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Molecular Characterization of Two Proximal Deletion Breakpoint Regions in Both Prader-Willi and Angelman Syndrome Patients

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Summary

Prader-Willi syndrome (PWS) and Angelman syndrome (AS) are distinct mental retardation syndromes caused by paternal and maternal deficiencies, respectively, in chromosome 15q11-q13. Approximately 70% of these patients have a large deletion of ~4 Mb extending from D15S9 (ML34) through D15S12 (IR10). To further characterize the deletion breakpoints proximal to D15S9, three new polymorphic microsatellite markers were developed that showed observed heterozygosities of 60%–87%. D15S541 and D15S542 were isolated from YAC A124A3 containing the D15S18 (IR39) locus. D15S543 was isolated from a cosmid cloned from the proximal right end of YAC 254B5 containing the D15S9 (ML34) locus. Gene-centromere mapping of these markers, using a panel of ovarian teratomas of known meiotic origin, extended the genetic map of chromosome 15 by 2–3 cM toward the centromere. Analysis of the more proximal S541/S542 markers on 53 Prader-Willi and 33 Angelman deletion patients indicated two classes of patients: 44% (35/80) of the informative patients were deleted for these markers (class I), while 56% (45/80) were not deleted (class II), with no difference between PWS and AS. In contrast, D15S543 was deleted in all informative patients (13/48) or showed the presence of a single allele (in 35/48 patients), suggesting that this marker is deleted in the majority of PWS and AS cases. These results confirm the presence of two common proximal deletion breakpoint regions in both Prader-Willi and Angelman syndromes and are consistent with the same deletion mechanism being responsible for paternal and maternal deletions. One breakpoint region lies between D15S541/S542 and D15S543, with an additional breakpoint region being proximal to D15S541/S542.

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Introduction

Prader-Willi syndrome (PWS) and Angelman syndrome (AS) are distinct mental retardation disorders, both involving cytogenetic abnormalities of chromosome 15q11-q13, with different parent-of-origin effects. The frequency of these disorders is ~1/10,000–1/25,000 for PWS (Holm 1981; Butler 1990) and 1/20,000 for AS (Clayton-Smith and Pembrey 1992). Approximately 70% of PWS cases are caused by a paternal deletion of 15q11-q13, with 20%–25% displaying maternal uniparental disomy (Nicholls et al. 1989; Butler 1990; Robinson et al. 1991). In AS a similar percentage are due to a maternal deletion (Beuten et al. 1993; Saitoh et al. 1994), <5% are due to paternal uniparental disomy (Knoll et al. 1991), and the remainder have no detectable chromosomal abnormality but are thought to include imprinting defects or mutations of a single imprinted gene (Reis et al. 1994). Most deletions are visible as the loss of 15q11-q13, by using high-resolution cytogenetic techniques; however, submicroscopic deletions have been detected by using molecular techniques (Saitoh et al. 1992; Greger et al. 1993; Buxton et al. 1994; Sutcliffe et al. 1994). The current hypothesis to explain the parent-of-origin effects of PWS/AS involves the presence of oppositely imprinted gene(s) within 15q11-q13 (Reis et al. 1994; Sutcliffe et al. 1994).

The size of the common deletion region in both PWS and AS is ~4 Mb. This recurring constitutional deletion is perhaps the most common that is observed in humans. Since PWS and AS each occur with frequencies of ~1/20,000, and since 70% have this deletion, the overall frequency of the deletion event is ~1/15,000. Interestingly, proximal 15q is involved in a number of other cytogenetic rearrangements. Inv dup(15) with breakpoints at 15q11.2 or 15q13 may account for ~50% of the small supernumerary marker chromosomes observed in humans (Robinson et al. 1993*a*; Webb 1994; Crolla et al. 1995). Two studies involving >21,000 newborns detected marker chromosomes in 0.024%–0.06%, making the frequency of inv dup(15) ~1/3,500–1/9,000 (Webb 1994). In addition to the common recurrence of

deletions and inv dup(15), other, less common abnormalities of this region have been observed, including duplications and triplications (Pettigrew et al. 1987; Clayton-Smith et al. 1993; Schinzel et al. 1994).

