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Branchiootorenal Syndrome and Oculoauriculovertebral Spectrum Features Associated With Duplication of *SIX1*, *SIX6*, and *OTX2* Resulting From a Complex Chromosomal Rearrangement

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We report on a 26-month-old boy with developmental delay and multiple congenital anomalies, including many features suggestive of either branchiootorenal syndrome (BOR) or oculoauriculovertebral spectrum (OAVS). Chromosomal microarray analysis (CMA) initially revealed a copy-number gain with a single BAC clone (RP11-79M1) mapping to 14q23.1. FISH analysis showed that the third copy of this genomic region was inserted into the long arm of one chromosome 13. The same pattern was also seen in the chromosomes of the father, who has mental retardation, short stature, hypernasal speech, and minor craniofacial anomalies, including tall forehead, and crowded dentition. Subsequent whole genome oligonucleotide microarray analysis revealed an ~11.79 Mb duplication of chromosome 14q22.3–q23.3 and a loss of an ~4.38 Mb sequence in 13q21.31–q21.32 in both the propositus and his father and FISH supported the apparent association of the two events. Chromosome 14q22.3–q23.3 contains 51 genes, including *SIX1*, *SIX6*, and *OTX2*. A locus for branchiootic syndrome (BOS) has been mapped to 14q21.3–q24.3, and designated as branchiootic syndrome 3 (BOS3). Interestingly, mutations in *SIX1* have been reported in patients with BOR/BOS3. We propose that the increased dosage of *SIX1*, *SIX6*, or *OTX2* may be responsible for the BOR and OAVS-like features in this family. © 2008 Wiley-Liss, Inc.

Key words: *SIX1*; *SIX6*; *OTX2*; branchiootorenal syndrome; oculoauriculovertebral spectrum; segmental trisomy; insertional translocation

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INTRODUCTION

Branchiootorenal syndrome (BOR; OMIM 113650) is an autosomal dominant disorder caused by mutations in the *EYA1* gene (OMIM 601653) [Abdelhak et al., 1997], a human homolog of the Drosophila eyes absent gene (*Eya*) [Bonini et al., 1993]. BOR is characterized by hearing loss, branchial cleft fistulas or cysts, ear pits, renal dysplasia, and otologic manifestations ranging from mild hypoplasia to complete absence with reduced penetrance and variable expressivity [Melnick et al., 1975; Fraser et al., 1978]. Clinical diagnosis of BOR is based on the presence of (1) at least three major criteria including branchial anomalies, deafness, preauricular pits, and renal abnormalities, (2) two major criteria, and at least two minor criteria including external ear anomalies, middle ear anomalies, inner ear anomalies, preauricular tags, facial asymmetry, and palate abnormalities, or (3) one major criterion and an affected first-degree relative who meets the criteria for BOR syndrome [Chang et al., 2004]. Branchiootic syndrome (BOS; OMIM 602588), a related disorder but without the renal anomalies, can also be caused by allelic defects in *EYA1* [Vincent et al., 1997]. A second locus for BOS was localized to chromo-

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some 1q31 (OMIM 120502) [Kumar et al., 2000]. Recently, Ruf et al. [2003] mapped a third gene locus for BOR/BOS to 14q21.3–q24.3 by linkage study and designated it as branchiootic syndrome 3 (BOS3; OMIM 608389). This chromosomal region contains the *SIX1*, *SIX4*, *SIX6* (OMIM 601205, OMIM 606342, OMIM 606326, respectively) gene cluster, the products of which are known to act in a developmental pathway of the *EYA* genes [Xu et al., 1999], and *OTX2* (OMIM 600037). Mutations in *SIX1* have been reported in patients with BOR/BOS, thus identifying *SIX1* as a gene causing BOR and BOS [Ruf et al., 2004].

Oculoauriculovertebral spectrum (OAVS; OMIM 164210) is a common birth defect pattern (1 in 5,600 live births) involving first and second branchial arch derivatives [Gorlin et al., 1990, 2001]. The phenotype of OAVS is extremely variable, with no generally accepted minimal diagnostic criteria [Regenbogen et al., 1982; Kaye et al., 1992]. The disorder includes the Goldenhar syndrome, which is defined as the combination of microtia, hemifacial microsomia, dermoids, and vertebral anomalies. The features of OAVS include unilateral malformation of the external ear and asymmetric facial hypoplasia with epibulbar dermoid and vertebral anomalies. Coloboma of the upper eyelid is frequent. The ear defects include preauricular tags of cartilagenous masses, atresia of the external auditory canal, anomalies in the size and shape of the external auricle, and anotia [Gorlin et al., 2001]. Cardiac, vertebral, and central nervous system defects have also been described [Rollnick et al., 1987]. The etiology of OAVS is unclear; autosomal dominant, autosomal recessive and multifactorial inheritance have been described [Rollnick and Kaye, 1983; Kelberman et al., 2001]. Chromosomal abnormalities described in patients with OAVS include trisomies 18 [Verloes et al., 1991] and 22 [Kobrynski et al., 1993], mosaic trisomies 9 [Wilson and Barr, 1983] and 17 [Hodes et al., 1981], duplication of 8pter [Josifova et al., 2004], deletions of 5p14 to 5pter [Choong et al., 2003] and 22qter [Herman et al., 1988], pericentric inversions of chromosome 1 with breakpoints at p13 and q21 [Stahl-Maugé et al., 1982] and chromosome 9 with breakpoints at p11 and q13 [Stanojević et al., 2000], and the recurrent unbalanced translocation der(11)t(11;22)(q23;q112) [Balci et al., 2006]. Furthermore, data from a family with features of hemifacial microsomia suggest linkage to a region of approximately 10.7 cM on chromosome 14q32 [Kelberman et al., 2001].

