We report on a mother and child with a paracentric inversion of the long arm of chromosome 18: 46,XX,inv(18)(q21.1q23). The child had findings in common with those seen in 18q- syndrome including: microcephaly, epicantal folds, midface hypoplasia, and abnormally modeled ears, dermatoglyphic whorls on fingertips, clubfeet, hearing loss, and developmental delay. The mother and several maternal relatives had mild mental retardation and hearing loss. Magnetic resonance imaging of the child’s brain showed abnormal myelination. Molecular studies including PCR-based markers for the MBP locus and fluorescent in situ hybridization with a P1 genomic clone on mother and child demonstrated only one copy of the MBP locus (18q23) with the deletion extending beyond the MBP locus. Therefore, the deletion in the MBP region may account for the abnormal myelination seen in the patient. The other clinical findings, including mental retardation and hearing loss in this family, may reflect disruption of distal or proximal genes within the deleted MBP region or at the more proximal breakpoint 18q21.1, and may represent a contiguous gene syndrome. Further study of this family may help define those genes functioning in the MBP region that contribute to the phenotype of 18q- syndrome. Am. J. Med. Genet. 76:372–378, 1998.

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KEY WORDS: 18q paracentric inversion; myelin basic protein (MBP); 18q- syndrome; abnormal myelination; hearing loss; mental retardation

INTRODUCTION

Over 100 individuals have been reported with 18q-syndrome. The incidence of 18q- syndrome is estimated to be 1 in 40,000. Although the clinical phenotype varies, the 18q- syndrome is generally characterized by growth deficiency, minor craniofacial anomalies, limb and genitourinary malformations, mental retardation, and neurologic abnormalities [Wertelecki and Gerald, 1971; Subrt and Pokorny, 1970; Miller et al., 1990; Kline et al., 1993]. Craniofacial anomalies include: midface hypoplasia, downturned corners of the mouth with protuberant lower lip, upslanting palpebral fissures, epicantal folds, and abnormally modeled ears. Limb abnormalities include clubfeet, tapered fingers, proximal thumbs and predominence of dermatoglyphic whorls on fingertips. Neurologic involvement can include hypotonia, seizures, deafness, enlarged ventricles, and variable psychomotor retardation. Abnormal central nervous system myelination on magnetic resonance imaging (MRI) scan of the brain has been demonstrated in many patients. [Miller et al., 1990; Struthdee et al., 1995; Gay et al., 1997]. Most patients with the 18q- syndrome have a deletion of the myelin basic protein (MBP) gene, which maps to 18q23 [Kamholz et al., 1987; Sparkes et al., 1987].

We report on a mother and child with a paracentric inversion of the long arm of chromosome 18: 46,XX,inv(18)(q21.1q23). Their physical findings are similar to those seen in 18q- syndrome, and they also had mild mental retardation and hearing loss.

CLINICAL REPORTS

The proposita, A.E., was a 3,300 g, 48-cm-long girl born by repeat Cesarean section to a 20-year-old woman. Pregnancy, labor, and delivery were uncomplicated. Neonatally the infant fed poorly; hyperbilirubinemia was treated with phototherapy. At age 8 months, her growth parameters were: weight 6,880 g (5th centile),
length 65.5 cm (25th centile), and head circumference (OFC) 42.5 cm (10th centile). Notable physical findings included a superficial midforehead lipoma, tall forehead, bitemporal narrowness, overlapping coronal sutures, epicanthal folds, upslanting palpebral fissures, apparently low-set ears, small external ear canals, prominent lower lip, accessory nipple, bilateral fifth finger clinodactyly, tapered fingers, predominence of dermatoglyphic whorls on fingertips, bilateral talipes equinus valgus, and anal stenosis. MRI scan of the brain at 15 months showed no abnormalities on T1 weighted images, but on T2 weighted images there were patchy areas of increased signal intensity in the deep white matter of the cerebral hemispheres, including the periatrial area and posterior centrum semiovale consistent with abnormal myelination. The corpus callosum appeared well formed (Fig. 1A,B). Audiograms and auditory brainstem-evoked response (ABR) testing demonstrated bilateral hearing loss. Renal ultrasound findings were normal. Chromosome analysis documented a paracentric inversion of chromosome 18 with breakpoints at q21.2 and q23: 46,XX, inv(18)(q21.1q23) (Fig. 2). Examinations at 16, 27.5, and 32 months showed poor weight gain and microcephaly. Her weight and OFC were 8.6 kg (5th centile) and 44.5 cm (<5th centile) at 16 months, and 10.3 kg (<5th centile) and 46 cm (<5th centile) at 27.5 months (Fig. 3). Additionally, she had manifestations of mild left ptosis, hyperextensible joints, mild generalized hypotonia, and rocker bottom feet. At 32 months, she had developmental delay: she said only 5 words and no sentences.

