

# Interstitial Duplications of Chromosome Region 15q11q13: Clinical and Molecular Characterization

Gabriela M. Repetto,<sup>1</sup> Lisa M. White,<sup>1</sup> Patricia J. Bader,<sup>2</sup> Debra Johnson,<sup>3</sup> and Joan H.M. Knoll<sup>1,4\*</sup>

<sup>1</sup>Division of Genetics, Children's Hospital, Boston, Massachusetts

<sup>2</sup>Indiana Genetics Clinic, Parkview Memorial Hospital, Fort Wayne, Indiana

<sup>3</sup>Health Partners, Minneapolis, Minnesota

<sup>4</sup>Department of Pathology, Beth Israel-Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts

Duplications of chromosome region 15q11q13 often occur as a supernumerary chromosome 15. Less frequently they occur as interstitial duplications [dup(15)]. We describe the clinical and molecular characteristics of three patients with de novo dup(15). The patients, two males and one female (ages 3–21 years), had nonspecific findings that included autistic behavior, hypotonia, and variable degrees of mental retardation. The extent, orientation, and parental origin of the duplications were assessed by fluorescent in situ hybridization, microsatellite analyses, and methylation status at D15S63. Two patients had large direct duplications of 15q11q13 [dir dup(15)(q11q13)] that extended through the entire Angelman syndrome/Prader-Willi syndrome (AS/PWS) chromosomal region. Their proximal and distal breaks, at D15S541 or D15S9 and between D15S12 and D15S24, respectively, were comparable to those found in the common AS/PWS deletions. This suggests that duplications and deletions may be the reciprocal product of an unequal recombination event. These two duplications were maternally derived, but the origin of the chromatids involved in the unequal crossing over in meiosis differs. In one patient, the duplication originated from two different maternal chromosomes, while in the other patient it arose from the same maternal chromosome. The third patient had a much smaller duplication that involved only D15S11 and parental origin could not be determined. There was no obvious correlation between pheno-

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## INTRODUCTION

Rearrangements involving the imprinted chromosome region 15q11q13 are common, suggesting increased instability. Deletions of this segment can be maternally or paternally derived and account for most findings in patients with Angelman or Prader-Willi syndromes (AS/PWS), respectively [Knoll et al., 1989; Hamabe et al., 1991; Robinson et al., 1991; Beuten et al., 1993; Chan et al., 1993; Zackowski et al., 1993; Woodage et al., 1994]. Translocations, inversions, and duplications and inversions in this region have also been described [see as examples Knoll et al., 1993a; Schinzel et al., 1994; Horsthemke et al., 1995; Greger et al., 1997]. The duplications include supernumerary dicentric chromosomes 15 [dic(15;15)] and interstitial duplications. Small dic(15;15) chromosomes have breakpoints similar to the proximal breakpoints of the common AS/PWS deletions, between the centromere and D15S541/D15S542/D15S18 or between the latter and D15S543/D15S9. The large dic(15;15) chromosomes frequently have breakpoints similar to the distal deletions; i.e., between D15S12 and D15S24 [Knoll et al., 1990; Cheng et al., 1994; Christian et al., 1995; Crolla et al., 1995; Huang et al., 1997]. Individuals with small dic(15;15) as the only abnormality have a normal phenotype, while those with large dic(15;15) containing additional copies of proximal chromosome 15q usually have mental retardation with clinical findings distinct from AS or PWS [Cheng et al., 1994; Leana-Cox et al., 1994; Blennow et al., 1995]. Interstitial duplications of 15q11q13 [dup(15)] and triplications [Schinzel et al., 1994] have been described less frequently than supernumerary duplications. Dup(15) chromosomes have been reported in karyotypes of in-

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\*Correspondence to: Joan H.M. Knoll, Ph.D., Children's Hospital, Division of Genetics, 300 Longwood Ave., Boston, MA 02115. E-mail: jknoll@bidmc.harvard.edu

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dividuals with normal and abnormal phenotypes [Ludowese et al., 1991], while those with confirmed molecular duplications are generally associated with abnormal clinical findings [Clayton-Smith et al., 1993; Baker et al., 1994; Bunday et al., 1994; Browne et al., 1997; Cook et al., 1997; Woods et al., 1997]. This apparent discrepancy is most likely due to the occurrence of a cytogenetic heteromorphism in proximal 15q [Knoll et al., 1993b].

