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Original investigations

Parental origin and mechanism of formation of polysomy X: an XXXXX case and four XXXXY cases determined with RFLPs

Han-Xiang Deng[1], Kyohko Abe[1], Ikuko Kondo[2], Masato Tsukahara[3], Haruyo Inagaki[4], Isamu Hamada[5], Yoshimitsu Fukushima[6], and Norio Niikawa[1]

[1]Department of Human Genetics, Nagasaki University School of Medicine, Sakamoto-Machi 12-4, Nagasaki, 852 Japan
[2]Departments of Human Ecology and Genetics, Faculty of Medicine, University of the Ryukyus, Okinawa, Japan
[3]Department of Pediatrics, Yamaguchi University School of Medicine, Ube, Japan
[4]Department of Pediatrics, Toyohashi City Hospital, Aichi, Japan
[5]Department of Pediatrics, Hakodate National Hospital, Hakodate, Japan
[6]Division of Medical Genetics, Saitama Children's Medical Center, Iwatsuki, Japan

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Summary. The parental origin and mechanism of formation of polysomy X were studied in five cases (one case of 49,XXXXX; four cases of 49,XXXXY), using various X-linked restriction fragment length polymorphisms as genetic markers. Segregation and densitometric analyses on the polymorphic DNA fragments revealed that, in all five cases, the additional X chromosomes are of maternal origin and the mechanism of formation is most probably a result of three non-disjunctions during maternal meiotic divisions: once at the first meiosis and simultaneously twice at the second meiosis. The identical origin and the identical mechanism of formation among the five cases are unlikely to be coincidental and suggest a common cause in the mothers of the five cases.

Introduction

The parental origin and mechanism of formation of chromosome abnormalities in man have been studied mainly in the autosomes, especially in the acrocentric chromosomes and a few other chromosomes bearing heteromorphic markers useful for segregation analysis (cf. Magenis 1988). It has been demonstrated that the additional chromosome in numerical autosomal abnormalities is predominantly maternal in origin (Juberg and Mowrey 1983; Ishikiriyama and Niikawa 1984; Hassold and Jacobs 1984; Hassold et al. 1984). Although we had little knowledge concerning the origin of numerical X chromosome abnormalities because of the lack of informative heteromorphisms on its arms, recent studies that used restriction fragment length polymorphisms (RFLPs) as markers have been successful in tracing the origin of additional or lost X chromosomes, i.e., a case of 49,XXXXY (Villamar et al. 1989), and cases of 47,XXY (Jacobs et al. 1988), 47,XXX (May et al. 1990), and monosomy X (Hassold et al. 1988).

Offprint requests to. H.-X. Deng

We report here the results of studies on the parental origin and the mechanism of formation of five polysomy X cases by the use of RFLPs.

Materials and methods

Cases

Patient I (S.I.), a one-year-old Japanese girl born to a 38-year-old, gravida-3, para-1 mother and a 44-year-old father, has multiple anomalies and a karyotype of non-mosaic 49,XXXXX. An elder brother is healthy. Detailed clinical data of this patient have been described elsewhere (Kassai et al. 1991). Patient II (F.A.) is a one-year-old Japanese boy with a karyotype of non-mosaic 49,XXXXY. He has peculiar facies, congenital heart defects (atrial septal defect and pulmonary stenosis), and mental retardation. The ages of the mother and the father at the patient's birth were 29 and 37 years, respectively. There are a healthy elder sister and two healthy elder brothers. The maternal grandparents, and the parents of the grandmother are first-cousins. Patient III (K.W.), a 21-year-old Japanese man, born to a 32-year-old, gravida-2, para-1 mother and a 31-year-old father, has a karyotype of non-mosaic 49,XXXXY. The patient has peculiar facies, webbed neck, deformed fingers and toes, a small penis with undescended testes and is of normal height, but has not developed secondary sexual characteristics. There is a 26-year-old healthy elder sister. Patient IV (N.A.), a 17-month-old boy with peculiar facies, clinodactyly of the fifth fingers, short stature (−2 S.D.) and developmental retardation, was born to a 29-year-old mother and a 30-year-old father. The patient's karyotype is a mosaic of 48,XXXY/49,XXXXY. The proportion of XXXY cells in peripheral blood lymphocytes was 10% and that of XXXXY cells was 90%. A three-year-old elder brother is healthy. Patient V (T.A.) is a 2-year-old malformed, mentally retarded (developmental quotient, 42) boy born to a 28-year-old mother and a 31-year-old father. There is no consanguinity in the parents or in the grandparents. An elder sister is healthy. The patient has a karyotype of non-mosaic 49,XXXXY.

