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Molecular and Cytogenetic Characterization of a De Novo t(5p;21q) in a Patient Previously Diagnosed as Monosomy 21

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Summary

Genomic single-copy DNA fragments were used to characterize an undetected chromosome translocation in an individual whose metaphase chromosome analysis revealed apparent monosomy 21. Eight RFLPs detected by six probes were used to identify homologous sequences from chromosome 21 in DNA digests from the proband and her parents. These family studies showed that the proband was disomic for the distal region of 21q. Reverse banding and in situ hybridization of chromosome 21–specific probes to metaphase chromosomes from the proband revealed a de novo translocation with breakpoints at 5p13 or 14 and 21q11 or 21. In situ hybridization permitted orientation of the translocated portion of chromosome 21 on the derivative chromosome 5 and, in conjunction with molecular analysis and previous mapping studies, refined the physical map for the long arm of chromosome 21.

Introduction

The occurrence of complete autosomal monosomy in man is rare and may be incompatible with life (Abeliovich et al. 1979). Patients with presumptive G monosomy vary widely in phenotypic features, leading to the suggestion that less severely affected cases may represent unrecognized translocations resulting in partial monosomy (Schinzel 1976).

Only one patient with apparent monosomy 21 has been examined using a DNA marker (Pellissier et al. 1987). A single probe, localized to 21q22.3, was hybridized to metaphase chromosomes from a newborn to confirm the cytogenetic diagnosis; no evidence for disomy or a translocation involving the terminus of 21q was found. However, while a translocation involving the most distal portion of chromosome 21 was excluded, disomy for the more proximal region of the chromosome was not ruled out.

In the present study, high-resolution chromosome analysis and molecular techniques, including RFLP analysis and *in situ* hybridization, were used to screen for chromosome 21 material in an individual previously diagnosed with monosomy 21 (Phelan and Stevenson 1984). The proband had several phenotypic abnormalities in common with previously reported cases of apparent monosomy 21. These features included profound mental retardation, growth deficiency, microcephaly, down-slanting palpebral fissures, prominent nasal bridge, hypertonia, skeletal defects, and dermatoglyphic abnormalities (Gripenberg et al. 1972; Halloran et al. 1974; Kaneko et al. 1975; Davis et al. 1976; Dziuba et al. 1976; Fryns et al. 1977). Screening the proband's genome for evidence of disomy for chromosome 21q material revealed a previously undetected translocation. The present report demonstrates the value of combined cytogenetic and molecular analyses to more precisely define cytogenetic rearrangements.

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Material and Methods

Case Report

D. E. was the 2.4-kg product of a full-term pregnancy of a 21-year-old primigravida and a 24-year-old father. At birth she had sparse hair, absent fingernails and toenails, and a weak cry. At 11 years of age her IQ was 27. At 25 years of age her height, weight, and head circumference were at the third percentile. Facial features included hypertelorism, bilateral epicanthal folds, down-slanting palpebral fissures, left exotropia, prominent nasal bridge, high-arched palate, prognathism, and low-set posteriorly rotated ears. She had bilateral fifth-finger clinodactyly, bilateral horizontal palmar creases, proximal cutaneous syndactyly of fingers 2–4, mild syndactyly of toes 2–4, pectus excavatum, curvature of the lumbar spine, and cubitus valgus.

Cytogenetic Analysis

Metaphase preparations from peripheral blood lymphocytes of D.E. and her parents were G-banded by the trypsin-Giemsa method of Seabright (1971). High-resolution analysis was performed on prometaphase spreads prepared by the amethopterin synchronization method of Yunis (1976). Reverse-banding (R-banding) was achieved by 5-bromodeoxyuridine incorporation followed by acridine orange staining (Pai and Thomas 1980).

RFLP Studies

Genomic DNA was prepared directly from blood samples from D.E. and her parents by standard methods of nuclear pellet isolation and lysis, protease digestion, phenol extraction, and ethanol precipitation. Five-microgram aliquots of DNA were digested according to supplier's recommendations, separated by horizontal agarose electrophoresis, and blotted onto nylon membranes (Plasco, Inc., Woburn, MA).

