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Molecular Analysis of the 18q− Syndrome—and Correlation with Phenotype

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Summary

Seven individuals with deletions of the distal long arm of chromosome 18 were evaluated at the clinical, cytogenetic, and molecular levels. The patients had varying degrees of typical clinical findings associated with the 18q− syndrome. Cytogenetic analysis revealed deletions from 18q21.3 or 18q22.2 to qter. Somatic cell hybrids derived from the patients were molecularly characterized using ordered groups of probes isolated from a chromosome 18-specific library. In general, the size of the deletion could be correlated with the severity of the phenotype. Based on the clinical pictures of these seven patients, a preliminary phenotypic map for the clinical features associated with deletions of the distal portion of the long arm has been generated. Furthermore, genes previously localized to 18q21 were mapped relative to the chromosome breakpoints present in these patients.

Introduction

The 18q− syndrome is a well-described partial aneusomy disorder resulting from the deletion of a portion of the long arm of chromosome 18. Classically, patients with this syndrome exhibit growth deficiency, dysmorphic facial features, extremity findings, genitourinary malformations, neurologic and ocular abnormalities, and developmental delay with mental retardation (MR) (Subrt and Pokorny 1970; Wertelecki and Gerald 1971; Wilson et al. 1979). Diagnostic findings are described in table 1. The dysmorphic features are mild and characteristically include midfacial hypoplasia, carp-shaped mouth, and abnormally folded ears. Neurologic findings include hypotonia, poor coordination, choreoathetotic movements, and ophthalmologic abnormalities (strabismus, nystagmus, myopia, and fundoscopic changes) (Schinzel 1984). Abnormal electroencephalograms and seizures have been described, as well as specific brain abnormalities such as dilation of the ventricles, hydrocephalus, porencephaly, cerebellar hypoplasia, decreased white matter, impaired or delayed myelination, and other migration defects (Wertelecki and Gerald 1971; Fryns et al. 1979; Wilson et al. 1979; Felding 1987; Miller et al. 1990; Vogel et al. 1990; Weiss et al. 1991). Normal brains have also been documented on autopsy or computed-tomography scan (Law and Masterson 1968; Fraccaro 1971; Abusrewil et al. 1988). Life expectancy is normal, and many affected adults have been reported (Subrt and Pokorny 1970; Wertelecki and Gerald 1971; Fryns et al. 1979; Schinzel 1984; Miller et al. 1990).

For all defined aneuploidies, clinical variation exists between patients. This may be due to genetic background as it relates to epistatic variations due to interactions with other genes not associated with the chromosomal alteration or the amount of chromosomal material deleted or duplicated. An extensive review of the literature suggests that there is a broad phenotypic spectrum in patients with 18q deletions, as summarized

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Table 1

Diagnostic Features of the 18q- Syndrome

Category	Findings
Growth	Decreased height and weight; microcephaly
Facies	Dysplastic ears; midfacial hypoplasia; down-turned corners of mouth; prognathism; protuberant lower lip
Limbs	Tapered fingers; proximal thumbs; single palmar crease; excessive whorls; abnormal toes; pes planus; clubfeet
Genitourinary system	Hypoplasia of labia or scrotum; micropenis; cryptorchidism; hypospadias
Skeletal system	Abnormal skull, ribs, vertebrae; atretic ear canals; vertical tali
Central nervous system	Hypotonia; seizures; sensorineural or conductive deafness; enlarged ventricles; hydrocephalus; ophthalmologic findings (strabismus, nystagmus, myopia, fundoscopic abnormalities); retardation of motor and mental development

in table 2. The range of severity of the 18q− syndrome appears to be variable; some affected individuals demonstrate involvement of all systems, with moderate to profound MR, and others have minimally dysmorphic features and no MR.

For other deletion syndromes, a chromosomal region has been able to be identified and subsequently correlated with molecular studies (Overhauser et al. 1986; van Tuinen et al. 1988; Driscoll et al. 1992). However, in most cases of the 18q− syndrome the extent of the cytogenetically determined deletion does not seem to correlate with the severity of the clinical findings (Wilson et al. 1979; Miller et al. 1990). This discrepancy between the clinical and cytogenetic results in the 18q− syndrome could be due to several factors: (1) a critical region for this syndrome exists near 18q21, but, since many of the cases were reported prior to high-resolution banding (Yunis 1976), the inherent limitations of cytogenetic analysis may have led to inaccuracies in reporting; (2) there is no specific critical region for this syndrome; (3) the background genome in the presence of a deletion plays a significant role in producing the clinical features; (4) an undetected low level of mosaicism mediates severity of the phenotype; and (5) differences in parental origin of the deletion affect the outcome. No previous study has investigated these hypotheses on a molecular level.