We describe a patient with features of BOR and OAVS in whom a chromosome segment from 14q22.3–q23.3 is duplicated and directly inserted into chromosome 13q21, resulting in a ~11.79 Mb trisomy of chromosome 14q22.3–23.3 that includes *SIX1*, *SIX6*, *OTX2*, and an associated deletion of an ~4.38 Mb gene-poor region on chromosome 13q21.31–q21.32. Interestingly, the patient's father and two other paternal relatives have the same unbalanced aberration and learning disability, significant developmental delays, and subtle dysmorphic features. We propose that increased dosage of *SIX1*, *SIX6*, or *OTX2* may be responsible for BOR- and OAVS-like phenotypes in this family.

CLINICAL REPORT

The propositus (Fig. 1A–C) is a 26-month-old male born to a primiparous 21-year-old woman at 39 weeks by cesarean section. Ultrasound studies at 21 weeks gestation suggested an occipital encephalocele or meningocele. Amniocentesis performed at 11 weeks showed a normal male karyotype. His birth weight was 1,997 g (1st centile) and head circumference was 31 cm (2nd centile). Initial evaluation at 6 weeks of age indicated a weight of 3.04 kg (<3rd centile), length of 48.1 cm (<3rd centile) and head circumference of 34.7 cm (1st centile). At 8 months weight was 5.49 kg (<3rd centile), length 61.7 cm (<3rd centile) and head circumference 41.1 cm (<3rd centile); he could smile and babble, reach for objects, and transfer objects from hand to hand. He was turning to voices, but was not yet sitting. Re-evaluation at 26 months showed persistent growth delays and microcephaly, with weight 7.65 kg (<3rd centile), length 76.3 cm (<3rd centile) and head circumference 43.5 cm (<3rd centile). He was walking with some assistance and using several single words to communicate.

Physical findings first noted in the newborn period included large fontanels and a tall prominent forehead. The right occiput appeared more prominent than the left occiput. Torticollis to the left was noted. He had a recessed jaw with a highly arched palate and possible short tongue. He had multiple skin tags in the preauricular and cheek regions as well as pits on the right cheek. Other dysmorphic features included short nose with broad nasal bridge, short palpebral fissures with copious tearing suggesting lacrimal duct stenosis (he underwent subsequent lacrimal duct stent placement bilaterally), low set ears with abnormal antihelix and ear lobe, prominence of the maxilla, severe hypoplasia of the mandible with broadening of the oral region, and macrostomia with bilateral Tessier number 7 clefts (with subsequent surgical reconstruction at 8 months of age) and micrognathia. His neck appeared short and abnormally shaped but with no webbing.

Cranial ultrasound showed normal sulcal pattern with no ventriculomegaly. MRI of the brain on day of life 4 showed no evidence of hydrocephalus or Chiari malformation. A 5 cm meningocele was noted with fibrous band tissue and dura but no brain parenchyma. Corpus callosum was thin.

Because of a deep sacral dimple, neonatal ultrasound of the spinal cord was performed and showed conus tip slightly low at L4 with normal filum