Molecular data on the extent of interstitial dup(15) chromosomes are limited. These abnormalities have been partially characterized in only a few patients. In this study, we have examined the extents and parental origin of interstitial duplications in three additional patients. We describe their clinical and molecular findings and demonstrate that duplications may arise by more than one mechanism.

## SUBJECTS AND METHODS

The three patients in this study were diagnosed with 46,XX or XY, dup(15)(q11q13) during evaluation for developmental delay and/or autistic behavior. GTG-banding resolution was at the 550-band level. Information regarding the patients' phenotype was obtained from clinical records, examination of photographs, and questionnaires completed by guardians and/or physicians. Peripheral blood samples were obtained from the patients, their parents, and the healthy brother of one patient.

### Cytogenetic and Molecular Studies

Duplications of 15q11q13 in the patients (JK326, JK339, JK439) were initially determined by GTG-banding [Seabright 1972]. The extent and orientation of the interstitial duplication were assessed by fluorescent in situ hybridization (FISH) with probes from the chromosome 15q11q13 region. The probes included cosmid clones D15S11, SNRPN, and GABRB3 (Oncor, Inc., Gaithersburg, MD), bacteriophage clones 34-10 (D15S9), JP3 (D15S63), 16 $\beta$ 3 (GABRB3), and 10-1-45 (D15S12) [Knoll et al., 1993a], and YACs 245B5(D15S9), 93C9 (D15S12), and B94H7 (D15S24) [Kuwano et al., 1992]. The probes were labeled with digoxigenin-16-dUTP or biotin-11-dUTP by nick translation and hybridized as previously described [Knoll and Lichter, 1994]. The YAC clones were inter-Alu amplified before nick translation [Baldini et al., 1992; Tagle and Collins, 1992]. Digoxigenin was detected with anti-digoxigenin-rhodamine antibody and biotin with avidin-FITC. Chromosomes were counterstained with 4', 6-diamino-2-phenylindole (DAPI) and viewed with an epifluorescence microscope equipped with triple- and single-band pass filters. At least ten hybridized metaphase cells were scored for the presence of tandem signals on a single chromosome 15. The normal chromosome 15 served as control and the presence of tandem signals on a chromosome was interpreted as a duplication of the region recognized by that probe. The orientation of the duplicated sequences was determined by two probe-two color FISH [Knoll and Lichter, 1994]. The color sequence patterns of 20 or more interphase cells with simultaneous dual probe hybridization

were scored to determine if the duplication was direct (red/green/red/green) or inverted (red/green/green/red).

### Parent of Origin

DNA was extracted from peripheral lymphocytes using the Puregene kit (Gentra, Inc., Research Triangle Park, NC). The parental origin of the duplication was determined by microsatellite analysis at multiple 15q11q13 loci and/or methylation analysis at D15S63. DNA was amplified by polymerase chain reaction (PCR) using radioactively end-labeled primers. PCR products were separated by polyacrylamide denaturing gel electrophoresis and detected by autoradiography [Albright and Slatko 1994]. Loci for microsatellite analysis included D15S541 (no. 328759), D15S18 (no. 190060), D15S543 (no. 340835), D15S11 (no. 190026), D15S128 (no. 188608), D15S210 (no. 200358), D15S113 (no. 355120), GABRB3 [Mutirangura et al., 1993], 155-CA [Glatt et al., 1994], D15S156 (no. 189402), D15S219 (no. 214861), D15S97 (no. 182257), D15S217 (no. 686877), and D15S165 (no. 191034).

