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Cytogenetic analysis of patients with primary and secondary amenorrhoea in Hong Kong: retrospective study

香港人口中原發性及繼發性無月經症患者的細胞遺傳學分析：回顧研究

Objective. To estimate the incidence and type of chromosomal abnormalities in patients with primary and secondary amenorrhoea in Hong Kong.
Design. Cytogenetic analysis and retrospective review.
Setting. Clinical Genetic Service, Department of Health, Hong Kong.
Patients. Case records of 549 patients with either primary (n=237) or secondary (n=312) amenorrhoea referred to the Clinical Genetic Service from 1 January 1991 to 30 April 2002 were reviewed. All these patients with amenorrhoea would have karyotyping (G banding) performed.
Main outcome measures. Clinical characteristics of patients, and incidence and type of chromosomal abnormalities in the local population.
Results. Sex chromosome anomaly was found in 24.5% and 9.9%, respectively, of women with primary and secondary amenorrhoea. In those with primary amenorrhoea, male karyotype was identified in 8.4% and X-chromosome abnormalities in 16.0%.
Conclusion. The incidence of chromosomal abnormalities in women with amenorrhoea is similar to that reported in the literature. Chromosomal abnormalities are identified often enough to warrant karyotyping of all women with amenorrhoea.

目的：評估染色體異常在香港原發性及繼發性無月經症患者的比率及類型分佈。
設計：細胞遺傳學分析及回顧研究。
安排：香港衛生署醫學遺傳科。
患者：從1991年1月1日至2002年4月30日期間，549名因原發性(n=237)及繼發性 (n=312) 無月經症而被轉介到醫學遺傳科的病人。所有病人均以G帶核型檢視染色體。
主要結果測量：患者的臨床特徵以及染色體異常在香港患者的比率和類型分佈。
結果：性別染色體異常分別佔原發性及繼發性無月經症患者的24.5%及9.9%。在原發性無月經症患者當中，8.4%擁有男性核型，16.0%則發現X染色體異常。
結論：染色體異常在香港無月經症患者的比率跟其他文獻報告十分相近。無月經症患者，不論是原發性或繼發性，都應為其進行核型排列。

Key words:
Amenorrhea;
Chromosome aberrations;
Karyotyping;
Ovarian failure, premature

關鍵詞：
無月經症；
染色體異常；
核型；
卵巢早衰

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Introduction

Hormonal disorders are the main causes of primary and secondary

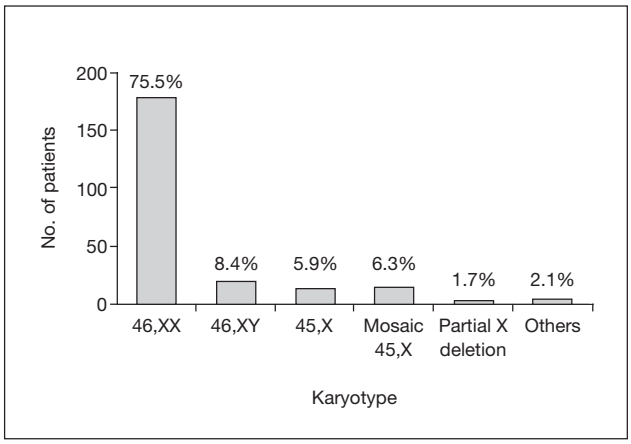

Fig 1. Karyotype distribution of patients with primary amenorrhoea

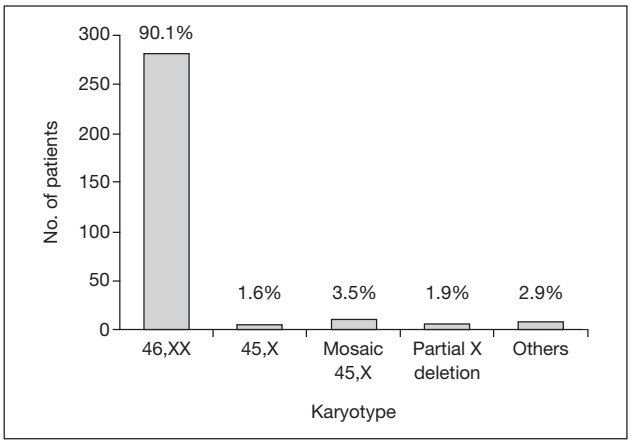

Fig 2. Karyotype distribution of patients with secondary amenorrhoea

amenorrhoea, although there are others. Common hormonal causes of primary amenorrhoea include constitutional delay, chronic systemic disease, hypothalamic-pituitary dysfunction, and absent ovarian function. Secondary amenorrhoea can be due to pregnancy, hypothalamic-pituitary disorders, polycystic ovarian disease, resistant ovarian syndrome, and absent or premature ovarian failure. Although there are many other reasons for ovarian failure, genetic or chromosomal causes are the most important as their presence affects subsequent management. For example, girls with XY gonadal dysgenesis have a high (30%) lifetime risk of gonadal malignancy and their testes should be removed as soon as possible.[1]