Some 50–100 metaphases from a culture of peripheral blood lymphocytes of each patient showed the karyotypes described above. Similar analysis of each parent of the five patients revealed a normal karyotype without mosaicism. Chromosome analyses were not available from any siblings or grandparents of the patients.

Southern blot analyses

Genomic DNAs were extracted from peripheral blood leukocytes or from Epstein-Barr virus (EBV)-transformed lymphoblastoid cells from the five patients and their parents. DNA samples (6 μg) were digested with various endonucleases of interest, according to the supplier's specifications. Electrophoresis, Southern blotting, hybridization, autoradiography, and all other experiments were performed according to standard techniques.

The following 14 probes were employed in this study, and all of them have been mapped to various regions of the short-arm of the X chromosome (Mandel et al. 1989): L782 (the identified locus, DXS85; located at Xp22.3–p22.2); pD2 (DXS43; Xp22.2); p99-6 (DXS41; Xp22.1); J66 (DXS268; Xp21.3-p21.2); cDMD1a (DMD; Xp21.3-p21.1); P20 (DXS269; Xp21.2); pERT87-1, pERT87-8 and pERT87-15 (DXS164; Xp21.2); J-Bir (DXS270; Xp21.2); L754 and 754-11 (DXS84; Xp21.1); XJ1.1 (DXS206; Xp21.1); L1.28 (DXS7; Xp11.4-p11.3.). The RFLPs already detected with these probes (Mandel et al. 1989) and our newly found cDMD1a/*Pvu*II RFLPs (Deng and Niikawa 1990) were used as markers for segregation analyses.

Allele doses in each patient were determined by calculating the density ratio (R1) of one polymorphic autoradiographic band to the other, compared with an average ratio (R2) among at least five normal individuals, i.e., R1/R2. For the allele dose in patient V, the ratio of a polymorphic band to an internal control probe band [p20.36 (the PTH gene, locus: 11pter-p15.4)] was calculated.

Results

The results of segregation and densitometric analyses of the 5 families are shown in Table 1 and Fig. 1.

Patient I

Analysis with the probe/enzyme combination of pERT87-1/*Xmn*I showed that the father is a hemizygote for a 7.5 kb allele and the mother a homozygote for an 8.7 kb allele. The pentasomy X patient seemed a heterozygote for both alleles, but densitometric analysis revealed that she has four copies of the 8.7 kb allele and one copy of the 7.5 kb allele (Fig. 1a). Another combination, P20/*Msp*I, detected two pairs of polymorphic fragments (6.8 kb/3.5 kb, and 2.1 kb/1.8 kb) in the members of this family, and showed that the father is a hemizygote both for the 3.5 kb allele and for the 2.1 kb allele whereas the mother is a 6.8 kb/3.5 kb heterozygote and a 1.8 kb/1.8 kb homozygote. The patient seemed heterozygous both for the 6.8 kb/3.5 kb and for the 2.1 kb/1.8 kb fragments; densitometry revealed two copies of the 6.8 kb allele, three copies of the 3.5 kb allele, one copy of the 2.1 kb allele, and four copies of the 1.8 kb allele (Fig. 1b). When the transmissions of the three RFLPs above were combined, it was concluded that four of the five X chromosomes in the patient had come from the mother, and two each of the four had been derived from each homolog of the mother.

