Am. J. Hum. Genet. 62:925–936, 1998

Molecular Cytogenetic Evidence for a Common Breakpoint in the Largest Inverted Duplications of Chromosome 15

A. E. Wandstrat,[1] J. Leana-Cox,[2] L. Jenkins,[3] and S. Schwartz[1]

[1]Department of Genetics, Case Western Reserve University School of Medicine, and Center for Human Genetics, University Hospitals of Cleveland, Cleveland; [2]Division of Human Genetics, Departments of Obstetrics/Gynecology and Pediatrics, University of Maryland, Baltimore; and [3]Kaiser Permanente Medical Group, San Jose

Summary

Chromosomes from 20 patients were used to delineate the breakpoints of inverted duplications of chromosome 15 (inv dup[15]) that include the Prader-Willi syndrome/Angelman syndrome (PWS/AS) chromosomal region (15q11-q13). YAC and cosmid clones from 15q11-q14 were used for FISH analysis, to detect the presence or absence of material on each inv dup(15). We describe two types of inv dup(15): those that break between D15S12 and D15S24, near the distal boundary of the PWS/AS chromosomal region, and those that share a breakpoint immediately proximal to D15S1010. Among the latter group, no breakpoint heterogeneity could be detected with the available probes, and one YAC (810f11) showed a reduced signal on each inv dup(15), compared with that on normal chromosomes 15. The lack of breakpoint heterogeneity may be the result of a U-type exchange involving particular sequences on either homologous chromosomes or sister chromatids. Parent-of-origin studies revealed that, in all the cases analyzed, the inv dup(15) was maternal in origin.

Introduction

Patients with a supernumerary pseudodicentric chromosome 15, more commonly known as an "inverted duplication of chromosome 15" ("inv dup[15]"), were initially described by Van Dyke et al. (1977). An inv dup(15) occurs in ~0.02% of liveborn individuals (Buckton et al. 1985), making this the most common autosomal anomaly in humans, after trisomy 21 (Speed et al. 1976). Initially, interest in the study of these supernumerary marker chromosomes focused on the dilemma faced by clinicians, as vast phenotypic variability among patients with an inv dup(15) in their karyotype (47,XX or 47,XY+inv dup[15]) has been reported. Although most inv dup(15) individuals have been ascertained through diagnosis of mental and/or developmental retardation (Schreck et al. 1977; Wisniewski et al. 1979; Maraschio et al. 1981), reported phenotypes of inv dup(15) patients have ranged from normal (Stetten et al. 1981; Knight et al. 1984) to Prader-Willi syndrome (PWS) (Fujita et al. 1980; Wisniewski et al. 1980; Ledbetter et al. 1982; Robinson et al. 1993*b*) or Angelman syndrome (AS) (Robinson et al. 1993*b*) to what has become known as "inv dup(15) syndrome" (Wisniewski et al. 1980; Zannotti et al. 1980). More recently, in instances in which PWS or AS has been observed, the phenotype has been demonstrated to be due either to uniparental disomy of two normal chromosomes 15 (Robinson et al. 1993*a,* 1993*b*) or to a deletion of one of the normal chromosomes 15 (Spinner et al. 1995), not to the presence of an inv dup(15). Inv dup(15) syndrome involves characteristics such as severe mental and developmental retardation, seizures, autism, abnormal dermatoglyphics, and strabismus (Zannotti et al. 1980; Plattner et al. 1993). Leana-Cox et al. (1994) demonstrated a positive correlation between the presence of the 3–4 Mb PWS/AS chromosomal region (15q11-15q13) (Mutirangura et al. 1993) on the inv dup(15) and mental retardation.

As chromosome-specific probes have been developed, the chromosomal origin of marker chromosomes can now be determined with relative ease and with a high degree of accuracy (Callen et al. 1990; Schwartz et al. 1990; Plattner et al. 1991). Intense study of the proximal portion of chromosome 15 has ensued in recent years, because this area of the chromosome often is detected in structural rearrangements, such as duplications, triplications, deletions, and translocations, as well as in an inv dup(15) (Mattei et al. 1984), and because this area contains several imprinted genes (Hall 1990; Nicholls 1993).

FISH analysis using probes that span the proximal

Received May 30, 1996; accepted for publication February 6, 1998; electronically published March 27, 1998.

Address for correspondence and reprints: Dr. Stuart Schwartz, Case Western Reserve University, Department of Genetics, Biomedical Research Building, 7th Floor West Administration, 10900 Euclid Avenue, Cleveland, Ohio 44106-4955. E-mail: sxs95@po.cwru.edu

0002-9297/98/6204-0025$02.00

portion of chromosome 15 has confirmed the existence of different-sized classes of inv dup(15) (Rauch et al. 1992; Cheng et al. 1994; Leana-Cox et al. 1994; Crolla et al. 1995; Mignon et al. 1996). There are several types of inv dup(15), based on the amount of heterochromatic and/or euchromatic material present (fig. 1*A*). However, to date, these classes have not been well defined molecularly, and most studies have relied on either RFLP or microsatellite-dosage analysis (Shibuya et al. 1991; Robinson et al. 1993*a;* Cheng et al. 1994; Crolla et al. 1995; Mignon et al. 1996). Although attempts have been made to define more precisely the inv dup(15) cases that contain little or no material from proximal 15q (Cheng et al. 1994; Crolla et al. 1995; Huang et al. 1997), previous studies have not systematically distinguished among the breakpoints in the inv dup(15)s that contain material from the PWS/AS chromosomal region. This is due, at least in part, to the fact that the region immediately distal to the PWS/AS chromosomal region is relatively poorly mapped, and few microsatellite markers have been placed in this area. However, some preliminary data, obtained using a limited number of YACs, have suggested more than one breakpoint in the larger inv dup(15) (Leana-Cox 1993), and a recent study has described several classes, based on isotopic in situ hybridization (Mignon et al. 1996).

In our study, we used a series of YAC clones from 15q11-q14 to classify more precisely inv dup(15) chromosomes that contain the PWS/AS chromosomal region. We show that there are at least two types of these chromosomes, on the basis of the amount of euchromatic material present. In order to further address the nature of the largest inv dup(15), we isolated a YAC that detects DNA at or near the breakpoint in this class of chromosomes. PCR microsatellite analysis, as well as methylation analysis at the SNRPN (small nuclear ribonucleoprotein polypeptide N) locus, confirmed that all the analyzed inv dup(15) cases that retained the PWS/AS critical region are maternal in origin.

Patients and Methods

Patients

Studies of 20 patients with an inv dup(15) were performed. Detailed clinical information on 15 cases has been published elsewhere, in the reports by Leana-Cox et al. (1994), for cases 1, 2, 4, 5, 8–14, 16, 17, 19, and 20 (in the original study, cases 15, 18, 24, 6, 3, 7, 8, 11, 13, 14, 21, 9, 26, 23, and 1, respectively), and Flejter et al. (1996), for cases 4 and 19 (in the original study, cases JB and MB, respectively). Table 1 lists case number, patient source, type of tissue used in this study, type of inv dup(15), and parent of origin of the inv dup(15). Table 2 lists case number, sex, age, and other phenotypic information for those patients who have not been described elsewhere.

Each patient in this study contains two copies of the PWS/AS chromosomal region, from D15S11 to GABRβ3, on the inv dup(15), as determined by FISH analysis performed either in our lab or in the lab in which the patient sample was ascertained. Peripheral blood samples and/or lymphoblast cell lines were obtained for routine cytogenetic studies and FISH. For FISH analysis, at least 5 or, typically, 10–20 cells were analyzed. DNA was isolated by routine methods.

