CYTOGENETIC STUDIES IN WOMEN WITH PRIMARY AMENORRHEA

S K Ten, Y M Chin, P Jamilatul Noor, K Hassan

ABSTRACT

Cytogenetic investigations were carried out on 117 women with primary amenorrhea who had been referred to our Genetics Laboratory by clinicians throughout Malaysia, after exclusion of other causes of the disorder. Thirty-six cases (31%) showed numerical or structural abnormalities of the sex chromosomes. These can be broadly classified into 4 main types, namely, presence of a Y chromosome (14%), X-chromosome aneuploidies (8%), structural anomalies of the X-chromosome (7%) and lastly, presence of a marker chromosome (2%). Mosaics constituted 17% of the abnormalities observed, always in association with a 45,X cell line. There was no observable correlation between the phenotype of the patients and their respective abnormal karyotypes.

The aetiological role of sex chromosomal abnormalities in these amenorrheic women is discussed.

Keywords: Cytogenetic, Primary amenorrhea

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INTRODUCTION

Primary amenorrhea is not a disease but a symptom that may result from several quite different causes. These include such conditions as endocrinological imbalance, gonadal anomalies and specific genetic disorders [1]. Cytogenetic investigations have shown the importance of chromosomal abnormalities as a cause of amenorrhea. Different surveys have implicated chromosomal aberrations in causing this symptom in 16-50 per cent of cases [2-11]. The wide variation in frequencies obtained is largely due to the degree of stringency in selection of the study population and, in part, to the small sample size studied in some surveys.

Although extensive studies have been conducted in the Caucasian population, the relative importance of chromosomal abnormalities as causes of primary amenorrhea in the Malaysian population has not been assessed. The purpose of our study is primarily to determine the frequency and types of chromosomal abnormalities among our Malaysian women with this disorder. Furthermore, it is our aim to see whether there might be any correlation between the karyotype and the phenotypic features.

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MATERIALS AND METHODS

A total of 117 women who failed to menstruate before 18 years of age were studied consecutively between 1980 and November 1985. Their age at presentation ranged from 18 to 36 years with a mean of 21 years. These women were referred to our cytogenetics laboratory by hospitals and private practitioners throughout Malaysia. The extent of clinical, laboratory and hormonal studies prior to referral varied considerably for all the patients. Information on these was obtained from the referring physicians.

For all chromosomal investigations of routinely cultured lymphocytes, we used G-banding [12], if necessary, C- [13] and Q-banding [14] were also done. For each case, 30 metaphase spreads were analysed. When mosaicism was suspected, at least 50 metaphases were examined.

RESULTS

The results are summarized in Fig 1 and Tables 1 to 4. The frequency of abnormal karyotypes was 31%. The chromosomal abnormalities can be broadly classified into 4 main types with or without mosaicism. The most

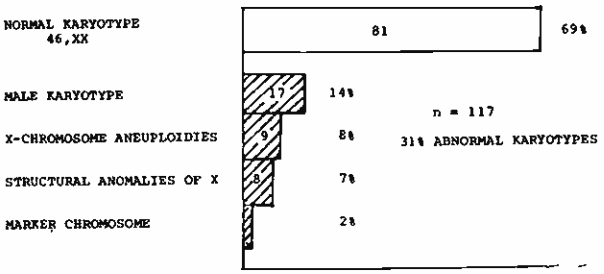

Fig 1
Karyotype distribution of patients with primary amenorrhea.

frequent karyotype was due to the presence of a Y chromosome (14%), followed by X chromosome aneuploidies (8%). Of the remaining patients, 7% had structural anomalies of one X chromosome and 2%, presence of a marker chromosome. Mosaics constituted 17% of the total chromosomal abnormalities observed, always in association with a 45, X cell line.