Patient II

Analysis with cDMD1a/*Pvu*II showed that the father is hemizygous for an 8 kb allele, the mother homozygous for a 15 kb allele, and the patient appeared homozygous for the 15 kb allele (Fig. 1c), indicating that the patient inherited all four X chromosomes from his mother. The L782/*Eco*RI combination revealed that the father is hemizygous for a 7 kb allele, the mother is heterozygous for 14 kb/7 kb alleles, and the patient heterozygous for 14 kb/7 kb alleles with a 1:1 density ratio (Fig. 1d). The combined data indicate that the cDMD1a/*Pvu*II genotype, and the L782/*Eco*RI genotype of the patient are 15 kb/15 kb/15 kb/15 kb, and 14 kb/14 kb/7 kb/7 kb, respectively. Thus, it was concluded that two of four X chromosomes of the patient had been derived from one homolog of the mother and the other two from the other maternal homolog.

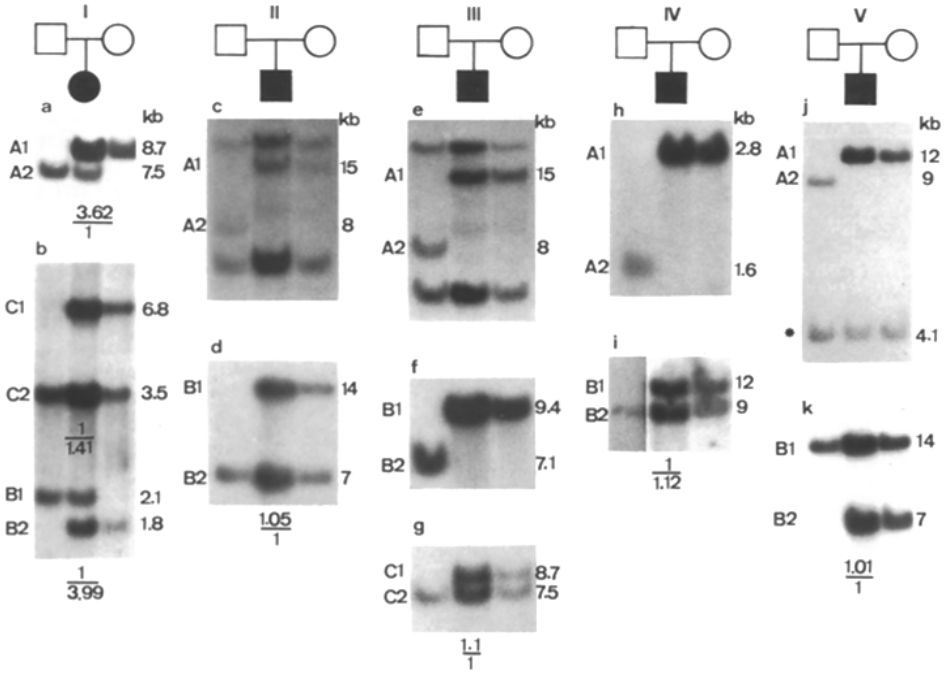

Fig. 1a–k. RFLPs in patients I-V and their parents. The density ratio of one polymorphic fragment (A1-C1) to the other (A2-C2) for each patient is shown at the bottom of each lane. Fragment sizes are shown in kb. Probes/enzymes used are pERT87-1/*Xmn*I (**a**, **g**), P20/*Msp*I (**b**), cDMD1a/*Pvu*II (**c**, **e**), L782/*Eco*RI (**d**, **k**), pERT87-15/*Bam*HI (**f**), pERT87-15/*Xmn*I (**h**), L1.28/*Taq*I (**i**, **j**), and p20.36/*Taq*I (**k**, *asterisk*) as an internal density control

Table 1. Genotypes of the present five patients and their parents at various loci, parental origin and mechanism of formation of polysomy X. Genotype symbols except for C-E in patient V are identical to those in Fig. 1