Although this region is involved in cytogenetic aberrations with a high frequency, there are little data addressing the mechanism of these rearrangements. One study of 19 AS patients that used RFLP analysis identified two deletion classes (Knoll et al. 1990): class I was deleted for D15S18 and D15S9-D15S13, while class II was deleted for only D15S9-D15S13 (Knoll et al. 1990). The development of a YAC contig extending through ~3.5 Mb of this region has facilitated the ordering of markers used in the RFLP analysis and definition of the common deletion breakpoints (Kuwano et al. 1992; Mutirangura et al. 1993*b*). FISH analysis using YACs from this region originally suggested that the proximal deletion breakpoint fell within a 370-kb YAC containing the D15S9 locus in seven PWS patients and three AS patients consistent with class II of the previous study, but no patients of class I were identified (Kuwano et al. 1992). On the distal side, breakpoints in eight of nine patients appeared to fall within a 200-kb YAC containing the IR10-1 (D15S12) locus (Kuwano et al. 1992). The purposes of the present study were (1) to develop new polymorphic microsatellite markers in proximal 15q near D15S9 (ML34) and D15S18 (IR39), (2) to map their genetic distance from the centromere by using ovarian teratomas of known meiotic origin to extend the genetic map of chromosome 15 and to examine the relationship of physical and genetic map distances in this region, and (3) to characterize at a molecular level the proximal deletion breakpoint regions in PWS and AS patients by expanding the number of patients analyzed with more informative proximal markers.

Patients and Methods

Patients

Studies were performed on 53 PWS and 33 AS deletion patients, some of which have been published previously (Kuwano et al. 1992; Robinson et al. 1993*b*; Zackowski et al. 1993). Each patient was known to be deleted for the interval from D15S11 to GABRB3, by FISH and/or other molecular techniques. Peripheral blood samples were obtained with appropriate informed consent from the patient and both parents, for either DNA isolation by routine methods or whole-cell lysis using the QIAamp blood kit (Qiagen).

Isolation of CA-Repeat Polymorphisms

We previously reported the development of a YAC contig spanning ~3.5 Mb on chromosome 15q11-q13 (Mutirangura et al. 1993*b*). To develop dinucleotide-repeat microsatellites proximal to D15S11, we used both yA124A3 isolated with a sequence-tagged site (STS) from D15S18 (IR39) and y254B5 isolated with an STS from D15S9 (ML34) (fig. 1). Two dinucleotide-repeat polymorphisms, D15S541 and D15S542, were identified within a single ~1-kb fragment isolated from yA124A3 by using Alu-CA PCR as described elsewhere (Mutirangura et al. 1993*a*).

D15S543 was developed from a cosmid library of y254B5 as described elsewhere (Nakao et al. 1994). Dot blot hybridization to 29 cosmids was performed by using the YAC right-end probe, which was predicted, on the basis of the previous FISH data, to be immediately proximal to the class II patient breakpoint. Cosmid 512 (c512) was positive for the YAC right end and was further characterized. A CA repeat within c512 was identified by dot blot hybridization and was isolated by using a modified vectorette-CA PCR method. In brief, 50 ng of cosmid DNA was cut with *Alu*I, *Hae*III, or *Hinc*II to give blunt ends. Reannealed vectorette oligonucleotides 5′-ACTGCAGAGACGCTGTCTGTCGAAGGTAAGGAACGGACGAGAGAAGGGAGAG-3′ (primer 1) and 5 ′-CTCTCCCTTCTCGAATCGTAACCGTTCGATCGAGAATCGCTGTCTCTGCAGT-3′ (primer 2) were ligated onto the blunt-ended DNA fragments overnight. PCR was performed as described elsewhere, by using a *Eco*RI-vectorette primer 5′-CGGAATTCCG AATGCTAACCGTCGATCGAGAATCGC-3′ with either a *Bam*-CA primer (5′-CCCGGATCCTGTGTGTGTGTGTGTG-3′) or a *Bam*-GT primer (5′-CCCGGATCCACACACACACACACACA-3′) (Feener et al. 1991; Mutirangura et al. 1993*a*). The products were double digested with *Eco*RI and *Bam*HI, were isolated, and were cloned into pBluescript SK(-) (Stratagene). After sequencing of the cloning sites, unique primers were designed for each product.

