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Monozygotic Twins with Discordant Sex

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Abstract. A nine-year-old girl with short stature was referred to the department of pediatrics at Kyushu University. The clinical diagnosis was Turner syndrome; karyotypic analysis performed on peripheral blood, using GTG techniques, demonstrated a 45,X/47,XYY (17:83) mosaicism. Her twin brother, a phenotypically normal male, had the same karyotype; 45,X/47,XYY (3:97) on peripheral blood. Their skin fibroblast karyotypes showed the same mosaicism, ie. 45,X/47,XYY (41:59 and 31:69 respectively). On eleven biochemical genetic markers the twin pair were concordant, thus the likelihood of monozygosity was 0.99527034. In addition, the analysis of variable number of tandem repeat (VNTR) markers revealed the likelihood of monozygosity to be 0.99944386. The most plausible explanation of the X/XYY mosaicism was nondisjunction of the Y in the first cleavage division of the 46,XY zygote. A disproportionate rate of cell populations with 45,X and 47,XYY in the twinning process of the X/XYY embryo, especially in the germ lines, would result in discordant sex in twin pairs.

Key Words: Monozygotic twins, Discordant sex, Variable number of tandem repeat (VNTR) markers, Nondisjunction

INTRODUCTION

Zygosity of twins is determined by placental conditions, sex, blood group systems, dermatoglyphics, and restriction fragment length polymorphisms (RFLPs). In general, twins with discordant sex are expected to be dizygotic. In recent literature, however, seven cases of twins with discordant sex were reported as monozygotic, all associated

with chromosomal aberrations [2,,4-6,8-11]. Different distributions of the Y chromosome in the gonads are believed to contribute to the mechanism of monozygotic twins with discordant sex, and in most cases the sex chromosomal aberrations are explained by nondisjunction or anaphase lag of the Y chromosome. Recently, in addition to blood group systems, a variable number of tandem repeat (VNTR) markers [8] have been applied for individual discrimination and diagnosis of zygosity [1]. VNTR markers are very useful to determine twin zygosity. In this report, we describe a pair of monozygotic twins of discordant sex both with 45,X/47,XYY mosaicism, diagnosed by blood group systems and VNTR markers.

CLINICAL REPORTS

A nine year-old female twin (II-2) was referred to the department of pediatrics of Kyushu University for evaluation of her short stature. She was born at 39 weeks' gestation to a 27 year-old, gravida 1, para 1, mother and 32-year-old father (Fig.1). Her birthweight was 2580 g. When she came for evaluation her height was 110 cm (−3.5 SD), and her weight 19.2 kg (−1.8 SD). Epicanthus and webbed neck were observed, but low posterior hairline was not evident. The chest was shield-like with widely spaced nipples. Heart murmur was not audible, Cubitus valgus was present, the external genitalia was normal. Breast development and pubic hair growth were in stage 1. Hematology, tumor markers, and blood chemistry resulted normal. The ovaries and uterus could not be identified by ultrasonography nor tomography of the abdomen.

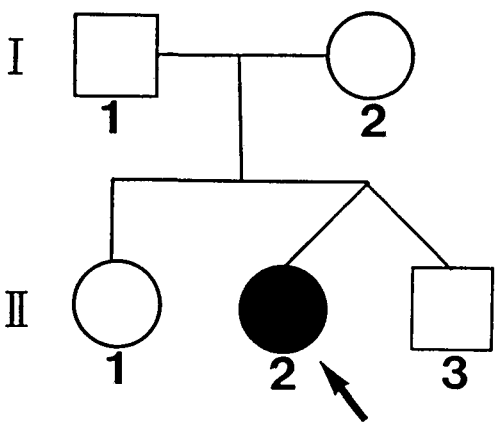

Fig. 1. Family pedigree.
I−1: Maxillary Cancer.