Patient no.	Sex chromosome constitution	Gene name	Probe	Genotype			Parental origin	Mechanism of formation
				Father	Mother	Patient		
I	XXXXX	DXS164	pERT87-1	A2	A1 A1	A1 A1 A1 A1 A2	Maternal	Three successive meiotic nondisjunctions
		DXS269	P20	B1	B2 B2	B1 B2 B2 B2 B2		
		DXS269	P20	C2	C1 C2	C1 C1 C2 C2 C2		
II	XXXXY	DMD	cDMD1a	A2	A1 A1	A1 A1 A1 A1	Maternal	Three successive meiotic nondisjunctions
		DXS85	L782	B2	B1 B2	B1 B1 B2 B2		
III	XXXXY	DMD	cDMD1a	A2	A1 A1	A1 A1 A1 A1	Maternal	Three successive meiotic nondisjunctions
		DXS164	pERT87-15	B2	B1 B1	B1 B1 B1 B1		
		DXS164	pERT87-1	C2	C1 C2	C1 C1 C2 C2		
IV	XXXY/XXXXY	DXS164	pERT87-15	A2	A1 A1	A1 A1 A1 A1	Maternal	Three successive meiotic nondisjunctions
		DXS7	L1.28	B2	B1 B2	B1 B1 B2 B2		
V	XXXXY	DXS7	L1.28	A2	A1 A1	A1 A1 A1 A1	Maternal	Three successive meiotic nondisjunctions
		DXS85	L782	B1	B1 B2	B1 B1 B2 B2		
		DXS270	J-Bir	C2	C1 C1	C1 C1 C1 C1		
		DXS164	pERT87-15	D2	D1 D2	D1 D1 D2 D2		
		DXS164	pERT87-1	E2	E1 E2	E1 E1 E2 E2		

Patient III

Analysis of the cDMD/*Pvu*II RFLP in this family gave a result identical to that in the family of patient II (Fig. 1e). The combination of pERT87-15/*Bam*HI indicated that the genotypes of the father, the mother, and the patient are 7.1 kb, 9.4 kb/9.4 kb, and 9.4 kb/9.4 kb/9.4 kb/9.4 kb, respectively (Fig. 1f). Their pERT87-1/*Xmn*I genotypes are 7.5 kb, 8.7 kb/7.5 kb, and 8.7 kb/8.7 kb/7.5 kb/7.5 kb, respectively (Fig. 1g). These results indicate that the XXXXY patient has four maternally derived X chromosomes; each of two pairs had come from each member of the homologous X-chromosomes of his mother.

Patient IV

Similar results were obtained in this family as those in patients II and III, although this patient is a mosaic consisting of 48,XXXY/49,XXXXY. The pERT87-15/*Xmn*I combination showed that all the X chromosomes of the patient had come from his mother, since the father is a hemizygote for the 1.6 kb allele, the mother a homozygote for the 2.8 kb allele, and the patient a homozygote for the 2.8 kb allele (Fig. 1h). The results of densitometric analysis of the L1.28/*Taq*I polymorphic fragments reflected the cytogenetic finding that a majority of lymphocytes of the patient are 49,XXXXY; a density ratio of his 8.7 kb fragment to the 7.5 kb fragment is 1:1 (Fig. 1i). These results indicate that the four X chromosomes in his XXXXY cell line had been transmitted from his mother; the pERT87-15/*Xmn*I genotype of the patient is 2.8 kb/2.8 kb/2.8 kb/2.8 kb, and his L1.28/*Taq*I genotype is 12 kb/12 kb/9 kb/9 kb. It was thus concluded that each of two pairs among four X chromosomes of the patient had been derived from each X-homolog of the mother.

Patient V

Densitometric and segregation analyses of the L1.28/*Taq*I fragments with an internal control probe [p20.36 (the PTH gene)] revealed that the genotypes of the father, the mother, and the patient are 9 kb, 12 kb/12 kb, and 12 kb/12 kb/12 kb/12 kb, respectively (Fig. 1j). Similar results were obtained with J-Bir/*Bam*HI, showing their genotypes to be 5 kb + 16 kb, 21 kb/21 kb, and 21 kb/21 kb/21 kb/21 kb, respectively. Analysis with L782/*Eco*RI suggested that their genotypes are 14 kb, 14 kb/7 kb, and 14 kb/14 kb/7 kb/7 kb, respectively (Fig. 1k). Likewise, the pERT87-1/*Xmn*I combination revealed their genotypes to be 7.5 kb, 8.7 kb/7.5 kb, and 8.7 kb/8.7 kb/7.5 kb/7.5 kb, and the pERT87-15/*Bam*HI indicated that they were 7.1 kb, 9.4 kb/7.1 kb, and 9.4 kb/9.4 kb/7.1 kb/7.1 kb, respectively. These results suggested that each of two pairs of alleles in the patient came from each of the alleles in the mother.

