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Restriction Fragment Length Polymorphisms Within Proximal 15q and Their Use in Molecular Cytogenetics and the Prader-Willi Syndrome

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Restriction fragment length polymorphisms (RFLPs) are described in detail for 6 DNA probes (D15S9–13, D15S18) that localize to the proximal long arm of human chromosome 15 (15q11–15q13: this report and Tantravahi et al., *Am. J. Med. Genet.* 33:78–87. Multiple RFLPs are detected by the probe that identifies locus D15S13, and these RFLPs are shown by genomic mapping to result from a nearby insertion or deletion of 1.8 kilobases (kb) of DNA. This set of RFLPs detected by proximal 15q probes can be used for studies on the Prader-Willi syndrome (PWS) and on mentally retarded individuals with a supernumerary inv dup(15) chromosome. Five of the polymorphic loci (D15S9–13) map to the region implicated in the cause of the PWS (15q11.2–15q12). Each of 4 families tested with these probes, as well as an additional "PWS-like" patient, was informative by RFLP analysis. The two PWS deletions studied, which occurred de novo, were inherited from the chromosome 15 provided by the father. By contrast, the 2 inv dup(15) chromosomes analyzed were of maternal origin. The use of RFLPs can also simplify the molecular determination of copy number in chromosomal aneuploidy, as exemplified by analysis of individuals with the PWS and a deletion, patients with an inv dup(15), and one patient with a more complex rearrangement involving chromosome 15. Our studies demonstrate the application of DNA probes for both molecular cytogenetic studies on this chromosome region and the development of diagnostic molecular markers to aid early clinical diagnosis of the PWS.

KEY WORDS: RFLPs, inv dup(15), parental origin

INTRODUCTION

The proximal region of chromosome 15 is of particular interest by virtue of its heterogeneous structural rearrangements, involving deletions, duplications, or translocations, all of which can be reflected in an abnormal clinical phenotype. The most common disorder involving chromosome 15 is a deletion of part of proximal 15q in the Prader-Willi syndrome (PWS) [Mattei et al., 1984; Pettigrew et al., 1987].

PWS is a congenital multisystem disorder, typically sporadic, with incidence 1/10,000–1/30,000 [Cassidy, 1984]. The clinical presentation is characterized by congenital hypotonia, with feeding problems, delayed motor development, and minor facial anomalies. Older children develop hyperphagia, with consequent obesity, and have hypogonadism, growth retardation, small hands and feet, and usually mild to moderate cognitive disorders and behavioral disturbances [Cassidy, 1984]. An interstitial 15q deletion, typically involving bands 15q11.2–q12, is described in approximately one-half of all PWS patients, with one-third reported to have apparently normal chromosomes [Ledbetter et al., 1981, 1982, 1987; Mattei et al., 1983, 1984; Cassidy et al., 1984; Fear et al., 1985; Labidi and Cassidy, 1986; Butler et al., 1986, Wenger et al., 1987] and the remainder exhibiting duplications or translocations (see below). However, Niikawa and Ishikiriyama [1985] suggest 100% correlation of a chromo-

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some 15q deletion (or unbalanced rearrangement leading to a net partial 15q1 deletion) and strictly defined PWS. A pronounced cytogenetic deletion [Hittner et al., 1982; Butler et al., 1986; Creel et al., 1986; Wiesner et al., 1987] or duplication [Pettigrew et al., 1987] may be associated with significant hypopigmentation. Normal chromosomes have also been reported in putative familial PWS [Burke et al., 1987; Lubinsky et al., 1987]. In addition, deletions of proximal 15q have also been reported in non-PWS patients [Schwartz et al., 1985; Kaplan et al., 1987; Reynolds et al., 1987; Greenberg and Ledbetter, 1987], Angelman syndrome [Kaplan et al., 1987; Magenis et al., 1987], and single patients with Williams syndrome [Kaplan et al., 1987] or hypomelanosis of Ito [Turleau et al., 1986]. This wide spectrum of phenotypic manifestations may reflect perturbation of multiple genetic determinants in the proximal 15q region. The characterization of these genetic variations by molecular biology techniques is of fundamental importance in an understanding of the disease phenotype and process.

Fewer than 5% of PWS patients have complex chromosome rearrangements [Ledbetter et al., 1987]. These include translocations that are generally unbalanced and thus equivalent to the deletions, although presumed balanced cases do occur [Charrow et al., 1983; Fraccaro et al., 1983; Hasegawa et al., 1984; Mattei et al., 1984]. Several authors [Lejeune et al., 1979; Mattei et al., 1984; Fraccaro et al., 1983; Greenberg et al., 1987] note that proximal 15q is frequently translocated to the telomere of another chromosome, perhaps hinting at compositional similarities between the segments undergoing exchange. Chromosome 15 is also involved [Schreck et al., 1977] in the formation of bisatellited marker chromosomes, representing an inverted duplication chromosome and commonly denoted inv dup(15). Patients with the largest inv dup(15) (q12 or q13) chromosomes usually have mental deficiency but not the PWS [Wisniewski et al., 1979; Maraschio et al., 1981; Mattei et al., 1984]. However, smaller inv dup(15) (q11.2) chromosomes are associated with the PWS [Fujita et al., 1980; Wisniewski et al., 1980; Mattei et al., 1984], sometimes as mosaics [Ledbetter et al., 1982; Wulfsberg et al., 1982]. The smallest inv dup(15) (q11.1) chromosomes described are from normal individuals [Stetten et al., 1981; McDermid et al., 1986]. Rare chromosome changes, including duplication of proximal 15q in either PWS [Pettigrew et al., 1987] or normal individuals [Brookwell and Veleba, 1987]; pericentric inversion in either the PWS [Winsor and Welch, 1983] or a syndrome of behavior disturbances [Crandall and Sparkes, 1970]; translocation and inv dup(15) involving the same 15q13 band [Wulfsberg et al., 1982; Murdock and Wurster-Hill, 1986]; and similar mosaic complex translocations also occur in PWS patients [Lejeune et al., 1979; Greenberg et al., 1987]. Molecular genetics should provide the means to resolve this cytogenetic heterogeneity and facilitate genotype–phenotype correlations in the PWS.

Molecular analysis of proximal 15q and the PWS was initiated by Donlon et al. [1986], when they isolated several clones from an inv dup(15)-enriched library obtained by flow sorting metaphase chromosomes. A gene dosage approach for mapping these probes and the analysis of abnormal chromosomes in PWS patients has been described (Tantravahi et al., this issue). Although useful in many instances, "molecular dose" analysis of chromosome alterations (deletion, duplication, etc.) can be further simplified by studying polymorphic systems. Family studies would also become possible. Restriction fragment length polymorphisms (RFLPs) [Botstein et al., 1980] also have applications in linkage analysis [White et al., 1985; Donis-Keller et al., 1987] and clinical diagnosis [Caskey, 1987; Martin, 1987]. We describe here a set of RFLPs for proximal 15q that add to those previously reported by others for this region [Pearson et al., 1987] as well as to a set of 15q probes positioned outside of the critical region for the PWS recently described by Tasset et al. [1988]. We illustrate and discuss the use of these probes for molecular biological analysis of cytogenetic alterations of proximal 15q in the PWS and other conditions. Preliminary results of these RFLPs have been presented elsewhere in abstract form [Latt et al., 1987; Nicholls et al., 1987].