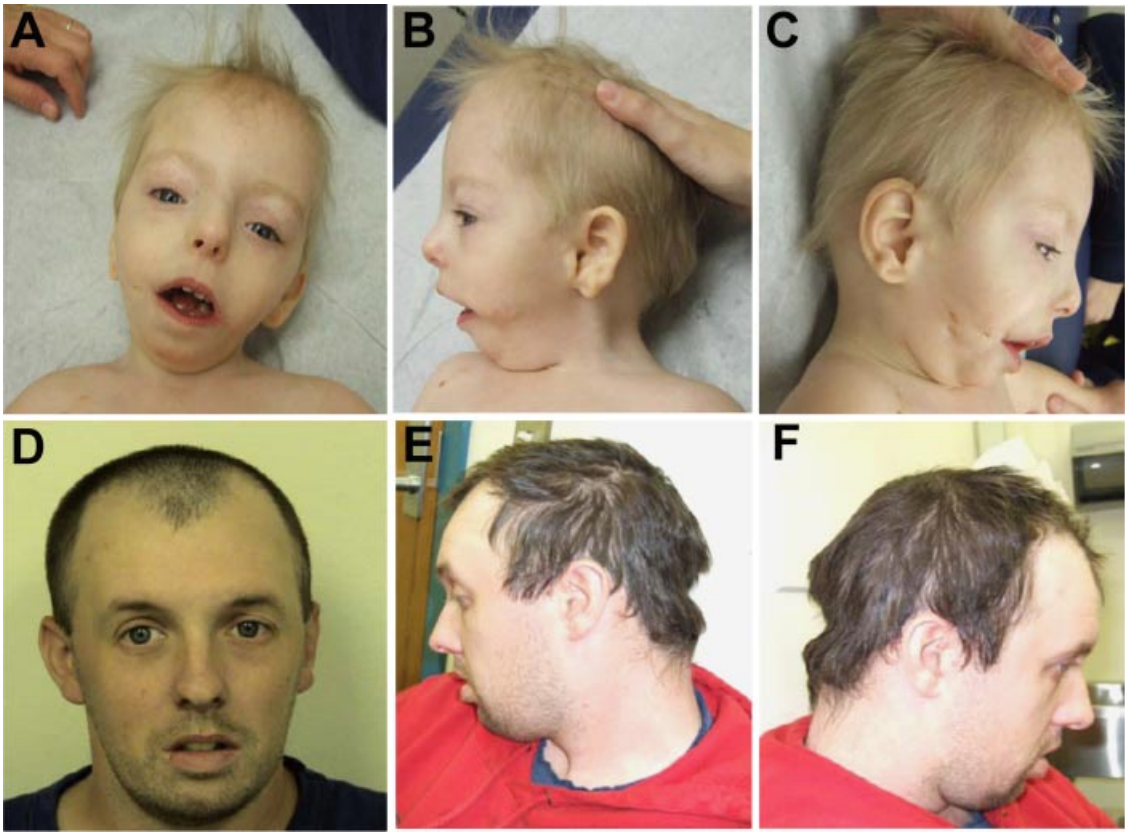

FIG. 1. Photographs of the propositus 26 months (**A–C**) and his father (**D–F**). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

terminali. Spine MRI at 6 months of age showed conus tip at approximately the L2–L3 level with no evidence of tethered cord, diastematomyelia, or a hydrosyringomyelia cavity. A repeat spine MRI at 10 months of age demonstrated conus tip at L3 with mild thickening and fatty infiltration of the filum terminale.

Renal usonogram showed small kidneys, the right kidney was three standard deviations below normal, and the left kidney was two standard deviations below normal with no hydronephrosis or cysts, and normal echogenicity. Genitalia were notable for a small, uncircumcised penis with absence of testes in the scrotum. He also had an anterior ventricular septal defect not requiring surgical repair.

Skeletal survey at day 7 showed poorly ossified and irregular skull shape, protuberance of the maxilla, and hypoplasia of the mandibles. Spine MRI at 6 and 10 months of age and cervical spine X-ray series at 26 months of age showed fusion of the pedicles and laminae on the left at C2–4. This produced a mild dextroconvex curve of the cervical spine with occipital tilt left side down, noted clinically as left torticollis (Fig. 1). There was no evidence of polydactyly or syndactyly.

Ophthalmology evaluation was significant for right-sided optic nerve hypoplasia that was not confirmed on re-evaluation. He passed his newborn hearing screen bilaterally but an auditory brainstem response evaluation at 2 years of age showed moderate to moderately severe hearing loss with a large conductive component; left ear was noted to have Eustachian tube dysfunction and the right ear had adhesive otitis media components. He was referred for fitting with hearing aids.

His father (Fig. 1D–F) is 29 years old and his mother is 21 years old. Both parents were reported with learning disabilities and significant developmental delay. The propositus' father has never been hospitalized or had any surgeries or chronic illnesses except asthma. He graduated from an Intermediate School District with special education services, but is unable to read or perform addition or subtraction. He has no hearing problems. On examination he demonstrated a height of 160 cm, weight of 58 kg and head circumference of 54 cm. His speech was hypernasal; hair was dark brown with coarse texture and normal distribution. Minor craniofacial anomalies included tall forehead, with level palpebral fissures. There were no ocular abnormalities and his gaze was conjugate. Dentition was crowded with slight decay with a normally arched palate. Uvula was single and midline. The neck was supple with no shortening, masses, or webbing; there were no

apparent digital or creasing pattern abnormalities. Neurologic, cardiac and pulmonary examinations revealed no abnormalities. By report from the father, the propositus' paternal aunt has a chromosome 13 abnormality and her 5-year-old daughter has agenesis of the corpus callosum and developmental delay. There is no evidence of consanguinity in the family.

SUBJECTS AND METHODS

We obtained DNA samples from the proband and his family members after acquiring informed consents approved by the Institutional Review Board for Human Subject Research at Baylor College of Medicine.

Cytogenetic and FISH Analyses

Chromosome analysis was performed using standard protocols for the GTG-banding technique. FISH was performed as previously described [Shaffer et al., 1997]. BAC clones for FISH analysis were chosen using the UCSC genome browser (http://genome.ucsc.edu).

