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Molecular Characterization of inv dup del(8p): Analysis of Five Cases

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We analyzed five patients with inverted duplication deletion of 8p [inv dup del(8p)] using fluorescence in situ hybridization (FISH) and short tandem repeat polymorphism (STRP) analysis. In all patients, inv dup del(8p) consisted of a deleted distal segment, an intact in-between segment, and a duplicated proximal segment. In all of them, the proximal breakpoint of the deletion and one of the breakpoints of the duplication were identical, each located at one of the two olfactory receptor gene clusters at 8p23. FISH analysis showed all their mothers to be heterozygous carriers of an 8p23 inversion [inv(8)(p23)]. STRP analysis indicated that the deletions occurred in maternally derived chromosomes. The duplicated segments had two copies of maternal, either heterozygous or homozygous alleles. These findings support and reinforce those in 16 patients with inv dup del(8p) and their parents by Floridia et al. [1996: Am J Hum Genet 58:785–796] and subsequent additional studies of 10 of them by Giglio et al. [2001: Am J Hum Genet 68:874–883]. Based on these findings, we propose a model for the inv dup del(8p) formation. The inverted segment and its normal counterpart in inv(8)(p23) heterozygous carrier mothers form a loop at the pachytene period of meiosis I. Inv dup del(8p) with heterozygous duplication is formed through at least one meiotic recombination within the loop. Inv dup del(8p) with the homozygous duplication arises through two meiotic recombinations on the inv(8)(p23) chromosome (one within the loop and the other between the loop and centromere). Subsequent rescue by eliminating a part of the duplicated segment and a centromere enables formation of viable inv dup del(8p). The frequency of the inv(8)(p23) allele is 39% in a normal Japanese population, comparable to 26% in Europeans Giglio et al. [2001: Am J Hum Genet 68:874–883]. The proposed mechanism of formation of inv dup del(8p) requires two independent events (a recombination within the loop and subsequent rescue), which may explain its rarity.

KEY WORDS: inv dup del(8p); 8p23 inversion; polymorphism

INTRODUCTION

Inverted duplication deletion of 8p [inv dup del(8p)] is a complex chromosome 8 rearrangement with an estimated prevalence of 1/10,000–30,000 newborns [Floridia et al., 1996; Giglio et al., 2001]. Its clinical manifestations include mental retardation, agenesis of the corpus callosum, facial abnormalities, congenital heart disease, orthopedic abnormalities, and hypotonia [de Die-Smulders et al., 1995; Guo et al., 1995]. The rearrangement consists of a deletion of the distal 8p23 region from D8S349 to 8pter, an intact segment from D8S252 to D8S265, and an inverted duplication of various extents from D8S552 to 8p or 8q, and was suggested to arise at maternal first meiosis [Floridia et al., 1996]. Two olfactory receptor gene-clusters exist at the breakpoints of inv dup del(8p) and a polymorphic 8p23 inversion [inv(8)(p23)] [Giglio et al., 2001]. Heterozygous inv(8)(p23) was found in 19 (26%) of 72 normal Europeans, as well as in all mothers of 8 reported patients with inv dup del(8p), suggesting that a maternal heterozygous inv(8)(p23) is causally related to inv dup del(8p) in a child [Giglio et al., 2001]. We have recently constructed a complete BAC/PAC contig covering the 8p23 inversion, and estimated the inversion-allele frequency of 27% in the normal Japanese population [Sugawara et al., 2003].

In this study, we analyzed the 8p23 genomic structure of five Japanese patients with inv dup del(8p) using BAC/PAC clones from the complete map. Here, we report on the result of analysis of short tandem repeat polymorphisms (STRP) in the patients and their parents to validate an underlying mechanism for inv dup del(8p). We also propose a model for the inv dup del(8p) formation.