Methylation analysis at locus D15S63 was performed when microsatellite analysis was not informative as to parental origin. DNA from patient JK439 and control individuals was digested with HindIII and HpaII, separated by agarose gel electrophoresis, and analyzed by Southern blot hybridization with radiolabeled probe PW71B (D15S63) [Dittrich et al., 1993]. The HpaII restriction site at D15S63 is methylated and not cut on the maternally inherited chromosome and results in a 6.0-kb HindIII-HpaII fragment. It is unmethylated and cut on the paternally inherited chromosome, resulting in a 4.4-kb HindIII-HpaII fragment. Normal individuals have 4.4- and 6.0-kb fragments of similar intensity. An increase in the intensity of one fragment relative to the other is consistent with the presence of an increased number of copies derived from that parent.

## RESULTS

### Clinical Findings

The clinical findings of these patients are summarized in Table I and compared with other cases with molecularly characterized duplications.

Case JK326 is a 3-year-old girl born at term after a normal pregnancy to a 23-year-old gravida 2 mother and a 27-year-old father. She had normal birth weight, length, and head circumference (OFC). At age one year, her OFC was at the 3rd centile and she had hypotonia, unusual hand movements, and global developmental delays. A muscle biopsy at age one year showed mild mitochondrial proliferation. Urine organic acids and plasma and urine amino acid profiles were normal. She had a normal head MRI and EEG. At age 3 years, she remains hypotonic, does not sit, has no recognizable words, has unprovoked laughter, and frequent hand-waving movements. No seizure activity has been reported.

Case JK339 is a 21-year-old man born at term to 22-year-old parents after a normal pregnancy. He was noted in early childhood to be hypotonic and to have global developmental delays, hand waving, and echolalia. He was diagnosed with autism at age 3 years. No

TABLE I. Manifestations of Patients With Dup(15) Characterized by Molecular Methods

Patient	JK326	JK339	JK439	Others <sup>a</sup> (N = 6)
Sex	F	M	M	2M, 4F
Age (years)	3	21	8	6–14
Mental retardation/developmental delay	+ <sup>b</sup>	+	+	6/6
Autism/autistic like	+	+	+	5/5
Seizures	– <sup>c</sup>	–	–	3/5
Speech delay	+	+	+	4/4
Unprovoked laughter	+	–	+	
Involuntary hand movements	+	–	+	1/3
Growth	Normal	Normal	Normal	3/3 Normal
Minor anomalies	–	–	–	1/6 (Minor)
Hypotonia	+	+	+	2/3 (1 with hypertonionia)
Parental age (maternal/paternal)	23/27	22/22	21/23	23–33/29–34
Extent of duplication	D15S11	D15S541–D15S12	D15S9–D15S12	See loci below
Parental origin	Not informative	Maternal	Maternal	Maternal in 4/4

<sup>a</sup>Clayton-Smith et al. [1993] (D15S10), Baker et al. [1994] (D15S11), Bunday et al. [1994] (D15S11 to D15S24), Schinzel et al. [1994] (triplication of D15S18 to D15S24), Cook et al. [1997] (D15S63 to D15S217). These loci represent minimum extent.

<sup>b</sup>Feature present.

<sup>c</sup>Feature absent.

seizure activity has been observed, but he had an abnormal EEG with focal left hemispheric discharges at age 19 years.

Case JK439 is an 8-year-old boy, born at term to a 21-year-old mother and a 23-year-old father. He had normal birth weight and length. At age 6 years, he was diagnosed with pervasive developmental disorder. He was noted to be hypotonic, has frequent hand-flapping movements, bouts of unprovoked laughter, and a normal OFC. No seizures have been observed.

The mean parental age at birth of the patients was  $22 \pm 1.0$  years for the mothers and  $24 \pm 2.6$  years for the fathers.