The results of the endocrinological investigations are shown in Table I. Her peripheral lymphocyte karyotype was 45,X/47,XYY (5:25) and so she was diagnosed as Turner syndrome. However, her brother (II-3), with normal male phenotype, also revealed 47,XYY (30 cells counted) in peripheral blood lymphocytes. Information available on the placenta and amnion was rather vague. To determine the zygosity of the twins we also investigated the blood groups and VNTR markers of the other family members.

Table 1 - Endocrinological findings in female twin (II-2)

Calendar age	9 2/12
Bone age	6 10/12
Somatomedin-C	0.64 U/ml
Triiodothyronine (T_3)	1.7 ng/ml
Thyroxine (T_4)	10.7 μg/dl
Thyroid Stimulating Hormone (TSH)	1.82 μU/ml
Testosterone	< 5.0 ng/dl
Growth Hormone	normal reaction pattern

INVESTIGATION AND RESULTS

1. *Cytogenetic Studies*

The peripheral blood lymphocyte karyotypes of the twins and their parents were investigated using the GTG technique. The Q-banding technique was performed to detect the Y chromosome, and skin fibroblasts of the twins were cultured in Earle's base MEM with 20% fetal bovine serum. The karyotype of fifth passage cells was investigated with the GTG technique (Table 2). The father and mother both showed normal karyotype in peripheral lymphocytes, 46,XY and 46,XX respectively (50 cells counted for each).

Table 2 - Cytogenetic findings

	Peripheral blood lymphocytes		Skin fibroblasts	
	45,X	47,XYY	45,X	47,XYY
II-2	17	83	41	59
II-3	3	97	31	69

Parents [father: 46,XY (50 cells counted) ; mother: 46,XX (50 cells counted)]

The peripheral lymphocyte karyotype of the female twin (II-2) was 45,X/47,XYY (17:83) mosaicism and her twin brother (II-3) also had the same mosaicism with a different ratio, 45,X/47,XYY (3:97). Furthermore, the karyotypes of their skin fibroblasts revealed the same mosaicism, with a different ratio between each twin. (See Annexes 1-3)

2. *Zygosity Determination*

Eleven blood group systems (polymorphic red cell antigen systems, and enzyme systems) of all family members were studied. In addition, RFLPs were analyzed by the Southern Blotting method using ^{32}P-labelled six VNTR probes (pYNZ22 [RsaI], pYNH24

[PvuII], pEFD52 [HaeIII], pCMM101 [MspI], pCI6-111 [MspI], pCMM86 [MspI]). The probability of monozygosity, Pr(MZ), was calculated according to the Essen - Möller formula:

$$Pr(Mz) = \frac{1}{1 + 1/q \cdot \Pi(Di/Mi)}$$

where Di or Mi is the probability that DZ or MZ twins have the same genotypes at a given genetic locus and q is the rate of monozygotic and dizygotic twinning in the population (q = 3.51 in Japan). On the eleven blood group systems, Pr(MZ) was 0.99527034. On the 6 VNTR markers, Pr(MZ) was 0.99944386 and in combination with all genotypes, Pr(MZ) 0.99999071, and Pr(DZ) was 0.00000928 <10-5 (Tables 3,4).

Table 3 - Blood group genotypes in the family and relative chance in favour of dizygotic twins (D/M value)

	I-1	I-2	II-2	II-3	D/M Values
Red cell antigen types					
ABO	AB	A	AB	AB	0.3336
MNSs	MNs	Ns	Ns	Ns	0.5
P	P_2	P_2	P_2	P_2	NI.
Rh	CCDee	CcDEe	CCDee	CCDee	0.5
Enzyme, Serum types					
PGM	1+2-	1+2+	1+1+	1+1+	0.5
PGD	A	A	A	A	NI.
AcP	—	—	B	B	0.8*
EsD	2-1	1-1	1-1	1-1	0.5
Hp	2-2	2-2	2-2	2-2	NI.
Gm	Gm(1+2-3-5-)	Gm(1+2-3-5-)	Gm(1+2-3-5-)	Gm(1+2-3-5-)	NI.
Km	Km(1-)	Km(1-)	Km(1-)	Km(1-)	NI.

