

# Turner syndrome diagnosed in northeastern Malaysia

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## ABSTRACT

**Introduction:** Turner syndrome affects about one in 2,000 live-born females, and the wide range of somatic features indicates that a number of different X-located genes are responsible for the complete phenotype. This retrospective study highlights the Turner syndrome cases confirmed through cytogenetic analysis at the Human Genome Centre of Universiti Sains Malaysia, from 2001 to 2006.

**Methods:** Lymphocyte cultures were set up using peripheral blood samples, chromosomes were prepared, G-banded, karyotyped and analysed in accordance to guidelines from the International System for Human Cytogenetic Nomenclature.

**Results:** The various karyotype patterns observed were 45,X; 46,X,i,(Xq); 45,X/45,X,+mar; 45,X/46,X,i,(Xq) and 45,X/46,XY. The mean age of our patients with Turner syndrome was 21 years, and the most common clinical features encountered in all these patients were short stature (100 percent), primary amenorrhoea (85.7 percent), absence of secondary sexual characteristics (57.1 percent), scanty pubic and axillary hair (50 percent), webbed neck (42.9 percent), wide carrying angle (42.9 percent), rudimentary uterus with bilateral streak ovaries (42.9 percent), underdeveloped breasts (35.7 percent) and wide-spaced nipples (21.4 percent).

**Conclusion:** Even though there is no causal therapy for Turner syndrome, management and treatment are possible for malformations and conditions associated with it. In addition, counselling of the parents and of the patients themselves are necessary. Hence, establishing an early diagnosis, educating and increasing awareness among doctors, and if possible, a prenatal diagnosis, will help in early intervention, genetic counselling and in improving the quality of life in these patients.

**Keywords:** cytogenetic analysis, genetic

counselling, gonadal dysgenesis, short stature, Turner syndrome

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## INTRODUCTION

Turner syndrome, gonadal dysgenesis or gonadal agenesis represents a special variant of hypergonadotrophic hypogonadism, and is due to the lack of the second sex chromosome or parts of it. This syndrome affects about one in 2,000 live-born females.<sup>(1)</sup> The wide range of somatic features in Turner syndrome indicates that a number of different X-located genes are responsible for the complete phenotype.<sup>(2)</sup> The syndrome includes those individuals with a phenotypic spectrum from female to male, with varying clinical stigmata of the syndrome, as described by Turner.<sup>(3)</sup> Though many karyotype abnormalities have been described in association with Turner syndrome, monoclonal monosomy X and its various mosaicisms, each with an X monosomic (XO) cell clone, are the most frequent karyotype anomalies. In other instances, the syndrome is due to a structural anomaly of the second X chromosome. The symptoms of this syndrome have been logically deduced to be caused by a single dosage of genes that are normally present and active in two dosages.

## METHODS

Peripheral blood was collected from the patients and lymphocyte cultures were set up using RPMI 1640 medium, foetal bovine serum, antibiotics, and phytohaemagglutinin (mitogen). The cultures were incubated at 37°C for 72 hours following which the cell division was arrested by the addition of colcemid. The cultures were then harvested and chromosomes were prepared by the conventional method, following which they were G-banded, karyotyped and analysed according to guidelines from the International System for Human Cytogenetic Nomenclature (ISCN,

**Table I. Various karyotype patterns of Turner syndrome.**

Karyotype	No. of cases
45,X	8
46X,i(Xq)	3
45,X/45,X,+mar	1
45,X/46,X,i(Xq)	1
45,X/46,XY	1

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**Table II. Clinical phenotypes of the 14 Turner patients.**