### FISH

All patients were found to have a de novo interstitial duplication of chromosome region 15q11q13 by FISH. The data are presented in Table II. Representative examples of duplication by FISH are shown in Figure 1. Patient JK326 has a small duplication involving only D15S11 but not D15S9, SNRPN, GABRB3, or D15S63. Patients JK339 and JK439 have large duplications that encompass the common AS/PWS region and include D15S9, D15S11, JP3, SNRPN, GABRB3, and D15S12, but not D15S24 as a single hybridization was observed on each chromosome 15 with YAC B94H7. The duplications were oriented in tandem in these two patients. The probe orders observed in two color–two probe hybridizations in greater than 80% of interphase cells were 34-10(D15S9)/10-1-45(D15S12)/34-10/10-1-45 and 254B5(D15S9)/GABRB3/254B5/GABRB3 for JK339 and JK439, respectively. The orientation in pa-

tient JK326 could not be determined because only D15S11 was duplicated.

### Parent of Origin

The results are summarized in Table III. Microsatellite analysis was informative for patient JK339 only. Three distinct alleles were detected at D15S541, D15S113, D15S18, GABRB3, D15S156, and D15S217. The latter four loci showed the presence of two maternal alleles and one paternal allele. Microsatellite analysis for D15S217 is shown in Figure 2. The presence of three alleles at D15S541 showed that the duplication extended proximal to D15S9 as had been determined by FISH. DNA polymorphisms were not informative for JK439. Only two distinct alleles (one maternal and one paternal) could be observed, despite parental heterozygosity at D15S543, D15S11, D15S128, D15S156, and D15S217 (Fig. 2). D15S210, 155-CA (GABRB3), and D15S97 were not informative for JK439 and are not included in Table III. Quantitative PCR analysis of the alleles was not used to infer the presence of a duplication or its origin. Parental origin in this patient was determined by methylation analysis at D15S63. Like JK339, this duplication was also maternally derived. The maternally derived 6.0-kb fragment was present with greater intensity than the paternally derived 4.4-kb fragment as compared with controls (not shown). The origin of the small duplication in JK326 could not be determined because microsatellite analysis at D15S11 was not informative and the duplication did not extend into the methylation-sensitive regions. A schematic of the molecular extents

TABLE II. FISH Results: Number of Hybridization Signals on Normal + Duplicated Chromosomes (15)

	Probe: 254B5 *Locus: D15S9	34-10 D15S9	D15S11 D15S11	JP3 D15S63	SNRPN SNRPN	16β3 GABRB3	GABRB3 GABRB3	10-1-45 D15S12	93C9 D15S12	B94H7 D15S24
JK326	—	1 + 1	1 + 2	1 + 1	1 + 1	1 + 1	—	1 + 1	—	—
JK339	—	1 + 2	1 + 2	1 + 2	1 + 2	—	1 + 2	1 + 2	1 + 2	1 + 1
JK439	1 + 2	1 + 2	1 + 2	1 + 2	1 + 2	—	1 + 2	1 + 2	1 + 2	1 + 1

\*Loci are listed from centromere to telomere (left to right); dash indicates probe not tested.