MATERIALS AND METHODS

Human DNAs, Cytogenetics, and Clinical Background

Cell lines from 5 Utah families (K-1329, K-1331, K-1333, K-1340, and K-1345), an Amish family (family 884), a control male (GM3798) and a control female (GM0131), and 2 individuals with an inv dup(15) (GM4347, GM6246) were obtained from the Human Genetic Cell Repository, Coriell Institute for Medical Research, Camden, New Jersey. GM4347 was established from a phenotypically normal individual, karyotype 47,XX+ inv dup(15) (q11.1) [Stetten et al., 1981]. The breakpoint of the inv dup(15) in the cell line GM6246 is reported [NIGMS catalog, 1986] to be at band 15q13.

Other cell lines were established from patients with PWS and their parents; subjects with an additional chromosome, i.e., an inv dup(15) (q13); and a patient initially examined for the PWS who has some characteristics but lacked others considered essential for the diagnosis of the PWS (DON5). This last child had hypotonia during infancy, with a normal suck reflex at birth. At present (age 6 years), she does not have short stature, abnormal eating habits, or obesity, but does have some characteristic facial anomalies and a learning disability [Tantravahi et al., in press]. Clinical characteristics of other patients, used to derive cell lines HS2 (from the same PWS patient as was DON10), HS3 (from the father of HS2), and HS27 (PWS), HS28, and HS29 (both from the parents of HS27), are described by Tantravahi et al. [in press], as are the cytogenetics of HS2 (DON10), HS3, DON5, and HS15 (from the same patient as was ALD24) [see also Donlon et al., 1986]. This last patient has moderate mental retardation, autism, a significant communication disorder, episodic irritability, hypotonia (which resolved at age 2), no feeding problems, and minor anomalies, including apparently low-set ears and bilateral epicanthal folds. Clinical data and a cytogenetic characterization of the PWS patient who was the source

of the cell line DMS918 are provided by Murdock and Wurster-Hill [1986].

We also report a new patient (HS38) with a 47,XX+ inv dup(15) karyotype. Her clinical manifestations include moderate psychomotor delay at age 2 years, hypotonia, congenitally dislocated hips, ventricular septal defect, large head, small hands and feet but normal length and weight, strabismus and nystagmus, minor facial anomalies (including bilateral epicanthal folds), and a slightly abnormal electroencephalogram (EEG). She does not appear to have the PWS. High-resolution analysis of chromosomes from peripheral blood lymphocytes [Yunis, 1976] from HS38 were evaluated by Giemsa-trypsin banding [Seabright, 1971].

DNA Studies

Ten clones (p34, p3–21, pIR4–3R, pIR10–1, p189–1, p190–2, p3–16, p135, pIR29–1, pIR39) of genomic segments defining loci D15S9–D15S18, respectively, were utilized for RFLP screening. All localize to 15q11–15q13, and at least 5 (and probably 6) of these to 15q11.2–15q12, by dosage mapping to cell lines bearing an inv dup(15) (q13) (ALD24) or a 15q11.2–15q2 deletion (DON 10), respectively [Donlon et al., 1986] (Tantravahi et al., this issue).

The probes, subcloned in plasmids, were purified by restriction enzyme digestion, gel electrophoresis, and electroelution [Nicholls, 1986a]. They were subsequently ^{32}P-labeled by the random primer method [Feinberg and Vogelstein, 1983]. Radiolabeled probe (0.5×10^6 cpm/3 μg DNA/track) was boiled for 3 min, rapidly cooled on ice, and mixed with hybridization buffer (see below).

Genomic DNA isolation [Aldridge et al., 1984], restriction enzyme digestion, fluorometric DNA quantitation [Brunk et al., 1979], and DNA blotting [Southern, 1975] were performed, the latter typically by transfer of DNA to Hybond N (Amersham Int.) membranes in 20× SSC (3 M NaCl, 0.3 M Na citrate). After transfer, DNA was covalently cross linked to dry (80°C, 30 min) membranes by near-ultraviolet (UV) irradiation for 1 min. Membranes were prehybridized [50% formamide, 5× SSC, 10× Denhardt's solution, 20 mg/liter denatured salmon sperm DNA, 0.5% sodium dodecyl sulphate (SDS)] at 42°C for 3–16 hr [Denhardt's solution is 0.02% each of bovine serum albumin (BSA), Ficoll, and polyvinylpyrrolidone (Denhardt, 1966)]. Hybridization was at 42°C, 16 hr, using 1×10^6 cpm probe/ml buffer (as prehybridization, except 1× Denhardt's and 5% dextran sulfate). Membranes were rinsed in 2× SSC and then washed at 56°C in 0.1× SSC, 0.1% SDS for 1 hr unless otherwise specified.

RFLP Analysis

DNA from 4 individuals was initially screened (stage I) with a large number of restriction enzymes to identify high-frequency RFLPs [Aldridge et al., 1984; Harris et al., 1987]. All probes except p34 were screened on the previously described panel of whites [Harris et al., 1987] using ApaI, BglI, BglII, EcoRV, KpnI, MspI, PstI, RsaI, SacI, TaqI, and XmaI, and several probes were screened against panels kindly provided by Drs. J. Gusella and R. Tanzi. The probes (p34, p190–2, pIR10–1, pIR4–3R, p189–1, p3–21, pIR29, and pIR39) were then screened against a panel of DNAs from 4 individuals from Amish (GM5993, GM5995a) and Utah (GM6991, GM7048) families cleaved with each of 24 enzymes: ApaI, BamHI, BanI, BclI, BglI, BglII, BstEII, BstNI, BstXI, DraI, EcoRI, EcoRV, HindIII, HinfI, KpnI, MspI, PstI, PvuII, RsaI, SacI, ScaI, TaqI, XbaI, and XmnI.

