A Girl with del(4)(q33) and Occipital Encephalocele: Clinical Description and Molecular Genetic Characterization of a Rare Patient

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ABSTRACT

We present clinical and developmental data on a girl with a \textit{de novo} terminal deletion of the long arm of chromosome 4, del(4)(q33). The patient was evaluated at birth and followed up until 5 years of age. She showed facial and digital dysmorphism, a complex congenital heart defect, a large occipital encephalocele, and postnatal growth deficiency. Her neuropsychomotor milestones were delayed, and she developed learning difficulties. Apart from standard Giemsa banding, a molecular genetic analysis was performed using a comparative genomic hybridization (CGH) array. This revealed a terminal deletion at the band 4q32.3, which is directly adjacent to 4q33. The clinical findings in our patient differ from those described previously in patients with del(4)(q33) and del(4)(q32), respectively. In particular, the prominent occipital encephalocele has not been observed before in a terminal 4q deletion.

INTRODUCTION

Chromosome 4q deletions are rare, with an estimated incidence of 1/100,000 (Strehle and Bantock 2003). Deletions of the terminal region of the long arm of chromosome 4 have been characterized as a distinct syndrome based on a number of common clinical features shared by previously reported cases (Townes \textit{et al.} 1979; Strehle \textit{et al.} 2001). Common characteristics are mild facial and digital stigmata (especially of the fifth finger), cardiac, skeletal, gastrointestinal, and renal anomalies, developmental delay, learning difficulties, growth failure, and a significant mortality (Mitchell \textit{et al.} 1981; Yu \textit{et al.} 1981; Lin \textit{et al.} 1988; Strehle and Bantock 2003).

Patients with large terminal deletions (4q31) are more severely affected with the characteristic facial, skeletal, and developmental anomalies (Giuffrè \textit{et al.} 2004). The region 4q31q34 may be critical for most of the clinical phenotype (Lin \textit{et al.} 1988). Some authors propose that the critical region for the 4q terminal deletion syndrome is 4q33, and that genes involved in facial, limb, cardiac, and central nervous system development must reside on 4q33 (Keeling \textit{et al.} 2001). Most deletions occurred \textit{de novo} and therefore carried a low recurrence risk (Strehle \textit{et al.} 2001).

The deletion 4q33 has been described in 15 patients; most of these cases presented with developmental delay and learning difficulties, facial and digital dysmorphisms, and congenital heart disease (Strehle and Bantock 2003). There are only 4 published cases of del(4)(q32), which will be discussed below (Lin \textit{et al.} 1988).

Here, we describe a patient evaluated repeatedly until the age of 5 years with a terminal deletion of chromosome 4, del(4)(q33), who in addition to the clinical features previously reported in patients with terminal 4q33 deletion, showed a prominent occipital encephalocele.

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MATERIALS AND METHODS

Patient

The patient was born at a gestational age of 38 weeks by caesarian section as the first offspring of healthy, nonconsanguineous parents. Her mother and father were 23 years old at the time of her birth. Vaginal bleeding was reported in the second trimester of pregnancy. Birth weight was 2.9 kg (< 50th centile) and her head circumference was 34 cm (> 50th centile). The newborn had Apgar scores of 8 at 1 min and 9 at 5 min. She was noted to have dysmorphic craniofacial features, including hypoplastic supraorbital ridges, large fontanelles, upwards slanting, short palpebral fissures, hypertelorism, glabellar haemangioma, over-folded ear helix, microstomia, and micrognathia (Fig. 1A). An occipital encephalocele was also present, which was surgically removed at the second day of life (Fig. 2). Magnetic resonance imaging of the brain performed at 3 months showed an Arnold–Chiari malformation type II, neuronal migration defects, and a supratentorial hydrocephalus. The patient was also noted to have hand and foot deformities: overlapping fingers and clinodactyly of the 5th digits, bilateral isodactyly of the toes, an overlap of the hypoplastic 5th toe over the 4th toe, and proximal implantation of the 2nd toes (Fig. 1C). Additional findings included abnormal labia minora, a prominent clitoris, and a sacrolumbar hemangioma. Chest X ray, echocardiogram, and cardiac catheterization revealed a complex cardiac defect with cardiomegaly, percutaneous ventricular septal defect, and preductal coarctation of the aorta, patent ductus arteriosus, and a perimembranous ventricular septal defect. Corrective heart surgery was performed on day 5 of life. The girl developed secondary pulmonary hypertension, which was controlled with medication.

On follow up she showed postnatal onset growth deficiency and feeding difficulties until 4 months of age. She displayed good head control at 5 months and sat alone at 1 year. At that time she had evidence of growth retardation with a weight of 5.9 kg (< 3rd centile) and a length of 70 cm (< 10th centile). The clinical features remained unchanged (Fig. 1B,C).