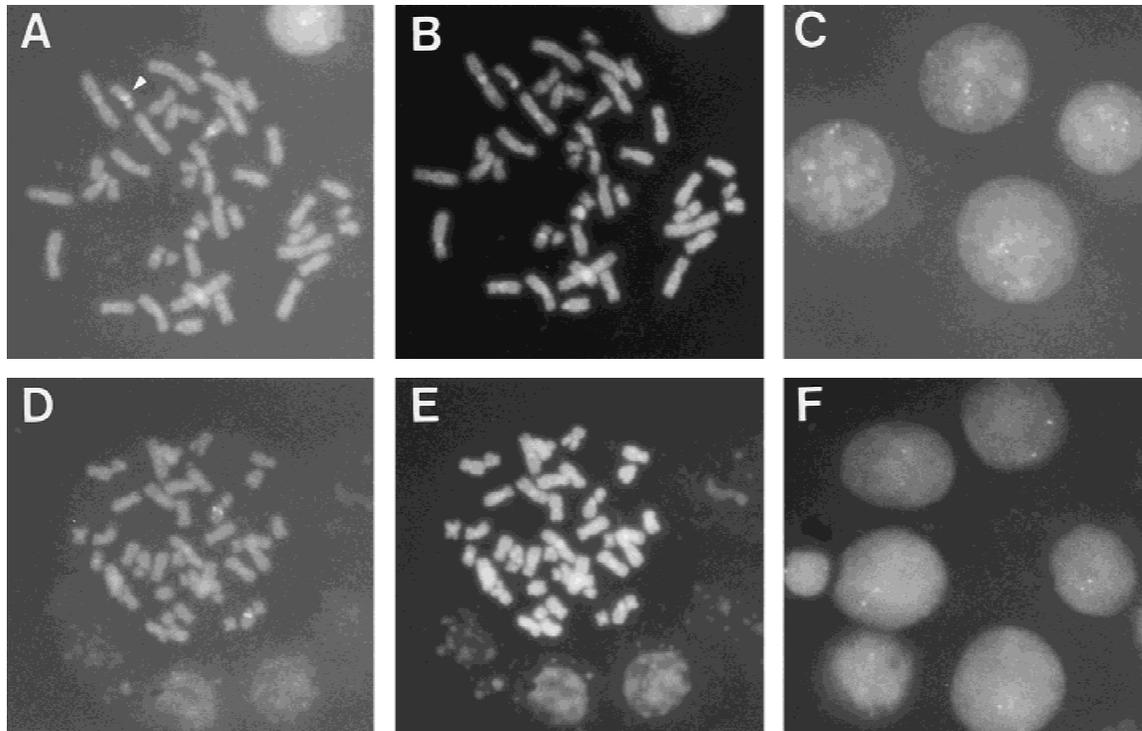


Fig. 1. FISH showing normal and duplicated signals on chromosomes 15. **A:** Metaphase cell from patient JK439 hybridized with probe GABRB3 showing the presence of the duplication (arrowhead). **B:** Corresponding DAPI image. **C:** Interphase nuclei from JK439 demonstrating the duplication of GABRB3. **D:** Metaphase cells from patient JK326 with probe SNRPN showing a normal pattern with one copy per chromosome 15. **E:** Corresponding DAPI image. **F:** Interphase nuclei of JK326 with probe SNRPN showing two copies per cell.

of the duplications that combines FISH and microsatellite data is presented in Figure 3.

**DISCUSSION**

We report on the clinical and molecular findings in three individuals with de novo interstitial duplications of 15q11q13. All patients had an abnormal phenotype. Their clinical findings are summarized in Table I and compared with other cases with molecular duplications or triplications. No major congenital anomalies were present; however, the patients had global developmental delay or mental retardation, autism or autistic-like behavior, hypotonia, normal growth, and no evidence of obesity. Autism and/or mental retardation have also been described in the patients recently reported by Woods et al. [1997] and Browne et al. [1997]. Micro-

cephaly was observed in JK326. Seizures were not observed in our patients but have been reported in others [Baker et al., 1994; Bunday et al., 1994; Schinzel et al., 1994] and some other individuals had abnormal EEGs (JK339) [see also Cook et al., 1997]. Other frequently occurring manifestations included echolalia, unprovoked laughter, and frequent hand movements. Mean maternal age was 22 years in our patients and 28 years in others reported.