The patient’s mother, J.E., was 20 years old and had an intelligence quotient of 70 on the Stanford-Binet Intelligence Scale. She had bilateral hearing loss with a small right ear canal and “atretic left eardrum.” J.E.’s brother and father both had a history of deafness and learning problems, and her sister had a history of mild mental retardation. On examination, J.E. was 152 cm tall, and had brachycephaly with mild frontal bossing, bitemporal narrowness, tall forehead, upslanting palpebral fissures, long, smooth philtrum, and protuberant lower lip (Fig. 4). She had the same paracentric inversion of chromosome 18 as her daughter, A.E. (Fig. 2).

MATERIALS AND METHODS

Molecular Genotypic Analysis

Molecular analysis, demonstrating a loss of material from the long arm of chromosome 18, was performed using polymerase chain reaction (PCR)-based microsatellite markers. High molecular weight genomic DNA was extracted from the peripheral blood leukocytes using the methods of Bell et al. [1981]. The DNA was analyzed using a PCR-based highly polymorphic marker for the MBP gene [Polymeropoulos et al., 1992].

PCR was performed in a total reaction volume of 10 μl, using 50 ng of genomic DNA, 50 ng of each primer, 200 mM dNTPs, and 0.5 U Taq polymerase (Perkin Elmer-Cetus, Norwalk, CT), 1.5 mM Mg and an annealing temperature of 55°C. One primer of the pair...
was end-labeled at the 5' end with \(^{32}\)P-dATP. PCR amplification consisted of 30 cycles of 1 min at 95°C, followed by 1 min at the annealing temperature of 55°C, and 1 min elongation at 72°C. PCR products were separated on a 7% polyacrylamide gel run at 65 W for 5 hr and visualized using Kodak (Rochester, NY) XAR-5 film and intensifying screens.

Fluorescent In Situ Hybridization (FISH)

Metaphase chromosome preparations were obtained from the patient and her mother after primary leukocyte culture utilizing the standard methods of Moorehead et al. [1960]. Ethidium bromide treatment [Ikeuchi, 1984] was used to produce prometaphase spreads.

A genomic clone for MBP was isolated by screening a human genomic P1 library [Shepherd et al., 1994] (obtained from DuPont Merck Pharmaceutical Company, Wilmington, DE) using MBP-specific PCR primers 4018 (5'-TCCTCTAATGGCTGAGTTCACCT-3') and 4017 (5'-TCCAGACCATCCAAGAAGACAGTG-3') (Genome Data Base, 1992). This gene was mapped to chromosome 18 band q23 [Sparkes et al., 1987]. The purified P1 DNA was labeled with biotin by nick translation using biotin-14-dATP (BRL/Gibco, Bethesda, MD).

Metaphase spread preparations were dropped onto slides and baked for 4 hr at 65°C. Hybridization buffer (50% formamide, 10% dextran sulfate, and 2 × SSC, pH 7.0), 10 μg human Cot-1 DNA with 40 ng labeled probe was denatured for 5 min at 70°C. Slides were dena-
tured at 70°C for 2 min in denaturation solution (70% formamide, 2 × SSC, pH 7.0). The denatured probe was added to the slides immediately and incubated overnight at 37°C in a humid chamber. The following day, the slides were washed in 50% formamide, 2 × SSC, pH 7.0, followed by washing in 2 × SSC, pH 7.0, at 42°C. The biotin-labeled probe was tagged using fluorescein isothiocyanate (FITC) labeled avidin. The chromosomes were counterstained with DAPI. The fluorescent probes were visualized using a Zeiss Axiosplan Fluorescence microscope (Zeiss, Thornwood, NY) equipped with FITC, DAPI, and triple band pass filter sets. Images were captured by computer using Applied Imaging Probevision (Applied Imaging, Santa Clara, CA) and photographs printed on a Kodak XL 7700 color image printer.