Identification of a putative RFLP resulted in the

TABLE I. Summary of RFLPs Detected by 15q11–15q13 Probes

Locus	Probe	RFLP enzyme	Constant bands (kb)	Size (kb)	Allele frequency	No. of chromosomes	PIC
D15S9	p34	ScaI	10	6.5	0.72	32	0.32
				6.3	0.28		
D15S10	p3–21	TaqI	0.6	9.0	0.83	36	0.28
				8.2	0.17		
		EcoRV	2.4	23.0	0.89	28	0.19
				7.0	0.11		
		ScrFI/BstNI	0.6	1.3	0.96	28	0.07
				0.9,0.3	0.04		
D15S11	pIR4–3R	RsaI	0.5	1.2	0.5	32	0.375
				1.0	0.5		
D15S12	pIR10–1	ScaI	–	17.5	0.16	32	0.4
				16.1	0.72		
				12.5	0.12		
D15S13	p189–1	TaqI Many others[a]	1.1	3.8	0.69	32	
				2.0	0.31		
D15S18	pIR39	BglII	1.9	9.0	0.21	28	0.28
				8.5,0.6	0.79		
		SacI	–	14	0.29	28	0.33
				8.5,5.5	0.71		

[a] Enzymes that can be utilized for the detection of the p189–1 RFLP are listed in the legend to Figure 4.

screening of 7 parental pairs of second-generation individuals from the Utah pedigrees studied (stage II). Stage III consisted of demonstrating Mendelian inheritance in 3-generation Utah pedigrees. Polymorphic information content (PIC) values were calculated for all unrelated individuals from stages II and III using $1-P_1^2-P_2^2-2(P_1^2P_2^2)$ or $1-P_1^2-P_2^2-P_3^2-2(P_1^2P_2^2 + P_1^2P_3^2 + P_2^2P_3^2)$ for 2 or 3 allele systems, respectively [Botstein et al., 1980; Skolnick and White, 1982]. Several probe/enzyme combinations require specific conditions for optimal RFLP detection. Most importantly, pIR10–1 and p34 require a 0.6% agarose gel, 40–48 hr electrophoresis at 2 V/cm; pIR4–3R requires a 1.2% agarose gel; and pIR39 hybridizations require a 65°C wash (as above). Probes pIR10–1 plus p34 (ScaI) and p189–1 plus p3–21 (TaqI) can be combined for haplotype analysis.

The genomic map for probe p189–1 was determined from single and double digests with EcoRI. This has been confirmed in cloned DNA using a cosmid spanning the allele containing the 1.8 kb insert (R.D. Nicholls, unpublished data).

Densitometric analysis of RFLP alleles for copy number determination was as described by Tantravahi et al. (this issue) except that normalization of test bands was not to a control band from another chromosome. Instead, the ratio of estimated hybridized ^{32}P counts per minute (cpm) for each allele in a test sample was normalized by dividing the test RFLP cpm ratio by the RFLP cpm ratio for a normal control heterozygote, known to have one copy of each allele, and run on the same blot. This was then converted to an allele copy number ratio.

RESULTS

Putative RFLPs were detected with 6 of the 10 DNA fragments previously mapped to 15q11–15q13. For 3 loci, more than one enzyme detected a polymorphism. Nine independent RFLPs for 6 probes were confirmed by demonstration of Mendelian inheritance in 1 to 3 Utah pedigrees, each consisting of 3 generations. The data are summarized in Table I and include allele frequencies and PIC [Botstein et al., 1980] values. The segregation of alleles for the 2 most polymorphic loci, D15S11 and D15S12, are shown in Figures 1 and 2.

Most of the 15q11–15q13 RFLPs appear to represent independent, single-site changes on the basis of restriction mapping in both cloned and genomic DNA (R.D. Nicholls, unpublished). The 3 allele system for pIR10–1 (D15S12) most likely results from 2 closely linked, independent ScaI site RFLPs (Fig. 2); the RFLP is not detected by any other enzyme tested. In contrast, probe p189–1, detecting locus D15S13, is polymorphic for many enzymes (Figs. 3, 4). With this probe, most enzymes (e.g., SacI, TaqI; Fig. 3) show an identical restriction fragment

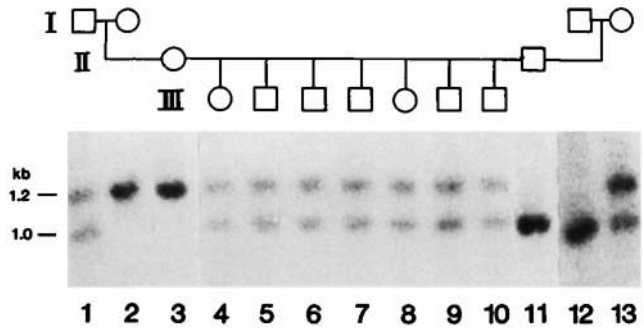

Fig. 1. Mendelian inheritance of a RsaI RFLP detected by probe pIR4–3R in Utah pedigree K-1345. Experimental conditions are given in Materials and Methods. Only the polymorphic 1.2 and 1.0 kb bands are shown here.

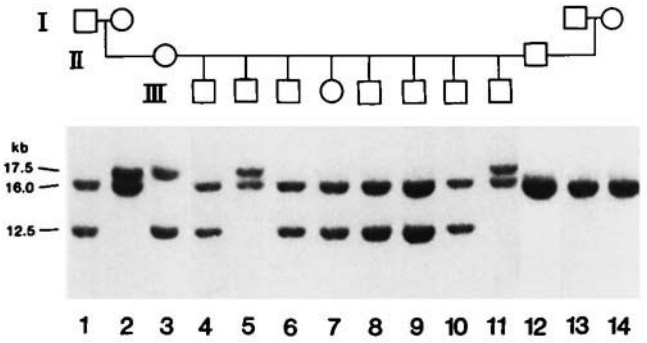

Fig. 2. Mendelian inheritance of a ScaI RFLP detected by probe pIR10–1 in Utah pedigree K-1333. The segregation of 3 alleles (no other band sizes have been observed in any individual) is observed in this family.

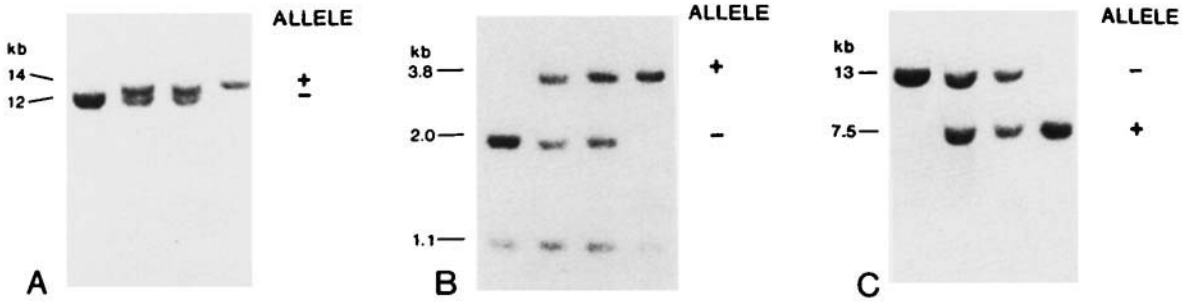

Fig. 3. DNA analysis of an insertion deletion RFLP detected by probe p189–1. **A:** SacI. **B:** TaqI. **C:** BglII. For each enzyme, the human DNA samples are (left to right) GM7038, GM6987, GM7057, GM6990. These are second-generation individuals from Utah pedigrees 1333 and 1331, respectively. The polymorphic alleles are identified by + (insertion) or − (deletion). A nonpolymorphic fragment at 1.1 kb is seen with TaqI. An identical pattern of alleles and size difference between alleles is seen with the 2 enzymes SacI and TaqI, whereas the allele pattern is reversed for BglII.

pattern and size difference between alleles, suggesting a 2 allele insertion (+) or deletion (−) polymorphism. However, BglII (Fig. 3) and several others (Fig. 4) display a reversed pattern of alleles compared to most enzymes, as well as heterogenous size differences. Genomic mapping allows construction of a detailed physical map, and this shows that the latter result is explained by the presence of recognition sites for these enzymes within a 1.8 kb insertion in the plus (+) allele (Fig. 4). Both the TaqI and BglII RFLPs for p189–1 were tested for and exhibited Mendelian inheritance (data not shown).