NI = not informative 0.01668
* = (1+p)/4 in no parental data

Table 4 - RFLP genotypes on VNTR markers and relative chance in favour of dizygotic twins (D/M Value)

Probes		I-1	I-2	II-2	II-3	D/M Values
pYNZ22	(RsaI)	2-2	1-2	1-2	1-2	0.5
pYNH24	(PvuII)	2-4	1-3	3-4	3-4	0.25
pEFD52	(HaeIII)	1-3	2-3	2-3	2-3	0.25
pCMM101	(MspI)	1-2	3-4	1-4	1-4	0.25
pCI6-111	(MspI)	1-4	2-3	1-2	1-2	0.25
pCMM86	(MspI)	1-1	2-2	1-2	1-2	NI.

Restriction enzymes in parentheses 0.00195313
NI: not informative

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DISCUSSION

Seven cases of MZ twins with discordant sex have been reported in recent years. In two of these cases, both twins were reported to have 45,X/46,XY mosaicisms [6,9]. In a further two cases, the female twin was reported to have a 45,X/46,XY mosaicism while the male twin had 46,XY only [2,10].

The mechanism in the first two cases is when the twinning division occurs after nondisjunction of the Y. While, in the latter two cases, it is possible that nondisjunction of the Y took place after the twinning event and so the female twins may have been chimera rather than 45,X/46,XY mosaicism. Edwards et al [4] reported on a female monozygotic twin with a 45,X/46,XY mosaicism and the male cotwin with 45,X only. In this case, the male twin may have had a germinal mosaicism of 45,X/46,XY. Fujimoto et al [5] reported a MZ pair of discordant sex, both having a 45,X/46,X, idic(Y) mosaicism caused by anaphase lag before the twinning event. In all of these cases, the female twins were Turner syndrome with 45,X cell lines. Nondisjunction, or anaphase lag, occurred before or after the twinning process and so the different distribution of the cell lines 45,X/46,XY or 46,X, idic(Y) in the germinal cells contributed to the occurrence of discordant sex.

Returning to our case at Kyushu University, the twin pair were concordant on eleven biochemical genetic markers thus indicating the likelihood of monozygosity to be 0.99527034. Analysis on 6 VNTR markers revealed likelihood of monozygosity to be 0.99944386. In combining the biochemical and VNTR markers the probability of monozygosity was determined as 0.99999071. This, we believe, is the first report of a discordant-sexed twin pair determined as monozygotic, using VNTR markers.

The most plausible explanation of X/XYY mosaicism was the nondisjunction of the Y chromosome in the first cleavage division of the 46,XY zygote. If the error had occurred later in cleavage, eg. after the establishment of the normal cell lines, then a multiple cell line mosaicism would have appeared. We could not detect any other cell lines, including a 46,XY. Only a few cases of 45,X/46,XYY mosaicism have been previously reported, see eg. Mulcahy et al [7]. The clinical features were widely variable: two cases had female external genitalia and short stature; one case had male external genitalia with gynecomastia; and another ambiguous external genitalia. There were no correlations between phenotype and the ratio of 45,X/47,XYY cell lines in lymphocytes and skin fibroblasts. Phenotypic sex would seem to be determined by the proportion of the germinal cell lines in the embryo see (Fig. 2).

In conclusion, the twinning event is normally expected to occur either before choriogenesis or between choriogenesis and amniogenesis [3]. In our case, the twinning event seems to have occurred in the 45,X/47,XYY zygote state and, consequently, the disproportionate rate of the cell populations, especially in the germ lines, would account for the within-pair sex discordancy.

