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Molecular Detection of Microscopic and Submicroscopic Deletions Associated with Miller-Dieker Syndrome

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Summary

Miller-Dieker syndrome (MDS), a disorder manifesting the severe brain malformation lissencephaly ("smooth brain"), is caused, in the majority of cases, by a chromosomal microdeletion of the distal short arm of chromosome 17. Using human chromosome 17–specific DNA probes, we have begun a molecular dissection of the critical region for MDS. To localize cloned DNA sequences to the MDS critical region, a human-rodent somatic cell hybrid panel was constructed which includes hybrids containing the abnormal chromosome 17 from three MDS patients with deletions of various sizes. Three genes (myosin heavy chain 2, tumor antigen p53, and RNA polymerase II) previously mapped to 17p were excluded from the MDS deletion region and therefore are unlikely to play a role in its pathogenesis. In contrast, three highly polymorphic anonymous probes, YNZ22.1 (D17S5), YNH37.3 (D17S28), and 144-D6 (D17S34), were deleted in each of four patients with visible deletions, including one with a ring chromosome 17 that is deleted for a portion of the single telomeric prometaphase subband p13.3. In two MDS patients with normal chromosomes, a combination of somatic cell hybrid, RFLP, and densitometric studies demonstrated deletion for YNZ22.1 and YNH37.3 in the paternally derived 17's of both patients, one of whom is also deleted for 144-D6. The results indicate that MDS can be caused by submicroscopic deletion and raises the possibility that all MDS patients will prove to have deletions at a molecular level. The two probes lie within a critical region of less than 3,000 kb and constitute potential starting points in the isolation of genes implicated in the severe brain maldevelopment in MDS.

Introduction

Miller-Dieker syndrome (MDS) is a multiple-malformation syndrome characterized by type I lissencephaly and characteristic facial appearance. Lissencephaly is a severe brain malformation whereby the typical convolutions (gyri and sulci) are absent and the brain surface is smooth, conferring a figure-eight shape to the brain (Miller 1963; Dieker et al. 1969; Jones et al. 1980);

type I refers to the classical form with a four-layered cortex. The specific sequelae of lissencephaly include bitemporal hollowing, a small jaw, and neurologic abnormalities. MDS can be differentiated from isolated lissencephaly syndrome (ILS) by its characteristic facies (Dobyns et al. 1984).

Because of occasional familial recurrence, the etiology of MDS was once thought to follow an autosomal recessive mode of inheritance. However, our studies have shown chromosomal microdeletions in band 17p13.3 in all three previously reported families with multiple affected children and in four of six patients/sibships in our series (Dobyns et al. 1983, 1984; Stratton et al. 1984; Greenberg et al. 1986). The smallest reported deletion, found in a patient with a ring chromosome 17 in which the deletion is clearly limited to subband 17p13.3, precisely defines the critical region for MDS

(Dobyns et al. 1983). Our subsequent experience indicates that de novo deletions are more common than unbalanced segregation of familial translocations in patients with cytogenetic deletions. As a result of these developments, more reliable estimates of recurrence can be made for genetic counseling, and prenatal diagnosis by cytogenetic analysis is now possible and has been accomplished (Stratton et al. 1984). However, several patients with the complete MDS clinical phenotype show no visible deletion on high-resolution analysis, raising the possibility of submicroscopic deletion or heterogeneous etiology (including autosomal recessive inheritance).

MDS is thus one of the newest additions to a category of genetic disorders termed "contiguous gene syndromes" (Schmickel 1986) that are characterized by subtle cytogenetic deletions detectable by high-resolution chromosome analysis. The recognizable phenotypes of these syndromes most likely result from deletion of several functionally unrelated genes physically contiguous on a chromosome. For Langer-Giedion (del 8q24), Aniridia-Wilms tumor (del 11p13), Prader-Willi (del 15q11–12), and diGeorge (del 22q11) syndromes, as well as for MDS, the deletion of multiple unrelated loci has been proposed as a general causative mechanism (Schmickel 1986; Francke 1987). Clinical variation within these syndromes may be attributable to different deletions involving overlapping sets of genes (Schmickel 1986; Francke 1987). This provides a plausible model for a genetic basis of isolated lissencephaly: lissencephaly and the other physical abnormalities of MDS may result from separate but contiguous loci on 17p, whereby mutation or deletion of only the putative lissencephaly locus would be a possible etiology for some cases of ILS.