Patient	Age (years)	Short stature	Webbed neck	Widely-spaced nipple	Under-developed breasts	Wide carrying angle	Scanty pubic and axillary hair	Rudimentary uterus with bilateral streak ovaries	Primary amenorrhoea	Absence of secondary sexual characteristics
1.	29	√	√	x	√	x	√	x	x	x
2.	47	√	√	x	x	√	x	√	√	x
3.	17	√	√	x	√	√	√	x	√	√
4.	19	√	x	x	√	x	√	√	√	√
5.	18	√	√	√	x	√	√	√	√	√
6.	34	√	x	x	√	√	√	x	√	√
7.	22	√	x	x	x	x	x	√	√	x
8.	17	√	x	x	x	x	x	x	√	√
9.	16	√	x	x	x	x	x	x	√	√
10.	28	√	√	x	√	x	x	√	√	x
11.	16	√	x	x	x	x	x	x	√	x
12.	23	√	x	√	x	x	x	x	√	√
13.	16	√	x	x	√	√	√	√	√	√
14.	8	√	√	√	x	√	x	x	x	x

**Table III. Mean and standard error (SE) of the different hormonal profiles of the Turner patients.**

Hormonal test	Mean ± SE	Range	Normal range	Age range (years)
FSH (mIU/ml)	79.79 ± 19.19	34.5–200	25.8–134.8	16–47
TSH (μIU/L)	2.26 ± 0.59	1–3.6	0.5–4.7	16–47
LH (mIU/L)	27.53 ± 6.04	8.2–72.2	7.7–58.5	16–47
Progesterone (nmol/L)	1.74 ± 0.26	1.12–2.2	0.3–2.5	16–47
Testosterone (nmol/L)	11.26 ± 9.14	0.3–52.1	0.2–2.9	16–47
Free T4 (pmol/L)	14.52 ± 1.41	11.5–19.07	9.1–23.8	16–47
Prolactin (μIU/ml)	410.32 ± 78.77	169–657.6	102–496	16–47
Oestradiol (pmol/L)	51.74 ± 18.37	18–132.3	< 145	16–47

1995).<sup>(4)</sup> In three cases, the sex-determining region-Y (SRY) test was also carried out to confirm the results of the routine cytogenetic analysis. For the SRY test, the DNA was extracted and amplified for the SRY gene using polymerase chain reaction (PCR). The PCR product was then run on gel electrophoresis for the detection of the bands.

## RESULTS

The cytogenetic analysis for the Turner syndrome cases showed different patterns of karyotypes (Table I). The mean age of our patients with Turner syndrome was 21 (range 8–47) years. The most common clinical features encountered in all these patients were short stature (100%, 14/14), primary amenorrhoea (85.7%, 12/14), absence of secondary sexual characteristics (57.1%, 8/14), scanty pubic and axillary hair (42.9%, 6/14), webbed neck (42.9%, 6/14), wide carrying angle (42.9%, 6/14), rudimentary uterus with bilateral streak ovaries (42.9%, 6/14), underdeveloped breasts (42.9%, 6/14) and widely-spaced nipples (21.4%, 3/14). A history of slow weight gain, as well as abnormality of the kidney, was observed in one case. Flat feet were present in another case. A third

case presented with an overlapping of the fourth toe on the fifth toe bilaterally with osteoporosis of the neck of the femur. Mental retardation was noticed in a single Turner case, and short metacarpal bones were seen in one patient. The clinical phenotypes are presented in Table II and the different hormonal profiles in Table III. The hormonal tests were not performed in two patients, aged eight and 34 years. The karyotypes as well as the metaphase spreads of Turner syndrome with various patterns are shown in Figs. 1a and 1b (45,X/45,X,+mar) and Fig. 2 (46,X,i,(Xq)). Two out of the three cases that were tested for the presence of SRY gene, indicated negative for the SRY gene as characterised by the presence of bands. However, in the third case, where the patient presented with a mosaic Turner syndrome, the test indicated positive for the presence of SRY gene.

