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Prenatal Diagnosis and Carrier Detection of a Cryptic Translocation by Using DNA Markers from the Short Arm of Chromosome 5

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Summary

DNA markers from the short arm of chromosome 5 were used to examine a large family in which a microscopically undetectable translocation was segregating. In addition to confirming that three retarded children were hemizygous for loci distal to 5p14, these analyses identified five individuals as being carriers of the balanced translocation. The use of molecular probes provided informed genetic counseling to the family for the first time. With the DNA mzarkers from 5p, prenatal diagnosis was performed on two fetal chorionic villus samples, both of which were found to have unbalanced karyotypes. The identification of translocation carriers was complicated by recombination between the small translocated segment of 5p and the corresponding region on the normal homologue, which changed the haplotype of the translocated 5p segment.

Introduction

The cri-du-chat or $5p^-$ syndrome is one of the most common human deletion syndromes, with an incidence of approximately 1 in 50,000 live births (Niebuhr 1978*a*, 1978*b*). Clinical features of the syndrome include developmental delay, microcephaly, epicanthic folds, severe mental retardation, and a high-pitched cry similar to the meowing of a cat, from which the disorder derives its name (Niebuhr 1978*a*, 1978*b*). The severe mental retardation characteristic of cri-du-chat is a significant health-care problem, as individuals with the disorder have long life spans. It has been estimated that as many as 1% of institutionalized individuals with IQs below 50 have cri-du-chat syndrome (Niebuhr 1978*a*, 1978*b*). The syndrome is associated with deletions of the short arm of chromosome 5 (5p) and invariably includes the midregion of 5p15, which must be rendered hemizygous for the phenotype of cri-du-chat to be apparent (Overhauser et al. 1986*a*). Although the majority of cases represent de novo deletions, 10%–15% of the cases arise from the segregation of a balanced translocation from one of the parents, resulting in partial monosomy for 5p and partial trisomy for the other region involved in the translocation (Niebuhr 1978*b*).

In recent years recombinant-DNA technology has become increasingly important in helping to confirm karyotype analysis when chromosome rearrangements are subtle. For many types of studies, DNA probes which reveal RFLP provide the best or only means to answer certain questions about chromosome rearrangements. These questions include the parental origin of the supernumary chromosome in trisomies (Davies et al. 1984), determining at which meiotic division nondisjunction occurs (Stewart et al. 1988), identifying microdeletions in certain congenital disorders (Schwartz et al. 1988; vanTuinen et al. 1988), and analysis of somatic chromosome rearrangements leading to reduction to homo- or hemizyosity for tumor-suppressor genes (Cavenee et al. 1983).

In the present report we describe DNA marker studies on a large family with a history of a congenital disorder resembling cri-du-chat but in which no chromosome rearrangement could be detected by light micros-

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copy. These studies revealed that three mentally retarded individuals in the family were monosomic for the region 5p15.1-5pter. In addition, five individuals who carried a balanced translocation were identified. The translocation exchanged the terminal portion of 5p with another, as yet unidentified, chromosomal segment which is the same size as and has banding characteristics similar to 5p15.1-5pter. Chromosome analysis at the 450–550-band stage could not detect the chromosome rearrangement in any individuals who, on the basis of the DNA marker studies reported here, were known to have balanced or unbalanced translocations. The combined use of DNA probes and interspecific somatic cell hybrids enabled us to determine the haplotype of the translocated segment of 5p for six DNA markers. This made it possible to identify balanced-translocation carriers and to do prenatal diagnosis on two fetuses that were at risk for having an unbalanced karyotype and that turned out to be chromosomally abnormal. Although these studies made counseling and prenatal diagnosis available to the family for the first time, one significant problem encountered in balanced carriers was meiotic recombination between the small translocated portion of 5p and the normal chromosome 5. That the haplotype of the translocated portion of 5p could differ from one carrier to the next made carrier detection and genetic counseling all the more difficult.

