Original Paper

Dvorah Abeliovich[a]
Judith Dagan[a]
Marion Werner[a]
Israela Lerer[a]
Yehuda Shapira[b]
Vardiella Meiner[a]

[a] Department of Human Genetics, and
[b] Neuropediatric Unit, Hadassah University Hospital, Hebrew University Medical School, Jerusalem, Israel

Simultaneous Formation of inv dup(15) and dup(15q) in a Girl with Developmental Delay: Origin of the Abnormal Chromosomes

Abstract

Two de novo abnormal derivatives of chromosome 15, inv dup(15) and dup(15q) were found in a girl with developmental delay and mild dysmorphological signs. Fluorescence in situ hybridization, using DNA probes of the Prader-Willi/Angelman syndromes (PWS/AS) critical region and chromosome-15-specific α-satellite, combined with molecular analysis using dinucleotide repeat polymorphisms within the PWS/AS region and the parent-of-origin specific methylation sites at the locus D15S63, shed light on how the abnormal karyotype was formed. We suggest that a translocation between the two homologues of maternal chromosomes 15 resulted in the formation of dup(15q) and two reciprocal products: an acentric fragment of 15q that was lost and a centric fragment that underwent U-type reunion to form inv dup(15).

Introduction

Patients with duplication of the proximal long arm of chromosome 15 have been reported in several publications [1], and inv dup(15) which is used to define the psu dic(15;15) marker chromosomes is one of the common findings in patients with a supernumerary chromosome [2, 3]. Recently, as a consequence of the wide application of the fluorescence in situ hybridization (FISH) technique and the molecular definition of the

Fig. 1. The patient at the age of 4 years.

Prader-Willi/Angelman syndromes (PWS/AS) critical region in 15q11–13, new interest has emerged in these chromosomal aberrations, both of which can be associated with either PWS or AS. It was suggested that the high frequency of inverted repeat sequences in 15q11–13 [4] are involved in an unequal crossing-over between two chromosomes 15 and that in intrachromosomal crossing-over, it results in inv dup(15), whereas interchromosomal crossing-over results in deletion or duplication of the proximal 15q [2].

In a mildly retarded girl with minor dysmorphological signs, we detected two de novo abnormal derivatives of chromosome 15; psu dic(15)(pter →q11.1::q11.1→pter), also designated inv dup(15), and dup(15)(q11.2q12).

Molecular analysis of the patient and her parents allowed us to draw a model of the origin of the abnormal chromosomes.

Patient and Methods

The Patient
The proband, a girl, was born as the fifth child to healthy parents; the mother was 28 years old and the father was 29 years old. The pregnancy was uneventful, although, in retrospect, a decrease in fetal movements was noticed. Delivery was induced because the child was after term; birth weight was 3,700 g. At the age of 6 months, hypotonia and motor delay were noted. At the age of 1 year she developed a convulsive disorder. There was a delay in the developmental milestones; she sat at the age of 1 year and walked at 3 years. Impaired cognition was also present, with poor language development. Computed tomography of the brain demonstrated mild widening of the parieto-occipital sulci. A thorough metabolic workup was completely normal. The child was reviewed by us at the age of 4 years. Her height and weight were on the third percentile, her occipitofrontal circumference was less than the third percentile. The dysmorphic features that were noted included an abnormal hair growth pattern, flat nasal bridge, deep-set eyes, and prominent ears (fig. 1). She was able to vocalize single words, functioning at an 18-month level at the age of 4 years, with mild behavioral problems.

Cytogenetic Analysis
Peripheral blood lymphocytes from the patient and parents were cultured according to standard techniques [5]. For high-resolution analysis banding, methotrexate was applied to the cultures. G and C banding were performed according to the standard techniques [6]. A chromosome-15-specific α-satellite probe (Oncor) and the Prader-Willi cosmid probe A (locus D15S11) with myl cosmid (Oncor) as a marker for chromosome 15 were used in FISH according to the manufacturer's instructions.

DNA Analysis
Genomic DNA was extracted according to standard techniques. Dinucleotide repeat polymorphisms at the loci GABRB3 [7], D15S97 [8], and D15S210 [9] were analyzed as previously described [10]. Parent-of-origin-specific DNA methylation sites of the locus D15S63 were analyzed in Southern hybridization using the DNA probe PW71/*Hind*III + *Hpa*II [11] and PW71/*Bgl*II + *Cfo*I [12].

