Turner syndrome

Relation between genotype and phenotype and long-term follow-up studies

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Cover: The cover picture illustrates two women with Turner syndrome phenotype, one with genotype 45,X and the other with 45,X/46,XX mosaic.

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To my beloved wife Maria,
and my children, Omar, Sara and Jonas

Abbreviations

ALP	=	alkaline phosphatase
ALT	=	alanine aminotransferase
AST	=	aspartate aminotransferase
BMD	=	bone mineral density
BMI	=	body mass index
CCDH	=	cell cycle delay hypothesis
DXA	=	dual energy X-ray absorptiometry
FISH	=	fluorescence in situ hybridization
GH	=	growth hormone
GT	=	γ-glytamyl transferase
HBA1c	=	glycosylated haemoglobin
HbsAg	=	hepatitis B surface antigen
HCV	=	hepatitis C virus
HDL	=	high-density lipoprotein cholesterol
HRT	=	hormone replacement therapy
IGF-1	=	insulin like growth factor-1
LDL	=	low density lipoprotein
MONICA	=	MONItoring of trends and determinants in CArdiovascular disease
PTAmid	=	pure tone averages at mid frequencies
PTAhigh	=	pure tone averages at high frequencies
SHOX	=	short stature homeobox gene
SMR	=	standardized mortality ratio
TPO	=	thyroid peroxidase antibody
TS	=	Turner syndrome
TSH	=	thyroid stimulating hormone
T4	=	thyroxine
WHO	=	World Health Organization
WHR	=	waist/hip ratio

Abstract
Turner syndrome - Relation between genotype and phenotype and long-term follow-up studies
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Turner syndrome (TS) is a chromosomal disorder with a prevalence of approximately 1/2 500 live female births. There is complete or partial absence of one of the two sex chromosomes, resulting in a genetic constellation of 45,X monosomy or 45,X/46,XX mosaic, respectively. In the present studies, using more accurate analysis with Fluorescence In Situ Hybridization (FISH), we investigated whether the international classification of the "genotype-phenotype correlation" should be different. TS women were compared with age-matched controls from the WHO MONICA study, carried out in Gothenburg, into cardiovascular risk factors and bone data. Stigmata were counted and balance and hearing were tested. Mosaics had fewer stigmata, no aortic dissection, were diagnosed 8 years later, had better balance and fine motor function and fewer cardiovascular risk factors compared with 45,X monosomy. The 45,X/46,XX mosaics were, thus, more similar to controls.

Mosaicism mitigated stigmata and the cardiovascular and fracture risk factor profile in TS.

Hypothyroidism and elevated liver enzymes are common in TS but no prospective studies have been performed. Thyroid function and liver enzymes were studied in TS patients during five years. The prevalence of hypothyroidism was 23% with an annual incidence of 3.2%, and the corresponding figures for elevated liver enzymes were 36% and 3.4%, respectively. Hypothyroidism was not associated with karyotype, family history or other metabolic factors but elevated thyroid peroxidase (TPO) antibodies were found in almost half of the TS cases with hypothyroidism. The most prevalently increased liver enzyme was gamma glutamyl transferase (GT) which was correlated with serum cholesterol, independently of obesity, waist/hip ratio and glucose level, but not with serum estradiol.

Every third TS woman developed hypothyroidism at five years and those with elevated TPO were at highest risk. Annual thyroid function control is mandatory. More than every second TS woman had elevated liver enzymes at five years. The elevated liver enzymes were benign. Estrogen replacement can be continued in TS.

Key words: Turner syndrome, chromosome, cardiovascular disease, body balance, fracture, hearing, hypothyroidism, liver
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Svensk sammanfattning

Relation mellan genotyp och fenotyp vid Turners syndrom

Turners syndrom (TS) innebär att en kvinnlig könskromosom helt (45,X) eller delvis, s.k. mosaik (45,X/46,XX), saknas. Det föds cirka 1/2500 flickor med TS. I denna studie användes en noggrannare genetisk analys med Fluorescens In Situ Hybridisering (FISH) på celler från munslemhinnan för att analysera graden av 46,XX celler. TS jämfördes med åldersmatchade kvinnor ur WHO MONICA studien, Göteborg avseende kardiovaskulära riskfaktorer och benmassa. Stigmata, balans och hörsel studerades. Mosaikerna hade färre stigmata, blev diagnostiserade 8 år senare än 45,X och hade bättre balans, finmotorik, hörsel och kardiovaskulär riskfaktorprofil och liknade mera kontrollerna.

Långtidsuppföljning av tyroidea och levervärden vid Turners syndrom

Vi studerade sköldkörtelvärden och leverenzymer hos kvinnor med TS under en 5 årsperiod. Prevalensen av hypotyreos vid starten var 23% med en årlig incidens av 3.2%, och förhöja levervärden 36%, respektive 3.4%. Hälften av dem med hypotyreos hade förhöjda TPO-antikroppar. Det fanns ingen relation till karyotyp, ärftlighet eller metabol faktor vid hypotyreos medan gamma GT, som var det vanligast förhöjda levervärdet, korrelerade positivt, oberoende av fetmagrad, till serum kolesterol.

Hypotyreos var vanligt vid TS oavsett karyotyp. Positiv TPO medför högst risk för utveckling av hypotyroes. Tyroideaprov bör tas årligen. Varannan TS kvinna, oavsett karyotyp, utvecklade förhöjda levervärden som verkade vara benigna. Östrogensubstitution kan fortsättas vid TS.

LIST OF PAPERS

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals.

I. El-Mansoury M, Barrenäs M-L, Bryman I, Hanson C, Larsson C, Wilhelmsen L, Landin-Wilhelmsen K. Chromosomal mosaicism mitigates stigmata and cardiovascular risk factors in Turner syndrome. *Clinical Endocrinology 2007;66:744-51.*

II. El-Mansoury M, Barrenäs M-L, Bryman I, Hanson C, Landin-Wilhelmsen K. Impaired body balance, fine motor function and hearing in women with Turner syndrome. *Clinical Endocrinology; 2009;71:273-8*

III. El-Mansoury M, Bryman I, Berntorp K, Hanson C, Wilhelmsen L, Landin-Wilhelmsen K. Hypothyroidism is common in Turner syndrome – results from a five-year follow-up. *Journal of Clinical Endocrinology and Metabolism, 2005;90:2131-2135.*

IV. El-Mansoury M, Berntorp K, Bryman I, Hanson C, Innala E, Karlsson A, Landin-Wilhelmsen K. Elevated liver enzymes in Turner syndrome during a five-year follow-up study. *Clinical Endocrinology 2008;68:485-90.*

CONTENTS **Page**

INTRODUCTION

In 1938, Henry Turner [1] and Otto Ullrich [2], and N.A. Šereševskij [3] before him, described the clinical features of Turner syndrome (TS), the most specific features of which are short stature and ovarian dysgenesis. TS is defined as the combination of these features and the complete or partial absence of the second sex chromosome, with or without cell-line mosaics [4,5]. Turner syndrome affects approximately one in 2 500 live born girls [6].

History

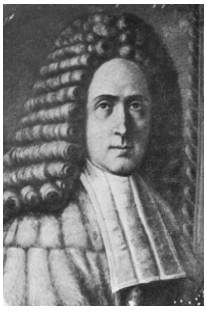
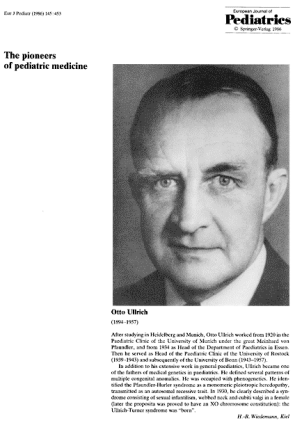


Figure 1. Giovanni Battista Morgagni (1682-1771)

Giovanni Battista Morgagni (1682-1771), Figure 1, an Italian anatomist and the father of modern anatomical pathology, was probably the first to describe a patient with TS (1768). On November 12, 1925, when addressing the question of a connection between congenital abnormalities and endocrinopathies at the Russian Endocrinological Society, N.A. Šereševskij [3] presented a 25-year-old woman who was the youngest of seven siblings. She was different from all the other siblings by always having been of shorter stature. She had consulted a physician about this problem many

times, but was repeatedly told that she would start growing once she started menstruating. Physical examination at the age of 20 revealed a girl 132 cm tall (expected mean height being 158 cm), with a low nuchal hair level, micrognathia, a high arched palate, short neck, and pterygium colli. Axillary or pubic hair growth was lacking and the papillae mammae were introverted and no mammae developed. The patient appeared to be mentally healthy. She had never had a boyfriend, and preferred to socialize with girlfriends. She felt lost when in a crowd, but quite at ease at home with her relatives.


Eur J Pediatr (1986) 145 :453

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The pioneers of pediatric medicine

Otto Ullrich
(1894–1957)

After studying in Heidelberg and Munich, Otto Ullrich worked from 1920 in the Paediatric Clinic of the University of Munich under the great Meinhard von Pfaundler, and from 1934 as Head of the Department of Paediatrics in Essen. There he served as Head of the Paediatric Clinic of the University of Rostock (1939–1940) and subsequently of the University of Bonn (1943–1957).

In addition to his extensive work in general paediatrics, Ullrich became one of the fathers of medical genetics in paediatrics. He defined several patterns of multiple congenital anomalies. He was occupied with phenogenetics. He identified the Pfaundler-Hurler syndrome as a monosomic pleiotropic hereditopathy, transmitted in an autosomal recessive trait. In 1930, he clearly described a syndrome consisting of sexual infantilism, webbed neck and cubiti valgi in a female (later the propositi was proved to have an XO chromosome constitution): the Ullrich-Turner syndrome was "born".

H.-R. Weidemann, Kiel

Figure 2. Otto Ullrich (1894-1957).

The German pediatrician Otto Ullrich (1894-1957), Figure 2, [2] was considered one of the fathers of medical genetics in pediatrics. In 1930, at a meeting of the Munich Pediatric Society, Ullrich presented an eight-year-old girl with webbed neck, stunted stature, cubitus valgus and an unusual facial appearance.