Array CGH

The chromosomal microarray analysis (CMA) was conducted on a clinically available microarray containing 853 BAC and PAC clones designed to cover genomic regions of 75 known genomic disorders, all 41 subtelomeric regions, and 43 pericentromeric regions [Cheung et al., 2005; Baylor College of Medicine, Chromosome Microarray Analysis, V.5, http://www.bcm.edu/cma/assets/abnormalities.pdf]. Procedures for DNA labeling and comparative genomic hybridization were described previously [Yu et al., 2003]. The fluorescent signals on the slides were scanned into image files using an Axon microarray scanner and ScanArray software (GenePix 4000B from Axon Instruments, Union City, CA). For each sample, two experiments were performed with reversal of the dye labels for the control and test samples, and the data from both dye-reversed hybridizations were integrated to determine inferences for each case. Microarray image files were quantified using GenePix Pro 4.0 software. The quantitation data were subjected to normalization and integration for all clones analyzed form a single patient sample as described [Shaw et al., 2004]. In addition, a single clone T-statistic and permutation based *P*-value were also computed, providing further criteria to determine whether a clone deviates significantly from the mean [Lu et al., 2007].

Whole Human Genome Oligo Microarray Kits 244K and 44K (Agilent Technologies, Inc., Santa Clara, CA) were used to analyze DNA from the proband and his father to further refine the identified genomic gains and losses. The procedures for DNA digestion, labeling, and hybridization were performed according to the manufacturer's instructions with some modifications [Probst et al., 2007].

RESULTS

Karyotype analysis of the proband at 500-band resolution showed extra material inserted into chromosome 13q21 (Fig. 2A). Analysis of parental chromosomes revealed that the father had the same abnormal karyotype, whereas the mother had a normal karyotype.

CMA in the propositus showed a gain of copy number with BAC clone RP11-79M1 on chromosome 14q23.1 (Fig. 2B). FISH analysis of the patient's father with RP11-79M1 showed the same aberration with the third copy inserted into 13q (Fig. 2C). Whole genome oligonucleotide microarray analysis using Agilent 244K (propositus), and 44K (father, data not shown) demonstrated that the proximal breakpoint of the duplication mapping to 14q22.3 at ~55.774 Mb and the distal breakpoint mapping to 14q23.3 at ~67.571 Mb (hg18 assembly), spanning an ~11.79 Mb genomic region (Fig. 3C,D). Surprisingly, the whole genome oligoarray also revealed a ~4.38 Mb deletion at 13q21.31–q21.32 with the proximal breakpoint mapping at ~62.339 Mb and the distal breakpoint mapping at ~66.718 Mb (Fig. 3A,B).

Using FISH with the clones flanking the breakpoints on chromosomes 13q and 14q, we confirmed that the insertion mapped within the deletion breakpoint positions, suggesting a single step event. To determine the orientation of the inserted fragment, the 13q21 specific BAC clones RP11-79D3 (centromeric and adjacent to the proximal deletion breakpoint), RP11-326B4 (telomeric and adjacent to the distal deletion breakpoint), and 14q22–q23 specific BAC clones RP11-10P7 (proximal end of the insertion), and RP11-305I24 (distal end of the insertion) were co-hybridized in metaphase FISH experiments in different combinations (Fig. 4). The results showed that the 14q22.3–q23.3 fragment was inserted in direct orientation into 13q21, with a loss of the 13q21.31–q21.32 segment. The propositus' karyotype was designated as 46,XY,der(13)del(13)(q21.31–q21.32)dir ins(13;14)(q21.32;q22.3q23.3).

DISCUSSION

Chromosomal insertions are rare structural chromosomal aberrations with an estimated frequency of 1 in 5,000 to 1 in 80,000 live births [Chudley et al., 1974; Van Hemel and Eussen, 2000]. Chromosomal insertions with associated deletion near or at the insertion breakpoint have been reported very rarely [Lukusa et al., 1999; Van Hemel and Eussen, 2000].

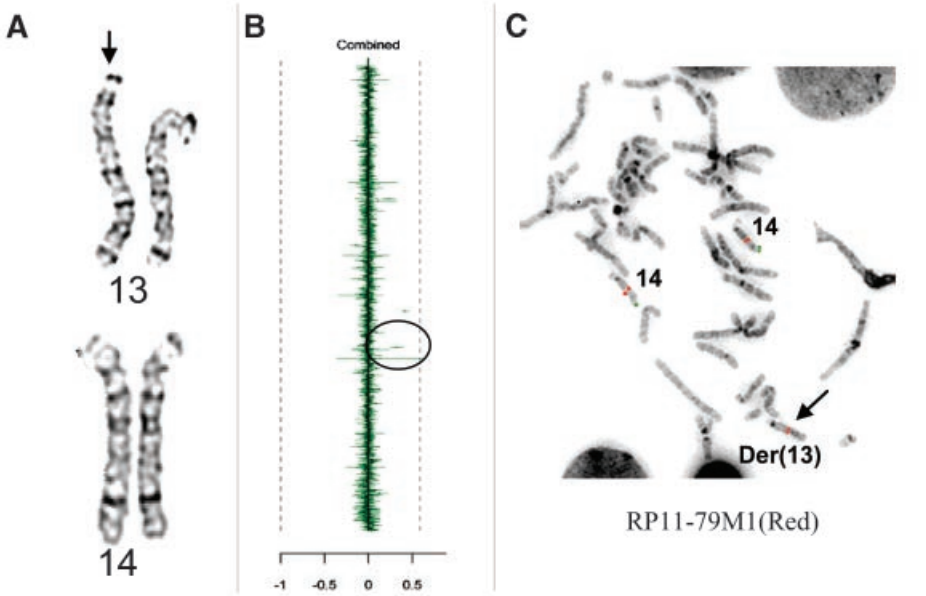
A
13
14
B
Combined
-1
-0.5
0
0.5
C
14
14
Der(13)
RP11-79M1(Red)
46,XY,der(13)del(13)(q21.31-q21.32)ins(13;14)(q21.32;q22.3q23.3)

FIG. 2.

