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Clinical Report

A Novel 8 Mb Interstitial Deletion of Chromosome 8p12-p21.2

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We report on a girl with delayed mental and motor development, ophthalmological abnormalities, and peripheral neuropathy. Chromosome analysis suggested a deletion within chromosome 8p. Further investigation by array-based comparative genomic hybridization (array-CGH) delineated an 8 Mb interstitial deletion on the short arm of chromosome 8. The breakpoints are located at chromosome bands 8p12 and 8p21.2. Forty-two known genes including gonadotropin-releasing hormone 1 (*GNRH1*), transcription factor *EBF2*, exostosin-like 3 (*EXTL3*), glutathione reductase (*GSR*), and neuregulin 1 (*NRG1*), are located within the deleted region on chromosome 8p. A comparison of our patient with the cases described in the literature is presented, and we discuss the genotype–phenotype correlation in our patient. This is the first report of array-CGH analysis of an interstitial deletion at chromosome 8p. © 2006 Wiley-Liss, Inc.

Key words: 8p; interstitial deletion; array-CGH

INTRODUCTION

In contrast to the terminal 8p- syndrome, the phenotype of interstitial 8p deletions is rather heterogeneous [Ostergaard and Tommerup, 1989; Tsukahara et al., 1995]. Both syndromes are still poorly defined and show non-specific clinical features. Up to now about 20 cases with interstitial 8p deletions have been described in the literature. The interstitial deletions in these cases range from chromosomal band 8p11.1 to chromosomal band 8p23.1. A critical region associated with congenital heart defects has been assigned to 8p23.1 [Devriendt et al., 1999].

We report on a 2½-year-old girl with psychomotor retardation and dysmorphic features. Initially, routine chromosome analysis was performed and an interstitial deletion of 8p was suspected. Using array-based comparative genomic hybridization (array-CGH) we confirmed an interstitial deletion of chromosome 8p12 to 8p21.2 and defined the deletion size to be 8 Mb.

CLINICAL REPORT

The patient is the second daughter of healthy and non-consanguineous parents. She was born spontaneously after $40 + 2$ weeks of gestation. The pregnancy was complicated by maternal parvovirus B19-infection with seroconversion in week 20. Fetal hydrops was excluded by ultrasound examination. Her birth weight was 3,300 g, her birth length 52 cm, and her APGAR-score 10/10. A difference between the palpebral fissure length (left < right) as well as nystagmus were noticed after birth. Sparse production of tears was reported.

The mental and motor development was delayed (sitting at 14 months, crawling at 16 months). First words were spoken at the age of 22 months. Physical examination at age $2\frac{1}{4}$ revealed a small, slender female with length 82 cm (4 cm < 3rd centile), weight 9.8 kg (1.5 kg < 3rd centile), head circumference 44.6 cm (1.8 cm < 3rd centile), and with the following

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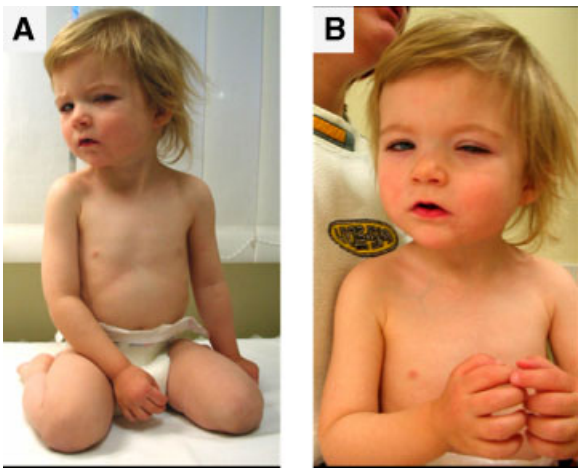

FIG. 1. Patient at age 2½ years. **A**: Frontal view of the patient. **B**: Facial aspect. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

dysmorphic features: thin upper lip, microphthalmia particularly of the left eye, strabismus convergens, and bifid uvula (Fig. 1A,B). At age 2½ she began to take tentative steps while standing on the back of her left foot. Reduced sense of pain and sleep disturbance were noticed. Because of feeding difficulties a percutanous stomach tube was recently placed. She has a generalized muscular hypotonia and no Achilles reflex. Echocardiographical examination and abdominal ultrasound were normal. Ophthalmological examination revealed hypoplastic papillae. Decreased conduction velocity of the left peroneal nerve and prolonged distal motor latency indicated peripheral neuropathy with dysmyelination. Cerebral magnetic resonance imaging showed dilatation of the inner ventricles and a small corpus callosum as signs of white matter hypoplasia. Central myelination was normal.

