

Clinical and Cytogenetic Findings in Seven Cases of Inverted Duplication of 8p With Evidence of a Telomeric Deletion Using Fluorescence In Situ Hybridization

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We report on the clinical and cytogenetic findings in 7 cases of inverted duplication of region 8p11.2-p23. The phenotype of inv dup (8p) compiled from this series and the literature (N = 29) consists of severe mental retardation (100%), minor facial alterations (97%), agenesis of the corpus callosum (80%), hypotonia (66%), orthopedic abnormalities (58%), scoliosis/kyphosis (40%), and congenital heart defect (26%). A telomeric deletion of region 8p23.3-pter was confirmed in 3 of our cases studied using fluorescent in situ hybridization with a telomeric probe for 8p. Thus, these karyotypes are inv dup del(8) (qter→p23.1::p23.1→p11.2:). Our findings suggest that most cases of inv dup(8p) probably have a telomeric deletion.

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KEY WORDS: chromosome 8, chromosome abnormality, fluorescence in situ hybridization (FISH), inverted duplication 8p, telomeric deletion

INTRODUCTION

Inverted duplication of the short arm of chromosome 8 (inv dup(8p)) is a relatively common chromosome abnormality associated with a well-delineated clinical picture. At least 39 cases have been reported [Weleber et al., 1976; Rethoré et al., 1977; Taylor et al., 1977; Hongell et al., 1978; Mattei et al., 1980; Polani et al., 1981; Jensen et al., 1982; Fryns et al., 1985; Kleczkowska et al., 1987; Dill et al., 1987; Nevin et al., 1990; Gorinati et al., 1991; Mitchell et al., 1991; Feldman

et al., 1993; Minelli et al., 1993; Barber et al., 1994]. Although there is a discrepancy in the reported breakpoints of the duplicated segment, cytogenetic analysis indicates that in most cases the duplicated region includes band 8p11.2 or 8p12 to 8p23. The locus for the glutathione reductase (GSR) gene has been located at 8p21.1. Levels of GSR in patients with inv dup(8p) are increased [Taylor et al., 1977; Mattei et al., 1980; Nevin et al., 1990; Gorinati et al., 1991; Minelli et al., 1993], showing a gene dosage effect and demonstrating that the duplicated portion of 8p includes band 8p21.1.

Cytogenetic studies of some cases of inv dup(8p) have also suggested a simultaneous distal deletion of 8p [Weleber et al., 1976; Rethoré et al., 1977; Mattei et al., 1980; Jensen et al., 1982; Nevin et al., 1990; Gorinati et al., 1991]. Dill et al. [1987] were the first to report a deletion of locus D8S7 located at band 8p23.1 in one patient with inverted duplication 8p. Molecular studies on 12 cases of inv dup(8p) have conclusively showed a deletion of region 8p23.1 in addition to the duplication 8p11.2-p23 [Mitchell et al., 1991; Minelli et al., 1993; Barber et al., 1994]. It appears that a distal 8p deletion almost always accompanies the inverted duplication.

We are reporting on the clinical and cytogenetic findings in 7 new patients with inv dup(8p) and compare our findings with those of previous reports. We also present the in situ hybridization studies on 3 of these patients which indicate there is an accompanying telomeric 8p deletion in patients with inv dup(8p).

MATERIALS AND METHODS

Patients

Seven patients with inv dup(8p) were examined by cytogenetic techniques in our laboratory (4 of them between 1982 and 1985, and 3 between 1993 and 1994). Clinical information on 6 of the patients was available (Table 1). Results of chromosome analysis on the parents of 3 patients were also available.

Cytogenetic Studies

Routine G-banding chromosome analyses were performed on phytohemagglutinin stimulated lymphocytes from 7 patients and the parents of 3 of them.

Received for publication September 6, 1994; revision received April 3, 1995.

