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Clinical and Molecular Analyses of Deletion 3p25-pter Syndrome

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Hemizygous deletion of 3p25-pter is associated with a phenotype of profound growth failure, microcephaly, characteristic facial changes, and mental retardation. Since the severity may be quite variable, we have studied 3 cases of del 3p25-pter to define the clinical manifestations and the critical chromosome region for phenotypic expression. The patient we now report died at age 6 months and provided an opportunity for a detailed necropsy analysis for only the second time in a del(3p) patient. He had marked hypoplasia of all organs, hypomyelination of white matter, and multiple renal cortical microcysts. Ordered genomic markers from the distal regions of chromosome 3p aided in determining the parent of origin of each deletion and in defining the boundaries of the deleted chromosomal segments. The deleted markers distal to the RAF1 oncogene in 2 of the 3 patients were consistently hemizygous. One patient had an interstitial deletion based on evidence of diploid inheritance of one of the most distal loci (D3S17). Available genetic linkage maps suggest that the deletion spans at least 19 centimorgans (cM).

KEY WORDS: chromosome 3, 3p deletion, growth retardation, mental retardation

INTRODUCTION

Deletion of region 3p25-pter has been described in individuals with a recognizable pattern of growth and developmental failure. The extended del(3p) phenotype includes failure to thrive due to feeding difficulties starting neonatally, severe respiratory distress, and increased susceptibility to infection throughout early childhood. These children have small stature, microcephaly, trigonocephaly and flat occiput, narrow forehead, hypertelorism, ptosis, epicanthal folds, upslanting palpebral fissures, bushy eyebrows with synophrys, narrow nose with prominent bridge, long philtrum, downturned mouth with thin upper lip, small and malformed ears with occasional preauricular pits and fistulas, postaxial polydactyly of fingers and, in rare instances, of toes. Other less frequently observed findings are renal cysts and kidney positional anomalies, cardiovascular anomalies, hiatus or umbilical hernia, genital hypoplasia, scoliosis, blindness, and deafness [Ramer et al., 1989]. Narahara et al. [1990] studied a patient with ring 3 chromosome and suggested that band 3p25.3 may be the "critical" region for unmasking the typical phenotype associated with this syndrome.

The clinical characteristics of previously reported cases have been compared to our 2 previously reported cases and our current patient. Since cytogenetic studies could not distinguish any differences in the size of the deletion among these patients, genomic DNA markers derived from 3p25→pter were used to determine the parent-of-origin of the deletion and the nature of the rearrangements in each patient. These studies define further the minimum genetic interval responsible for the del 3p25 phenotype.

MATERIALS AND METHODS

Patient Ascertainment

Propositi were ascertained through the Genetics Clinic at The Milton S. Hershey Medical Center. Three children presented with failure to thrive with pre- and postnatal growth deficiency, mental retardation, and unusual craniofacial findings. Each was found to have a deletion of 3p. Twenty to 45 ml of peripheral blood were obtained by venipuncture from patients and parents in each family. DNA was purified from 10–30 cc of blood [Marcadet et al., 1989] while the remainder of blood provided lymphocytes which were immortalized with Epstein-Barr Virus (EBV) [Neitzel, 1986] for continuous

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sources of DNA. The immortalized lines of probands LD and DZ were made available to the research community through the Human Genetic Mutant Cell Repository, Camden (GM10922 and GM10985). Fibroblast cultures from TR were derived from multiple tissues obtained at autopsy.

DNA Probes

Ten DNA clones were used as probes to identify restriction fragment length polymorphisms (RFLPs) on restriction-digested genomic DNA. Cos LIB-I was isolated by a chromosome walk from D3S18. Other probes were purchased from American Type Culture Collection and Collaborative Research, Inc. (CRI), or derived in our laboratories [Table I: Hosoe et al., 1990; Naylor and Bishop, 1989].

RFLP Studies

Genomic DNA was isolated from the peripheral lymphocytes, lymphoblastoid cell lines, or tissues from each patient and parents. Ten micrograms were digested with the appropriate restriction endonuclease corresponding to the polymorphism detected by each probe. DNA digestions were carefully quantitated, fractionated on 0.8% agarose gels, and blotted onto nylon membranes [Southern, 1975]. Genomic probes were labelled by the random hexamer priming method [Feinberg and Vogelstein, 1983], denatured, and hybridized (in 50% formamide, 5X SSPE, 1% sodium dodecyl sulfate [SDS], 0.1% Denhart's solution, 5% dextran sulfate) at 42°C for 12–14 hours. Labelled probes containing repetitive elements were first incubated with denatured human genomic DNA [Sealy et al., 1985] in order to quench the repeat sequence hybridization. Blots were washed at high stringency (0.1 × SSC, 1% SDS, 65°C).