Six single-copy anonymous DNA sequences, cloned from the long arm of chromosome 21, were used as hybridization probes (table 1). pPW233F, pPW228C, pPW236B, and pPW267C were isolated from a library constructed from a somatic cell hybrid line containing human chromosome 21 as its only human chromosome (Watkins et al. 1985). pGSH8 and pGSB3 were isolated from a flow-sorted library enriched for chromosome 21 (Stewart et al. 1988). These six clones detect eight chromosome 21–specific RFLPs (table 1). Physical mapping of the clones was achieved by both *in situ* hybridization to metaphase chromosomes (Munke et al. 1985; present report) and hybridization to somatic cell mapping panels (Van Keuren et al. 1985; Stewart et al. 1988). These loci were ordered by two-point linkage analysis, and the genetic distances between them were determined to be D21S4-(7cM)-D21S1/D21S11-(7cM)-D21S12-(20cM)-D21S17-(35cM)-D21S19 (Tanzi et al., in press).

Plasmid DNAs were labeled with (alpha-^{32}P)dCTP

Table 1

Chromosome 21–specific Probes

Clone	Location	Locus	Polymorphism			
			Enzyme	Allele	Length	Frequency
pPW233F.....	21cen-q22.2	DS1S4	*Pst*I	A1	8.2	.87
				A2	6.9	.13
pPW228C	21cen-q21	D21S1	*Bam*HI	A1	6.1	.71
				A2	7.0	.29
			*Msp*I	B1	4.6	.62
				B2	7.8	.38
pPW236B	21cen-q21	D21S11	*Taq*I	A1	4.1	.90
				A2	5.1	.10
			*Eco*RI	B1	1.8	.75
				B2	2.6	.25
pPW267C	21q11-q21	D21S12	*Taq*I	A1	8.6	.91
				A2	23.0	.09
pGSH8.....	21q22	D21S17	*Bgl*II	A1	18.0	.57
				A2	12.0	.43
pGSB3	21q22.3	D21S19	*Pst*I	A1	1.6	.80
				A2	1.5	.20

(3,200 Ci/mmol; New England Nuclear) to a specific activity of greater than 10^7 cpm/μg by using the methods of Rigby et al. (1977). Membranes were prehybridized, hybridized, washed, and exposed to Kodak XAR-7 film according to a method described elsewhere (Amos et al. 1984).

Dosage of specific chromosome sequences in D.E. relative to her parents was quantified using densitometry, as described by St. George-Hyslop et al. (1987), for loci that were homozygous in this family. Membranes that had first been probed with chromosome 21–specific sequences were stripped and rehybridized to a genomic probe for the human gamma-globin gene, which has been localized to chromosome 11 (Tuan et al. 1979). The intensity of signal in response to the second hybridization was used to normalize the total amount of DNA between gel lanes to allow subsequent estimation of the dosage of genomic sequences in the patient.

Chromosomal In Situ Hybridization

Metaphase chromosome preparations from peripheral blood lymphocytes from D.E. and from normal human males (control samples) were hybridized according to a method described elsewhere (Morton et al. 1984) with either of two recombinant plasmids, pPW267C and pGSB3. Plasmids were labeled by nick-translation using all four ^{3}H-dNTPs to a specific activity of greater than 10^7. Following autoradiography, the chromosomes were stained in quinacrine mustard dihydrochloride. Data were collected at the light microscope by visualization of metaphases with a combination of incident UV and transmitted visible light.

Results

Cytogenetic Analysis

Analysis of 200 metaphase spreads from peripheral blood and of 180 spreads from fibroblast cultures from the proband revealed 45,XX,-21 in all cells (fig. 1*A*). Parental karyotypes were normal.

High-resolution analysis with G-banding also revealed 45 chromosomes with absence of one chromosome 21. R-banding (fig. 1*B*) demonstrated a previously undetected translocation involving the short arm of chromosome 5 and the long arm of chromosome 21 (Phelan 1985; Schinzel 1985).