Probe Preparation

Several YAC clones localized on 15q, at or distal to 15q13, were used in this study. Total YAC DNA was isolated by preparation of yeast spheroplasts, with yeast lytic enzyme (ICN Biochemicals), lysing of the spheroplasts with Sarkosyl (Sigma), deproteinization of the resulting solution with proteinase K, and purification of the resultant DNA with phenol-chloroform extractions and ethanol precipitation. Human-specific YAC DNA sequences then were amplified by use of *Alu* PCR, with *Alu* consensus-sequence primers CL1, 5′-TCC CAA AGT GCT GGG ATT ACA G-3′, and CL2, 5′-CTG CAC TCC AGC CTG GG-3′. The probes were labeled either with biotin, by use of the Bionick Kit (Gibco BRL), and additional DNaseI or with digoxygenin, by use of the Genius 2 Kit (Boehringer Mannheim). Unincorporated nucleotides were separated from labeled probed DNA, on a Sephadex G-50 spin column, and the labeled probes were stored at −20°C.

Probe Application and Hybridization

FISH was performed in accordance with the technique described by Pinkel et al. (1986). The slides used ranged in age from a few days to several months and were stored at −20°C prior to hybridization. FISH was performed by use of standard procedures, which have been described elsewhere (Sullivan et al. 1993). Hybridizations for probes D15S11, GABRβ3, and D15Z1 (Oncor) were performed in accordance with the manufacturer's instructions. The slides were counterstained with propidium iodide or 4′6 diamidino-2-phenylindole, were visualized under a Zeiss fluorescence microscope, and were photographed with Kodak ektachrome ASA 400 color-slide film (Eastman Kodak). A digital multicolor-image–analysis system also was used, with a Zeiss Axiophot microscope equipped with a cooled charge-coupled–device camera.

PCR and Microsatellite Analysis

Primers for the following loci were used: D15S18 and GABRβ3 (Mutirangura et al. 1993), D15S122 and D15S128 (Richard et al. 1994), D15S165 and D15S144

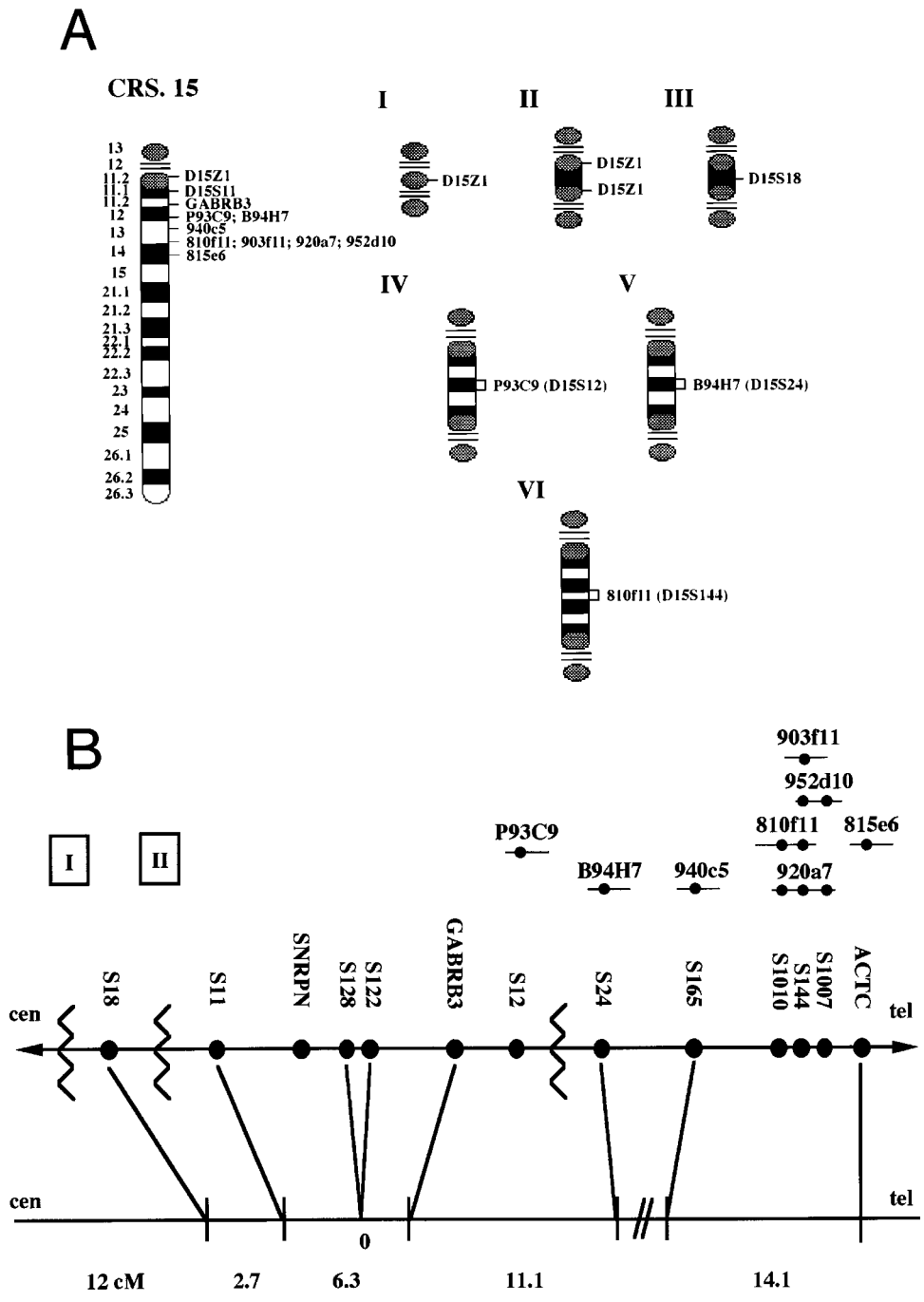

Figure 1 Types of inv dup(15) chromosomes. *A,* Normal chromosome 15, with band designations (*left*) and probes utilized in this study (*right*). Types I–III represent those inv dup(15) chromosomes that are associated with a normal phenotype. Type I represents the inv dup(15) that is monocentric and that has only one copy of the D15Z1 locus. Type II represents the inv dup(15) that is dicentric, that has two copies of the D15Z1 locus, and that breaks in 15q11, proximal to the D15S18 locus (Cheng et al. 1994; Huang et al. 1997). Type III represents the inv dup(15) that is dicentric, that has two copies of the D15Z1 locus, and that breaks in 15q11, distal to D15S18 (Huang et al. 1997). Types IV–VI represent those inv dup(15) chromosomes that contain euchromatic material from the PWS/AS commonly deleted region and that are associated with an abnormal phenotype. Type IV represents the inv dup(15) that breaks in 15q13, distal to P93C9 (D15S12) and proximal to B94H7 (D15S24) (cases 1–6 in this study; Mignon et al. 1996). Type V represents the inv dup(15) that breaks in 15q13, distal to B94H7 (D15S24) and proximal to pIR29-1 (D15S17) (Mignon et al. 1996). Type VI represents the inv dup(15) that breaks at or near sequences detected by YAC 810f11 (cases 8–20 in this study). *B,* All the microsatellite markers and YAC clones used in this study are listed (based on information from the Whitehead (http://www-genome.wi.mit.edu) and Genome (http://gdbwww.gdb.org) databases, as well as from the study by Christian et al. [1995]), and lines between the physical and genetic maps have been used to connect loci. The jagged lines indicate the breakpoint regions seen in the common deletion found in PWS/AS patients. The two classes of proximal deletions have been designated "I" and "II" (*boxed*).