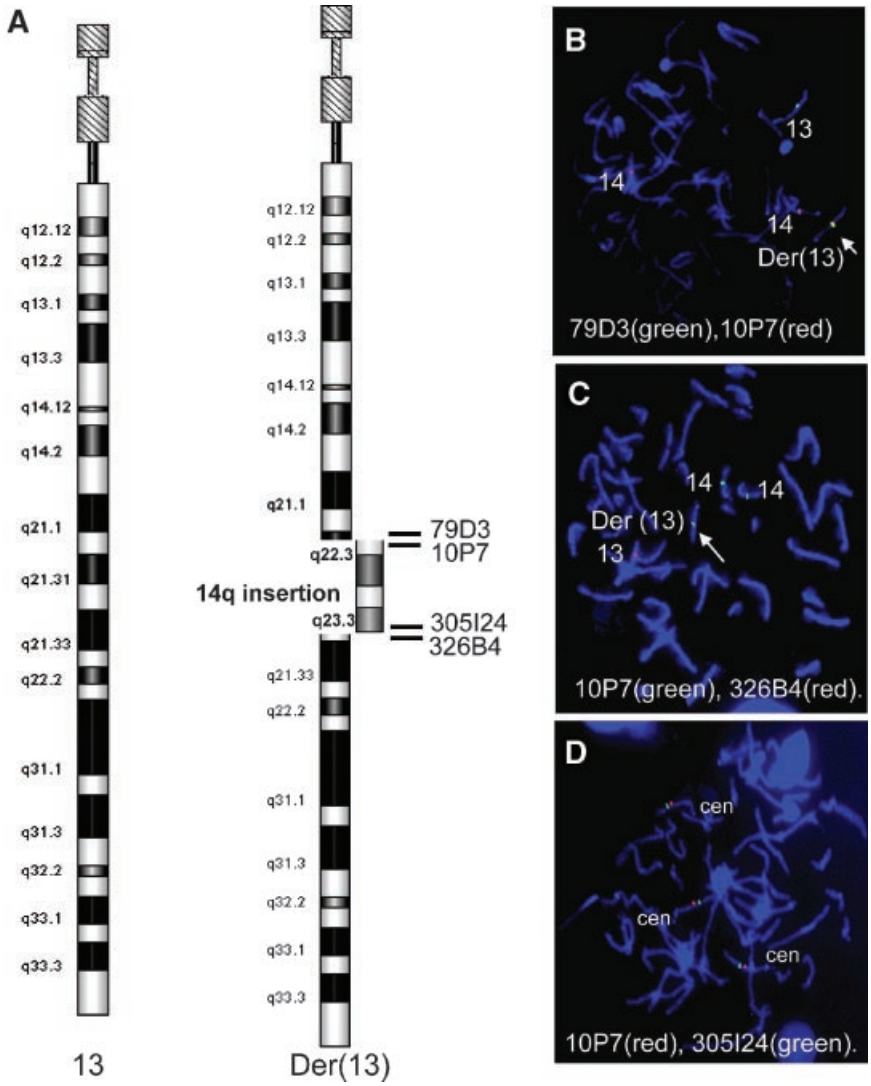
A
q12.12
q12.2
q13.1
q13.3
q14.12
q14.2
q21.1
q21.31
q21.33
q22.2
q31.1
q31.3
q32.2
q33.1
q33.3
13
q12.12
q12.2
q13.1
q13.3
q14.12
q14.2
q21.1
q22.3
79D3
10P7
14q insertion
q23.3
305I24
326B4
q21.33
q22.2
q31.1
q31.3
q32.2
q33.1
q33.3
Der(13)
B
13
14
14
Der(13)
79D3(green),10P7(red)
C
14
14
Der (13)
13
10P7(green), 326B4(red).
D
cen
cen
cen
10P7(red), 305I24(green).

FIG. 4.