Figure 3. Henry Turner (1892-1970).

In 1938, Henry Turner (1892-1970), Figure 3, [1], born in Harrisburg, Illinois, US, but living and working in Oklahoma, published a paper in The Journal of Endocrinology where he described seven young TS women with sexual infantilism, laxity of the skin of the neck, short stature and retarded bone age. He attributed all these clinical features to a primary defect in the anterior pituitary gland. In 1942, however, Fuller Albright [7] redefined TS as a syndrome of ovarian insufficiency. The first to discover the absence of an X-chromosomal body was the Italian-British genetician Paolo Polani [8], (London, UK) and his collaborators (1954), while the basic chromosomal aberration was described by the Oxford genetician Charles Ford and his collaborators [9].

Later on, as some authors believed that the girl described in 1930 by Ullrich actually had the Noonan syndrome, a controversy arose about whether Ullrich's contribution warranted eponymous recognition. This issue was not settled until 1991, when Hans-Rudolf Wiedemann [10], and J. Glatzl, who restudied the very same patient, then 66 years of age, demonstrated that her chromosomal constitution was 45,X.

Consequently, the Russian literature uses the name Šereševskij syndrome, some Europeans call it Ullrich-Turner syndrome and in American-English countries, the condition is named Turner syndrome.
In Sweden, Jan Lindsten reported on chromosomal mosaicism in 1961 [11] and treatment with growth hormone (GH) in TS in 1964 [12].

GENOTYPE IN TURNER SYNDROME

TS is a disorder caused by the loss of genetic material from one of the two sex chromosomes (monosomy 45,X) or in a proportion of cells (mosaicism 45,X/46,XX). The genotype is usually specified as, for example, 45,X(10)/46,XX(90). Monosomy 45,X is found in 40-50%, mosaicism, 45,X/46,XX, in 20-25%, and iso- or ring chromosome and marker X or Y in about 20-25% **[**13**]**, Figure 4.

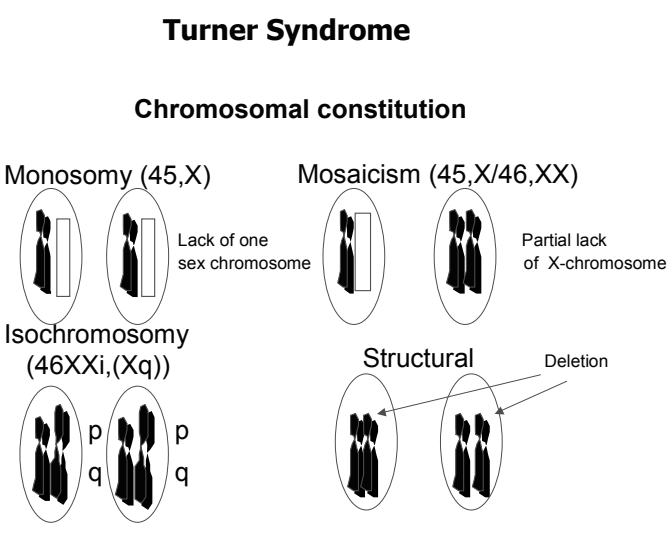

Figure 4. Chromosomal constitution of monosomy 45,X, mosaic (45,X/46,XX), 46,XXi,(Xq10) and structural abnormalitiy in TS.

The short stature homeobox (SHOX) gene is located on the short (p) arm of the X chromosome (Xp11.33), Figure 5. An area called “the pseudoautosomal region” has been shown to escape X inactivation. TS is considered to be a result of haplo-insufficiency of genes that escaped inactivation, e.g. loss of one copy of this gene reduces the amount of SHOX protein by half. This deficiency explains some of the phenotypic characteristics in TS, principally short stature [14,15].

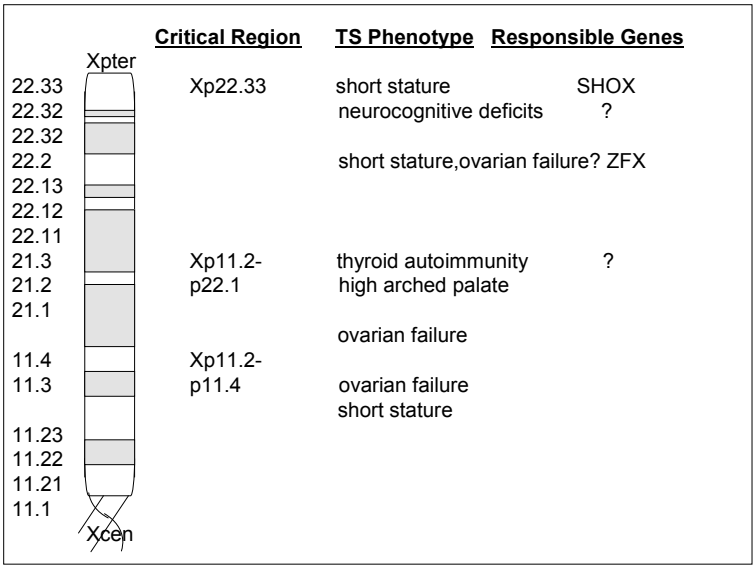

Figure 5. Map of X chromosome short arm showing critical regions, associated TS phenotypes, and candidate genes.

The SHOX gene acts as a transcription factor during early embryonic development to control the formation of many body structures. It is essential for the development of the skeleton and is found predominantly in bone fibroblasts. SHOX gene deficiency causes a wrist bone abnormality named Madelung in Leri-Weill dyschondrosteosis, Langer mesomelic dysplasia and other abnormalities like micrognathia [16,17].

A locus in the Xp11.2-p22.1 region has been found to influence height. Furthermore, there is evidence that genes in the interval "Xp11.2-p22" are involved in autoimmune thyroid diseases and high arched palate [18].

- The Cell Cycle Delay Hypothesis

The findings, from which the Cell Cycle Delay Hypothesis (CCDH) emanates, were that ear and hearing disorders in TS were related to genotype, serum concentrations of insulin like growth factor-1 (IGF-1) and height [19]. Moreover, a prolonged cell cycle time was shown, both for 45,X and trisomy 21 cells, leading to a slower growth rate and growth retardation of the fetuses [20-23]. Finally, the cell cycle time is further prolonged and the growth retardation more pronounced if the chromosomal damage involves specific growth regulating genes, such as the SHOX gene [16,20,23].

When applying the CCDH to TS specifically, it is assumed that the greater the proportion of genetically aberrant cells lacking the SHOX gene at the second p-arm of the X chromosome, the greater the number of cells with a prolonged cell cycle. The SHOX gene regulates skeletal muscles, the heart and brain, bone marrow fibroblasts, osteoblasts and chondrocytes, which are all dependent on IGF-1 and growth hormone also in adulthood. For this reason, both growth retardation and age-related disorders are expected to be

most pronounced in those fetal SHOX-regulated mesodermal tissue types that have a very short time window available for developing complex structures, such as the face, the neck/throat region, the ear, the heart, the brachial arches, the aorta and the great thoracic arteries, the lymphatic ducts, the kidney and the gonads. So, if the up-regulation to the required cell cycle rate fails, the number of cell cleavages drops and, due to the smaller number of cells, the size of that organ is reduced or the skeletal bones become shorter. In the worst-case scenario, the organ does not reach its final architecture, leaving a cleft palate or a cardiac defect.

CLINICAL FEATURES OF THE TURNER SYNDROME (PHENOTYPE)

- External stigmata

The clinical features range from a severe phenotypic character with short stature, gonadal dysgenesis and different malformations to an isolated mild reduction in final height or premature ovarian failure, Figure 6.

The most visible phenotype is the short stature, which has been reported in up to 98 % of all TS patients [6,24]. According to studies, cubitus valgus is seen in 45%, a low posterior hairline in about 40%, and short metacarpals and high arched palate in 35% [4,24]. Peripheral lymphedema dorsally of the hands and feet may be the initial presenting sign of TS and is found in approximately one-third of affected infants [6,25].

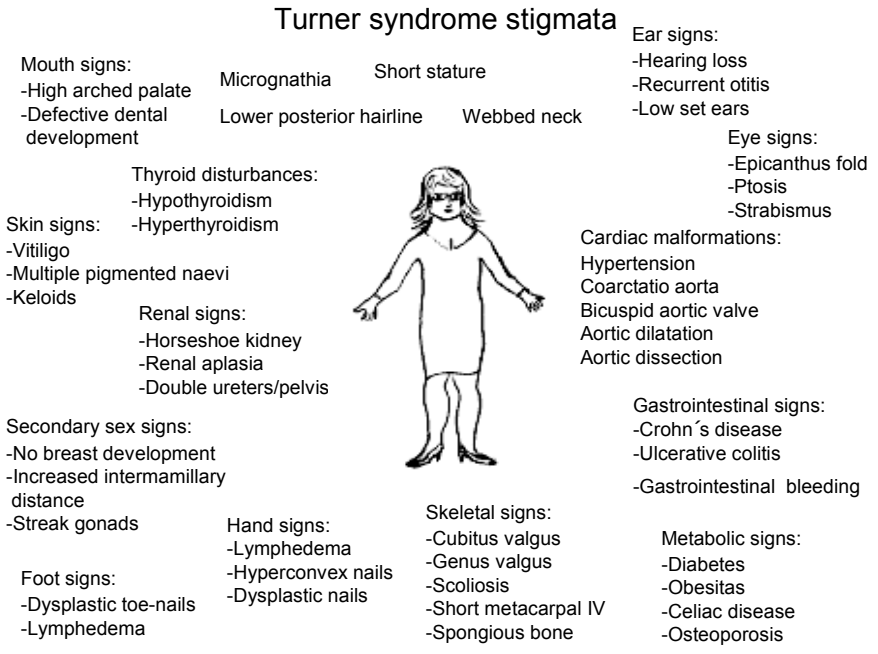

Figure 6. Stigmata that may be present in Turner syndrome. Modified from original figure, kindly provided by Dr Lisskulla Sylvén.