Microsatellite PCR Analysis

One oligonucleotide of each primer set was end-labeled for 90 minutes at 37°C in a 10-μl reaction containing 10 μM primer, 0.025 mCi γ[^{32}P] ATP (Amersham) at 3,000 Ci/mmol, 10 mM $MgCl_2$, 5 mM DTT, 50 mM Tris-HCl pH 7.6, and 5 units of T4 polynucleotide kinase. The resulting labeled mix was either used immediately or stored at −20°C without further purification.

The PCR reactions were performed in a reaction mixture containing 40 ng of genomic DNA or whole-cell lysate, 200 μM of each dNTP, 10 mM Tris-HCl pH 8.4, 50 mM KCl, 1.2 mM $MgCl_2$, and 0.5 units of *Thermus aquaticus* DNA polymerase (Perkin Elmer Cetus) in a total volume of 10 μl. Typically, 1 μl of labeled probe plus 1 μl of 10 μM unlabeled complementary primer was added per 100 μl of PCR reaction mix.

The PCR reactions were performed in a Perkin-Elmer 9600 thermocycler as follows: initial denaturation at 95°C for 4 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, extension

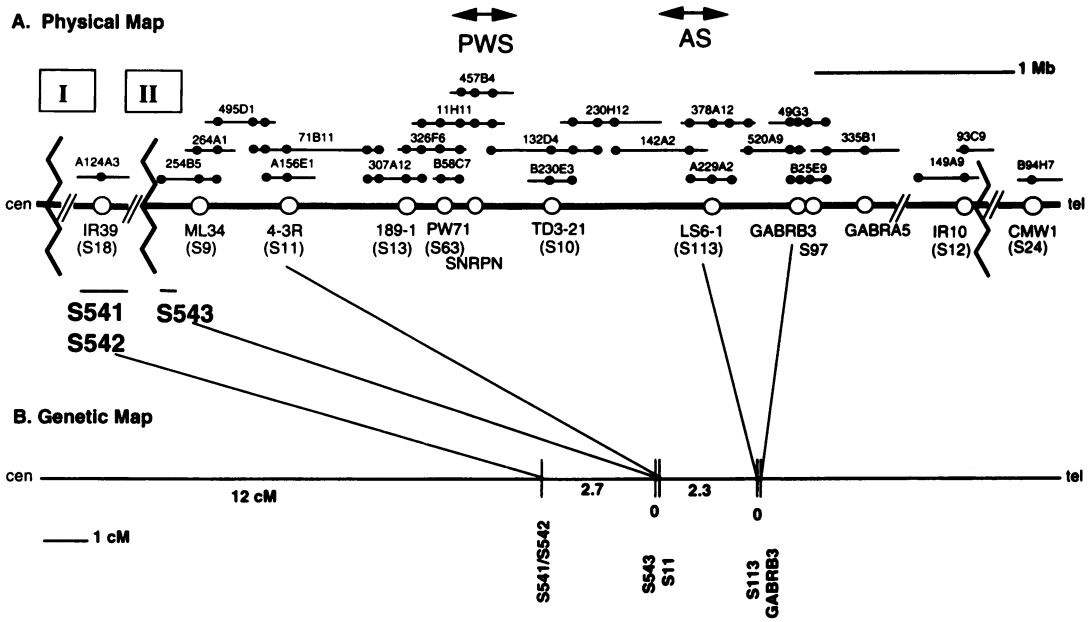

Figure 1 *A*, Physical map: summary of chromosome 15q11-q13 including a 4-Mb YAC contig (Kuwano et al. 1992; Mutirangura et al. 1993*b*). The jagged lines represent the regions of breakage in the common deletion found in PWS/AS. The PWS and AS critical regions are indicated by double-pointed arrows (Buxton et al. 1994; Sutcliffe et al. 1994). The two classes of proximal deletions are indicated by the "I" and "II" within the squares. The new microsatellites D15S541, D15S542, and D15S543 are listed below the line. *B*, Genetic map. The genetic distance (in cM) was determined by using ovarian teratomas of known meiotic origin (table 2). Lines have been used to connect loci on the physical and genetic maps.

at 72°C for 30 s, and a final extension at 72°C for 5 min. The PCR product mixture was diluted 2:3 with formamide loading buffer (Sambrook et al. 1989), denatured at 95°C for 2 min, and was placed on ice. One to three microliters of the product was separated on a 35-cm × 45-cm 6% acrylamide (19:1 bis)/5.6 M urea/32% formamide gel (Litt et al. 1993). The gels were prewarmed to 50°C prior to being loaded and were allowed to run for ~2–3 h. The wet gels were transferred to Whatman filter paper or old film, were covered with plastic wrap, and were exposed to Kodak XAR-5 film for 6–18 h at −70°C with two intensifying screens.

Gene-Centromere Mapping