To demonstrate the use of these proximal 15q RFLPs for molecular cytogenetic analysis, most importantly of the PWS, initial studies were performed with the 5 most informative RFLPs, which mapped to 15q11.2–q12 (pIR10–1, ScaI; pIR4–3R, RsaI; p189–1, TaqI; p34, ScaI; p3–21, TaqI; Table I). Two families with a PWS child were studied. Each was informative for one of the RFLPs in the set of proximal 15q RFLPs. The segregation of alleles for each family (Fig. 5) clearly demonstrates 2 phenomena: detection of a deletion at the molecular level and parental inheritance of the mutation. Each child should inherit one allele from each parent. Thus, in family I (Fig. 5A), the PWS child (lane 1, HS27) inherits only a 12.5 kb allele (probe pIR10–1, ScaI) from his heterozygous mother (lane 2). The homozygous father (lane 3; 2 16.0 kb alleles) does not donate an allele at this locus. Although the second family is uninformative for this probe (Fig. 5A), they are informative for probe pIR4–3R at the D15S11 locus (Fig. 5B). The individual with PWS (lane 4, HS2) inherits only one maternal allele (lane 2), but no paternal allele (lane 3), again demonstrating a deletion of paternal origin. A preferential paternal origin for de novo deletions associated with the PWS has been demonstrated previously by cytological analysis [Butler and Palmer, 1983; Butler et al., 1986].

It should be noted that the 2 PWS patients described above have cytogenetically visible 15q11.2 deletions [Donlon et al., 1986] (Tantravahi et al., this issue). Although the molecular data only confirm cytogenetics in these cases, it is expected that DNA studies would ultimately allow detection of smaller, submicroscopic

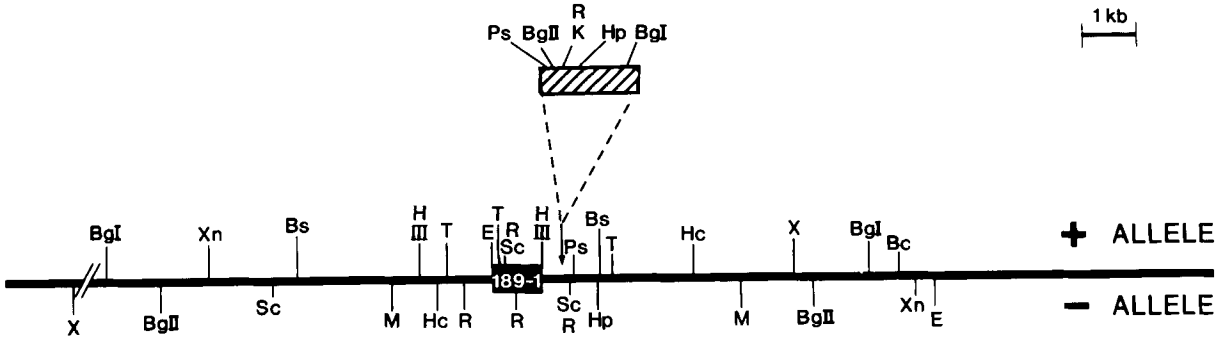

Fig. 4. Genomic map and molecular basis for the insertion–deletion RFLP detected by probe p189–1. The probe is shown by a dark box and the extra DNA in the + allele by a hatched box. All enzymes that cut within or to the left (as shown) of the probe p189–1 and to the right of the insertion will detect the RFLP. HindIII (H) cuts between the probe and insert and does not detect the RFLP. BglII (BgII), BglI (BgI), and KpnI (K) cut within the insert, and this alters the size and pattern of alleles detected (see Fig. 3). Symbols for other enzymes shown are: Bc, BclI; Bs, BstEII; E, EcoRI; Hp, HphI; Hc, HincII; M, MspI; Ps, PstI; R, RsaI; T, TaqI; X, XbaI; Xn, XmnI. Sites for BamHI, EcoRV, KpnI, SacI, and XmaI occur outside the E sites.

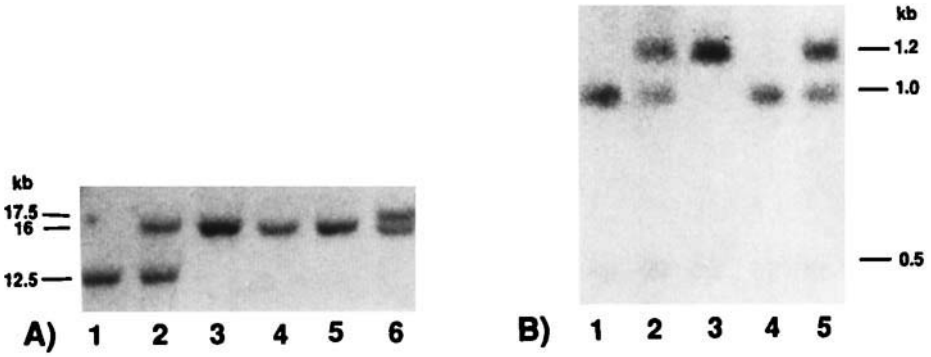

Fig. 5. Segregation of RFLPs in PWS families. **A:** Segregation of ScaI alleles detected by probe pIR10–1 is shown for 2 families with a PWS child. The affected individual with the PWS (**lane 1**, HS27) in the first family inherits the 12.5 kb allele from the mother (**lane 2**, HS28) but does not inherit a paternal (16 kb) allele (**lane 3**, HS29). A second family is uninformative at this locus (proband HS2, father, and mother, **lanes 4–6**, respectively). **B:** Segregation of the pIR4–3R RsaI RFLP in this second family (**lanes 2–4**). The PWS individual (**lane 4**, HS2) inherits the maternal (1.0 kb) allele (**lane 2**, DON12) but no allele from the father (**lane 3**, HS3). Two controls, a heterozygote (**lane 5**, GM131) and a homozygote (**lane 1**, GM3798) for the lower allele, are also shown. The 0.5 kb band is invariant.