CLINICAL RESULTS

We have previously reported the manifestations of patients LD and DZ [Ramer et al., 1989]. A new patient, proband TR, had a phenotype typical of del 3p25 syndrome. Consistent pre- and postnatal growth retardation with microcephaly and profound mental and developmental retardation were the primary findings.

Clinical Report: Patient TR

Patient TR was delivered by cesarean section at 35 weeks gestation due to fetal distress. Mother and father, ages 32 and 42 years, respectively, were healthy and unconsanguineous. Birthweight, length, and head circumference were 3.5 standard deviations below the mean for age. The newborn period was complicated by pneumonia and apneic episodes. A heart murmur was noted, but a specific congenital heart defect was not identified. The infant had microcephaly, flattened nasal bridges, hypertelorism, blepharophimosis, narrow palpebral fissures, long philtrum, micrognathia, and small

TABLE I. Probe/Enzyme Combinations With Allele Frequencies*

Probe	Locus	Enzyme	Allele	Size	Frequency
(1) p627	C-*Raf*1	*Bg*/I	A1	4.0	0.54
			A2	3.3	0.46
		*Taq*I	A1	6.8	0.74
			A2	6.3	0.26
(2) LIB28-2	—	N/A			
(3) Cos LIB-1	—	*Bam*HI	A1	12.0	0.69
8 kb fragment			A2	5.9	0.31
(4) CRI-L162,	D3S18	*Dra*I	A1	3.8	0.3
5 kb fragment			A2	1.5	0.7
			B1	2.8	0.2
			B2	2.5	0.8
(5) LIB42-26	D3S225	*Hind*III	A1	9.8	0.72
			A2	5.5	0.28
(6) LIB38-96	D3S191	*Msp*I	A1	6.8	0.26
			A2	4.7	0.74
(7) LIB11-31	D3S215	*Msp*I	A1	20.5	0.71
			A2	12.5	0.29
(8) CRI-R532	D3S22	*Msp*I	A1	11.5	?
			A2	9.1	?
		*Pvu*II	A1	11.0	?
(9) CRI-L892	D3S17	*Taq*I	A1	9.5	?
			A2	8.6	?
			A3	8.4	?
			A4	8.0	?
		*Sac*I	A1	10.5	?
			A2	9.5	?
(10) LIB31-17	D3S211	*Bam*HI	A1	7.3	0.23
			A2	3.4	0.77
		*Hind*III	A1	9.8	0.72
			A2	3.8	0.28

*Source of probe/source of table data: (1) ATCC/Bonner et al. [1984,1986]; (2) Lerman, National Cancer Institute (NCI)/personal communication, Latif, Lerman, NCI; (3) see (2); Glenn et al. [1990]; (4) CRI and see (2), Hosoe et al. [1990], Glenn et al. [1990]; (5) see (2), Lerman et al. [1991]; (6) Lerman, NCI/Latif et al. [1991]; (7) see (5); (8) (9) CRI/Donis-Keller et al. [1987]; (10) see (5); ? = not reported.