RFLP Studies

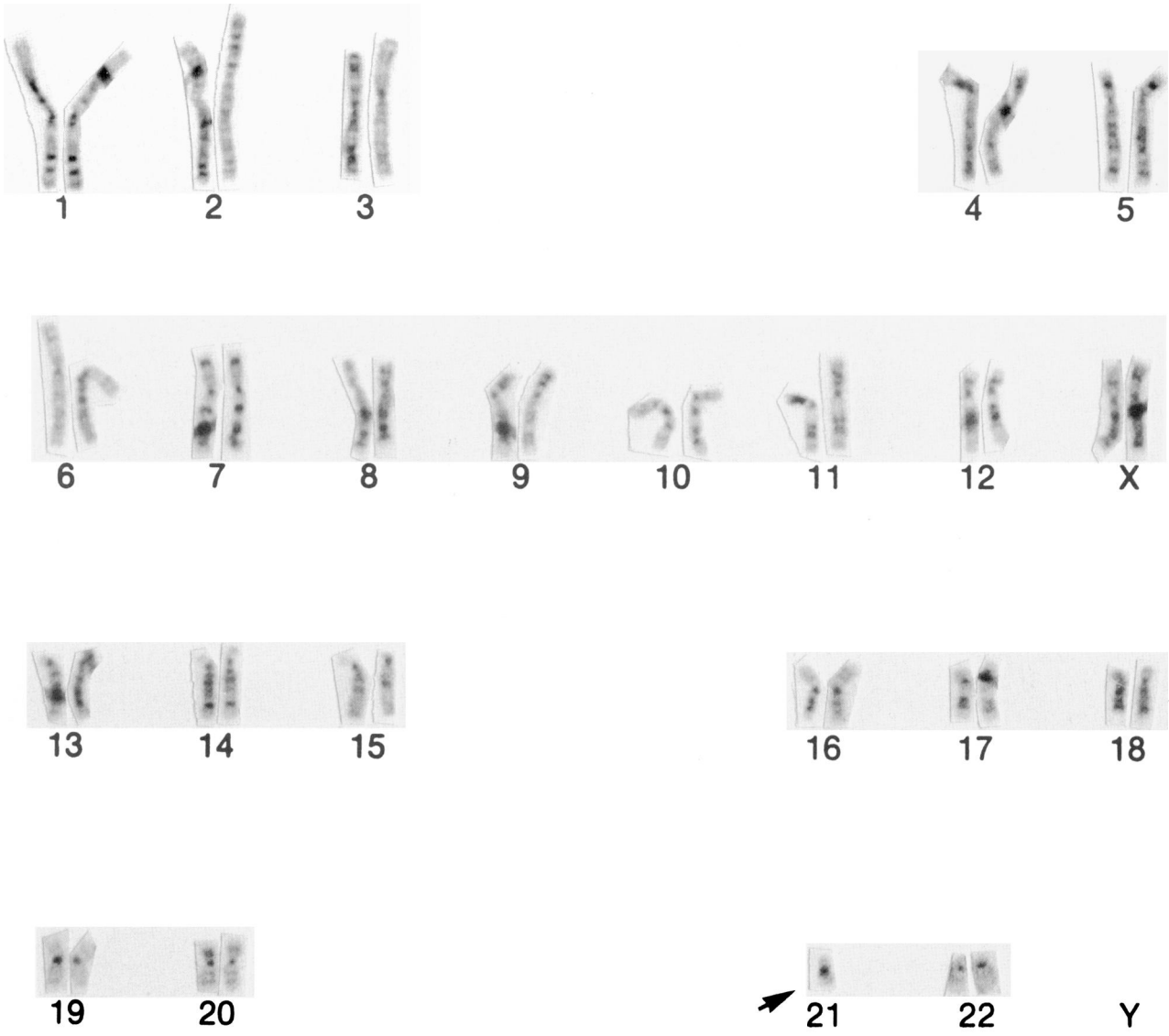
The family was uninformative for the loci detected by pPW233F, pPW236B, and pPW228C. This result is shown in figure 2*A*, in which all three family members exhibit only the major allele for the *Msp*I RFLP detected by pPW228C. Densitometry of this autoradiograph and of a second film (fig. 2*B*) representing rehybridization of the original membrane to a chromosome 11 clone, gamma-globin, was used to compare the intensity of D.E.'s hybridizing chromosome 21 fragment to that of her parents. After normalization of the total amount of DNA in each lane on the pPW228C autoradiograph, the relative intensity of her hybridizing fragment was calculated to be 62% and 52% of that of her mother and father, respectively. Densitometric calculations were made with similar results for pPW233F and pPW236B (data not shown), indicating that D.E. was hemizygous for the loci detected by these three probes (pPW228C, pPW223F, and pPW236B).