She was re-evaluated at aged 4 years (Fig. 3); weight was 14.2 kg (10th centile), length was 92 cm (< 3rd centile), and head circumference was 47 cm (< 3rd centile). Her neurodevelopmental milestones were significantly delayed. Unaided walking was possible from the age of 2.5 years. She was eating using her fingers and a spoon, but had problems with toilet training. She could say single words and carry out simple orders. Vision and hearing were normal. She did not attend nursery, but enjoyed playing by herself or with other children.

Cytogenetic and molecular analyses

Chromosome analysis was performed on peripheral blood lymphocytes from the patient and her parents. GTG-banding was performed using standard protocols. A total of 20 GTG-banded metaphases were examined with the complete analysis of four karyotypes. For molecular analysis, the Agilent Human Genome CGH microarray kit 44B (Agilent Technologies, Inc.) was used. The microarray contains approximately 43,000 60-mer oligonucleotide probes, which provide genome-wide coverage for >30,000 mapped genes with a spatial resolution of

FIG. 1. Facial features of the patient in the neonatal period (A) and at 1 year of life (B). Note the dysmorphic craniofacial features described in the text. C: Details of her feet at birth and at 1 year of life, respectively.
30–35 kb (calculated using the nonrepeated sequences of the genome). The experiment was performed as recommended by the manufacturer. Briefly, patient genomic DNA was extracted from peripheral blood using the Pure Gene kit (Genta Systems, Minneapolis, MN). Both reference DNA (Promega, Madison, WI) and test DNA were digested with Rsal and Alul (Invitrogen, Carlsbad, CA), and then purified using a Clean and Concentrator™ kit (Zymo Research, Orange, CA). Purified test and reference DNA samples were labeled with Cy5- and Cy3-labeled dCTPs (GE Healthcare Bio-Sciences Corp. Piscataway, NJ), respectively, using a BioPrime random labeling kit (Invitrogen, Carlsbad, CA). The labeled test and reference DNA samples were combined and hybridized to the microarray slide. After hybridization and washing, the slide was scanned on an Agilent Microarray Scanner and captured images were analyzed with Feature Extraction Software v8.1 and CGH Analytics 3.1 (Agilent Technologies, Inc.).

RESULTS

Figure 4 shows a G-banded metaphase from peripheral blood lymphocytes. The normal and deleted chromosomes 4 are shown with their corresponding ideogram. A de novo terminal deletion with breakpoints at 4q33 was found. Parental chromosomes were normal, indicating that the deletion had arisen de novo and carried a low recurrence risk. Molecular analysis using an array CGH kit revealed a terminal 25.7-MB deletion at the breakpoint 4q32.3 (Fig. 5). The bands 4q33 and 4q32.3...
could not be differentiated from each other by light microscopy alone.

**DISCUSSION**

So far more than 100 patients have been reported with terminal or interstitial deletions of chromosome 4q. According to Strehle and Bantock, the 4q deletion syndrome falls under the category of multiple congenital anomalies and mental retardation syndromes and has growth failure as a leading phenotypical characteristic (Strehle and Bantock 2003). The phenotype and the severity of clinical manifestations are variable and depend on the exact site and extension of the deleted 4q segment (Giuffrè et al. 2004). Comparison of the clinical signs in our patient with those published by other research groups showed that our patient has the key features present in the 15 patients described with 4q33 deletion (Strehle and Bantock 2003). She had most of the manifestations like facial and digital anomalies, congenital heart defect, and postnatal onset growth deficiency. She did not have gastrointestinal or renal anomalies. Pediatric reviews until 5 years of age revealed growth failure and delayed neuropsychomotor milestones. Our patient also had a cardiovascular abnormality similar to those described in 4q deletions with identical breakpoints (Evers et al. 1993; Grammatico et al. 1997).

In their literature review of 101 children with interstitial and terminal 4q deletion syndrome, Strehle and Bantock reported central nervous system involvement in 34% of cases. Brain anomalies were more common in interstitial than in terminal deletions of 4q (Strehle and Bantock 2003). Epilepsy was a frequent feature (Kempen 1975; Raczenbeck et al. 1991; Suwa et al. 1998) and several children had structural abnormalities, for instance an absent or hypoplastic corpus callosum (Fagan and Gill 1989; Fukushima et al. 1992), cerebral or cerebellar atrophy (Hoo et al. 1986; Koppitch et al. 1990; Slavotinek et al. 1997; Strehle et al. 2001) or dilated ventricles/macrocephaly (Frappaz et al. 1983; Beall et al. 1998; Rose et al. 1991; Kulhary et al. 1995). The only published case of a 4q deletion associated with an encephalocele was described by Nowaczyn et al. (1997). Their patient of Italian origin was born with a high occipital encephalocele, which was excised at 7 days of age, cerebellar hypoplasia, and a ventricular septal defect. Karyotype analysis revealed an interstitial deletion of chromosome 4, del(4)(q13.2q23). The boy died following a respiratory arrest at the age of 7 months.