- Cardiac malformations

Bicuspid aortic valve is common in TS, affecting up to 40% of patients [26]. It is usually an isolated abnormality but may unfortunately be found in combination with other anomalies such as aortic coarctation, Figure 7. The latter is evident in about 10% of TS patients and is an important contributor to hypertension [27-29].

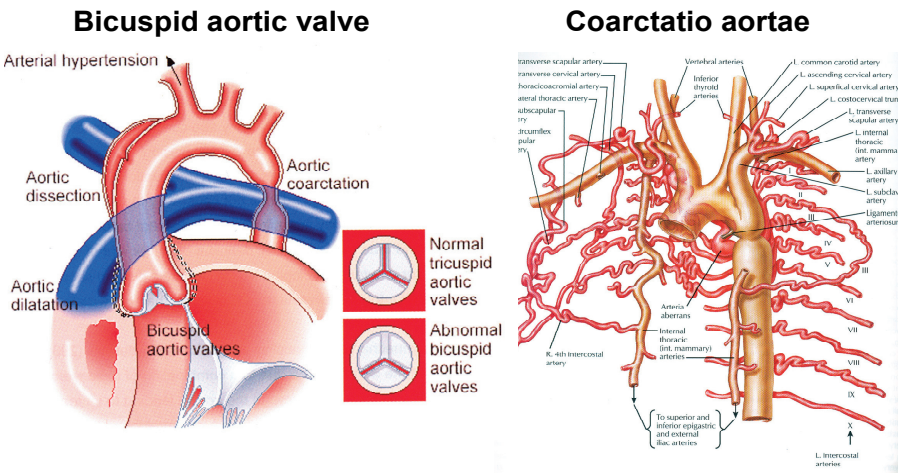

Figure 7. Bicuspid aortic valve to the left and coarctatio aortae and collaterals to the right. Courtesy of Associate Professor Claus Gravholt and Netter's Cardiology.

In the last decade, the association between aortic dissection and TS has been increasingly recognized with several reports of sudden death [30-33]. Aortic root dilatation is the main cause, with a prevalence of approximately 8 to 42% [27,31,33,34]. In a study by Elsheikh et al., it was found that 42% of adult TS women examined by echocardiography had significant aortic root dilatation [35]. The authors of another study on Marfan's syndrome patients [30], confirmed that the combination of hypertension, abnormal aortic valve, and other left-sided cardiac malformations has been shown to predispose for aortic dissection. These risk factors in combination are commonly present in TS women [27,33].

- Body balance and fractures

Gross and fine motor dysfunctions have been observed already in childhood [36], but studies on falling, balance and motor function in adult TS women are scarce. Davies et al. [37], reported a fracture frequency of 45% in women with TS, while Landin-Wilhelmsen and colleagues [38], evidenced a lower prevalence of about 16%, mainly in TS >45 years of age, which is more than the 5% prevalence in the general population. Most TS professionals agree that women with TS have reduced bone mass or bone density [39,40], with an increased risk of fractures, but the importance of the peak bone density gained during skeletal development is somewhat contradictory. Ross et al. evidenced a fracture incidence about 3 times higher in TS women compared with controls and low bone density in girls with TS compared with age-matched controls, but normal bone density when TS girls were compared with height-matched controls [41].

Shore and colleagues [42], on the other hand, showed that women with TS have an approximately 25% reduction in peak bone mass and that the bone density remains low even after correction for height and skeletal maturation. Whether the reduction in bone mass or bone density is secondary to poor mineralization or a consequence of a delay in skeletal maturation is not fully understood. Serum vitamin D levels were lower in TS than controls and might contribute to poor skeletal mineralization in TS [38].

- Ear and hearing problems

In TS, all three parts of the ear (external, middle and inner) are affected and this is more common among 45,X and isochromosome cases than among 45,X/46,XX mosaics. The prevalence of these problems is similar in all parts of the world. One third of girls and women exhibit a minor anomaly of the

auricular, which is smaller and often posteriorly rotated. The external ear canal is often slanted and narrow. Most girls with TS (approximately 75%) suffer from recurrent otitis media. Mosaics are less affected by ear problems [19,43,44]. Apart from temporary conductive hearing loss due to serous otitis media, sensorineural hearing function is usually normal among young TS patients. With increasing age, a sensorineural high frequency hearing loss develops. It is estimated that women with a 45,X karyotype suffer presbyacusis 10-20 years earlier than the population as a whole [45].

- Hypothyroidism

Hypothyroidism is a major problem in TS. A relationship between thyroid disease and TS was first suggested by Atria et al. [46], in 1948, when they reported post mortem findings of a small thyroid gland with lymphocytic infiltration in a young TS woman. The association was later confirmed in TS and in gonadal failure with a high incidence of Hashimoto's disease and elevated thyroid antibodies [47]. A high prevalence of thyroid peroxidase (TPO) antibody titer in TS patients aged about 10 years has been reported [48-50], and also a positive family history with an elevated TPO in both TS patients and their mothers [51]. Hypothyroidism affects growth development negatively, especially the spurt phase, and is also thought to increase the risk of coronary artery disease due to concomitant hypercholesterolemia [52,53]. A causal relationship between aberrations of the X chromosome and the risk of autoimmune hypothyroidism was also proposed [54-56].

- Liver function

Elevated liver enzymes have frequently been detected in routine investigations of TS women, but it is uncertain whether elevated liver enzymes are accompanied by any signs or symptoms of liver disease [57,58]. No long-term follow-up studies have been performed in adult TS patients. A vascular pathogenesis has been suggested, but the clinical significance of elevated liver enzymes in TS is not known [59].

- Psychological aspects

The clinical picture of TS girls is diverse. The majority of girls and women with TS have normal intelligence [60]. A small number with ring or marker X chromosomes may have mental retardation [61]. However, TS girls and women often function well and independently with regard to work and social life, although they may still have some learning difficulties [29,62].

Morbidity and mortality in adulthood

Mortality in TS is 3 times higher than in the general population, [63], (SMR=3.0 [95% CI:2.7-3.4]), and was raised for nearly all major causes of death. Life expectancy is shortened by 13 years [25]. This high mortality can be expected, primarily due to the cardiovascular complications [64], and especially in those with 45,X [65]. Circulatory disease accounted for 41% of the excess mortality, with the greatest SMRs for aortic aneurysm (SMR=23.6, 95% CI: 13.8-37.8) and aortic valve disease (SMR=17.9, 95% CI: 4.9-46.0) [63].

In a series investigated by VP Sybert [66], hypertension occurred in 17% of children and 24-40% of adults with TS [29,67]. Obesity, predominantly centrally distributed, is common in TS women and could contribute to an increased risk of atherosclerosis and cardiovascular disease. However, the plasma insulin concentration is unexpectedly low [68]. There is epidemiological evidence of an increased incidence of coronary heart disease in TS [69]. Diabetes and hyperlipidemia have been suggested as possible causes [69], but are not general signs in TS [29]. High cholesterol levels have been demonstrated in some, but not in all women with TS [29,70,71].

DIAGNOSTIC ISSUES

TS is the most common sex chromosome abnormality in females. Short stature and ovarian failure are important characteristic features [6], but there is a broad phenotypic spectrum with less severely affected patients, especially in the mosaic karyotype. In general, there are five periods, pre- and post-natally, when TS is usually diagnosed, Figure 8.

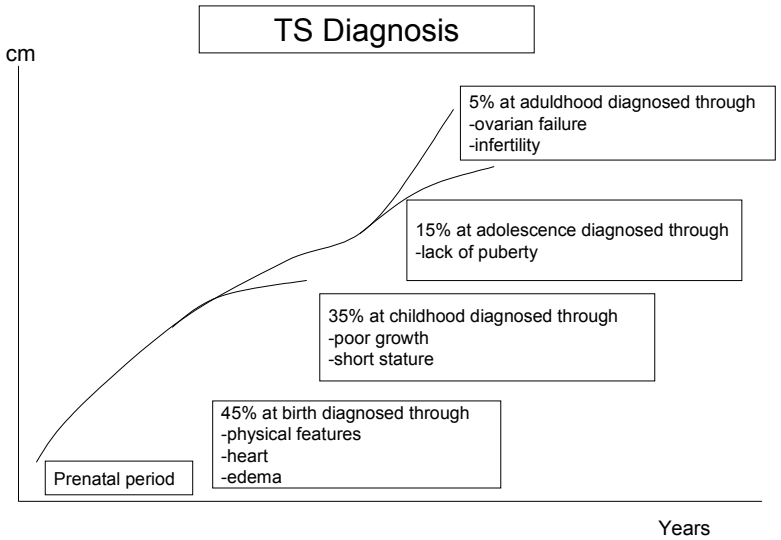

Figure 8. Stages in life when TS is suspected and diagnosed. Courtesy of Professor Kerstin Albertsson-Wikland

- *Prenatal period*

Sex chromosome abnormalities are increasingly detected prenatally through chorionic villous sampling and amniocentesis. Certain ultrasound findings are suggestive of TS, such as nuchal translucency, cystic hygromas, coarctation of the aorta, left-sided cardiac defects and renal anomalies [72]. Moreover, a high concentration of maternal serum levels of ά-fetoprotein, human chorionic gonadotropin, and inhibin A, further increases the probability of the diagnosis of TS [73].

As postnatal outcome could not be ascertained with prenatal chromosome analyses, especially in mosaic cases, reevaluation is mandatory [74]. The TS

phenotype severity cannot be anticipated from the degree of mosaicism detected prenatally [75]. Therefore, physicians and geneticians should familiarize themselves with this controversy before informing parents, as recent reports show that fetuses diagnosed prenatally are electively aborted [74]. TS featuses with severe affections are often spontaneously aborted.

-At birth

Due to their characteristic features, about 45% of TS patients are diagnosed at birth. Swollen hands and feet are mostly noticed, but a nuchal hygroma may also be present. Physicians should be observant of other congenital anomalies as well; in particular, cardiac and renal conditions. Reevaluation with karyotype analyses is the principal method used to ascertain the TS diagnosis.