A total of 105 ovarian teratomas that (*a*) arose by either whole-genome meiosis I nondisjunction (type I) or by meiosis II nondisjunction (type II) or endoreduplication (type III) or (*b*) were of mitotic origin (type IV), as well as their corresponding host tissues, were available for analysis. Cytogenetic analysis of host-teratoma pairs at nine chromosomal heteromorphisms and genetic markers at >50 loci were used to identify the mechanism of origin (Deka et al. 1990; Surti et al. 1990). Type III and IV teratomas are uninformative for linkage mapping. Thus, 65 types I and II ovarian teratomas and their hosts were genotyped for S541 and S543. Samples uninformative for S541 were then typed for S542, to achieve the maximum number of informative samples. Of these samples, 24 type I teratomas and 41 type II teratomas were informative and thus were utilized for map analysis. The determination of the interlocus distances and associated lod scores was performed as described elsewhere (Chakravarti et al. 1989; Deka et al. 1990; Mutirangura et al. 1993*a*).

Results

Development of New Dinucleotide-Repeat Polymorphisms

Three dinucleotide repeats—D15S541, D15S542, and D15S543—were developed from the two most proximal YACs in the contig (fig. 1). Alu-CA PCR identified a single ~1-kb fragment from yA124A3 with two internal CA repeats: D15S541 contained $(CA)_{18}$ repeats, and D15S542 contained $(CA)_{14}TA(CA)_9$ repeats. Analysis of ~100 chromosomes demonstrated observed heterozygosity values of 72% for S541 and 87% for S542 (table 1).

Our previous FISH data had suggested a common breakpoint within y254B5 (Kuwano et al. 1992). Therefore, we identified a cosmid from the proximal (right) end of y254B5, to develop a new polymorphic microsatellite marker, D15S543. This locus is unusual in that a single set of primers amplified two different polymorphic systems on different chromosomes. A second reverse primer was created 22 bp downstream, in an attempt to eliminate the non–chromosome 15 polymor-

Table 1

New Chromosome 15 Microsatellites

Locus	Primer Sequences	Size (bp)	Heterozygosity
D15S541	F: GCATTTTTGGTTACCTGTATG R: GTCTTCCAGGTTTATGGTTGTC	~150	72%
D15S542	F: AGCAGACTCCGGAACCTCATC R: CCTGCCTTCTTGCTGGGGCTG	~140	87%
D15S543	F: GCTGTGTTCACTTTCCAGAG R 1: GCTGTGATCTGTTTCAACAGAG R 2: GGCGCCATGAGTGTTTGTCG	~140	61%

phism, but both loci were still amplified (table 1). Analysis of a monochromosomal hybrid mapping panel (Ledbetter et al. 1990) demonstrated that the "upper" polymorphism mapped to chromosome 16 and that the "lower" polymorphism mapped to chromosome 15 (data not shown). Previous reports have identified duplicated regions between chromosomes 15 and 16 (Buiting et al. 1992; Nagaoka et al. 1994; Tomlinson et al. 1994). The chromosome 15 polymorphism has been given a designation of "D15S543," with an observed heterozygosity of 61%.