DNA from human genomic clones for the markers D18S19 from distal 18q22.3 or proximal 18q23 and D18S553 from distal 18q23 (this marker is present in the half-YAC clone, which is known to contain the 18q telomere) were isolated from the P1 genomic library and used for two-color FISH on samples from the mother and child. This confirmed the presence of a paracentric inversion involving the long arm of chromosome 18 (46,XX, inv(18)(q21.1q23)).

The two P1 probes were labeled by nick-translation. D18S19 was labeled with biotin-14-dUTP (BRL/Gibco, Grand Island, NY), and D18S553 was labeled with digoxigenin-11-dUTP (Boehring Mannheim Biochemical, Indianapolis, IN). Slides for FISH were prepared from the mother and daughter by the standard methods. Labeled probes (40 ng each) were hybridized to the metaphase spreads overnight. Following washing, the probes were visualized using Texas Red labeled avidin to detect D18S19 and FITC labeled antibodies to digoxigenin to detect D18S553. The chromosomes were counterstained with DAPI and visualized on a Zeiss Axioscop microscope.

RESULTS

Molecular analyses of the DNA from the mother and the child were performed using a PCR-based highly polymorphic marker at the MBP gene [Polymeropoulos et al., 1992] to determine if they had a deletion in this region. Figure 5 illustrates that the mother and the child do not share a common allele at this locus. Therefore, we hypothesized that the chromosome they have in common has a deletion in the region of the MBP gene and that the MBP allele observed was derived from their intact chromosome 18.

To test this hypothesis, we performed FISH on metaphase chromosomes prepared from both the child and mother. At least 20 cells were analyzed for the presence of one or two copies of the MBP locus. Both mother and child were found to have only one copy of MBP (Fig. 5A,B). This result is consistent with the interpretation of the molecular PCR analysis.

The paracentric inversion involving the long arm of chromosome 18 was confirmed in the mother and child using two-color FISH with probes from the telomere and a proximal marker known to be present in the patients (data not shown).

DISCUSSION

The 18q- syndrome was described by de Grouchy et al. [1964] and Lejeune et al. [1966]. Since then, over 100 individuals have been reported. Table I shows that the proposita and her mother had many findings of the 18q- syndrome, the proposita having acquired microcephaly, epicanthal folds, midface hypoplasia, and abnormally modeled ears, dermatoglyphic whorl patterns, clubfeet, hearing loss, and developmental delay. Rarely 18q- patients have biochemical abnormalities such as IgA deficiency [Finley et al., 1969], hypothyroidism [Henrot et al., 1989], and pernicious anemia [Stricker and Linker, 1982]. Life expectancy may be nearly normal [Law and Masterson, 1969; Wilson et al., 1979; Miller et al., 1990].

It was reported previously that loss of the critical region, 18q21.3, is required for expression of the syndrome [Wilson et al., 1979]. However, other studies have shown individuals with typical findings and deletion of more distal segments such as 18q22.3 to qter [Felding et al., 1987; Miller et al., 1990; Kline et al., 1993]. Therefore, it was suggested that the variability in the clinical phenotype of the 18q- syndrome may be more representative of a contiguous gene syndrome with baseline deficit of 18q22.2 to qter than of loss of a
single critical region within 18q21.3 [Silverman et al., 1995]. In addition, molecular studies showed that the size of the deletion correlates with severity of the phenotype and that certain anomalies may be associated with the location of the deletions in the distal portion of 18q [Kline et al., 1993]. They assigned microcephaly and moderate-severe mental retardation to band q21.2-q21.3 and abnormal brain MRI findings to band q21.2-q22.1. However, in a more recent review of 26 patients by Strathdee et al. [1995], there was not a good correlation between size of the deletion and severity of the phenotype. Their data were based only on molecular analysis and clinical data gathered from charts. Brkanac et al. [1996] found that 14% of patients with 18q-deletion syndrome have more complex rearrangements, with many having partial trisomy. Inclusion of partial trisomy patients may account for why Strathdee et al. [1995] did not find a clear correlation between size of deletion and clinical severity.

