Oxandrolone in growth hormone-treated girls with Turner syndrome

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1

General introduction

History

Turner syndrome (TS) was called after Henry Turner, an American clinical endocrinologist who lived from 1892 to 1970. In 1938, he described seven females with short stature, sexual infantilism and anatomical abnormalities such as webbed neck and increased angle of the elbow (cubitus valgus).[1] Although the syndrome is nowadays most frequently named after Turner,[2] he was not the first to describe the syndrome.

The German pediatrician Otto Ulrich already reported an 8-year old girl with the syndrome in 1930.[3] Many Europeans therefore refer to the syndrome as the 'Ullrich-Turner syndrome'. But even before the publication of Ullrich, the syndrome had been described. An association between short stature and defective ovarian development was noted in 1922 by Rossle,[4] as well as by Funke in 1902.[5] In the Russian literature, Seresevskij published about gonadal dysgenesis with short stature,[6] which led to the occasional use of the term "Seresevskij-Turner's syndrome".[7] It is however likely that Morgagni already noticed the syndrome in 1768.[2] He described an autopsy of a small woman, which revealed renal malformations, an extremely small uterus, and no identifiable gonadal tissue.[8]

Although many had thus preceded Turner's publication in 1938, Turner was the first to describe multiple patients, to emphasize the lack of sexual development due to gonadal failure, and to treat patients with exogenous sex steroids and 'pituitary growth hormone (GH)' therapy.[1, 9] Forty-four years after his article "*A Syndrome of Infantilism, Congenital Webbed Neck, and Cubitus Valgus*", his index patient was re-examined and established to have the TS specific 45,X karyotype.[10]

Etiology

In 1959, Ford *et al.* established the chromosomal basis of TS by describing a patient with a 45,X karyotype.[11] Subsequently, many TS patients were noted to actually only *partly* lack one of the X chromosomes. We now know that up to 60% of the patients show a mosaicism (consisting of one cell line containing 45,X) and/or a structural aberration of the second X chromosome (i.e. an Xp or Xq deletion, isochromosome Xq,

or ring X).[12] Furthermore, cytogenetic examination reveals Y chromosome material in about 5% of women with TS. The Y chromosome fragment of these patients misses the testis determining gene *SRY*, thereby leading to the female phenotype.[13]

Prevalence and clinical features

Turner syndrome is one of the most common chromosomal disorders, affecting approximately 1 in 2000 to 2500 live-born girls.[14] As mentioned earlier, the main characteristics are gonadal dysgenesis and short stature. Other possible features include congenital heart diseases, autoimmune disorders, hearing disorders, renal anomalies, specific psychological characteristics, and a number of dysmorphic features (table 1). The degree of TS-related morbidity seems to be partially dependent on the underlying chromosome disorder, and is usually most marked when there is a complete loss of one X chromosome.

Cardiovascular complications are the main cause of the increased mortality in TS women, in whom life expectancy may be reduced by up to 13 years. The most common congenital anomalies are a bicuspid aortic valve (13-34%) and coarctation of the aorta (7-14%). There is also an elevated risk of aortic root dilatation,[15, 16] which is associated with an elevated risk of aortic dissection and rupture. Besides structural cardiac abnormalities, the syndrome is associated with potentially hazardous electrocardiogram changes, including a prolonged QTc interval, right axis deviation, accelerated AV conduction, shortened PR interval and T-wave abnormalities.[18, 19] Furthermore, coronary artery disease is approximately twice as common as in the general population. As early as at age 25, women with TS have several risk factors for atherosclerosis, including higher body mass index, higher waist-to-hip ratio, higher blood pressure and a disadvantageous lipid profile. Girls with TS have a high incidence of impaired glucose tolerance and an increased risk of developing overt diabetes mellitus in adulthood.