Material and Methods

Cell Lines

Epstein-Barr virus–transformed lymphoblast cultures were established from most family members. These cells were used to make interspecific somatic cell hybrids, as described below, and as a source of DNA for genotyping of RFLP markers. For a few individuals, DNA was extracted directly from fresh peripheral leukocytes.

The procedures used to make interspecific (human-Chinese hamster) cell hybrids which retain human chromosome 5 under selective pressure have been described in detail elsewhere (Dana and Wasmuth 1982; Overhauser et al. 1986*a*). Independent hybrids with a chromosome 5 were grown into mass cultures for extraction of DNA. At the time cells were harvested for DNA, chromosomes were analyzed from replicate cultures by using trypsin-Giensa banding and G-11 staining, according to a method described elsewhere (Dana and Wasmuth 1982). Each hybrid examined had what appeared to be an intact chromosome 5.

Extraction of DNA and Southern Blot Hybridization

The procedures for extraction of high-molecular-weight DNA from cells, restriction-endonuclease digestion, agarose-gel electrophoresis, and transfer of DNA to nylon blotting membranes have been described elsewhere in detail (Overhauser et al. 1987). DNA was extracted from chorionic villus tissue according to a method described by Old (1986). DNA fragments to be used as probes were labeled by the random primer method of Feinberg and Vogelstein (1983, 1984). Prehybridization, hybridization, and washing of filters were performed according to a method described elsewhere (Overhauser et al. 1987). The DNA probes from chromosome 5 that were used in these studies and that are listed in table 1 have been characterized elsewhere (Overhauser et al. 1987). Each of these probes reveals one or more RFLPs (Overhauser et al. 1987), and most are available from the American Type Culture Collection.

Results

Description of the Family

The pedigree of the family is shown in figure 1. The propositus, III-14, was suspected soon after birth of having cri-du-chat, on the basis of catlike cry, microcephaly, epicanthic folds, and failure to thrive. However, chromosome analysis at the 450–550-band stage of resolution with use of both G-banding and R-banding revealed no abnormality in either chromosome 5. During counseling it was discovered that by a previous marriage the father (II-7) had had two sons (III-11, and III-12), both of whom were in institutions for the mentally retarded. At least one son was remembered as having an unusual cry. Both sons and the father had been karyotyped elsewhere on two occasions and had all been reported as having normal 46 XY karyotypes. On the basis of this

Table 1

Polymorphic DNA Markers from Chromosome 5

Probe	HGM D Number	Localization
1. 213-274E-C	D5S10	5p15.3
2. J0214H-B	D5S13	5p15.3
3. J0209E-B	D5S12	5p15.2
4. M647E-C	...	5p15.2
5. J0120H-B	D5S18	5p15.2
6. J056E-F	D5S17	5p15.1
7. J044E-B	D5S19	5p14
8. J0205E-C	D5S22	5q