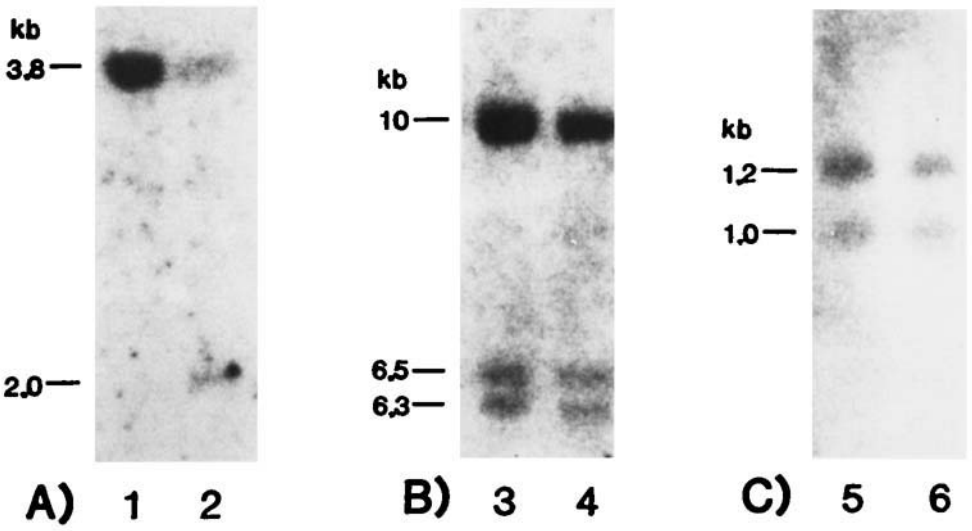

Fig. 6. Use of RFLPs to detect 2 chromosome 15 alleles in DNA from a patient (DON5) with some manifestations of PWS. This individual was reported to have a small deletion in 15q11.2 at the cytological level [Donlon et al., 1986]. However, she is heterozygous (**lanes 2, 4, 6**) at 3 loci mapped to 15q11.2–12, those detected by probes p189–1, TaqI (**A**); p34, ScaI (**B**); and pIR4-3R, RsaI (**C**). A normal homozygous control (**lane 1**) or heterozygous control (**lanes 3, 5**) is shown in each case. The 10 kb band detected in **B** is invariant.

deletions. We next studied DNA from the cell line DON5, from a patient who has some manifestations of the PWS (see Materials and Methods) and who was previously described as having a small deletion in 15q11.2 from cytological techniques [Donlon et al., 1986]. Three of the five loci (p189–1, p34, pIR4–3R) in the 15q11.2–12 RFLP set were heterozygous in DNA from this patient (Fig. 6), clearly demonstrating inheritance of 2 alleles for at least these segments in 15q11.2–15q12. All other polymorphic probes (data not shown) were uninformative, but dosage hybridization (Tantravahi et al., this issue) was consistent with the presence of 2 copies per diploid genome. Thus a deletion in this patient has yet to be demonstrated at the DNA level.

To show further the utility of a molecular cytogenetic approach to disease loci and chromosome rearrangements in proximal 15q, we next studied several unusual bisatellited chromosomes that have been shown to derive from an inverted duplication of chromosome 15pter to various points within proximal 15q [Schreck et al., 1977; Wisniewski et al., 1979, 1980; Fujita et al., 1980; Maraschio et al., 1981; Stetten et al., 1981; Ledbetter et al., 1982; Wulfsberg et al., 1982; Mattei et al., 1984]. One new patient (HS38) had a 47,XX+ inv dup(15) chromosome abnormality, but the exact breakpoints of the inv dup(15) remain ambiguous (Fig. 7). Both breakpoints could lie in 15q12 or at the 15q12–q13 junction (Fig. 7, left); alternately, one may be in 15q11 and one in 15q13 (Fig. 7, right). This difficulty in interpretation has been discussed by other authors [Maraschio et al., 1981]. The availability of molecular analysis should now allow some differentiation between these alternatives. DNA segments from chromosome 15 that are proximal to an isodicentric (mirror) breakpoint will be present in 4 copies; if breakpoints of the inv dup(15) are in different chromosome bands, then some probes will be present in

Fig. 7. GTG-banded normal chromosome 15 and an inv dup(15) from a non-PWS patient (HS38). The breakpoint of the inv dup(15) chromosome appears to be either q12 or q13 in some cells (e.g., left); other cells could be alternatively interpreted as having one breakpoint at 15q11 and one at 15q13 (e.g., right). Thus the karyotype is given as 47,XX,inv dup(15) (15pter → 15q11 or 15q12::15q13 or 15q12 → 15pter).

triplicate, others quadruplicate, whereas the distal loci will remain disomic.

The feasibility of this approach was investigated using cell lines from 3 individuals with an inv dup(15) chromosome and a breakpoint in 15q13 (HS13, HS15, GM6246), a smaller inv dup(15) (q11) (GM4347) [Stetten et al., 1981; Donlon et al., 1986], and a control male (Fig. 8). Each case shows that RFLP analysis simplifies the determination of copy number in the aneuploid state (Fig. 8) as compared to analysis in the absence of polymorphisms (Tantravahi et al., this issue). Copy numbers were estimated by scanning densitometry (Table II). Allele ratios of 1:1 (Table II) with the control GM3798 and the small inv dup(15) (q11) (GM4347), as well as the previous data suggesting 2 copies for GM4347 (Tantra-