D.E. was heterozygous for three loci known to be distal to D21S1. All family members were heterozygous for D21S12, the *Taq*I RFLP detected by pPW267C (fig. 3*A*). Both D.E. and her mother were heterozygous for D21S17, the *Bgl*II RFLP detected by pGSH8 (fig. 3*B*). D.E. and both parents were heterozygous for D21S19, the *Pst*I polymorphism detected by pGSB3 (fig. 3*C*).

Chromosomal In Situ Hybridization

Hybridization of pPW267C to metaphase chromosomes of D.E. revealed two peaks of hybridization (fig. 4*A*). One peak occurred at 21q11-q21, a more precise position than to which this probe had previously been assigned (Van Keuren et al. 1985). Another peak occurred on the short arm of chromosome 5, representing the rearranged chromosome 21 material. Of the 91 silver grains scored in 50 cells, six (6.6%) were located on or beside chromosome 21 at 21q11-q21. These six grains represented 85.7% of the hybridization to chromosome 21. Nine silver grains (9.9%) were located on the short arm of chromosome 5 corresponding approximately to the positions of bands p13-14. It was not possible to definitively identify the der(5) chromosome by QFQ-banding. These nine grains are 69.2% of the total grains recorded on chromosome 5. Hybridization of pPW267C to metaphase chromosomes from a normal male was performed for comparison and revealed a single peak of hybridization at 21q11-q21. In this experiment a total of 129 silver grains were counted in 50 cells. Sixteen grains (12.4%) were located at 21q11-q21, representing 89% of grains on that chromosome (data not shown). One grain was seen at 5p13-p14, consistent with random background exposure.

Hybridization of the pGSB3 clone to metaphases



(continued)

from D.E. and from a normal male demonstrated a profile similar to that obtained for pPW267C (fig. 4*B*). A total of 71 spreads were analyzed from D.E., and 172 silver grains were recorded. Seven grains (4.1%) were located on or beside 21q22, representing 77.8% of the grains on chromosome 21. Eleven grains were clustered on the short arm of chromosome 5 at a region corresponding to 5p15. This cluster of grains represented 6.4% of the total number of silver grains counted and 57.9% of grains on chromosome 5. Hybridization of the pGSB3 was thus distal to D21S12 on chromosome 21. The localization of D21S19 was also distal to D21S12 on chromosome 21, permitting orientation of the translocated material on the der(5). For comparison, 72 metaphases from a normal male were analyzed after hybridization with pGSB3 and a sole peak was noted at 21q22 (data not shown). Fifteen (9.9%) of 152 grains scored were located at this site and represented 88.2% of silver grains on chromosome 21. A single grain was seen at 5p15, consistent with a random background disintegration.