CYTOGENETIC AND MOLECULAR CYTOGENETIC ANALYSIS

Metaphase chromosome preparations were obtained from PHA-stimulated lymphocyte cultures from the patient and her mother according to standard procedures. Chromosome analysis was carried out on GTG banded chromosomes at a resolution of 500 bands.

A genome wide 1 Mb resolution BAC array was used for the array CGH analysis [Fiegler et al., 2003] (clones kindly provided by Nigel Carter, Wellcome Trust Sanger Centre). CGH arrays were produced at the Max-Planck Institute for Molecular Genetics, Berlin, Germany. Detailed protocols are available at the institute's webpage (http://www.molgen.mpg.de/~abt_rop/molecular_cytogenetics/ProtocolsEntry.html). In brief, BAC/PAC inserts were isolated based on a standard alkaline lysis protocol. Remaining genomic *E. coli* DNA was removed by exonuclease treatment. BAC insert DNA was amplified by Ligation-Mediated PCR [Klein et al., 1999]. Following ethanol precipitation the PCR products were dissolved in 3×SSC/1.5 M betaine and printed on epoxy slides (Advalytix, Brunnthal, Germany).

For array CGH analysis genomic DNA was extracted from peripheral blood of the patient by standard methods. Prior to hybridization, the patient's DNA and reference DNA were labeled with Cy3 and Cy5, respectively, using the BioPrime Array CGH labeling kit (Invitrogen, Carlsbad, CA). The array was hybridized for 24 hr at 42°C under humidified conditions in a SlideBooster™ hybridization station (Advalytix). Following post-hybridization washes the array was scanned with a GenePix Personal 4100A scanner and the spot intensities were measured by GenePix Pro 6.0 software (Axon Instruments, Union City, CA). Further analysis and visualization of the data was performed using CGHPRO software developed at the Max-Planck Institute for Molecular Genetics [Chen et al., 2005]. Raw data were normalized by "Subgrid LOWESS". The log2ratio of test to reference was calculated. Copy number gains and losses were determined by a conservative threshold of 0.3 and −0.3, respectively. A gain or loss was regarded as genomic aberration only if two or more neighboring clones showed a log2ratio above or below the threshold.

BAC clone RP11-395I14, which maps to chromosome band 8p21.2 was used as probe for FISH. BAC DNA was fluorescently labeled using nick translation, and hybridized to metaphase spreads of the patient's lymphocytes using standard procedures. Cep8 (Vysis, Downers Grove, IL) was used as control probe. Chromosomes were counterstained with DAPI.

RESULTS

GTG-banding chromosome analysis of peripheral blood lymphocytes indicated a possible deletion in chromosome 8p in all metaphases analyzed (Fig. 2B). To verify a loss of genomic material from chromosome 8p the patient's genomic DNA was analyzed by array-CGH with a genome wide resolution of approximately 1 Mb. Array-CGH analysis revealed an interstitial deletion spanning from 8p12 to 8p21.2 (Fig. 2A). The patient's karyotype was thus interpreted as 46,XX,del(8)(p12p21.2). The deletion encompasses an 8 Mb interval on chromosome 8p (Fig. 3). Deletion breakpoints are located between BAC clones RP11-11N9 and RP11-75P13 (8p12) and between BAC clones RP11-561E1 and RP11-395I14 (8p21.2). Array-CGH results were confirmed by FISH using BAC clone RP11-395I14 which maps within the deleted region to 8p21.2 (Fig. 2C). We mapped the deletion on the

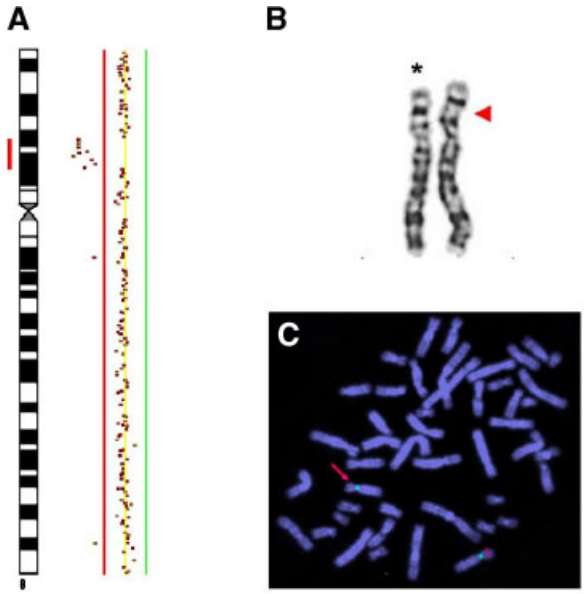

FIG. 2. **A**: Array CGH profile of chromosome 8. The log2 values of the test/reference ratios are shown. The red bar next to the ideogram marks the deleted region on chromosome 8p. **B**: Partial karyotype showing G-banded chromosomes 8 of the patient. Derivative chromosome 8 (*) shows a partial deletion of band 8p21 (red arrowhead). **C**: FISH analysis with RP11-395I14 mapping to 8p21.2 (RP11-395I14 shown in red; control probe cep8 shown in green). The deletion of the second signal on chromosome 8 is indicated by the red arrow. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

