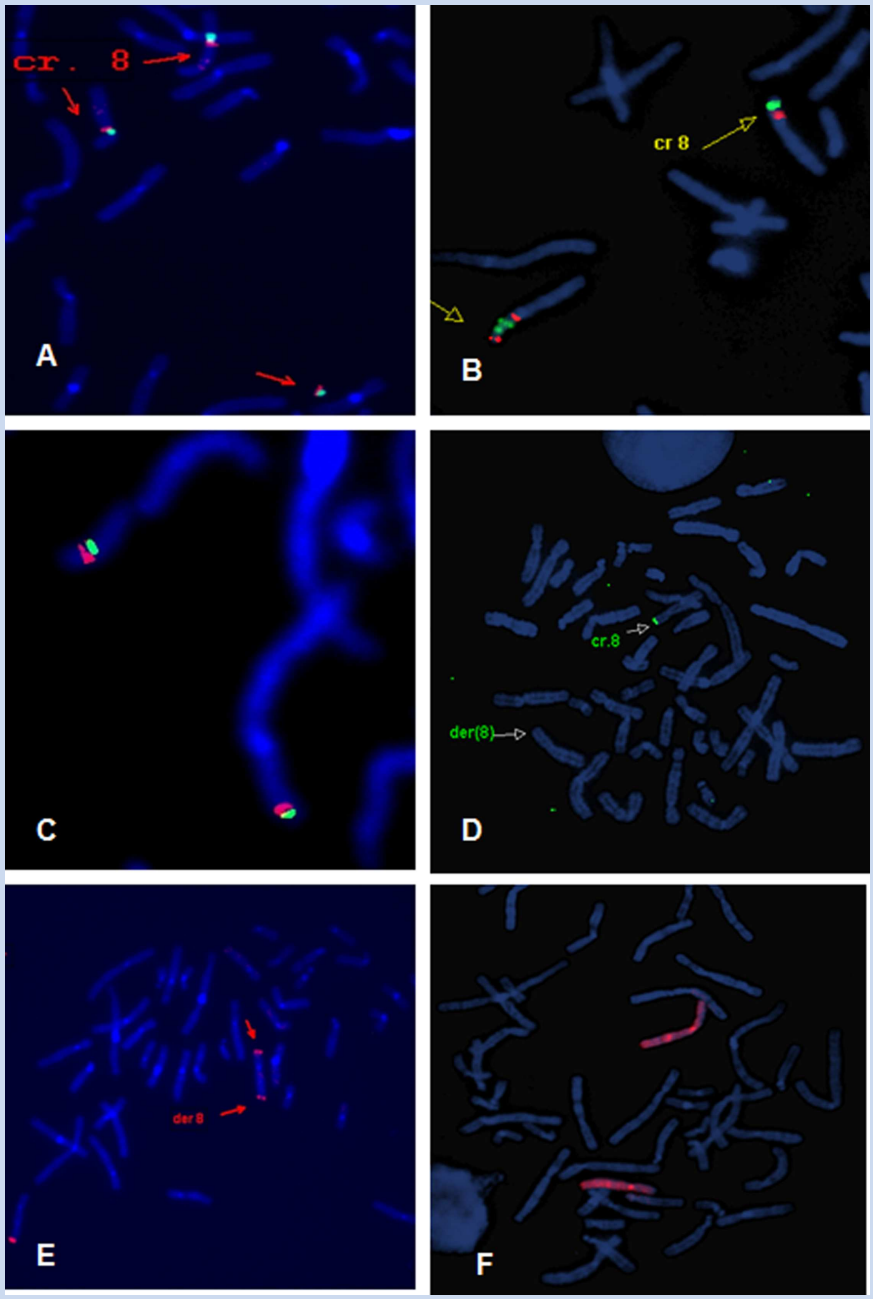

FIG. 2. FISH analysis of Patients 1 to 6. A: Origin of the small de novo supernumerary marker chromosome in Patient 1 was located on chromosome 8 pericentromeric region by using BAC clones RP11–524J7 red/RP11–65O11 green (8q11.1/8p11.1). B: (Patient 2) FISH using BAC clones RP11–366G13 (8p12) red and RP11–255E13(8p22) green performed on Patients 2 to 6 showed an inverted duplication encompassing those clones. C: (Mother of Patient 4) BAC clones (RP11–399J23 and RP11–589N15) showed that the mothers of Patients 2–6 were heterozygous for inv(8) (p23) D: (Patient 2) Subtelomeric FISH studies showed an 8p deletion in Patients 1 to 6. E: subtelomeric signal in both arms for the RP11–356M23 (8q24.23) probe in Patient 1. F: Chromosome painting for Patient 2, showing that the extra material was from chromosome 8.