A number of surveys in various parts of the world have endeavoured to ascertain the contribution of sex chromosomal abnormalities to the problem of amenorrhoea. The percentage of chromosomal abnormalities reported varies greatly, from 15.9% to 63.3% for primary amenorrhoea[2-7] and from 3.8% to 44.4% for secondary amenorrhoea.[3,6,7,8-10] The wide variation is likely due to the different selection criteria of different studies.

This study was undertaken to determine the frequency and type of chromosomal abnormalities that result in primary and secondary amenorrhoea in the local population.

Patients and methods

All women with primary or secondary amenorrhoea who were referred to the Clinical Genetic Service of the Department of Health from 1 January 1991 to 30 April 2002 were recruited. Primary amenorrhoea was defined as the absence of menstruation and secondary sexual characteristics in phenotypic women aged 14 years or older, or aged 16 years or older if secondary sexual characteristics were present. Patients with secondary amenorrhoea had at least one spontaneous bleeding episode, followed by no menstruation for a minimum of 12 months at or before the age of 42 years. Patients were referred from different local hospitals or clinics for genetic analysis after exclusion of non-genetic causes. The diagnosis of primary or secondary amenorrhoea was ascertained at the patient's initial visit. Physical examination was performed to identify any secondary sexual characteristics or syndromal features. Clinical information and laboratory investigations were obtained from hospital records or the referring doctor.

Chromosomal analysis of all patients was performed on routinely cultured lymphocytes after Trypsin-Leishman banding. A minimum of 15 metaphases were examined in each patient. If abnormal karyotype was found in this first analysis, further cells (up to 100) could be examined.

Results

There were 549 women referred for primary or secondary amenorrhoea during the study period: 237 (43.2%) presented with primary amenorrhoea and 312 (56.8%) with secondary amenorrhoea. Most were referred from local hospitals, only three were referred by private practitioners. Age at referral to the Clinical Genetic Service ranged from 14 to 43 years (median, 19 years) for primary amenorrhoea and 16 to 48 years (median, 32 years) for secondary amenorrhoea.

A pathological or male karyotype was present in 58 (24.5%) of the patients with primary amenorrhoea (Fig 1). Age at first presentation ranged from 14 to 39 years. Male karyotype accounted for 20 (8.4%) patients: five had pure XY gonadal dysgenesis, two of whom had a gonadal tumour at presentation. Fourteen were suspected to have testicular feminisation syndrome in the absence of any internal female sex organs. One patient had end-stage renal failure and was also suspected to have Drash syndrome.

X-chromosome abnormalities (either structural or numerical) were found in 38 (16.0%) patients: Turner's syndrome (n=14), mosaic Turner (n=15), partial X-chromosome deletion (n=4), X isochromosome (n=1), X-autosome translocation (n=2), 46,X, +mar,der(Y) (n=1), and mosaic 47,XXX/48,XXXX (n=1). The percentage of mosaicism ranged from 1% to 85%. All except two patients with X-chromosome abnormalities showed poor development of breasts, genital organs, or secondary sexual characteristics. The two patients who were considered to have normal secondary sexual characteristics had mosaicism: 45,X/46,XX/47,XXX/48,XXXX in a ratio of 6:85:8:1 and 45,X/46,X,del(X)(p11.4) in a ratio of 38:62.

There were 312 patients referred with secondary amenorrhoea. Age of menopause ranged from 11 to 40 years (median, 30 years). An abnormal karyotype was present in 31 (9.9%) patients (Fig 2). These comprised the following: monosomy X (n=5, 1.6%), mosaic Turner karyotype (n=11, 3.5%: percentage of mosaicism ranged from 2% to 89%), partial X-chromosome deletion (n=6, 1.9%), isochromosome X (n=1), X-autosome translocation (n=2), trisomy X (n=3), mosaic triple X (n=2), and mosaic 48,XXXX/49,XXXXX (n=1). These last three all had a small percentage (2-3%) of abnormal cells.