MATERIALS AND METHODS

Subjects

The subjects studied are five patients (Cases 1–5) with inv dup del(8p) and their parents. Their duplicated segments involved respectively 8p23 → p11.21, 8p23 → p11.1, 8p23 → p11.23, 8p23 → p12, and 8p23 → p12 in Cases 1–5. All five

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TABLE I. Results of Fluorescence in Situ Hybridization (FISH) in Patients With Inverted Duplication 8p

Clone (probe) used	Distance (Mb) from 8pter	Band	Case 1	Case 2	Case 3	Case 4	Case 5
GS-77L23	8p telomere	8p23	–	–	–	N.D.	–
RP1-5K2	5.8	8p23	–	–	–	–	–
RP11-399J23	8.2	8p23	+	+	+	+	+
RP11-589N15	11.6	8p23	+	+	+	+	+
RP11-813L8	12.4	8p22	++	++	++	++	++
RP11-138H14	14.1	8p22	++	++	++	++	++
RP11-236O1	15.6	8p22	++	++	++	++	++
RP11-90I3	16.7	8p22	++	++	++	++	++
RP11-685B14	18.2	8p22	++	++	++	++	++
RP11-51C1	19.3	8p21.3	++	++	++	++	++
RP11-76B12	25.5	8p21.2	++	++	++	++	++
RP11-116F9	28.6	8p21.1	++	++	++	++	++
RP11-79H13	35.2	8p12	++	++	++	++	++
RP11-210F15	36.8	8p12	++	++	++	N.D.	++
RP11-265K5	38.2	8p12	++	++	++	N.D.	++
RP11-63F20	38.6	8p11.23	++	++	++	N.D.	+
RP11-495O10	38.7	8p11.23	++	++	++	N.D.	+
RP11-723D22	38.8	8p11.23	++	++	++	N.D.	+
RP11-1084M23	39.1	8p11.22	++	++	+	N.D.	+
RP11-147I9	39.2	8p11.22	++	++	+	N.D.	+
RP11-749M23	39.5	8p11.22	++	++	+	N.D.	+
RP11-262I23	39.7	8p11.21	+	++	+	N.D.	+
RP11-691P15	40.7	8p11.21	+	++	+	N.D.	+
RP11-282J24	41.6	8p11.21	+	++	+	N.D.	+
RP11-73M19	43	8p11.1	+	++	+	N.D.	+
RP11-217N16	46.7	8q11.1	+	+	+	N.D.	+

–, deletion; +, single copy; ++, duplication; N.D., not done. Shadow indicates regions of a single copy.

patients showed mental retardation, craniofacial, ocular, cardiac, and skeletal anomalies.

Fluorescence in Situ Hybridization (FISH) Analysis

FISH using BAC DNA as a probe was performed on metaphase chromosomes of the patients with inv dup del(8p) and their parents. BAC clones mapped to 8p23.3-p12 were selected using the UCSC genome browser database (http://genome.ucsc.edu) and the contig map covering 8p23 [Sugawara et al., 2003]. An 8p subtelomere clone, GS-77L23 was also used [Knight et al., 2000]. Chromosome slides were preincubated in 2× SSC at 37°C for 30 min, denatured in 70% formamide/2× SSC at 72°C for 2 min, and then dehydrated at −20°C in ethanol. Cloned DNA was labeled with SpectrumGreen™-11-dUTP or SpectrumOrange™-11-dUTP (Vysis, Downers Grove, IL) by nick translation and denatured at 76°C for 10 min. Probe-hybridization mixture (10 μl) was applied on the chromosomes, and incubated at 37°C for 16 hr. Slides were washed three times in 4× SSC, 0.1% Tween-20 at 45°C and mounted in antifade solution (Vector, Burlingame, CA) containing DAPI. Photomicroscopy was performed under a Zeiss Axioskop microscope equipped with a quad filter set with single band excitation filters (84000, Chroma Technology Corp., Brattleboro, VT). Images were collected and merged using a cooled CCD camera (TEA/CCD-1317-G1, Princeton Instruments, Trenton, NJ) and IPLab/MAC software (Scanalytics, Inc., Fairfax, VA). To confirm the polymorphic inv(8)(p23), BAC clones, RP11-399J23 and RP11-589N15, were used as probes for two-color FISH analysis on maternal chromosomes as previously described [Sugawara et al., 2003].

