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Molecular Characterization of Patients with 18q23 Deletions

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Summary

The 18q$^-$ syndrome is a deletion syndrome that is characterized by mental retardation, hearing loss, midfacial hypoplasia, growth deficiency, and limb anomalies. Most patients with this syndrome have deletions from 18q21-qter. We report on three patients with deletions of 18q23. A mother and daughter with identical deletions of 18q23 have many of the typical features of the 18q$^-$ syndrome, including midfacial hypoplasia and hearing loss. In contrast, the third patient has few of the symptoms of the 18q$^-$ syndrome. A contig of the 18q23 region was generated to aid in the mapping of the breakpoints. FISH was used to map both breakpoints to the same YAC clone. Furthermore, somatic-cell hybrids from the daughter and the third patient were isolated. The mapping results of sequence-tagged sites relative to the two breakpoints were identical, suggesting that the two deletion breakpoints map very close to one another. The analyses of these patients demonstrate that the critical region for the 18q$^-$ syndrome maps to 18q23 but that a deletion of 18q23 does not always lead to the clinical features associated with the syndrome. These patients demonstrate the wide phenotypic variability associated with deletions of 18q.

Introduction

The 18q$^-$ syndrome is associated with a terminal deletion of the long arm of chromosome 18. The clinical features associated with this syndrome include mental and developmental delay, hypotonia, growth retardation, midface hypoplasia, flat philtrum, carp-shaped mouth, broad nasal bridge, hearing loss, ophthalmic abnormalities, tapered fingers, and excess whorls on the fingertips (de Grouchy et al. 1964; Subrt and Pokorny 1970; Werteleki and Gerald 1971; Wilson et al. 1979; Felding et al. 1987). Less frequent findings include cleft palate, prognathism, and IgA deficiency (Feingold et al. 1969; Wilson et al. 1979; Lewkonia et al. 1980; Stricker and Linker 1982). A majority of patients have deletions from 18q21 to qter; however, larger and smaller deletions have been reported (Wilson et al. 1979; Qazi et al. 1980; Felding et al. 1987; Kline et al. 1993; Silverman et al. 1995; Strathdee et al. 1995).

Although many of the patients described in the literature have the recognizable set of clinical features associated with the 18q$^-$ syndrome, there is nevertheless a wide clinical spectrum associated with this disorder (Wilson et al. 1979; Felding et al. 1987). Indeed, a number of patients have been described who would not be diagnosed with this syndrome on the basis of their clinical features, and some of these patients have virtually none of the typical features associated with this syndrome (Qazi et al. 1980; Kohonen-Corish et al. 1996).

There have been several investigations on large numbers of patients with 18q deletions, in order to generate phenotype-genotype correlations. Using bivariate flow karyotyping, Silverman et al. (1995) concluded that the critical chromosomal region involved in the clinical features associated with the typical 18q$^-$ syndrome mapped to 18q22.2-qter, a region that was estimated to be 9 Mb in size.

Strathdee et al. (1995) analyzed 26 patients with deletions of 18q, many of whom had been initially karyotyped, not to rule out 18q$^-$ syndrome but because of other clinical indications (e.g., segmental spinal muscular atrophy [Weiss et al. 1991]). It was determined that the frequency of the clinical features associated with 18q terminal deletions was reduced when these atypical patients were included in the analysis. Furthermore, the extent of the deletion in the leukocytes from each patient was determined by a FISH-based approach with numerous chromosome 18–specific lambda phage clones. Several patients with the smallest deletions had most of the clinical features associated with the 18q$^-$ syndrome. Therefore, Strathdee et al. concluded that, for the 18q$^-$ syndrome, the critical chromosomal region that is asso-

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ciated with presentation of the clinical features maps to 18q22.3-qter. However, a deletion of this region leads only to a susceptibility to the clinical features, since not all patients with this region deleted had the clinical findings.

In this report we describe three patients with small terminal deletions of 18q23. Two of the patients, a mother and a daughter, present with typical clinical findings associated with the $18q^-$ syndrome. In contrast, the third patient, who has a similar-sized deletion, has almost none of the features of the syndrome. The clinical description of these patients exemplifies both the clinical variability observed in individuals with 18q terminal deletions and the difficulty in giving a prognosis for infants who have an 18q deletion. A contig of 18q23 has been generated to map the deletions of these patients in more detail, and the analysis has narrowed the susceptibility critical region for the $18q^-$ syndrome to a single chromosomal band, 18q23.

Material and Methods

Cytogenetics

Cytogenetic determination of the 18q deletion was made prior to the analysis by a clinical cytogenetic laboratory. Blood samples were obtained from the daughter and patient 3, and the leukocytes were transformed with Epstein-Barr virus, according to established procedures. Metaphase spreads were obtained from Epstein-Barr virus–transformed lymphocytes or from peripheral blood samples, and slides were prepared according to standard cytogenetic procedures.