JK326 had a small duplication encompassing only D15S11 while JK339 and JK439 had duplications encompassing the common AS/PWS deletion region (i.e., D15S541/proximal of D15S9 through D15S12). There was no obvious correlation between the size of the interstitial duplication and the severity of the clinical findings. The occurrence of a similar phenotype in the

TABLE III. Microsatellite Data

Subject	*D15S541	D15S18	D15S543	D15S11	D15S128	D15S113	GABRB3	D15S156	D15S219	D15S217	D15S165
JK326 (patient)	—	—	b	ab	ab	ad	bd	a	b	—	ab
JK325 (mother)	—	—	bc	b	ac	cd	cd	a	ab	—	b
JK324 (father)	—	—	ab	ab	b	ab	ab	ab	b	—	a
JK339 (patient)	abc	<b>cdb</b>	ac	ab	ab	abc	<b>abd</b>	<b>abc</b>	ab	<b>cdb</b>	ab
JK340 (brother)	ab	cb	bc	b	bc	ac	—	ab	ab	cb	ab
JK338 (mother)	bc	cd	c	b	ab	ac	ab	bc	ab	cd	a
JK337 (father)	ab	ab	ab	ab	bc	ab	cd	a	b	ab	b
JK439 (patient)	—	ac	bc	b	cd	ac	ab	b	—	bc	ab
JK438 (mother)	—	bc	cd	bc	ac	ac	b	bc	—	cd	ab
JK437 (father)	—	a	ab	ab	bd	ab	a	ab	—	ab	a

\*Loci are listed from centromere to telomere. Bold face indicates informative loci, and a dash indicates not tested.

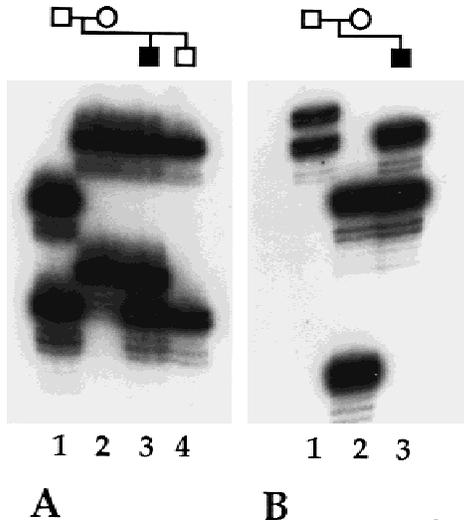


Fig. 2. Microsatellite analysis at locus D15S217 in families of JK339(A) and JK439(B). The duplication is of maternal origin in JK339 (A, lane 3) as determined by the presence of two maternal alleles and one paternal allele. The origin of the duplication in JK439 (B, lane 3) was not informative because only two alleles were observed despite parental heterozygosity. Therefore, this duplication was derived from one parental chromosome and not from both as in JK339.

patient with the smaller duplication as compared with those with the larger duplications may define the critical region for the constellation of anomalies. This duplication may be coincidental with the clinical findings,

but alterations in copy number of D15S11 have not been reported in normal individuals. Further characterization of the duplication in JK326 may help elucidate genes on chromosome 15 involved in the pathogenesis of autism and/or the other manifestations. Data suggesting linkage of autism to more distal 15q11q13 markers have been reported [Pericak-Vance et al., 1997]. Necdin, an imprinted gene near D15S11, was identified recently [McDonald and Wevrick, 1997]; whether this gene is altered in JK326 remains to be determined.

Patients JK339 and JK439 had larger duplications that extended from D15S541 through D15S12 and from at least D15S9 through D15S12, respectively. The exact proximal extent in JK439 could not be determined since the duplication was derived from a single homolog and microsatellite analysis was therefore not informative. The two proximal and the single distal regions of breakage are similar as those observed for the common AS/PWS deletions [Knoll et al., 1990; Christian et al., 1995]. The similar regions of breakage in the duplications and the common deletions suggest a potential relationship between the two as described in Charcot-Marie-Tooth disease type IA and hereditary neuropathy with liability to pressure palsies on chromosome 17 [Chance et al., 1994]. Misalignment of repetitive sequences has been implicated in the rearrangements that cause these two disorders [Pentao et al., 1992; Reiter et al., 1996]. Repetitive sequences such as END repeats [Amos-Landgraf et al., 1994, 1997;