STRP Analysis

To confirm the parent-of-origin and types of rearrangements of inv dup del(8p), STRP analysis was performed on DNA from the patients with inv dup del(8p) and their parents using

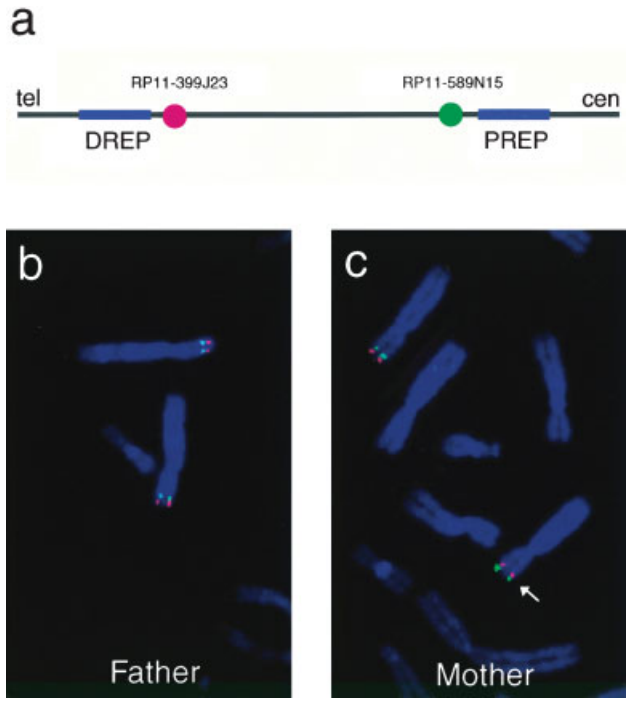

Fig. 1. Two-color fluorescence in situ hybridization (FISH) of polymorphic inv(8)(p23). **a**: Schematic presentation of two probes, RP11-399J23 labeled with SpectrumOrange (red) and RP11-589N15 with SpectrumGreen (green), mapped within the inversion. **b**: The father of Case 1, a normal homozygote. Telomere-red-green (T-R-G) orientation was observed in both chromosomes 8. **c**: The mother of Case 1, heterozygous inversion [inv(8)(p23)] with T-G-R orientation (arrow).

28 markers derived either from the ABI PRISM Linkage Mapping Set-MD10 (PE Applied Biosystems, Foster, CA) or from the UCSC genome browser database. DNA was extracted from fixed lymphoblastoid cells by the SepaGene kit (Sanko Junyaku, Tokyo, Japan) or directly from peripheral blood leukocytes with the standard method. PCR was performed for 45 cycles at 94°C for 10 sec, 55°C for 30 sec, and 72°C for 30 sec in a 20 μl reaction volume containing 50–100 ng genomic DNA, 1 U Ex Taq polymerase (Takara shuzo, Shiga, Japan), 1 μM primers, 1.5 mM $MgCl_2$, 250 μM dNTP, and 1× PCR buffer. PCR products were electrophoresed on the ABI Prism 377 Autosequencer (PE Applied Biosystems) and analyzed with software, GeneScan Analysis v.3.1.2 and Genotyper v.2.5 (PE Applied Biosystems).

RESULTS AND DISCUSSION

FISH study using clones from the complete map covering the inversion breakpoint we constructed and other clones available through genome databases defined the regions for deletion, intact segment, and for duplication in the five patients with inv dup del(8p) [Sugawara et al., 2003] (Table I). The distal deletion of the five patients spanned a 5.8-Mb region from the 8p telomere to the breakpoint, the intact segment encompassed 4.7 Mb [Sugawara et al., 2003], and the duplication included 25.7–30.5 Mb regions. The deletion breakpoint was located between RP1-5K2 and RP11-399J23, and the distal duplication breakpoint was between RP11-589N15 and RP11-813L8 in all five patients studied. The other duplication breakpoint, on the other hand, varied in each patient. These findings support previous data that deletion, intact segment, and the distal edge of duplication were consistent, but the proximal edge varied in inv dup del(8p) [Floridia et al., 1996; Giglio et al., 2001].