## DISCUSSION

This is probably the first report on the incidence of Turner syndrome in northeastern Malaysia. The cases presented here are those studied from 2001 to 2006 in the Human Genome Centre of Universiti Sains Malaysia, Kelantan, Malaysia. The first case of probable Turner syndrome was

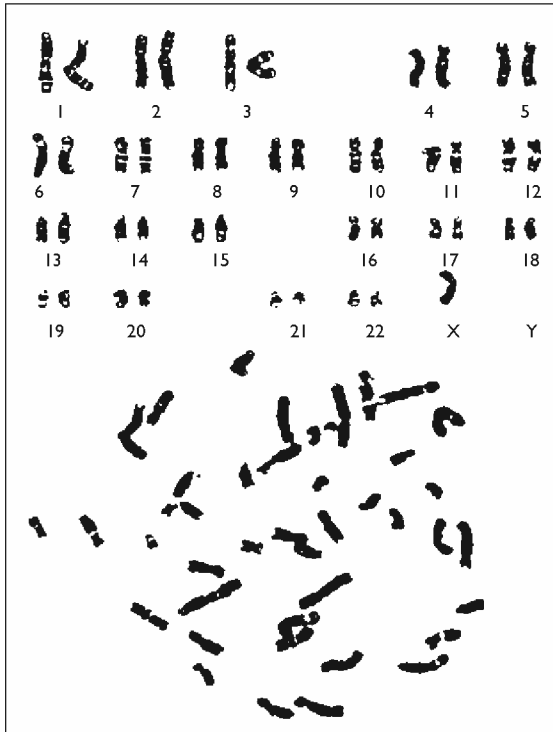


Fig. 1a Metaphase spread and karyotype of a Turner 45,X/45,X,+mar (45,X).

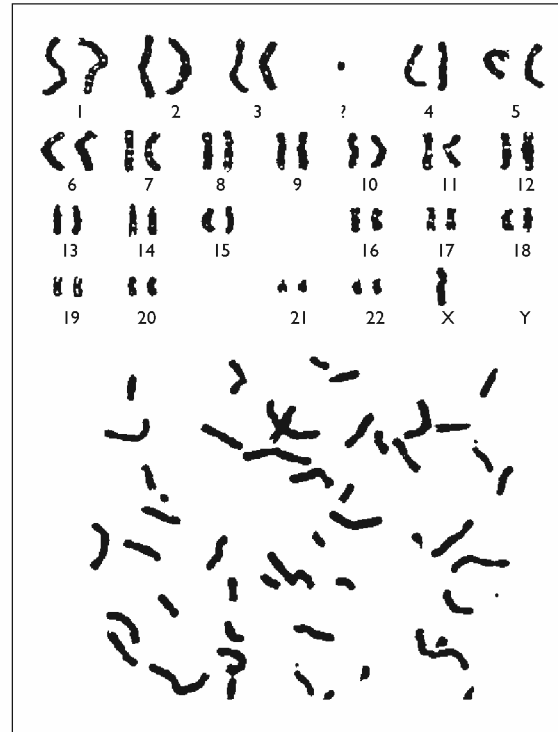


Fig. 1b Metaphase spread and karyotype of a Turner 45,X/45,X,+mar (45,X,+mar).

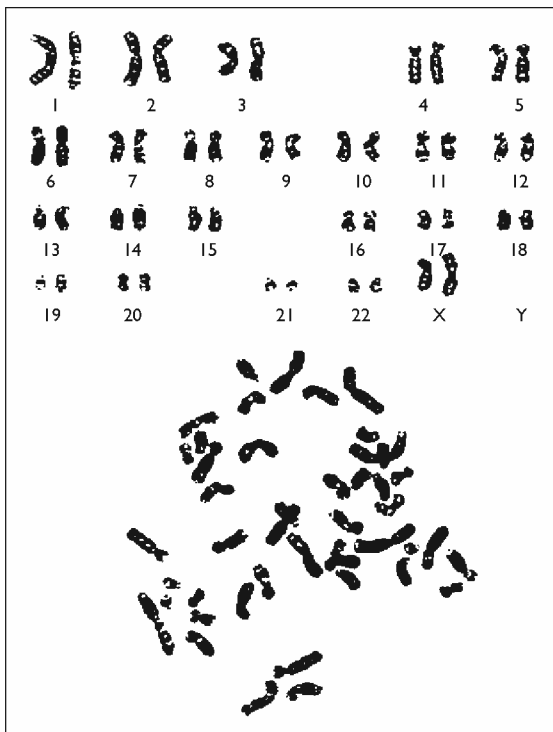


Fig. 2 Metaphase spread and karyotype of a Turner 46,X,i(Xq).