8 followed by anomalous crossover at the regions delimited by D8S552 and D8S35 and by D8S252 and D8S349, which presumably contain inverted repeated sequences. The resulting dicentric chromosome, 8qter-8p23.1(D8S552)::8p23.1-(D8S35)-8q ter, due to the presence of two centromeres, breaks at anaphase I, generating an inverted duplicated 8p, dicentric if the breakage occurs at the centromere or monocentric if it occurs between centromeres. Yu and Graf [2010] proposed different mechanisms for the formation of inv-dup and its stabilization by telomere capture. According to this study, an inv-dup production requires the formation of a

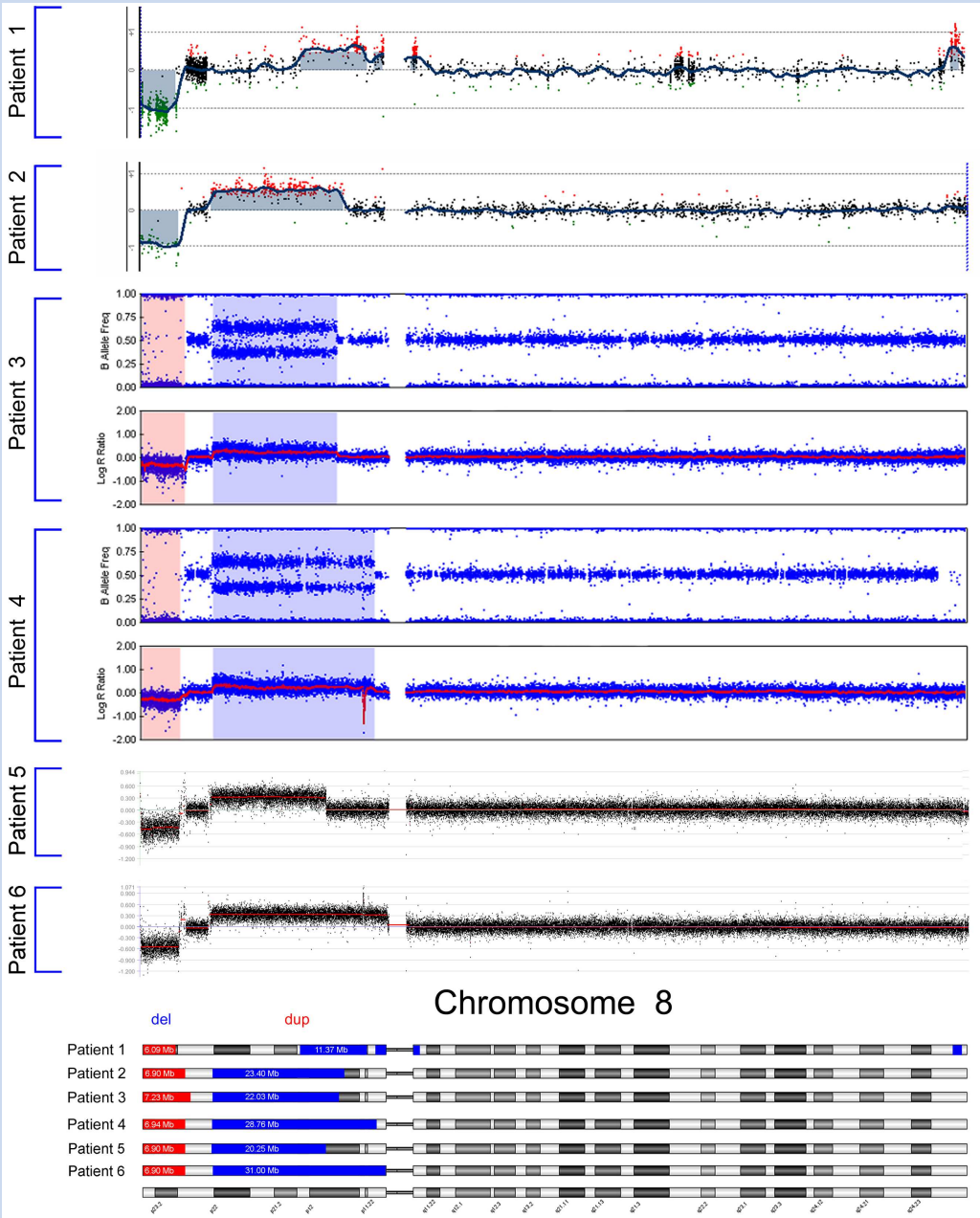

FIG. 3. CMA of Patients 1–6. Duplicated fragments are depicted in blue and deleted in red. The CMA with the custom KaryoArray® platform showed in Patient 1: a 6.09 Mb terminal 8p deletion (GRCh37 position 330,897–6,420,809), an 11.37 Mb duplicated proximal region (GRCh37 position 28,529,348–39,899,187), and a 22.11 Mb normal region between duplicated and deleted regions. CMA also showed in Patient 1 a 7.54 Mb centromeric duplication (GRCh37 position 41,348,847–48,885,448), and a 2.53 Mb 8q telomeric duplication (GRCh37 position 143,626,319–146,157,954). The CMA showed in Patient 2: a 6.9 Mb terminal 8p deletion (GRCh37 position 1–6,901,486), a 23.4 Mb duplicated proximal region (GRCh37 position 12,627,630–36,027,465), and a 5.73 Mb normal region between duplicated and deleted regions. Analysis of the SNP data with the Illumina Human610-Quad BeadChip showed in Patient 3: a 7.2 Mb terminal 8p deletion (GRCh37 position 1–7,233,949), a 22.03 Mb duplicated proximal region (GRCh37 position 12,554,743–34,577,042), and a 5.31 Mb normal region between duplicated and deleted regions. SNP data also showed in Patient 4: a 6.9 Mb terminal 8p deletion (GRCh37 position 