Women with TS also have an increased prevalence of auto-antibodies and are at an increased risk of developing autoimmune diseases. Especially hypothyroidism and celiac disease are common: half of all middle-aged Turner patients suffer from Hashimoto's thyreoiditis,[20, 21] and the incidence of celiac disease is increased 11-fold (affecting approximately 6% of patients).[22]

Table 1. Physical features and their frequency in Turner syndrome (adapted from Ranke, 1989.[56])

Feature	Frequency*	Feature	Frequency*
Eyes	20-39%	**Chest**	60-79%
Ptosis		Scutiform thorax	
Epicanthus		Widely spaced nipples	
Myopia		Inverted nipples	
Strabismus			
Nystagmus		**Skeleton**	40-59%
		Cubitus valgus	
Ears	40-59%	Short metacarpal bones	
Deformed auricles		Spongiose bone structure	
Otitis media		Scoliosis	
Impaired hearing			
		Heart vessels	40-59%
Mouth, jaw	60-79%	Aortic coarctation	
High, arched palate		Bicuspid aortic valve	
Micrognatia (small lower jaw)		Aortic dilatation/aneurysm	
Abnormal dental development		elongated transverse aortic arch	
		anomalous pulmonary venous	
Skin, skin appendages	60-79%	connection	
Lymphoedema of hands and		persistent left superior vena	
feet		cava	
Numerous pigmented naevi			
Increased body hair growth		**Kidneys**	40-59%
Nail dysplasia		Renal malformation (e.g.	
Increased skin ridge patterns		horseshoe kidney)	
(dermatoglyphics)		Renal aplasia	
Alopecia		Vessel abnormalities	
Vitiligo			
		Ovaries	80-100%
Neck	60-79%	Gonadal dysgenesis	
Short, thick neck			
Low nape of neck/hair-line		**Growth**	80-100%
Pterygium colli		Small for gestational age	
		Short stature	

*Frequencies reported in the literature show some variation; ranges are therefore given for frequency, rather than exact figures.

The majority of individuals with TS have normal intelligence although verbal IQ scores are typically higher than performance IQ scores. Visual-spatial skills, social recognition, problem solving and motor functions may be impaired. There is an increased risk of social isolation and anxiety.[23]

Dysmorphic features include webbed neck, cubitus valgus and lymphedema of the hands and feet.[24] These physical characteristics may however not be immediately obvious, and may even be absent in several patients.

Because of the diverse disorders that may be present in TS, a life-long multidisciplinary approach is warranted. In 2007 a consensus statement about clinical care for girls and women with TS was published after an interdisciplinary meeting of geneticists, pediatricians, cardiologists, internists, behavioral health specialists, and gynecologists. Table 2 shows the suggested guidelines for evaluation of newly diagnosed patients with TS, and table 3 the schedule for ongoing care.[25]

Table 2. Screening at diagnosis of Turner syndrome in children and adults (with permission adapted from Bondy *et al.,*[25] *Copyright 2007, The Endocrine Society*).

All patients
Evaluation by cardiologist with expertise in congenital heart disease
Comprehensive exam including blood pressure in all extremities
ECG
Clear imaging of heart, aortic valve, aortic arch, and pulmonary veins
• Echocardiography is usually adequate for infants and young girls
• MRI and echo for older girls and adults
Renal ultrasound
Hearing evaluation by an audiologist
Evaluation for scoliosis and/or kyphosis
Evaluation for knowledge of Turner syndrome; referral to support groups
Evaluation for growth and pubertal development
Ages 0–4 years
Evaluation for hip dislocation
Eye exam by pediatric ophthalmologist (if age ≥ 1 years)
Ages 4–10 years
Thyroid function tests (T_4, TSH) and celiac screen (anti-Ttg antibodies or HLA typing; no further testing necessary if DQ2 and DQ8 negative)
Educational and psychosocial evaluations
Orthodontic evaluation (if age ≥ 7 years)
Age > 10 years
Thyroid function tests (T_4, TSH) and celiac screen (anti-Ttg antibodies or HLA typing; no further testing necessary if DQ2 and DQ8 negative)
Educational and psychosocial evaluations
Orthodontic evaluation
Evaluation of ovarian function/estrogen replacement
Complete blood count, creatinine, blood urea nitrogen, liver function tests, fasting blood glucose, lipids
Bone mass density (if age ≥ 18 years)

Table 3. Ongoing monitoring in Turner syndrome (with permission adapted from Bondy *et al.,*[25] *Copyright 2007, The Endocrine Society*).