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TABLE I. Summary of the Cytogenetic Findings and Clinical Findings of Patients With Inverse Duplication of Chromosome 8p

Case	Duplication	Sex	Race	Age ^a	Main clinical findings
1	p12p23.1 ^{b,c}	F	B	2 mo	Dextrocardia without situs inversus, ASD, VSD, hypotonia, agenesis of corpus callosum, hydronephrosis, umbilical hernia, facial anomalies
2	p12p23 ^{b,c}	F	W	1 yr	Developmental delay, hypotonia, facial asymmetry, facial anomalies, partial agenesis of corpus callosum
3	p12p23 ^b	F	W	19 mo	Developmental delay, hypotonia, facial anomalies, agenesis of corpus callosum, cranial asymmetry
4	p11.2p23.2 ^c	M	W	10 yr	Severe mental retardation, neonatal hypotonia, spasticity, contractures, absent speech, unilateral conductive hearing loss, kyphosis, facial anomalies
5	p21.3p23.3 ^d	F	B	12 yr	Severe mental retardation, absent speech, clubfeet, dextrocardia without situs inversus, neonatal hypotonia, spasticity, contractures, severe scoliosis and kyphosis, unilateral sensorineural hearing loss, precocious puberty, facial anomalies
6	p11.2p23.1	M	W	20 yr	Severe mental retardation, limited speech, clubfeet, contractures, seizures, bilateral microphthalmia, retinal colobomas, cataracts
7	p11.2p23.1 ^c	F	U ^e	12 yr	No clinical information

^aCurrent age of patients.^bPatients who had FISH analysis^cPatients whose parents' chromosomes were studied.^dPatient was diagnosed in 1982 as 47,XX, inv dup(8)(p23.3p21.3)?, +r(?). The abnormal banding pattern on the derivative 8p is similar to other cases.^eU = unknown.

Standard procedures were used for culture and harvest. Three cases were studied using the methotrexate/thymidine synchronization high resolution method.

Fluorescence in situ hybridization (FISH) using a whole chromosome 8 painting probe and the cosmid telomeric probe (mapped to 8p23.2-pter) (ONCOR) was performed on the 3 most recent cases. The telomeric probe was a digoxigenin-labeled probe without the chromosome 8 control marker. To identify both chromosome 8's, the biotinylated chromosome 8 α -satellite probe located at the centromeric region (ONCOR) was used at the same time the telomeric probe was added to the slide for hybridization. The detection process for the telomeric probe was done first according to the manufacturer's procedure. Then the slide was mounted with a coverslip and read under fluorescent microscope as routine. Only one positive signal was shown at the end of the short arm of a C-group chromosome on the patient's slide. The coordinates of the spreads were

recorded. The slide was washed twice in phosphate buffered detergent (PBD) buffer for 10 minutes. The detection process for α -satellite of chromosome 8 was performed, and both chromosome 8's were identified in each of the spreads previously recorded.

RESULTS

Clinical

Table I is a summary of the cytogenetic findings and clinical findings of our 7 cases, and Table II lists the frequency of specific clinical features in our patients plus 29 patients reported previously [Feldman et al., 1993; Minelli et al., 1993]. The age at diagnosis in our patients ranged from 1 month to 9 years. All 6 patients had some minor anomalies, including facial asymmetry, frontal bossing, mandibular hypoplasia, bulbous nose, and wide mouth (Fig. 1).

Agenesis of the corpus callosum was discovered through magnetic resonance imaging (MRI) in our 3

TABLE II. Clinical Findings in Our Patients and in Previously Reported Patients

Clinical findings	Our cases (N = 6)	Cases in literature (N = 29)	Total (N = 35)
Hypotonia	6/6	17/27	23/35 (66%)
Mental retardation	5/5	27/27	32/32 (100%)
Minor facial anomalies	6/6	28/29	34/35 (97%)
Scoliosis and/or kyphosis	2/6	12/29	14/35 (40%)
Agenesis of corpus callosum	3/3	5/7	8/10 (80%)
Congenital heart disease	3/6	6/29	9/35 (26%)
Orthopedic problems	3/6	12/20	15/26 (58%)