1–6,925,869), a 28.7 Mb duplicated region (GRCh37 position 12,554,743–41,232,360), and a 5.61 Mb normal region between duplicated and deleted regions. Nimblegen array showed in Patient 5: a 6.9 Mb terminal 8p deletion (GRCh37 position 1–6,900,000), a 20.25 Mb duplicated region (GRCh37 position 12,296,000–32,800,000), and a 5.39 Mb normal region between duplicated and deleted regions. Array data also showed in Patient 6: a 6.9 Mb terminal 8p deletion (GRCh37 position 1–6,900,000), a 31 Mb duplicated proximal region (GRCh37 position 12,296,000–43,700,000), and a 5.39 Mb normal region between duplicated and deleted regions.

symmetric dicentric chromosome in the prophase of the meiosis. In the absence of functional telomeres, broken chromosome ends from the breakage of the dicentric intermediate can result in severe genomic instability if not repaired [McEachern et al., 2000].

In Patient 1, microsatellite analysis demonstrated that both the derivative chromosome 8 and the marker chromosome were paternal in origin; the mother and the father of this patient do not have the inversion polymorphism associated to the ORDR clusters, thus suggesting a different mechanism. This patient also showed a 8qter duplication, with extra material located on the short arm of chromosome 8. Patient 1 additionally has a duplication of the pericentromeric region of chromosome 8 due to an extra mosaic (56%) small ring. The absence of a single copy region at 8p23.1 excludes the involvement of OR clusters in this rearrangement. In this case, this stabilization might be carried out by the telomeres of the acentric fragment resulting from the dicentric formation and subsequent rupture. In the telomere healing mechanism, the telomeric sequences can be acquired de novo by direct addition of telomeric repeats onto the end of a broken chromosome [Yu and Graf, 2010].