To understand more fully the variation in phenotype displayed by individuals with 18q deletions, we have analyzed seven patients with 18q deletions and different presentations. We have obtained clinical information from these patients, performed cytogenetic analysis, and derived somatic cell hybrids containing the deleted chromosome 18 for molecular studies. In this report we describe the results of physical mapping of the 18q deletions and correlate these with the clinical manifestations.

Patients and Methods

Patient Population and Samples

Patients with deletions of 18q were referred, either by the Chromosome 18 Registry and Research Society, a support group for individuals with chromosome 18 abnormalities and their families, or from nonaffiliated genetic centers. For each patient the available medical history, developmental status, physical examination, reports of diagnostic procedures, and photographs were reviewed and compared with those of patients with the 18q− syndrome described in the literature.

Cytogenetic determination of an 18q deletion was made prior to referral. Peripheral blood samples were obtained from the patients, in sodium heparin tubes. Cytogenetic analysis of banded chromosomes was repeated using standard methods for high-resolution banding (Yunis 1976). Peripheral blood was also collected in ACD tubes (Fisher Scientific; Pittsburgh) for Epstein-Barr virus transformation of lymphocytes according to established procedures (Buchwald 1984).

Somatic Cell Hybrid Analysis

Somatic cell hybrids were derived from each transformed lymphoblast cell line by polyethylene glycol–mediated fusion with UCW206, a Chinese hamster ovary mutant cell line that contains a temperature-sensitive mutation in the asparaginyl-tRNA synthetase gene. The complementary human gene is located on 18q, thus enabling only hybrids containing a human

Table 2

Features of Reported Patients with Terminal Deletions of Chromosome 18q

Clinical Feature	Wertelecki and Gerald 1971	Faulkner et al. 1983	Faed et al. 1972	Wilson et al. 1979	Weiss et al. 1991	Vogel et al. 1990	Hoo 1986	Felding et al. 1987	Miller et al. 1990
	del(18)(q21)	del(18q)(21)	del(18)(q21)	del(18)(q21.3)	del(18)(q21.3)[a]	del(18)(q22)	del(18)(q22.2)[b]	del(18)(q22.3)	del(18)(q22.3)
Growth retardation	+	+	+	+	−	+	−	+	+
Microcephaly	−	−	+	+	−	+	−	+	+
Facial dysmorphism[c]	+	+	+	+	−	+	+	+	+
Extremity findings:[c]									
Upper	+	−	−	+	−	−	NM	+	+
Lower	+	−	−	+	+	+	NM	+	+
Hypoplastic genitalia	+	+	+	+	−	+	NM	−	−
Neurologic findings:									
Seizures	−	−	−	−	−	−	−	−	−
Hypotonia	+	+	+	−	+	+	−	+	+
Deafness	Conductive	−	Sensorineural	−	−	Conductive	NM	−	Conductive
Ophthalmologic findings	+	+	+	+	+	−	NM	+	+
Abnormal CNS myelination	−	−	−	−	+	+	NM	+	+
Developmental delays	++	+	++	+++	−	+++	−	+++	++
Degree of MR or intelligence quotient	40–50	Mild	Mild-moderate	20–50	LDs	42	−	Moderate	Mild-moderate

NOTE.—A minus sign (−) denotes absence or that feature was not mentioned; a single plus sign (+) denotes presence; and multiple plus signs (++ and +++) denote increased levels of severity.

[a] LDs = learning disabilities.

[b] NM = not mentioned in report.

[c] Includes three or more features as listed by category in table 1.

chromosome 18 to survive at the restrictive temperature (Cirullo et al. 1983; Kline et al. 1992). Initial screening of the somatic cell hybrids for the presence of the derivative chromosome 18 was performed by PCR as described elsewhere (Kline et al. 1992). The cell hybrids that appeared to have retained only the deleted human chromosome 18 were analyzed by G11 staining and G-banding to confirm the PCR results (Worton and Duff 1979; Dana and Wasmuth 1982). Attempts were made to obtain at least two independent somatic cell hybrids from each lymphoblast cell line containing the deleted chromosome 18, to confirm that this chromosome represented that of the patient. This was performed because the process of making fusions can produce chromosomal breakage. The amount of chromosomal breakage observed after the screening of 2–10 somatic cell hybrids from individual fusions involving UCW206 ranged from none to five aberrant events.

DNA Analysis

A set of chromosome 18–specific DNA probes were obtained from a lambda phage library derived from HHW324, a somatic cell hybrid containing a normal chromosome 18 as its only human component. The lambda phage inserts had been mapped to specific chromosomal regions by using a panel of somatic cell hybrids as reported elsewhere (Kline et al. 1992). A subset of 70 of these lambda phage were used as the DNA probes and were labeled using the random primer method for this project (Feinberg and Vogelstein 1983).