Table 1

Patients in This Study

Case No.	Source[a]	Sample Type[b]	Type of Inv Dup(15)	Parent of Origin[c]
1	Leana-Cox et al. (1994)	PB	IV	N/A
2	Leana-Cox et al. (1994)	L	IV	N/A
3	CWRU	L	IV	Maternal[d]
4	Leana-Cox et al. (1994); Flejter et al. (1996)	L	IV	Maternal[e,f]
5	NIGMS	F	IV	Maternal[e]
6	CWRU	L	IV	Maternal[d,e]
7	Kaiser Permanente Medical Group	DNA only	IV or V or VI	Maternal[d,e]
8	Leana-Cox et al. (1994)	F	VI	Maternal[d,e]
9	NIGMS	L	VI	Maternal[e]
10	NIGMS	L	VI	Maternal[e]
11	Leana-Cox et al. (1994)	A	VI	N/A
12	Leana-Cox et al. (1994)	L	VI	Maternal[e]
13	Leana-Cox et al. (1994)	L	VI	Maternal[e]
14	Leana-Cox et al. (1994)	F	VI	Maternal[e]
15	CWRU	L	VI	Maternal[d,e]
16	NIGMS	A	VI	Maternal[e]
17	Leana-Cox et al. (1994)	L	VI	Maternal[d,e]
18	Kaiser Permanente Medical Group	PB	VI	Maternal[e]
19	Leana-Cox et al. (1994); Flejter et al. (1996)	L	VI	Maternal[e,f]
20	Leana-Cox et al. (1994)	DNA only	VI	Maternal[d]

[a] NIGMS = National Institute of General Medical Sciences Genetic Mutant Cell Repository; CWRU = Case Western Reserve University. Respository numbers are GM02729 (case 5), GM06246 (case 9), GM10183 (case 10), and GM02662 (case 16).

[b] PB = peripheral blood; L = lymphoblast; F = fibroblast; and A = amniocyte.

[c] N/A = DNA not available for study.

[d] Determined by PCR analysis.

[e] Determined by methylation analysis at the SNRPN locus.

[f] Determined by PCR analysis, as reported by Flejter et al. (1996).

(Beckmann et al. 1993), and D15S1010 and D15S1007 (Hudson et al. 1995). One oligonucleotide of each primer set was end-labeled at 37°C for 60 min, in a 10-μl reaction containing 10 μM primer; 0.050 mCi [^{32}P]ATP (Amersham), at 3,000 Ci/mmol; 50 mM imidazole-HCl (pH 6.4); 12 mM $MgCl_2$; 1 mM 2-mercaptoethanol; 70 μM ADP; and 3 units T4 polynucleotide kinase. The resulting labeled mix either was used immediately or was stored, at −20°C, without further purification.

The PCR-reaction mixture consisted of 25–50 ng ge-

Table 2

Phenotypic Features for Inv Dup(15) Patients Not Reported Elsewhere

Case No.	Sex	Age (years)	Mental Retardation	Other Features
3	M	17	Yes	Normal growth and development, no dysmorphic features, autism, ecolalia, aggression, seizures, and IQ = 36
6	M	3	Yes	Pregnancy of gravida 5 para 2 mother was complicated by gestational diabetes; developmental delay, no speech, seizures, abnormal electroencephalogram, and hypotonia
7	F	2	Yes	Developmental delay, strabismus, deep-set eyes, decreased muscle tone, and speech delay
15	F	6	Yes	Autism, behavioral and learning problems, and hypotonia
18	F	20	Yes	Strabismus but good vision, able to care for herself, reads at an ~1st-grade level, quite verbal and socially quite mature, poor sense for numbers and finances, tight thigh muscles, flat feet, and walks slightly bent over but orthopedic intervention has not been needed

Table 3

FISH Results for Inv Dup(15) with a Breakpoint between D15S12 and D15S24

	FISH Results[a]		
Case No.	Cosmid GABRβ3	YAC P93C9	YAC B94H7
1	++	++	−
2	++	++	−
3	++	+/++	−
4	++	++	−
5	++	++	−
6	++	+/++	−

[a] The number of signals present on the inv dup(15) chromosome is indicated for each hybridization: a double plus sign (++) denotes two signals; a plus/double plus sign (+/++) denotes one or two signals; and a minus sign (−) denotes no signal.

nomic DNA, 1.25 mM each dNTP, 50 mM $MgCl_2$, 1 × *Taq* buffer, and 0.32 units *Taq* DNA polymerase, in a total volume of 25 μl. PCR analysis also was performed on YAC DNA, to assess YAC sequence-tagged site (STS) content. For these experiments, ~1.1 μl labeled probe plus 1.1 μl 10-μM unlabeled complementary primer were added per 25 μl PCR-reaction mixture.

After initial denaturation at 94°C for 1 min, samples were amplified in an MJ Research PTC-200 thermocycler, using a high touchdown program of 11 cycles of 94°C for 30 s, 70°C for 20 s, and 72°C for 1 min; 4 cycles of 94°C for 40 s, 60°C for 20 s, and 72°C for 1 min; 10 cycles of 94°C for 40 s, 58°C for 20 s, and 72°C for 1 min; and 10 cycles of 94°C for 40 s, 56°C for 20 s, and 72°C for 1 min, with a final cycle at 72°C for 2 min. After amplification, the reactions were mixed with an equal volume of formamide-loading dye (95% formamide, 20 mM EDTA, 0.05% bromophenol blue, and 0.05% xylene cyanol), were denatured at 95°C for 5 min, and were placed on ice. Each sample was loaded directly onto a 0.4-mm–thick denaturing 5% polyacrylamide gel (40-cm length). Electrophoresis was performed at 40 V/cm for 3.5 h. The gel was dried and was autoradiographed, on Fuji RX x-ray film, for 12–48 h at room temperature.

Alu *Fingerprinting*

YAC DNA was prepared, and human-specific sequences were amplified with *Alu* consensus-sequence primers, as described above (see Probe Preparation above). The PCR-amplified products were separated on 2% agarose gels and were stained with ethidium bromide.

Methylation at SNRPN Exon 1

Analysis of the methylation status of exon 1 of SNRPN was performed as described elsewhere (Glenn et al. 1996). The band intensities on the autoradiographs were measured on a Sci Scan 500 scanning densitometer. For standardization, the ratio of the two alleles was compared with the ratio in normal controls, as well as to that in PWS/AS-deletion patients. Methylation analysis was repeated at least once for each patient, to verify the results.

Results

Breakpoint Analysis

FISH analysis was performed on 19 of the 20 cases known to contain the PWS/AS chromosomal region, by use of probes located in 15q11-q14, to define the breakpoints in the inv dup(15). Case 7 could not be analyzed by FISH, because only a DNA sample was available. Whereas figure 1*A* lists all the loci used in the FISH analysis, figure 1*B* is a genetic map of proximal 15, listing the loci contained in the FISH probes as well as several of the PCR microsatellite markers in the results described below. As described below, two types of inv dup(15) that include the PWS/AS chromosomal region could be identified. For 6 cases, the inv dup(15) included material from D15S12 but not from D15S24. The remaining 12 cases contained both D15S12 and D15S24 and appeared to share a breakpoint near D15S144 (fig. 1 and table 1). Table 3 summarizes data from our analysis of the 6 cases in which, by use of standard cytogenetic techniques, an inv dup(15) was shown to break in 15q13. In all 6 cases, YAC P93C9 (containing D15S12) hybridized to both the normal chromosomes 15 and to the inv dup(15) (fig. 2*a*), whereas YAC B94H7 (containing D15S24) hybridized only to the normal chromosomes 15 and not to the inv dup(15) (fig. 2*b*).