There are several aspects of MDS amenable to molecular genetic analysis, and we address them in the present study. We investigate the possibility that, as an augment to high-resolution cytogenetic analysis, DNA probes in this region may offer an easier and more reliable approach to laboratory diagnosis. Some or all MDS patients with normal chromosomes may possess submicroscopic deletions detectable only by molecular techniques, thus increasing the sensitivity of laboratory diagnosis. Second, molecular genetic analysis of 17p in ILS patients is conducted in order to test the hypothesis that a common link exists between the pathophysiology of MDS and ILS. Ultimately, identification of expressed sequences within the MDS critical region may lead to understanding of the developmental defects in MDS, in particular those that cause the severe brain malformation.

To initiate a molecular characterization of this region, we have used a detailed panel of somatic cell hybrids to precisely map cloned sequences to 17p and to identify those that are specific to the MDS critical deletion region. We have regionally mapped cloned sequences from two categories: (1) genes already localized to 17p and (2) a series of polymorphic anonymous clones recently isolated by hybridization to minisatellite core sequences (Nakamura et al. 1987). As an extension of earlier findings (vanTuinen et al. 1987a; vanTuinen and Ledbetter 1987), we now report three independently isolated, highly polymorphic clones that more narrowly define the MDS critical region.

Material and Methods

Clinical and Cytogenetic Evaluation

MDS and ILS criteria have been modified from our previous criteria (Dobyns et al. 1984) on the basis of subsequent experience. The minimal diagnostic criteria for MDS that are used in the present study include (1) type I lissencephaly (widespread with only a few abortive gyri), (2) grossly normal or mildly dysplastic cerebellum, and (3) characteristic facial appearance. The most consistent facial changes include bitemporal hollowing; upturned nares; long, usually protuberant upper lip with thin vermillion border; relatively flattened midface; and small jaw. Other facial changes, postnatal growth deficiency, and microcephaly are often but not always observed. When present, midline calcification adjacent to the third ventricle is probably pathognomonic.

Diagnostic criteria for ILS are less strict and include (1) type I lissencephaly similar to or less severe than that in MDS, (2) grossly normal or mildly dysplastic cerebellum, a criterion that excludes patients with "cerebro-cerebellar" lissencephaly (Dobyns et al. 1985), and (3) relatively normal facial appearance except for bitemporal hollowing, small jaw, and sometimes microcephaly. A few patients have had other minor facial changes, ones not typical of MDS. None have had a midline calcification.

High-resolution chromosome analysis (approximately the 850-band stage) of lymphocytes from MDS patients was performed prior to construction of somatic cell hybrids or analysis of DNA.

Table 1 summarizes the clinical diagnosis and cytogenetic results in our MDS and ILS patients and in three other cell lines used for mapping purposes. Clinical descriptions and high-resolution cytogenetic results for cases MDS-1, MDS-5, MDS-6, and MDS-A have been reported elsewhere (Dobyns et al. 1983, 1984;

Table I

Summary of Clinical Diagnosis and Cytogenetic Results

Case	Clinical Diagnosis	Karyotype
MDS-9	MDS	Normal
MDS-6	MDS	Normal
MDS-1	MDS	ring(17)(p13.3q25.3)
MDS-11	MDS	del(17)(p13.108:)
MDS-A	MDS	t(15;17)(q26;p13.108)
MDS-5	MDS	del(17)(p13.105:)
HO	Fetal demise	del(17)(p13.100:)
HB	SMS[a]	del(17)(p11.2p11.2)
GM1139	Normal	t(15;17)(q15;p11.2)[b]
ILS-3	ILS	Normal
ILS-7	ILS	?del(17)(p13.3:)

[a] SMS = Smith-Magenis syndrome (HGM9), the newly proposed eponym for "17p11 deletion syndrome" reported by Smith et al. (1986) and Stratton et al. (1986).

[b] The reported breakpoints in GM1139, originally assigned to 17p13, were not determined from high-resolution cytogenetic analysis and were found to be in error. Analysis with molecular probes showed that the actual breakpoint is 17p11.2 within the interstitial deletion HB (vanTuinen et al. 1987b).

Stratton et al. 1984), while MDS-9, MDS-11, ILS-3, and ILS-7 have not been published previously. Patient MDS-9 was born to parents that were double third cousins (coefficient of inbreeding = 1/64), and he has two normal siblings. Pregnancy was complicated by diminished fetal movements and polyhydramnios. Facial changes, although more subtle than usual, were consistent with MDS (Dobyns 1987, fig. 4; Dobyns, in press, figs. 1B, 1E). He also has typical, severe neurologic abnormalities. CT scan showed severe type I lissencephaly but no midline calcification. Patient MDS-9, like MDS-6, revealed no cytogenetic deletion of 17p after high-resolution analysis.