Results

The patient's karyotype included 47 chromosomes with two abnormal chromosomes (fig. 2): 15q+ apparently dup(15)(q11.2q12) and a small extra bisatellited chromosome. The supernumerary marker chromosome exhibited, in C banding, two heterochromatic regions, one constricted representing the active centromere and the other without a constriction representing the inactive centromere. FISH, using the chromosome-15-specific α-satellite DNA probe (not shown) revealed two signals on the bisatellited chromosome in addition to one signal on each chromosome 15. The bisatellited marker chromosome is thus psu dic(15) or as it is widely called in the literature inv dup(15) [3, 13]. FISH, using a cosmid probe A (locus D15S11) of the PWS region, gave two adjacent signals (fig. 3) on the chromosome 15q+, confirming the interpretation of this chromosome according to the G banding. In addition, there was one signal on the normal chromosome 15. Cosmid probe A did not hybridize to the small extra chromosome. The parents both had a normal karyotype: 46,XX and 46,XY. There was no heteromorphism that could be used to differentiate between the parental chromosomes 15.

(CA)$_n$ polymorphisms of the loci GABRB3, D15S97 and D15S210 (PWS/AS region) were fully informative in the family; both parents were heterozygous and did not share any of their alleles. For these polymorphisms, the child had three different alleles, two maternally derived and one paternally. In the (CA)$_n$ polymorphism of locus GABRB3, the mother had alleles 2/8, the father had alleles 4/6, and the child had 2/6/8 (fig. 4); in D15S210, the mother had alleles 4/5, the father had alleles 1/6, and the child had alleles 4/5/6. In the (CA)$_n$ polymorphism of locus D15S97, the mother had alleles 4/5, the father
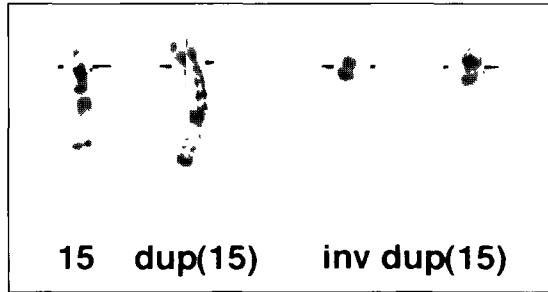
had alleles 3/6, and the child had alleles 4/5/6 (not shown). Polymorphisms of the loci D15S11, D15S113, and GABRA5 were not informative because the parents shared one of their alleles. The locus D15S63 was analyzed at two parent-of-origin-dependent methylation sites, the *Hpa*II sensitive and the *Cfo*I sensitive [11, 12]. Double digestion of genomic DNA with the restriction enzymes *Hind*III + *Hpa*II or *Bgl*II + *Cfo*I and Southern hybridization with the DNA probe PW71 normally detects two fragments of equal intensity; the larger of 6.0 or 8 kb representing the methylated sites on the maternal chromosome and the smaller of 4.2 or 6.4 kb representing the unmethylated sites on the paternally derived chromosome. In the DNA sample of the patient, the intensity of the hybridization signal of the 6.0- (*Hind*III + *Hpa*II) and 8-kb (*Bgl*II + *Cfo*I) fragments, the maternally derived alleles, was much stronger than that of the 4.2- and 6.4-kb fragments (fig. 5a, b).