The largest inv dup(15) chromosomes seemed to share a common breakpoint distal to the PWS/AS chromosomal region, as defined by probes that are currently available. FISH analysis revealed that at least 11 of the remaining 12 cases share a breakpoint within or near sequences detected with YAC 810f11 (case 20 could not be analyzed with YACs from D15S144, as sufficient patient material was not available for study; however, in a previous study, Leana-Cox [1993] demonstrated that YAC B94H7 [D15S24] hybridized to the inv dup[15] in this case, indicating that case 20 may share a breakpoint at or near sequences detected by YAC 810f11). In addition to YAC 810f11, YACs from several other loci were used in our analysis, to determine the boundaries of the breakpoint region.

In all cases tested, D15S165, detected by YAC 940c5, was found to be present on the inv dup(15), whereas the cardiac-muscle actin (ACTC) gene (Kramer et al. 1992), detected by YAC 815e6, was found to be absent. This led us to extend our FISH analysis, to four YACs containing D15S144, located ~6 cM distal to D15S165 and ~3.4 cM proximal to ACTC, based on information from the Whitehead database (http://www-genome.

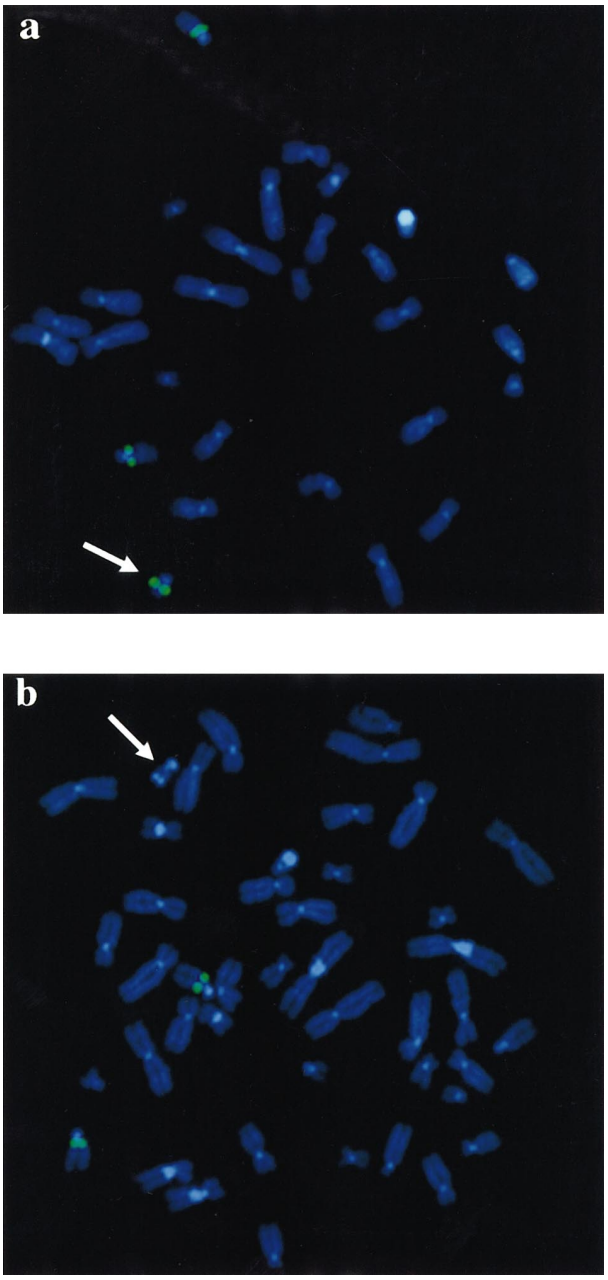

Figure 2 FISH results for the inv dup(15) that breaks between D15S12 and D15S24. All chromosomes were counterstained with DAPI and appear blue, and all probes were labeled with fluorescein isothiocyanate (FITC) and appear green. *a*, Partial metaphase spread from case 3. P93C9 (D15S12) appears on both normal chromosomes 15, as well as on the inv dup(15) (indicated by the arrow). *b*, Partial metaphase spread from case 5. B94H7 (D15S24) appears on both normal chromosomes 15 but not on the inv dup(15) (indicated by the arrow).

wi.mit.edu). Of the four YACs (810f11, 903f11, 920a7, and 952d10), only 810f11 hybridized to the marker chromosome, in each case (table 4 and fig. 3*a* and *b*). Interestingly, in all 11 cases, the signal seen on the marker was observed to be reduced in intensity when compared with the signal from the normal chromosomes 15 in the same cell. Figure 3*a* and *b* shows examples of metaphase analysis of two different cases (18 and 12, respectively). Interphase analysis of a third case (8) also was performed, by use of dual-color FISH with YAC 810f11 and a cosmid located proximal at D15S11 (fig. 3*c*). Again, only one signal was seen from YAC 810f11, between two signals from the D15S11 cosmid, and the YAC 810f11 signal was visibly reduced, when compared with that from the normal chromosomes 15. Figure 3*d* shows an example of a partial metaphase spread, from case 8, hybridized with YAC 920a7. The signal from YAC 920a7 is seen clearly on the two normal chromosomes 15 but is absent from the inv dup(15) chromosome, indicating that, in each case, the breakpoint lies proximal to D15S144 and to the other sequences contained in this YAC.

Of the four YACs containing D15S144, only YACs 810f11 and 920a7 were found to be nonchimeric, by FISH analysis. We concluded that YAC 810f11 contains sequences, absent from YAC 920a7, that are homologous to sequences present on the inv dup(15) chromosomes in the cases in this study, since these two YACs showed a characteristic difference in all the largest inv dup(15) chromosomes tested. Figure 4 shows the order of the three STSs (D15S1010, D15S144, and D15S1007) used to establish the order of YACs 810f11 and 920a7. To estimate the extent of overlap between these two YACs, we next used interspersed repeat-element "fingerprinting," with PCR primers derived from the *Alu* family of repeats. As shown in figure 5, these two YACs have very similar banding patterns, indicating a high degree of overlap. This indicates that a small portion of YAC 810f11 is accountable for the signal observed on the inv dup(15) in these cases.

The most prudent explanation for these data is that the inv dup(15) breakpoint in these cases lies in the small segment of YAC 810f11 located centromeric to YAC 920a7 or immediately distal to the centromeric end of YAC 920a7, with so little of YAC 810f11 hybridized to the inv dup(15) that YAC 810f11 was not visible by FISH. Consistent with this explanation, STS-content analysis for D15S1010, D15S144, and D15S1007 indicated that YAC 810f11 extends centromeric to YAC 920a7, and, thus, YAC 810f11 is located in the correct position to span the inv dup(15) breakpoint. These data suggest that, in fact, YAC 810f11 spans the breakpoint in the largest inv dup(15). Alternatively, however, it is theoretically possible that all the D15S144 YACs lie distal to the breakpoint but that this portion of YAC 810f11 contains low-copy repetitive elements (not suppressed by Cot-1 DNA, during the FISH analysis) that lie at or near the breakpoint. These alternative expla-

Table 4

FISH Results for the Largest Inv Dup(15) Chromosomes

	FISH Results[a]					
	D15S165		D15S144		ACTC	
Case No.	YAC 940c5	YAC 810f11	YAC 903f11	YAC 920a7	YAC 952d10	YAC 815e6
8	+	+	−	−	−	−
9	N/T	+	−	−	−	−
10	N/T	+	−	−	−	−
11	N/A	+	N/A	N/A	N/A	N/A
12	+	+	−	−	−	−
13	N/T	+	−	−	−	−
14	+	+	−	−	−	−
15	+	+	−	−	−	−
16	N/T	+	−	−	−	−
17	+	+	−	−	−	−
18	N/T	+	−	−	−	N/T
19	N/T	+	−	−	−	N/T

[a] The number of signals present on the inv dup(15) chromosome is indicated for each hybridization: a plus sign (+) denotes one signal; a minus sign (−) denotes no signal; N/A = tissue not available for study; and N/T = not tested.

nations will be explored in more depth in the Discussion section.