Parents of MDS-11 are unrelated and have an older son who is normal. At 6 mo, growth was low normal (weight 5th percentile, length and head circumference 10th percentile). Facial appearance and neurologic problems were typical of MDS. CT scan showed severe type I lissencephaly with a small midline calcification adjacent to the third ventricle. High-resolution cytogenetic analysis was abnormal: 46,XY,del(17)(p13.108:).

The parents of ILS-3 are unrelated and have no other children. ILS-3's facial appearance was normal except for bitemporal hollowing, small jaw, and epicanthal folds (Dobyns 1987, fig. 2; Dobyns, in press, figs. 2A, 2D). CT and MRI scans show type I lissencephaly with symmetric macrogyria posteriorly and no calcification (Dobyns 1987, fig. 2). ILS-3 had no visible deletion of 17p by high-resolution analysis.

The parents of ILS-7 also were unrelated without other children. ILS-7's facial appearance was normal except for bitemporal hollowing and slight prominence of the metopic region. CT scan showed rather severe type I lissencephaly but without calcification. High-resolution analysis of ILS-7 was not definitive in that a subtle discrepancy between the two 17 homologues was suspected in the region 17p13.3 in some cells. This discrepancy was near the range expected for normal contraction variation; thus, the possibility of a small cytogenetic deletion in this patient could not be ruled out.

Somatic Cell Hybrids

Previously described (Su et al. 1984) polyethyleneglycol methods of somatic cell fusion were used to generate hybrids between mouse thymidine kinase–deficient (Tk⁻) cells and human fibroblast or lymphoblast cell lines bearing a variety of deletions or translocations of 17. Included are three MDS cell lines with deletions of 17p useful for direct mapping of probes to the MDS critical region. An additional set of hybrids (BR-4, BR-7, and BR-8) was constructed from an MDS male showing no visible deletion, a result affording a direct test for submicroscopic deletions in this patient. Hybrids were isolated in hypoxanthine-aminopterin-thymidine (HAT) to retain chromosome 17 or derivatives thereof. One hybrid (MH-22) retained a normal chromosome 17 as the only human material (vanTuinen et al. 1987b) and was useful in rapid confirmation of assignment to chromosome 17. Cytogenetic analysis of somatic cell hybrids was performed as described elsewhere (vanTuinen et al. 1987b). Twenty cells from each hybrid were analyzed from G-banded preparations. Selected cells were destained and sequentially G-11 stained to identify all human material (Alhadeff et al. 1977).

Probes

Cloned genes and anonymous clones already mapped to 17p were investigated for localization within MDS deletions. Three cloned genes previously mapped to distal 17p were localized: RNA polymerase II (RNA PO2A) (Cannizzaro et al. 1986), myosin heavy-chain 2 (MYH2) (Rappold and Vosberg 1983), and tumor antigen TP53 (TP53) (McBride et al. 1986). HF12–2 (D17S1), YNZ22.1 (D17S5), YNH37.3 (D17S28), and MCT35.1 (D17S31) are polymorphic anonymous probes previously mapped to 17 by linkage analysis (Nakamura et al., in press). 144-D6 (D17S34) was mapped to 17 by somatic cell hybrids and in situ hybridization by Kondoleon et al. (1987). S12–30 is an alphoid satellite clone that detects the locus D17Z1 (Willard et al., 1986); its

isolation has been described elsewhere (vanTuinen et al. 1987*b*).

Plasmid DNA was isolated by the alkaline lysis method (Birnboim and Doly 1979). Labeling with [^{32}P]dCTP for hybridization to Southern blots was by the random primer method (Feinberg and Vogelstein 1984), yielding specific activities of 0.2–1.0 × 10^9 cpm/µg.

DNA Analysis

DNA from cell pellets of lymphoblast lines and somatic cell hybrids was extracted by routine methods (Maniatis et al. 1982). DNA from whole blood was extracted by the method of Grunebaum et al. (1984). Genomic DNAs were digested with 4 U/µg of restriction endonucleases, as recommended by the manufacturer (Boehringer-Mannheim). Agarose-gel electrophoresis, capillary DNA transfer, hybridization, and washing were done as described elsewhere (vanTuinen et al. 1987*b*). In clinical cases not analyzed by somatic cell hybrids and not fully informative for RFLPs, dosage analysis was performed by simultaneous hybridization of the experimental probe and a single-copy loading control probe that maps to chromosome 6p (H. Zoghbi, personal communication). Kodak XAR-5 film was used for quantitation according to the criteria of Laskey and Mills (1975, 1977). A Helena Laboratories Auto Scanner was used for densitometry.