human genome sequence using the Ensembl database (www.ensembl.org; Fig. 3).

The chromosome analysis and the array-CGH profile of the patient's mother were normal. The patient's father was not available for investigation. Molecular analysis using three markers from the aberrant region showed that all markers were hemi-zygous in the patient. The remaining allele of 8p12p21.2 could be of maternal origin but we cannot rule out that our patient is homozygous for one or more of the markers. Therefore, a definite statement according to the origin of the deletion is not possible.

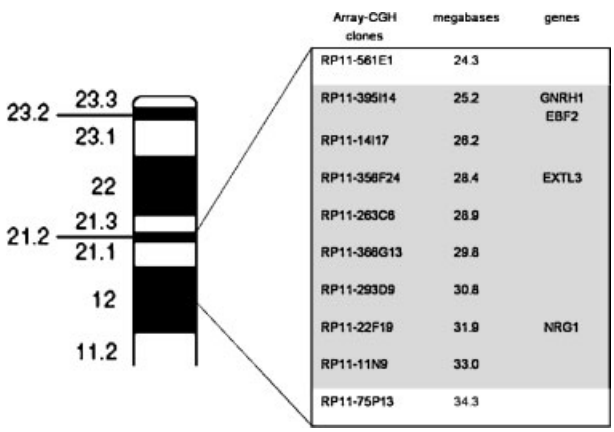

FIG. 3. Localization of the breakpoints on chromosome 8p according to array-CGH and FISH analyses. The deletion (shown in grey) is located between 24.3 and 34.3 Mb. Some of the genes located within the deleted regions are shown on the right side. For a complete list of known genes located within the deletion refer to Table II (see the online Table II at http://www.interscience.wiley.com/jpages/1552-4825/suppmat/index.html). Position of BAC clones according to Ensembl version 31, April 2005 (http://www.ensembl.org).

DISCUSSION

In the present study, an interstitial deletion 8p12-p21.2 was identified in a girl with delayed mental and motor development, ocular abnormalities (papillar hypoplasia), and peripheral neuropathy. Chromosome analysis suggested an aberrant chromosome 8p. However, the exact determination of the deletion size was not possible by conventional cytogenetic analysis. Subsequent analysis by array-CGH revealed an 8 Mb deletion with breakpoints in chromosome bands 8p12 and 8p21.2. This demonstrates the value of the higher resolution of array-CGH for cytogenetic analysis.

To our knowledge, 14 cases of interstitial deletion 8p with an overlap with 8p12-p21.2 have been described in the literature summarized in Table I. Deletions in these cases range from chromosomal band 8p11.1 to band 8p23.1. Microcephaly, mental retardation and genital hypoplasia are common findings in patients with interstitial deletion of 8p (Table I). Heart defects are commonly associated with distal 8p deletions. In the present case no heart defects were detected which is in concordance with the interstitial deletion being located proximal to the critical deletion region for congenital heart defects. On the other hand hereditary spherocytic anemia has been described as common feature in patients with a proximal 8p deletion including chromosomal band p11.2 which is thought to be due to hemi-zygosity for *ANK1*. Mutations of *ANK1* are associated with hereditary spherocytosis. As expected from the phenotype of our patient the deletion described here does not overlap with the critical region in 8p11.2 containing *ANK1*.

Compared to conventional cytogenetics a further advantage of array-CGH is the possibility to directly map the deletion on the human genome sequence thereby facilitating the identification of candidate genes. Within the deleted region on chromosome 8p lie ~42 known genes (see the online Table II at http://www.interscience.wiley.com/jpages/1552-4825/suppmat/index.html) including gonadotropin-releasing hormone 1 (*GNRH1*), transcription factor *EBF2*, exostosin-like 3 (*EXTL3*), glutathione reductase (*GSR*), Werner syndrome helicase (*WRN/RECQL2*), and neuregulin 1 (*NRG1*). Homozygous mutations in the gene for *GSR* are a cause of hemolytic anemia related to low levels of glutathione. Werner syndrome is an autosomal recessive disorder caused by mutations in the *RECQL2* gene, which encodes a homolog of the *E. coli* RecQ DNA helicase. Affected individuals usually have a prematurely aged appearance as well as premature occurrence of arteriosclerosis and diabetes mellitus.