Genomic DNA isolation from the somatic cell hybrids, restriction-enzyme digestion, and DNA electrophoresis were performed using standard methods (Sambrook et al. 1989). Southern blotting and hybridization with probes from the library were performed as described elsewhere (Kline et al. 1992).

PCR Analysis

PCR reactions were performed in a 25-μl volume containing 100 ng genomic DNA, 1 μM each primer, 250 μM dNTP, 2% formamide, 0.6 U AmpliTaq DNA polymerase (Perkin Elmer Cetus, Norwalk, CT) in buffer containing 10 mM Tris-HCl pH 8.3, 50 mM KCl, 0.01% (w/v) gelatin. The sequences of the primers, the size of each amplified region, the concentrations of $MgCl_2$ used, and the annealing temperatures are listed in table 3. Amplification was performed for 35 cycles of denaturation at 96°C for 90 s, followed by annealing at the appropriate temperature for 45 s, extension at 72°C for 30 s, and a final extension time of 10 min at 72°C in a Coy thermocycler. The amplification products were separated on a 1.5% agarose gel.

Results

Clinical Features

A clinical summary of the seven patients is shown in table 4. Features of patient JL111 have been reported elsewhere (Weiss et al. 1991). There were four females and three males, with ages ranging from 2 to 23 years. All of the patients demonstrated a tall forehead and midfacial hypoplasia. All except two (JL 111 and JL143) had other typical features, including down-turned corners of the mouth, especially when younger. Three had dysplastic ears, and all except two patients (JL156 and JL111) had varying degrees of sensorineural or conductive deafness, of whom only one (JL209) had atretic ear canals. Four of the patients had normal growth. Of the others, one (JL156) had microcephaly with head circumference well below the 5th percentile for age and with both height and weight at the 25th percentile for age; one (JL143) had retardation of weight and height, which resolved after deficiency of human growth hormone was diagnosed and treated; and the third (JL172) had failure to thrive (well below the 5th percentile for age) for all three parameters. All of the patients demonstrated typical limb abnormalities, including tapered fingers, brachydactyly, proximally placed thumbs, clubfeet, abnormal toes, pes planus, or abnormal dermatoglyphics. Two of the three males (JL184 and JL172) had cryptorchidism, and all three had a small penis; none of the females had hypoplastic genitalia.

Neurologically, all of the patients had varying degrees of hypotonia. One patient (JL111) had segmental spinal muscular atrophy. Two (JL184 and JL172) had a history of seizures. Four of the individuals (JL111, JL209, JL184, and JL172) had abnormalities found on ophthalmologic examination, including myopia, nystagmus, and strabismus. None of the patients had a history of hydrocephalus or enlarged ventricles. Moderate to severe MR was seen in two patients (JL156 and JL172), mild MR was found in three patients (JL209, JL184, and JL143), one patient (JL111) had normal intelligence with learning disabilities, and the last patient (JL181) had a normal intelligence quotient. Magnetic resonance imaging (MRI) studies of the brain were available on five of the seven patients, and, of these five, two (JL181 and JL143) were reported as normal. The third MRI (JL111) demonstrated lack of normal differentiation between grey and white matter, with increased sig-

Table 3

Genes Located on Chromosome 18

Gene	Location	PCR Primers	PCR Size (bp)	Mg (mM)	Temperature (°C)	Reference
DCC	18q21.2	ACCTAATTCACCCCCTATATT TGTAAACCGACATATCTGATG	550	3.0	55	B. Vogelstein, personal communication
PLANH2	18q21.2-22.2	GAGGATCTTTGTGTGGCAAAC TCGGCCCAAATGTGGAGCTTC	200	3.0	65	Silverman et al. 1991
GRP	18q21.3	ATCCTTCTTTCCAAATATTAC AATGCCAACACAAGTGCGAGA	200	2.0	55	Silverman et al. 1991
BCL2	18q21.3	ATTGTGATGGTCATATTATTG ATATCATATGTCCTTTATGCA	420	3.0	55	Silverman et al. 1991
MBP	18q22.3	GATAAGTAATAGAGTTTGGAGAG GGAATTCCTCAGTTACTCGAGAT	583	3.0	55	Boylan et al. 1990

nal intensity on T2 weighted images in white matter, consistent with abnormal myelination. A fourth MRI (JL184) showed increased signal intensity on T2 weighted images in selected white-matter tracts, consistent with hypomyelination in these areas. Most notable was an abnormality within a white-matter tract in the basal ganglia. The last MRI (JL156) had markedly increased signal intensity on T2 weighted images in all white-matter tracts, suggesting absent or greatly reduced myelination. On the basis of these comparisons, patients JL172 and JL156 were considered severely affected, patients JL209 and JL 184 were considered moderately involved, and patients JL143, JL111, and JL181 were considered mildly affected.