Results

Hybrid Mapping Panel

Somatic cell hybrids used to localize chromosome 17p probes are listed in table 2. Two hybrids contain deriva-

tive or deletion 17's bearing the largest (MDS-5) and the smallest (MDS-1) MDS deletions described so far; a third (MDS-A) contains an intermediate-sized deletion. The idiogram in figure 1 depicts the breakpoints on 17p that we have isolated in somatic cell hybrids and that are therefore useful in the regional mapping of clones to 17p.

Mapping Cloned Genes

A summary of the results of hybridization of 17p probes to somatic cell hybrids is provided in table 2 and illustrated in figure 1. Three cloned genes, MYH2, TP53, and RNAPO2A, were mapped by the panel to evaluate their relationship to the MDS deletions. Representative mapping blots are shown in figure 2. All three MDS deletion hybrids (lanes 3–5) show positive hybridization to MYH2, as shown by presence of the characteristic 2.3-kb human band in these lanes. The signal is present in HO-11 and HB-9 (lanes 6 and 7) but negative in SA-5 (not shown), indicating an assignment in the region 17p11.2-p13.100. For TP53 the diagnostic human band at 2.5 kb is positive in all three MDS deletion hybrids but negative in HO-11 (fig. 2*B*). Thus, TP53 maps to the next most distal region, p13.100-p13.105. The results for RNAPO2A were identical to those for TP53. Thus, all three loci map in mid or distal 17p, close to but not within the largest MDS deletion (fig. 1). This is a significant physical distance from the critical region defined by the smallest visible deletion, the ring 17. Therefore, these three genes are not being studied further with regard to a role in MDS.

Localization of Anonymous Clones

Five anonymous clones (HF12-2, MCT35.1, YNH-37.3, YNZ22.1, and 144D6) were known to map to

Table 2

Concordance Patterns of Cloned Sequences and Regional Somatic Cell Hybrids

	PROBE							
HYBRID (case)	MYH2	HF12-2 (D17S1)	TP53	RNAPO2A	MCT35.1 (D17S31)	YNZ22.1 (D17S5)	YNH37.3 (D17S28)	144-D6 (D17S34)
BR-7,-8 (MDS-9)	+	+	+	+	+	−	−	+
MH-74 (MDS-1)...........	+	+	+	+	+	−	−	−
FW-1(MDS-A)	+	+	+	+	+	−	−	−
JW-4 (MDS-5)	+	+	+	+	+	−	−	−
HO-11 (fetal demise)	+	+	−	−	−	−	−	−
HB-9 (SMS)	+	+	+	+	+	+	+	+
SA-5 (GM1139)	−	ND	−	−	−	−	−	−

NOTE.—A plus sign (+) denotes presence of hybridizing signal; a minus sign (−) denotes absence of a hybridizing signal. ND = not determined.
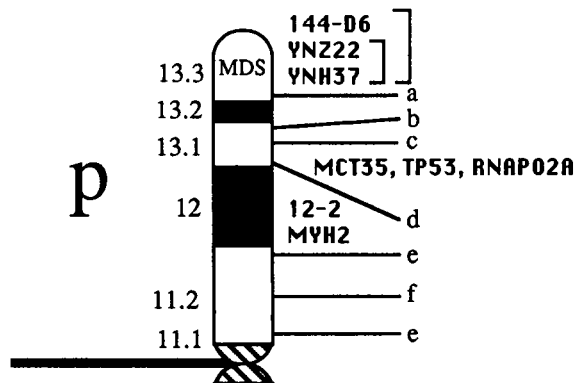
Figure I   Idiogramatic representation of breakpoints in 17p used for high-resolution mapping in the MDS region. MDS patients with deletion of 17p13 are MDS-1 (breakpoint a), MDS-A and MDS-11 (breakpoint b), and MDS-5 (breakpoint c). The next most proximal breakpoint (d) was found in a fetal demise (Greenberg et al., in press) that has a deletion larger than that seen in MDS-5. Two breakpoints (e) in p11.2 represent the interstitial deletion 17p11.2 of a patient with Smith-Magenis syndrome (patient 1 of Stratton et al. 1986). GM1139 provided a breakpoint (f) allowing discrimination of probes distal or proximal to the interstitial deletion (both hybridize positively to the latter). The large and small brackets indicate the submicroscopic deletion of target sequences from MDS-6 and MDS-9, respectively, i.e., cases with normal chromosomes. The order of probes in the clusters MYH2/HF12–2 and YNH37/YNZ22/144-D6 was previously determined by linkage analysis (Nakamura et al., in press). Our physical localization of MCT35 is consistent with its linkage relationship to these five probes, whereas linkage studies have not been reported for TP53 and RNAPO2A.