-During childhood

The most striking feature that brings TS girls to the pediatrician is growth retardation. To avoid a short final stature, TS girls are offered growth hormone treatment. Therefore, physicians dealing with TS patients are concerned about a delayed diagnosis [24], and wish to identify these patients earlier, especially the mosaic cases [76]. In Denmark, the diagnostic prevalence was increased when the registers were reviewed 10 years later [65].

-During adolescence and adulthood

Approximately 20% of TS patients are diagnosed during adolescence and adulthood. The most agonizing problem is lack of puberty and, in adulthood, infertility, especially in 45,X cases. There are reports of cases of

spontaneous pregnancy in TS women, many of them being mosaics [77,78]. At present, women with TS can get pregnant by oocyte donation [79]. In the future, cryopreservation of follicles or oocytes could be an option for patients suffering from infertility [80].

Indication for karyotyping

The diagnosis of TS should be considered in any female with unexplained growth failure, pubertal delay or any constellation of the previously mentioned stigmata. According to the American College of Medical Genetics, a standard of a 30-cell karyotype should be analyzed, which identifies at least a 10% mosaicism with 95% confidence.

However, FISH studies are warranted to detect low levels of mosaicism for an XY or XX cell population or an X or Y structural construction and should be performed if there is strong suspicion of undetected mosacism [13,81]. Conventional cytogenic studies identify sex chromosome mosaicism in only approximately 55% of patients [14]. A second cell line population with 46,XY or a structural rearrangement of the Y chromosome is important to identify the risk of gonadoblastoma [82]. Hook and Warburton postulated that all TS patients could be mosaics with either a Y or a second X in some cells [13].

In Sweden, the conventional karyotype analysis of 30 cells costs around SEK 4 000, and FISH costs just over SEK 4 000.

GENERAL AIM OF THIS THESIS

The aim was to study the relation between the genotype and phenotype in TS regarding stigmata, cross-sectionally, as well as long-term follow-up studies on thyroid and liver function.

- Working hypotheses and specific aims:

I. In the traditional studies on genotype and phenotype correlation in TS, women with 45,X are worse off, compared with the group of all other karyotypes combined. The present working hypothesis is that the greater the proportion of 46,XX cells, the milder the TS phenotype (Paper I).

II. The poorer the fine motor function and body balance, the poorer the bone density and hearing function, and the fewer the 46,XX cells (Paper II).

III. Hypothyroidism is more frequent among TS patients than in the general population. Our hypothesis was that TS women with a lower frequency of 46,XX had a higher risk of developing hypothyroidism during the five-year follow-up (Paper III).

IV. Elevated liver enzymes may be associated with metabolic syndrome factors, such as body weight, waist/hip ratio, glucose and blood lipids and genotype. The aim was to study the change in liver enzymes over five years and the outcome of the elevated liver enzymes (Paper IV).

SUBJECTS AND METHODS

- Patients

During 1995–2007, women with TS, mean age 31; range 16-71 years, were recruited from the gynecology and endocrinology out-patient clinics at Sahlgrenska University Hospital in Göteborg, (Paper I-IV), the University Hospital in Malmö (Paper III +IV), and the University Hospitals in Umeå and Uppsala, Sweden (Paper IV). Patients were also recruited by means of an advertisement in the national TS patient newspaper, Table 1.

Paper	TS (n)	TS (mean age, years)	Controls (n)	Controls (mean age, years)
I Stigmata study	126	31	45	30
II Fine motor & balance study	75	30	31	37
III Thyroid study	91	37	228	37
IV Liver study	218	33	0	-

Table 1. The number and mean age of the participating TS patients and controls in the 4 papers.

- Controls

A random population sample of women was recruited from the World Health Organization (WHO) MONItoring of trends and determinants in CArdiovascular disease (MONICA) Project, Göteborg, Sweden.

The MONICA Project is a screening study for cardiovascular risk factors and comprises 38 centers around the world.
In 1995, 1 200 men and 1 200 women aged 25–64 years, selected at random from the population census of Göteborg, Sweden, were invited to participate in the third MONICA screening study [83]. Randomly selected, age-matched women were used as controls in Papers I and III. In Paper II, the controls were recruited from the hospital staff, Table 1.

- Ongoing examination program

The Swedish Turner Academy was established in 1993 and consists of pediatricians, gynecologists and endocrinologists at all university clinics in Sweden with a special interest in TS. The Turner Academy aims at treating and following girls and women with TS in an organized interdisciplinary way; Figure 9.

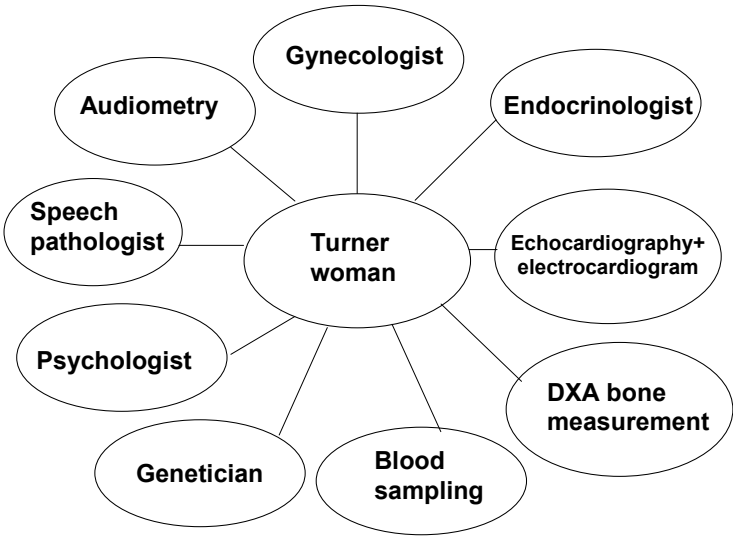

Figure 9. The interdisciplinary examinations of TS women according to the national Swedish guidelines [84].

TS women in the cross-sectional studies participated in a voluntary screening program over 1-2 days. In the longitudinal studies the same program was performed after five years.

Examinations regarding gynecological and medical status, blood pressure and thyroid function were assessed every year, and cardiac evaluation, bone mineral density and audiogram every five years; Figure 10.

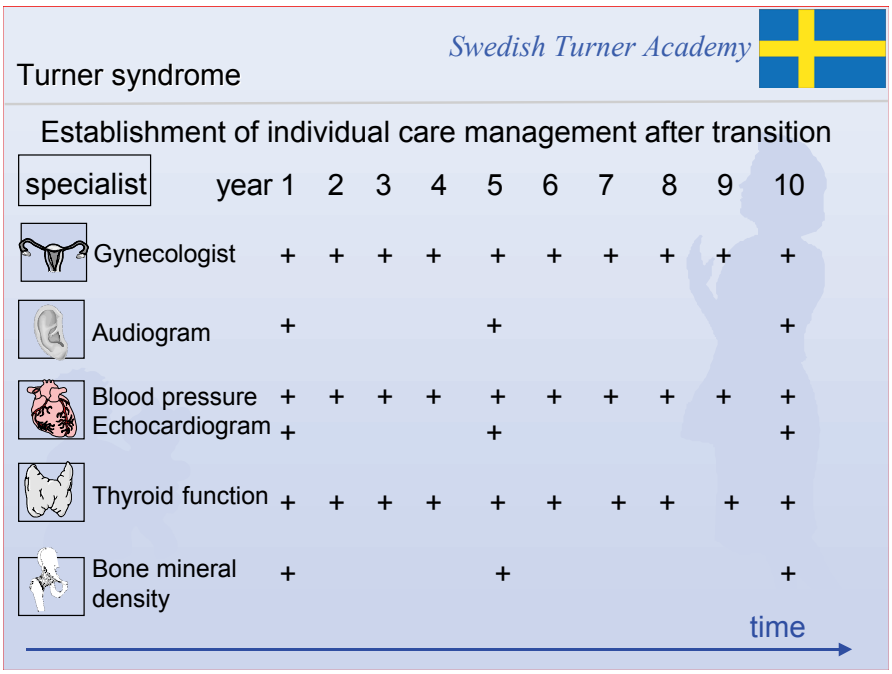

Figure 10. The examination program for TS over time according to the national Swedish guidelines [84].

One special aim is to help these teenagers through the transition period from puberty to adulthood, and to introduce them to the adult clinic.
This unique project, with the systematic, interdisciplinary investigation stations (Figure 10), has been a good example to follow worldwide and serves as a basis for structural, disciplined clinical guidelines internationally [85-86].

- Anthropometry

Body height was measured barefoot to the nearest 1 cm, while body weight was measured to the nearest 0.1 kg in the fasting state with the subjects in underwear and without shoes. Body mass index (BMI) was calculated as body weight divided by height squared (kg/m^2). Waist circumference was measured with a soft tape midway between the lowest rib margin and the iliac crest in the standing position. The hip circumference was measured over the widest part of the gluteal region, and the waist/hip ratio was calculated.

- Blood pressure and cardiac examinations

Blood pressure was measured with a mercury sphygmomanometer to the nearest 2 mmHg on the right arm in the sitting position after 10 min of rest. Disappearance of Korotkoff sounds (phase V) was used to determine diastolic pressure. A cuff size corresponding to the circumference of the right arm was chosen. Hypertension was defined as > 140/90 mmHg [87] and/or if the medical records indicated treatment for hypertension. Electro- and echocardiography were performed on all patients. Coarctatio aortae and cardiac valve malformations were asked after.

- Bone mineral density and body composition

Bone mineral density was assessed by Dual energy X-ray Absorptiometry (DXA) in TS women and with Achilles ultrasound in controls (both from LUNAR, Wisconsin, MI, USA), Figure 11. Osteoporosis was defined according to the WHO criterion with a t-score <-2.5 SD of young adults at the lumbar spine or the femoral neck in TS and at the left calcaneus in controls [88]. The Achilles ultrasound was well correlated with bone measurements according to the DXA [89]. Fractures and treatment for osteoporosis were recorded. Lean body mass and body fat were estimated using DXA in TS women and by impedance measurements (SEAC Multiple frequency bioimpedance meter model SFB 2, UniQuest Ltd, Queensland, Australia) based on total body resistance and reactance in controls [90].