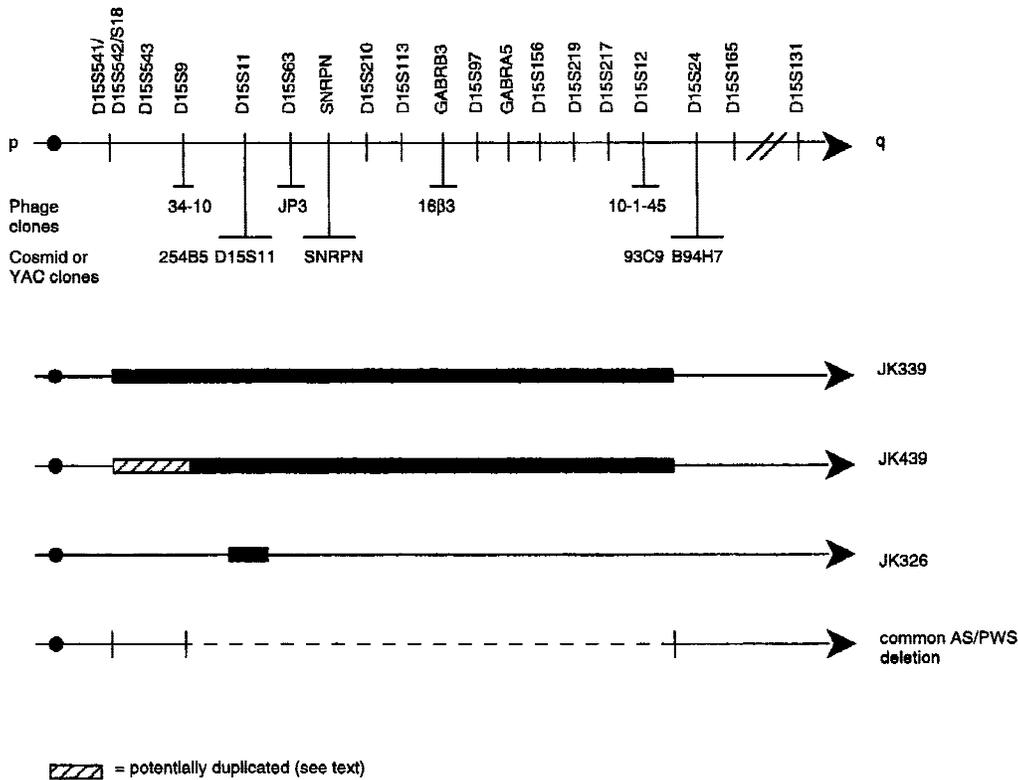


Fig. 3. Schematic showing the extent of duplications in the three individuals. The involvement of locus D15S541 in JK339 was detected by microsatellite analysis (see Table III) and it could not be ruled out in JK439 (see text). The common AS/PWS deletion region is shown for reference. Similar regions of breakage are evident.