Cytogenetic Analysis

Each patient had been previously karyotyped and diagnosed with a de novo deletion of 18q21.3. Cytogenetic analysis performed elsewhere showed normal chromosomes for all of the parents of the patients. Complete cytogenetic analysis on each patient was repeated, with close attention to the deleted region on 18q. Partial karyotypes of the seven individuals are shown in figure 1, and the results are listed in table 4. Four of the patients had deletions at 18q21.3, while three appeared to have a more distal breakpoint at 18q22.2. The latter findings differed from the initial reports, illustrating differences in interpretation between laboratories, even with high-resolution banding. All of the deletions appeared to be terminal; however, the possibility of an interstitial deletion could not be ruled out on the basis of only cytogenetic analysis.

Somatic Cell Hybrid Analysis

Somatic cell hybrids containing the deleted chromosome 18 obtained from each of the seven transformed cell lines were analyzed at the molecular level. The 70 DNA markers that initially mapped to 18q21-qter were tested to determine whether each probe was located within, proximal to, or distal to the deleted segment of chromosome 18 for each patient. The previous location of the DNA markers is shown in the top panel of figure 2. The somatic cell hybrids JH226 and JH251 shown in the top panel of figure 2 are derived from patients with balanced translocations involving chromosome 18. Thus, the probes that map to the most distal portion of 18q have been localized using somatic cell hybrids that should not have an undetectable interstitial deletion.

Examples of autoradiographs and the amount of chromosome 18 present in each hybrid derived from the seven patients are shown in the bottom panel of figure 2. Different molecular breakpoints were detected in all of the patients, except that the cell hybrid lines representing JL156 and JL172 demonstrated identical hybridization patterns for all of the probes screened. On the basis of these resultant patterns, the DNA markers were further subdivided, and their locations relative to each breakpoint are shown in the bottom panel of figure 2. All the distal DNA markers (region 7) were found to be deleted in all of the patients.

Localization of Genes Relative to the 18q Deletions

Several genes have been previously localized within 18q21-qter. Four have been mapped to 18q21.3: gastrin-releasing peptide (GRP); BCL2, a gene involved in a

Table 4

Clinical Features of Patients with 18q Deletions

Clinical Feature	JL181	JL111	JL143	JL184	JL209	JL172	JL156
	46,XX,del(18)(q22.2)	46,XX,del(18)(q22.2)[a]	46,XX,del(18)(q22.2)	46,XY,del(18)(q21.3)	46,XX,del(18)(q21.3)[b]	46,XY,del(18)(q21.3)[b]	46,XY,del(18)(q21.3)
Growth retardation	−	−	+	−	−	+	−
Microcephaly	−	−	−	−	−	+	+
Facial dysmorphism[c]	+	−	+	+	+	+	+
Extremity findings:[c]							
Upper	+	+	−	+	+	−	+
Lower	+	+	+	+	+	+	+
Hypoplastic genitalia	−	−	−	+	−	+	−
Neurologic findings:							
Seizures	−	−	−	+	−	+	−
Hypotonia	+	+	+	+	+	+	+
Deafness	Sensorineural	−	Conductive	Sensorineural	Conductive	Sensorineural	−
Ophthalmologic findings	−	+	−	+	+	+	−
Abnormal MRI brain	−	+	−	+	ND	ND	++
Developmental delays	−	+	+	++	+	+++	+++
Degree of MR or intelligence quotient	83	LDs	Mild	Mild-moderate	Mild	Severe	Moderate

NOTE.—A minus sign (−) denotes absence; a single plus sign (+) denotes presence; and multiple plus signs (++ and +++) denote increased levels of severity.

[a] LDs = learning disabilities.

[b] ND = not determined.

[c] Includes three or more features as listed by category in table 1.