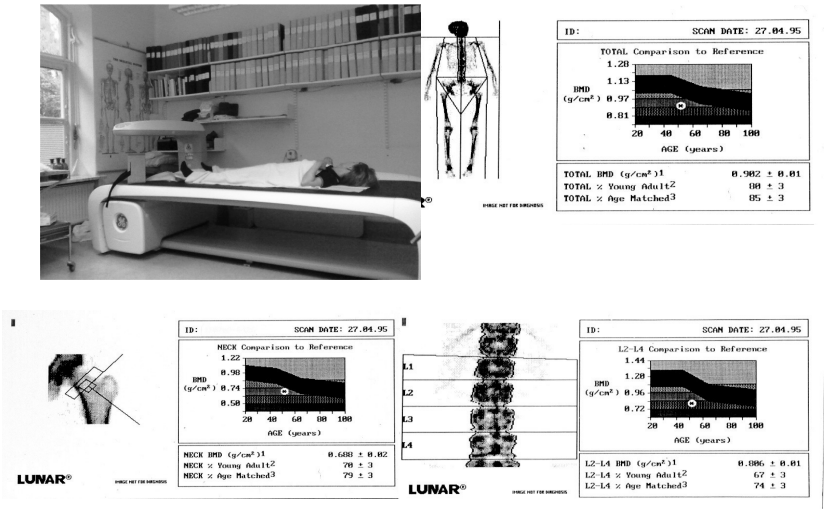

Figure 11. DXA measurement and example of the protocols showing bone mineral density in relation to the manufacturer's reference levels.

- Stigmata evaluation

All TS women were examined by an endocrinologist (KLW +MEM together), a gynecologist (IB) and an ear, nose and throat specialist (MLB) who recorded external stigmata independently of each other. Thirty-eight external stigmata, reported in text books and in the literature [4], were listed and looked for, including subjectively impaired vision (wearing glasses) and hearing (with or without hearing aids). The karyotype was blinded to the observers.

- Biochemical analyses

Fasting venous blood samples were drawn from an antecubital vein in the morning after an overnight fast. Samples from menstruating women were collected on cycle day 7-9. The same laboratory was used for patients and controls in study I+II. In study III+IV, analyses were performed by the accredited laboratories at each university hospital. The reference levels were similar for thyroid hormones, glucose, lipids and liver enzymes at the 4 hospitals. After centrifugation, all samples were frozen and stored at –70° C until analysis, which was performed within one year. Blood samples were drawn in a similar way throughout the five years. Blood glucose was determined with a glucose-6-phosphate, dehydrogenase method (Kebo Lab, Stockholm, Sweden). Diabetes mellitus was defined as fasting blood glucose ≥ 6.7 mmol/l, according to WHO criteria or if the patient had treatment for diabetes. Plasma insulin was determined with a RIA method (Phadebas, Pharmacia). Concentrations of total serum cholesterol, high-density lipoprotein cholesterol (HDL) and triglycerides were determined enzymatically (Boehringer, Mannheim, Germany). Low-density lipoprotein

cholesterol was calculated according to Friedewald´s formula [91]. Free thyroxine (T4), thyroid stimulating hormone (TSH) and follicle stimulating hormone (FSH) were measured with an immunometric method with luminometry (Johnson&Johnson, La Jolla, CA). Hypothyroidism was defined as TSH > 4 mU/l and as those who already had thyroxine substitution. Serum testosterone was determined by non-extraction competetive radioimmunoassay using an antiserum against a T-19-carboxymethyl adduct to bovine serum albumin (Radioassay System Laboratories ^{125} IT: ICN Biochemicals Inc. Diagnostics Division, Costa Mesa, CA, USA), serum estradiol by radioimmunoassay (Sorin Medical, Boule, Italy), sex hormone binding globuline (SHBG) by immunoradiometric assay (Farmos Group Ltd, Oulunsalo, Finland), serum IGF-1 and serum IGF-1 binding protein –3 (IGFBP-3) according to Nichols Institute Diagnostics (San Juan Capistrano, CA, USA).
Aspartate (AST) and alanine aminotransferase (ALT), alkaline phosphatase (ALP), γ-glutamyl transferase (GT), bilirubin, vitamin B12, endomysium and gliadin antibodies were measured. Reference values were < 0.60 μkat/l for AST and ALT, < 2.5 μkat/l for ALP, < 0.80 μkat/l for GT and < 20 μkat/l for bilirubin. HBsAg and anti-HCV were checked in subjects with AST and ALT > 5 μkat/l on two repeated measurements. HBsAg, anti-HCV, antibodies against smooth muscle, mitochondria and antinuclear factor were analysed in every fourth TS woman.

- Fluorescence In Situ Hybridization (FISH)

The FISH procedure was performed using buccal mucosal cells, Figure 12. In those cases in which FISH revealed another cell line, which was not diagnosed with the conventional karyotype, another karyotyping of 100 cells was performed, to (1) confirm the FISH analysis, and (2) characterize the second sex chromosome. Mosaicism was defined as > 5% content of a healthy 46,XX cell line [14,15]. In this way, the degree of mosaicism could be quantified according to FISH. The chromosome status was based on the combined picture of FISH and karyotyping (CH). The conventional karyotype was calculated on at least 40 lymphocytes.

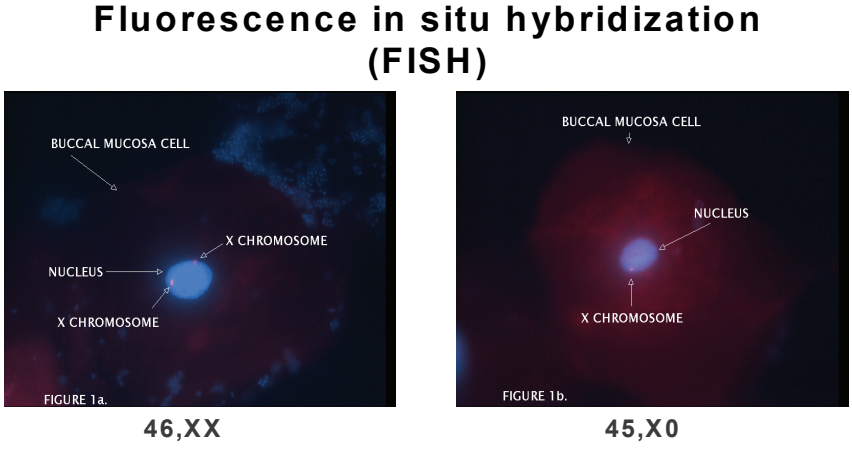

Figure 12. Chromosomal constitution of normal (46,XX) and Turner syndrome (45,X) subjects. Courtesy of Associate Professor Charles Hanson.

- Audiometry

Hearing measurements were carried out by a trained audiologist in sound-insulated test booths with background levels well below the accepted standards, Figure 13. Pure tone hearing thresholds were determined according to international standards by air and bone conduction for the frequencies 0.25, 0·5, 1, 1·5, 2, 3, 4, 6 and 8 kHz. The pure tone averages (PTA) at the mid frequencies 0·5, 1 and 2 kHz (PTAmid) and the high frequencies 3, 4 and 6 kHz (PTAhigh) were used as summary statistics. The higher the PTAmid and PTAhigh, the worse the hearing function.

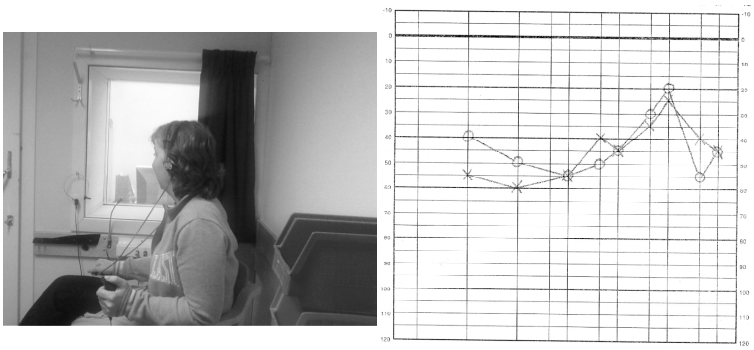

Figure 13. A TS patient performing audiometry in a test booth to the left and an example of hearing test protocol to the right.

Fine motor function and balance test

The Bruininks–Oseretsky test for fine motor function (5 items), and motor proficiency [92,93] were administered to each patient without knowledge of

the subject's karyotype (MLB, MEM). The subject performed five out of eight items in the upper limb speed and dexterity subtest protocol; (i) number of pennies placed in a box with preferred hand in 15 s; (ii) time to place 12 pairs of pennies in two boxes with both hands simultaneously; (iii) number of shape cards sorted with preferred hand in 15 s; (iv) number of beads stringed with preferred hand in 15 s; and (v) number of pegs placed on a pegboard with preferred hand in 15 s; Figure 14. The maximum outcome for each subtest was 8, 10, 10, 7 and 8 points, respectively, giving a maximum score of 43 points.

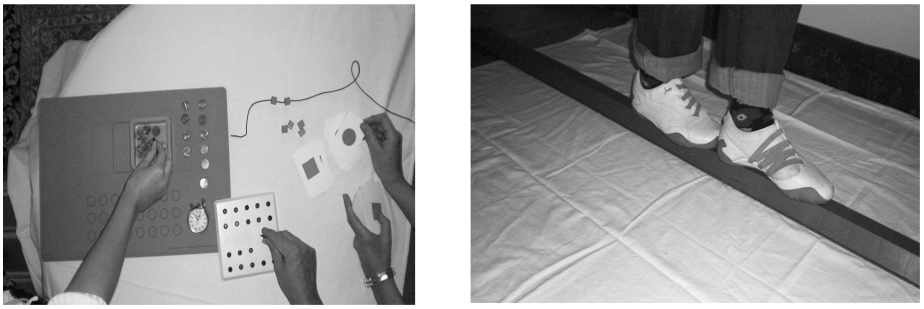

Figure 14. Fine motor function (left) and body balance test (right).