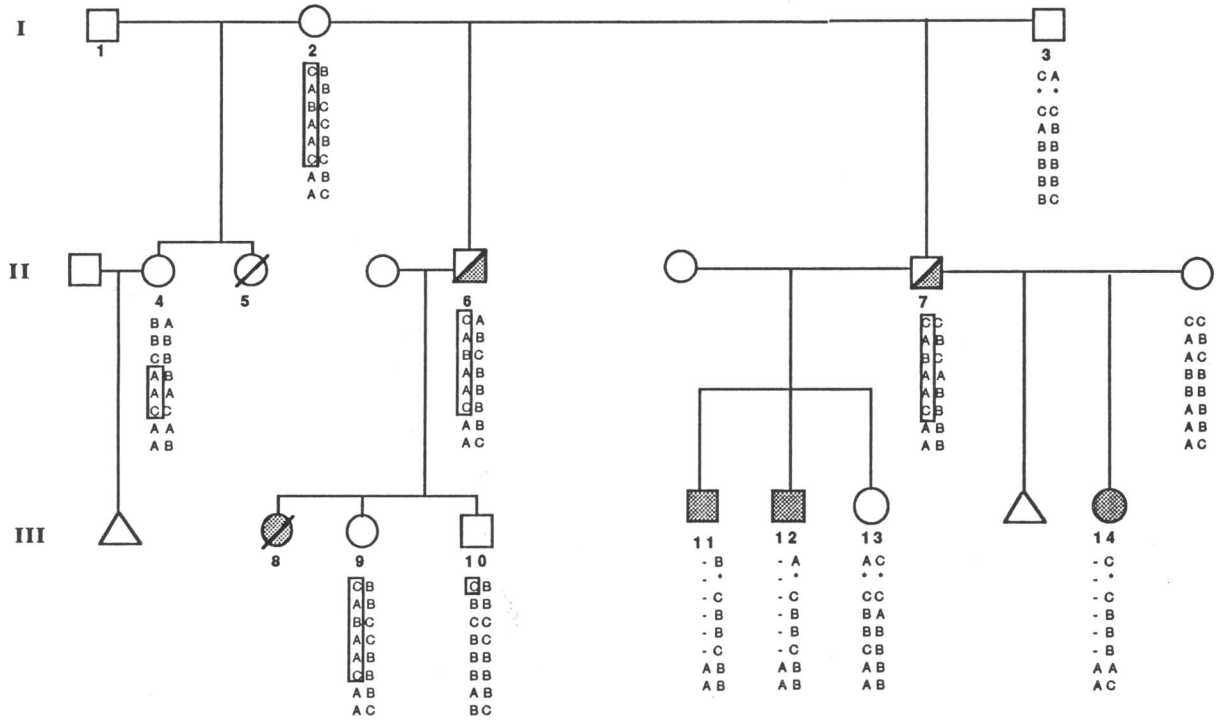

Figure 1 Family in which a cryptic translocation of 5p is segregating. Half-closed symbols denote individuals who on the basis of the family history, were obligate carriers for the balanced translocation. Fully closed symbols represent individuals known or suspected of having cri du chat. Two fetuses on which prenatal diagnosis was performed are indicated by triangles. The propositus is individual III-14. For the eight loci listed in table 1, the genotypes and haplotypes of family members for DNA markers on chromosome 5 are shown below each person. The order of the loci is the same as in table 1. The alleles associated with the translocated segment of 5p are enclosed in boxes for individuals I-2, II-6, and II-7. An asterisk (*) indicates that the genotype or haplotype for that locus was not determined.

information, the father had originally been assured before his second marriage that there was little risk of recurrence of what appeared to be an autosomal recessive disorder. However, given the complete family history, it seemed very likely that the father carried a cytogenetically undetectable balanced translocation involving 5p and another chromosome.

Cell Hybrid and DNA Probe Analysis

To determine directly whether the father was a translocation carrier, his chromosomes 5 were segregated in somatic cell hybrids by using a selection that requires retention of a human chromosome 5 (see Material and Methods). Southern blot analysis was performed on DNA extracted from several independent hybrids with normal appearing chromosomes 5 derived from lymphoblastoid cells from the father. The eight DNA markers from chromosome 5 that are listed in table 1 were used as the probes. Each of these probes has been regionally mapped, and the order listed in table 1 is their order from pter to qter (Overhauser et al. 1987). Of eight hybrids examined, five gave positive hybridization signals to all eight probes. The remaining three hybrids were positive for probes D5S19 and D5S22 but were negative for all the rest. Figure 2 shows autoradiograms of Southern blots of three of the probes hybridized to one hybrid of each type. The results of these analyses with all eight probes, which are summarized in figure 3, confirmed that the father is missing DNA markers distal to 5p14 (numbers 1–6 in table 1) from one chromosome 5 homologue, even though both homologues appear normal under the light microscope. Exactly the same results were obtained with hybrids with the different homologues of chromosome 5 from the propositus. If this much of 5p was simply deleted, it would have been very obvious by chromosome analysis. Therefore, the missing segment of 5p must be replaced with material from another chromosome which is the same size and has the same banding properties as the missing segment of 5p.