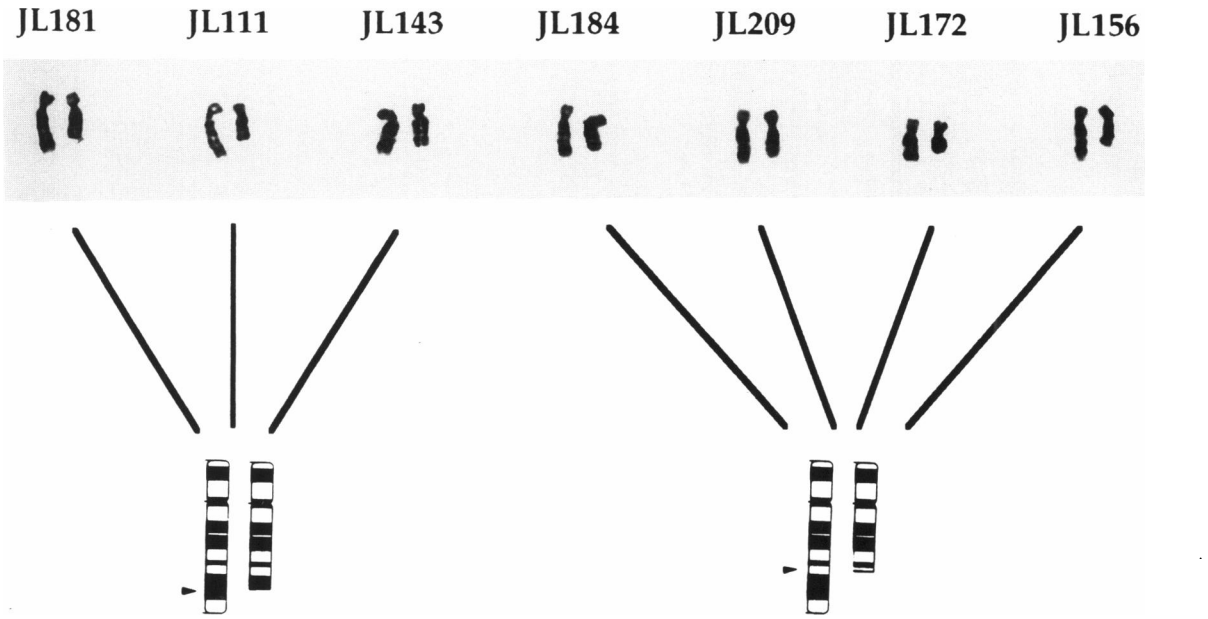

Figure 1 Partial karyotypes of seven patients with distal 18q deletions. The chromosome 18 homologues are shown for each patient. An ideogram with the approximate location of each breakpoint is shown.

translocation predisposing to follicular lymphoma; plasminogen activator inhibitor type-2 (PLANH2); and DCC, a possible tumor-suppressor gene (Tsujimoto et al. 1984; Lebacq-Verheyden et al. 1987; Ye et al. 1988; Fearon et al. 1990). Little has been determined about their relative order, except that BCL2 maps proximally to PLANH2 (Silverman et al. 1991). The myelin basic protein gene (MBP) has been mapped to 18q22-q23 (Kamholz et al. 1987). Since it was not known where these genes map with respect to chromosomal regions deleted in patients with the 18q− syndrome, it has been unclear whether they affect the etiology or phenotype of the syndrome.

To determine the location of these genes relative to the molecular breakpoints present in our patients and to each other, PCR amplification was performed using primer sets specific for each of the five genes previously described. The sets of primers are listed in table 3. The results of the PCR amplification of genomic DNA from the seven representative somatic cell hybrids, as well as the region to which each gene maps, are shown in figure 3. On the basis of these results, the gene order could be determined to be cen–DCC–GRP–BCL2–PLANH2–MBP–tel.

Comparison of Phenotype with Physical Map

As described above, the seven patients demonstrated a range of clinical involvement. After the molecular order of the breakpoints was determined, the severity of the phenotype was found to correlate, in general, with the size of the deletion. A preliminary phenotype-genotype correlation map has been constructed on the basis of the above clinical, cytogenetic, and molecular data and is shown in figure 4. The two patients with the most proximal molecular breakpoints (JL172 and JL156) were the most severely affected clinically, exhibiting microcephaly, the greatest degree of MR, and, on one of the two (JL156), the most abnormal brain MRI. The moderately affected individuals (JL209 and JL184) displayed mild MR but normal head size and had smaller deletions than did JL172 and JL156. Thus, the genes within region 2, the chromosomal segment deleted in the first two patients but present in all of the others, appear to be the most significant in terms of brain growth and mental development. The chromosomal regions defined by regions 3 and 4 may also be involved in mental development. From the five brain MRI results available, a deletion encompassing region 4 appears to result in severe defects of myelination, while a deletion of region 5 appears to produce some abnormal white-matter findings and ophthalmologic abnormalities. Some of the dysmorphic facial features (midfacial hypoplasia and tall forehead), hypotonia, and lower-extremity abnormalities seen in most of the patients may result from deletions within region 7. Since