Body balance was performed with static and dynamic tests [92] with the subject performing toe-to-heel Romberg's test with closed and open eyes, respectively, and standing and walking on a rib, Figure 14. The maximum score for the static test was 29 points and for the dynamic balance test 8 points, in total 37 points; see appendix, Paper II.

STATISTICAL METHODS

Means, medians and SD values were calculated by conventional methods. Differences in stigmata and non-continuous variables were tested with odds ratio, the Mantel–Haentszel's Chi-squared test and Fisher's exact test. Yates' correction was used when no stigmata were found in one of the groups. Differences between groups regarding continuous variables were tested with Student's t-test. Differences within TS subjects after follow-up were tested with Wilcoxon's signed rank test. Simple correlations were calculated using Pearson's method. Multiple stepwise regression models were used to test interactions between factors. Stepwise, logistic regression was also used to study predictors of liver enzymes at follow-up. A p-value of less than 0.05 (two-sided test) was considered statistically significant.

RESULTS

Chromosomal distribution in relation to stigmata, cardiovascular risk factors and body balance (Papers I+II).

- *Stigmata and cardiovascular risk factors (Paper I).*

The chromosomal distribution is seen in Table 2. Half of the TS women had a monosomy, 45,X. Twenty-five per cent of the whole group were true mosaics (45,X/46,XX) and the remaining 25% were labelled as "others," including iso-ring-marker X and a Y chromosome. Five of the 60 patients with 45,X in the conventional karyotype were reclassified as 45,X/46,XX mosaics and one with 45,X had a Y marker according to the FISH analysis.

N=126	Karyotyping	FISH
Chromosomal distribution	n (%)	n(%)
45,X	60 (48%)	55 (44%)
45,X/46,XX	29 (23%)	34 (27%)
45,X/46,XXi(Xq10) isochromosome	17 (14%)	17 (14%)
45,X/46,+marker Y	13 (10%)	14 (11%)
46,X,+marker X	3 (2%)	3 (2%)
45,X/46,X,ring (X)	4 (3%)	4 (3%)

Table 2. Chromosomal distribution according to conventional karyotyping and FISH, respectively.

The larger the number of 46,XX cells; i.e. true mosaicism, the fewer the external stigmata in TS. Cardiac abnormalities were more frequent in TS

with monosomy 45,X than in mosaicism, 45,X/46,XX. Nor did mosaics differ from controls regarding BMI, waist/hip ratio, blood pressure, blood lipids, bone mineral density or diabetes prevalence, Table 3.

	45,X n=55	**45,X/46,XX n=34**	**Other N=37**	**Controls n=45**
Body mass index, kg/m^2	25.2±4.8**	23.9±2.8	26.1±4.7***	22.7±2.6
Waist, cm	78±10**	74±7	79±13*	72±10
Hip, cm	95±10	93±6	96±9	93±9
Waist/hip ratio	0.81±0.05***	0.79±0.06	0.82±0.10**	0.77±0.05
Systolic BP, mmHg	124±14*	120±13	124±15*	117±11
S-cholesterol,mmol/l	5.4±1.4**	4.6±0.9 ##	5.4±1.0**	4.7±0.9
t-score L_2-L_4, SD	-0.87±1.5**	0.08±1.1#	-0.55±1.4*	0.05±0.08
Osteoporosis, %	15***	5	13**	0

*=p<0.05, **=p<0.01. ***=p<0.001 vs controls
#=p<0.05, ##=p<0.01 vs 45,X

Table 3. Cardiovascular risk factors and bone data in relation to chromosomal constitution in women with TS and controls. Means±SD. BP=blood pressure, SD=standard deviation from young adults for bone measurement. Other=TS with iso, ring, marker X or Y-chromosome. Ovals show the similarities between mosaics and controls.

TS women with 45,X/46,XX, were diagnosed 8 years later, at a mean age of 18 years, compared with 10 years for TS women with 45,X and others, such as iso, ring, marker X or Y.

In general, TS women were shorter with lower lean body mass and serum testosterone, but with greater BMI, waist circumference, waist/hip ratio,

systolic blood pressure, TSH and total cholesterol than controls. Hypertension, hypothyroidism and osteoporosis were more common in TS, although the TS women smoked less.

The most frequently found stigmatas were low hairline (68%), webbed neck (60%), increased intermamillary distance (60%), high arched palate (56%), keloid (19%), epicanthus fold (19%) and cardiac malformations (51%), which were more common in TS with a 45,X karyotype than in TS with 45,X/46,XX mosaicism.

The six TS women with aortic dissection were 45,X and one harbored a Y chromosome; Table 4. The latter woman (= number 2) had 45,X with the conventional karyotype but a marker Y on the FISH. She had a fairly normal phenotype and gynecological history with two spontaneous pregnancies at age 36 and 38. Her aortic dissection occurred in the 7th month of the second pregnancy [78].

Pat	Age, years	Karyo -type	Hyper- tension	Other CHD	Other information	Status	Autopsy PAD	Year
1	28	45,X	Yes	Aortic insuff Ø 3.5 cm	Psoriasis	Dead	Yes	1996
2	38	45,X/ 46,XY	Yes	Surgery coarc- tatio	2-para, Pheochrom Hypothyr	Alive	No	1997
3	61	45,X	No		Vit B12 deficiency	Alive	No	2005
4	28	45,X	No	Aortic insuff Ø 4.5 cm	Epilepsy	Dead	Yes	2006
5	36	45,X	No	Surgery coarctatio Bicuspid valve	Atopic dermatitis	Dead	Yes	2006
6	29	45,X	No	Bicuspid valve	Vitamin D deficiency	Dead	Yes	2009

Table 4. Data for the six TS women with aortic dissection. CHD= Cardiovascular heart disease. PAD=Pathologic anatomic diagnosis.

We conclude that mosaicism mitigated stigmata and cardiovascular risk factors in TS and that mosaic Turner patients were healthier than TS women with monosomy. Turner should be suspected if these external stigmata are present together with short stature and hypogonadism.

- *Fine motor function and body balance (Paper II)*

Fine motor function and body balance were impaired in TS women compared with controls. Balance and fine motor function were poorer in TS with 45,X compared with 45,X/46,XX mosaics; Figure 15.

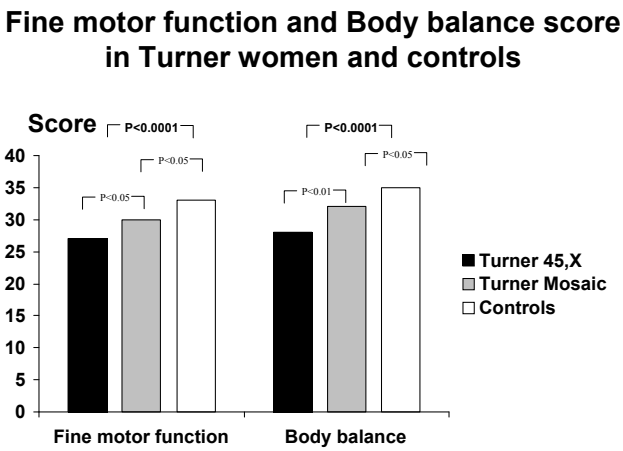

Figure 15. Fine motor function and body balance score in TS and controls.

Fine motor function and body balance were similar in TS with iso- and ring chromosome and TS with 45,X, Table 5.

Chromosomal distribution	Fine motor function	p-value vs. 45,X	p-value vs. mosaic	Body balance	p-value vs. 45,X	p-value vs. mosaic
TS(45,X/46,XX)	33 ± 2.2	p<0.05		35 ± 2.4	p<0.05	
TS (45,X)	27 ± 4.8		p<0.05	28 ± 6.8		p<0.05
TS (iso)	19.2 ± 7.2	ns	p<0.01	20.2 ± 7.2	ns	p<0.01
TS (ring)	25.5 ± 7.8	ns	ns	22.0± 14.8	ns	ns
TS (Y-marker)	33.0 ± 3.4	p<0.05	ns	25.5 ± 3.5	ns	p<0.01

Table 5. Fine motor function and body balance scores in TS with iso-, ring and marker Y chromosome in comparison with 45,X and mosaics, respectively. ns = not significant.

Mosaics had better hearing function than non-mosaics ($P<0{\cdot}05$). Fractures had occurred in 13% of the TS women and their fine motor function and body balance tended to be poorer than in the patients without fractures. Glasses were worn by 69% of the non-mosaics and 40% of the mosaics [OR = 3·4, (95% CI = 1·2 – 9·7), $P< 0{\cdot}05$], but fine motor function and body balance did not differ between users and non-users of glasses.

Both fine motor function and body balance were negatively correlated with age, waist circumference and waist/hip ratio, and positively correlated with bone mineral density and hearing function (i.e. inversely to PTAmid and PTAhigh) in all TS women. Body balance was positively correlated with the degree of physical activity in all TS and in mosaics.

Long-term follow-up of thyroid and liver function (Papers III+IV)

- *Hypothyroidism (Paper III)*

Autoimmune hypothyroidism was common in TS; in 25% of subjects at the start of the study and in 37% at 5 years, compared with 2% in the population. There was no relationship between hypothyroidism and karyotype or family history, respectively, in TS. The yearly incidence of hypothyroidism in TS was 3.2%.

An elevated TPO antibody concentration was found more frequently in TS women with hypothyroidism than in women without hypothyroidism, ($p<0.05$). The TPO antibody titer varied widely, range 0-4200 U/ml (kU/l). The TPO concentration was positively correlated with serum TSH; $r=0.18$, ($p=0.03$). TSH was not correlated with serum cholesterol. There was no correlation between the frequency of 45,X cells and serum TPO, TSH or free T4 concentrations, respectively. Elevated TPO concentrations were evenly distributed between the karyotypes. Body mass index was higher among TS women with hypothyroidism than in TS women without hypothyroidism.