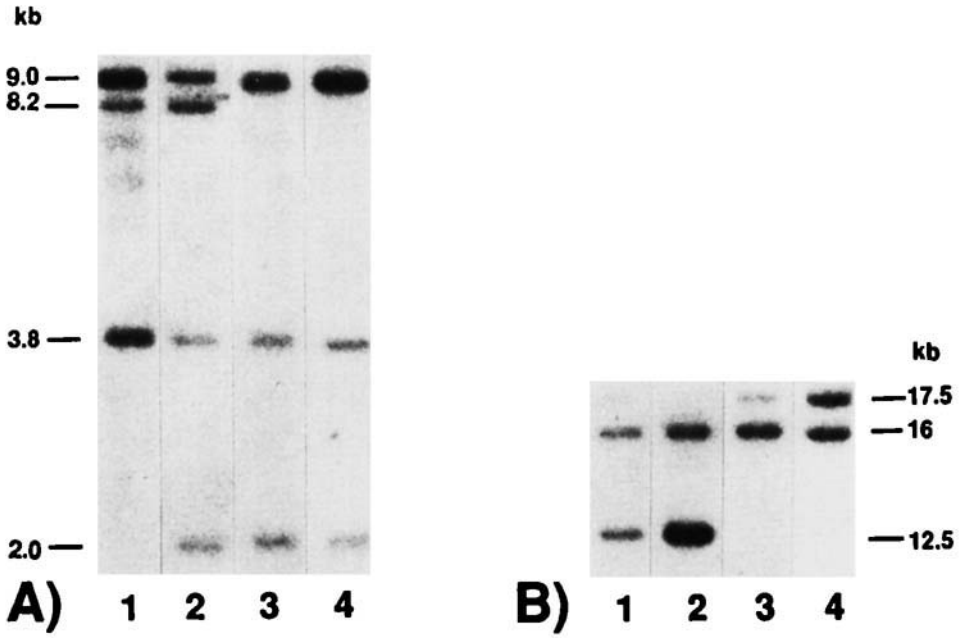

Fig. 8. Simplification of the determination of copy number for inv dup(15) chromosomes using RFLPs. **A:** DNA from 3 patients with inv dup(15) (q13) chromosomes and a normal control (GM3798, **lane 4**) is shown hybridized simultaneously to probes p3–21 (8.2 kb, 9 kb alleles) and p189–1 (2.0 kb, 3.8 kb alleles). Both probes recognize a TaqI RFLP. Two patients are heterozygous for alleles detected by probe p3–21. GM6246 (**lane 1**) and HS13 (**lane 2**) show a 2:1 or 1:1 ratio (Table II) for the 9 and 8.2 kb bands, respectively. At the p189–1 locus, the 2 patients (HS13, **lane 2**; HS15, **lane 3**) and control have a 1:1 ratio (Table II) for bands at 3.8 and 2.0 kb. The amount of DNA loaded per track is not absolutely equal, and thus interlane comparison of band density cannot be made. **B:** ScaI alleles detected with probe pIR10–1 in 3 individuals from **A** and one with an inv dup(15) (q11) are shown. The latter (GM4347, **lane 1**) and control (GM3798, **lane 4**) show 1:1 ratios (Table II) of 16/12.5 and 17.5/16 kb alleles, respectively. GM6246 (**lane 2**) and HS13 (**lane 3**) display 1:2 or 1:3 ratios (Table II) for the 16/12.5 and 17.5/16 kb bands, respectively.

TABLE II. Densitometric Analysis of Copy Number for inv dup(15) Chromosomes*

		p3–21 (TaqI)			p189–1 (TaqI)			pIR10–1 (ScaI)			
DNA Source	Karyotype	cpm 8.2 kb/ 9.0 kb	Copy no. ratio	Copies/ genome	cpm 2.0 kb/ 3.8 kb	Copy no. ratio	Copies/ genome	cpm 12.5 kb/ 16 kb	cpm 16 kb/ 17.5 kb	Copy no. ratio	Copies/ genome
GM3798	46,XY	—	—	ND	0.709 1.13	1:1 1:1	2 2	—	1.001	1:1	2
GM4347	47,XX+ inv dup(15)(q11)	—	—	ND	—	—	ND	1.142[a]	—	1:1	2
GM6246	47,XX+ inv dup(15)(q13)	0.498	1:1.88[b]	3	—	—	ND	2.45[a]	—	2.18:1[c]	3
HS13	47,XX+ inv dup(15)(q13)	0.934	1:1.07	4	0.873	1:1.23	4	—	2.615	2.62:1[c]	3–4
HS15	47,XX+ inv dup(15)(q13)	—	—	ND	0.95[a]	1:1.122[d]	4	—	—	—	ND
HS16	46,XX	—	—	ND	0.87	1:1	2	—	—	—	ND
HS36	46,XX	1.124	1:1	2	—	—	ND	—	1.019[e]	1:1	2
HS37	46,XY	0.853	1:1	2	—	—	ND	0.953[e]	—	1:1	2
HS38	47,XX+ inv dup(15)	1.95	2.15:1[f]	3	0.528	1:2.16[g]	3	—	2.625[e]	2.58:1[e,f]	3–4

* From data shown in Figures 8 and 9. Method of analysis is given in Materials and Methods. Values shown are the ratios of hybridized counts (cpm) for each allele before normalization (sizes in kb), the corresponding normalized copy ratios, and inferred copy number of the probe. —, Not polymorphic; ND, not determined. Normalized copy number ratios for the control heterozygotes (GM3798, HS16, HS36, HS37) are assumed to be 1:1, and the test cpm ratios have been normalized to controls run on the same gel. Values are the average of two autoradiogram exposures per gel, except as indicated.

[a] Values are the average of two gels.

[b] Normalized relative to HS13 (assumed to have an even number of copies per genome).

[c] Normalized to the control possessing the same two alleles.

[d] Average value after normalization to GM3798 and HS16.

[e] Values are from single autoradiogram exposures.

[f] Normalized to the average value of controls HS36, HS37 (p3–21 only) and DON12 (cpm ratio 1.019, not shown; pIR10–1 only).

[g] Normalized to GM3798 (cpm ratio 1.145).

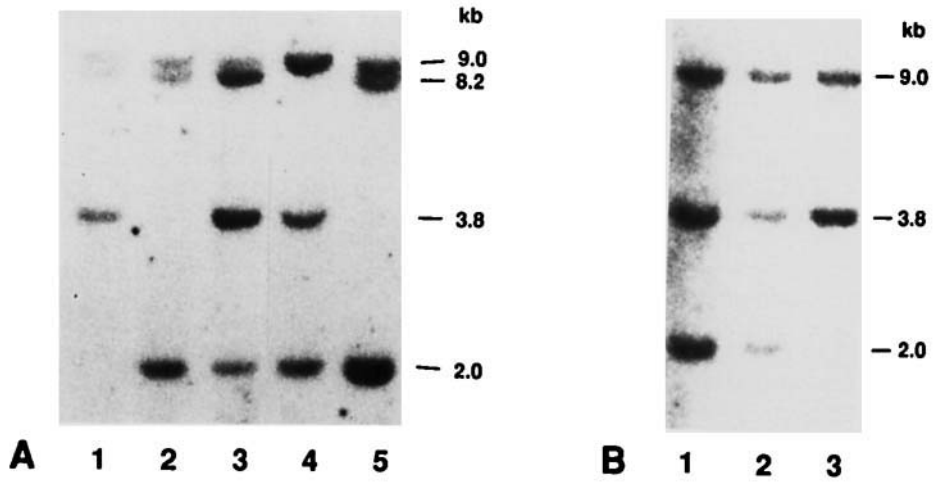

Fig. 9. Parental origin of inv dup(15) chromosomes in 2 non-PWS families with a child carrying an inv dup(15) by RFLP analysis. **A:** Family I (**lanes 1–3**) and two controls (GM3789, **lane 4**; GM7038, **lane 5**) hybridized simultaneously with probes p3–21 and p189–1 to identify TaqI alleles (see Fig. 8). All 3 relatives [**lane 1**, HS36, mother; **lane 2**, HS37, father; **lane 3**, HS38, inv dup(15)] are heterozygous with probe p3–21, HS38 showing a 1:2 ratio (Table II) for the 2 alleles. This DNA displays the same ratio for p189–1 alleles (2/3.8 kb, respectively) and is informative for parental (maternal) origin of the extra chromosome 15 DNA. **B:** Inheritance of alleles detected by probes p3–21 + p189–1 in family II. The affected individual (**lane 1**, HS15) with an inv dup(15) (q13) is heterozygous with probe p189–1 and displays maternal inheritance of the additional chromosome 15 allele. The other samples are **lane 2**, HS16, mother; **lane 3**, DS31, father.