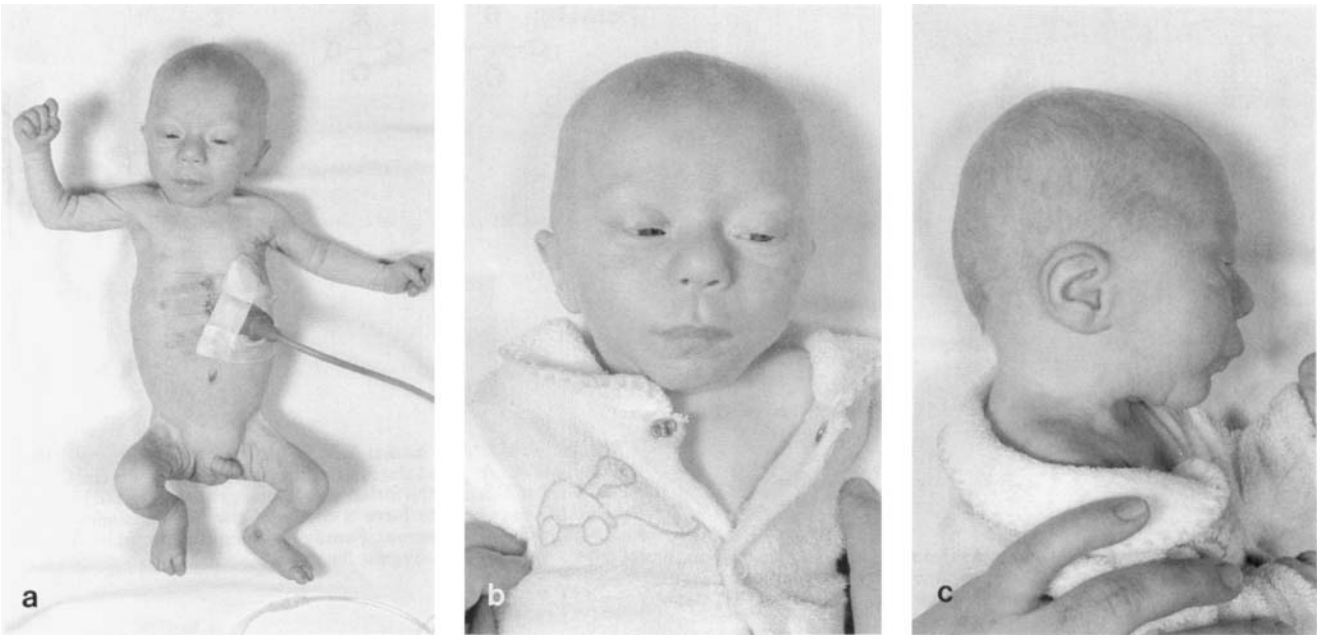

Fig. 1. **a:** Patient TR at 2 months with gastrostomy tube. **b:** Patient TR with flat nasal bridge, bilateral ptosis, apparent hypertelorism, long philtrum with thin upper lip and "carp-like" mouth. **c:** Patient TR, with view of head shows micrognathia, small ears with simple folded helix and long philtrum.

C-shaped ears (Fig. 1a-c). Retinal exam documented bilateral macular hypoplasia.

During the first 2 months of life, TR required nasogastric tube feedings because of poor suck. He had persistent central and obstructive apnea, and was hospitalized repeatedly for pulmonary toilet and antibiotic therapy. At age 2.5 months, he had profound failure to thrive as a complication of repeated episodes of gastroesophageal reflux and aspiration pneumonia. Nissen fundoplication and gastrostomy tube placement were carried out in an effort to prevent further respiratory insult and to improve nutrition.

For the remaining 4 months of life, he was hypotonic and lethargic with persistent respiratory distress and abundant upper airway secretions. Although adequate nutrition was provided via gastrostomy, the patient failed to gain weight and had decreased subcutaneous tissue for his age.

At age 5.5 months, TR was admitted for increased somnolence and severe upper respiratory secretions with difficulty breathing. Vigorous pulmonary toilet was performed with minimal improvement. Parents elected to take TR home with antibiotic therapy and nasal oxygen. Two weeks later without substantial change, he was put to bed and several hours later was found dead in his crib.

Autopsy disclosed a small brain (weight 460 g [normal 700–800 g]) with agenesis of the corpus callosum, poorly developed frontal lobes, and a dilated right lateral ventricle. Histological studies documented hypomyelination of the white matter in the cerebral hemispheres with extensive astrocytosis. The kidneys were well formed but small (combined weight 52 g [normal single kidney weight 48–76 g]) as were the adrenals and thymus. Kidney sections showed dilated glomeruli and numerous diffuse cortical microcysts (results not shown). Renal cortical tubules also showed focal cystic changes. Testes were found high in the inguinal canals with no accompanying changes in the urogenital tract.

CYTOGENETIC STUDIES

Chromosomal analysis using GTG-banding (Giemsa/trypsin) of metaphase chromosomes showed a partial deletion of the short arm of one chromosome 3. Study of prometaphase chromosomes indicated that the deletion included all of region 3p26 and most or all of 3p25 (Fig. 2). The parents of each child had normal chromosomes.