FISH

Chromosome painting was performed according to the manufacture's protocol (Oncor). FISH analysis was performed as described elsewhere (Mewar et al. 1992), with the following modifications: DNA to be labeled was sonicated to a size of <10,000 bp and was labeled with biotin by use of a Bionick Kit (Gibco BRL), according to the manufacturer's protocol. The labeled DNA was then resonicated to a size of <500 bp. A total of 400 ng of labeled DNA, 5 μg of human cot-1 DNA, and 5 μg of sonicated salmon-sperm DNA was dried and resuspended in 10 μl of 50% formamide and 2 × SSC with 10% dextran sulfate, for each hybridization reaction. After overnight hybridization at 37°C, the slides were washed for 20 min at 42°C in 50% formamide and 2 × SSC, for 20 min at 42°C in 1 × SSC, and for 15 min at 65°C in 0.1 × SSC. Probes were detected with two rounds of avidin-conjugated fluorescein in alternation with biotinylated anti-avidin, and the chromosomes were counterstained with propidium iodide. Slides were viewed by use of a Nikon epifluorescence microscope, and photographs were taken with Kodak Gold 100 ASA film.

Somatic-Cell-Hybrid Isolation

Somatic-cell hybrids were derived from the transformed lymphoblast cell lines by polyethylene glycol–mediated cell fusion with UCW206, as described elsewhere (Kline et al. 1992). UCW206 allows for the selection of somatic-cell hybrids that have retained a human chromosome 18, since it has a temperature-sensitive arginyl tRNA synthetase whose complementary human gene maps to 18q21.1. The resulting somatic-cell hybrids were initially screened by PCR to identify the clones containing the del(18), by use of previously mapped sequence-tagged sites (STSs) (Overhauser et al. 1993). Confirmation of the presence of the deletion of the del(18) was determined by trypsin-Giemsa banding.

YAC Clone Analysis

YAC clones from the CEPH large-insert YAC library were obtained from Los Alamos Laboratory. For STS-content analysis, 5-ml preparations of YAC clones were grown in minimal media lacking uracil, for 3 d at 30°C. The number of cells in each culture was estimated by measurement of the optical density at 600. Aliquots of $\sim 1 \times 10^7$ cells were placed in 1.5-ml centrifuge tubes and were centrifuged for 5 min at 14,000 *g* to pellet the cells. The cell pellets were then resuspended in 100 μl of dH_2O, were boiled for 10 min to release the DNA, and immediately were frozen. PCR analysis was performed by use of 5 μl (equivalent to 5×10^5 cells) of the boiled preparation, for each reaction.

PCR Analysis

PCR analysis was performed in an MJ-Device thermocycler. A typical 25-μl PCR reaction contained 1.5 mM $MgCl_2$, 40 ng genomic DNA, 200 μM NTP, 1.2% formamide, 0.75 U AmpliTaq DNA polymerase, and 0.4 μM of each primer. Each reaction was heated to 94°C for 4 min, followed by 35 cycles of denaturation at 94°C for 1 min and a combined annealing and extension at 52°C–63°C for 30 s, with a final extension for 6 min at 72°C. Amplification products were separated on a 1.5% agarose gel.

YAC-End Cloning

Cloning the ends of YAC inserts was performed by use of Alu-vector PCR (Breukel et al. 1990) or recircularization (Burke et al. 1987) approaches. For Alu-vector PCR, the reactions were performed by use of either the left end– or right end–specific primers in addition to the Alu primers, as well as by use of the Alu primers alone. The primer 5′-AGTCGAACGCCCGATCTCAA-3′ (Breukel et al. 1990) for the right end of the YAC vector and the primer 5′-CGCAAGACTTTAATTTAT-

CACTACG-3′ (from sequence in Silverman 1993) for the left end of the YAC vector were used. The primers 5′-GGATTACAGGYRTGAGCCA-3′ and 5′- RCCAYTGCACTCCAGCCTG-3′ (Liu et al. 1993) were used for amplification from Alu sequences. For amplification, each reaction was heated to 94°C for 4 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 3 min, with a final extension time of 10 min at 72°C. The products were run on a 1.5% agarose gel, and only Alu primers/vector-specific products were excised and cloned into the T/A cloning vector (version 2.3; Invitrogen).

For recircularization, 1 μg of YAC DNA was digested with *Xho*I for 1 h at 37°C and then was heated to 65°C for 20 min to inactivate the enzyme. Recircularization was achieved by incubation of the digested DNA at a concentration of 20 ng/μl in the presence of 1 U of T4 DNA ligase in the recommended buffer at 14°C overnight. DH5α cells were transformed with the recircularized DNA and were plated on ampicillin plates to select for the recircularized vector.

Several of the end clones were sequenced by use of an ABI sequencer. Oligonucleotides suitable for PCR amplification were generated for several YAC ends: for the distal end of YAC clone 742-B12, the primers were 5′-GGTTTCAGCATGTTAGCCAG-3′ and 5′-GCCCACCTATTTGCAGTTTC-3′; for the distal ends of YAC clone 752-G11, the primers were 5′-CTGGCAACAGAATGAGACTC-3′ and 5′-GAAGCCATGTGTAACCTGG-3′. PCR conditions were as described above.