Table I
Male Chromosome Constitution

Karyotype	No. of patients	Hormonal statues	Clinical features
46, XY	14	increased FSH/LH (3)	Normal Stature Female external genitalia Poor sec sexual characteristics Streak gonads (4): absence of uterus/ fallopian tubes (2) rudimentary uterus (2) Dysgerminoma (1) Leydig cell tumour of right ovary (1)
45, X/46, XY	1	–	Short stature Streak gonad Absent uterus
45, x/47, XY, +13	1	–	Short stature Poor sec sexual characteristics
45, X/46, XY/ 45, X, t (Y;17)/ 46, XY, t(Y;17)	1	–	Short stature Webbing of neck Poor sec sexual characteristics

( ) : Number of patients
sec : secondary
FSH : follicle stimulating hormone
LH : luteinising hormone

Of the 17 patients with a male chromosome constitution, 3 were mosaics (Table I). The 14 patients

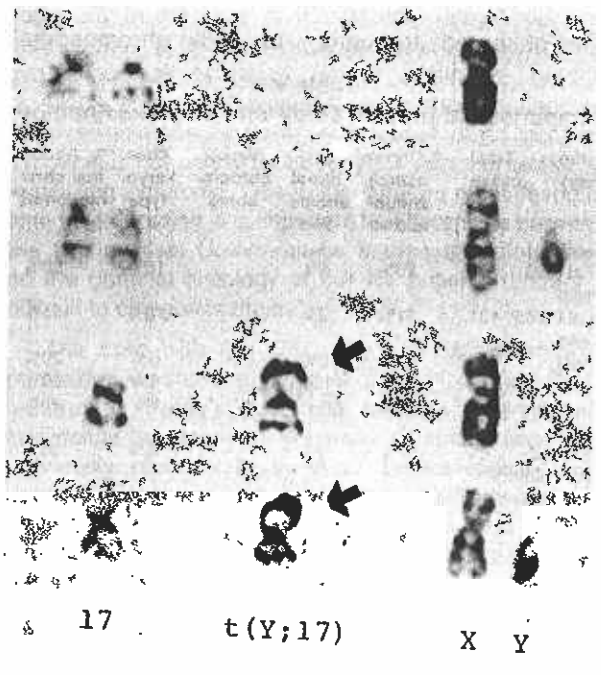

Fig 2
Partial karyotypes of patient with (Y; 17) translocation having four cell lines: 2n= 45, X/46, XY/45, X, t(Y;17)/46, XY, t(Y;17)

Table II
X-Chromosome Aneuploidies

Karyotype	No. of patients	Hormonal status	Clinical Features
45, X	8	Increased FSH/LH Decreased Estradiol	TURNER'S STIGMATA Short stature Poor sec sexual characteristics Small, atrophied uterus Streak gonads
45, X/47, XXX	1	Increased FSH Decreased estradiol	TURNER'S STIGMATA Mentally retarded

with 46,XY karyotypes had normal stature, female external genitalia but poor secondary sexual characteristics. Laparoscopy done on four patients showed the presence of streak gonads with rudimentary or absent uterus and fallopian tubes. One of the 4 patients had dysgerminoma and another Leydig cell tumour of

Table III
Structural Anomalies of X

Karyotype	No. of patients	Hormonal status	Clinical Features
ISOCHROMOSOMES			
46, X, i (Xq)	4	Increased FSH/LH Decreased estradiol	Short stature Sexual infantilism Mentally retarded (1)
45, X/46, X, i (Xq)	1	Increased FSH/LH	Short stature Poor sec sexual characteristics
DELETIONS			
46, X, del (Xq)	2	Increased FSH/LH	Short stature Poor sec sexual characteristics Mentally retarded (1)
ANOMALY IN Xq			
46, X, Xq+	1	–	Short stature Female external genitalia Poor sec sexual characteristics Small hypoplastic uterus