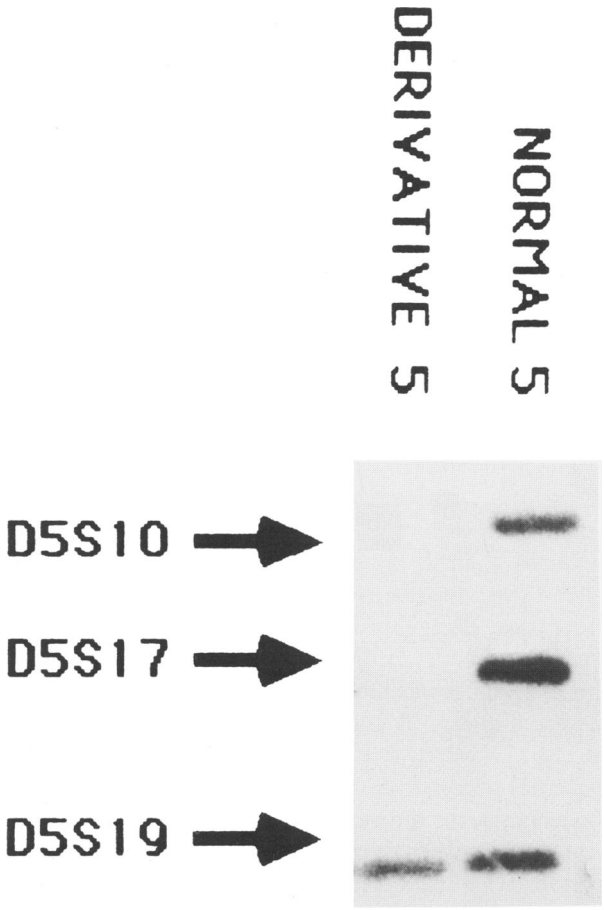

Figure 2 Southern blot analysis of cell hybrids containing the normal or derivative chromosome 5 from the father (II-7) of the propositus. DNA from the two hybrids was digested with *Eco*RI and then was analyzed by Southern blotting by using three DNA probes derived from 5p15.3 (D5S10), 5p15.1 (D5S17), and 5p14 (D5S19). A single filter was hybridized sequentially to all three probes.

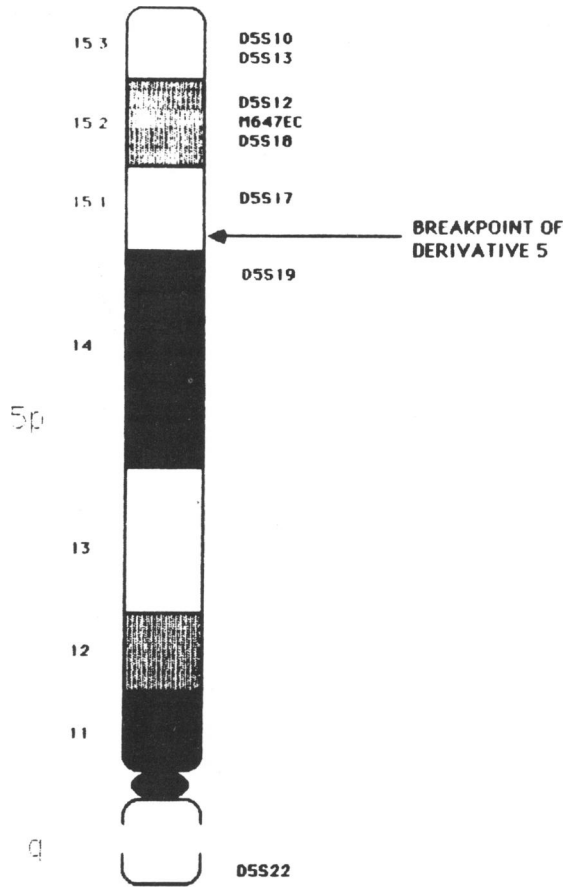

Figure 3 Location of translocation breakpoint on 5p. The eight DNA markers shown have been regionally mapped previously. Each was used as a probe on Southern blots of DNA from cell hybrids that contained either the normal or derivative chromosome 5 from the father (II-7) and the propositus (III-14), as described in the text. On the basis of these analyses, the breakpoint of the translocation on 5p was placed between loci D5S19 and D5S17.