Gene-Centromere Mapping with Ovarian Teratomas

A variety of genetic studies require the determination of the meiotic origin of nondisjunction, for which polymorphic genetic markers that map close to the centromere are necessary. Although conventional genetic mapping in reference pedigrees can be used for such centromeric mapping, the lack of known and easily assayable centromeric polymorphisms, such as that for chromosome 15, hamper this approach. Instead, gene-centromere mapping in nondisjunction products, such as in benign ovarian teratomas, for which every centromere is informative, is easily implemented (Chakravarti et al. 1989). Only those ovarian teratomas that arise by whole-genome meiosis I (type I) or II (type II) nondisjunction are useful for mapping purposes; ovarian teratomas that arise by whole-genome endoreduplication or by mitotic proliferation cannot be used for gene-centromere mapping (Chakravarti et al. 1989).

Human ovarian teratomas are parthenogenetic, usually have a normal 46, XX karyotype, and, in >70% of cases, arise by whole-genome nondisjunction at meiosis I or II (Surti et al. 1990). Type I teratomas have heterozygous centromeres; genetic markers close to the centromere are also heterozygous unless crossing-over occurs, in which case markers distal to the chiasma are reduced to homozygosity 50% of the time. Type II teratomas have homozygous centromeres, as do genetic markers close to it; crossing-over maintains markers as heterozygous distal to the chiasma. Quantitative methods have been developed that allow the use of information on heterozygosity at marker loci to map them relative to the centromere and with respect to each other (Chakravarti et al. 1989; Deka et al. 1990; Mutirangura et al. 1993*a*).

PCR analysis on the marker loci D15S541, D15S542, and D15S543 was performed to genotype 24 informative type I and 41 informative type II benign ovarian teratomas, as well as their corresponding hosts. Since D15S541 and D15S542 were developed from the same ~1-kb fragment and showed an identical heterozygosity/homozygosity pattern in all teratomas that were jointly informative, they were haplotyped. Genotype data on D15S541/542 and D15S543, as well as our previously published data on D15S11, D15S113 and GABRB3 (Mutirangura et al. 1993*a*), were combined and jointly analyzed for relative distances from the centromere. For constructing the map, we used the results of physical mapping, which suggests the order 15cen–D15S541/542–D15S543–D15S11–D15S113–GABRB3. Based on this map, table 2 presents the female genetic distances (in cM) and the associated multipoint lod scores (see Materials and Methods in Mutirangura et al. 1993*a*).

The results show that the new markers D15S541/542 and D15S543 map very close to each other (distance 2.7 cM; lod score 6.58) but that, in genetic terms, D15S541/542 is at a considerable distance from the chromosome 15 centromere (distance 12 cM; lod score 3.98). Not unexpectedly, D15S11, which is ~1 Mb distal to D15S543, shows no detectable recombination with D15S543. The other genetic distances are not significantly different from those published by us earlier (Mutirangura et al. 1993*a*). It is still surprising that D15S541/542, which is physically close to the chromosome 15 centromere, demonstrates a genetic distance of 12 cM. Our previous mapping showed D15S11 to be 13 cM distal to the centromere: the current data show the same interval to be 14.7 cM—a difference that is not statistically significant. These mapping data suggest that there may be a female recombination hotspot in the pericentromeric long arm of human chromosome 15.