Figure 2   Hybridization of cloned sequences to Southern blots of the somatic cell hybrid mapping panel. For ease of interpretation hybrid lanes are identified by "deleted" idiograms representing the portions of 17 retained in hybrids. Lane 1, human DNA; lane 2, mouse Cl1D; lanes 3, 4, and 5, MDS deletion hybrids made from MDS-1, MDS-A, and MDS-5, respectively; lane 6, HO-11; lane 7, HB-9 (from SMS). The probes and the restriction endonucleases used to reveal diagnostic human fragments are indicated on the right, while the molecular weight (in kb) of human bands is indicated on the left. In A and B, sequences that hybridize to mouse are present in positions omitted from the figure. Absence of signal for YNZ22.1 in all three MDS deletion hybrids indicates that it maps to the MDS critical region.

17p and were therefore tested for potential localization within MDS deletions. All were confirmed to be on 17, by hybridization to the mapping panel. HF12–2 was present in all of the MDS and regional hybrids and therefore maps to 17p11.2-p13.100. MCT35.1 maps distal to the breakpoint at p13.100 but is present in all three MDS hybrids. Hence it maps to the same location as RNAPO2A and TP53 and is similarly excluded from the MDS critical region (fig. 1).

YNZ22.1, YNH37.3, and 144-D6 mapped to a region distal to MCT35.1, HF12–2, and the cloned genes, a result consistent with the linkage relationships of these markers (Nakamura et al., in press). Regional mapping for YNZ22.1 is shown in figure 2C. The 21-kb band characteristic of the control human DNA in lane 1 is present in lane 7 (which contains a hybrid DNA [HB-9] with intact distal 17p), while it is absent in all three MDS deletion hybrids (lanes 3–5). The results were identical for YNH37.3 and 144-D6 (fig. 1; table 2), demonstrating that all three clones map to a region within the smallest visible MDS deletion, within the terminal prometaphase band p13.3.
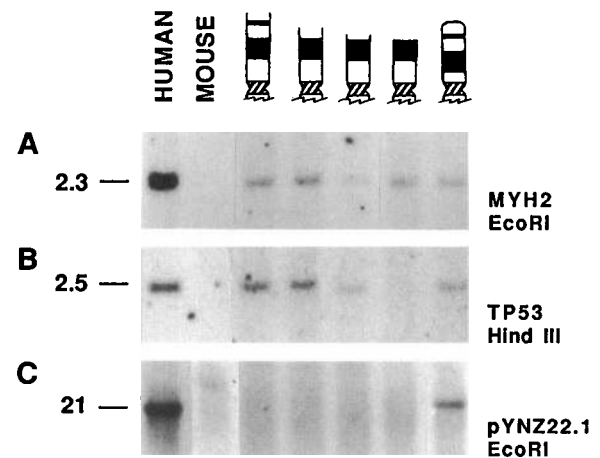
Deletion analysis of a fourth MDS case (MDS-11) with a visible cytogenetic deletion was performed without construction of somatic cell hybrids. Dosage analysis of YNH37.3 and YNZ22.1 indicated that only single copies of the target sequences were present in the proband (table 3). RFLP analysis was conclusive for 144-D6, which was hemizygous in the proband and indicated failure to inherit a paternal allele (not shown).

Anonymous Clones Detect Submicroscopic Deletions

Since these three anonymous probes lie within the critical region defined by visible MDS deletions, we next tested the possibility that they would reveal submicroscopic deletions in two cytogenetically normal MDS patients. We took advantage of the highly polymorphic nature of these probes and attempted to answer this question by using RFLPs at these loci. Failure to inherit an allele from one parent would indicate a de novo deletion in the chromosome 17 contributed by that parent.