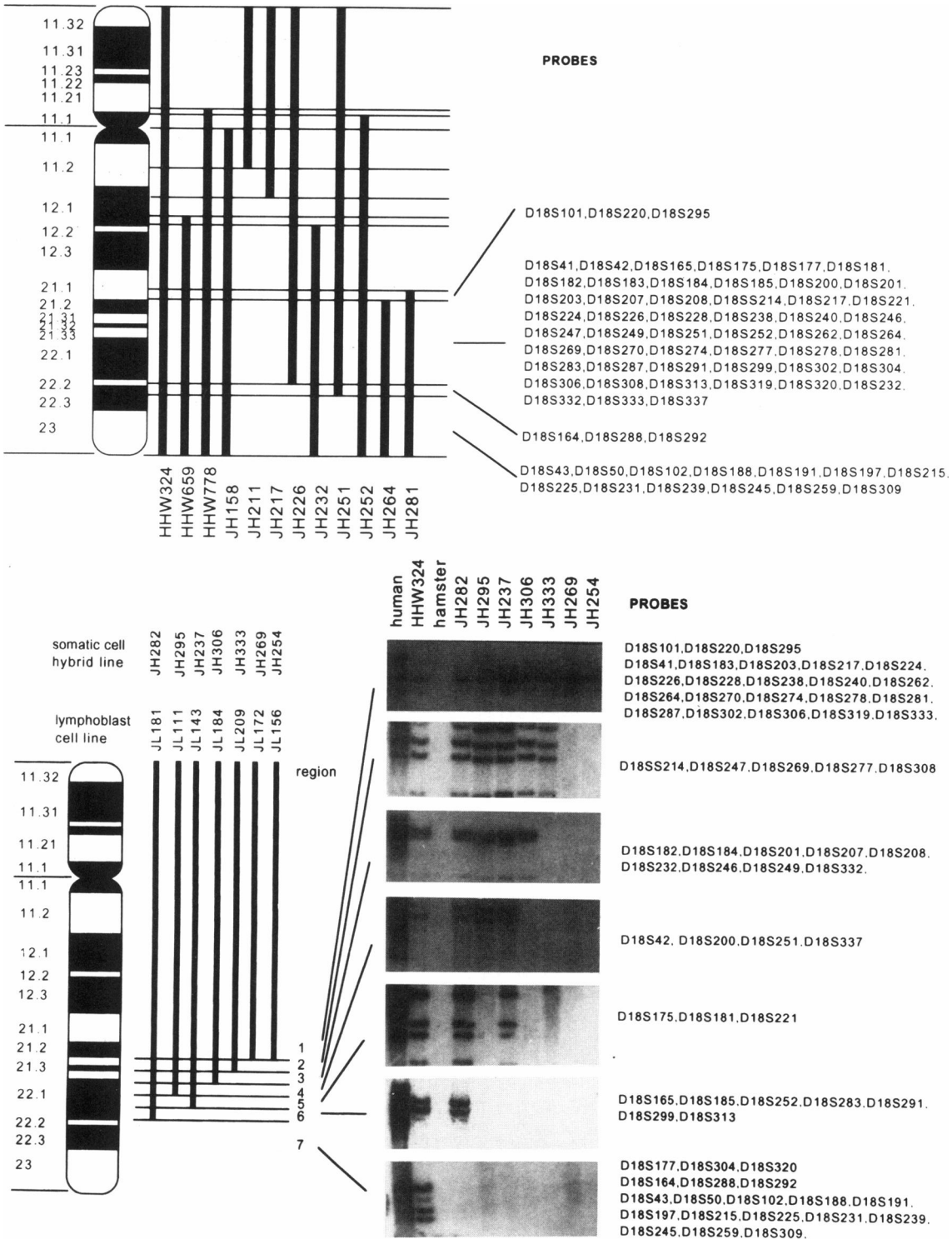

Figure 2 Southern blot hybridization to somatic cell hybrids with chromosome 18 rearrangements. An ideogram of a normal chromosome 18 is shown on the left. The vertical black bars represent the amount of chromosome 18 material present in each somatic cell hybrid, on the basis of the hybridization patterns. *Top,* Names and initial location of the 70 probes used for mapping. *Bottom,* Southern blot analysis of somatic cell hybrids with 18q deletions. The names of the lymphoblast cell lines and the corresponding somatic cell lines are shown. Genomic DNAs digested with *Eco*RI were electrophoresed, blotted, and hybridized to each lambda phage clone. The probes used to represent each of the regions identified are as follows: region 1, D18S87; region 2, D18S332; region 3, D18S247; region 4, D18S42; region 5, D18S221; region 6, D18S299; and region 7, D18S164.