In an effort to provide counseling for the rest of the family, the genotypes of most family members were determined for each of the loci listed in table 1, all of which reveal RFLP (Overhauser et al. 1987). The loci will be referred to as 1–8, which is the order both listed in table 1 and under each genotyped member of the family shown in figure 1. By comparing the genotype of the father (II-7) of the propositus with the haplotype of his normal chromosome 5 in a cell hybrid, it was simple to deduce the haplotype for the six markers on the translocated segment of 5p. The alleles on the translocated segment of 5p are shown within the box in figure 1.

Even though a sample for DNA analysis was not available from the first wife of II-7, the genotypes of both sons who were mentally retarded (III-11 and III-12) confirm they are hemizygous for loci on distal 5p. As shown in figure 4, the father is C,C at locus 1 (D5S10) while the sons are A-, and B-, respectively. Thus, neither inherited a paternal allele at this locus. The same result was obtained for another probe, M647E-C. The normal daughter (III-13) of II-7 by his first wife inherited paternal alleles associated with normal chromosome 5 at loci 3 (C) and 5 (B), indicating she is most likely not a translocation carrier. One last point that was apparent from analyzing this portion of the family is that the translocation carrier in the first generation is I-2, not I-3. Thus, at locus 5 (D5S18) the allele associated with the translocated segment of 5p is A but I-3 is BB.

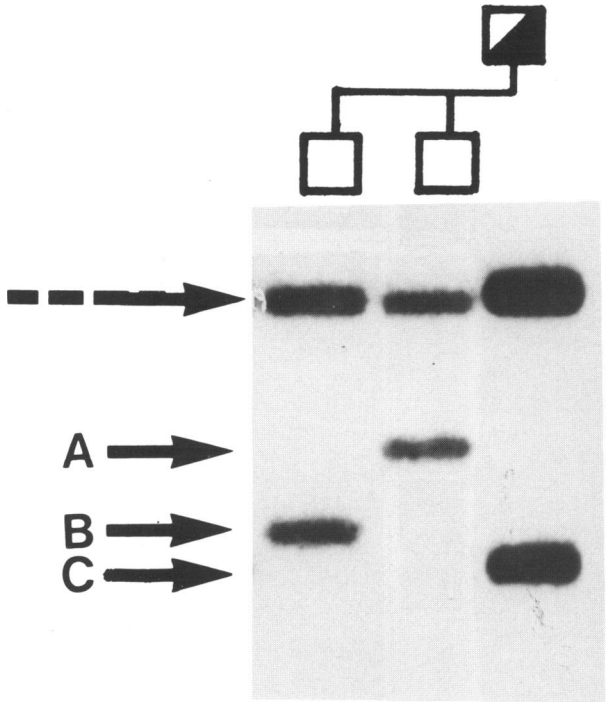

Figure 4 Hemizygosity of two retarded children of II-7 at the D5S10 locus. DNA extracted from lymphoblast cultures of II-7 and his two retarded sons, III-11 and III-12, was digested with *Hin*cII and was subjected to Southern blot analysis by using D5S10 as a probe. The restriction fragments corresponding to alleles A, B, and C are indicated. The largest restriction fragment, indicated with a dashed arrow, is an invariant fragment. The father is homozygous (C,C), but neither son inherited this allele.

This result placed the I-2's second-marriage offspring at risk for being affected or carriers of the translocation.