144-D6 was fully informative in the family of MDS-9 and is heterozygous in the proband (table 3); therefore it is not deleted. For YNH37.3 and YNZ22.1 the DNA

Table 3

Deletion Analysis by RFLPs and Densitometry

			PROBE		
CASE	KARYOTYPE	DIAGNOSIS	YNZ22.1	YNH37.3	144-D6
MDS-11	del(17)(p13.108:)	MDS	1.02X	1.11X	Hemizygous (paternal del)
MDS-6	Normal	MDS	0.97X	Hemizygous (paternal del)	0.87X
MDS-9	Normal	MDS	Hemizygous (paternal del)	. . .[a]	Heterozygous
ILS-3	Normal	ILS	2.40X	2.27X	Heterozygous
ILS-7	?del(17)(p13.3:)	ILS	Heterozygous	Heterozygous	Heterozygous

NOTE.—Inheritance of alleles from both parents (heterozygous) or from one parent only (hemizygous) in informative families is so indicated. Parental origin is indicated in parentheses. In probands with inconclusive RFLP results, the numbers represent copy number, standardized from densitometric scanning of the test probe relative to a standard probe.

[a] Patient shared a band with both parents that could not be separated from the other alleles of the parents, precluding densitometry (see fig. 3). Paternal deletion for YNH37.3 was demonstrated by somatic cell hybrid analysis (table 2).

analysis of the affected male, his parents, and two siblings is shown in figure 3. Both parents are heterozygous for YNH37.3 and share an allele at 2.9 kb (fig. 3A). Since the child has one band at 2.9 kb, he could be either hemizygous or homozygous for the probe. The RFLP data are therefore inconclusive regarding deletion of probe YNH37.3 from this patient. Dosage analysis could not be performed in MDS-9 with YNH37.3, owing to the inability to discriminate the closely spaced parental alleles.

For YNZ22.1 the mother is heterozygous for alleles at 1.7 and 1.9 kb, and the father is homozygous for an allele at 1.1 kb (fig. 3B; table 3). The child has a single band, at 1.7 kb, from the mother but no paternal

Figure 3 Hybridization of YNH37.3 (A) and YNZ22.1 (B) to DNAs of a cytogenetically normal MDS patient (MDS-9), indicated by a black square, and his family members. In A, the proband has a single band at 2.9 kb for YNH37.3 shared by both parents, a result consistent with homozygosity or hemizygosity for the locus. In B, RFLP analysis for YNZ22.1 is fully informative. Both normal siblings inherited a 1.7-kb allele from their mother and a 1.1-kb allele from their father. The proband inherited the 1.7-kb allele from his mother but failed to inherit a 1.1-kb allele from the father, suggesting paternal deletion. All genomic DNAs were digested with BamHI.

band at 1.1 kb, indicating he failed to receive a paternal allele. Although these data are consistent with a de novo deletion of the paternal 17, they could also be explained by nonpaternity. To exclude the latter possibility and to clarify the results of YNH37.3, two approaches were pursued. First, paternity testing (conducted by Dr. M. Pollack, Baylor College of Medicine) was performed using a standard panel of HLA and six red cell genetic markers. The results showed a probability of paternity of 99.8%, making paternal deletion the more likely explanation for the YNZ22.1 data.

Second, to demonstrate deletion by the most direct means, somatic cell hybrids were constructed from the affected child and tested for the presence of the three probes. Since the two 17's in the subject cannot be distinguished cytogenetically, we used the highly polymorphic alphoid probe S12–30 to discriminate the paternal and maternal 17 homologues in the hybrids. In terms of the major haplotype categories suggested by Willard et al. (1986) and further illustrated by Willard et al. (1987), the father of the affected boy has haplotypes 1/1 (prominent 2.7-kb band only) while the mother has haplotypes 1/2 (2.7-kb band/2.0- and 1.5-kb bands) (fig. 4A). The affected child has haplotypes 1/2; therefore the paternal (haplotype 1) and the maternal (haplotype 2) homologues can be easily distinguished.