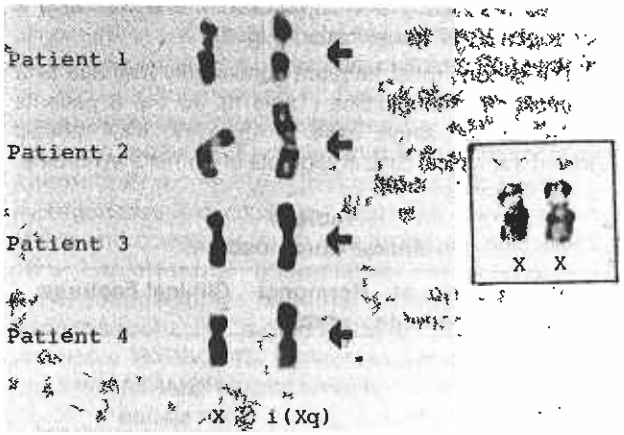

Fig 3
Partial karyotypes of 4 patients with isochromosome of the long arm of X: 2n= 46, X, i(Xq). Insert:Normal XXs.

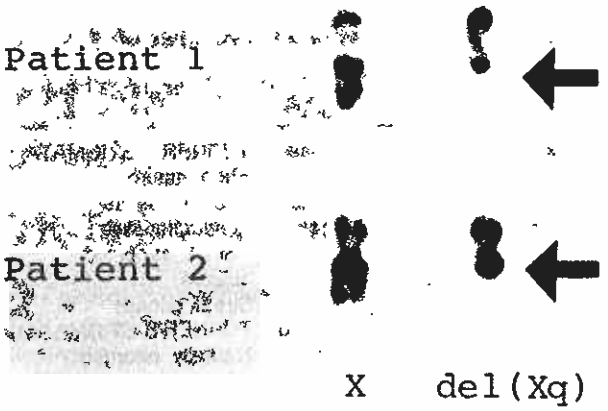

Fig 4
G-banded X-chromosome pairs from 2 patients with partial long arm deletions: 2n = 46, X, del (Xq).

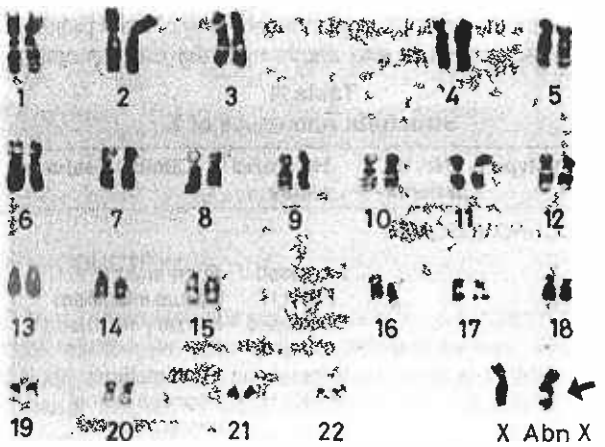

Fig 5
G- banded karyotype of a patient with an abnormal X chromosome.

the right ovary. The 3 mosaics were comparatively shorter than their pure 46, XY counterparts. The patient with the 45, X/47, XY, +13 mosaicism did not show any dysmorphic features associated with trisomy 13. Of 61 cells analyzed, 7 cells contained additional chromosome 13. Another very interesting case was one of mosaicism with four cell lines present in her lymphocyte culture. G- and Q-banding of the patient's metaphases indicated that besides having a Y chromosome, she was also a carrier of a Y; 17 translocation (Fig 2).

The second most frequent abnormality was due to X chromosome aneuploidies (Table II). All the 8 patients as well as the single case of 45, X/47, XXX mosaic showed the typical clinical stigmata of Turner's syndrome.