The uncle of the propositus, II-6, was suspected of being a translocation carrier, since one of his children (III-8) died in infancy and was reported by the parents to resemble the propositus. The chromosomes 5 from II-6 were segregated in cell hybrids and were analyzed with the DNA probes as described above for II-7. These studies confirmed II-6 also carries the balanced translocation. Again, by comparing the genotype of II-6 with the haplotype of his normal 5 in a cell hybrid, the haplotype associated with the translocated 5p segment was determined. As expected, it was the same as for his brother (II-7). Although no sample was obtained from the wife of II-6, it was possible to determine the chromosomal status of both living children (III-9 and III-10). The genotype of III-9 was consistent with her having inherited the balanced translocation as indicated in figure 1. At loci 1 (D5S10) and 4 (M647) this child definitely had the paternal alleles on the translocated 5p segment (C and A, respectively). The second child, III-10, gave a surprising result. At loci 3, 5, and 6 the child inherited the paternal alleles from the normal chromosome 5 (C, B, and B, respectively). However, at locus 1 (D5S10) this individual clearly had the paternal allele (C) from the translocated portion of 5p. The only explanation for this result is a meiotic recombination event, in the father, between the translocated 5p segment and the corresponding region of the normal 5, as diagrammed in figure 5*A*. Thus, individual III-10 inherited a normal chromosome 5 from his father—but it was one on which the distal portion of 5p is derived from the translocated segment.

The first child (II-5) of I-2 by a second husband was mentally retarded and died at age 34 years. The retardation was thought to have been due to brain damage, at birth, incurred by a forceps delivery, but in retrospect it could have been due to her having an unbalanced karyotype. The genotype of II-4 (the half-sister of II-6 and II-7) was determined for loci 1 and 2 first. From this analysis, it was found that she clearly inherited the maternal alleles (B and B) from the normal chromosome 5. However, at loci 4, 5, and 6 she inherited the maternal alleles from the translocated portion of 5p. Once again, the only explanation is that a recombination event between the translocated 5p segment and the normal 5 had occurred in the mother and had transferred alleles from the normal 5 onto the translocated segment of 5p (fig. 5*B*). Thus, we predicted II-4 was a balanced carrier. That this interpretation is correct was shown by the woman's having a fetus with an unbalanced karyotype, as described below.

Prenatal Diagnosis

Just as the studies on the entire family were completed, both II-4 and the wife of II-7 became pregnant. Prenatal diagnosis of the chromosomal status of both fetuses was attempted using the 5p DNA markers to analyze DNA extracted from chorion villus biopsies. The results of this analysis using the probe D5S12 on DNA from the fetus of II-7 and his wife are shown in figure 6*A*. The fetus clearly had three alleles, including both paternal alleles, at the D5S12 locus. The fetus was therefore unbalanced and trisomic for distal 5p. At the time this finding was made no fetal heartbeat was detectable, and a therapeutic abortion was performed. The result obtained on DNA from the fetus of II-4 by using D5S10 is shown in figure 6*B*. The mother is A, B, and the father is B,C; but the fetus had only the C allele. Thus, no maternal allele was inherited at locus