As for the phenotypic characteristics associated with invdup-(8p), the 8p23.2-pter region appears to be a critical region associated with autism, intellectual disability and impaired language [Chien et al., 2010; Nucaro et al., 2011]. This region contains the genes *ARHGEF10* (OMIM 608236) and *CSMD1* (OMIM 608397), associated to central nervous system development, *CLN8* (OMIM 607837) responsible for epilepsy and progressive intellectual disability, and *DLGAP2* (OMIM 605438), postulated as a candidate gene for intellectual disability. Glancy et al. (2009 described an inherited 8p23.1–p23.2 duplication of 6.78 Mb in a family with speech delay, autism, epilepsy and learning difficulties. This duplication disrupted *CSMD1* causing haploinsufficiency of this gene. In all our patients these genes are deleted. Patient 1 has features similar to the patient described by Glancy et al. [2009]; comprising a milder phenotype than the other children. All patients except Patient 1 have practically the same telomeric 8p deletion size, thus the loss of material in this region does not allow us to clarify the phenotypic difference between them. Similarly, the less severe mental impairment in Patient 1 does not seem to be explained by the reduced number of terminally deleted genes compared with the other cases.

On the other hand, we found a correlation between the duplication size and the clinical features observed in our patients. With exception of Patient 1 whose clinical manifestations are clearly less severe, all duplications exceed 20 Mb. While it is true that all our patients showed speech delay, poor social interaction, and stereotyped movements, the two patients with the largest duplications (28.6 Mb in Patient 4 and 31 Mb in Patient 6) also showed severe intellectual disability and a larger number of malformations including abnormal face, brain alterations and limb malformations. These two duplicated regions include many genes involved in brain development and may be affected by overdoses. Fisch et al. [2011] linked the size of the duplication with IQ and autism in a series of patients with this anomaly; unfortunately phenotypic data are lacking from this publication. *MTMR7* (OMIM 603562), *CTSB* (OMIM 116810), *SGCZ* (608113), *MTMR9* (OMIM 606260), *MTMR7* (OMIM 603562) and *ATP6V1B2* (OMIM 606939) are localized in the 8p21–23 region, all of which are involved in brain development or with a putative role in epilepsy [Baulac et al., 2008]. *NRG1* (OMIM 142445) (8p12) is also duplicated in our patients. This gene has an important role in muscle spindle differentiation, and in fetal heart it has been detected in endothelial cells [Hippenmeyer et al., 2002; Lemmens et al., 2004]. Muscle spindles are complex mechanoreceptors that provide sensory information critical for proprioception and maintenance of muscle tone, and the overexpression of this gene could be responsible for the severe hypotonia observed in these patients.

All duplications, except Patient 1 also include the fibroblast growth factors *FGF17* (OMIM 603725) and *FGF20* (OMIM 605558), both of which are essential instructive signals produced by the apical ectodermal ridge and the zone of polarizing activity as the respective ectodermal and mesenchymal key signaling centers coordinating proximodistal and anteroposterior limb axis development [Benazet and Zeller, 2009]. The two patients with the larger duplications (Patients 5 and 6) also have duplication of *FGFR1* (OMIM 136350). It is well known the important role of the *FGF-FGFR* family in neuronal migration and in craniofacial anomalies including cleft lip and palate [Riley et al., 2007a, b]. The overexpression of these genes may deregulate this pathway and might explain the severe limb malformations and facial anomalies observed in these individuals. Interestingly, only Patient 1, whose duplication does not include *FGF17, FGF20,* or *FGFR1*, does not present skeletal and limb malformations.

To our best knowledge, there are no reports regarding the duplication of *FGF17, FGF20,* or *FGFR1* in patients with cardiac defects. Further, *GATA4* is in the interstitial normal region, and it is not duplicated in any patient. Thus, it is not possible to establish a direct association between the different heart anomalies found on these patients and the specific genes altered in this chromosomal aberration.

Hand et al. [2010] described a girl with a mosaic del(8p)/invdup(8p) characterized by CMA, with a similarly size deletion to that found in our cases, and the duplication is similar to that observed in our Patient 1 (11 Mb). This girl had psychomotor and speech delay without congenital anomalies, similar to our Patient 1. They suggested that the attenuated phenotype in their case may be due to compensation of one cell line for imbalances in the other cell line [Hand et al., 2010]. However, since our Patient 1 also has an attenuated phenotype without an 8p mosaic region, we hypothesize that genes responsible of physical anomalies could be located outside of this duplicated region.