Table 2

Gene-Centromere Mapping of Chromosome 15 Polymorphisms in Ovarian Teratomas

Marker Locus[a]	No. of Informative Teratomas/No. of Heterozygous Teratomas: Type I (n = 24)	Type II (n = 41)	Multipoint Lod Score[b]	Map Distance[b] (cM)
15cen	24/24	41/0	...	
D15S541/D15S542 (IR39)	20/19	35/9	3.98	12.0 ± 3.1
D15S543 (ML34)	8/7	25/8	6.58	2.7 ± 2.2
D15S11 (4-3R)	18/16	26/7	7.28	.0
D15S113 (LS6-1)	16/14	31/13	6.77	2.3 ± 2.0
GABRB3	18/16	32/11	7.82	.0

[a] Loci are arranged in their presumed physical order relative to the centromere.

[b] For the three intervals 15cen-(S11), (S11)-(S113), and (S113)-GABRB3; data were estimated from all teratomas, by likelihood analysis as described in Patients and Methods.

Analysis of PWS/AS Deletion Patients

The three microsatellites were used to analyze the position of the proximal deletion breakpoint on 86 PWS or AS patients known to be deleted from D15S11 through GABRB3 (Kuwano et al. 1992; Robinson et al. 1993*b*; Zackowski et al. 1993). Figure 2 shows examples of the genotype and family data on two PWS and two AS patients, for the three loci. Of 86 patients (53 PWS and 33 AS) analyzed for D15S541/D15S542, 80 were informative for at least one of the two markers. The data on the informative patients confirmed the presence of two classes of deletion as described by Knoll et al. (1990) (table 3). Overall, 44% (35/80) were deleted for S541/S542, indicating that the deletion breakpoint is proximal to this locus and similar to class I (fig. 2). The other 56% (45/80) were heterozygous at S541/S542, indicating that the deletion breakpoint in this group is distal to this locus. Comparison of the PWS patients versus the AS patients (table 3) showed no significant differences, in relative frequency of the two breakpoint regions, between paternally and maternally derived deletions.

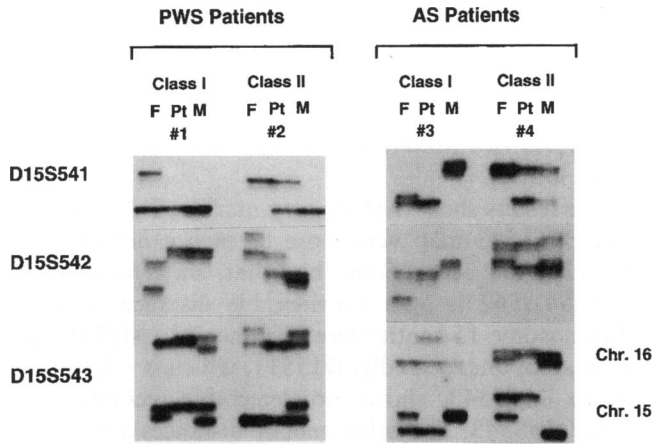

Figure 2 Microsatellite analysis. Representative data for S541, S542, and S543 are illustrated for four families; F = father; Pt = patient; and M = mother. *A*, PWS deletion patients. In patient 1, S541 and S543 were uninformative, while S542 showed a paternal deletion, indicating class I. Patient 2 was uninformative for S543, while S541 and S542 were both informative for biparental inheritance, indicative of class II. *B*, AS deletion patients. Patient 3 was fully informative for all three markers, showing a maternal deletion for S541, S542, and S543, indicative of class I. Patient 4 was also fully informative for all three markers, showing biparental inheritance for S541 and S542 with a maternal deletion for S543, indicative of class II.