Age of menarche and menopause for patients with monosomy X, varied from 12 to 20 years and 18 to 26 years, respectively. Two of five patients had been reported to have normal secondary sexual characteristics. At presentation, only one was married and she had not conceived. Age of menarche and menopause in patients with mosaic Turner or Turner variants, ranged from 11 to 17 years and 18 to 40 years, respectively. Three of 10 patients who had married had no children. All of them had mosaic Turner karyotype, with 4%, 6%, and 23% monosomy cells, respectively. Age of menarche and menopause in patients with triple X syndrome ranged from 11 to 14 years and 23 to 38 years, respectively. All three patients had normal secondary sexual characteristics.

A total of 16 patients (primary or secondary amenorrhoea) had cell lines with X-chromosome deletions or translocations. The long arm of the X chromosome in the region from q13 to q26.3 was involved in 13 patients. The short arm of the X chromosome at p11 was involved in the other three.

As a reference, abnormal karyotypes other than pure 45,X, 46,XX, 46,XY and 47,XXX and patient characteristics are shown in Tables 1a and 1b.

Discussion

A large number of surveys have been undertaken worldwide in a bid to ascertain the frequency of sex chromosomal anomalies in patients who present with primary or secondary amenorrhoea. Most have had small patient numbers. In this study it was possible to include a large number of patients since most patients in Hong Kong are referred to our centre for cytogenetic studies and counselling. Nonetheless this does not take account of those who are unwilling to attend the clinic because of embarrassment or cultural issues. In addition, patients with primary or secondary amenorrhoea due to other causes were not referred and thus excluded from the study. The actual frequency of sex chromosome abnormalities in patients with amenorrhoea in Hong Kong thus remains undetermined.

Previous estimates of the frequency of sex chromosomal abnormalities vary from 15.9%[4] to 63.3% for primary amenorrhoea, with the majority falling between 20% and 30% (Table 2a). The estimated frequency following this study of 24.5% is thus comparable. Estimations for the frequency of secondary amenorrhoea show a wider variation: 3.9% to 44.4% (Table 2b). This may be due to the wide variation in patient selection criteria of different studies. Studies carried out in the last 5 years have quoted rates of 9.9% and 11.1%.[3,8] Nonetheless these studies involved only a small number of women (30 and 9, respectively). This was in accordance with the figure obtained by this study (9.9%) and thus may represent the actual frequency.

Male karyotype presented in a significant percentage (8.4%) of patients with primary amenorrhoea although they appeared physically normal with some just appearing tall for their age. Physical presentation of the male karyotype may occur later on. Two patients presented to a gynaecologist when they were over the age of 30 years and about to get married. This emphasises the importance of an early diagnosis

Table 1a. Patients with primary amenorrhoea and karyotype other than pure 45,X, 46,XX, 46,XY and 47,XXX and their characteristics

Case No.	Karyotype*	Age at presentation (years)	Body height (cm)	Secondary sexual development†
A40	45X/47XXX (87%:13%)	17	139	No
A143	45X/46XY (85%:15%)	18	150	No
A53	45X/46X,r(x)(p11.2q24) (77%:23%)	18	137	Poor
A28	45X,inv(9)(p11q13)/46X,r(X),inv(9)(p11q13) (76%:24%)	21	144	Poor
A155	45X/46X,+mar der(X)(DX21+) (67%:33%)	16	136	No
A14	45X/46X,i(Xq) (82%:18%)	28	138	Poor
A24	45X/46X,i(Xq) (77%:23%)	22	138	Poor
A202	45X/46X,i(Xq) (61%:39%)	21	140	Poor
A63	45X/46X,Xq- (58%:42%)	16	155	No
A140	45X/46X,del(X)(q27) (65%:35%)	18	154	No
A134	45X/46X,del(X)(p11.4) (38%:62%)	30	149	Yes
A42	45X/46XX/47XXX/48XXXX (6%:85%:8%:1%)	42	160	Yes
A22	45X/46XX/47XXX (6%:92%:2%)	30	147	Poor
A139	45X/46XX (9%:91%)	34	152	No
A33	45X/46XX (1%:99%)	43	150	Yes
A66	46X,+mar,der(Y)	16	149	Poor
A215	46X,del dup(X) (pter→q22::q22→q11.1)	16	152	Poor
A174	46X,del(X)(p11.1)	37	140	Poor
A153	46X,del(X)(q22.2)	30	174	Poor
A49	46X,del(X)(q26-qter)	17	156	Poor
A92	46X,i(Xq)	20	140	Poor
A191	46X,t(15;X)(p11.2;q21.2)	28	152	Poor
A1	46X,t(X;9)(p11;q11)	21	168	Poor
A186	46XX/47XXX/48XXXX (75%:24%:1%)	30	141	Poor