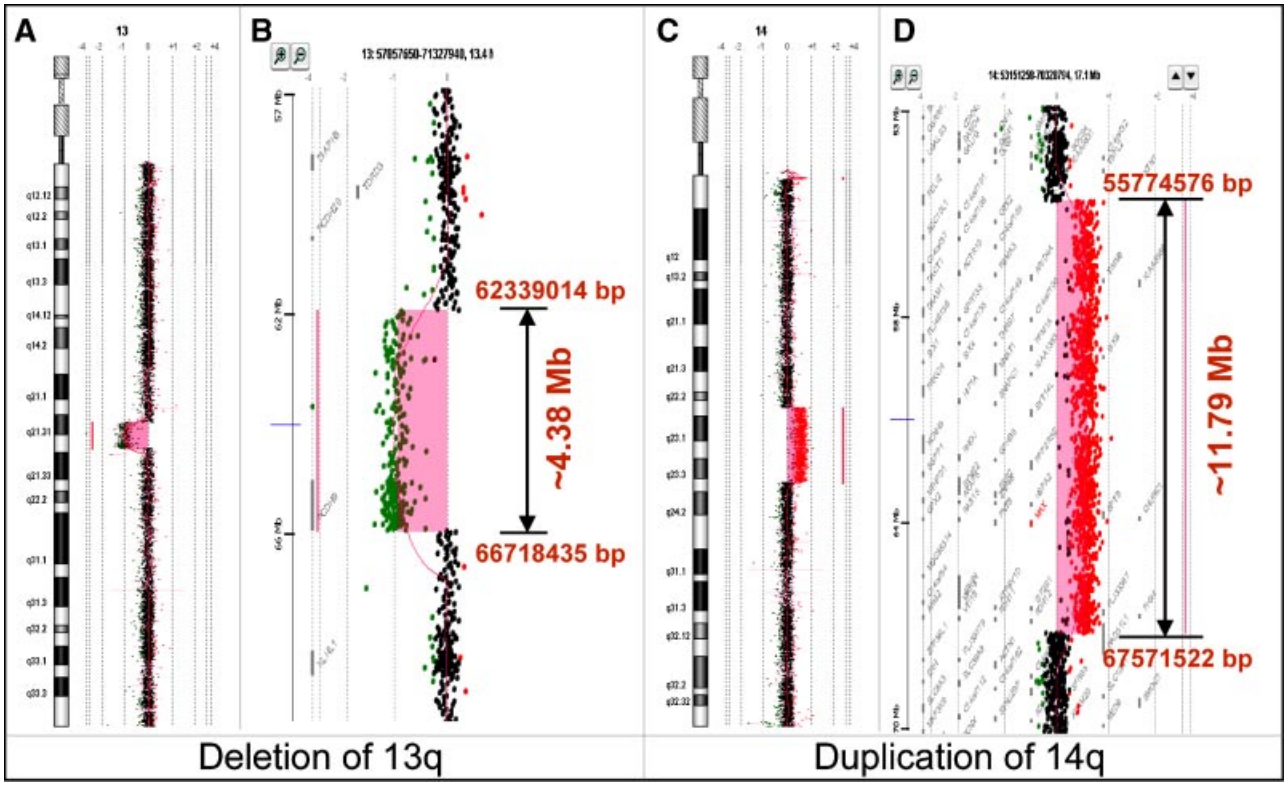

FIG. 3. Results of the whole genome oligonucleotide microarray analysis (244K) showing an ~4.38 Mb deletion at 13q21.3 (**A**) and an ~11.79 Mb duplication at 14q23 (**C**). **B**,**D**: Show enlargements of the deletion region at 13q21.3 and duplication region at 14q23, respectively. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

The propositus presented with a severe phenotype, including ear tags, renal dysplasia, lop-ear defect, and lacrimal duct stenosis, suggesting BOR. In addition to the features typical for BOR, he had some traits for OAVS, including prominent forehead, meningocele, protuberance of the maxilla, severe hypoplasia of the mandible, macrostomia, short palpebral fissures, micrognathia, heart defect, and vertebral anomalies (Table I). Combined with other typical dysmorphic features, CNS defects such as hydrocephalus, occipital encephalocele, cerebellar hemisphere/vermis hypoplasia, and lipoma of the corpus callosum may be clues to the prenatal diagnosis of OAVS [Castori et al., 2006].

As reported previously, a number of patients with BOR have characteristics overlapping with OAVS [Cohen et al., 1989]. Both syndromes are associated with malformations of the external ears, preauricular tags, pits, or sinuses, and conductive or mixed hearing loss [Rollnick and Kaye, 1985]. Hearing loss is a major clinical finding which has been observed in 93% of BOR patients [Hone and Smith, 2001]. The overlapping clinical features suggest that OAVS may constitute the severe end of the spectrum of BOR in some families [Rollnick and Kaye, 1985; Sensi et al., 1996].

The phenotypes of patients with the 13q deletions proximal to 13q32 are mild [Ballarati et al., 2007].

FIG. 2. **A**: Partial G-banded karyotype of the patient, with an arrow indicating the extra material on chromosome 13q. Chromosomes 14 are normal. **B**: CMA profile of patient. One clone on 14q23 showed displacement to the right (circled), indicating a gain of 14q23 material in the patient versus the reference DNA. **C**: FISH analysis of patient's father with probe RP11-79M1 specific for chromosome region 14q23 (red) demonstrating the presence of 14q23 material on der(13q21; shown by arrow).