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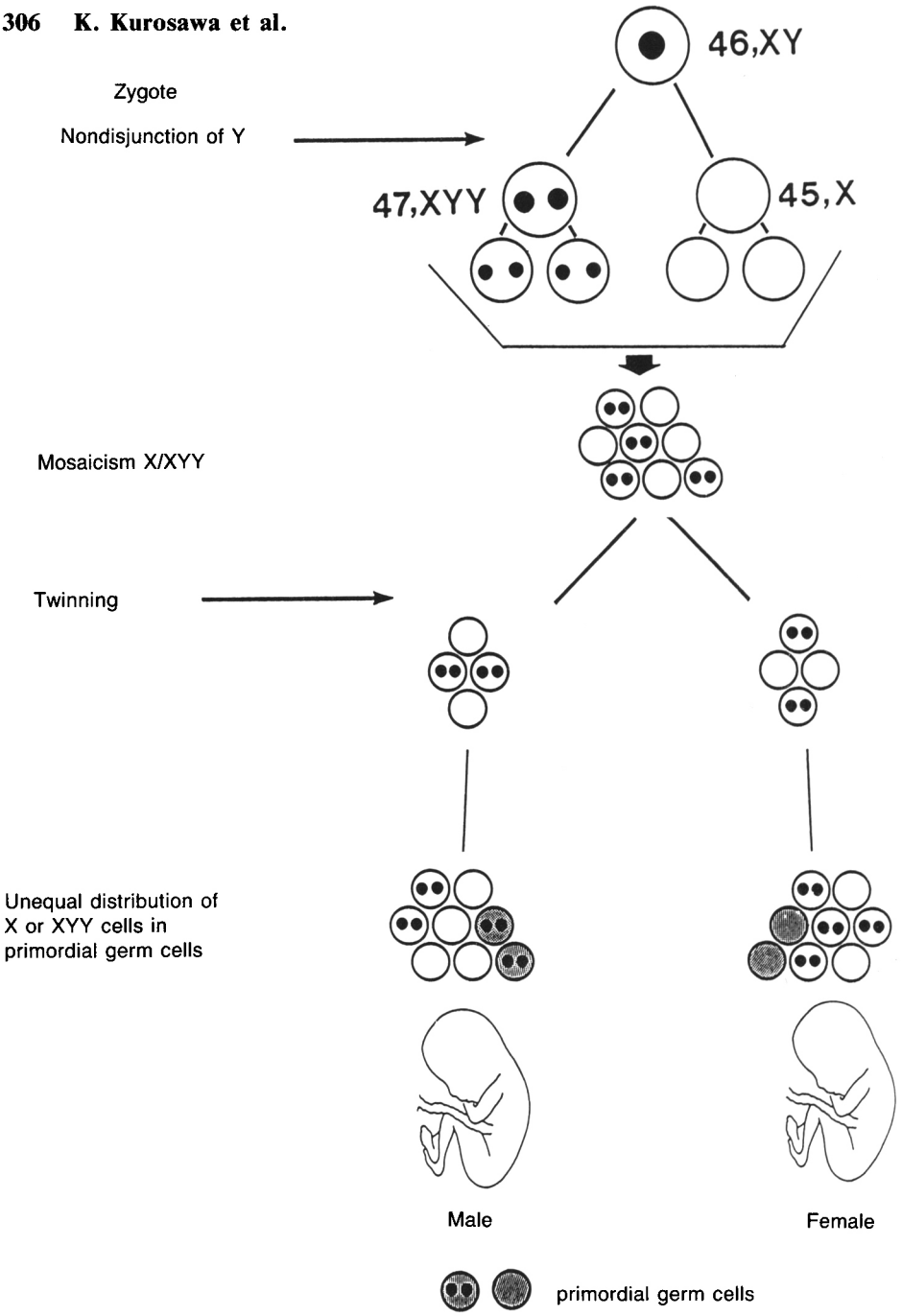

Fig. 2. Mechanism of the occurrence of monozygotic twins with discordant sex.