Three hybrids were identified that retained intact chromosome 17's, and the results of Southern hybridization of these DNAs are shown in figure 4A. Hybrid BR-4 retained both chromosome 17's on cytogenetic analysis, consistent with a Southern blot pattern (1/2) that was identical to the genomic pattern of the affected male. It was therefore uninformative in deletion analysis. Hybrids BR-7 and BR-8 both had a single intact
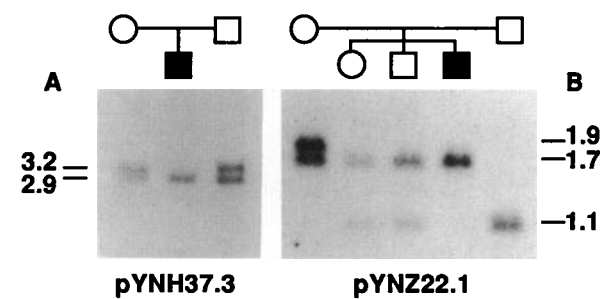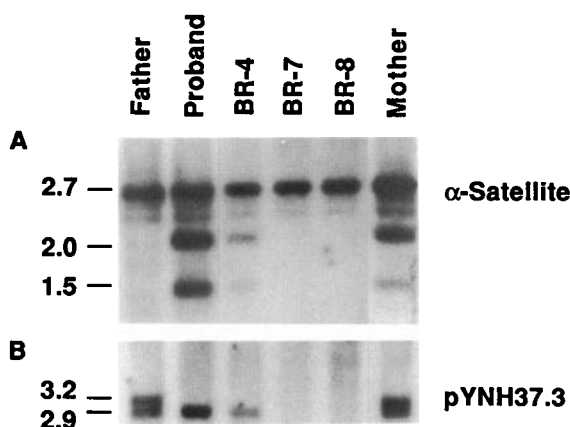
Figure 4 Hybridization of the alpha satellite probe S12–30 (A) and YNH37.3 (B) to hybrids containing chromosome 17's from cytogenetically normal MDS patient MDS-9. In A, comparison of the parents' and the affected child's alpha satellite banding patterns show that the paternal and maternal haplotypes of the child's chromosome 17's can be distinguished (see text). Hybrid BR-4 retained both parental 17's on cytogenetic analysis, consistent with the hybridization pattern, while hybrids BR-7 and BR-8 retained the paternal 17 only. In B, YNH37.3 is detected only in hybrid BR-4 retaining both chromosome 17's but is absent from both hybrids retaining only the paternal 17. Genomic DNAs in A were digested with EcoRI; those in B were digested with BamHI.

chromosome 17 and haplotype 1 (2.7-kb band only) on Southern analysis, consistent with retention of only the paternal chromosome 17. These two hybrid clones show no hybridization to probe YNH37.3 (fig. 4B), establishing that the affected child's paternal 17 is deleted for sequences detected by this probe. This filter was stripped and rehybridized with YNZ22.1 with identical results (table 2), confirming the RFLP data suggesting deletion of this probe.

In the family of MDS-6, both parents were heterozygous for different alleles of YNH37.3; 3.2 kb and 3.3 kb in the mother and 3.0 kb and 3.25 kb in the father. Distinction between these alleles was confirmed by comparison of their migration to that of a nonpolymorphic standard probe detecting a target sequence of 4.0 kb which was hybridized simultaneously to the filter. The proband inherited the 3.3-kb allele from the mother but inherited no paternal allele. For both YNZ22.1 and 144D6, RFLP analysis was not completely informative, as the proband possessed a band shared by both parents. Densitometric analysis (table 3) indicated that both probes are present in only one copy in the proband, confirming a deletion. Thus, all three probes are deleted in MDS-6, and, as in MDS-9, the deletion is paternal in origin.

No Deletion Detected in ILS Patients

In contrast to the results of deletion analysis for MDS, analysis of two ILS patients showed that neither was deleted for YNZ22.1, YNH37.3, or 144-D6 (table 3). Patient ILS-7 was heterozygous for all three probes; therefore no deletion of target sequences occurred. Patient ILS-3 was heterozygous for 144-D6 but not fully informative for the other two probes. However, dosage analysis indicated no deletion of either probe.

Discussion

We have constructed a somatic cell hybrid regional mapping panel for the efficient mapping of cloned sequences to the MDS critical region. Using the panel, we excluded three cloned genes from the critical region, precluding their use in diagnosis and arguing against their direct involvement in this disorder. We have, however, identified three anonymous clones that are deleted in each of four cytogenetically visible deletions associated with MDS, including the smallest cytogenetic deletion described in this disorder. In addition, a combination of three independent methods revealed a de novo deletion for two of these (YNZ22.1 and YNH-37.3) in two MDS patients with normal chromosomes. This finding confirms and more narrowly defines an MDS critical region previously postulated on cytogenetic grounds. It establishes the existence of submicroscopic deletions in MDS and suggests that the incidence of deletion in MDS is higher than that revealed by cytogenetic analyses alone. MDS thus represents a mental retardation/microdeletion syndrome in which molecular detection is achieved in the absence of visible cytogenetic deletion.