A subset of 48 patients (21 PWS and 27 AS) were analyzed for D15S543. Of these, 13/48 were informative for a deletion of this locus, while the remaining 35/48 demonstrated only a single allele (fig. 2). No patients were heterozygous for D15S543, suggesting that this marker is deleted in the majority of PWS and AS cases. This result was unexpected on the basis of the previous FISH data, which had suggested that the right end of y254B5, used to identify c512 from which S543 was derived, extended proximally across the deletion breakpoint for class II patients (Kuwano et al. 1992). For 8/10 patients previously analyzed by FISH, DNA was available for microsatellite analysis on the patient and both parents. Of five informative cases, two were in class I and represented discrepancies with the YAC FISH results. These two patients were deleted for S541/S542 and had a single allele for S543 but had previously shown a positive FISH signal for y254B5 of reduced intensity on the deleted homologue relative to the normal homologue. In an attempt to clarify this discrepancy, FISH was performed by using c512, from the proximal end of y254B5, on the one patient available for study (data not shown). This result showed a dele-

Table 3

PWS/AS Deletion Data

	PWS	AS	Total
Class I	20 (42%)	15 (47%)	35 (44%)
Class II	28 (58%)	17 (53%)	45 (56%)
Total	48	32	80

tion for c512, indicating that the original YAC FISH result was a "false positive." Possible explanations for such a false-positive FISH result are discussed below (see Discussion). The three class II patients were heterozygous (not deleted) at S541/S542, but they had a single allele at S543, consistent with a deletion at this locus. Since S543 is contained within c512, this result suggests that the class II breakpoint may also be proximal to y254B5 and not contained within the YAC as originally proposed. Further FISH evaluation, using c512 and other individual cosmid clones in this region, is currently underway to clarify the precise location of the class II breakpoint in relation to y254B5.

Discussion

Although many molecular markers have been developed within the PWS/AS region, and although most of this region has been cloned in a YAC contig, there is still a paucity of genetic markers in the most proximal region of chromosome 15. In this study, three dinucleotide-repeat polymorphisms proximal to D15S11 have been developed to allow further characterization of this region. Our previous estimate of the genetic distance between the centromere and S11 was 13.0 ± 3.2 cM, on the basis of ovarian teratoma mapping (Mutirangura et al. 1993*a*). The current genetic maps available for chromosome 15 contain no mapping data proximal to S11. On the EUROGEM genetic map of chromosome 15, when the CEPH reference families are used, S128 is the most proximal marker utilized (Spurr et al. 1994). We have mapped S128 very close to SNRPN by using our YAC contig, placing S128 between S11 and S113 (authors' unpublished results). Beckman et al. (1993) generated a genetic map of 55 microsatellite markers by using S11 as the most proximal marker. The markers described here were analyzed by using the same panel of ovarian teratomas as were used in our previous study (Mutirangura et al. 1993*a*), and genetic distances were recalculated. These data place S541/S542 at 12.0 cM and S543/S11 at 14.7 cM from the centromere, making them the most proximal markers currently available for chromosome 15 (table 2).

Although there is no direct measurement of the physical distance between the centromere and S11, the cytogenetic distance is estimated to be much less than one metaphase band (<5 Mb). The observed genetic distance of 12.0 cM from the centromere to S541/S542 was unexpectedly large, suggesting the presence of a female recombination hotspot in the pericentromeric long arm of human chromosome 15. High male meiotic recombination in proximal chromosome 15q is also inferred from cytological observations of meiotic chiasmata (Saadallah and Hultén 1983). These data represented an exception to the common assumption that recombination is decreased at human centromeric regions. Further efforts are needed to complete the physical contig from S541/S542 to the centromere and to identify additional polymorphic markers within this region. It will be of interest to further refine the mapping information regarding the relationship of genetic and physical map distances in this pericentromeric region and to compare this region with the pericentromeric region of other human acrocentric and nonacrocentric chromosomes.