Parent-of-Origin Studies

To determine the parent of origin of the inv dup(15) chromosomes, PCR analysis of microsatellite markers was performed for the cases for which parental DNA was available. For cases 4 and 19, such data were published previously (Flejter et al. 1996), and those results are repeated here. In all nine cases (3, 4, 6, 7, 8, 15, 17, 19, and 20) for which analysis was possible, PCR analysis established that the patient inherited both maternal alleles and only one paternal allele (fig. 6 and table 5). Thus, in each case, the inv dup(15) appeared to be of maternal origin.

To extend this analysis to cases for which parental DNA was not available, we performed methylation analysis at SNRPN exon 1, as described by Glenn et al. (1996), for 15 inv dup(15) patients (cases 4–10 and 12–19). Dosage analysis revealed that the 4.3-kb band showed increased dosage, compared with the 0.9-kb band (fig. 7). As the 4.3-kb band is of maternal origin (on the basis of analysis of PWS/AS-deletion–patient controls [fig. 7; Glenn et al. 1996]), this information is consistent with the inv dup(15) being maternal in origin in all cases examined. Importantly, methylation data proved to be consistent for the seven cases for which both methylation analysis and PCR microsatellite analysis was performed. When considered together, our parent-of-origin studies indicate that, in all 17 cases analyzed, the inv dup(15) is maternal in origin (table 1).

Phenotype/Karyotype Correlations

We analyzed our patient population for traits reported elsewhere to be associated with the presence of an inv dup(15) chromosome, such as mental retardation, autism, seizures, and any notable dysmorphic features. Although the number of patients for whom specific information was available varied by feature, some consistent clinical features were apparent. For example, all 16 patients for whom clinical information was available exhibited some degree of mental retardation, and this finding was independent of whether the inv dup(15) had a breakpoint between D15S12 and D15S24 or near D15S1010. Similarly, autism was noted in eight of nine patients for whom this type of clinical information was available, and, again, this finding apparently was unrelated to the size of the inv dup(15). Dysmorphic features such as strabismus, hypotelorism, microcephaly, and low-set ears were reported in two of three clinically evaluated cases with the proximal breakpoint and in all seven cases with the distal breakpoint.

On the other hand, suggestions of characteristic differences between patients with different types of inv dup(15) were noted. Thus, among patients with a breakpoint between D15S12 and D15S24, four patients had experienced seizures, whereas no notation was made for the fifth patient. In contrast, among patients with a larger inv dup(15), only one of nine patients for whom complete information was available reported having experienced seizures. A much more thorough and consistent clinical evaluation of inv dup(15) patients clearly will be required, to address the nature of any specific phenotype/karyotype correlations.