as reported by Funke, a patient who presented many of the criteria for the syndrome, including lymphoedema, small stature and amenorrhoea at the age of 16 years and pterygium colli. Ullrich collated several cases reported thereafter and identified the first case as the Turner syndrome. In 1938, Turner described a variant of primary

amenorrhoea in seven adolescent and young adult females with sexual infantilism, short stature, webbed neck and cubitus valgus.<sup>(5)</sup> However, it was Sharpey-Shafer who described further cases of this syndrome and coined the term 'Turner syndrome'.<sup>(5)</sup> Since then, a number of other karyotype anomalies, such as mosaicisms and structural anomalies of the second X chromosome, have been found in association with Turner syndrome.

Monosomy X is the most frequent karyotype of the syndrome; next in frequency are the mosaicisms XO/XX, XO/XXi and XO/XY. Their relative frequencies in the series are 15.5%, 9.3% and 9.0%, respectively.<sup>(6)</sup> Other karyotype anomalies are rare, and XX Turner syndrome with intrachromosomal changes of the genetic material, such as inversions, is exceptional. In the present study, the incidence of the most frequent karyotype of the Turner syndrome was found to be 45,X (57.1%), followed by 46,X,i(Xq) (21.4%), 45,X/45,X,+mar (7.1%), 45,X/46,X,i(Xq) (7.1%) and 45,X/46,XY (7.1%). In previous studies, the relative frequencies in terms of the most commonly-occurring karyotypes reported in Turner syndrome were 45,X (45%), 45,X/46,XX (13%), 45,X/46,X,i(Xq) (8%), 46,X,i(Xq) (7%) and 45,X/46,XY (7%); although some correlations between karyotype and phenotype have been made, phenotypic predictions for a given patient that are based on karyotypic analysis are unreliable in patients with Turner syndrome.<sup>(7)</sup>

Two pathogenic mechanisms, which have been described in the literature, lead to monoclonal monosomy

X: meiotic non-disjunction and chromosomal lag or loss. However, a great variety of X chromosome abnormalities have been described in this syndrome. The following mosaicisms are due to mitotic loss of a second normal X or a structurally abnormal X in some cells: XO/XX; XO/XY; XO/X,i (Xp); XO/ X,i (Xq); XO/XXp-; and XO/XXq-. Different karyotypes are associated with varying phenotypic expression. The Xq isochromosome is associated with autoimmune disorders but not congenital abnormalities. The clinical picture of Turner syndrome varies from case to case. Phenotype is not well predicted by genotype, particularly in the case of mosaicism.<sup>(8)</sup> This is particularly true of the various mosaicisms when the picture depends on the ratio of the different cell populations and their distribution in various tissues and organs.

The mean age of our patients with Turner syndrome was 21 years, with a range from eight to 47 years, and a wide range of somatic features have been observed in these cases. All the patients belonged to the Malay ethnicity and were from the state of Kelantan. Short stature was the most common clinical feature present in the cases of Turner syndromes observed at this centre, followed by primary amenorrhoea, absence of secondary sexual characteristics, scanty pubic and axillary hair, webbed neck, wide carrying angle, rudimentary uterus with bilateral streak ovaries, underdeveloped breasts and widely-spaced nipples. In addition to the above characteristics, a history of slow weight gain, as well as abnormality of the kidney, flat feet, overlapping of the fourth toe on the fifth toe bilaterally with osteoporosis of the femoral neck, short metacarpal bones and mental retardation were noticed. Ultrasonographical findings also indicated hypoplastic left heart in one of our patients.