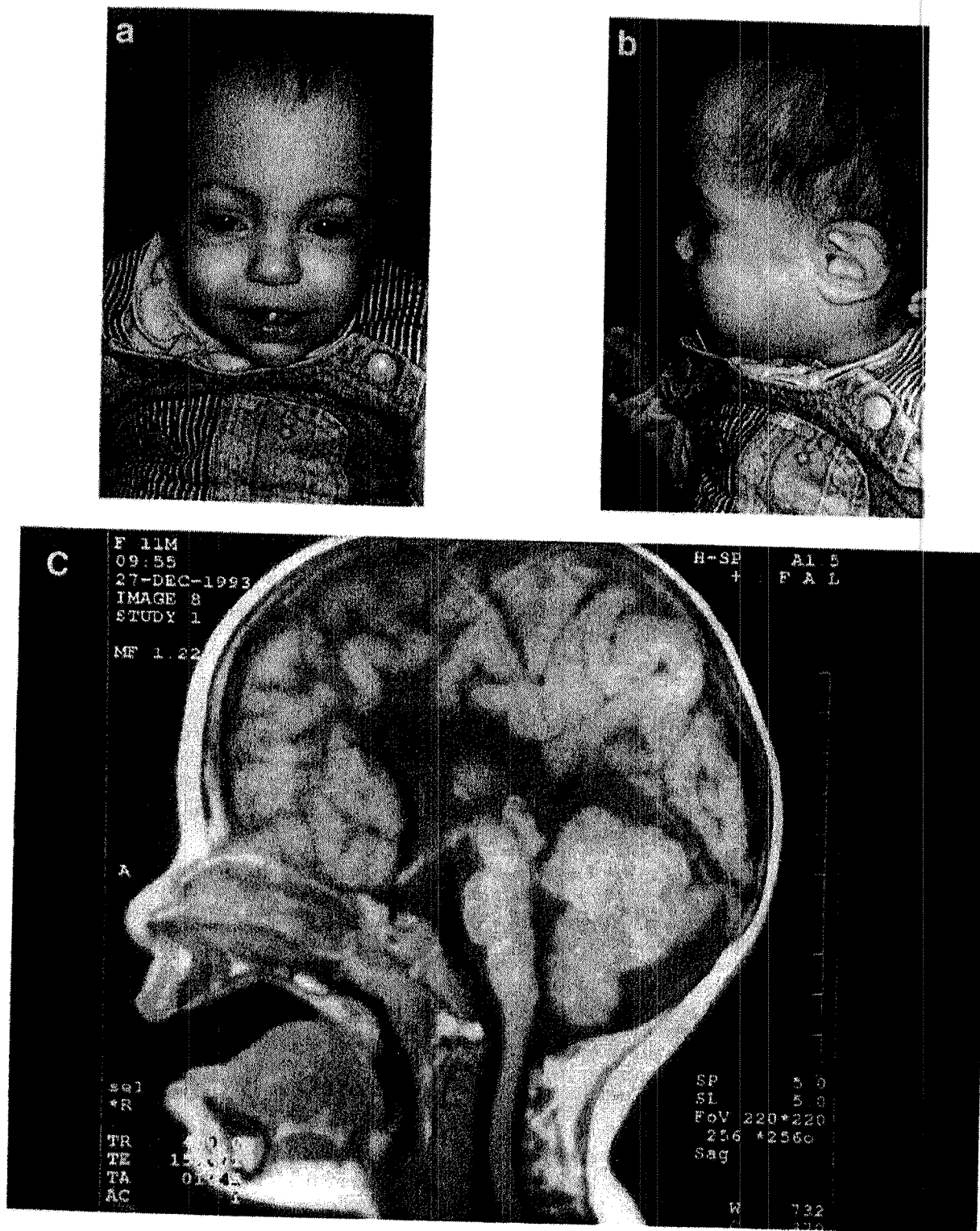


Fig. 1. a: Patient 3. Note frontal bossing and bulbous nose. b: Side view of patient 3. Note large and simple ear. c: Patient 3. Note agenesis of corpus callosum on head MRI.

youngest patients. Our older patients did not have brain imaging studies, so agenesis of the corpus callosum may be more common in our population. Two of our

patients had dextrocardia without situs inversus. One of these patients also had septal defects. A third patient had cardiomegaly with possible atrial septal defect.

Hypotonia was followed by spasticity and contractures in our 3 older patients. Two of our patients had severe, progressive scoliosis and/or kyphosis and two patients had clubfeet. None of our older patients could walk independently.