After five years, a further 16% had developed elevated serum TSH concentrations ($\geq$4 mU/l). More than one in three TS women had hypothyroidism, 36% of whom had an elevated TPO antibody titer. The distribution of hypothyroidism by age is seen in Figure 14.

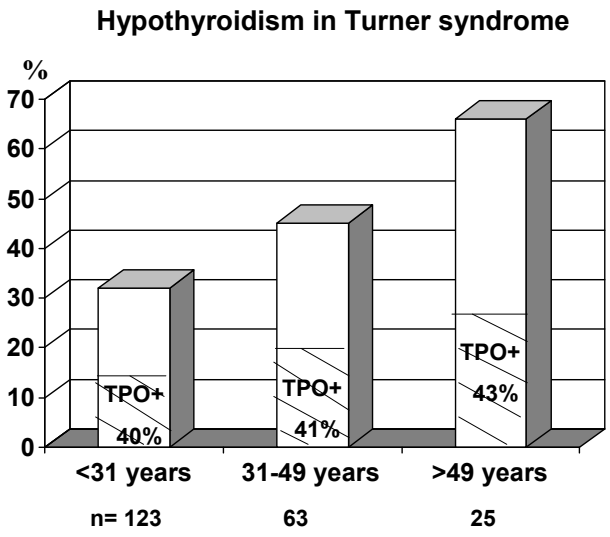

Figure 14. Frequency (%) of TS women with hypothyroidism and elevated TPO (striped area) by age.

- *Liver enzymes (Paper IV)*

At start, 79 women (36%) had one or more elevated liver enzymes above the reference level. The most common elevation was GT (30%). Elevated ALT was more common (27%) than elevated AST (18%); Figure 15. Any elevated liver enzyme was present in 6% of age-matched women in the WHO MONICA study (kindly provided by Dr Pinelopi Trimpou); Table 6.

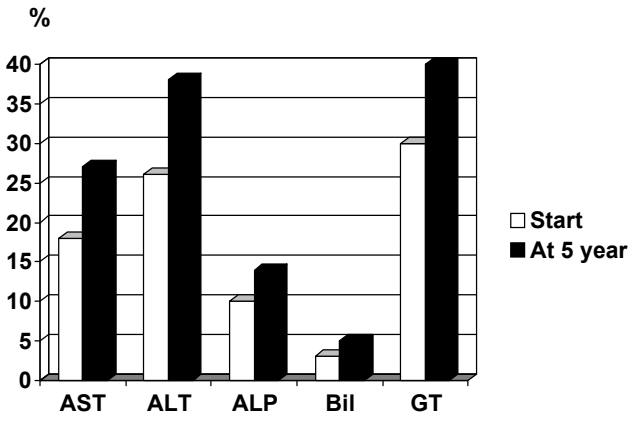

Figure 15. TS women (%) with elevated liver enzymes at start (white bars) and at the five-year follow-up (black bars).

No differences were seen with regard to age, body composition, glucose metabolism, hepatic antibodies, growth hormone treatment or HRT.
At five years, the mean levels of AST, ALT and GT had increased, and another 23% had liver enzymes above the reference levels; in total, 59% (36% at start + 23% at five years), Figure 15.

Elevated liver enzymes, and particularly elevated GT, was most commonly found in the age group 36–45 years and associated with obesity and high serum cholesterol concentrations; Figure 16.

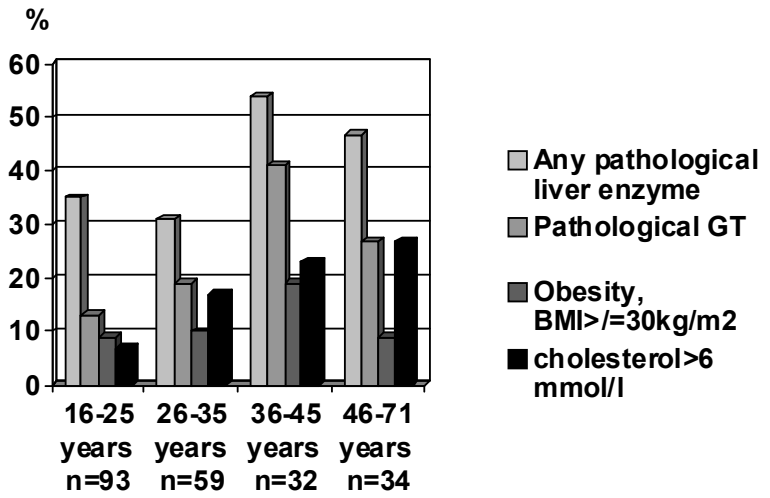

Figure 16. TS women (%) with one or more elevated liver enzymes, pathological GT, obesity and hypercholesterolemia by age group.

Height, body fat, lean body mass, LDL, insulin, TSH, estradiol and testosterone were unaltered during follow-up.
Multivariable analysis was performed for GT vs. BMI, waist/hip ratio, total cholesterol, triglycerides, blood glucose and HbA_1c. Only total cholesterol was correlated with GT; p= 0.0032 at start, and p= 0.0005 at five years.

In the 139 women with normal liver enzymes at baseline, stepwise logistic regression analysis was performed with liver enzymes being the dependent variable and BMI, waist/hip ratio, total cholesterol, blood glucose and HbA_1c at baseline used as independent variables. HbA_1c and total serum cholesterol at baseline were significant predictors of an increased value of any of the enzymes AST, ALT or GT (p=0.0257 and p= 0.0382 versus GT,

respectively), when analyzed in separate models. In a multivariable model only total cholesterol predicted an increase in GT.

Liver biopsies were performed in six women, showing one with cholangitis, one with hepatitis C, two women had liver steatosis, and two had normal liver biopsies and liver ultrasonography. Withdrawal of estrogen HRT did not influence the liver enzymes in any of these six subjects.

- *Hypothyroidism, elevated liver enzymes and other metabolic aberrations*
In the Swedish TS population from Paper IV, 9% had both elevated TSH and liver enzymes at start and 8% at five years. Eighteen per cent of TS patients had elevated TSH with normal liver enzymes at start and 20% at five years. The 9% who had received thyroxine supplementation after the initial investigation still had elevated liver enzymes at five years. The only factor that was associated with normalized liver enzymes at follow-up was a lowering of cholesterol levels. In fact, two TS women were put on simvastatin due to serum cholesterol of 7 mmol/l and GT of 12 μkat/l. After one month the cholesterol level was 5 mmol/l and GT 1.2 μkat/l.

Metabolic data for the Swedish TS cohort, n=218, are given in Table 6. Only 4% of the women of similar age (35-45 years) in the random population sample from the WHO MONICA study had elevated TPO and this figure was increased in the age group 65-74 years (unpublished data kindly provided by Dr Pinelopi Trimpou, 2009). Vitamin B12 deficiency, but not diabetes mellitus, was more common in TS than in controls, Table 6.

	At Start TS	Controls WHO (MONICA)	*P*-value	After 5 years TS
Elevated liver enzymes, %	36	6	0.000033	53
Hypothyroidism, %	22	6	0.000082	31
Elevated TPO, %	34	4	0.000028	36
Vitamin B12 deficiency, %	15	2	0.035	10
Obesity, %	11	6	0.530	16
Diabetes mellitus type II, %	3	2	0.640	3.5
Celiac disease, %	2	0	0.790	2

Table 6. Metabolic disturbances in the Swedish TS women, n=218, from Paper IV compared with a random population sample, n=51, of age-matched women 35-44 years from the WHO MONICA study, Gothenburg, 2009 (kindly provided by Dr Pinelopi Trimpou, unpublished data).

DISCUSSION

Relationship between genotype and phenotype (PAPER I+II)

This is the first study where genotype-phenotype correlations were performed on the basis of the hypothesis that the 45,X/46,XX is the deviant karyotype by being healthier (the Gothenburg classification), and not the 45,X monosomy, being more severely affected than all other karyotypes clustered together as one group. This is also the first study to use a more specific, accurate genetic analysis, FISH, aimed at making it easier to diagnose TS mosaic patients, thereby finding stronger genotype-phenotype correlations than has so far been the case. The traditional international karyotype classification designating TS patients as 45,X monosomy versus all other karyotypes included in the 45,X/46,XX mosaicism has depicted monosomy as the vulnerable group, with high morbidity and mortality rates. The present study shows that it is the true 45,X/46,XX women who diverge from the 45,X and all other karyotypes by being more healthy with fewer stigmata, including better hearing, balance and bone quality and cardiovascular risk factor profile.

According to the CCDH, most stigmata in TS can be ascribed to the growth disturbances, caused by a delayed cell cycle time in the chromosomally abnormal 45,X cells. The webbed neck is probably caused by lymphatic obstruction and weaknesses in the lymphatic vessel walls.
The TS literature usually focuses on describing TS with a 45,X monosomy or an isochromosomy, simply because they show the most typical clinical features and medical problems and may therefore be diagnosed at a glance by a trained pediatrician, gynecologist or endocrinologist. In Sweden, at present, we know from the Swedish genetic Turner register (unpublished

data), and also the Danish cytogenetic register that we detect only 50% of the total number estimated to be present if all TS cases were found [94]. We expect the undiagnosed TS cases to be mosaics. We therefore wish to highlight the mosaic group, which - due to the number of 46,XX cells - have fewer and less visible signs of their syndrome. These patients may feel well, but they are doubtlessly afflicted by somewhat higher morbidity, as shown in this study. Furthermore, the mosaics have hypertension, aortic valve abnormalities, hypothyroidism and elevated liver enzymes that require care and pharmacological treatment.

Many studies have discussed the diagnostic delay [76,94,95], but the problem of identifying the mosaics still remains. The necessity of measuring girls' body height longitudinally according to protocol is important, as well as using genetic testing whenever growth is affected and keeping up the awareness and knowledge among all health professionals of TS.