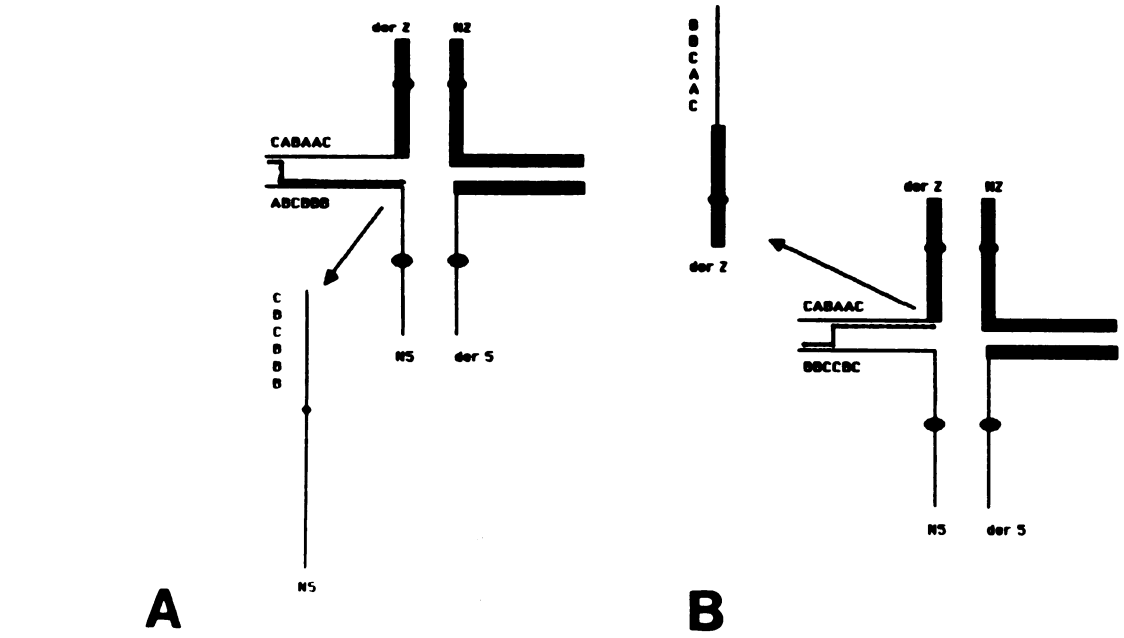

Figure 5 Diagram of recombination between the translocated segment of 5p and the normal chromosome 5 homologue observed in individuals III-10 (*A*) and II-4 (*B*). The other chromosome involved in the reciprocal translocation, which has not yet been identified, is designated Z.