Mental retardation was severe in all cases, characterized by global developmental delay in our younger patients, and absent speech and completely dependent functioning in our older patients.

Cytogenetics

Chromosome analysis indicated the presence of extra material on the short arm of one chromosome 8 in all the patients studied. This extra material was interpreted, as *inv dup(8)(p11.2-p23)*, except in patient 5 (Table I). The absence of band 8p23.2 was noticed by high resolution evaluation (Fig. 2). Cytogenetic analyses on the parents of 3 patients showed normal chromosomes.

In the 3 patients studied, the chromosome 8 painting probe showed consistent fluorescence staining through the entire length of both chromosomes 8 excluding involvement of any other chromosome in the aberration. All 3 patients had a deletion of the telomeric region 8p23.3-pter of the abnormal chromosome 8, detected by the cosmid telomeric probe of locus 8p23.3-pter. In the metaphases examined by both the telomeric probe and the chromosome 8 α -satellite probe, the signal in the

telomeric region was observed only on the normal chromosome 8p; it was absent on the abnormal 8p (Fig. 3). The positive signal in the telomeric region was shown on both chromosome 8p's in normal controls.

DISCUSSION

Two recently reported case series with inverted duplication of 8p suggest that this chromosome abnormality is not as rare as previously expected and is a recognizable clinical entity [Feldman et al., 1993; Minelli et al., 1993]. The clinical aspects of our cases are similar to those reported previously, as shown in Table II. The finding of dextrocardia without situs inversus in 2 of our 6 patients is noteworthy. Dextrocardia was noted only once in the previously reported cases of *inv dup(8p)* and was associated with situs inversus [Feldman et al., 1993].

Kleczkowska et al. [1987] characterized the changes in phenotype from birth to adulthood. The findings in our older patients concur with their descriptions of adult *inv dup(8p)* patients with severe mental retardation, inability to express themselves verbally, and limited mobility. Thus the neurologic picture of *inv dup(8p)* evolves from neonatal hypotonia to spasticity and contractures and severe mental retardation in older children. This prognostic information may be useful in the genetic counseling of parents whose children have inverted duplication of 8p.

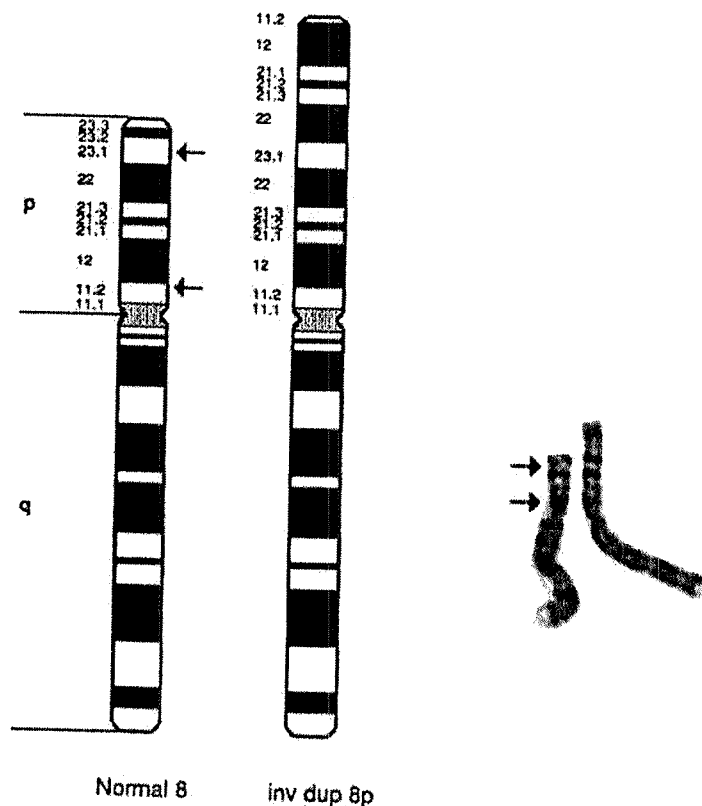


Fig. 2. Idiograms and high resolution banding of normal chromosome 8 and inverted duplication chromosome 8.