Clinical Implications of MDS Probes

All eight MDS cases studied in our laboratory have deletions detected cytogenetically and/or by molecular analysis, arguing against a monogenic etiology for MDS. The deletion probes will therefore be a valuable adjunct to high-resolution cytogenetic analysis in the diagnosis of MDS. Since small deletions are particularly difficult (or impossible) to detect even with high-resolution chromosome analysis, use of molecular probes for RFLP or dosage analysis may in some cases be easier and more reliable than cytogenetic analysis. However, RFLP or dosage analysis cannot completely replace cytogenetic analysis, for a significant proportion of MDS cases are due to parental translocation or inversion, which are not detectable by molecular methods. Therefore, in determining the nature and ori-

gin of an MDS deletion, cytogenetic analysis is necessary to distinguish inheritance of an unbalanced familial rearrangement from de novo rearrangement.

Prior to the recognition of MDS as a chromosome deletion syndrome, a 25% recurrence risk was given to parents of MDS patients, under the assumption of autosomal recessive inheritance. With the availability of cytogenetic analysis, de novo deletions can be assigned a negligible risk of recurrence. Although the risk in translocation or inversion families remains substantial, prenatal diagnosis by amniocentesis or chorionic villus sampling can be offered (Stratton et al. 1984).

The results of molecular analysis that are presented here further reduce the number of MDS cases that could be attributable to a hypothetical autosomal recessive phenocopy with a 25% recurrence risk. In our two cytogenetically normal MDS cases molecular detection of a de novo submicroscopic deletion implies instead a negligible recurrence risk, with dramatically contrasting implications for genetic counseling. The possibility remains that all future cases studied with probes will prove to have deletions, completely excluding autosomal recessive inheritance as a cause of MDS.

Molecular Dissection of a Contiguous Gene Syndrome

We estimate that the four MDS visible deletions range in size from less than 3×10^6 to 7×10^6 bp. Although we do not have a direct estimate of the size of the submicroscopic deletions (MDS-6 and MDS-9) detected by molecular methods only, YNZ22.1 and YNH37.3 exhibit a recombination fraction of .01 (average of both sexes) (Nakamura et al., in press). If 1 cM = 1×10^6 bp in this region of the genome, then a cytogenetically undetectable deletion encompassing both probes may be of the order of 1×10^6 bp in extent—or less if the 17p terminus is highly recombinogenic. This is below the threshold ($2-3 \times 10^6$ bp) of microscopic detection.

The high incidence of demonstrable deletion in MDS (eight of eight patients or sibships in our experience) is perhaps best explained by a requirement for deletion of more than one locus for expression of the syndrome. The uniformity of the MDS phenotype despite different deletion sizes suggests that few, perhaps only two, genes separated by 1×10^6 bp or less must be deleted for expression of the complete MDS phenotype.

If MDS and ILS may be thought of as related syndromes with a shared, major manifestation of lissencephaly, an analogy with other microdeletion syndromes is appropriate. In our experience, MDS is a clinically homogeneous syndrome, except that patients with large deletions and/or associated duplications have

more abnormalities. ILS is probably heterogeneous (Dobyns 1987). However, the phenotype of ILS patients with relatively severe lissencephaly is almost identical to that of MDS, except for the facial changes. Thus, some cases of ILS could be due to dysfunction of a major "lissencephaly" gene located within the MDS critical region. If so, the different mechanisms involved in production of the MDS versus the ILS phenotypes may be separable at a molecular level. However, in testing the possibility that ILS patients might manifest submicroscopic deletion of 17p13, we found no deletion, in either of our two patients, with any of the three probes that detected MDS deletions.

Two alternative explanations may be offered for this negative finding. ILS will not be expected to reveal deletion of 17p if it has an etiology distinct from that of MDS. However, since there may be a large portion of the MDS critical region not directly covered by only two plasmid probes, ILS deletions very much smaller (i.e., $\ll 1 \times 10^6$ bp) than those seen in MDS might escape detection on conventional Southern blots using these probes. These results are therefore not inconsistent with the existence of small undetectable deletions affecting loci associated with lissencephaly yet not large enough to include loci responsible for the facial dysmorphology characteristic of MDS. Saturation of this area with additional probes could rule out deletions in this lower size range. The amount of DNA covered could also be increased by one or two orders of magnitude by applying pulsed-field gel electrophoresis after DNA cleavage by infrequently cutting restriction endonucleases (Anand 1986).

Note added in proof: Three additional MDS patients without cytogenetically detectable deletions have been analyzed, all of whom demonstrate deletions for YNZ22.1 and YNH37.3. To date, 13 of 13 MDS patients in our sample series have molecular deletions.

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