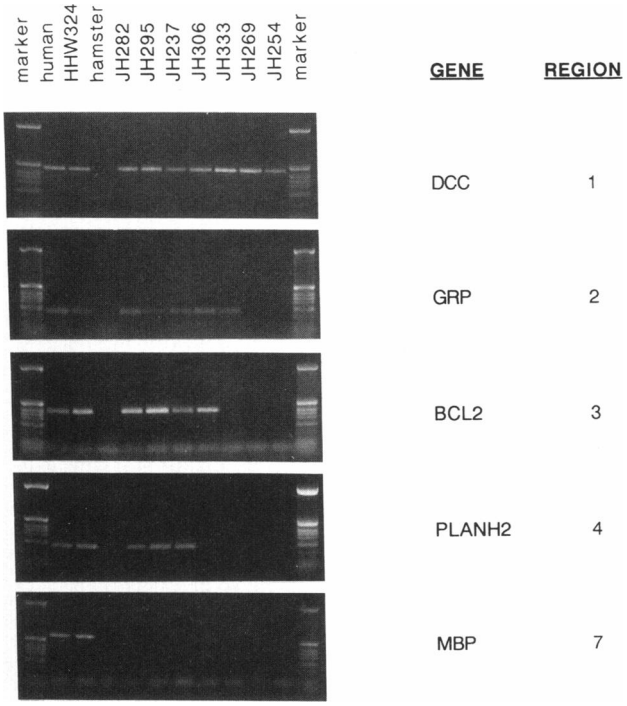

Figure 3 PCR amplification mapping of genes previously localized to 18q. The PCR primers and conditions are listed in table 2. Genomic DNA used for each amplification is shown. The marker is pBR322 digested with *Hin*fI. The region to which each gene mapped is shown on the right.

the three males had some degree of genital hypoplasia, a gene that plays a role in male sexual development may reside in regions 4–7. Of the three females, only one (JL111) had reached puberty and was clinically normal with respect to sexual development. Thus, the extent of involvement of this region in female sexual development could not be evaluated at this time. Specific abnormalities on neurologic examination and overall somatic growth (height and weight) did not appear to correspond to a deletion of a defined chromosomal region. Multiple genes residing on different regions of chromosome 18 or other chromosomes may be involved in the appearance of these clinical findings.

Discussion

Seven patients with 18q deletions have been evaluated at the clinical, cytogenetic, and molecular levels. Clinically, the patients were found to be mildly, moderately, or severely affected. Cytogenetically, three of the patients (JL181, JL111, and JL143) had more distal breakpoints at 18q22.2, compared with the other four patients, who had chromosomal breakpoints at 18q21.3. Our interpretations were different from some of the initial karyotype readings. This may have been due to a more consistent approach to the analysis of the deletions in our laboratory, since all of the preparations were scanned by a single person, aware of the presence of an 18q deletion but not aware of the previously determined breakpoints. Discrepancies of karyotype interpretation are inevitable, and this underscores the limitations of cytogenetic analysis, especially when there is an attempt to make genotype-phenotype correlations for aneusomy syndromes. Because of this limitation, the breakpoints were evaluated utilizing a molecular approach.

With somatic cell hybrids derived from the seven patients and a set of chromosome 18–specific genomic DNA probes, the deletion breakpoints in the patients were further characterized. Molecular analysis of the deletions confirmed our cytogenetic findings and, in addition, identified subtle differences in sizes of the breakpoints. The distal to proximal order of the breakpoints was determined to be JL181, JL143, JL111, JL184, JL209, and both JL172 and JL156. The relative order could not be ascertained for the deletions present in the latter two, although, clinically, JL172 appeared slightly more severe. It is unlikely that the two patients have exactly the same molecular breakpoints; this more likely reflects the lack of probes that could differentiate between them.

To initiate the identification of functionally important genes within the critical region for the 18q− syn-

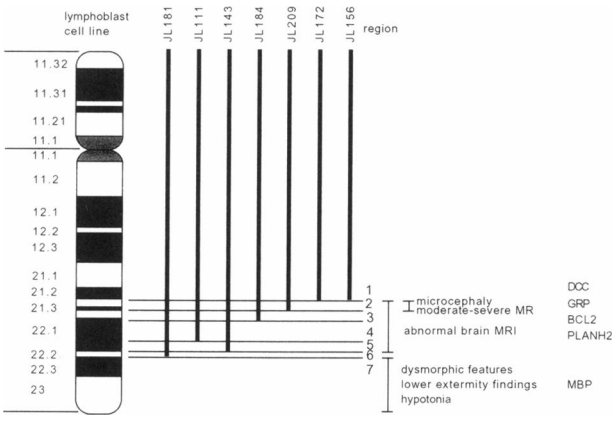

Figure 4 Preliminary phenotypic map for 18q deletions. The size of the deleted chromosome 18 is represented by the vertical black bars. The clinical characteristics that seem to map to specific chromosomal segments are shown. The location of the genes with respect to the phenotypic map are shown.

drome, genes previously mapped to 18q21.3 were localized relative to the same chromosomal breakpoints. GRP mapped to region 2, BCL2 to region 3, and PLANH2 to region 4. Further analysis will be needed to determine whether these genes play a role, if any, in the etiology or phenotype of this syndrome.

Since, on the basis of brain MRI scans, abnormalities in central nervous system myelination have been described in individuals with 18q deletions, the location of the MBP gene relative to the breakpoints observed in our patients was important. Because the MBP gene mapped to region 7, a region deleted in all of our patients, it is unlikely that the presence of only one copy of this gene is solely responsible for producing abnormalities in myelination, seen on MRI, which has been suggested in previous reports (Weiss et al. 1991). The degree to which myelination is affected appears to correlate with severity of other features, and, therefore, it is possible that having one deleted MBP gene in conjunction with other more proximal genes within the deleted region may deleteriously affect brain structure and, subsequently, central nervous system function. Further brain MRI studies in these individuals should provide more information about myelination effects.