Discussion

The parental origin of polysomy X was successfully ascertained in all of the present five cases (Table 1). All of the additional X chromosomes in all the cases studied are of maternal origin. All their mothers have normal karyotypes with no evidence of somatic mosaicism, such as XX/XXX. Therefore, the additional X chromosomes in all the cases are most probably the result of three nondisjunctions at the maternal meiosis, once between homologous X chromosomes at the first meiosis, and twice between sister chromatids in both X chromosomes in the secondary oocyte at the second meiosis (Fig. 2). An identical mechanism of formation has been reported in an XXXXY case (Villamar et al. 1989). An alternative explanation includes a nondisjunctional error occurring at the maternal first meiosis and the other error(s) in early cell division(s) of the zygote followed by sub-

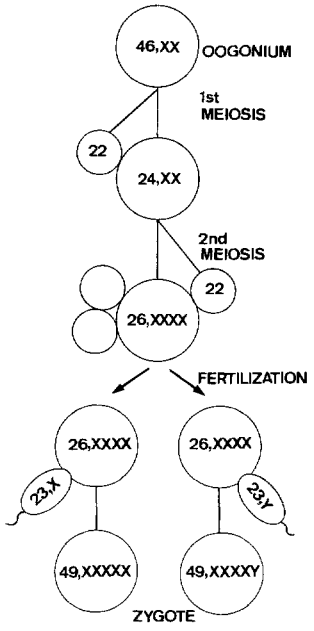

Fig. 2. Scheme for nondisjunctions occuring in patients I-V

sequent exclusions of all cell lines except that with 49 chromosomes. However, this mechanism is less likely, since there is no evidence for the presence of such cell lines in any of the patients except patient IV, although those cells are viable. Patient IV is a mosaic consisting of both 48,XXXY and 49,XXXXY cells. In this patient, the absence of a 47,XXY cell line may refute the possibility of somatic nondisjunction, but postzygotic anaphase lag of an X chromosome in a 49,XXXXY cell may have produced his 48,XXXY cell line.

Polysomy X is very rare and the prevalence of XXXXX and XXXXY may be at most double the estimate value, 1/85,000, for 49,XXXXY (Kleczkowska et al. 1988). There are three possible meiotic errors that produce XXXXX or XXXXY sex chromosome constitutions in a child: three nondisjunctions in the ovum mentioned above (mechanism I); fertilization of an XXX-bearing ovum by an XX- or XY-bearing sperm (mechanism II); fertilization of an XX-bearing ovum by an XXY-bearing sperm (mechanism III). It is significant that in spite of such a rare abnormality, the additional X chromosomes in six of the six cases so far studied resulted from an identical mechanism (mechanism I), suggesting a common fundamental cause among the six poly-X cases.

The average of maternal and paternal ages at the births of our five poly-X cases were 30.2 and 34.6 years, respectively. The maternal age seems high, but is not significantly different from that (28.4 ± 4.2 years for maternal age) of most Japanese mothers (Kuroki and Konishi 1984). None of the five mothers had a history of exposure to radiation, drugs/chemicals, or viral infections during the relevant pregnancies. A specific recessive gene leading to nondisjunction is known in *Drosophila melanogaster;* aneuploid offspring are frequently produced from homozygous females (Gowen 1933). In humans, the existence of such a gene is controversial. Epidemiological studies on consanguinity in maternal grandparents of Down syndrome patients have provided data against the hypothesis (Matsunaga 1966; Forssman and Akesson 1967; Hamamy et al. 1990), but some in favor of it (Alfi et al. 1980). At least in the family of our patient II, the nondisjunctions for this patient might be causally related to the presumed mother's homozygosity, since the maternal grandparents were first-cousins.

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