Results

Clinical Analysis

The first two patients, a 28-year-old mother and her 18-mo-old daughter, were referred to the genetics clinic for evaluation of dysmorphic features and hearing loss in the daughter. The daughter was delivered at 32 wk of gestation by Cesarean section because of placenta previa, and the early postnatal clinical course was complicated by prematurity. On examination at 18 mo of age, her height was close to the 5%-ile, and her weight and head circumference were between the 10%- and 25%-ile. She had many of the facial features associated with the 18q$^-$ syndrome, including midfacial hypoplasia, carp-shaped mouth, and a flat nasal bridge. A list of clinical features associated with the 18q$^-$ syndrome, the reported frequency of these clinical features, and the features present in the daughter and the other patients are presented in table 1. In addition to the clinical features mentioned in table 1, the daughter had hypertelorism, epicanthal folds, and periauricular pits. Furthermore, on both feet her second and third toes were overlapping. She had moderate bilateral hearing loss secondary to structural external and middle ear defects that were strikingly similar to her mother's abnormalities. The daughter also had mild to moderate global developmental delay; however, it was unclear how much the hearing loss and poor social circumstances were contributing factors. A karyotype of 46,XX,del(18)(pter→q22::q23→qter) was reported.

The mother's height and weight were at the 10%-ile, and her head circumference was below the 3%-ile. She also had many of the facial features associated with the syndrome, as shown in table 1. The mother also had significant hearing loss. She had complex structural middle and external ear defects, including bilateral stenosis of the external auditory canals. These defects have been described in detail elsewhere (see case 7 of Sataloff 1991, pp. 103–106). After several surgical operations, her conductive-hearing loss was repaired, and her hearing is now close to normal, with only intermittent need for a hearing aid. She has finished 1 year of college. She is reported to have psychiatric problems, possibly a multiple-personality disorder. High-resolution analysis revealed a karyotype identical to that of her daughter. No mosaicism was detected. Because of the psychiatric problems, additional blood samples could not be obtained from the mother.

Patient 3 has been described elsewhere (Kohonen-Corish et al. 1996), and the clinical features of this patient are listed in table 1. In brief, hypotonia was observed for only a few months after birth. Of the clinical features associated with the 18q$^-$ syndrome, decreased growth, midface hypoplasia, and developmental delay were observed at 2 years of age, but all were absent by 4 years of age. She did have increased whorls on her fingers. A karyotype of 46,XX,del(18)(q23) was reported. This cytogenetic interpretation was confirmed with polymorphic markers mapping within the 18q21-q23 region (Kohonen-Corish et al. 1996).

FISH Analysis of the Patients

Blood samples from the daughter and patient 3 were obtained, and transformed lymphoblastoid cell lines were generated. Chromosome 18 painting was performed on both cell lines to rule out a cryptic translocation. Fluorescence could be detected only on the chromosome 18 homologues (data not shown), demonstrating that the 18q23 region had not been translocated to another chromosome. Lambda phage clones that mapped to the distal portion of 18q (Strathdee et al. 1995) were used initially to analyze the extent of the deletion. Although the deletions were determined to be smaller than those previously reported by Strathdee et al. (1995), the small number of clones available for mapping limited the ability to more precisely determine the extent of the deletions. Therefore, a contig of 18q22.3-

Table 1

Clinical Features of Patients with 18q23 Deletion

Clinical Feature	Frequency (%)		Status in[c]		
	A[a]	B[b]	Mother	Daughter	Patient 3
Decreased growth	>80	54	−	+	+/−
Microcephaly	50–80	42	+	−	−
Face:					
Midface hypoplasia	50–80	69	+	+	+/−
Up- or downslanting palpebral fissures	30–50	31	+	+	−
Malformed ears	50–80	50	+	+	−
Flat philtrum	<10	38	+	+	−
Carp-shaped mouth	50–80	50	+	+	−
Prognathism	<10	19	+	+	−
High or cleft lip/palate	30–50	50	−	−	−
Flat nasal bridge	>80	23	+	+	−
Limbs:					
Tapered fingers	>80	8	−	+	−
Proximal thumbs	>80	15	−	−	−
Clubfeet	<10	11	−	−	−
CNS:					
Hypotonia	>80	73	−	+	+/−
Seizures	<10	31	−	−	−
Hearing loss	50–80	60	+	+	−
Strabismus	30–50	38	−	−	−
Nystagmus	50–80	27	−	+	−
Developmental delay	>80	96	−	+	+/−

[a] From Wilson et al. (1979), Qazi et al. (1980), and Felding et al. (1987).
[b] From Strathdee et al. (1995).
[c] A plus sign (+) denotes presence of the feature; a minus sign (−) denotes absence of the feature; and a plus/minus sign (+/−) denotes that the feature was present at age 2 years but absent at age 4 years.

qter was generated to facilitate the characterization of the chromosomal deletions represented by the daughter and patient 3.

Contig Construction of 18q22.3-qter

yRM2050, a 1/2YAC clone that contains the 18q telomere and that had been reported elsewhere (Strathdee et al. 1994), was used to anchor the end of the YAC contig. YAC clones mapping to the 18q22.3-qter region initially were identified from the Whitehead database and were obtained. STSs mapping to the terminal region of 18q were identified from various databases (see table 2) and were mapped to the YAC clones. To fill gaps in the contig, the ends of YAC inserts that flanked gaps in the contig were cloned by use of Alu-vector PCR (Breukel et al. 1990) or recircularization (Burke et al. 1987) approaches. After the determination, on the basis of hybridization to the YAC clone in the region, of which ends of the YAC had been cloned, the end clones were sequenced and used to generate additional STSs. These STSs were used to screen the CEPH YAC library (Longmire et al. 1993). After several screenings of the CEPH library by use of additional STSs, three gaps remained in the YAC contig. One was filled by cosmid walking from the end of the YAC insert next to the gap, by use of an arrayed chromosome 18 flow-sorted library (Games et al. 1994). Two gaps still remain, on either side of YAC 717-F12. These regions do not seem to be represented within the CEPH library. The contig generated by YAC and cosmid screening is shown in figure 1.