It is clinically significant that none of the seven patients had a deletion of the DCC gene, which mapped to region 1. This suggests that, compared with the general population, they should be at no increased risk of developing colon cancer, which is a potential concern for individuals with any 18q deletion. Since the reported life span of individuals with 18q deletions appears to be normal (Subrt and Pokorny 1970; Wertelecki and Gerald 1971; Fryns et al. 1979; Schinzel 1984; Miller et al. 1990), the exact location of the DCC gene relative to the breakpoint in these patients would be important, especially in those with larger distal or more proximal interstitial deletions than the ones described in the present report.

The molecular analysis of the cell lines suggests that all of the deletions are terminal. This is based on the observation that all of the DNA probes (including MBP) mapping to 18q22.3-qter were absent in the seven somatic cell hybrids. Two of these probes, D18S50 and MBP, have been placed at the terminal end of the genetic map (Straub et al., in press). The probe D18S17, which is the most distal q arm marker on the genetic map, was also found to be missing in all seven cell lines (data not shown). This is highly suggestive that the deletions are terminal rather than interstitial. However, until a clone that includes the telomeric sequences for 18q is obtained and the somatic cell hybrids can be analyzed, it will be impossible to conclusively prove that the deletions are terminal.

With the order of the breakpoints determined, the size of the deletions was compared with the phenotype. Statistical comparisons of the clinical findings among the seven patients could not be made, because of small sample size; however, some trends were apparent. The four individuals with the largest deletions would be described as having the classic 18q− syndrome, with two more severely affected. As a group, smaller deletions produced a milder phenotype. One patient (JL111) was the least affected clinically but did not have the smallest deletion. The two patients with the smallest deletions (JL143 and JL181) were, however, younger in age than was JL111, and it is difficult to ascertain full developmental status at this time, as well as other potentially progressive features. Evidence has shown that, over time, these individuals perform better than initially anticipated (Wertelecki and Gerald 1971).

Specific regions could be implicated in some clinical manifestations. Regions 2–4 appear to be of greatest significance with respect to brain growth and neurologic status, especially with regard to level of developmental achievement. Terminal deletions that include region 4 seem to produce the major features associated with this syndrome. This region will need to be further analyzed in order to determine the validity of the phenotypic mapping. Deletions of 18q that encompass regions 4–7 could be significant for producing some degree of male genital hypoplasia but appear to have no effect on female development. Changes in somatic growth did not appear to correlate with a specific region. Growth hormone deficiency, seen in one of our mildly affected patients, has been documented in only one previous report of an 18q deletion (Abusrewil et al. 1988). No chromosomal region could be implicated in producing the extremity anomalies, deafness, and seizures.

An undetected low level of mosaicism could contribute to variation in presentation, but this is less likely when the chromosome abnormality is fully present in the lymphocytes. Karyotyping of skin fibroblasts could be performed to further ascertain this aspect but, again, may not be conclusive.

Parental origin of de novo deletions can be determined by procuring parental samples and performing molecular analysis using RFLPs. The importance of this has been demonstrated in other partial aneuploidy syndromes. Deletions in patients with Prader-Willi syndrome are of paternal origin, whereas maternally derived deletions of the same chromosomal region lead to

Angelman syndrome (Butler and Palmer 1983; Knoll et al. 1989; Williams et al. 1990). In contrast, although most of the de novo deletions seen with the cri-du-chat syndrome are paternal in origin, no difference in the severity of phenotype is seen between these patients and patients with maternally derived deletions (Overhauser et al. 1990). It should be possible to determine whether the parental origin of the chromosome 18 with the 18q deletion correlates with the clinical findings of studies using standard RFLPs or microsatellite markers located on the distal portion of 18q (Straub et al., in press).

Variation in genetic background can be investigated using families in which one parent carries a balanced translocation and multiple offspring inherit an 18q deletion. However, at present, no such family is available. The analysis of multiple affected offspring resulting from the unbalanced inheritance of a balanced translocation involving other chromosomal regions has shown variations in clinical severity (Overhauser et al. 1989).

From the analysis of these seven individuals, it appears that subtle differences in the size of deletions on the long arm of chromosome 18 may have great impact on prognosis. On the basis of this limited number of patients, a brain MRI scan may be one diagnostic test in determining the degree of involvement and prognosis of a patient with an 18q deletion. Developmental assessments would also be helpful. Clinical and molecular analysis of additional patients will be crucial. Further studies on the subregions determined, especially region 4, will provide clarification of their significance.

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