RFLP ANALYSIS

The most centromeric marker, the c-raf-1 proto-oncogene, has been localized to band 3p25 [Bonner et al., 1984]. Southern blot analysis of genomic DNA digested with several restriction enzymes disclosed that the c-raf-1 locus was intact in TR (Fig. 3). Each parent was found to be homozygous for a different Bg1I allele, while the patient inherited one copy of both alleles. Density calibration of a nonpolymorphic band in Taq1 digests showed that this locus was also intact in families D and Z (Fig. 4). A closely linked marker, telomeric to c-raf, LIB28-2, was also intact in each del 3p patient (results not shown). These results suggested that the deletion breakpoint was distal to c-raf and LIB28-2 in all 3 patients.

The next most telomeric marker, Cos LIB-1 [Hosoe et al., 1990], has been localized to band 3p26 near the 3p25 boundary by in situ hybridization [Modi, unpublished]. The 7.9 kb EcoRI subfragment of Cos LIB-1 was subcloned and found to be informative for a BamHI poly-

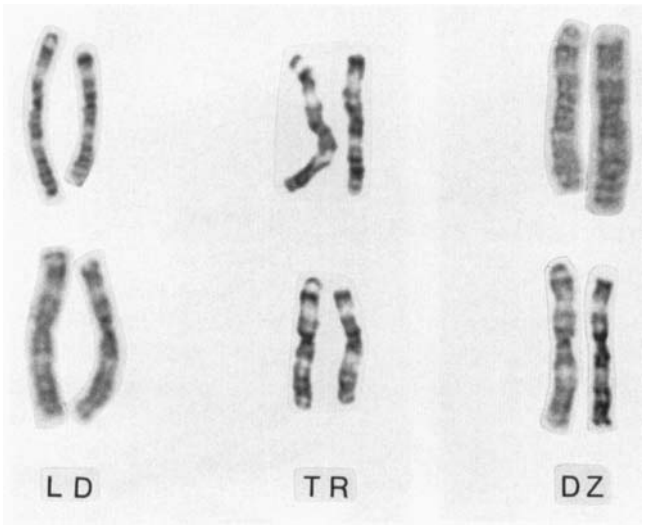

Fig. 2. Representative chromosomes from patients showing deleted segment. Two pairs of representative chromosome 3 homologs are illustrated for each patient as indicated below each set. The abnormal chromosome is on the right in each pair.

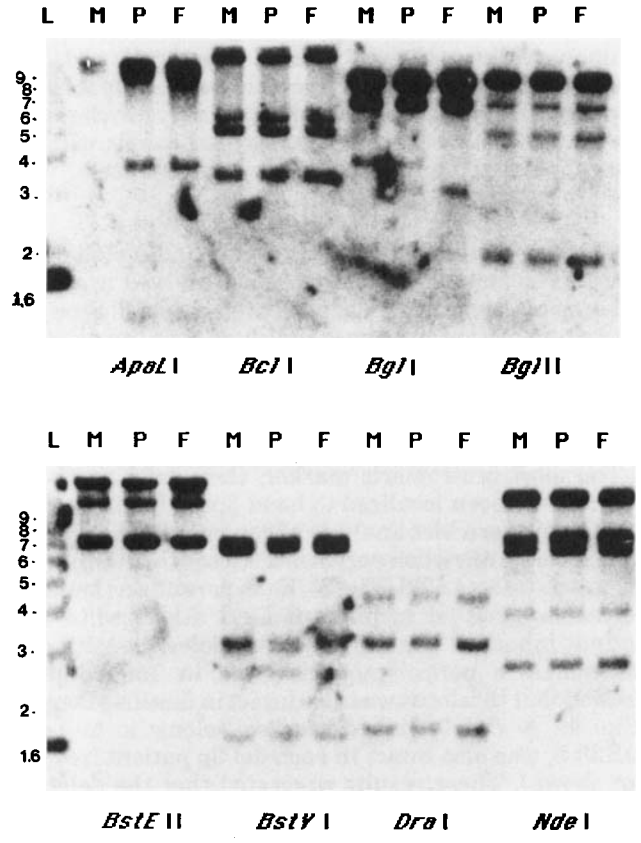

Fig. 3. RFLP analysis of the *c-raf*-1 locus in family R. Southern blot of human DNAs (restriction enzyme digests indicated below the lanes). The mother and father were homozygous for different *BglI* RFLPs. The patient inherited one copy each of the parental polymorphic bands. Approximate size markers (kb) are indicated along the left side (L). M, mother; P, patient; F, father.