Deletion Analysis of Patients with Small Terminal Deletions of 18q

The construction of the contig of the 18q23 region was instrumental for the characterization of the deletions in the two patients, in that an ordered set of clones and PCR-based markers were generated. The samples first were hybridized with the 1/2YAC yRM2050 to test for the presence of an interstitial deletion. Both patients were found to have a terminal deletion, on the basis of the absence of any hybridization of yRM2050 on the del(18) (data not shown). YAC clones mapping more proximal to yRM2050 also detected hybridization to only one chromosome 18 homologue. These results were consistent with a karyotype interpretation of a terminal deletion. Hybridization on another chromosome, which would be

Table 2

STSs Mapping to 18q23

STS	Product Size (bp)	Reference or Database
D18S553	151	http://gdbwww.gdb.org/gdb
D18S70	111–126	Weissenbach et al. (1992)
D18S497	118	Van Kessel et al. (1994)
WI-10807	204	http://www-genome.wi.mit.edu
SHGC-11871	231	http://shgc.stanford.edu
WI-6554	227	http://www-genome.wi.mit.edu
752-E	226	Present study
742-E	170	Present study
WI-3741	124	http://www-genome.wi/mit.edu
D18S461	160–169	Gyapay et al. (1994)
D18S871	150	http://gdbwww.gdb.org/gdb
D18S994	162	http://www-genome.wi.mit.edu
D18S462	179–193	Gyapay et al. (1994)
D18S50	176–190	Straub et al. (1993)
D18S554	220	Gyapay et al. (1994)
MBP	583	Overhauser et al. (1993)
SHGC-8084	96	http://shgc.stanford.edu
SHGC-8023	204	http://shgc.stanford.edu
D18S844	194	http://www-genome.wi.mit.edu
242YF2	188	http://www-genome.wi.mit.edu
D18S380	150	Gerken et al. (1994)
D18S1121	161–174	http://gdbwww.gdb.org/gdb
D18S489	374	Gerken et al. (1994)
D18S1161	100–108	http://gdbwww.gdb.org/gdb
D18S58	144–160	Weissenbach et al. (1992)
D18S486	107	Gyapay et al. (1994)
CYTB5	170	Shepard et al. (1991)
D18S533	150	Francke et al. (1994)
D18S469	234–244	Gyapay et al. (1994)

suggestive of a cryptic translocation event, was not detected. The location of the breakpoint in both patients was mapped to the DNA present in 943-C11. As shown in figure 2, a much-reduced hybridization signal was observed on the del(18), for both the daughter and patient 3, compared with the undeleted homologue. The intensities of the hybridization signals on the del(18)s of both patients were very similar, suggesting that the locations of the breakpoints were near each other. On the basis of these results, the karyotypes of the mother and daughter were revised, to be 46,XX,del(18)(q23).

Additional patients who possess small deletions of 18q and have features of the 18q$^-$ syndrome have been reported (Strathdee et al. 1995). The location of the deletion endpoints for cases 25 and 26, which had delineated the critical region for the 18q$^-$ syndrome as reported by Strathdee et al. (1995) were determined by use of YAC clones from the contig. Both cases were found to have larger deletions than were found in the patients in the report, as determined by FISH analysis (data not shown). The locations of all the deletions are shown in figure 1. On the basis of the sizes of the YAC clones in the contig and on the basis of the size of the deletion in the mother and daughter, the size of the 18q$^-$ syndrome critical region has been reduced to ~6 Mb.

In an attempt to obtain a more precise localization of the deletion breakpoints in the patients, somatic-cell hybrids were generated from the daughter and patient 3, as well as from case 25 and case 26. STS mapping was performed on all of the somatic-cell hybrids. As shown in figure 3, STSs that mapped near the telomere were deleted in all four cases, further confirming that the deletions were terminal in nature. The marker D18S489 was found to be present in both the daughter and patient 3 but was deleted in case 25 and case 26. For all of the STSs mapping in the region, the locations of the chromosomal break in the daughter and patient 3, relative to the STSs, were identical. Therefore, similar to what was shown by the FISH analysis, the STS mapping demonstrates that the deletions in the new patients are smaller than those in patients previously reported and also suggests that the breakpoints in these two patients are in close proximity.