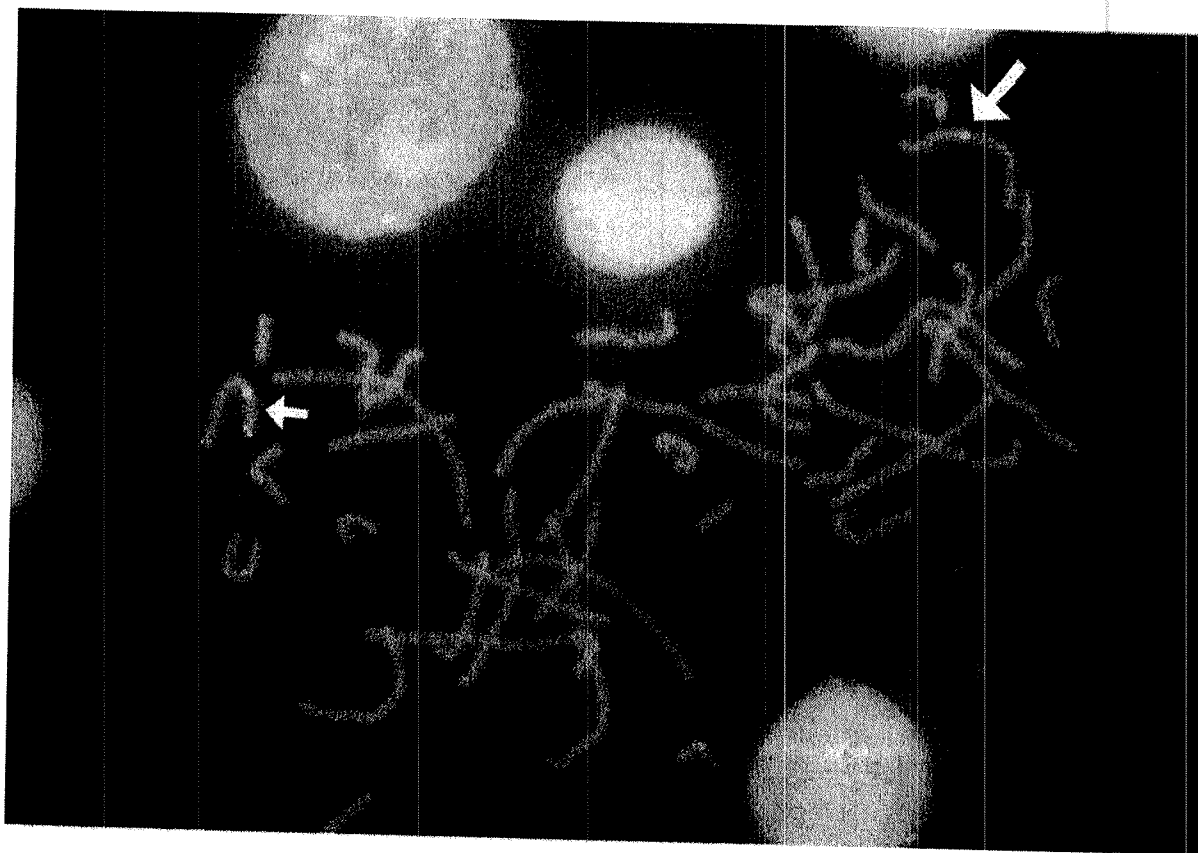


Fig. 3. In situ hybridization with telomeric 8p probe and chromosome 8 α -satellite probe. Small arrow indicates the normal chromosome 8 with normal positive telomeric region; large arrow indicates the inverted duplication of 8p with deletion of telomeric region.