Occipital encephalocele and ocular abnormalities are characteristic for patients with Knobloch syndrome. This syndrome is caused by mutations of the gene COL18A1, which maps to chromosome 21q22.3. COL18A1 encodes collagen XVIII, which is a component of basement membranes. Various cell types use basement membranes as a structure to which they can adhere and for the transport of nutrients. It is possible that other genes responsible for collagen production reside on chro-

**FIG. 4.** G-banded partial karyotype of the patient.

**FIG. 5.** Chromosome 4 ideogram and plot as obtained by array CGH. The 25.7 MB deletion is indicated by a vertical gray bar.
mosome 4q (Marneros and Olsen 2005; Passos-Bueno et al. 2006).

Chromosomal deletion syndromes with slightly differing breakpoints and different phenotypes contribute to the localization of genes with specific functions. A review of the features reported in patients with del(4)(q32), del(4)(q33), and del(4)(q34) showed that there was a clear difference in the frequency and severity of anomalies in children with either of these deletions (Keeling et al. 2001). In their review of 4q deletions, Lin and co-workers (1988) compared 2 new patients with del(4)(q32) with 2 cases of del(4)(q32) described by Rethore et al. (1979) and Fryns et al. (1981). All 4 patients had the Pierre–Robin sequence, were hypotonic at birth, and developed moderate to severe learning difficulties. Three of them had a congenital heart defect and absent digital flexion creases, and 1 died at the age of 3 months. Other reports of patients with interstitial deletions inside the terminal region 4q may contribute to an understanding of genotype–phenotype correlations and to an identification of the critical region and the genes responsible for the main manifestations (Sarda et al. 1992; Calabrese et al. 1997; Robertson et al. 1998; Keeling et al. 2001; Strehle et al. 2001; Giuffré et al. 2004).

In less than 10% of published cases with 4q syndrome, molecular genetic techniques have been applied, for instance fluorescent in situ hybridization (FISH), comparative genomic hybridization (CGH), representational oligonucleotide microarray analysis (ROMA), or multiplex amplifiable probe hybridization (MAPH) (Armour et al. 2004; Jain 2004; Jobanputra et al. 2005; Ylstra et al. 2006). These relatively new techniques have enabled researchers to determine the chromosomal breakpoints in 4q deletions more accurately than previously possible with Giemsa banding (Becker et al. 2003; Pickard et al. 2004; Van Buggenhout et al. 2004; Eggermann et al. 2005). In addition, they have resulted in the mapping of the genes for piebaldism and Rieger syndrome to chromosome 4q (Spritz et al. 1992; Flomen et al. 1997; Schinzel et al. 1997) and to the identification of a candidate gene for autism (Ramanathan et al. 2004). More than 200 genes were deleted in our patient as shown in our array CGH study, including 34 genes with well-known function (a list of deleted genes can be obtained from the author). Many of them are important for embryo development. For example, *dHAND* is a basic helix–loop–helix transcription factor. It is expressed in the developing heart and may play an important role in cardiogenesis (Srivastava et al. 1995). Thus far, there is no definitive correlation between *dHAND* deletion and congenital heart disease (Huang et al. 2002; Vogt et al. 2006). In mouse models, knockout of *dHAND* causes cardiac defects (Firulli et al. 1998). Another gene associated with cardiovascular development is *VEGF-C*, which is active in angiogenesis and cell growth (Cao et al. 1998).

Previously, it was reported that chromosome 4q deletions are associated with cervical cancer (Backsch et al. 2005), sporadic basal cell carcinomas (Sironi et al. 2004), and hepatocarcinoma (Bluteau et al. 2002). Several tumor suppressor genes reside in this region. The inhibitor of growth family member-1 is able to modify the function of histone acetylase and histone deacetylase (HDAC) and functions in DNA repair and cellular apoptosis (Wang et al. 2006). This raises the question whether patients with 4q deletion syndrome should be surveyed for cancer. *Cas-

pase 3* is also deleted in our patients. This protein plays a central role in apoptosis, which is an essential process for embryological development. Another gene deleted in our patient is the FAT tumor suppressor homologue 1. This gene is highly expressed in fetal epithelia and plays an important role in the developmental process (Dunne et al. 1995). We would like to point out that coagulation factor XI is also deleted in our patients. However, patients with 4q deletion syndrome have never been reported in association with a coagulopathy.

In conclusion, the findings in our patient differ from those previously found in patients with 46,XX,del(4)(q33) and del(4)(q32), respectively. To our knowledge, this is the first description of a patient with a terminal 4q deletion associated with an encephalocoele. It could be more than coincidence that two cases of 4q deletions were associated with an occipital encephalocoele, and therefore detailed research should be performed to establish a possible link. We recommend that molecular genetic analyses be performed in all future case reports of children with 4q deletion syndrome.

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