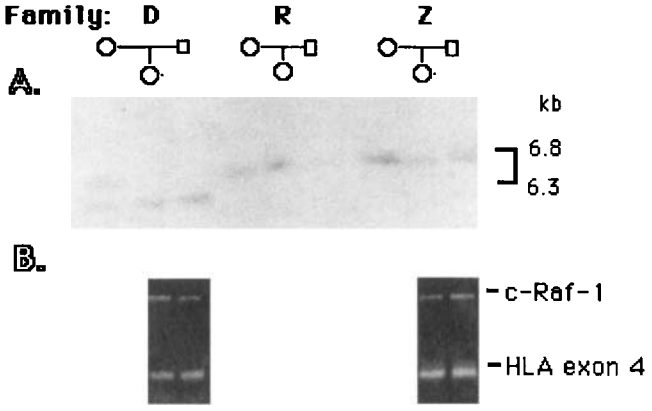

Fig. 4. *c-raf*-1 locus intact. **A,B:** RFLP analysis of the *c-raf*-1 locus in Family D showed that the mother was heterozygous for the 6.8 and 6.3 kb *TaqI* alleles and the father was homozygous for the 6.3 kb allele. Patient LD appeared to have 2 copies of the 6.3 kb allele, having received one from each parent. Family R was uninterpretable. Family Z members were all homozygous for the 6.8 kb allele.

morphism in family R. Paternal homozygosity for the 12 kb allele and maternal homozygosity for the 5.9 kb allele is evident (Fig. 5). The patient inherited only the maternal 5.9 kb fragment, suggesting a loss of the paternal complement at this locus. Southern analysis of BglI and HindIII polymorphisms detected by this probe demonstrated hemizygosity in each affected individual, but these digests were not informative for the parental origin of the deletion.

The extent of the deletion was delineated with several probes which mapped to band 3p26. The most centromeric of these probes, D3S18, detected an informative DraI RFLP in 2 families (Fig. 6). In family D, the patient received a single copy of the maternal 1.5 kb allele. In family Z, the patient inherited a single copy of the father's 3.8 kb allele. D3S225 and D3S215 loci were also hemizygous in each patient (results not shown). In each family, one of the parents was homozygous for the 9.8 kb HindIII allele of D3S225 and the other parent was heterozygous for 9.8 and 5.5 kb alleles. Parental origin could not be determined at this locus since each patient inherited a single copy of the common 9.8 allele. D3S215 was similarly uniformative.

To define the distal boundary of the deletion, a series of more telomeric probes were tested. D3S22 was hemizygous in PvuII digests of each of the patients (data not shown). At D3S211, TR inherited a single copy of the maternal BamHI 7.3 and HindIII 3.8 kb alleles (Fig. 7). Locus D3S17 showed diploid inheritance only in patient TR, suggesting that it is the most telomeric marker in the panel (Fig. 8). Patients LD and DZ each received a single copy of parental alleles of indeterminant origin. This result was replicated independently by different authors (P.N.M. and P.K.R.).

In each patient, the parental chromosome carrying the deletion was identified with one or more genetic markers. Probes Cos LIB-I and D3S211 documented loss of the paternal complement in proband TR. The 5 kb EcoR1 fragment of D3S18 showed loss of paternal and

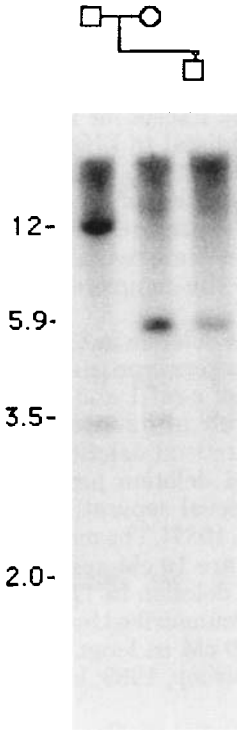

Fig. 5. Paternal deletion at the Cos LIB-1 locus in family R. RFLP analysis was informative for *Bam*HI-digested DNA. The father was homozygous for a 12 kb band while the mother was homozygous for a 5.9 kb band. The patient inherited only the 5.9 kb fragment demonstrating a deletion within the paternally inherited chromosome. Approximate allele sizes (kb) are indicated along the left side.