Table IV
Marker Chromosome

Karyotype	No. of patients	Hormonal status	Clinical Features
46, x, mar	1	–	TURNER'S STIGMATA
45, X/46, X, mar	1	–	Short stature Hypogonadism Shield chest Clitoris not enlarged

mar: marker chromosome

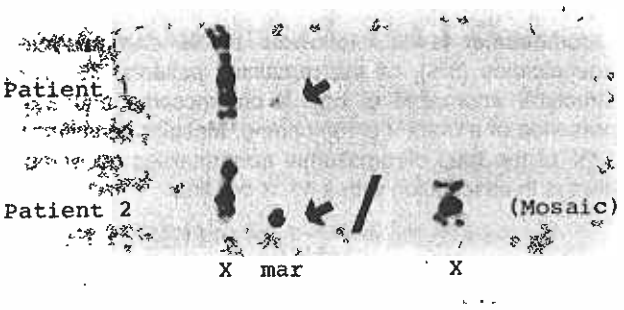

Fig 6
Partial G-banded karyotypes of 2 patients with a marker chromosome: 2n = 46, X, mar. Patient is a mosaic with a 45, X cell line as well.

In addition, all had increased serum gonadotrophin levels and decreased plasma estradiol level. The mosaic patient was also mentally retarded.

Structural anomalies of the X chromosome were detected in 8 patients (Table III). Five patients were found to have an isochromosome of the long arm of X (Fig 3), two had deletion of the long arm of X (Fig 4) and another structural defect in the long arm of X (Fig 5). The nature of the extra chromosomal material at the distal region of Xq in the case with the Xq+ anomaly could not be determined with G- or Q- banding. The abnormal X is probably an unbalanced translocation product. Although the clinical features of these patients varied considerably, all of them showed at least one or more 'Turner-like specific features (like short stature, poor development of gonads or secondary sexual characteristics). Serum follicle stimulating hormones and luteinising hormones measured in 7 of these patients were always increased.

Marker chromosomes, differing both in morphology and size, were found in 2 patients (Table IV, Fig 6). Both patients showed features of Turner's syndrome.

DISCUSSION

The observed frequency of 31% chromosomal

Table V
Comparison of present study with previous findings

Authors (year)	Total cases	X-Chromosome aneuploidies	Structural anomalies	Marker chromosome	Male karyotype	% Abnormal chromosomes
Jacobs et al (1961)	32	25%	9%	-	16%	50%
Philip et al (1965)	101	21%	2%	-	18%	41%
Kallio (1973)	100	11%	3%	-	6%	20%
Sarto (1974)	50	22%	10%	-	6%	38%
Van Niekerk (1978)	77	20%	-	-	5%	25%
Sulewski et al (1980)	11	9%	9%	-	18%	36%
Joseph & Thomas (1982)	63	8%	3%	2%	3%	16%
Mulye et al (1983)	120	9%	6%	-	7%	22%
Opitz et al (1983)	88	17%	7%	-	4%	28%
Anglani et al (1984)	145	25%	10%	-	11%	46%
Combined series	787	17%	5%	.001%	7%	33%
Present study	117	8%	7%	2%	14%	31%

abnormalities is comparable to the overall frequency of 33% deduced from the pooled data of ten independent studies conducted between 1961 - 1984 (Table V). The abnormalities always involved the sex chromosomes.

In contrast to previous surveys, our study showed very distinctly that the presence of a Y chromosome was the most frequent abnormal karyotype observed. Furthermore, marker chromosomes which constituted 2% of the abnormalities observed was previously detected in only one study by Joseph and Thomas [8].

Generally, the differential diagnosis of XY females can be classified into four types, based on the clinical features, hormonal profiles and histology of the gonads. The two commonest and best known are testicular feminizing syndrome (androgen insensitivity syndrome) and pure XY gonadal dysgenesis (Swyer's syndrome) [15]. It can be considered that the ovarian failure is not directly caused by the chromosomes as such, since these patients have the normal chromosome constitution of the male sex. Rather, it results from a disorder of sexual differentiation. Pedigree studies have shown that testicular feminizing syndrome is inherited through a gene whose expression is limited to the male sex, either a recessive X-linked or a dominant autosomal [5]. The outstanding features of this syndrome is feminizing of the patients, absence of fallopian tubes, uterus and cervix with immature gonards resembling foetal testes [4,15].