Two-color FISH using RP11-399J23 and RP11-589N15 revealed that all five mothers were heterozygous inv(8)(p23) carriers (inversion heterozygotes) (Fig. 1), whereas none of the fathers had the inversion. Because the allele frequency of inv(8)(p23) is 27% among the Japanese [Sugawara et al., 2003], the frequency of inversion heterozygotes in the Japanese is estimated at 39%. Therefore, all five mothers to be heterozygotes by chance would be less than 0.1%. The high frequency of heterozygotes in the normal population suggests that inv(8)(p23) itself may not have any significant, pathological effects on its carriers.

STRP analysis using 28 markers clearly demonstrated that all deletions in the five patients had arisen in the maternally derived chromosomes 8 (Table II). This finding supports the causal relationship between inv(8)(p23) and inv dup del(8p). The duplicated regions in Cases 1, 2, and 4 contained both heterozygous maternal alleles and a single paternal allele at D8S1731 (Case 1), at D8S258 (Case 2), and at D8S560 and D8S1752 (Case 4), while those in Cases 3 and 5 were not informative as to heterozygous maternal contribution. Interestingly, Case 1 inherited both of maternal alleles at D8S1731 but only one maternal allele, apparently duplicated, at D8S258, D8S282, D8S560, D8S1786, D8S1733, D8S1752, D8S1771, and D8S1769. Similarly in Case 5, only one duplicated maternal allele was detected at D8S1790 and D8S258.

TABLE II. Allelotyping in Five Families With inv dup del(8p) Patients by Short Tandem Repeat Polymorphism (STRP) Analysis

Location	STRP marker	Distance (Mb) from 8pter	Case 1	Case 2	Case 3	Case 4	Case 5
Deletion							
8p23.3	D8S264	2.0	**A/A/BC**	**BD/D/AC**	AC/A/AB	**BC/C/A**	**AD/A/BC**
8p23.1	D8S277	6.7	**A/A/BC**	A/A/A	AB/A/A	**B/B/A**	**A/A/B**
8p23.1	D8S1819	7	**AB/B/A**	AB/A/A	**B/B/A**	**AC/AC/B**	**BC/C/AB**
Single copy segment							
8p23.1	D8S1825	8.9	A/AB/AB	AB/A/A	A/A/A	A/AB/AB	AB/A/A
8p23.1	D8S550	10.8	AC/AC/BC	**AC/BC/B**	AB/AB/AC	**BC/CD/AD**	**BD/BC/AC**
8p23.1	D8S265	11.2	A/A/A	AB/A/A	AB/AB/A	A/A/AB	A/A/AB
Duplication							
8p22	D8S552	12.5	AB/B/B	—	A/AB/AB	AC/BC/B	AB/AB/AB
8p22	D8S1754	12.9	—	—	—	A/AB/AB	A/A/A
8p22	D8S1790	13	—	—	—	AB/AB/AB	BC/B/AB
8p22	D8S1827	14.7	AB/AB/A	—	—	AB/AB/AB	—
8p22	D8S1731	15.1	**BD/ACD/AC**	—	—	AC/ABC/AB[a]	—
8p22	D8S549	15.6	AB/B/B	AB/A/A	AB/AB/AB	—	—
8p22	D8S261	17.8	A/A/A	—	A/AB/AB	A/AB/AB	B/AB/A
8p21.3	D8S1715	19.8	—	—	—	A/A/A	A/A/A
8p21.3	D8S258	20.4	B/B/AB	**B/ABC/AC**	AB/AB/AB	A/AB/B	A/A/AB
8p21.3	D8S282	21.7	AB/A/AC	—	—	—	—
8p21.3	D8S560	21.9	AB/AB/AC	—	BC/AC/A	**A/ABC/BC**	B/AB/AB
8p21.3	D8S298	22.1	AB/AB/A	—	—	B/AB/A	—
8p21.3	D8S1786	22.7	AC/BC/AB	—	—	BC/AC/AC	—
8p21.3	D8S1733	22.8	AB/B/AB	—	—	AB/AC/AC	—
8p21.2	D8S1752	23	AB/B/BC	—	—	**BC/ACD/AD**	—
8p21.2	D8S1734	23.1	A/A/A	—	—	AB/AB/AB	—
8p21.2	D8S1771	25.7	BC/AC/AB	AB/AB/AB	B/AB/AB	AB/A/A	—
8p21.1	D8S1839	27.8	AB/A/A	—	—	AB/AB/AB	—
8p21.1	D8S1820	28.5	—	—	A/AB/AB	A/A/A	—
8p12	D8S1769	31.5	AC/BC/AB	—	—	—	—
8p12	D8S1810	32.1	AC/BC/B	—	—	AB/B/B	—
8p12	D8S283	34	BC/AB/AB	—	—	BC/AC/A	—