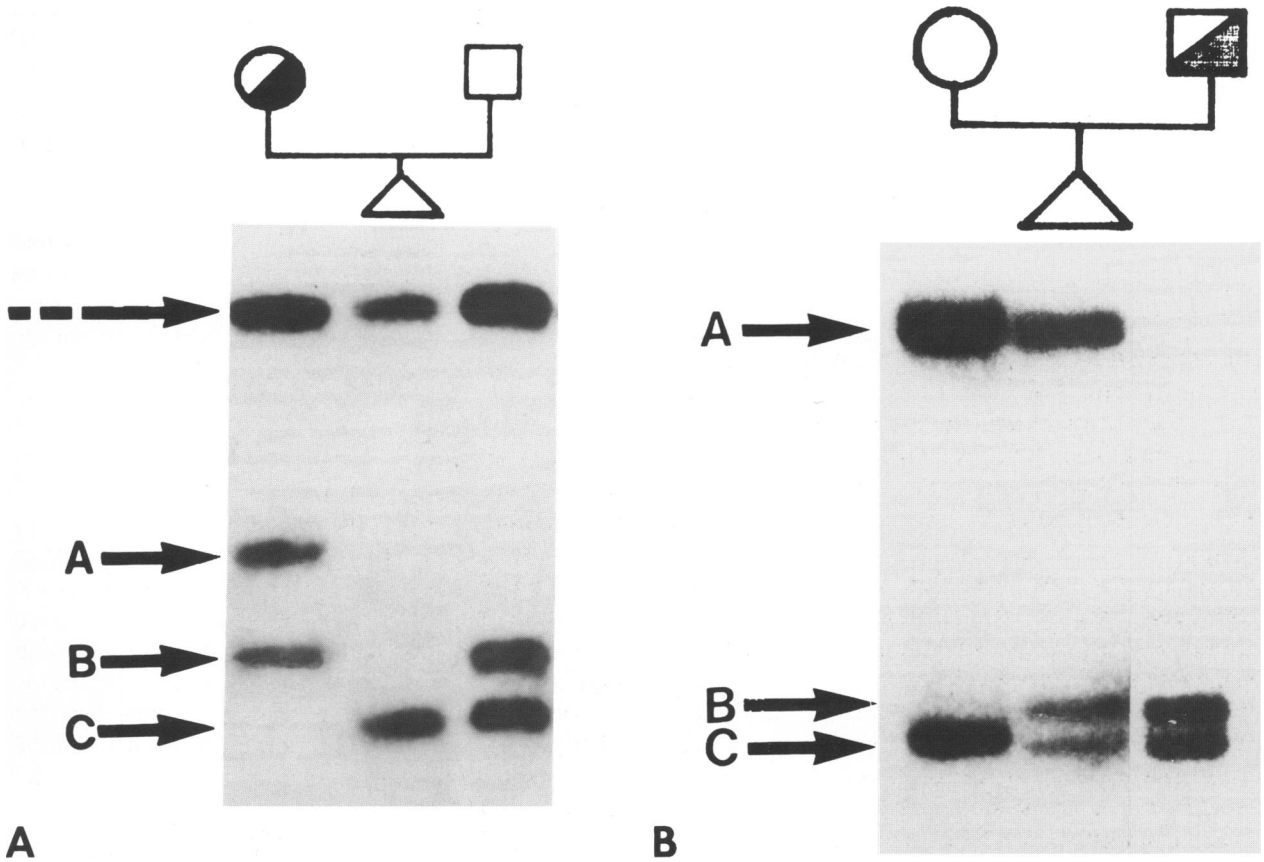

Figure 6 DNA marker analysis on chorionic villus samples. *A*, DNA from II-7, from his wife, and from the fetal chorionic villus sample was digested with *MspI* and was analyzed by Southern blotting by using D5S12 as a probe. The three allelic restriction fragments A, B and C are indicated. The fetal sample has all three alleles, including both from the father. *B*, DNA from II-4, from her husband, and from the fetal chorionic villus sample was digested with *Hin*cII and was analyzed by Southern blotting by using D5S10 as a probe. The dashed arrow indicates a constant band. The fetal sample has only the C allele and none from the mother.

D5S10. The same result was obtained for a second locus, D5S18. The fetus was therefore unbalanced and hemizygous for distal 5p loci.

Discussion

The analysis of the family described in this report is indicative of the increased reliance on DNA markers to determine "molecular karyotypes." With the increased use of molecular probes to examine chromosome rearrangements, it is becoming apparent that there are a significant number of cases of subtle chromosome rearrangements which can be identified using recombinant-DNA technology but which are not detectable by chromosome banding. Elsewhere we have described individuals with an unbalanced karyotype (deletion of most of 5p14) who have no unusual phenotype (Overhauser et al. 1986*b*). Without DNA marker studies, several members of this family would have been suspected of being balanced-translocation carriers and at significant risk for having offspring with congenital anomalies. Recently, another family with a subtle translocation involving 5p has come to our attention. As in the family described here, the chromosomal status of individuals having unbalanced karyotypes could only be sorted out using DNA markers. In addition, we are presently using polymorphic DNA markers from the short arm of chromosome 4 to analyze individuals from a family with both a cryptic translocation involving 4p and a history of Wolf-Hirschhorn syndrome.

The experience with this family points out the importance of repeated clinical evaluations for counseling purposes as advances are made in DNA technology and molecular karyotyping. Identifying individuals with unbalanced karyotypes was very straightforward and required only RFLP studies on offspring and on the parent suspected of being a translocation carrier. However, the ability to unequivocally identify translocation carriers was dependent on segregating chromosome 5 homologues from at least one carrier from the second generation in cell hybrids. This made it possible to determine the haplotype of the translocated segment of 5p. Once the haplotype of the translocated portion of 5p was known, it was possible to predict with reasonable certainty which other at-risk individuals were balanced carriers. In this regard, the loci closest but distal to the breakpoint were the most critical, since the haplotype for these loci is less likely to be changed by recombination than are more distal loci. Thus, the genotype for one individual (II-4) at the two most distal loci indicated she was not a translocation carrier, while the genotypes for the three more-proximal loci indicated she did have the balanced translocation. Had the proximal loci not been examined or not been informative, this individual could have been mistakenly counseled that she was not at risk for having children with unbalanced karyotypes.

Acknowledgments

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