On the other hand, pure XY gonadal dysgenesis is a genetically heterogeneous condition. It appears to be inherited either as a sex-linked or an autosomal - linked gene [5]. The presence of a cervix or Mullerian system, sexual infantilism and low-normal testosterone levels in these patients serve to differentiate XY gonadal dysgenesis from testicular feminizing syndrome [16].

The pathological significance of these two forms is that they are at risk of developing malignant tumours of the gonads as was demonstrated by our two patients. Hence it is important that the rudimentary streak gonads especially in the case of XY gonadal dysgenesis and intra-abdominal testes in the case of testicular feminizing syndrome are removed before malignant growth develops. It is recommended that the streak gonads be removed as soon as possible for cases of XY gonadal dysgenesis but only after puberty for cases of testicular feminizing syndrome. Due to the different recommended time for extirpation, it is important to differentiate between the two entities. Unfortunately, inadequate information on the gonadal histology of our XY patients renders it difficult to categorize them.

The case with 45, X/47, XY, +13 mosaicism was perplexing in showing none of the typical phenotypic features of Patau's syndrome and having a normal intellectual development. A somewhat similar case was previously reported in a 46,XY female mosaic with gonadal dysgenesis and normal intellectual development having double autosomal trisomies 8 and 21 [7]. Haemopoietic chimerism rather than mosaicism was then offered as an alternative explanation for the peripheral blood karyotype.

To the best of our knowledge, no cases of 46, XY female mosaic with a Y;17 translocation have been reported before. The presence of somatic features of Turner's syndrome and ovarian failure in our patient with this chromosomal anomaly is probably accounted for by the 45, X cell line. It is of interest to note here that Ferguson-Smith [17] hypothesized that short stature and other somatic abnormalities of Turner's syndrome can be produced by a deletion from a Y chromosome because the Y may carry genetic material which prevents the expression of Turner's features in a normal male. Whether our patient's phenotype is related to a break in the Y chromosome resulting from the Y;17 translocation remains unresolved. The mechanism through which the mosaicism could arise is also not apparent as cytogenetic data of the patient's family is lacking.

The clinical spectrum of our patients with abnormalities of the X chromosome is wide. In general, patients with monosomy X showed typical Turner's stigmata while those with structural anomalies showed one or more Turner-like features. The difference in clinical manifestation may be explained by the theories proposed by Fraccaro et al [18] and Wyss et al [19]. It is postulated that genes whose absence determine the somatic features of Turner's syndrome are distributed along all of Xp and the middle of Xq, specifically between q13 - q26. Based on this hypothesis, it follows that our five patients with isochromosome of Xq (and hence, monosomy for Xp) were of short stature and showed only certain features of Turner's, such as sexual infantilism, streak gonads and associated congenital malformations.

On the other hand, genes essential for gonadal function are located on the proximal part of Xp, the long arm of X proximal to Xq13 and/or the long arm of X distal to Xq26 [17-20]. Large deletions of Xq with breakpoints at or proximal to q13 are expected to produce gonadal dysgenesis with primary amenorrhea; half of the patients with such deletions have Turner's syndrome [18]. This is found to be so in our two patients with deletions of Xq with breaks presumably at or after Xq13, and also by the single case with Xq+ involving an apparent break at or distal to Xq26.

The cause of the mental retardation in three of our patients is not clear. The presence of the 47, XXX cell line in one of the cases could possibly be the cause as it has been found that an extra X can slow down embryonic cell development in a special way [21]. As for the case with del (Xq) anomaly, partial monosomy of Xq could have exposed a hemizygous recessive gene present on the intact X chromosome.