In addition to their utility as the most proximal markers on the genetic map for linkage studies, these markers will be useful in the molecular characterization and diagnosis of PWS and AS patients. Because of their close proximity to the centromere, they will be particularly useful in distinguishing between meiosis I and meiosis II nondisjunction errors in the origin of uniparental disomy 15. For deletion patients, previous molecular data defining the proximal and distal deletion breakpoints have been limited. In a study of 13 patients, Knoll et al. (1990) defined two major classes of deletion in AS, on the basis of different proximal deletion breakpoints. Class I patients (6/13) showed deletions for D15S18 and D15S9 through D15S13, while class II patients (7/13) were deleted for only D15S9–D15S13. Also using RFLP analysis, Robinson et al. (1993*b*) demonstrated that 4/10 PWS patients were deleted for D15S9 but not for D15S18, consistent with class II above. Microsatellite analysis using S541/S542 has allowed the confirmation of these two classes of deletion breakpoints by using a much larger sample of both PWS and AS patients and has provided data indicating no difference in frequency of these classes in the two disorders. This is consistent with the same mechanism of deletion being involved in both paternal and maternal deletions of chromosome 15.

In a previous study using FISH with YAC clones from this region, we obtained results suggesting a precise localization of a common breakpoint within the single YAC clone y254B5 in seven PWS and three AS patients, which would be consistent with the class II characterization given by Knoll et al. (1990). In each of these 10 deletion patients, y264A1 (fig. 1*A*) was completely deleted by FISH. The overlapping proximal clone, y254B5, showed positive hybridization to both chromosome 15 homologues, but with significantly reduced intensity on one homolog compared with the other. This was inter-

preted as a partial deletion of the YAC clone, implying that the breakpoint in all 10 patients was contained within this single YAC. Eight of these patients had parental bloods available and were reanalyzed by using S541, S542, and S543. Of the five informative cases, two patients showed class I breakpoints and represent obvious discrepancies with the previous FISH results. Recent FISH studies on one of these patients, with cosmid clones, are consistent with the microsatellite data and indicate that the interpretation of the original YAC FISH data was incorrect. The three other patients remain classified as class II breakpoints; however, results of analysis of them were consistent with a deletion at S543. Their breakpoints are therefore likely to be proximal to y254B5 and not contained within this YAC as originally proposed.

The most likely explanation for the "false positive" result by FISH with y254B5 is the presence of a low-copy repeat sequence within the YAC, which cross-hybridizes to homologous sequences located more proximally on 15q. A complete deletion of the YAC sequences would show a reduced signal by FISH, corresponding to the cross-hybridization to the homologous sequences. Evidence for the presence of numerous repeated sequences in proximal 15q was first demonstrated by Donlon et al. (1986) using 15q11-q13 specific markers isolated from flow-sorted inv dup(15) chromosomes, where DNA segments were difficult to clone in this region when propogated in recombination-proficient hosts. In addition, Horsthemke and colleagues (Buiting et al. 1992) have described a gene family with multiple loci on chromosome 15 and 16, which could contribute to the instability in this region.

There are other examples of human chromosome rearrangements in which low-copy repeat sequences predispose to abnormal chromosome pairing and unequal crossing-over, producing deletion and duplication events (Ledbetter and Ballabio 1995). For example, X-linked ichthyosis due to steroid sulfatase (STS) deficiency is characterized by a high frequency (>85%) of submicroscopic deletions of the STS gene at Xp22.3, involving interspersed low-copy repeats (Ballabio et al. 1990; Yen et al. 1990; Ballabio and Andria 1992). Another important example is chromosome 17, in which reciprocal duplication and deletion events involving a 1.5-Mb segment of 17p11.2-p12 lead to Charcot-Marie-Tooth disease type 1A (CMT1A) and hereditary neuropathy with liability to pressure palsies (HNPP), respectively (Pentao et al. 1992; Chance et al. 1994). In this case, a large repeated DNA sequence is normally present in two copies located 1.5 Mb apart on chromosome 17, but unequal meiotic recombination leads to duplication or deletion of this segment. The high frequency of interstitial deletions of chromosome 15 and clustering of breakpoints into two defined regions on the proximal side of the deletion suggests that there will be a specific predisposing molecular mechanism for this deletion event. It is also of interest to determine whether patients with inv dup(15) have breakpoints similar to those of class I and/or class II deletion patients and involve a similar predisposing mechanism. Completion of the physical map across these two breakpoint regions will facilitate the molecular characterization of these common chromosomal rearrangements.

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