The proposita, A.E., and her mother, J.E., both had a paracentric inversion of chromosome 18 with breakpoints at q21.1 and q23 with no cytogenetically apparent gain or loss of material. Both had mild mental retardation, hearing loss, and the minor anomalies commonly seen in 18q- syndrome including: short stature, decreased weight, microcephaly, downturned corners of the mouth, midface hypoplasia, protuberant lower lip, small ear canals, dermatoglyphic whorl patterns, clubfoot, and tapered fingers. These findings suggested probable loss or disruption of genes at a molecular level in the distal portion of the long arm of chromosome 18.

Another consideration is that the patient had more severe manifestations than the mother including microcephaly, tapered fingers, clubfoot, epicanthal folds, and decreased weight although they appeared to have the same cytogenetic paracentric inversion and the same molecular deletion involving the MBP gene locus. Possibilities for this clinical variation could be due to (1) differences in parental origin of the deletion assuming the mother may have inherited the inversion from her father because of his similar clinical history, while the patient inherited the inversion from her mother,
(2) further molecular deletions or disruptions not yet detected, or (3) genetic background attributed to interaction with other genes not associated with the chromosome alteration.

Most patients with 18q- syndrome have de novo deletions [Schinzel, 1984]. Others may have a familial translocation [Fryns et al., 1979], direct transmission [Subrt and Pokorny, 1970; Sulzer and Zierler, 1976; Miller et al., 1990], mosaicism [Pagon et al., 1979], or ring chromosome [Schinzel, 1984]. There was only one other case in addition to the present report with familial inversion; however, it was a pericentric inversion [Wertelecki and Gerald, 1971]. To our knowledge, there have been no reports of a familial 18q paracentric inversion having similar findings to individuals with 18q- syndrome. In general, paracentric inversions are rare with an incidence of less than 1% in human populations. They are difficult to detect and crossing over results in a dicentric and acentric chromosome and, therefore, in fewer viable offspring [Therman and Susan, 1993]. The patient’s family history of mental retardation and/or hearing loss suggested that there was familial inversion of the long arm of chromosome 18, in addition to the transmission of the inversion from the mother to the patient. However, there may be other causes for the familial mental retardation.

Many patients with 18q- syndrome have evidence of dysmyelination of the cerebral white matter. One of the genes that maps to distal 18q23 is the myelin basic protein (MBP) [Kamholz et al., 1987]. The shiverer mouse, which does not express MBP due to a recessive mutation at 18q22-23, has a defect in the CNS myelination that causes a generalized action tremor and convulsions [Chernoff, 1981]. The MBP heterozygous mouse is not reported to be hypomyelinated, but there are no MRI data on the mouse. It has been suggested that delayed myelination in the 18q- syndrome is associated with deletion of only 1 copy of MBP [Weiss et al., 1991]. A recent report by Gay et al. [1997] showed that all patients who had abnormal MRI scans with prolonged white matter T1 and T2 relaxation times lacked one copy of the MBP gene, which supports the conclusion that incomplete myelination in the 18q- syndrome is associated with haplinsufficiency of the gene for MBP. Our propositus had abnormal T2-weighted images on MRI scanning of the brain, consistent with impaired or delayed myelination of the central white matter tracts. The molecular studies, including PCR-based markers for the MBP locus and FISH hybridization with a P1 genomic clone on both mother and child, demonstrated only one copy of the MBP locus (18q23) with the deletion extending beyond the MBP locus. Therefore, the deletion in the MBP region may account for the abnormal myelination seen in the proposita.

The other clinical findings including mental retardation and hearing loss in this family may reflect disruption of distal or proximal genes within the deleted MBP region, or at the more proximal breakpoint 18q21.1, and may represent a contiguous gene syndrome. Further study of the family may help define those genes functioning in the MBP region that contribute to the phenotype of 18q- syndrome.