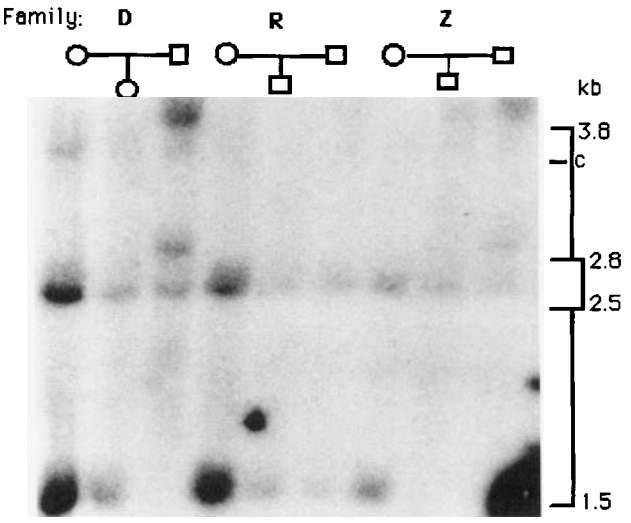

Fig. 6. D3S18 informative for parental origin of del(3p). A 5 kb *Eco*RI subfragment detected an informative *Dra*I RFLP with the 3.8 and 1.5 kb allele system: Family R: Mother was homozygous for the 1.5 kb allele and father was heterozygous for the 1.5 kb and 3.8 kb alleles. Patient TR had a single copy of the common 1.5 kb allele. Family D: Mother was homozygous for the 1.5 kb allele and father was homozygous for the 3.8 kb allele. Their daughter received only a single copy of mother's 1.5 kb allele, revealing a paternal deletion of the D3S18 locus. Family Z: Mother was homozygous for the 1.5 kb allele while father was homozygous for the 3.8 kb allele. Their son inherited a single copy of the father's 3.8 kb allele revealing a maternal deletion of this locus. The 2.8 kb/2.5 kb allele system was uninformative. Approximate allele sizes (kb) are indicated along the right side. c denotes a constant band.

maternal information in families D and Z, respectively. D3S17 confirmed loss of the maternal complement in family Z.

Table II summarizes the results of all of the Southern analyses performed on the 3 families. It includes additional markers which confirm results on deleted markers which were not informative as to parental origin.

FLUORESCENCE IN SITU HYBRIDIZATION

In situ hybridization with probe D3S18 confirmed the deletion of this locus (data not shown). *DAPI*-stained metaphase cells of proband DZ were evaluated to identify both normal and truncated chromosome 3. Fluorescence was not evident at band 3p26 on the deleted metaphase chromosome. On the normal chromosome 3, chromosomal material extended telomerically beyond the fluorescence supporting the interstitial location of D3S18.

DISCUSSION

Deletion 3p25-pter, first reported by Verjaal and De Nef in 1978, presents as a specific phenotype of small stature, profound microcephaly and mental retardation, characteristic craniofacial changes, multiple congenital anomalies, and failure to thrive. TR represents the second report of an autopsy of a del (3p) patient, and the findings in our patient are very similar to those reported by Beneck et al. [1984]. Both patients studied, in addition to the typical del (3p) phenotype, had severe respiratory compromise and died several months after birth. Cortical dilation of tubules and glomeruli with increased interstitial connective tissue was seen in the kidneys of both patients. No other obvious urogenital malformations were found. Isolated renal cystic changes are not uncommon in many different dysmorphogenetic conditions and may be nonspecific; however, it is possible that the deleted region may harbor one or more genes specifically involved in renal development [Kovacs and Kung, 1991]. The proximal breakpoint in TR appears to be near the putative Von Hipple Lindau (VHL) locus (B. Zbar and M. Lerman, unpublished results), but none of the 3p patients has shown evidence of VHL. It is presumed that such anomalies generally would not develop until later in life.

Despite having nearly identical cytogenetic deletions, age-matched patients show some variation in phenotype [Mowrey, 1991]. Consistent with this clinical finding, the observation that some del(3p) patients are less severely affected than others raised the possibility that the size of the deletion might possibly be correlated with the severity of disease. At least 2 patients with del(3p) syndrome exhibit few of the features common to other affected individuals [Tazelaar et al., 1991].