In summary, the majority of our patients carry a similar deletion and different duplication; the latter probably explaining the phenotypic variability among then. Exhaustive clinical evaluation and CMA (oligos and/or SNPs) for fine narrowing of the deletion and duplication size is recommended for genetic counseling and follow-up of individuals with invdupdel(8p).

REFERENCES

Baulac S, Gourfinkel-An I, Couarch P, Depienne C, Kaminska A, Dulac O, Baulac M, LeGuern E, Nabbout R. 2008. A novel locus for generalized epilepsy with febrile seizures plus in French families. Arch Neurol 65: 943–951.

Benazet JD, Zeller R. 2009. Vertebrate limb development: moving from classical morphogen gradients to an integrated 4-dimensional patterning system. Cold Spring Harbor Persp Biol 1:a001339.

Buysse K, Antonacci F, Callewaert B, Loeys B, Frankel U, Siu V, Mortier G, Speleman F, Menten B. 2009. Unusual 8p inverted duplication deletion with telomere capture from 8q. Eur J M Genet 52:31–36.

Caglayan AO, Engelen JJ, Ghesquiere S, Alofs M, Saatci C, Dundar M. 2009. Luorescence in situ hybridization and single nucleotide polymorphism of a new case with inv dup del (8p). Genet Couns 20:333–340.

Chien WH, Gau SS, Wu YY, Huang YS, Fang JS, Chen YJ, Soong WT, Chiu YN, Chen CH. 2010. Identification and molecular characterization of two novel chromosomal deletions associated with autism. Clin Genet 78:449–456.

de Die-Smulders CE, Engelen JJ, Schrander-Stumpel CT, Govaerts LC, de Vries B, Vles JS, Wagemans A, Schijns-Fleuren S, Gillessen-Kaesbach G, Fryns JP. 1995. Inversion duplication of the short arm of chromosome 8: clinical data on seven patients and review of the literature. Am J Med Genet 59:369–374.

Devriendt K, Matthijs G, Van Dael R, Gewillig M, Eyskens B, Hjalgrim H, Dolmer B, McGaughran J, Brondum-Nielsen K, Marynen P, Fryns JP, Vermeesch JR. 1999. Delineation of the critical deletion region for congenital heart defects, on chromosome 8p23.1. Am J Hum Genet 64:1119–1126.

Fisch GS, Davis R, Youngblom J, Gregg J. 2011. Genotype-phenotype association studies of chromosome 8p inverted duplication deletion syndrome. Behav Genet 41:373–380.

Floridia G, Piantanida M, Minelli a, Dellavecchia C, Bonaglia C, Rossi E, Gimelli G, Croci G, Franchi F, Gilgenkrantz S, Grammatico P, Dalprá L, Wood S, Danesino C, Zuffardi O. 1996. The same molecular mechanism at the maternal meiosis I produces mono and dicentric 8p duplications. Am J Hum Genet 58:785–796.

Giglio S, Broman KW, Matsumoto N, Calvari V, Gimelli G, Neumann T, Ohashi H, Voullaire L, Larizza D, Giorda R, Weber JL, Ledbetter DH, Zuffardi O. 2001. Olfactory receptor-gene clusters, genomic-inversion polymorphisms, and common chromosome rearrangements. Am J Hum Genet 68:874–883.

Glancy M, Barnicoat A, Vijeratnam R, de Souza S, Gilmore J, Huang S, Maloney VK, Thomas NS, Bunyan DJ, Jackson A, Barber JC. 2009. Transmitted duplication of 8p23.1–8p23.2 associated with speech delay, autism and learning difficulties. Eur J Hum Genet 17:37–43.

Guo WJ, Callif-Daley F, Zapata MC, Miller ME. 1995. Clinical and cytogenetic findings in seven cases of inverted duplication of 8p with evidence of a telomeric deletion using fluorescence in situ hybridization. Am J Med Genet 58:230–236.