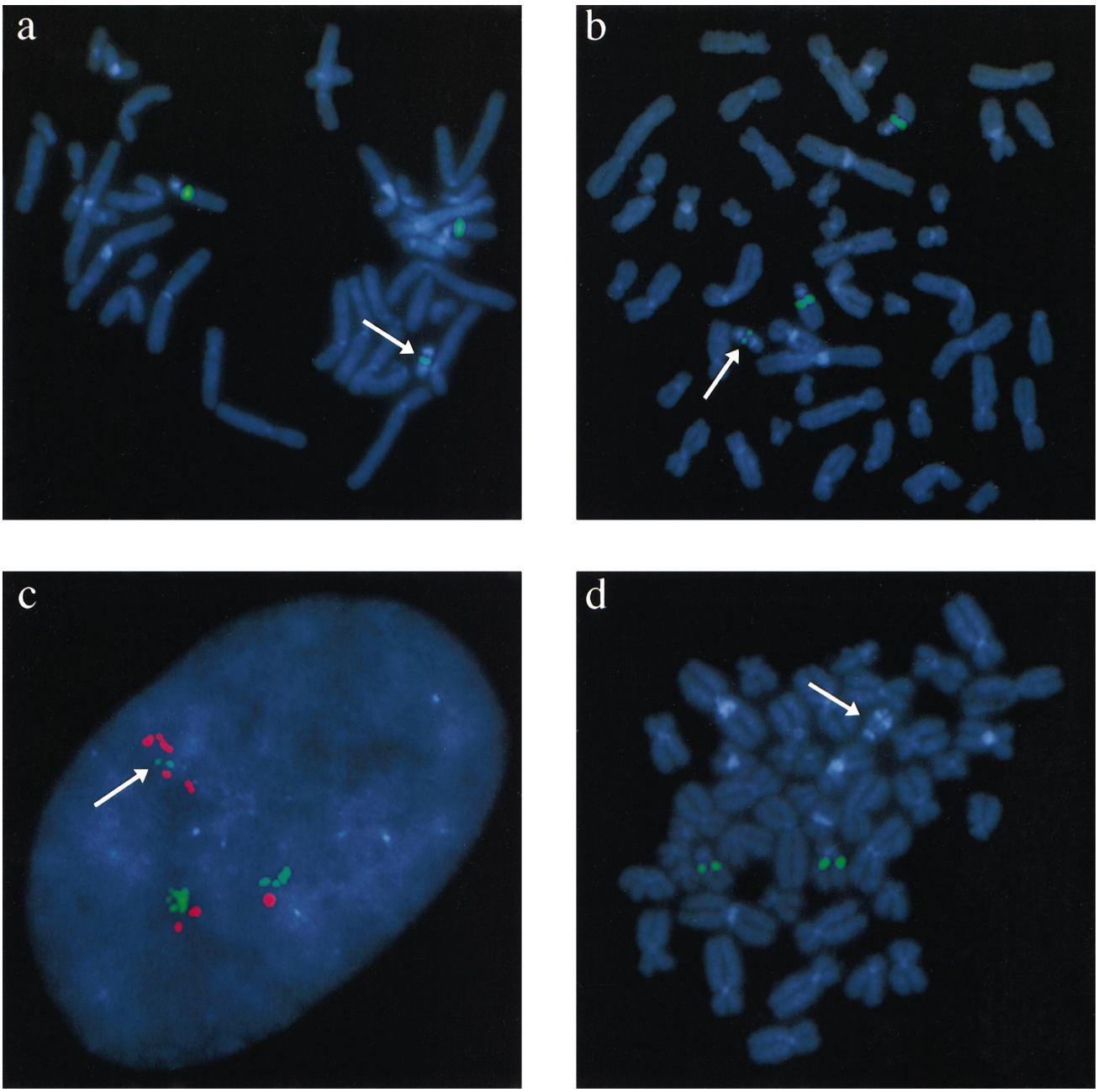

Figure 3 FISH results for the largest inv dup(15)s. All chromosomes and cells were counterstained with DAPI and appear blue. *a,* Partial metaphase spread from case 18, hybridized with YAC 810f11 (FITC labeled [green]) from the D15S144 locus. The signal from YAC 810f11 appears on both normal chromosomes 15, as well as on the inv dup(15) (indicated by the arrow). The signal intensity from the inv dup(15) appears to be reduced, when compared with that from the normal chromosomes 15. *b,* Another example of a partial metaphase spread, from case 12, hybridized with YAC 810f11 (FITC labeled [green]) from the D15S144 locus. The signal from YAC 810f11 appears on both normal chromosomes 15, as well as on the inv dup(15) (indicated by the arrow). Again, the signal intensity appears to be reduced, when compared with that from the normal chromosomes 15. *c,* Interphase cell from case 8, hybridized with both YAC 810f11 (FITC labeled [green]) and the cosmid from the D15S11 locus (rhodamine labeled [red]). The signal from the inv dup(15) is clear, and two signals from the D15S11 cosmid and only one, reduced signal from YAC 810f11 (indicated by the arrow) are shown. *d,* Partial metaphase spread from case 8, hybridized with YAC 920a7 (FITC labeled [green]), also from the D15S144 locus. The signal from YAC 920a7 appears on both normal chromosomes 15 but not on the inv dup(15) (indicated by the arrow).

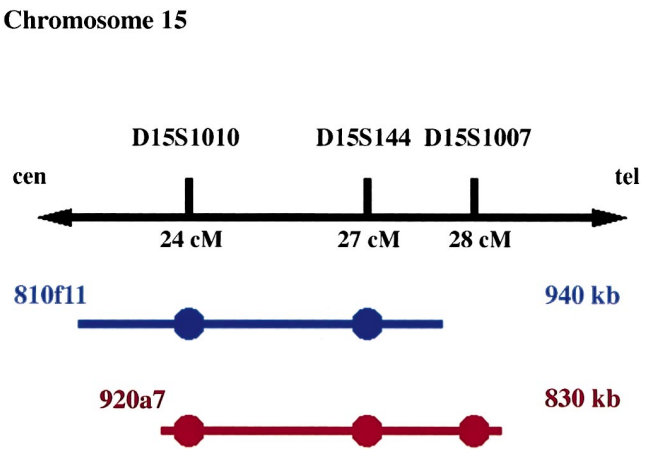

Figure 4 Diagram showing the order of STSs (D15S1010, D15S144, and D15S1007) and YACs 810f11 and 920a7, which are located in the breakpoint region of the largest inv dup(15).

Discussion

Phenotype/genotype correlations in our study were consistent with those in the study by Leana-Cox et al. (1994) and in the more recent study by Mignon et al. (1996), in that no major phenotypic differences were found among patients with different types of inv dup(15) that contain the PWS/AS chromosomal region. Traits, such as autism, mental retardation, and dysmorphic features, that are commonly reported to be associated with inv dup(15) syndrome were found to occur in both classes defined molecularly in this study. In addition, it should be noted that many patients were evaluated when they were quite young; therefore, traits such as seizures may not have been reported, and the patient may have developed the trait later in life. Mignon et al. (1996) hypothesized that imprinting effects among patients with an inv dup(15) may explain the variability in phenotype, but they could not find evidence to support this hypothesis, during their study. However, they did note that the severity of the seizure phenotype seemed to correlate with the severity of the other phenotypic traits, including the degree of mental retardation.

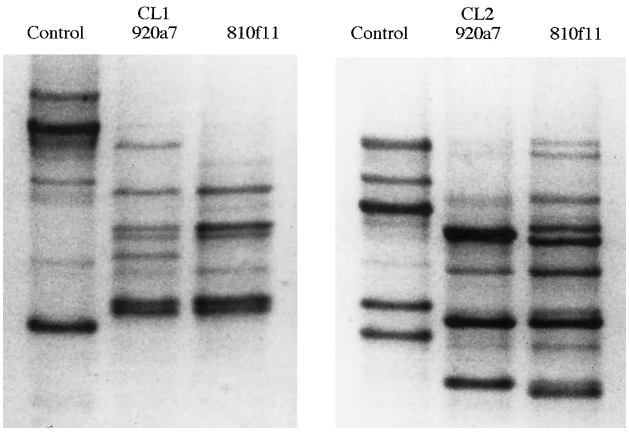

Figure 5 *Alu* fingerprinting of YACs 810f11 and 920a7, using PCR primers CL1 and CL2, which were derived from the *Alu* family of repeats. A negative control YAC from the X chromosome is shown for comparison. Clearly, YACs 810f11 and 920a7 have many bands in common, indicating a high degree of homology.

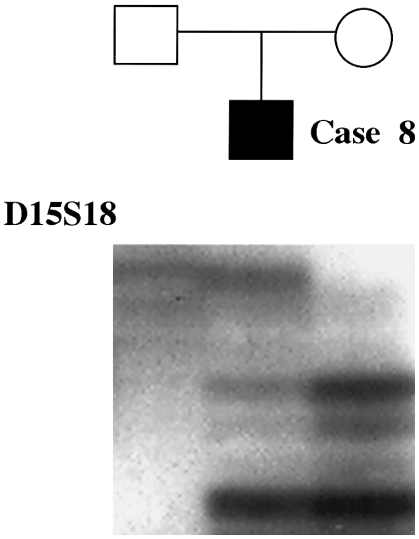

Figure 6 PCR microsatellite analysis, with polymorphic CA repeats for D15S18, of case 8. The proband has inherited only one allele from the father and two alleles from the mother.

In our study, we identified at least two types of inv dup(15) that include material from the PWS/AS chromosomal region. Six cases have a breakpoint between D15S12 and D15S24, whereas twelve cases have an inv dup(15) with a breakpoint in 15q13-14, near the D15S144 locus detected by YAC 810f11. Of the four YACs containing D15S144, only one, 810f11, hybridized to the inv dup(15) chromosome in all the cases tested. In each case, the signal intensity from 810f11 was reduced on the inv dup(15), when compared with the signal intensity from the normal chromosomes 15 in the same cell (fig. 3*a–c*). This observation raises the possibility that the breakpoint(s) in this class of inv dup(15) lies at the centromeric end of YAC 810f11.

However, given the fact that several low-copy repeat sequences have been found on proximal chromosome 15 and have been implicated in the common deletion found in PWS and AS patients (Buiting et al. 1992; Amos-Landgraf et al. 1994), it is possible that YAC 810f11 contains a low-copy repeat without actually spanning the breakpoint. If so, such a putative element must reside in the relatively small centromeric portion

Table 5

PCR Analysis of Microsatellite Markers

Case No. and Family Member	Genotype at Locus					
	D15S18	D15S122	D15S128	GABRβ3	D15S165	D15S144
3:						
Father		AB	BC	AD		
Proband		BC	ACD	BCD		
Mother		BC	AD	BC		
4:[a]						
Father				EF		
Proband				ADF		
Mother				AD		
6:						
Father		BC	AB		AC	AC
Proband		ABC	ACD		AC	AB
Mother		AB	CD		BC	BC
7:						
Father		AC	BB	CD	AB	AB
Proband		ABD	AB	ABD	BC	A
Mother		BD	AB	AB	CC	AA
8:						
Father	AA	CD	AB	BB	AB	AA
Proband	ABC	ADE	ABC	AB	BC	A
Mother	BC	AE	AC	AB	AC	AA
15:						
Proband		BD	AB	C	ABC	D
Mother		BD	AB	CC	BC	DD
17:						
Father	CD	AC	AB	BB		AC
Proband	ABC	BCD	AC	AB		AB
Mother	ABC	BCD	AC	AB		B
19:[a]						
Father				CF		
Proband				BDF		
20:						
Father	AB	CD	BC			AB
Proband	BC	ABC	ABC			AB
Mother	BC	AB	AC			A

Note.—Allele designations are meant to represent the different alleles present and do not indicate locus copy number.