vahi et al., this issue), were utilized as points of reference. The cell line HS13 shows a 1:1 ratio for alleles at p3–21 and p189–1 (Fig. 8A; Table II). The even number of copies, a 1:3 ratio with pIR10–1 (Fig. 8B; Table II) and the absolute values determined by Tantravahi et al. (this issue) suggest the presence of 4 copies of all loci tested that lie proximal to the 15q13 breakpoint. Similarly, the results for HS15 (Figs. 8A, B, 9; Table II) suggest 4 copies after comparison with the absolute copy number (Tantravahi et al., this issue). In contrast, GM6246 displays a 1:2 ratio for alleles at 2 polymorphic loci (Fig. 8A, B; Table II; p3–21, TaqI; pIR10–1, ScaI), suggesting only 3 copies at each locus.

In addition to the simplified molecular detection of aneuploidy, RFLPs should allow the parental origin of the nondisjunction event to be determined. This is clearly evident from the data in Figure 9. Within family I, the propositus (HS38) with an inv dup(15) (Fig. 7), inherits a single copy of the 2 kb allele for p189–1 (TaqI) from her homozygous father but 2 copies of a 3.8 kb allele from her homozygous mother (Fig. 9A; Table II), demonstrating a maternal origin of the inv dup(15). Probes p3–21 (Fig. 9A; Table II) and pIR10–1 (ScaI; Table II) are also informative for copy number determination in this family but not for parental origin. The data suggest that 2 probes are present in triplicate (p189–1, p3–21), whereas one probe (pIR10–1) may be present in HS38 in 4 copies. A second cell line with an inv dup(15) (q13) (HS15) shows a 2:2 ratio of alleles (see above) at the p189–1 TaqI RFLP (Figs. 8A, 9B; Table II). A 3.8 kb allele is donated by each parent, but the 2 copies of the 2.0 kb allele can come only from the single maternal 2.0 kb allele (Fig. 9B), again demonstrating maternal inheritance of the extra chromosome by molecular genetic techniques. Finally, a

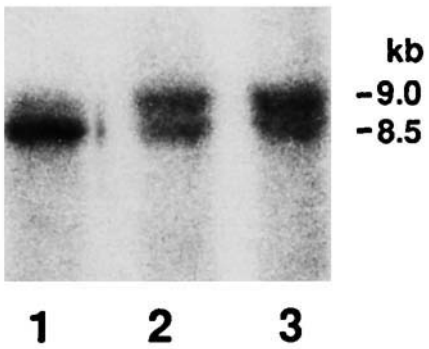

Fig. 10. Copy number analysis for cell line DMS918. A BglII RFLP is detected by probe pIR39 for DMS918 (**lane 1**), with a 47,XY+ t(5;15),+inv dup(15) karyotype, and 2 controls (**lane 2**, GM7009; **lane 3**, GM7011). All 3 cell lines are heterozygous for the BglII RFLP. Densitometry data are given in Table III.

PWS patient (DMS918) with a more complex rearrangement [Murdock and Wurster-Hill, 1986], t(5;15)(q35↔3) + inv dup(15) (?q13), was analyzed by RFLP analysis. The only informative RFLP was pIR39 BglII, in which the 2 alleles showed an approximately 1:3 ratio (Fig. 10), suggesting the presence of 4 copies.

DISCUSSION

We have described 9 independent RFLPs for 6 probes within proximal 15q, a significant increase in the number of informative genetic markers for this region of the genome. Prior to this study, few polymorphic DNA segments were localized in chromosome 15 [O'Connell et al., 1987; Pearson et al., 1987; Tasset et al., 1988]. The

satellite and centromeric heterochromatin polymorphisms detected cytogenetically have been informative in some studies [Schreck et al., 1977; Wisniewski et al., 1979, 1980; Maraschio et al., 1981; Mattei et al., 1983, 1984; Smith, 1983; Niikawa and Ishikiriyama, 1985; Butler et al., 1986]. Two probes detecting RFLPs within or distal to 15q15 [Barker et al., 1984; Brissenden et al., 1986], an infrequent RFLP localized to 15q12–q24 [Feder et al., 1985], a hypervariable locus mapped to 15pter–q13 [Rich et al., 1988], and 5 polymorphic probes from a linkage study of the human genome [Donis-Keller et al., 1987] have been previously described in the vicinity of proximal 15q. Seven of the new RFLPs in the present report have sufficient heterozygosity (PIC > 0.24) to be generally useful as independent genetic markers. In addition, the 15q11.2–q12 set of RFLPs comprising 5 of these markers that we have described has been informative in all family studies to date. Only 3 individuals homozygous for all 5 15q11.2–12 loci have been found (and D15S18 or the rare D15S10 polymorphisms were not studied in 2 of these). Among a small set of 13 individuals, 9 were heterozygous for at least one RFLP at these latter 2 loci. Thus, the complete set of RFLPs for proximal 15q will be useful in molecular and linkage studies of putative familial and "nondeletion" PWS (see Introduction for references), Angelman syndrome [Kaplan et al., 1987; Magenis et al., 1987], possibly factor XI deficiency [McKusick, 1986], and perhaps a group of families who show chromosome 15 linkage for specific learning disability [Smith, 1983; Smith et al., 1986; Bisgaard et al., 1987].

These studies have established the feasibility of determining the parental origin of chromosome 15 alterations using informative RFLPs, as demonstrated here for the 4 families studied. Two cases of inv dup(15) were maternal in origin, consistent with the preferential maternal origin shown cytogenetically for this marker chromosome [Wisniewski et al., 1979; Maraschio et al., 1981]. Although a maternal origin is preferential for nondisjunction events in general, including trisomy 21 [Hassold and Jacobs, 1984], the derivation of inv dup(15) chromosomes [Schreck et al., 1977; Maraschio et al., 1981] and the inv dup(22) in the cat eye syndrome [Magenis et al., 1988] involves breakage and nondisjunction. In contrast, the deletions in 2 PWS patients were paternal in origin. Combined with 22 cases from the cytological literature, 21 of 24 are paternal in origin [Butler and Palmer, 1983; Butler et al., 1986; Mattei et al., 1983; Niikawa and Ishikiriyama, 1985]. Preferential paternal origin of chromosome 16 deletions in the α-thalassemia/mental retardation syndrome is also found [Nicholls, 1986b], and in general for de novo structural rearrangements [Chamberlin and Magenis, 1980; Olson and Magenis, 1987]. The differences in parental origin for deletions in the PWS and generation of inv dup(15) chromosomes suggest differences in the mechanism of chromosome breakage in each case.