Discussion

Numerous cases of presumptive monosomy 21 have been described, although many of these were studied

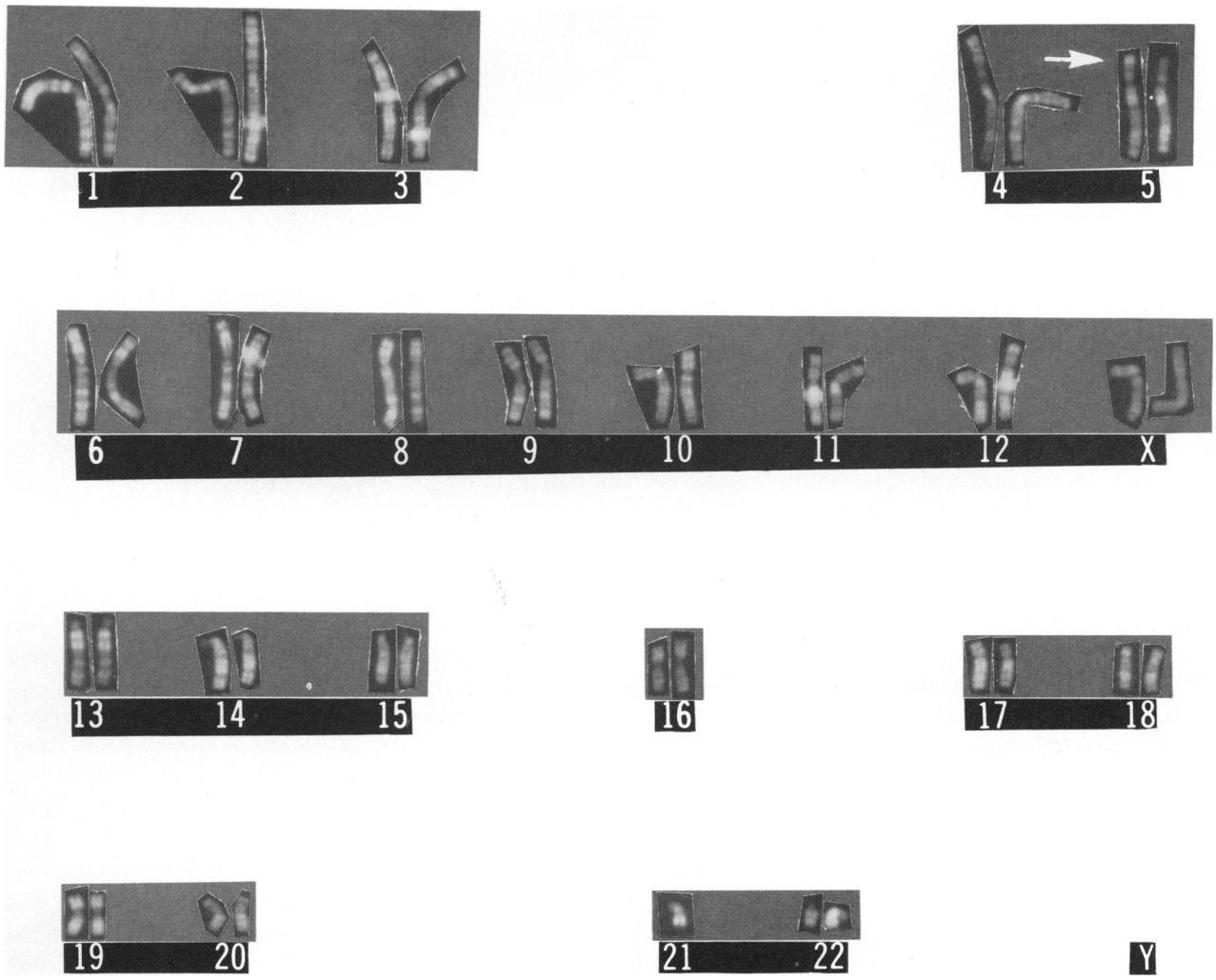


Figure 1 Representative karyotypes of D.E. with (*A*) G-banding, showing absence of one chromosome 21, and (*B*) R-banding, showing the translocation of a portion of 21q to the short arm of chromosome 5.

before the use of chromosome banding techniques. Reexamination of several cases of presumed monosomy 21 with higher-resolution cytogenetic techniques has revealed unbalanced translocations leading to only partial deletion of this chromosome in many of the cases (Wyandt et al. 1971; Cohen and Putnam 1972; Cooksley et al. 1973; Dutrillaux et al. 1973; Ikeuchi et al. 1976). Schinzel (1976) suggests that all cases of apparent monosomy 21 may, in fact, be only partially monosomic. An undetected translocation resulting in partial monosomy for chromosome 21 and partial monosomy for a second involved chromosome may account for the widely variable phenotypes in such cases. Current methods of high-resolution chromosome analysis may fail to detect a cryptic translocation in an individual with presumptive monosomy 21.