Discussion

In this report, we describe three patients with deletions of 18q23. Two patients, a mother and daughter, have many of the typical clinical features of the 18q$^-$ syndrome, which include facial features (e.g., midface hypoplasia and carp-shaped mouth) and nervous-system involvement (e.g., hypotonia and hearing loss). In contrast, patient 3, whose deletion, by both FISH and STS analysis, is nearly identical in size to the daughter's deletion, has few of the clinical features associated with terminal deletions of 18q. On the basis of results from these patients, there are several possible interpretations to explain the difference in clinical presentation, including the following: (1) patient 3 has a slightly smaller deletion than do the other two patients, and the material that is deleted in the mother and daughter but that is present in patient 3 contains a gene(s) that is critical in the presentation of the clinical features associated with the syndrome; or (2) wide clinical variation is common in patients with very similar-sized deletions, and a deletion of the critical region for the 18q$^-$ syndrome (18q23) confers a susceptibility only to the clinical abnormalities. Strathdee et al.'s (1995) characterization of patients with larger deletions supports the second interpretation, since patients with terminal deletions from 18q21 have been identified who lack many of the typical features, such as the midfacial hypoplasia or carp-shaped mouth.

Another possible explanation for the phenotypic variability observed in the three patients is that there is an imprinting effect on one or more genes within the deletion. However, previous analysis of a larger sample of patients suggested that there was no correlation between

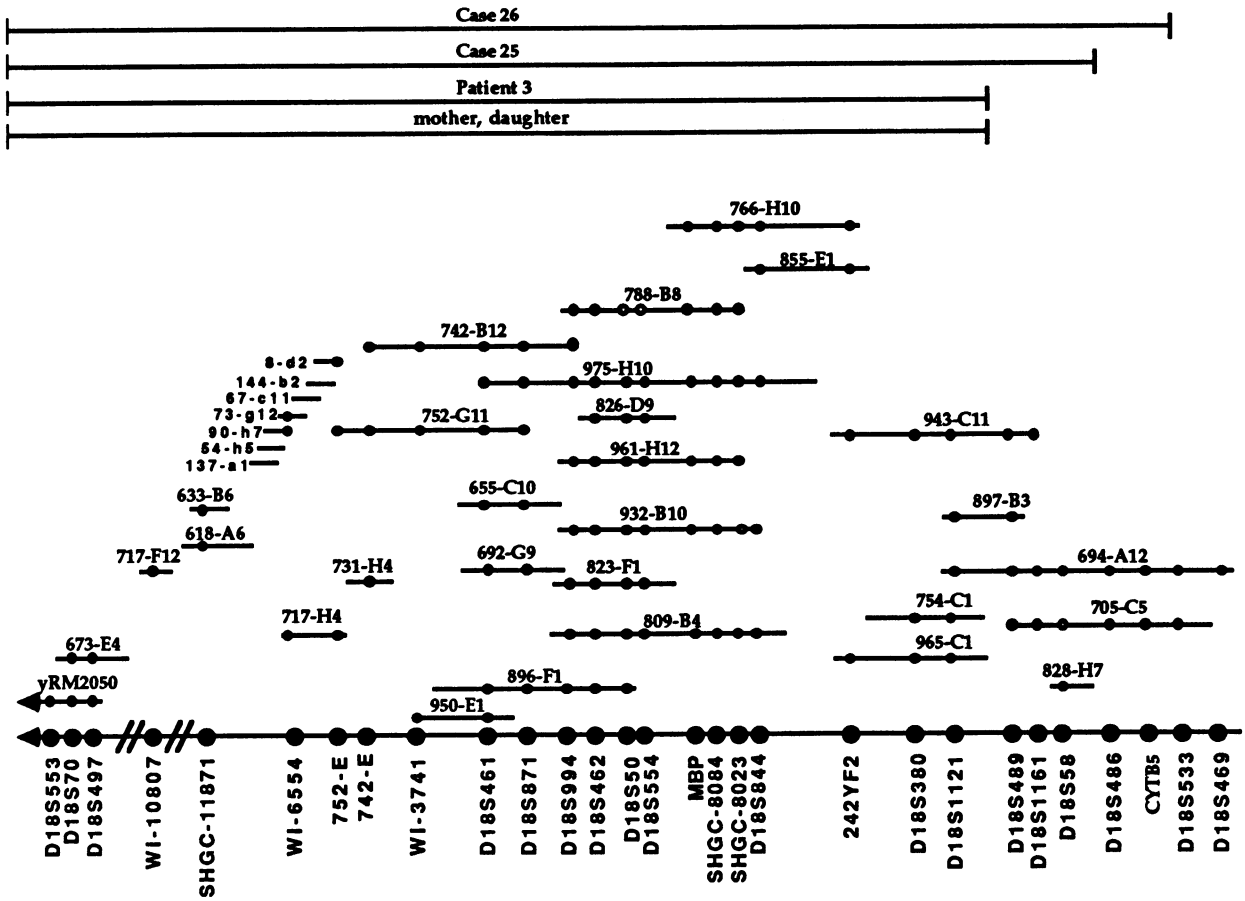

Figure 1 Contig of the 18q⁻ syndrome critical region. YAC and cosmid clones are represented by horizontal lines, with names of the YAC clones in uppercase letters and with names of cosmids in lowercase letters. The STSs used to construct the contig are listed along the bottom, with a blackened circle indicating the presence of the STS, and with an unblackened circle indicating its absence, in a particular YAC or cosmid clone. In addition, the positions of the two gaps in the contig are indicated by the double diagonal lines. The horizontal lines above the contig indicate the DNA that is deleted in the patients who define the new critical region (mother, daughter, and patient 3) and in the patients who define the old critical region (case 25 and case 26, taken from Strathdee et al. 1995). The black arrow indicates the position of the 18q telomere.

the parental origin of 18q terminal deletions and the clinical severity (Strathdee et al. 1995).