All ages
Cardiovascular monitoring: Follow-up depends on clinical situation
For patients with apparently normal cardiovascular system and age-appropriate blood pressure
• Reevaluation with imaging at timely occasions, e.g. at transition to adult clinic, before attempting pregnancy, or with appearance of hypertension. Girls that have only had echocardiography should undergo MRI when old enough to cooperate with the procedure
• Otherwise, imaging about every 5–10 years
For patients with cardiovascular pathology, treatment and monitoring determined by cardiologist
Blood pressure annually
Ear nose throat evaluation and audiology every 1–5 years
Girls < 5 years
Social skills at age 4–5 years
School age
Liver and thyroid screening annually
Celiac screen every 2–5 years (anti-Ttg antibodies or HLA typing once; no further testing necessary if DQ2 and DQ8 negative)
Educational and social progress annually
Dental and orthodontic care as needed
Older girls and adults
Fasting lipids, blood sugar, liver and thyroid screening annually
Celiac screen every 2–5 years (anti-Ttg antibodies or HLA typing once; no further testing necessary if DQ2 and DQ8 negative)
Age-appropriate evaluation of pubertal development and psychosexual adjustment

Short stature and growth hormone therapy

The average untreated adult height of a woman with TS is approximately 20 cm below the height predicted by the height of the parents and ethnical origin. Average adult height in north European women with TS is 147 cm versus an average height of 171 cm in healthy Dutch women (Fig. 1).[26] Four distinct phases have been recognized in the pattern of growth of girls with TS.[27, 28] First, growth is disturbed in utero resulting in a length approximately 3 cm shorter and a weight about 500 grams less than in normal female newborns. Second, postnatal growth appears to be in the low-normal to low female range during infancy and early childhood.[28, 29] Thereafter,

height velocity shows a gradual decrease leading to a progressive loss in height.[30] Finally, there is a lack of the pubertal growth spurt.[27] Due to the delayed closure of the epiphysial growth plates, a number of patients continue to grow until their early twenties.[27]

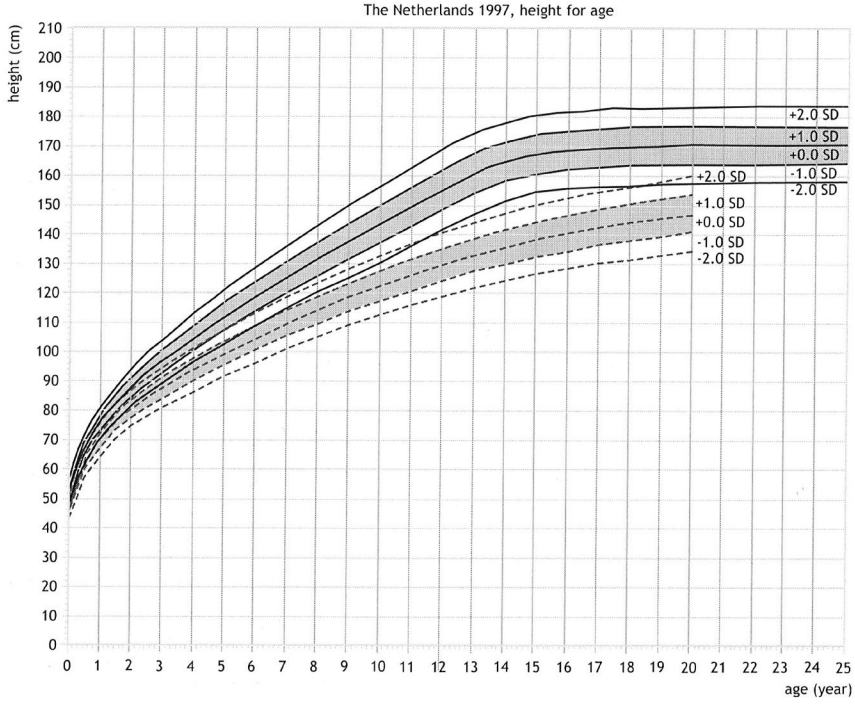

Figure 1. Growth chart for healthy Dutch girls (straight lines) and untreated north European girls with TS (dashed lines). (Figure adapted from Growth Analyser, www.growthanalyser.org)