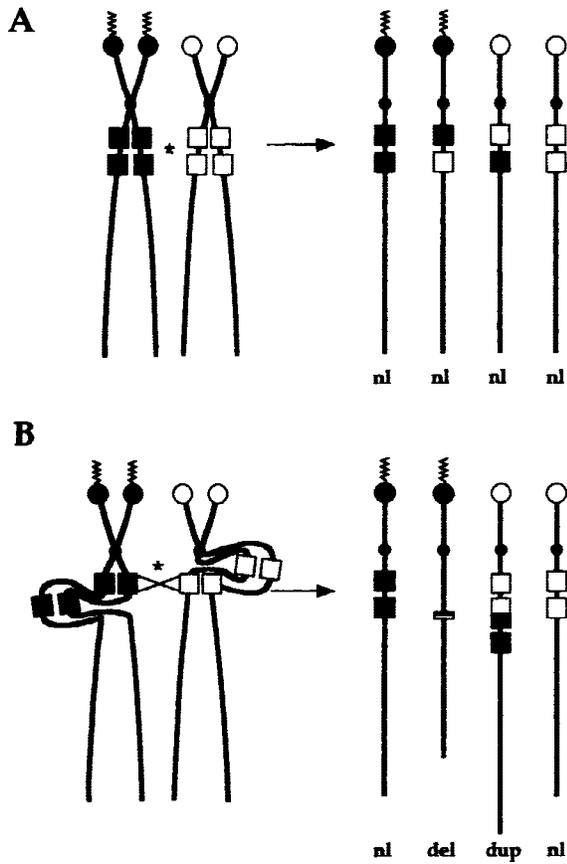


Fig. 4. Proposed mechanism of formation of interchromosomal deletions and duplications. Boxes represent proximal and distal repeat units such as END or other ones so far unidentified. **A:** Recombination between properly aligned units would result in normal homologs (nl) without loss or gain of material. **B:** Recombination between misaligned repeat units would result in a homolog with a deletion (del) and one with a duplication (dup). Nonrecombined normal homologs are also shown. Recombination between sister chromatids would result in intrachromosomal duplications and deletions. Variations in the structure of the repeat units involved could account for differences observed in duplication size (see Table I).

Christian et al., 1997] have been localized to the proximal and distal ends of the common AS/PWS deletion [see Robinson et al., 1997]. Mismatching of these repeat units followed by unequal recombination would result in one homolog with a deletion and one with a duplication as indicated in Figure 4. Such a model is consistent with the interchromosomal duplication observed in JK339. Similar events between sister chromatids could explain the intrachromosomal duplication as observed in JK439. Both inter- and intrachromosomal rearrangements have been demonstrated in the mechanism of 15q11q13 deletions in PWS [Carrozzo et al., 1997]. This model would predict similar frequencies of deleted chromosomes and duplicated chromosomes; however, significantly more cases with deletions have been reported. The apparent discrepancy in deletion versus duplication frequencies may be due to factors such as parental origin, underascertainment due to nonspecific manifestations, difficulty in detection by routine cytogenetics, instability of the duplications, and/or additional mechanisms of formation.

Individuals with maternally derived duplications

have an abnormal phenotype [Clayton-Smith et al., 1993; Baker et al., 1994; Schinzel et al., 1994; Browne et al., 1997; Cook et al., 1997; this report] while paternally derived ones do not [Browne et al., 1997, Cook et al., 1997]. Differential expression of imprinted genes are likely to account for this finding. At least seven imprinted genes have been detected in 15q11q13: ZNF127, SNRPN, PAR-5, IPW, PAR-1, and Necdin are *paternally* expressed and UBE-3A is *maternally* expressed in brain [McDonald and Wevrick, 1997; Rougeulle et al., 1997; Vu and Hoffman, 1997; see Robinson et al., 1997]. Studies of gene expression patterns in tissue samples of patients with duplications may improve our understanding of the effect of parental origin on phenotype.

Underascertainment of duplications could be also related to the referral patterns of patients with autism and subtle clinical findings. As the clinical phenotype becomes better defined and recognized, samples from more individuals may be submitted to the cytogenetics laboratory and clinical indications will prompt FISH testing of 15q11q13 for detection of duplications. Such studies are crucial in confirming the presence of a duplication since centromere proximity, differential homolog condensation, and heteromorphisms in the region make accurate interpretation by routine cytogenetics impossible [Browne et al., 1997; Mignon et al., 1997]. In clinically diagnosed AS and PWS patients, FISH testing has substantially increased the detection frequency of 15q11q13 deletions over routine cytogenetics. Undoubtedly, a similar increase will be observed for duplications of this same region.

Finally, not all duplications may be the result of unequal recombination events and thus would not have a relationship with the deletions. Some, especially smaller ones as in JK326, may result from errors in DNA replication, DNA repair, or other as of yet unidentified mechanisms. Duplications may be relatively unstable and selected against or converted to an acceptable cellular phenotype (normal or deleted) [Monnat et al., 1992]. The characterization of additional patients will improve our understanding of the genetic constitution of 15q11q13, its effects on phenotype, and the mechanism of its apparent instability.

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