The deletion of critical chromosomal regions on other chromosomes invariably results in a distinct clinical phenotype associated with that chromosomal deletion, as exemplified by 5p deletions that result in the cri-du-chat syndrome (Overhauser et al. 1994), 17p deletions that result in the Miller-Dieker syndrome (Ledbetter et al. 1989), and 4p deletions that result in Wolf-Hirschhorn syndrome (Estabrooks et al. 1995). In contrast, deletions of the terminal end of 18q can result in the complete absence of clinical features associated with the typical 18q⁻ syndrome phenotype. This has become more apparent when patients who have been ascertained through cytogenetics rather than via clinical phenotype are included within the study (Strathdee et al. 1995). This is the likely reason why the incidence of specific clinical findings associated with 18q deletions are higher when clinical genetic case reports that describe patients with the 18q⁻ syndrome are used (see table 1), as compared with the incidence when reports that include clinical findings of patients with 18q deletions are used.

On the basis of the clinical presentation of the mother and daughter, both of whom have many of the typical clinical features associated with the syndrome, the critical chromosomal region for the 18q⁻ syndrome maps within 18q23. The size of the critical region is ~6 Mb, on the basis of the size of YAC clones that minimally overlap and span the 18q23 critical region.

The lack of clinical features present in patient 3 demonstrates that a deletion of this 18q23 critical region will not always result in a clinically recognizable 18q⁻ syndrome phenotype. Although this patient may represent the extreme end of the clinical spectrum that is associated with terminal deletions of 18q, this case demonstrates that genotype-phenotype–correlation studies

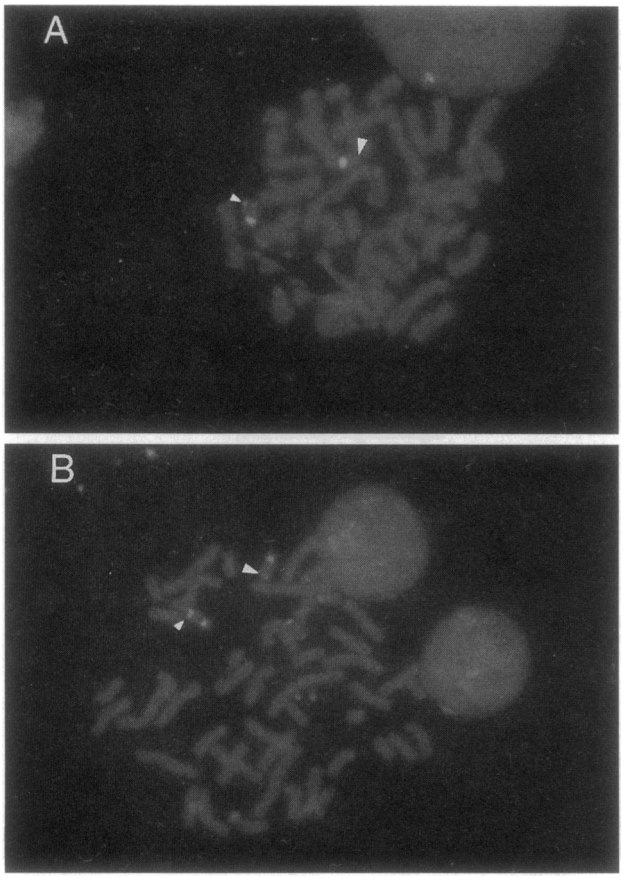

Figure 2 FISH analysis of 18q⁻ patients: patient 3 (*A*) and her daughter (*B*). Metaphase spreads were hybridized simultaneously with both genomic DNA from the YAC clone 943-C11 and a chromosome 18–specific alpha-satellite probe. The small arrow points to the normal chromosome 18 homologue, and the large arrow points to the deleted chromosome 18 homologue.

on the 18q⁻ syndrome must be based on the presence of clinical features, rather than on the lack of clinical features in a patient with a deletion. This case also demonstrates the dilemma that exists in the categorization of patients with 18q deletions. Although this patient has an 18q deletion that likely encompasses the genes involved in the presentation of the 18q⁻ syndrome, this patient would not be diagnosed with "the 18q⁻ syndrome" on the basis of the clinical phenotype but would be diagnosed with the 18q⁻ syndrome on the basis of cytogenetic results. Only through the identification of the genes that are involved in the etiology of the syndrome will it be possible to begin to unravel the molecular basis of this clinical variability.