In the present case, the initial karyotypic analysis revealed apparent monosomy 21 in both peripheral blood and fibroblast cultures. To investigate the possibility of an undetected translocation involving chromosome 21, both molecular and cytogenetic analyses were performed. Genomic DNA prepared from D.E. and her parents was systematically searched by Southern blot analysis using single-copy, anonymous DNA fragments. Chromosome preparations from the proband were examined further by R-banding, prometaphase analysis,

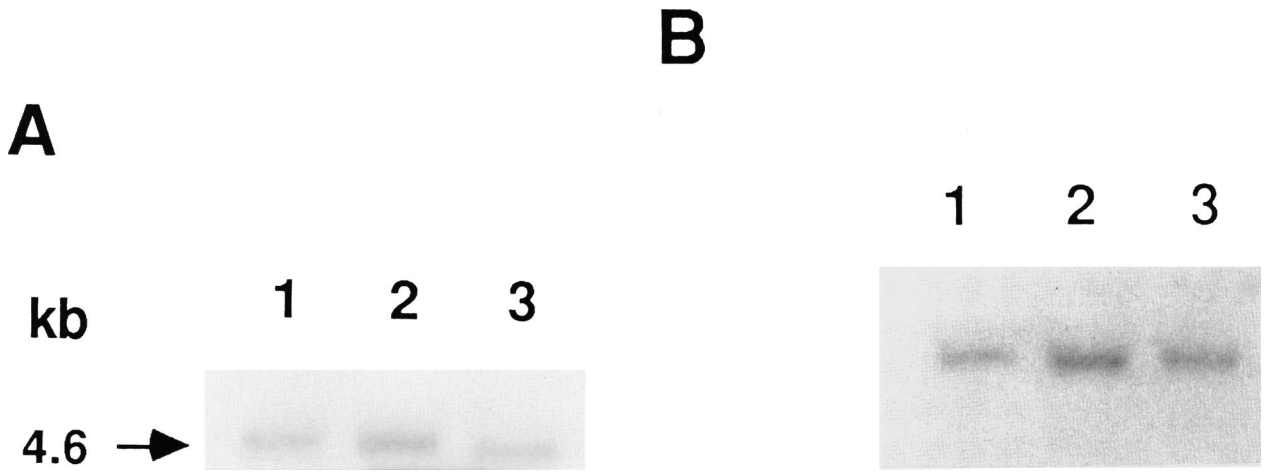


Figure 2 Autoradiographs of genomic *MspI* digestions hybridized initially to pPW228C (*A*) and subsequently hybridized to gamma-globulin (*B*). Lane 1, father; lane 2, mother; lane 3, D.E.

and *in situ* hybridization of chromosome 21–specific probes.

The proband and at least one parent were heterozygous for three loci, D21S12, D21S17, and D21S23, that have been localized to the long arm of chromosome 21 (Munke et al. 1985; present report). These data demonstrate that D.E. is disomic for at least this region of chromosome 21.

The family was uninformative for several other, more proximal probes. Densitometry was used to estimate dosage of the proximal region of 21q in the patient. The proband was monosomic for proximal 21q extending possibly to 21q21, as shown by hemizygosity for D21S4, D21S1, and D21S11. Additionally, these results indicate tht D21S4, D21S1, and D21S11 are proximal to D21S12, excluding a more complex chromosome rearrangement in the proband.

High-resolution chromosome analysis with G-banding revealed absence of one chromosome 21. R-banding was performed because an unbalanced translocation involving chromosome 21 might be undetected in a G-banded karyotype if the bands on the two involved chromosomes are of similar size and staining intensity. R-banding revealed that the short arm of one chromosome 5 fluoresced more brightly than its homologue. This segment corresponds to the bright fluorescence on chromosome 21 and confirms that the distal segment of 5p has been lost and has in its place a portion of 21q. Parental karyotypes were normal on G-banding and R-banding.