Hand M, Gray C, Glew G, Tsuchiya KD. 2010. Mild phenotype in a patient with mosaic del(8p)/inv dup del(8p). Am J Med Genet A 152A:2827–2831.

Hippenmeyer S, Shneider NA, Birchmeier C, Burden SJ, Jessell TM, Arber S. 2002. A role for neuregulin1 signaling in muscle spindle differentiation. Neuron 36:1035–1049.

Lemmens K, Fransen P, Sys SU, Brutsaert DL, De Keulenaer GW. 2004. Neuregulin-1 induces a negative inotropic effect in cardiac muscle: role of nitric oxide synthase. Circulation 109:324–326.

McEachern MJ, Krauskopf A, Blackburn EH. 2000. Telomeres and their control. Ann Rev Genet 34:331–358.

Mehmet A, Ergün A, Kula S, Karaer K, Ferda Perçin E. 2010. A case with de novo inv dup del(8p) associated with dextrocarida and corpus callosum agenesis. Pediatr Intern 52:2.

Nucaro A, Pisano T, Chillotti I, Montaldo C, Pruna D. 2011. Chromosome 8p23.2-pter: a critical region for mental retardation, autism and epilepsy. Clin Genet 79:394–395.

Riley BM, Mansilla MA, Ma J, Daack-Hirsch S, Maher BS, Raffensperger LM, Russo ET, Vieira AR, Dode C, Mohammadi M, Marazita ML, Murray JC. 2007a. Impaired FGF signaling contributes to cleft lip and palate. Proc Nat Acad Sci 104:4512–4517.

Riley BM, Schultz RE, Cooper ME, Goldstein-McHenry T, Daack-Hirsch S, Lee KT, Dragan E, Vieira AR, Lidral AC, Marazita ML, Murray JC. 2007b. A genome-wide linkage scan for cleft lip and cleft palate identifies a novel locus on 8 p11–23. Am J Med Genet Part A 143A:846–852.

Rodriguez-Revenga L, Vallespin E, Madrigal I, Palomares M, Mur A, Garcia-Minaur S, Santos F, Mori MA, Lapunzina P, Mila M, Nevado J. 2013. A parallel study of different array-CGH platforms in a set of Spanish patients with developmental delay and intellectual disability. Gene 521:82–86.

Shimokawa O, Kurosawa K, Ida T, Harada N, Kondoh T, Miyake N, Yoshiura K, Kishino T, Ohta T, Niikawa N, Matsumoto N. 2004. Molecular characterization of inv dup del(8p): Analysis of five cases. Am J Med Genet Part A 128A:133–137.

Sugawara H, Harada N, Ida T, Ishida T, Ledbetter DH, Yoshiura K, Ohta T, Kishino T, Niikawa N, Matsumoto N. 2003. Complex low-copy repeats associated with a common polymorphic inversion at human chromosome 8p23. Genomics 82:238–244.

Vallespin E, Palomares Bralo, Mori M, Martin MA, Garcia-Minaur R, Fernandez S, de Torres L, Garcia-Santiago ML, Mansilla F, Santos E, Crespo MM, Martin MC, Martinez-Glez S, Delicado V, Lapunzina A, Nevado P. 2013. Customized high resolution CGH-array for clinical diagnosis reveals additional genomic imbalances in previous well-defined pathological samples. Am J Med Genet Part A 161A:1950–1960.

Vermeesch JR, Thoelen R, Salden I, Raes M, Matthijs G, Fryns J-P. 2003. Mosaicism del(8p)/inv dup(8p) in a dysmorphic female infant: A mosaic formed by a meioticd error at the 8p OR gene and an independent terminal deletion event. J Med Genet 40:e93.

Yu S, Graf WD. 2010. Telomere capture as a frequent mechanism for stabilization of the terminal chromosomal deletion associated with inverted duplication. Cytog Gen Res 129:265–274.

Zuffardi O, Ciccone R, Giglio S, Pramparo T. 2006. Inversion chromosomes In Genomic disorders: The genomic basis of disease. In: Lupski J Stankiewicz P, editors. Totowa, New Jersey: Humana Press. pp 289–299.