The inverted duplication 8p is a complex chromosome rearrangement that includes deficiency of the telomeric region. We found that the segment of the duplication involved the region of 8p11.2 (or 8p12)-8p23.1 in all our cases (except in patient 5, see Table I). High resolution banding allowed us to identify the absence of the distal region 8p23.2 in 3 cases. These findings are consistent with the cytogenetic interpretation in previous reports. Since Dill et al. [1987] confirmed the deletion of region 8p23.1 using a DNA probe of locus D8S7, similar results have been reported separately in recent years. Mitchell et al. [1991] reported on a second case with deletion of DEF 1 gene which also mapped on 8p23.1. Ten patients with inv dup(8p) investigated by using a DNA probe of locus D8S7 also showed the same deletion [Minelli et al., 1993]. Comparing the deletion of region 8p23.1, Barber et al. [1994] described the result of the protelomeric primer sequences LPL3GT (mapped to 8p21.3-8p22) which detected two alleles indicating that the deletion did not extend to the locus 8p22. The results of all these previous studies seem to indicate that the rearrangement of inv dup(8p) was formed by the duplication of region 8p11.2-p23.1 and the deletion of region 8p23.1.

In 3 of our cases, FISH analysis using whole chromosome painting probes confirmed that the extra material is from chromosome 8. To clarify the nature of the

telomeric region of inv dup(8p), we used a telomeric probe (mapped to 8p23.3-pter, ONCOR) which revealed a consistent deletion. This observation suggests that a new telomeric region was established on the short arm of the abnormal 8, while the original telomeric region was deleted. Based on our results and previous reports, the rearrangement of inverted duplication of 8p should be interpreted as inverted-duplication of region 8p11.2-8p23.1 with a concomitant deletion of region 8p23.1-8pter. The karyotype is then 46,XX(Y),inv dup del(8) (qter→p23.1::p23.1→p11.2:).

The origin of inverted duplication 8p is uncertain; however, two mechanisms have been proposed. The first involves end-to-end fusion between the short arms of chromosome 8 homologs due to the unequal recombination between homologous DNA sequences that lie in an opposite orientation to each other (Fig. 4a) [Weleber et al., 1976; Dill et al., 1987]. This event would occur at meiosis I between homologous chromosomes or alternatively at meiosis II between sister chromatids. The resulting dicentric chromosome would subsequently break during anaphase and generate a duplication-deficiency chromosome with an inverted duplication of most of the short arm of chromosome 8 and deletion of the small distal region. The telomeres would be reconstituted in "some" manner. Recent molecular studies [Morin, 1991; Lamb et al., 1993] indicate that the