Discussion

The rare finding of the simultaneous appearance of inv dup(15) and dup(15) (q11.2q12) in a daughter of normal parents
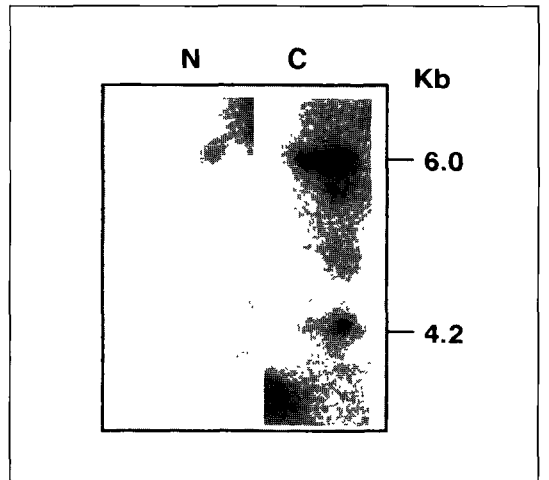
Fig. 3. FISH. Prader-Willi cosmid probe of the locus D15S11 (Oncor probe A), and myl cosmid as a marker for chromosome 15. Note the double signal on one chromosome 15 and the lack of signal on the inv dup(15).
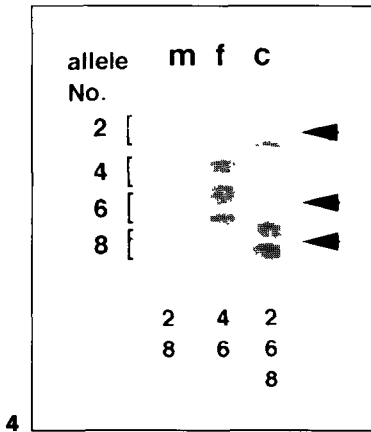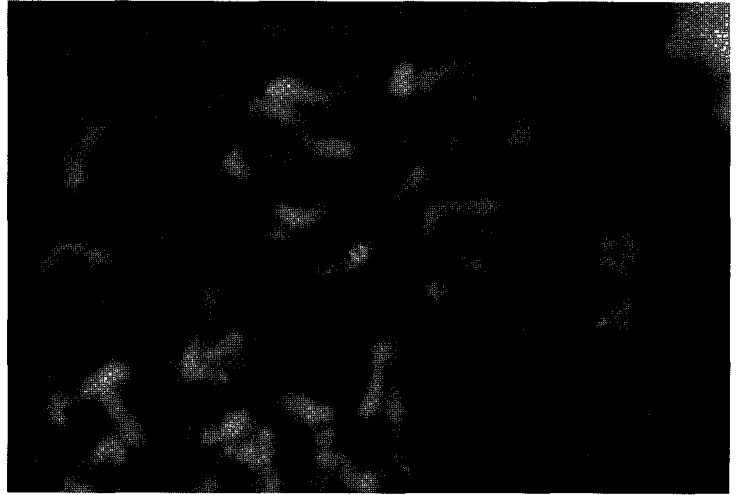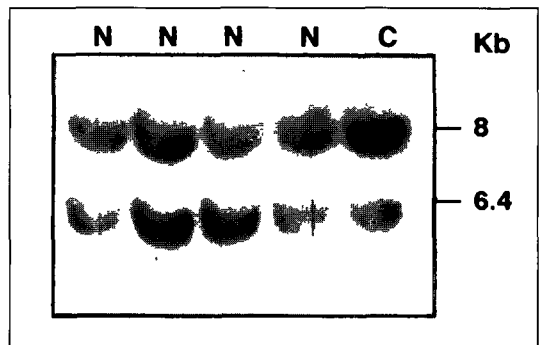

Fig. 4. (CA)$_n$ dinucleotide repeat polymorphisms of locus GABRB3. Note that the difference in the intensity of alleles 8 and 2 of GABRB3 that was observed in the mother and child is probably a PCR artifact and not a dosage effect. The intensity of the various alleles in the loci D15S210 and D15S97 was equal. m = Mother; f = father; c = child.

Fig. 5. Southern hybridization. N = Normal individuals; C = the patient. **a** PW71/*Hin*dIII + *Hpa*II. **b** PW71/*Bgl*II + *Cfo*I.
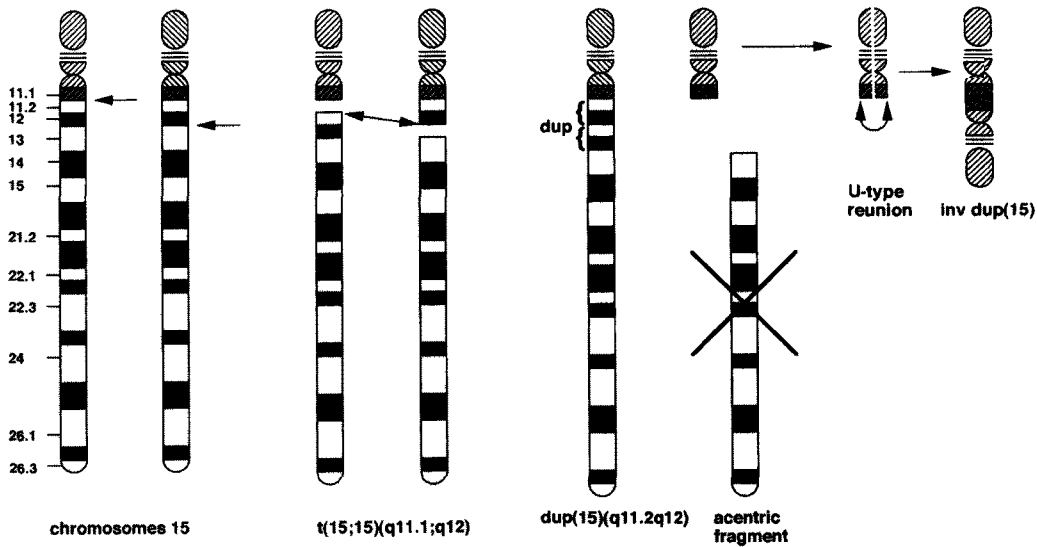
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Shapira/Meiner

Simultaneous Formation of inv dup(15)
and dup(15q)