Nondisjunction of Y had occurred in the first cleavage division of the 46,XY zygote. Disproportionate rate of cell populations with 45,X and 47,XYY in the twinning process of the X/XYY embryo, especially in the germ lines, would result in discordant sex of the twin pair.

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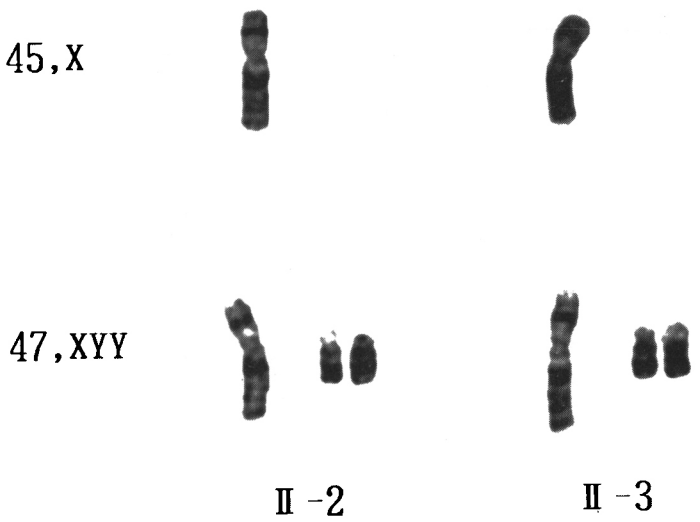
Annexe 1



Karyotypes of female twin (II–2) and her twin brother (II–3).

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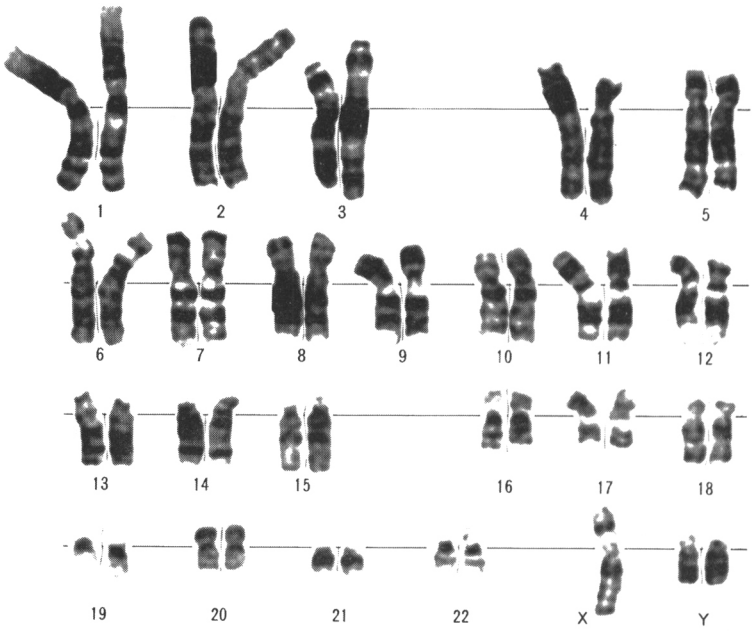
Annexe 2



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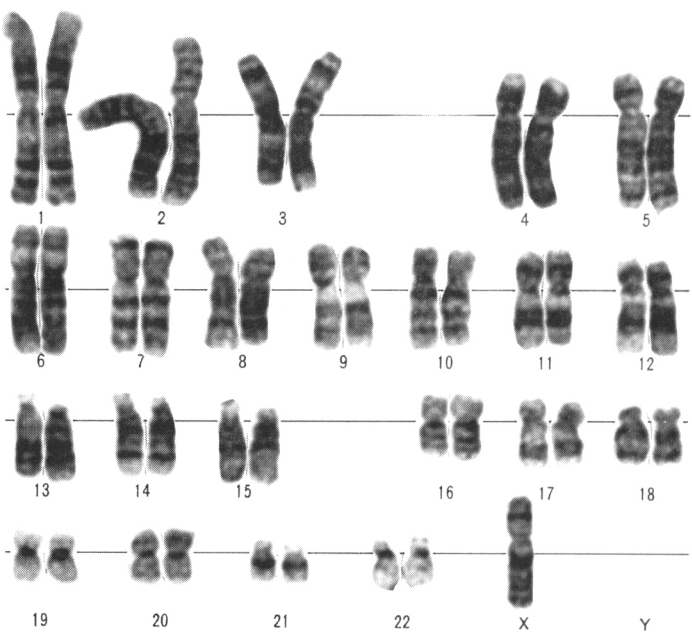