The wide range of somatic features in Turner syndrome indicates that a number of different X-located genes are responsible for the complete phenotype. Short stature in Turner syndrome has been associated with haploinsufficiency of a critical chromosomal region (distal of Xp22.2), which escapes inactivation (pseudoautosomal region of X and Y) and in which the short stature-homeobox (SHOX) gene resides (Xp22.33).<sup>(2)</sup> As a result, most women with Turner syndrome have only one copy of the SHOX gene, instead of the usual two copies, which reduces the amount of SHOX protein by half. Researchers believe that this deficiency is at least partly responsible for the short stature and skeletal abnormalities in women with this condition. In addition, genes USP9X (DFRX) on the p arm, RPS4X, and DIAPH2 on the q arm of the X chromosome have also been identified as the candidate genes for Turner syndrome.<sup>(2)</sup>

In young women presenting with primary amenorrhoea, the single most common cause is primary

ovarian failure due to gonadal dysgenesis. These patients have significantly elevated gonadotropin levels due to ovarian failure. Ovarian failure is most commonly associated with Turner syndrome, followed numerically by 46,XX gonadal dysgenesis, and rarely, 46,XY gonadal dysgenesis.<sup>(9)</sup> Growth retardation is less pronounced and the clinical picture of the two most frequent mosaicisms—XO/XX and XO/XY—varies between normal female phenotype and typical Turner syndrome. Moreover, male phenotypes and intersexes with ambiguous genitalia may be found in association with XO/XY mosaicism. Women with Turner syndrome also can have a mosaic pattern with additional Y chromosome material (45,X/46,XY). Although this mosaic pattern is uncommon, these women have an increased risk for the development of gonadoblastoma, dysgerminoma, and masculinisation, and prophylactic gonadectomy is warranted.<sup>(10)</sup> The presence of a Y cell line in a patient with Turner syndrome brings with it an increased risk of developing gonadoblastomas and malignant germ cell tumours within the streak gonads.<sup>(9)</sup>

Ultrasonographical findings suggestive of Turner syndrome include increased nuchal translucency, cystic hygroma, coarctation of the aorta or other left-sided cardiac defects, brachycephaly, renal anomalies, polyhydramnios, oligohydramnios, and growth retardation.<sup>(11)</sup> An abnormal maternal serum screen (elevated  $\alpha$ -foetoprotein, dimeric inhibin A, and free  $\beta$ -human chorionic gonadotropin) may also suggest Turner syndrome. Ultrasonographical findings and abnormal maternal serum screen are not sufficient to diagnose Turner syndrome; a karyotype is required for definitive diagnosis. Chromosomes should always be re-evaluated postnatally because abnormal prenatal karyotypes may reflect cytogenetic abnormalities found only in the placenta, but not in the foetus.<sup>(12)</sup> In addition, inexpensive high-throughput screening is possible for Turner syndrome using quantitative genotyping approaches.<sup>(13)</sup> During the newborn period, physical features characteristic of Turner syndrome include lymphoedema of the hands and feet, nuchal folds, left-sided cardiac anomalies (coarctation or hypoplastic left heart), webbed neck, and low hairline. During childhood, declining growth velocity with short stature and elevated levels of follicle-stimulating hormone (FSH) should prompt evaluation for Turner syndrome.<sup>(14)</sup>

There is no causal therapy for Turner syndrome. However, there are several forms of treatment for affected patients, which include management of cardiovascular, intestinal and renal malformations; treatment of microsomia; treatment of the gonadal infantilism; cosmetic surgery of the pterygium and counselling of the parents and of the patients themselves. Psychological counselling about the patient's womanhood should be

of utmost importance; they should be advised that they can marry but will need to adopt children if they want to raise a family.<sup>(8)</sup> The delay in the diagnosis of Turner syndrome in the present scenario could be attributed to the lack of antenatal screening and early neonatal screening. Early diagnosis will enable early intervention and early psychological counselling to the patient as well as the parents, which in turn will help to enhance their quality of life. The institution of societies and referral centres to cater exclusively to the needs of the Turner syndrome patients will also help them to seek advice and improve their outlook towards the society. Hence, establishing early diagnosis, educating and increasing awareness among doctors, as well as prenatal diagnosis, would be an effective measure in alleviating the social trauma related to Turner syndrome patients in this population.

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