We also verified that 45,X TS women were severely affected by cardiovascular disease. The fact that 4 of 6 young TS women died from aortic dissection is worrying. Therefore, intensive treatment of hypertension and regular cardiac examinations, preferably by magnetic resonance imaging, are warranted [32,33,86,96,97]. Further studies are needed to find out the pathogenesis of the aortic dissection in TS.

The number of fractures was quite low in these fairly young TS women. It will be of great interest to follow the fracture incidence in these TS women, who have received modern growth promoting treatment. If they continue to fracture, it could be due to balance problems rather than osteoporosis, as we

believe that GH, androgens and continuous HRT will prevent osteoporosis from developing. However, body balance and motor function were strongly related to hearing function, indicating vestibular dysfunction. Still, a more central nervous mechanism cannot be excluded. There was, however, no relationship with metabolic factors such as glucose, lipids, or vitamin B or D.

The early onset of the high-frequency hearing loss in TS is a consequence of a reduced number of sensory hair cells from birth. A shortened cochlea has been observed, in a TS mouse model, and its estrogen receptor-beta were important for hearing function [98,99]. In fact, the size of the auricle in the fetus is used as an ultrasonographic marker, especially when growth retardation is combined with a webbed neck, as in TS [100,101]. In this thesis, the CCDH explains the mitigation of the clinical phenotype among the mosaics with a healthy 46,XX cell line.

We conclude that TS women with a healthy cell line, 45,X/46,XX mosaic, had a milder phenotype and fewer cardiovascular and fracture risk factors and better balance than TS women who lacked healthy cells (45,X monosomy, iso- and ring chromsome, marker X and Y fragments). This has to be considered in future genotype-phenotype comparisons.

Long-term studies on thyroid and liver function (PAPER III +IV)

A high prevalence and incidence of hypothyroidism was found, independent of karyotype or family history. Hypothyroidism was associated with a higher BMI and underscores the need for physicians to be more alert to this problem and initiate early treatment with thyroxine hormone. This is

important as thyroid disturbances are present already at young age [48-50,55] and as untreated girls could fail in growth during their early years of growth development.
Autoimmunity seems to be the most probable etiology as one third of all TS patients had a positive TPO at start. At the five-year follow-up we found that more than every third TS woman had developed hypothyroidism. Those with a positive TPO seemed to be at higher risk. One might postulate that all TS women will develop hypothyroidism during their lifetime. This is not, however, the case, as seen in Figure 14. Some of the oldest women, 45,X, had neither elevated TPO, nor hypothyroidism.
Quite a few TS women had vitamin B12 deficiency and celiac disease, which speaks in favor of an increased autoimmune susceptibility in TS [100].

A high prevalence and incidence of pathological liver enzymes was found in women with TS. Every third TS patient had high liver enzymes at start and more than every second patient at the five-year follow-up. It is tempting to assume that there should be a common denominator for hypothyroidism and elevated liver enzymes in TS. We could not see any such associations and liver enzymes did not fall after thyroxine replacement. Furthermore, there was no relationship between the pathological liver values and any other factor, such as hepatic autoimmunity, alcohol, hepatitis or karyotype.

Our findings indicate that increased serum cholesterol was the strongest correlate to the liver enzymes. This, in turn, could be associated with increased abdominal fat, as TS women commonly have an increased waist/hip ratio [29,101]. The latter is a well-known cause of liver steatosis or

a non-alcoholic fatty liver. GT was significantly correlated with serum cholesterol independently of other factors. It appeared that this condition was benign with no serious progress, so far, in our TS women. Histological, structural hepatic changes, but no fatal cases have been reported [59].

We could not find any relationship with serum estradiol levels. Over 90% were taking estrogen HRT, mainly 2 mg 17 β-estradiol orally. Others have suggested estrogen deficiency as the cause of the elevated liver enzymes and recommended that the HRT dose be increased [68,101-103].

As a conclusion of the five-year follow-up, we found that more than every third TS woman developed hypothyroidism independently of karyotype. Those with a positive TPO had the highest risk. Hence, thyroid function should be controlled annually in TS. Furthermore, every second TS woman, independent of karyotype, developed elevated liver enzymes, which seemed to be benign. Estrogen HRT can be continued in TS.

Limitations

Limitations of the study were the absence of data on TPO and liver enzymes in the WHO MONICA study, until recently (kindly provided by Dr P. Trimpou). Controls could also have been selected at random in the balance study (Paper II). The strengths of the study are the continuity of the doctors in the project since 1993, the random population sample as controls, and the fairly large adult TS cohort during follow-up.

Management and treatment of TS women

GH has been available for the treatment of short stature in TS girls since 1988. GH treatment starts very early during infancy, followed by androgens. Estrogen is given to induce puberty, usually at an age similar to that of their age-matched peers. Hence, the adult TS women, below 30 years age, have received what is believed to be the optimal growth promoting therapy for well-being and prevention of osteoporosis; Figure 17.

The cardiovascularly protective roll of estrogen HRT is debated. The use of HRT in postmenopausal women is likely to reduce the risk of ischemic heart disease [104]. It is still debated whether TS women should retain HRT throughout life or stop hormone replacement at an age corresponding to the menopause [86]. A low incidence of mammary cancer has been shown in TS, indicating that life-long HRT is fairly safe in this respect [105]. Estrogen HRT can be continued in TS, despite pathological liver enzymes.

We recommend yearly thyroid controls and that thyroxine substitution be initiated already at a TSH level in the upper normal range, especially when TPO is positive. Life-style advice should be given to avoid weight gain. A physical exercise program could improve body balance and prevent fractures.

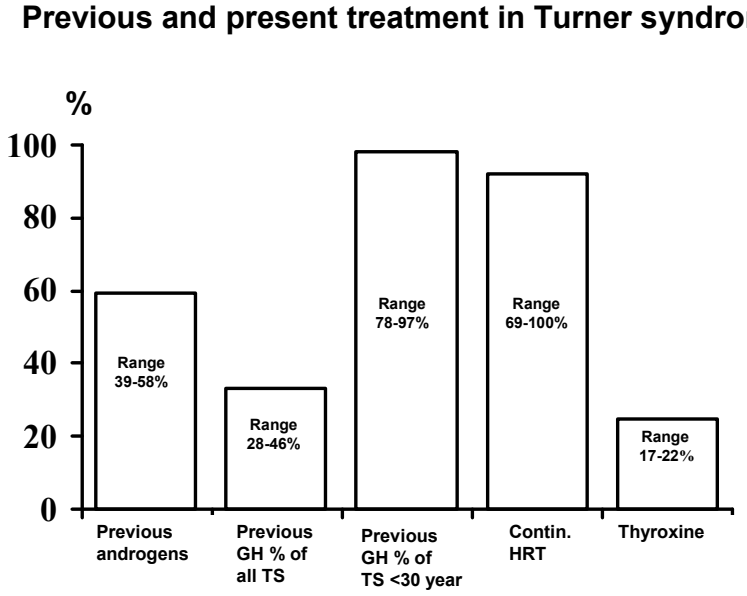

Figure 17. Previous and present treatment with different hormone supplementation in Swedish TS women, n=218. HRT= estrogen hormone replacement therapy, GH=growth hormone.

Patients with left-sided cardiac anomalies are at high risk of endocarditis in connection with infection; prophylactic antibiotics are therefore mandatory before surgery. As TS patients are at risk of cardiovascular complications, meticulous cardiological follow-up is essential in the long term. Hypertension must be treated aggressively. A lower target blood pressure than the common recommendations for the general population is warranted if aortic root dilatation is present [106]. Prophylactic ß-blockers or calcium

antagonists have been used in Marfan's syndrome to halt the progression of aortic root dilatation with some success [107], and would probably be effective in TS women as well.

Echocardiography has hitherto been the main principle in the diagnosis of aortic root dilatation. Studies justify the use of magnetic resonance imaging for its higher precision quality in detecting defects missed by echocardiography [96,108]. This is of the utmost importance before pregnancy induction. According to the national and recent international recommendations [84,86], women with normal echocardiograms have to be followed up every five years, but those with abnormal cardiac findings should be monitored annually by an expert cardiologist, usually at a Grown Up Congenital Heart disease (GUCH) clinic.

CONCLUSION

- Mosaicism seemed to mitigate the TS phenotype and the cardiovascular and fracture risk factor profile. Mosaics were diagnosed 8 years later than 45,X cases. Our findings could explain the high morbidity and mortality among Turner women with 45,X, despite less smoking.

- Fine motor function and body balance were poorer in adult TS women on HRT than in controls. Higher age, hearing impairment, osteoporosis, abdominal obesity, a sedentary lifestyle and the Turner syndrome *per se* were strong determinants, and mosaicism mitigated fine motor function, body balance and hearing in TS.

- More than every third TS woman had hypothyroidism at the five-year follow-up, irrespective of karyotype. The annual incidence was 3.2%. Patients with elevated TPO antibodies had the highest risk. Thyroid function should be checked yearly in women with TS.

- Every second TS woman had elevated liver enzymes at the five-year follow-up. The annual incidence was 3.4%. No relationship with karyotype, alcohol, viral hepatitis, estradiol or autoimmunity was found. Total serum cholesterol was a significant predictor of elevated liver enzymes at five years, independently of other factors. Elevated liver enzymes seemed to be benign during this follow-up. Estrogen HRT can be continued.

Take home message!

Suspect Turner syndrome when two or more of the following are present:

-Short stature
-Lack of puberty
-Infertility
-Hearing impairment
-Hypothyroidism
-Elevated liver enzymes
- >3 external stigmatas
-Cardiac malformation

If strong suspicion of TS, FISH is recommended.

Future perspectives

Based on the results from the present studies it would be of great interest to:

-Treat blood pressure with different agents to lower target levels, in order possibly to diminish the risk of aortic dissection in TS;

-Study the effects of lipid lowering agents on liver enzymes in TS;

-Study the incidence of fractures in the future when the "modern" TS women who have received the growth promoting therapy enter their 40s and the "fracture age". Do they still fracture?

-Follow other possible autoimmune diseases prospectively in TS.

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