Fig. 6. Model describing the sequence of events that brough about the simultaneous formation of the dup(15q) and inv dup(15).

immediately raised the possibility of a common cause in the formation of the two abnormal chromosomes. The FISH results, the segregation of the $(CA)_n$ dinucleotide repeat polymorphisms, and the dosage effect on the parent-of-origin-dependent methylation sites at the D15S63 locus confirmed the following points: (a) the chromosome 15q+ was a dup(15)(q11.2q12); (b) the chromosome dup(15) was maternally derived; (c) the dup(15) chromosome included chromosomal material of the two maternal homologous of chromosome 15; (d) the bisatellited small extra chromosome was inv dup(15), and (e) the PWS/AS region was not included in the inv dup(15). We did not analyze the duplicated chromosome with additional DNA probes and, therefore, could not determine the extent of the duplication beyond the PWS/AS region. On the basis of these findings,

we suggest (fig. 6) that a translocation between the two maternal chromosomes 15, t(15;15)(q11.1;q12), resulted in the formation of the dup(15q) and two reciprocal products, an acentric fragment 15(q13-qter) and a centric chromosome 15(pter-q11.1) which, if joined, could give a deleted chromosome 15 or, alternatively, could get lost. However, the centric fragment was rescued by U-type reunion, and the acentric fragment was lost. Nondisjunction brought about the cosegregation of the two abnormal chromosomes 15. The same sequence of events, but without nondisjunction, could form a karyotype with one abnormal derivative of chromosome 15, either the inv dup(15) or the dup(15q). It is also possible that the products of a translocation were a centric del(15q) and inv dup(15) including the PWS/AS region; both are known among PWS patients [14]. This model is an

alternative, or additional mechanism, to the model of unequal crossing-over that was suggested for the formation of the dup(15) patients [2, 15].

The finding of an inv dup(15) chromosome is not rare; it has frequently been found as a supernumerary chromosome and in most of the cases that were analyzed, it was maternally derived, as in the present case. Robinson et al. [14] suggested that the first error in the formation of some inv dup(15) could be a disomic 15 ovum and the rescue out of it, in the trisomy 15 fetus, was the formation of the inv dup(15). This model, like ours, predicts that the formation of the inv dup(15) is a consequence of chromosomal breakage rather than unequal crossing-over.

Several cases with dup(15q) have been reported [for a summary see ref. 1], and based on the molecular analysis in two reported cases, the duplication derived from homologous chromosomes, maternal or paternal [15], as also observed in the present case. Mutirangura et al. [15] suggested that inverted repeats which might exist in proximal 15q could predispose it to an unequal crossing-over resulting in the formation of proximal dup(15q) or del(15q), namely, misalignment of tandemly repeated sequences during meiosis I. MN7, a putative gene family with 4–5 copies within 15q11–13 [16], was a possible candidate exerting instability in the PWS/AS region and the clinical syndrome associated with it. However, to date there is no evidence that any large sequence homology exists in the deletion breakpoints associated with PWS. We, therefore, prefer to use the term translocation that is not confined to meiosis I and provides a wide and more general explanation for rearrangements in the proximal long arm of chromosome 15. It should be emphasized that the 'translocation' and the 'unequal crossing-over' models are not mutually exclusive, and a translocation can be a product of an unequal crossing-over. In the present case, if the abnormal chromosomes were not derivatives of homologous chromosomes, it would be considered a classical translocation. The 'translocation model' provides an explanation for the difference in frequency of inv dup(15) and dup(15q). For the formation of inv dup(15), one break in 15q is required followed by U-type reunion, while for the formation of dup(15), two breaks, one on each of the two homologues, are needed.

The clinical presentation of the patient was mainly developmental delay. Clayton-Smith et al. [1] summarized the phenotypes of 18 patients with dup(15q), Wenger et al. [17] added more cases and summarized 38 cases with dup(15)(12q), 19 of whom had a normal phenotype. The clinical variability among the patients with dup(15q) can be accounted for by differences in the breakpoints and possible involvement of chromosomal material other than 15 in the 15q+. In addition, it was suggested [15] that the parental origin of the duplicated 15q in the PWS/AS region has an effect on the clinical phenotype, with a PWS phenotype if the extra material is paternally derived and an AS phenotype if it is maternally derived. In our patient, however, although duplication of maternal origin in the PWS/AS region is evident, the clinical features were nonspecific, and we could not identify resemblance to AS. The contribution of the inv dup(15) chromosome to the clinical manifestation of the patient is difficult to assess; however, according to Leana-Cox et al. [13], a normal phenotype is more often associated with the absence of the PWS region in the inv dup(15).

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