In situ hybridization of the two distal 21q probes, pPW267C and pGSB3, was used to confirm the translocation to 5p. As expected, the results demonstrated association of both probes with the normal chromosome 21 in the patient. The probes also hybridized to the distal short arm of chromosome 5, demonstrating translocation of chromosome 21 material to 5p.

In the present case, the use of high-resolution chromosome analysis and molecular techniques allowed the diagnosis of a previously undetected translocation involving chromosomes 5 and 21. The proband was monosomic for the distal short arm of chromosome 5 and for the short arm, centromere, and proximal long arm of chromosome 21. Her karyotype was thus designated 45,XX,t(5;21)(p13;q11) or (p14;q21). A similar 5;21 translocation has been reported by Schinzel (1983) in monozygotic twin females with features characteristic of the cri-du-chat syndrome. Many clinical features of our patient, such as long narrow face, prominent nasal bridge, high-arched palate, hypertelorism, epicanthal folds, low-set ears, microcephaly, short stature, and hypertonia, are suggestive of a diagnosis of cri-du-chat syndrome.

High-resolution chromosome analysis in association with molecular technology permitted a more precise definition of the chromosome abnormality in this patient than was possible by cytogenetic evaluation alone. Furthermore, these results demonstrate the power of combining cytogenetic methods, chromosomal *in situ* hybridization, and RFLP analysis for the precise physical mapping of human chromosomes.