The marker order derived by linkage studies of 3-generation Utah kindreds is consistent with the deletion map generated from patients TR, LD, and DZ. The proximal markers c-raf-1 and LIB28-2 are intact in each

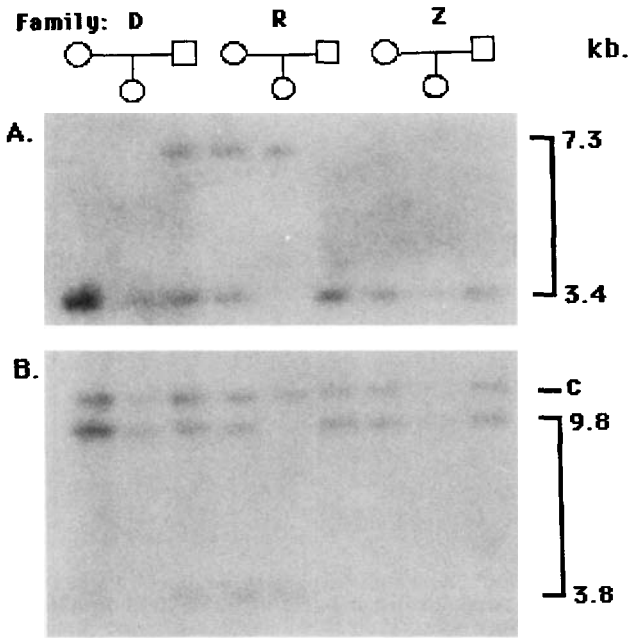

Fig. 7. D3S211 informative for paternal origin in family R. **A:** RFLP analysis of *Bam*HI-digested DNA revealed deletion of this locus. In families D and Z, studies were uninformative for parental origin, however, dosage calibration revealed that the patient inherited a single copy of the common 3.4 kb allele. In family R, the mother was heterozygous while the father was homozygous for the 3.4 kb allele. Patient TR received a single copy of the 7.3 kb allele which could only be inherited from his mother. **B:** RFLP analysis of *Hind*III-digested DNA revealed that probands LD and DZ each received a single copy of the common parental allele. Proband TR received a single copy of the unique 3.8 kb maternal allele, confirming the paternal origin of the deleted segment. c denotes a constant band.

patient. The proximal deletion breakpoints therefore occur within the 5 cM interval bounded by c-raf-1 and the closest telomeric markers, Cos LIB-1 and D3S18 [Hosoe et al., 1990]. Except for locus D3S17 in patient TR, all of the loci distal to LIB28-2 were hemizygous (Cos LIB-1, D3S18, D3S225, D3S191, D3S215, D3S22, D3S211). The distal extent of the deletion in patients DZ and LD cannot be defined with this set of markers, and it is unknown whether these rearrangements are interstitial or resulted in the complete loss of the 3p25-pter domain.

Delineation of the deletion in TR suggests that each of the hemizygous markers is proximal to D3S17. Chromosomal localization of c-raf-1 and D3S17 to bands 3p25 and 3p26, respectively, also supports the conclusion that TR carries an interstitial deletion (Modi, unpublished results). The distal deletion junction in TR is found within a 3 cM interval separating D3S22 and D3S17 [Donis-Keller et al., 1987]. The most distant deleted loci, D3S22 and D3S18 are 19 cM apart, defining the minimum length of the deletion in TR. The deletion in this patient, which is circumscribed by the c-raf-1 and D3S17 loci, is less than 30 cM in length [Donis-Keller et al., 1987; Naylor and Bishop, 1989; Hosoe et al., 1990; Tory et al., 1991].

The molecular genetic studies presented here distinguish at least 2 different classes of deletions in del 3p25 syndrome. In patient TR, the most distal probe, D3S17, is intact, suggesting that TR carries the smallest deletion. Since each of the patients has the same overall phenotype, sequences distal to D3S17 are presumed not to be involved in expression of the syndrome. Thus, the deleted segment in TR appears to define the minimal