* Shaded boxes denote mosaic Turner karyotype
† Breast and pubic hair development: No=Tanner stage 1; Poor=Tanner stage 2-4; and Yes=Tanner stage 5

Table 1b. Patients with secondary amenorrhea and karyotype other than pure 45,X, 46,XX, 46,XY and 47,XXX and their characteristics

Case No.	Karyotype*	Age at menopause (years)	Body height (cm)	Secondary sexual development†
B264	45X/46X,del(X)(q25) (50%:50%)	27	147	Poor
B173	45X/46X,idic(X)(p11.2) (34%:66%)	22	142	Poor
B115	45X/46XX (89%:11%)	30	139	Poor
B153	45X/46XX (40%:60%)	18	150	Poor
B87	45X/46XX,inv(9)(p11q13) (23%:77%)	31	143	Poor
B138	45X/46XX (22%:78%)	32	137	Yes
B52	45X/46XX/46XY (10%:20%:70%)	27	161	Poor
B306	45X/46XX (6%:94%)	40	158	Yes
B67	45X/46XX (4%:96%)	33	154	Yes
B77	45X/46XX (4%:96%)	36	148	Yes
B74	45X/46XX/47XXX (2%:97%:1%)	30	162	Yes
B23	46X,del(X)(q13q26)	29	150	Yes
B226	46X,del(X)(q21.2q26)	30	151	Yes
B125	46X,del(X)(q23)	19	150	Poor
B233	46X,del(X)(q24)	26	160	Yes
B44	46X,del(X)(q26)	28	147	Yes
B58	46X,del(X)(q26.3)	30	155	Yes
B143	46X,i(Xq)	22	139	Poor
B272	46XX, FXA:91-92 repeats	26	154	Poor
B66	46XX,t(1;8)(p31.1;q22.3)	24	158	Yes
B55	46XX,t(14;X)(p11.2;q26.3)	21	144	No
B105	46XX,t(X;22)(q26;q11.2)	18	156	Yes
B262	46XX/47XX,+mar (44%:56%)	27	161	Yes
B41	46XX/47XXX (97%:3%)	33	152	Yes
B49	46XX/47XXX (98%:2%)	34	161	Yes
B56	46XX/48XXXX/49XXXXX,inv(9)(p11q13) (96%:2%:2%)	20	155	Yes

* Shaded boxes denote mosaic Turner karyotype
† Breast and pubic hair development: No=Tanner stage 1; Poor=Tanner stage 2-4; and Yes=Tanner stage 5

Table 2a. Chromosomal abnormalities in primary amenorrhoea*

	Present study	Temocin et al,[3] 1997	Roy and Banerjee,[5] 1995	Ten et al,[2] 1990	van Niekerk,[6] 1978	Series compiled,[6] before 1978
Study population	Hong Kong	Turkey	India	Malaysia	South Africa	-
No. of cases	237	68	60	117	77	336
46,XX	179 (75.5)	50 (73.5)	22 (36.7)	81 (69.2)	56 (72.7)	239 (71.1)
Abnormal karyotype	58 (24.5)	18 (26.5)	38 (63.3)	36 (30.8)	21 (27.3)	97 (28.9)
46,XY	20 (8.4)	-	2 (3.3)	16 (13.7)	4 (5.2)	29 (8.6)
45,X	14 (5.9)	-	16 (26.7)	9 (7.7)	7 (9.1)	39 (11.6)
Mosaic 45,X	15 (6.3)	-	20 (33.3)	-	8 (10.4)	23 (6.8)
46X,del(X)	4 (1.7)	-	-	-	-	-
46X,i(Xq)	1 (0.4)	-	-	-	-	-
X-A translocation	2 (0.8)	-	-	-	-	-
Mosaic triple X	1 (0.4)	-	-	-	-	-
46,X,+mar	1 (0.4)	-	-	2 (1.7)	-	-