FIG. 4. The location and orientation of the insertion fragment 14q22–q23 in the 13q21 region are illustrated by FISH analysis. **A**: A diagram depicting the derivative chromosome 13 with the insertion of chromosome 14q22.3–q23.3 into chromosome 13q21, resulting in the deletion of chromosome 13q21.31–q21.32. The position of the four BACs used for the combination FISH are shown on the right in panel A. **B**: BACs RP11-79D3 (green signal) from chromosome 13 hybridized to both the normal chromosomes 13 and the der(13), while BAC RP11-10P7 from chromosome 14 (red signal) hybridized on both chromosomes 14 and der(13). The overlapping green and red signals on der(13; indicated by the white arrow) demonstrate one end of the insertion. **C**: RP11- 10P7 from chromosome 14 (green signal) was hybridized to both chromosomes 14 and der(13), while RP11-326B4 (red signal) hybridized to normal chromosomes 13 and der(13). Both red and green signals on the der(13) showed two separate distinct signals (white arrow), revealing the distal end of the insertion. **D**: RP11-10P7 labeled in red and RP11-305I24 labeled in green both hybridized on chromosomes 14 and der(13) in the same direction with respect to the centromere of both chromosomes 14 and der(13); the same red–green orientation are shown indicating the direct insertion of 14q22–q23 fragment into 13q21.

TABLE I. Phenotypic Features of BOS3 and OAVS Compared to Those Found in the Presented Patient

Clinical features	BOR	OAVS	Our patient
Branchial cleft fistulas	+		−
Renal anomalies	+		+
Lacrimal duct stenosis	+		+
Hearing impairment	+	+	+
Ear defect	+	+	+
Preauricular pits	+	+	−
Preauricular tag	+	+	+
Facial asymmetry	+	+	+
Development delay		+	+
Microsomia		+	+
Impaired speech		+	+[a]
Cleft lip/palate		+	−
Tracheoesophageal fistula		+	−
Heart defect		+	+
Genito-urinary anomalies		+	+
Irregular skull shape		+	+
Mandibular hypoplasia		+	+
Micrognathia		+	+
Recessed jaw		+	+
Facial nerve paralysis		+	−
Microphthalmia		+	−
Coloboma		+	−
Epibulbar dermoid		+	−
Vertebrae abnormality		+	+
Central nervous system defects		+	+
Limb anomalies		+	−

[a]Noted at 26 months of age.

PCDH9, the only gene in the 13q21 deleted interval in the proband, encodes for a protocadherin, a member of the subfamily of calcium-dependent cell–cell adhesion and recognition proteins of the cadherin superfamily [Strehl et al., 1998]. Like other protocadherins, *PCDH9* is predominantly expressed in brain, but *PCDH9* is also expressed in a broader variety of tissues, and the expression patterns appear to be developmentally regulated [Strehl et al., 1998]. To date, no clinical anomalies have been attributed to this gene and the 13q21–q22 deletions have been reported to be compatible with normal phenotypes [Couturier et al., 1985; Knegt et al., 2003]. Epigenetic silencing by hypermethylation of the CpG-rich promoter region of protocadherin 20 (PCDH20, 13q21.2, GeneID 64881) has been demonstrated [Imoto et al., 2006]. Whether this region upstream (i.e., more centromeric) of the 13q21.31–q21.32 deletion is involved in genomic imprinting and exerts an epigenetic effect on the inserted 14q22.3–q23.3 fragment in der(13) resulting in the variable phenotype between the proband and his father is unknown. It is possible that the dysmorphic features and multiple congenital anomalies in our patient result from an epigenetic effect on region(s) near the 14q insertion in der(13), with subsequent dosage effects from genes in 14q insertion, but this remains to be determined.

In the duplicated 14q22–q23 segment, there are 51 annotated genes, including *SIX1*, *SIX6*, and *OTX2*. Of these 51 genes, 26 with known gene functions are listed in Table II.

SIX1 haploinsufficiency has been proposed in patients with BOR/BOS3 [Ruf et al., 2004]. Interestingly, in mice, *Six1* is required for development of the kidney, muscle, and inner ear, and exhibits synergistic genetic interactions with *Eya* factors. $Eya1^{+/-}$ $Six1^{+/-}$ double heterozygous mice have a defect in kidney development that is not observed in the single heterozygotes, suggesting that *Six1* and *Eya1* act in the same genetic pathway; interactions between *Six1* and *Eya1* are essential for specific *Six1*–DNA binding [Li et al., 2003; Xu et al., 2003; Zheng et al., 2003]. Since *SIX1* mutations play a major role in BOR/BOS3, we suggest that duplication of *SIX1* may contribute to the patient's phenotype.

SIX6 is approximately 140 kb proximal to *SIX1*. *SIX6* is expressed in the retina, optic nerve, hypothalamic and pituitary regions, and has been postulated as a candidate gene for anophthalmia [Gallardo et al., 1999]. *Six6* was shown in mice to regulate early progenitor cell proliferation during mammalian retinogenesis and pituitary development [Li et al., 2002]. Interestingly, disruption of the *Six6* gene in mice results in a hypoplastic pituitary gland and variable degrees of retinal hypoplasia, often with absence of optic chiasm and optic nerve [Li et al., 2002]. Similar to *SIX1*, *SIX6* has been proposed to be haploinsufficient [Gallardo et al., 1999, 2004].