The present studies on parental origin by recombinant DNA techniques are preliminary in nature but provide a powerful alternative to cytological techniques, which focus primarily on the pericentromeric region and short arm of 15. Parental studies are equally important for patients with normal chromosomes and those with deletions, to determine the general applicability of this hypothesis and the notion that paternal exposure to environmental agents may be a causal factor in the PWS [Butler et al., 1985; Strakowski and Butler, 1987]. Collaborative studies have been initiated with Dr. M. Butler to investigate the parental derivation of the deleted 15 at a molecular level more definitively.

In addition to the demonstration of parental origin of inv dup(15) chromosomes, we have shown that RFLPs can also simplify determination of the copy number of probes in DNA from cells with inv dup(15) chromosomes.[1] RFLPs have also been utilized to facilitate probe copy number determination by McDermid et al. [1986] for chromosome 22 in the cat eye syndrome. One patient (HS38) with an inv dup(15) showed 3 copies of 2 probes (p189–1, p3–21) but perhaps as many as 4 of another (pIR10–1). This result suggests that molecular analysis can detect heterogeneity in chromosome breakpoints. Direct cloning and mapping of this region in HS38 might help to substantiate this suggestion. In a second inv dup(15) patient, HS15, at least 2 of the probes detected 2 extra maternal copies in the abnormal 15. If the maternal grandparents were available, genetic phase could be determined, and this would determine whether the inv dup(15) was derived from 1 or 2 chromosomes. However, centromeric analysis would still be required to identify the meiotic stages of nondisjunction and centromere inactivation. This was previously demonstrated cytologically for inv dup(15) chromosomes [Schreck et al., 1977; Maraschio et al., 1981]. Additionally, RFLPs at or near the centromere, and hence unlikely to be influenced by recombination with the centromere, would improve identification of the meiotic stage of the error leading to the inv dup(15) [Hassold and Jacobs, 1984].

Combined with cytogenetics, RFLPs are of great utility for studies of copy number and genomic organization in diseases associated with chromosome aberrations, as we have demonstrated here by detection of proximal 15q deletions in PWS patients and duplications in patients with an additional bisatellited inv dup(15) (Figs. 5, 8–10). The unexpected observation of 4 copies of at least 4 15q11–15q13 probes [3 probes by dosage (Tantravahi et al., in press), one probe by RFLPs plus dosage (Table III)] in the 47,XY+ t(5;15)+ inv dup(15) patient, DMS918, suggests that the chromosome rearrangements in this patient are unusually complex.

Using the present probes and additional probes that we (S. Ringer, K. Kupke, et al., unpublished) and others [Rich et al., 1988; Donis-Keller et al., 1987; Tasset et al., 1988] have isolated will allow systematic deletion and rearrangement mapping of chromosome 15 in PWS individuals, with the goal of identifying the disease locus or region. Detection of submicroscopic deletions in PWS

[1] This is especially the case when dosage hybridization studies indicate that extra copies of the probe are present and the allele ratio in the patient's DNA is close to unity. It should be noted that, in the alternative situation, differentiation between 2:1 and 3:1 allele ratios is difficult, as is the corresponding distinction between a total of 3 or 4 copies of DNA (per cell) hybridizing with the probe.

TABLE III. Densitometric Analysis for Cell Line DMS918*

DNA source	pIR39 (BglII) RFLP 8.5 kb/9 kb cpm	Copy no. ratio	Copies/ genome
GM7009	0.803	1:1	2[a]
GM7011	0.885	1:1	2[a]
DMS918	2.302	2.73:1[b]	4

* From data shown in Figure 10. See Materials and Methods for method of analysis and Table II for abbreviations.
[a] A copy number of two is assumed for each normal control.
[b] Normalized to the average of the controls.

patients with apparently normal chromosomes [Ledbetter et al., 1987] may also be possible, as was recently demonstrated for patients with the α-thalassemia/mental retardation syndrome [Weatherall et al., 1981; Nicholls, 1986b] or Miller-Dieker syndrome [van Tuinen et al., 1987]. Combined with deletion mapping, the technique of pulsed field gel electrophoresis (PFGE) [see e.g., Schwartz and Cantor, 1984] will be indispensable for physical mapping of proximal 15q [Nicholls et al., 1987] and perhaps for pinpointing the gene(s) responsible for PWS, the latter by identifying breakpoints of the deletions or other chromosome rearrangements in PWS. This approach also applies to other disease loci that may reside within proximal 15q (see above).

Identification by DNA studies of the genes responsible for PWS will be necessary for phenotypic correlation. This should then help resolve the controversies surrounding the syndrome, derived from the difficulty of making a clinical diagnosis and the variety (or existence) of chromosome aberrations detected cytogenetically [Ledbetter et al., 1981, 1982, 1987; Mattei et al., 1983, 1984; Cassidy et al., 1984; Fear et al., 1985; Labidi and Cassidy, 1986; Butler et al., 1986; Wisniewski et al., 1980; Fujita et al., 1980; Kousseff, 1982; Fraccaro et al., 1983; Charrow et al., 1983; Winsor and Welch, 1983; Niikawa and Ishikiriyama, 1985; Pettigrew et al., 1987; Kousseff et al., 1987; Zellweger, 1988; Hockey, 1988] as well as the possibility of recessive, familial PWS [Burke et al., 1987; Lubinsky et al., 1987].

PFGE analysis and DNA cloning may also provide answers to questions regarding the molecular basis for the structural lability of proximal 15q. From observations of a patient mosaic for 3 different translocations involving 15q and a different autosome, Lejeune et al. [1979] suggested that the lability of proximal 15q may involve inverted repeats. Donlon et al. [1986] provided molecular evidence for inverted duplication of sequences in 2 clones. However, we have not yet detected any deletion breakpoints with our probes, and further study is required to identify the sequences and molecular mechanisms involved in the chromosome rearrangements.

Clinical diagnosis of the PWS may be difficult, and often it depends on a natural progression from neonatal hypotonia and failure to thrive to hyperphagia and obesity, with mental retardation and behavioral disturbances [Cassidy, 1984; Greenberg et al., 1987]. Early diagnosis of the PWS would allow prospective dietary management and genetic counseling of patients and families [Cassidy, 1984; Greenberg et al., 1987]. Greenberg et al. [1987] have recently demonstrated an early presumptive diagnosis of PWS by combining chromosome analysis with observation of clinical manifestations. The molecular genetic techniques we have discussed and begun to apply to PWS may eventually assist an early clinical diagnosis of PWS patients.

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