Polymorphic alleles (A, B, C, D) are shown as those in father/patient/mother. The pair in boldface characters is informative for the parent-of-origin of the deletion, intact segment, or duplication, and the pair in underlined characters indicates homozygous maternal contribution.
[a]Heterozygous maternal contribution if allele C is inherited from the father. —: not done.

From these findings, we propose here a model to explain how inv dup del(8p) is formed. Usually, during the pachytene period of meiosis I in heterozygous carriers for inv(8)(p23), the 4.7-Mb inverted segment and its normal counterpart form a loop (Fig. 2). A recombination (crossing-over) within the loop at the maternal meiosis I is essential for the inv dup del(8p) formation (Fig. 2). A single recombination at site "a" or "b" within the loop results in heterozygous maternal alleles at duplication (type A in Fig. 2). If two recombinations occur, one within the loop (site "a") and another between the loop and centromere (site "c"),

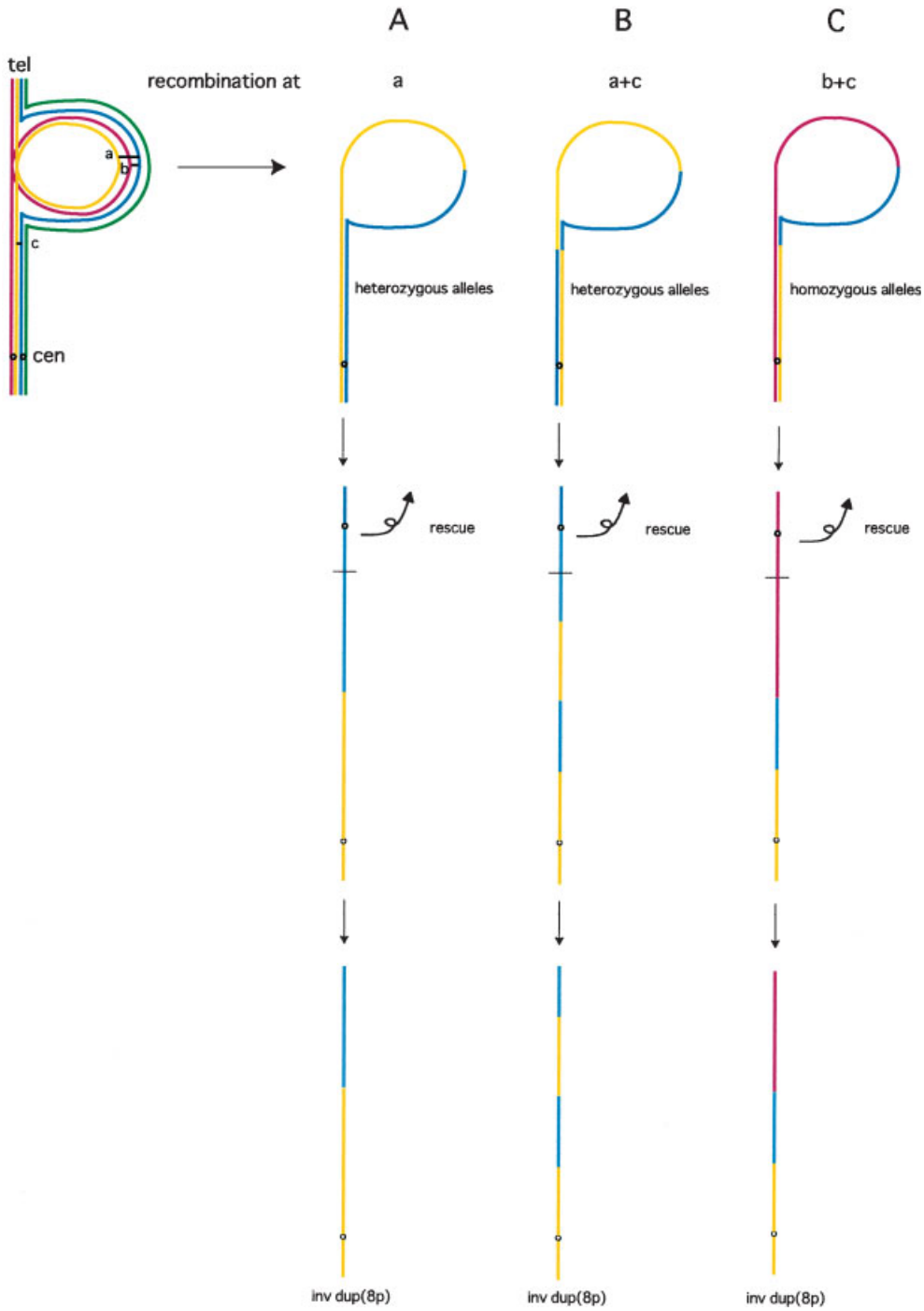

Fig. 2. A model for the formation of inv dup del(8p). Sister chromatids of one homologous chromosome are delineated in red and orange, and other sister chromatids of another homologous chromosome in blue and green. A loop is formed to align at the heterozygous 8p23 inversion and a meiotic recombination within the loop at the maternal meiosis I is essential for the inv dup del(8p) formation. A single recombination at site "a" or "b" within the loop results in heterozygous maternal alleles at duplication (**type A**). If two recombinations occur, one within the loop (site "a") and another between the loop and centromere (site "c"), a dicentric chromosome with maternal heterozygous alleles can be formed (**type B**). Instead, two recombinations within the loop (site "b") and another site "c" between the loop and centromere, can form a different dicentric chromosome mixed with heterozygous and homozygous maternal alleles or that with maternal homozygous alleles, depending on the site of "c" (**type C**). Although such dicentric chromosomes may be lethal, rescue by eliminating a part of a duplicated segment and a centromere may result in the inv dup(8) formation.