Ⅱ-2

Peripheral lymphocyte karyotype of female twin 45,X/47,XYY mosaicism (17:83).

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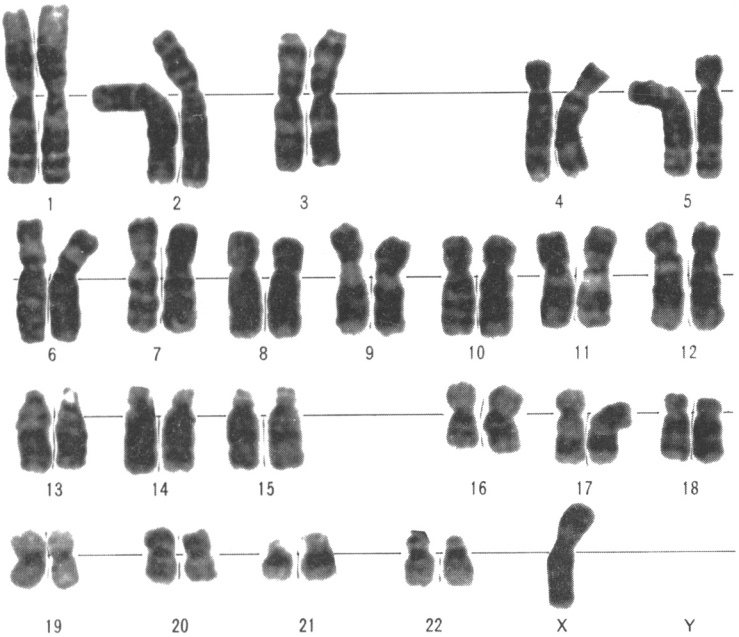
Annexe 3



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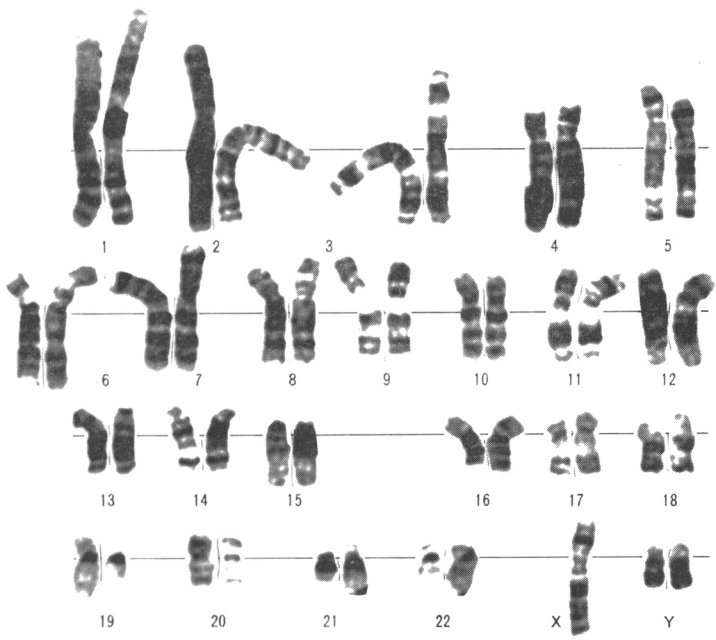

Ⅱ-3

Peripheral lymphocyte karyotype of male twin 45,X/47,XYY mosaicism (3:97).

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