* Values are expressed as No. (%), unless otherwise stated

Table 2b. Chromosomal abnormalities in secondary amenorrhoea*

	Present study	Devi and Benn,[8] 1999	Temocin et al,[3] 1997	Lin and Yu,[10] 1996	Castillo et al,[9] 1992	Opitz et al,[7] 1983	van Niekerk,[6] 1978
Study population	Hong Kong	United States	Turkey	Shanghai	Chile	Germany	South Africa
No. of cases	312	30	9	18	47	15	103
46,XX	281 (90.1)	26 (86.7)	8 (88.9)	10 (55.6)	32 (68.1)	10 (66.7)	99 (96.1)
Abnormal karyotype	31 (9.9)	4 (13.3)	1 (11.1)	8 (44.4)	15 (31.9)	5 (33.3)	4 (3.9)
45,X	5 (1.6)	-	-	-	5 (10.6)	-	-
Mosaic 45,X	11 (3.5)	-	-	-	-	-	-
46X,del(X)	6 (1.9)	-	-	-	-	-	-
46X,i(Xq)	1 (0.3)	-	-	-	-	-	-
X-A translocation	2 (0.6)	-	-	-	-	-	-
47,XXX	3 (1.0)	-	-	-	-	-	-
Mosaic triple X/ quadruple X	3 (1.0)	-	-	-	-	-	-

* Values are expressed as No. (%), unless otherwise stated

as prophylactic gonadectomy can be advised. Two other patients already had a gonadal tumour at presentation; early diagnosis of XY gonadal dysgenesis may have prevented their occurrence.

A study of fertility in Danish women with Turner's syndrome revealed that 7.6% of them could achieve spontaneous pregnancy, but 48% of the fertile women registered with 45,X/46,XX mosaicism had 45,X in less than 10% of the analysed cells.[11] In our study, patients with pure monosomy X were invariably infertile. Three (30%) of 10 married women with mosaic Turner karyotype did reproduce. The percentage of monosomy cells ranged from 4% to 23%. This indicates that pregnancy is possible in patients with X-chromosome abnormalities, especially when there is a low percentage of mosaicism. The reverse also applies: a history of pregnancy in a woman with later unexplained amenorrhoea should not exclude her from cytogenetic investigation. There have been case reports of familial Turner syndrome,[12-16] although the reproductive life is short, with premature menopause, and an increased risk of miscarriage and abnormal offspring.[11,17] Counselling should include the condition on future offspring, for examples, infertility, premature menopause; and its consequences such as osteoporosis, coronary heart disease, etc.

Premature ovarian failure may be secondary to X-chromosome deletions or translocations. Reports of patients with premature ovarian failure and Xq deletions suggest that there is a gene (*POF1*) localised to Xq21.3-q27 or within Xq26.1-q27[18-20] and a gene (*POF2*) localised to Xq13.3-q21.1.[19] In this study, most patients had cell lines with X-chromosome deletions or translocations at Xq13-q26.3, comparable with the suggested *POF* genes. Three patients had an Xp11 deletion, indicating that Xp11 or the proximal part of the short arm is also crucial for normal ovarian function as suggested by other studies.[21]

Three (1.0%) patients with secondary amenorrhoea

had triple X karyotype. It has been suggested that girls with karyotype 47,XXX have a higher incidence of ovarian failure.[22] As triple X syndrome is thought to be quite common in the general population, the true frequency cannot be determined. Such a karyotype may represent the aetiology of amenorrhoea or be just a coincidental finding. Further evaluation might be necessary to clarify the situation.

A significant number of patients had sex chromosomal abnormalities, thus early cytogenetic investigation is prudent to guide further management. Patients with amenorrhoea should be initially screened by primary physicians and gynaecologists for non-genetic causes. After exclusion of non-genetic causes, patients with amenorrhoea, in particular primary amenorrhoea, should receive prompt referral for genetic study. The reason for referral should be explained to the patient. If cytogenetic abnormalities are detected, a full explanation should be given to the patient by a geneticist or gynaecologist with experience in genetics. Counselling should include the risk of premature menopause for patients with Turner's syndrome and the use of hormonal replacement therapy, the possibility of infertility in the future children of patients with mosaic Turner, and the risk of gonadal malignancy for patients with XY gonadal dysgenesis. Counselling should be performed tactfully, bearing in mind that sensitive issues related to femininity are involved. An experienced counsellor and clinical psychologist would be helpful.

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