To date, only two genes have been mapped to the 18q23 region: myelin basic protein (MBP) and cytochrome b5 (CYTB5). CYTB5 maps outside the deletions in the three patients reported here, but MBP does map within the three deletions. A role for MBP in the clinical features associated with the 18q⁻ syndrome has been postulated. Weiss et al. (1991) have suggested that hemizygosity of the MBP gene results in delayed myelination and may be at least partly responsible for the mental retardation and delay observed in some patients with 18q⁻ syndrome. Loss of both copies of the MBP gene is responsible for the *shiverer* phenotype in mice, which is characterized by generalized tremors, seizures, and a shortened life span (Chernoff 1981). In contrast, mice that are hemizygous for the MBP gene are phenotypically normal. More-detailed analysis of myelination in mice with reduced MBP expression has produced conflicting results. Barbarese et al. (1983) reported that heterozygous *shiverer* mice that express MBP at ~50% of wild-type levels have a corresponding 50% reduction in the level of MBP present in myelin of the CNS from both 15- and 90-d-old animals. However, in two more recent studies, Roch et al. (1987) found only a slight delay in MBP accumulation in mouse CNS, with MBP reaching normal levels by 85 d, and Shine et al. (1992) found no reduction of MBP in heterozygous *shiverer* mice at 60 d. These results suggest that longitudinal studies of patients who are hemizygous for MBP are needed in order to determine both the levels of MBP present in the myelin and whether MBP accumulation is delayed in patients with terminal deletions of the long arm of chromosome 18. The observation of patients with deletions of MBP who are of normal intelligence and development (Strathdee et al. 1995; present report) suggests that hemizygosity of MBP is not the only factor

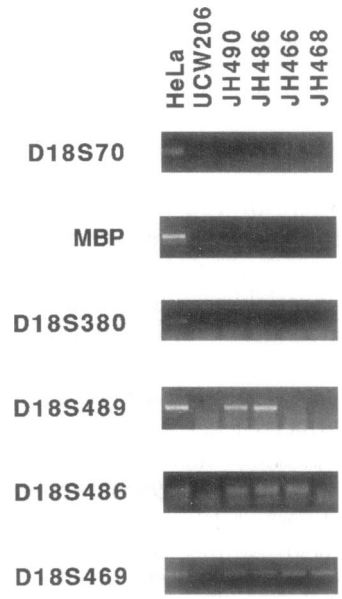

Figure 3 STS analysis of somatic-cell hybrids. Somatic-cell hybrids derived from patients with small terminal deletions of chromosome 18 are shown. HeLa = human control; UCW206 = hamster control; JH490 = patient 3; JH486 = daughter; JH466 = case 25; and JH468 = case 26.

involved in the phenotype of mental retardation and developmental delay in these patients. In addition, it is unlikely that hemizygosity of the MBP gene plays a causative role in the other clinical features associated with terminal deletions of chromosome 18. Therefore, the mapping of additional genes to 18q23 is needed to identify candidate genes that may be involved in the facial features, hearing loss, and limb abnormalities.

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References

Barbarese E, Nielson ML, Carson JH (1983) The effect of the shiverer mutation on myelin basic protein expression in homozygous and heterozygous mouse brain. J Neurochem 40:160–168

Breukel C, Wijnen J, Tops C, van den Klift H, Dauwerse H, Meera Khan P (1990) Vector-Alu PCR: a rapid step in mapping cosmids and YACs. Nucleic Acids Research 18:3097

Burke DT, Carle GF, Olson MV (1987) Cloning of large segments of exogenous DNA into yeast by means of artificial chromosome vectors. Science 236:806–812

Chernoff GF (1981) Shiverer: an autosomal recessive mutant mouse with myelin deficiency. J Hered 72:128

de Grouchy J, Roper P, Salmon C (1964) Deletion partielle des bras longs du chromosome 18. Pathol Biol (Paris) 12: 579–582

Estabrooks LL, Breg WR, Hayden MR, Ledbetter DH, Myers RM, Wyandt HE, Yang-Feng TL, et al (1995) Summary of the 1993 ASHG ancillary meeting "Recent Research on Chromosome 4p Syndromes and Genes." Am J Med Genet 55:453–458

Feingold M, Robert SS, Atkins L, Anderson R, Bartsocas CS, Page DL, Littlefield JW (1969) IgA deficiency associated with partial deletion of chromosome 18. Am J Dis Child 117:129–136

Felding I, Kristoffersson U, Sjostrom H, Noren O (1987) Contribution to the 18q− syndrome: a patient with del(18)(q22.3qter). Clin Genet 31:206–210

Francke U, Chang E, Corneau K, Geigl E-M, Giacalone J, Li X, Luna J, et al (1994) A radiation hybrid map of human chromosome 18. Cytogenet Cell Genet 66:196–213

Games J, McNinch J, Alleman J, Amemiya C, Ioannou P, Wong B, Kroisel P, et al (1994) Preparation of large insert libraries for chromosome 18 mapping using lambda, cosmid and P1 vectors. Cytogenet Cell Genet 65:160

Gerken S, Fish K, Uyar D, Polymeropoulos MH, Bradley P, White R, Overhauser J, et al (1994) Integration of 28 STSs into the physical map of human chromosome 18. Genomics 24:612–613

Gyapay G, Morissette J, Vignal A, Dib C, Fizames C, Millasseau P, Marc S, et al (1994) The 1993–1994 Généthon human genetic linkage map. Nat Genet 7:246–339

Kline AD, Rojas K, Mewar R, Moshinsky D, Overhauser J (1992) A deletion mapping panel of human chromosome 18. Genomics 13:1–6

Kline AD, White ME, Wapner R, Rojas K, Biesecker LG, Kamholz J, Zackai E, et al (1993) Molecular analysis of the 18q− syndrome—and correlation with phenotype. Am J Hum Genet 52:895–906