OTX2, a homeobox family gene, encodes a DNA binding protein that regulates transcription in the neural and ocular development [Hever et al., 2006] and is required for anterior brain, eye, and antenna formation [Finkelstein and Boncinelli, 1994]. *OTX2* loss-of-function mutations have been associated with a broad spectrum of ocular and neurological phenotypes, ranging from bilateral anophthalmia to mild microphthalmia and from severe developmental delay to normal cognitive development [Ragge et al., 2005]. We suggest that duplications of *SIX6* and *OTX2* may contribute to optic nerve hypoplasia and developmental delay of the proband.

Interstitial duplication of 14q22–q23 has not been reported previously, although several interstitial deletions involving the 14q22–q23 region have been described [Bennett et al., 1991; Elliott et al., 1993; Lemyre et al., 1998; Ahmad et al., 2003; Nolen et al., 2006]. Interestingly, the common features of patients with these deletions are bilateral anophthalmia, ear anomalies, microretrognathia, arched palate, facial asymmetry, and microcephaly. Some rarely observed anomalies include absent pituitary, hypoplastic adrenal glands and kidneys, undescended testes with a micropenis, and preaxial polydactyly. Nolen et al. [2006] reported an individual with a 9.66 Mb deletion in 14q22–q23, who had bilateral anophthalmia, high forehead, ear anomalies, limb anomalies, and central nervous system anomalies.

TABLE II. Genes and Related Disorders Contained in Duplication Region of 14q22.3–q23.3

Gene	MIM no.	Name	Disorder
OTX2	600037	Orthodenticle, Drosophila, homolog of, 2	Microphthalmia, syndromic 5
PSMA3	176843	Proteasome (prosome, macropain) subunit, alpha type, 3	
DAAM1	606626	Dishevelled-associated activator of morphogenesis 1	
RTN1	600865	Reticulon 1 (neuroendocrine-specific protein)	
SIX6	606326	Sine oculis homeo box, Drosophila, homolog of, 6	Microphthalmia, isolated, with cataract 2
SIX1	601205	Sine oculis homeo box, Drosophila, homolog of, 1	Brachiootic syndrome 3, deafness
SIX4	606342	Sine oculis homeo box, Drosophila, homolog of, 4	
MNAT1	602659	Menage a trois 1	
PRKCH	602659	Menage a trois 1	
HIF1A	603348	Hypoxia-inductible factor 1, alpha subunit	
KCNH5	605716	Potassium voltage-gated channel, subfamily H, member 5	
PPP2R5E	601647	Protein phosphatase-2, regulatory subunit B (B56)	
SYNE2	608442	Synaptic nuclear envelope protein 2	
ESR2	601663	Estrogen receptor-2 (ER beta)	
ZNF46	194541	Zinc finger protein-46 (KUP)	
HSPA2	140560	Heat-shock 70kD protein-2	
SPTB	182870	Spectrin, beta, erythrocytic	Elliptocytosis-3; spherocytosis-1
GPX2	138319	Glutathione peroxidase-2, gastrointestinal	
FNTB	134636	Farnesyltransferase, CAAX box, beta	
MAX	154950	MAX protein	
FUT8	602589	Fucosyltransferase 8	
MPP5	606958	Membrane protein, palmitoylated 5	
PIGH	600154	Phosphatidylinositol glycan, class H	
ARG2	107830	Arginase II	
RDH11	607849	Retinol dehydrogenase 11	
RDH12	608830	Retinol dehydrogenase 12	Leber congenital amaurosis, type III

This deletion had about 5 Mb overlap with the present 14q duplication, and included the *OTX2*, *SIX1*, and *SIX6* genes. The authors proposed that monosomy of *OTX2*, *SIX6*, as well as *BMP4* which is not duplicated in our patient, contributed to the ocular phenotype and abnormal pituitary development, while monosomy *SIX1* was responsible for the ear and other craniofacial features.

Although the propositus' father has the same chromosome aberration as the child, his phenotype is less severe, with learning disability, significant developmental delay, hypernasal speech and minor craniofacial dysmorphisms including tall forehead. Two other paternal relatives with mild phenotypes, also reported have the same karyotypes. Our findings confirm previous observations about the intrafamilial variability of BOR and OAVS [Melnick et al., 1975; Fraser et al., 1978; Heimler and Lieber, 1986].

In summary, we have characterized a familial insertion–deletion in detail. The relation between phenotype and genotype of the 11.79 Mb duplication region at 14q22.3–q23.3 in the present patient and his family members is very complex and could be affected by other genes, or environmental or epigenetic factors. Genes located in the 14q22.3–q23.3 duplication region are affected by dosage alterations which may explain some of the symptoms found in our patients. Previous studies of *SIX1* mutations suggested that the increased dosage of *SIX1* may contribute the most to the abnormal phenotypes in the family presented here. Other genes in this region, such as *OTX2* and *SIX6*, with expression patterns and murine mutant phenotypes involving eye development and neurological phenotypes may contribute to optic nerve hypoplasia, and/or development delay of the propositus.

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