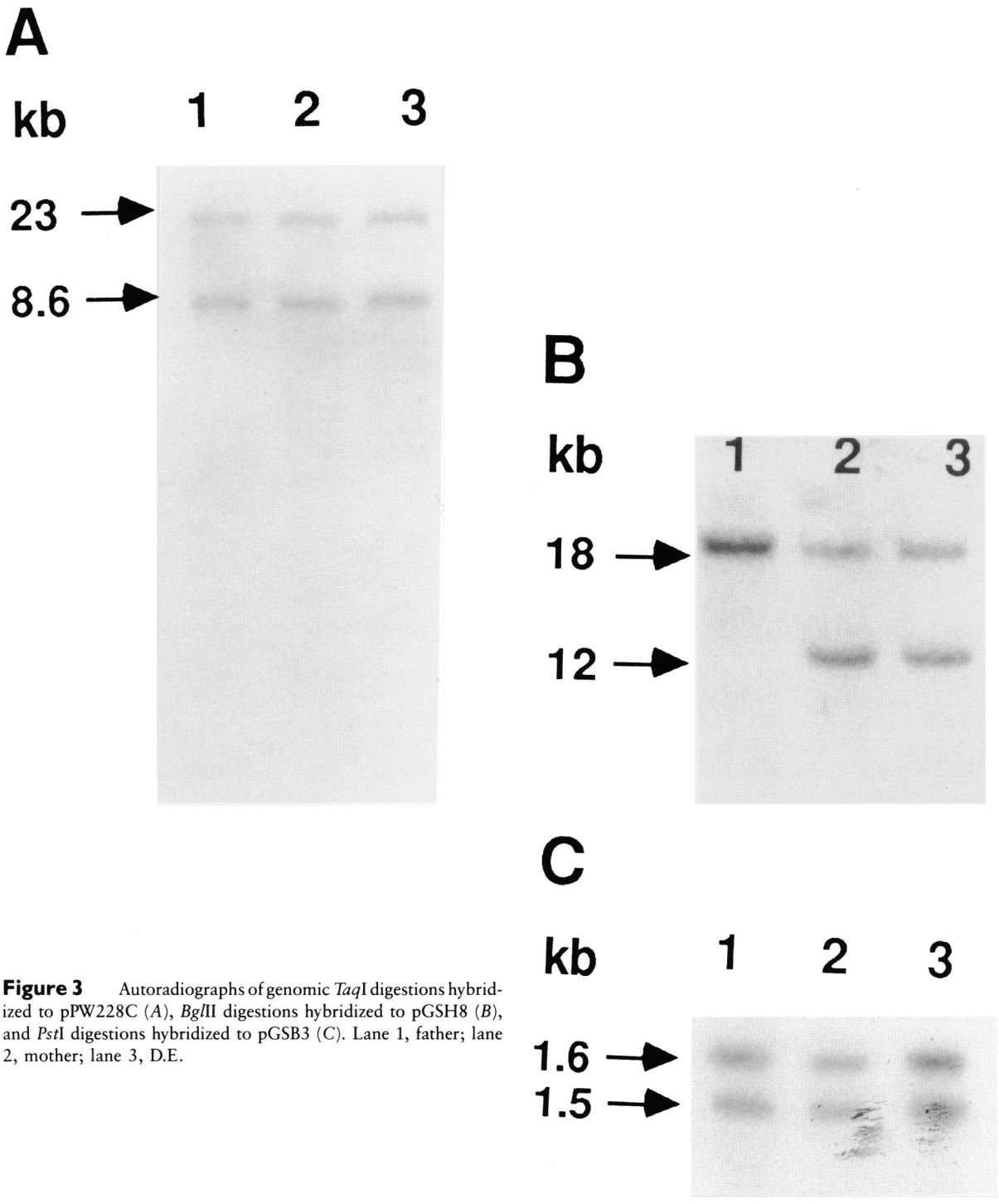


Figure 3 Autoradiographs of genomic *TaqI* digestions hybridized to pPW228C (*A*), *BglII* digestions hybridized to pGSH8 (*B*), and *PstI* digestions hybridized to pGSB3 (*C*). Lane 1, father; lane 2, mother; lane 3, D.E.

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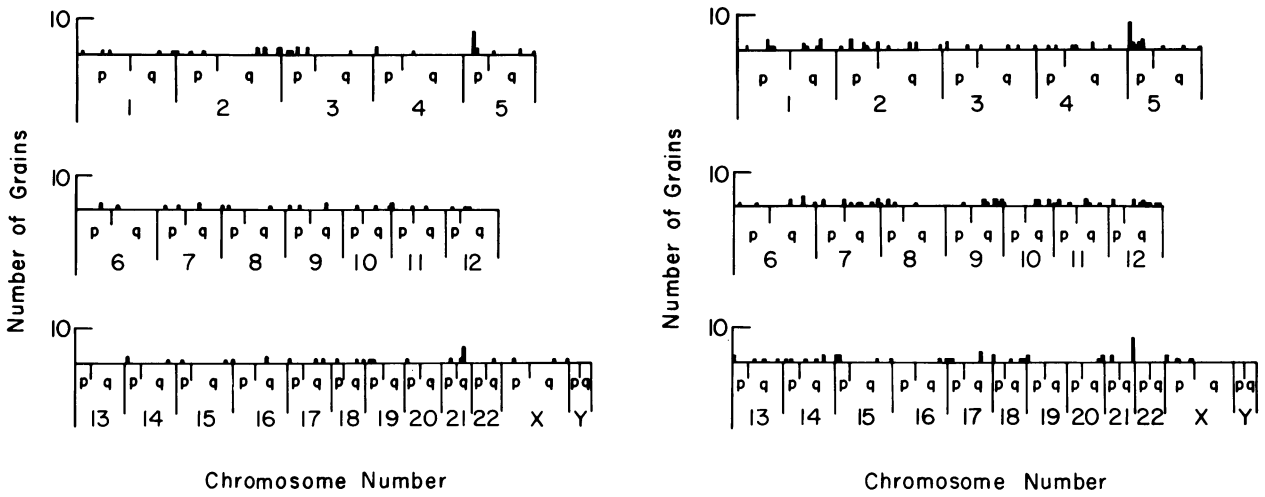

Figure 4 Histogram of silver grain distribution corresponding to hybridization of pPW267C (*A*) and pGSB3 (*B*) to metaphase chromosomes from D.E.

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