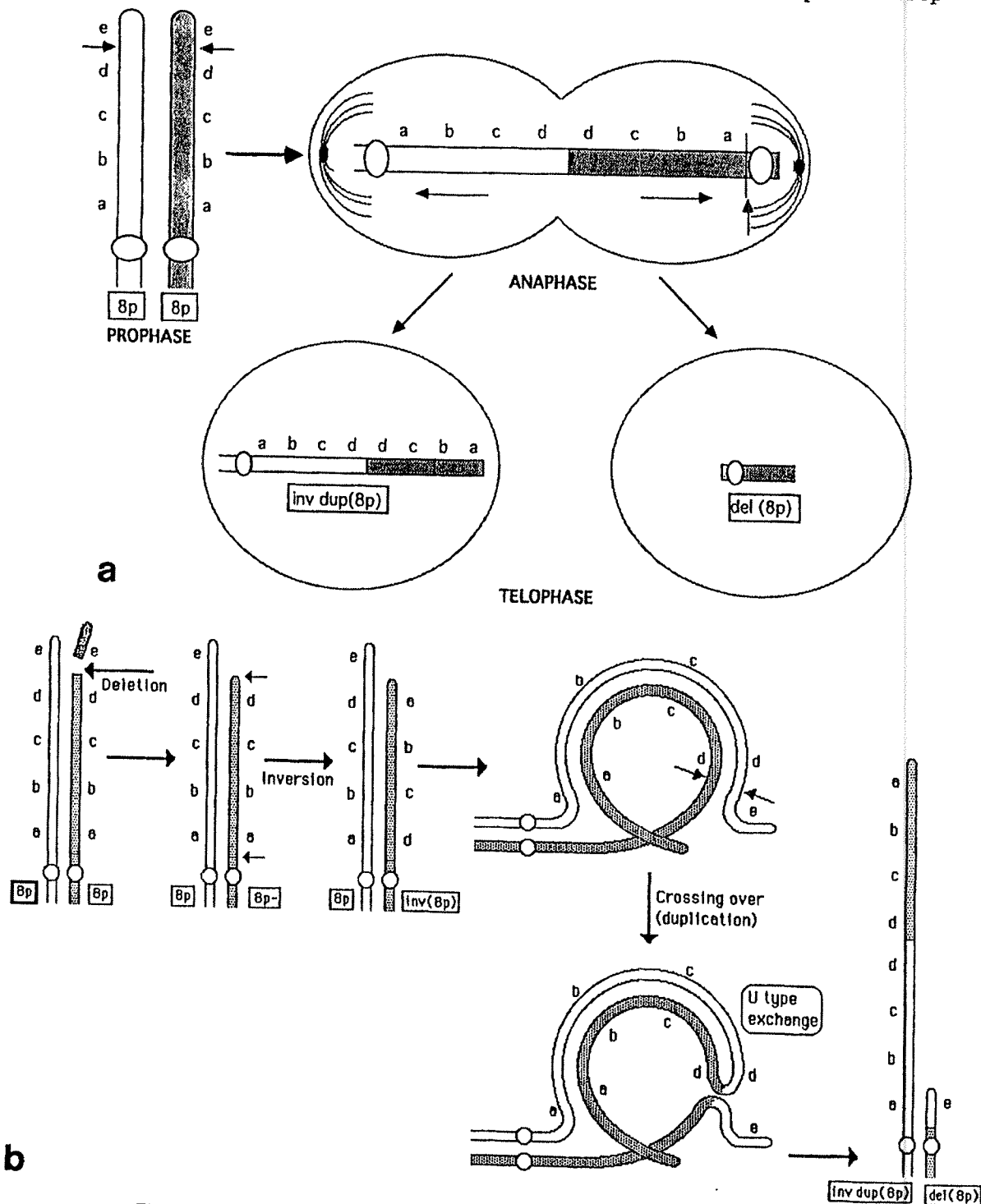


Fig. 4. Proposed mechanisms of formation of inv dup 8p. **a:** End-to-end fusion between the short arms of chromosome 8 homologs due to aberrant crossing over. The resulting dicentric chromosome would break during anaphase and generate a duplication-deficiency chromosome with an inverted duplication of 8p and a deletion of telomeric region. **b:** Telomeric deletion followed by a paracentric inversion of 8p, the U-type exchange in the inversion loop leading to an inverted duplication-deficiency 8p with a new telomeric region [modified from diagram of reference Mitchell et al., 1994].

telomere can be reconstituted at deletion breakpoints [Wilkie et al., 1990] with only minimal complementarity of 2–4 nucleotides to the proposed RNA template of

human telomerase. We favor this mechanism which requires only a single primary recombination to produce the inverted duplication-deficiency chromosome.

An alternative mechanism has been suggested [Mitchell et al., 1994] involving a paracentric inversion of chromosome 8p. A U-type abnormal exchange occurs in the inversion loop during meiosis I and creates an inverted duplication 8p. This model could generate both the inverted duplication and the loss of a distal segment of 8p23.1 but would also maintain the normal telomeric structure. Based on our findings of the complete deletion of telomeric region (8p23.1-pter) on the inv dup(8p), we postulate that the deleted telomere on the paracentric inversion of 8p may form first, followed by the U-type exchange in the inversion loop, leading to an inverted duplication-deficiency 8p with a new telomeric region (Fig. 4b).

The inverted duplication of 8p is the most common unbalanced chromosome abnormality in our laboratory. At least 39 cases have been published previously in the literature with the same inverted duplication and a recognizable phenotype. This suggests there is something unique about the DNA structure of 8p region which predisposes it to inverted duplication. This type of duplication is not commonly found in other chromosomes. We postulate that both No. 8 chromosomes may have either very similar or repeat sequences near the telomeric and centromeric regions which would predispose to crossing over errors between the homologous and sister chromatids on the chromosome 8 during meiosis. Advances in molecular genetics may elucidate the basis of this uncommon occurrence.

ACKNOWLEDGMENTS

We wish to thank Dr. Harold Chen for expert advice and encouragement.

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