[a] Results from Flejter et al. (1996). The mother of Case 19 is deceased.

of YAC 810f11 that does not overlap YAC 920a7. On the basis of our *Alu*-fingerprinting data (fig. 5), this region would appear to be no larger than ~10% of the 930-kb YAC. If such a family of low-copy repeats exists in this region of chromosome 15, FISH analysis would be expected to show another, smaller signal adjacent to the larger signal on the normal chromosomes 15. Our failure to see such a signal (e.g., fig. 3*a* and *b*) indicates that any such elements detected by YAC 810f11 must be confined to a relatively small region, on chromosome 15q, that is not resolved in interphase nuclei. In either case, our data indicate that further analysis of the centromeric end of this YAC should be informative for the determination of the basis of inv dup(15) formation.

The heterogeneous nature of the breakpoints among the different types of inv dup(15), as well as at the proximal boundary of the common deletion region in PWS/AS-deletion patients (Christian et al. 1995), suggests that several low-copy repeat sequences may be involved in the genesis of these chromosomal abnormalities. Such sequences may confer DNA instability at these regions, by facilitating illegitimate recombination during replication. A favored theory for the formation of an inv dup(15), originally proposed by Schreck et al. (1977), suggests that illegitimate recombination between homologous chromosomes, involving a U-type exchange (as opposed to a normal, X-type exchange), generates the inv dup(15) and a duplicated, acentric fragment, followed by nondisjunction and centromere inactivation. Analysis of cytogenetic heteromorphisms has supported the interchromosomal nature of the recombination event (Zannotti et al. 1980; Stetten et al. 1981; Plattner et al. 1993; Robinson et al. 1993*a*), although it is possible that such U-type exchanges can occur either between sister chromatids or between homologous chromosomes.

Nondisjunction must play a role in any mechanism

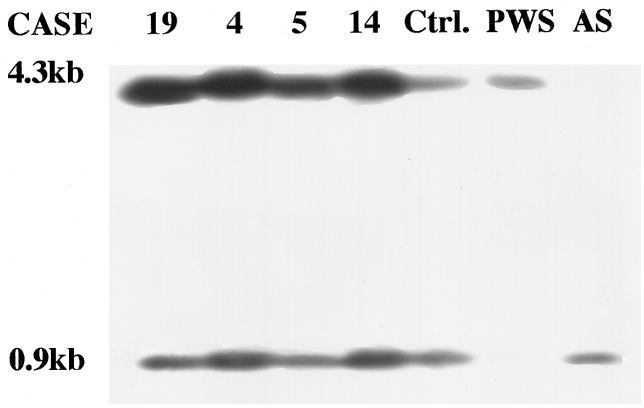

Figure 7 Methylation analysis at SNRPN exon 1. The 4.3-kb and 0.9-kb bands correspond to the maternal and paternal alleles, respectively, in normal individuals. Clearly, the 4.3-kb band is increased in intensity, compared with the 0.9-kb band, in the inv dup(15) cases, when compared with the intensity ratios generated from normal individuals, as well as with those from PWS/AS-deletion–patient controls.

resulting in the additional inv dup(15) (Schreck et al. 1977). As assessed by a variety of cytogenetic or molecular methods, the majority of instances of inv dup(15) that contain the PWS/AS chromosomal region are maternal in origin (Wisniewski et al. 1979; Maraschio et al. 1981; Nicholls et al. 1989; Shibuya et al. 1991; Robinson et al. 1993*a;* Crolla et al. 1995; Flejter et al. 1996), and all the markers examined in this study also appeared to be maternal in origin (table 1 and figs. 6 and 7). Not surprisingly, therefore, increased maternal age has been associated with an increased probability of having a child with an inv dup(15) (Wisniewski et al. 1979; Maraschio et al. 1981). This finding underscores the importance of understanding the nature and mechanism of formation of these marker chromosomes, as well as of clarifying the phenotypic consequences of different classes of inv dup(15), for the purpose of providing appropriate genetic counseling, especially in the context of prenatal diagnosis.

In summary, our study demonstrates that there are at least two classes of inv dup(15) that include the PWS/AS chromosomal region. By analysis of the proximal region of chromosome 15, which has both a large number of low-copy repeat sequences and a high proportion of structural abnormalities, a paradigm for other, less common structural rearrangements may emerge.

Acknowledgments

We wish to acknowledge Laurie Becker and J. Marie Haren, of the cytogenetics laboratory at the Center for Human Genetics, for their expert technical assistance. We would like to express thanks to Dr. Rob Nicholls, for his helpful discussions and for providing us with YACs P93C9 and B94H7 and the SNRPN exon 1 probe used in our methylation studies. Additional collaborators, who coordinated the acquisition of patient samples, were Drs. Joleen Zackowski (Eastern Virginia Medical School, Roanoke), Wendy Flejter (University of Utah, Salt Lake City), Catherine Palmer (Indiana School of Medicine, Indianapolis), Fern Tsien (Tulane University School of Medicine, New Orleans), and Linda Sheppard (Palo Verde Laboratory, Chandler, AZ). We also thank Drs. Terry Hassold and Huntington Willard, for their critical comments on the manuscript. This work was supported, in part, by National Institutes of Health grant PO1 HD32111 and by a grant, from the Wilson Foundation, to the Center for Human Genetics at University Hospitals of Cleveland.

References

Amos-Landgraf J, Gottlieb W, Rogan PK, Nicholls RD (1994) Chromosome breakage in Prader-Willi and Angelman syndrome deletions may involve recombination between a repeat at the proximal and distal breakpoints. Am J Hum Genet Suppl 55:A38

Beckmann JS, Tomfohrde J, Barnes RI, Williams M, Broux O, Richard I, Weissenbach J, et al (1993) A linkage map of human chromosome 15 with an average resolution of 2 cM and containing 55 polymorphic microsatellites. Hum Mol Genet 2:2019–2030

Buckton KE, Spowart G, Newton MS, Evans HJ (1985) Forty four probands with an additional "marker" chromosome. Hum Genet 69:353–370

Buiting K, Greger V, Brownstein BH, Mohr RM, Voiculescu I, Winterpacht A, Zabel B, et al (1992) A putative gene family in 15q11-13 and 16p11.2: possible implications for Prader-Willi and Angelman syndromes. Proc Natl Acad Sci USA 89:5457–5461

Callen DF, Ringenbergs ML, Fowler JCS, Freemantle CJ, Haan EA (1990) Small marker chromosomes in man: origin from pericentric heterochromatin of chromosomes 1, 9, and 16. J Med Genet 27:155–159

Cheng S-D, Spinner NB, Zackai EH, Knoll JHM (1994) Cytogenetic and molecular characterization of inverted duplicated chromosomes 15 from 11 patients. Am J Hum Genet 55:753–759

Christian SL, Robinson WP, Huang B, Mutirangura A, Line MR, Nakao M, Surti U, et al (1995) Molecular characterization of two proximal deletion breakpoint regions in both Prader-Willi and Angelman syndrome patients. Am J Hum Genet 57:40–48

Crolla JA, Harvey JF, Sitch FL, Dennis NR (1995) Supernumerary marker 15 chromosomes: a clinical, molecular and FISH approach to diagnosis and prognosis. Hum Genet 95: 161–170