Kohonen-Corish M, Strathdee G, Overhauser J, McDonald T, Jammu V (1996) A new deletion of 18q23 with few typical features of the 18q− syndrome. J Med Genet 33:240–243

Ledbetter DH, Ledbetter SA, VanTuinen P, Summers KM, Robinson TJ, Nakamura Y, Wolff R, et al (1989) Molecular dissection of a contiguous gene syndrome: frequent submicroscopic deletions, conserved sequences, and a hypomethylated "island" in the Miller-Dieker syndrome. Proc Natl Acad Sci USA 86:5136–5140

Lewkonia RM, Lin CC, Haslam RHA (1980) Selective IgA deficiency with 18q+ and 18q karyotypic anomalies. J Med Genet 17:453–456

Liu P, Siciano J, Seong D, Craig J, Zhao Y, de Jong PJ, Siciliano MJ (1993) Dual alu polymerase chain reaction primers and conditions for isolation of human chromosome painting probes for hybrid cells. Cancer Genet Cytogenet 66:93–99

Longmire JL, Brown NC, Meincke LJ, Campbell ML, Albright KL, Fawcett JJ, Campbell EW, et al (1993) Construction and characterization of partial digest DNA libraries made from flow-sorted human chromosome 16. Genet Anal Tech Appl 10:69–76

Mewar R, Kline AD, Jackson L, Overhauser J (1992) Confirmation of a cryptic unbalanced translocation using whole chromosome Fluorescence in situ hybridization. Am J Med Genet 44:477–481

Overhauser J, Huang X, Gersh M, Wilson W, McMahon J, Bengtsson U, Rojas K, et al (1994) Molecular and phenotypic mapping of the short arm of chromosome 5: sublocalization of the critical region for the cri-du-chat syndrome. Hum Mol Genet 3:247–252

Overhauser J, Mewar R, Rojas K, Lia K, Kline AD, Silverman GA (1993) STS map of genes and anonymous DNA fragments on human chromosome 18 using a panel of somatic cell hybrids. Genomics 15:387–391

Qazi QH, Madahar C, Alvi S, McGann B (1980) Distal 18q deletion without clinical findings of 18q− syndrome. Ann Genet 23:60–62

Roch JM, Cooper BJ, Ramirez M, Mattieu JM (1987) Expression of only one myelin basic protein allele in mouse is compatible with normal myelination. Mol Brain Res 3:61–68

Sataloff RT (1991) Embryology and anomalies of the facial nerve and their surgical implications. Raven Press, New York

Shepard EA, Povey S, Spurr NK, Phillips IR (1991) Chromosomal localization of a cytochrome b5 gene to human chromosome 18 and a cytochrome b5 pseudogene to the X chromosome. Genomics 11:302–308

Shine HD, Redhead C, Popko B, Hood L, Sidman RL (1992) Morphometric analysis of normal, mutant and transgenic CNS: correlation of myelin basic protein expression to myelinogenesis. J Neurochem 58:342–349

Silverman GA (1993) Isolating vector-insert junctions from

yeast artificial chromosomes. PCR Methods Appl 3:141–150

Silverman GA, Schneider SS, Massa HF, Flint A, Lalande M, Leonard JC, Overhauser J, et al (1995) The 18q$^-$ syndrome: analysis of chromosomes by bivariate flow karyotyping and the PCR reveals a successive set of deletion breakpoints within 18q21.2-q22.2. Am J Hum Genet 56:926–937

Strathdee G, Harrison W, Riethman HC, Goodart SA, Overhauser J (1994) Interstitial deletions are not the main mechanism leading to 18q deletions. Am J Hum Genet 54:1085–1091

Strathdee G, Zackai EH, Shapiro R, Kamholz J, Overhauser J (1995) Analysis of the clinical variation seen in patients with 18q terminal deletions. Am J Med Genet 59:476–483

Straub RE, Speer MC, Luo Y, Rojas K, Overhauser J, Ott J, Gilliam TC (1993) A microsatellite genetic linkage map of human chromosome 18. Genomics 15:48–56

Stricker RB, Linker CA (1982) Pernicious anemia, 18q deletion syndrome, and IgA deficiency. JAMA 248:1359–1360

Subrt I, Pokorny J (1970) Familial occurrence of 18q−. Humangenetik 10:181–187

Van Kessel AG, Straub RE, Silverman GA, Gerken S, Overhauser J (1994) Report of the Second International Workshop on Human Chromosome 18 Mapping. Cytogenet Cell Genet 65:141–165

Weiss BJ, Kamholz J, Ritter A, Zackai EH, McDonald-McGinn DM, Emanuel B, Fischbek KH (1991) Segmental spinal muscular atrophy and dermatological findings in a patient with chromosome 18q deletion. Ann Neurol 30: 419–423

Weissenbach J, Gyapay G, Dib C, Vignal A, Morissette J, Millasseau P, Vaysseix G, et al (1992) A second-generation linkage map of the human genome. Nature 359:794–801

Wertelecki W, Gerald PS (1971) Clinical and chromosomal studies of the 18q− syndrome. J Pediatr 78:44–52

Wilson MG, Towner JW, Forsman I, Siris E (1979) Syndromes associated with deletion of the long arm of chromosome 18 [del(18q)]. Am J Med Genet 3:155–174