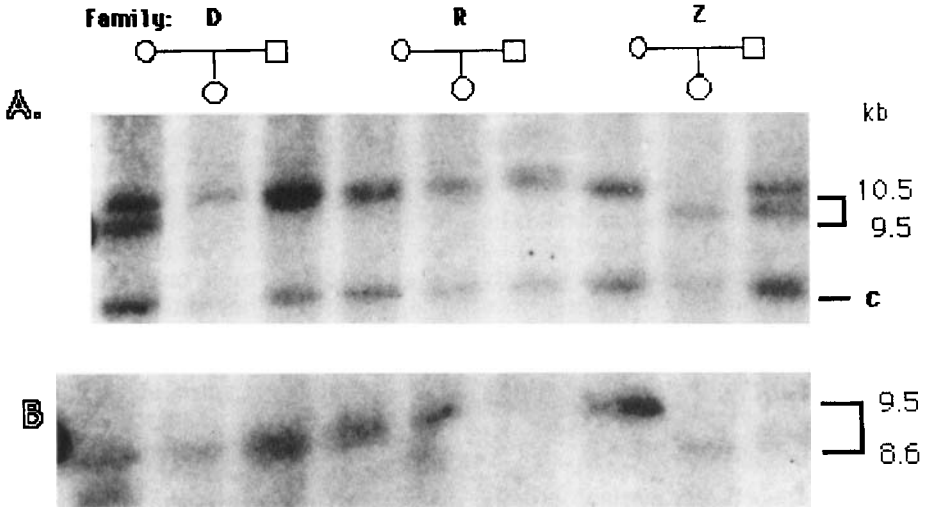

Fig. 8. D3S17 locus. **A:** RFLP analysis of *Sac*I digested DNA: Family D demonstrated a deletion by intensity of the common 10.5 kb allele. In family R, both parents were homozygous for the 10.5 kb allele and the patient appeared to have inherited a double dose of the common allele, as evidenced by a similar intensity in each lane. In family Z, mother was homozygous for the 10.5 kb and father was heterozygous for the 9.5 and 10.5 kb alleles. Patient DZ inherited only the paternal 9.5 kb allele with no copy of the mother's allele. **B:** RFLP analysis of *Taq*I-digested DNA: Family D demonstrated a deletion by intensity of the common 10.5 kb allele. Family R was uninterpretable. In family Z, the mother was homozygous for the 9.5 kb allele and the father was heterozygous for the 9.5 kb and 8.6 kb alleles while their son inherited only the paternal 8.6 kb allele, suggesting a maternal deletion at this locus. Approximate size (kb) are indicated along the right side. c denotes a constant band.

TABLE II. Results of Southern Analyses*

Family			
Locus			
p627	1,2 2,2 2,2 (+)U[a]	1,1 1,2 2,2 (+)PM[a]	1,1 1,1 1,1 (+)U[a]
LIB28-2	NT	1,1 1,1 1,1 +	NT
Cos LIB-1	1,1 1 1,2 (−)U	2,2 2 1,1 (−)P	1,1 1 1,2 (−)U
D3S18	2,2 2 1,1 (−)P[b]	2,2 2 1,2 (−)U	2,2 1 1,1 (−)M[b]
D3S225	1,1 1 1,2 (−)U	1,1 1 1,2 (−)U	1,2 1 1,1 (−)U
D3S215	1,2 2 1,2 (−)U	1,1 1 1,1 (−)U	1,2 1 1,1 (−)U
D3S22	1,1 1 1,1 (−)U	1,1 1 1,1 (−)U	1,1 1 1,1 (−)U
D3S17	1,2 1 1,1 (−)U	1,1 1? 1,1 (+)U	1,1 2 1,2 (−)M
D3S211			
(A)	2,2 2 1,2 (−)U	1,2 1 2,2 (−)P	2,2 2 2,2 (−)U
(B)	1,1 1 1,2 (−)U	1,2 2 1,1 (−)P	1,1 1 1,1 (−)U

*Numbers 1 and 2 refer to alleles at a given locus; (−), locus deleted; (+), locus present; P, paternal; M, maternal; U, uniformative RFLPs, deletion inferred when careful band density analysis suggested a single dosage; NT, not tested.
[a]Confirmed by polymerase chain reaction (PCR) analysis.
[b]Deletion confirmed by *in situ* hybridization analysis.

critical region of hemizygosity necessary for expression of the phenotype.

Half-dosage of genes and reduced levels of gene products in this region probably define the del(3p) phenotype. Since both maternal and paternal deletions are found, del(3p) syndrome is not likely to be modified by genomic imprinting. Hemizygosity may instead unmask one or more aberrantly expressed, developmentally regulated recessive alleles. It is also possible that loss of a normal allele results in a null phenotype for mutant growth regulators at critical developmental periods. Experiments designed to test these possibilities will begin with the physical characterization of the breakpoint boundaries in del(3p) patients.

ACKNOWLEDGMENTS

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