Flejter WL, Bennett-Baker PE, Ghaziuddin M, McDonald M, Sheldon S, Gorski JL (1996) Cytogenetic and molecular analysis of inv dup(15) chromosomes observed in two patients with autistic disorder and mental retardation. Am J Med Genet 61:182–187

Fujita H, Sakamoto Y, Hamamoto Y (1980) An extra idic(15p)(q11) chromosome in Prader-Willi syndrome. Hum Genet 55:409–411

Glenn CC, Saitoh S, Jong MTC, Filbrandt MM, Surti U, Driscoll DJ, Nicholls RD (1996) Gene structure, DNA methyl-

ation, and imprinted expression of the human *SNRPN* gene. Am J Hum Genet 58:335–346

Hall JG (1990) Genomic imprinting: review and relevance to human disease. Am J Hum Genet 46:857–873

Huang B, Crolla JA, Christian SL, Wolf-Ledbetter ME, Macha ME, Papenhausen PN, Ledbetter DH (1997) Refined molecular characterization of the breakpoints in small inv dup(15) chromosomes. Hum Genet 99:11–17

Hudson T, Stein LK, Gerety SS, Ma J, Castle AB, Silva J, Slonim DK et al (1995) An STS-based map of the human genome. Science 270:1945–1954

Knight LA, Lipson M, Mann J, Bachman R (1984) Mosaic inversion duplication of chromosome 15 without phenotypic effect: occurrence in a father and daughter. Am J Med Genet 17:649–654

Kramer PL, Luty JA, Litt M (1992) Regional localization of the gene for cardiac muscle actin (ACTC) on chromosome 15q. Genomics 13:904–905

Leana-Cox J (1993) Molecular cytogenetic analysis of inv dup(15) chromosomes. PhD thesis, University of Maryland, Baltimore

Leana-Cox J, Jenkins L, Palmer CG, Plattner R, Sheppard L, Flejter WL, Zackowski J, et al (1994) Molecular cytogenetic analysis of inv dup(15) chromosomes, using probes specific for the Prader-Willi/Angelman syndrome region: clinical implications. Am J Hum Genet 54:748–756

Ledbetter DH, Mascarello JT, Riccardi VM, Harper VD, Airhart SD, Strobel RJ (1982) Chromosome 15 abnormalities and the Prader-Willi syndrome: a follow-up report of 40 cases. Am J Hum Genet 34:278–285

Maraschio P, Zuffardi O, Bernardi F, Bozzola M, De Paoli C, Fonatsch C, Flatz SD, et al (1981) Preferential maternal derivation in inv dup(15): analysis of eight new cases. Hum Genet 57:345–350

Mattei MG, Souiah N, Mattei JF (1984) Chromosome 15 abnormalities and the Prader-Willi syndrome: cytogenetic analysis. Hum Genet 66:313–334

Mignon C, Malzac P, Moncla A, Depetris D, Roeckel N, Croquette M-F, Mattei M-G (1996) Clinical heterogeneity in 16 patients with inv dup 15 chromosome: cytogenetic and molecular studies, searching for an imprinting effect. Eur J Hum Genet 4:88–100

Mutirangura A, Jayakumar A, Sutcliffe JS, Nakao M, McKinney MJ, Buiting K, Horsthemke B, et al (1993) A complete YAC contig of the Prader-Willi/Angelman chromosome region (15q11-q13) and refined localization of the SNRPN gene. Genomics 18:546–552

Nicholls RD (1993) Genomic imprinting and uniparental disomy in Angelman and Prader-Willi syndromes: a review. Am J Med Genet 46:16–25

Nicholls RD, Knoll JH, Glatt K, Hersh JH, Brewster TD, Graham JM Jr, Wurster-Hill D, et al (1989) Restriction fragment length polymorphisms within proximal 15q and their use in molecular cytogenetics and the Prader-Willi syndrome. Am J Med Genet 33:66–77

Pinkel D, Straume T, Gray JW (1986) Cytogenetic analysis using quantitative, high-sensitivity fluorescence hybridization. Proc Natl Acad Sci USA 83:2934–2938

Plattner R, Heerema NA, Howard-Peebles PN, Miles JH, Soukup S, Palmer CG (1993) Clinical findings in patients with FISH identified marker chromosomes. Hum Genet 91: 589–598

Plattner R, Heerema NA, Patil SR, Howard-Peebles PN, Palmer CG (1991) Characterization of seven DA/DAPI positive bisatellited marker chromosomes by in situ hybridization. Hum Genet 87:290–296

Rauch A, Pfeiffer RA, Trautmann U, Liehr T, Rott HD, Ulmer R (1992) A study of ten small supernumerary chromosomes identified by fluorescence in situ hybridization (FISH). Clin Genet 42:84–90

Richard I, Broux O, Chiannilkulchai N, Fougerousse F, Allamand V, Bourg N, Brenguier L, et al (1994) Regional localization of human chromosome 15 loci. Genomics 23: 619–627

Robinson WP, Binkert F, Gine R, Vazquez C, Muller W, Rosenkranz W, Schinzel A (1993*a*) Clinical and molecular analysis of five inv dup(15) patients. Eur J Hum Genet 1:37–50

Robinson WP, Wagstaff J, Bernasconi F, Baccichetti C, Artifoni L, Franzoni E, Suslak L, et al (1993*b*) Uniparental disomy explains the occurrence of the Angelman or Prader-Willi syndrome in patients with an additional small inv dup(15) chromosome. J Med Genet 30:756–760

Schreck RR, Breg WR, Erlanger BF, Miller OJ (1977) Preferential derivation of abnormal human G-group-like chromosomes from chromosome 15. Hum Genet 36:1–12

Schwartz S, Wolff DJ, Zackowski JL (1990) Utilization of fluorescence *in situ* hybridization for chromosome identification and elucidation of structural rearrangements. Am J Hum Genet Suppl 47:A40

Shibuya Y, Tonoki H, Kajii N, Niikawa N (1991) Identification of a marker chromosome as inv dup(15) by molecular analysis. Clin Genet 40:233–236

Speed RM, Johnston AW, Evans HJ (1976) Chromosome survey of total population of mentally subnormal in North-East of Scotland. J Med Genet 13:295–306

Spinner NB, Zackai E, Cheng SD, Knoll JH (1995) Supernumerary inv dup(15) in a patient with Angelman syndrome and a deletion of 15q11-q13. Am J Med Genet 57:61–65

Stetten G, Sroka-Zaczek B, Corson VL (1981) Prenatal detection of an accessory chromosome identified as an inversion duplication (15). Hum Genet 57:357–359

Sullivan BA, Leana-Cox J, Schwartz S (1993) Clarification of subtle reciprocal rearrangements using fluorescence in situ hybridization. Am J Med Genet 47:223–230

Van Dyke DL, Weiss L, Logan M, Pai GS (1977) The origin and behavior of two isodicentric bisatellited chromosomes. Am J Hum Genet 29:294–300

Wisniewski LP, Hassold T, Heffelfinger J, Higgins JV (1979) Cytogenetic and clinical studies in five cases of inv dup(15). Hum Genet 50:259–270

Wisniewski LP, Witt ME, Ginsberg-Fellner F, Wilner J, Desnick RJ (1980) Prader-Willi syndrome and a bisatellited derivative of chromosome 15. Clin Genet 18:42–47

Zannotti M, Giovanardi PR, Dallapiccola B (1980) Extra dicentric 15pter-q21/22 chromosomes in five unrelated patients with a distinct syndrome of progressive psychomotor retardation, seizures, hyper-reactivity and dermatoglyphic abnormalities. J Ment Defic Res 24:235–242