In conclusion, our study confirms that chromosomal abnormalities are significant aetiological factors in gonadal dysgenesis resulting in primary amenorrhea. Furthermore, an appreciable proportion of these individuals with sex chromosomal abnormalities are mosaics. When hormonal data was available, it was found that the chromosomal abnormalities occur in association with a hypergonadotropic condition. This was also noted by Mulye and coworkers [9] who observed an incidence of 48% chromosomal abnormalities in a group of hypergonadotropic patients versus none in a hypogonadotropic hypogonadism group. Certain anomalies involving the X chromosome may in addition result in mental retardation as well.

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REFERENCES

1. McDonough PG: Amenorrhea - Etiologic approach to diagnosis. Fertil Steril 1978; 30: 1-15.
2. Jacobs PA, Harnden DG, Buckton KE, et al: Cytogenetic studies in primary amenorrhea. Lancet 1961; i: 1183-8.
3. Philip J, Sele V, Trolle D: Primary amenorrhea. A study of 101 cases. Fertil Steril 1965; 16: 795-804.
4. Kallio H: Cytogenetic and clinical study on 100 cases of primary amenorrhea. Acta Obstet Gynecol Scand Suppl 1973; 24: 7-78.
5. Sarto GE: Cytogenetics of fifty patients with primary amenorrhea. Am J Obstet Gynecol 1974; 119: 14-23.
6. van Niekerk WA: Chromosomes and the gynecologist. Am J Obstet Gynecol 1978; 130: 862-75.
7. Sulewski JM, Dang TP, Ferguson KA, Ward SP, Ladda RL: Chromosomal abnormalities associated with infertility. Obstet Gynecol 1980; 55: 469-75.
8. Joseph A, Thomas IM: Cytogenetic investigations in 150 cases with complaints of sterility or primary amenorrhea. Hum Genet 1982; 61: 105-9.
9. Mulye V, Ambani L, Shring M, Peter J, Motashaw N: Cytogenetic studies in primary amenorrhea. Indian J Med Res 1983; 78: 53-8.
10. Opitz O, Zoll B, Hansmann I, Hinney B: Cytogenetic investigation of 103 patients with primary or secondary amenorrhea. Hum Genet 1983; 65: 46-7.
11. Anglani F, Baccichetti C, Artifoni L, Lenzini E, Tenconi R: Frequency of abnormal karyotypes in relation to the ascertainment method in females referred for suspected sex chromosome abnormality. Clin Genet 1984; 25: 242-7.
12. Seabright M: A rapid banding technique for human chromosomes. Lancet 1971; ii: 971-2.
13. Chandley AC, Fletcher JM: Centromere staining at meiosis in man. Humangenetik 1973; 18: 247-52.
14. Caspersson T, Zech L, Johansson C, Modest EJ: Identification of human chromosomes by DNA-binding fluorescent agents. Chromosoma 1970; 30: 215-27.
15. Madan K, Schoemaker J: XY females with enzyme deficiencies of steroid metabolism. A brief review. Hum Genet 1980; 53: 291-5.
16. Swyer GIM: Male pseudohermaphroditism: a hitherto undescribed form. Br Med J 1955; 2: 709-12.
17. Ferguson - Smith MA: Karyotype - phenotype correlations in gonadal dysgenesis and their bearing on pathogenesis of malformations. J Med Genet 1965; 2: 142-56.
18. Fraccaro M, Maraschio P, Pasquali F, Scappaticci S: Women heterozygous for deficiency of the (p21 -> pter) region of the X chromosome are fertile. Hum Genet 1977; 39: 283-92.
19. Wyss D, DeLozier CD, Daniell J, Engel E: Structural anomalies of the X chromosome: personal observation and review of non-mosaic cases. Clin Genet 1982; 21: 145-59.
20. De la Chapelle A, Schroeder J, Haahtela T, Aro P: Deletion mapping of the human X chromosome. Hereditas 1975; 80: 113-20.