TABLE I. Comparison of Present Case to Previously Reported Cases of 8p Interstitial Deletion

Clinical characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Present case
Deletion 8p	p21-p23	p11-p21	p11.1-p21.1		p11.22-p21.1	p11.1-p21.1	p11-p21		p11.2-p21	p21.1-p23.1	p11.23-p21.3	p11.23-p21.1	p12-p22	p11.2	p12-p21.2
Sex	M	F	F	F	M	M	F	M	M	M	F	M	M	M	F
Psychomotor retardation	+	+	+	+	+	+	+	+		+	+	+	+	–	+
Growth retardation	+	+	+	+	+	+				+		+	–	+	+
Microcephaly	–	+	–	–	+	+	+	+		+	+	+	+	–	+
Facial dysmorphism	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ocular abnormality		+	+	+		+					–	+	+	–	+
Hypogonadism		+			+	+	+	+	+	+		+	+	+	+
Neuropathy				+			+					+		–	+
Cardiac anomaly	–	–								+	+	+	–	–	–
Spherocytosis			+	+	+	+	+	+	–		–	+		+	–

1, Orye and Craen [1976]; 2, Beighle et al. [1977]; 3, 4, Chilcote et al. [1987]; 5, Kitatani et al. [1988]; 6, Lux et al. [1990]; 7, 8, Cohen et al. [1991]; 9, Stratton et al. [1992]; 10, Marino et al. [1992]; 11, Tsukahara et al. [1995]; 12, Okamoto et al. [1995]; 13, Devriendt et al. [1999]; 14, Vermeulen et al. [2002].

Pulsatile secretion of GNRH1 by the hypothalamus is responsible for the initiation and maintenance of the reproductive axis in humans. GNRH-deficiency due to failure of GNRH1 secretion is the main cause for hypogonadotropic hypogonadism [Seminara et al., 1998]. Signs of hypogonadotropic hypogonadism (e.g. micropenis, cryptorchidism) were observed in 10 of 14 patients (71%) who show deletions overlapping with our case including the *GNRH1* gene (8p21.2) [Beighle et al., 1977; Cohen et al., 1991; Marino et al., 1992; Stratton et al., 1992; Devriendt et al., 1999].

A second gene, *EBF2*, located proximal to the *GNRH1* gene, has been suggested as a candidate gene for idiopathic hypogonadotropic hypogonadism. Lack of EBF2 transcription factor expression in mice causes defective migration of GNRH-synthesizing neurons, thereby leading to secondary hypogonadism [Corradi et al., 2003]. In addition, *EBF2*-null mice exhibit peripheral neuropathy caused by defective axon sorting, hypomyelination, segmental dysmyelination, axonal damage, and a decrease in motor nerve conduction velocity. A more proximal gene, *NRG1*, also plays an essential role in the nervous system, as well as in heart development [Falls, 2003]. Reduced *NRG1* expression in mutant mice caused hypomyelination and reduced nerve conduction velocity [Michailov et al., 2004]. Neurological findings such as muscle hypotonia or absent/decreased reflexes were described in four other patients with interstitial 8p deletions [Chilcote et al., 1987; Cohen et al., 1991; Okamoto et al., 1995]. Neurophysiologic investigation of our patient revealed a decreased conduction velocity and prolonged distal motoric latency due to dysmyelination similar to the phenotype of *EBF2*-null mice described by Corradi et al. [2003]. These observations suggest that haploinsufficiency of *EBF2* and *NRG1* might be the cause of the peripheral neuropathy observed in our patient.

EXTL3 encodes a glycosyltransferase implicated in heparan sulfate biosynthesis. By studying zebrafish mutants (ext2/dackel and extl3/boxer) Lee et al. [2004] demonstrated two distinct roles for heparan sulfate in retinal axon guidance: (1) sorting of dorsal axons within the optic tract; (2) path finding to the tectum. The papillar hypoplasia of our patient could result from a defective axon sorting within the optic tract due to impaired heparan sulfate biosynthesis. One case described by Okamoto et al. [1995] with a deletion encompassing the *EXTL3* gene had retinal dysplasia and was virtually blind. Ocular abnormalities like strabismus and nystagmus have also been described in five other patients with interstitial deletions which show overlap with our case [Beighle et al., 1977; Chilcote et al., 1987; Lux et al., 1990; Devriendt et al., 1999].

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