a dicentric chromosome with maternal heterozygous alleles can be formed as observed (type B in Fig. 2). Cases 2 and 4 with heterozygous maternal contribution to duplication can be explained by type A or B. Instead, two recombinations within the loop (site "b") and another site "c" between the loop and centromere, can form a different dicentric chromosome mixed with heterozygous and homozygous maternal alleles as observed in Case 1 or that with maternal homozygous alleles as observed in Case 5, depending on the site of "c" (type C), as described previously [Madan, 1988]. Although such dicentric chromosomes may be lethal, rescue by eliminating a part of a duplicated segment and a centromere may result in the inv dup(8) formation. Since the whole length of the short arm of chromosome 8 is about 43 Mb, the probability of a meiotic recombination within the 4.7 Mb inversion extent may be about 0.11 (4.7/43), under an assumption that a recombination occurs once in the 8p arm. However, since the formation of inv dup del(8p) needs at least two independent events, i.e., once for the recombination within the loop and second for the subsequent rescue, a chance of its formation may be very small.

In conclusion, we provided another line of evidence for the causal relationship between inv dup del(8p) and maternal inv(8)(p23). Such submicroscopic inversions may exist elsewhere in the human genome. In fact, a similar chromosome abnormality, inv(4)(p16), has been found as an inversion polymorphism, and double heterozygous state for inv(4)(p16) and inv(8)(p23) may cause the formation of recurrent translocations, [t(4;8)(p16;p23)] [Giglio et al., 2002].

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