In 1997, haploinsufficiency of the so-called 'Short-stature Homeobox-containing gene' (*SHOX*) gene was identified as the main cause of the short stature in TS.[31] The *SHOX* gene encodes a homeodomain transcription factor expressed during early fetal life in developing skeletal tissue of the radius and ulna, the tibia and distal femur, and the first and second pharyngeal arches.[32] The protein is specifically expressed in the growth plate and appears to play an important role in regulating chondrocyte differentiation and proliferation during growth.[33, 34] The *SHOX* gene is located in the pseudoautosomal region (PAR1) on the distal end of the X and Y chromosomes at

Xp22.3 and Yp11.3.[31, 35] Healthy female individuals express two copies of the *SHOX* gene because genes in PAR1 do not undergo X inactivation. Women with TS however lack all or part of their second sex chromosome, and thus have only a single copy of the *SHOX* gene. This state of *SHOX* haploinsufficiency appears to be mainly responsible for the average 20-cm height deficit in untreated women with TS.[36] It is also believed to be the main cause of the disproportionate growth, resulting in relatively broad shoulder and pelvic widths, and short legs.[37, 38] However, other factors such as aneuploidy or chromosomal imbalance have also been suggested to contribute to the short stature in TS.[39-41]

Even though patients with TS are not GH deficient,[42] GH therapy increases adult height by 5 to 12 cm.[43-45] Studies in the early 1970s with small numbers of older girls with TS were disappointing probably due to the low doses used, and the low frequency of GH administration (2-3 times weekly).[46] With the availability of recombinant human GH after 1985, larger numbers of girls were treated in formal clinical trials. In a multicenter dose-response study in the Netherlands in which GH doses were given varying from 1.33 to 2.67 mg/m^2/day (~ 0.045 to 0.090 mg/kg/day), the mean gain in adult height ranged from 12.5 to 16.0 cm.[47] TS is now an accepted indication for GH treatment in many countries, but some controversy still exists about the effect of GH treatment in girls who start GH therapy at a relatively advanced age. Early diagnosis and thereby early GH treatment seems important to achieve a normal adult height. Overall, GH therapy seems to be safe, although long-term follow-up data on safety are still awaited.

Ovarian dysfunction and estrogen replacement therapy

Because the ovaries usually start to involute within 4 or 5 months of gestation,[48] the majority of patients are infertile, have diminished ovarian estrogen and androgen production,[49, 50] and need estrogen replacement therapy to induce pubertal maturation. The optimal age to start estrogen therapy has been a point of discussion. It has been suggested to postpone estrogen therapy to delay closure of the epiphysial growth plate and, consequently, to prolong the growth phase. However, delay of pubertal development may have serious psychosocial consequences. The Dutch

growth hormone dose-response study showed that starting GH treatment at a relatively young age, i.e. beginning low dose estrogen therapy from the age of 12 years, resulted in a normalization of height in most girls and pubertal development in conformity with healthy peers.[47] Starting estrogens at very low doses at an even younger age has been shown to even increase adult height in TS.[51]

Oxandrolone therapy in Turner syndrome

For decades, the growth promoting effect of androgens has been tested, but there has always been reluctance to use these agents because of undesirable side effects, including excessive virilization and acceleration of bone maturation. Anabolic steroids were synthesized to dissociate anabolic from androgenic effects and one of the best studied compounds is oxandrolone (Ox). Ox is a synthetic, nonaromatizable anabolic androgenic steroid with the chemical name 17β-hydroxy-17α-methyl-2-oxa-5α-androstane-3-one. It is derived from testosterone, in which a carbon atom (at position 2 in the phenanthrene nucleus) is replaced by an oxygen atom. In comparison with testosterone, Ox has a high anabolic to androgenic ratio (10:1) that has the advantage of giving less virilizing effects than testosterone.[52]

In the past decades, several studies suggested that the addition of the weak androgen oxandrolone (Ox) to GH therapy may further increase adult height.[53-55] However, Ox dosages of ≥ 0.1 mg/kg/day had to be lowered to 0.05 and 0.06 mg/kg/day on the frequent findings of virilizing side effects and increased bone maturation.[53-55] Although the recommended Ox dosage is nowadays ≤ 0.05 mg/kg/day,[25] the efficacy and safety of such dosage is unclear. We hypothesized that, due to the effect of Ox on bone maturation, the optimal dosage with respect to final height gain could be lower than 0.06 mg/kg/day, and therefore performed a randomized dose-response study.

The Dutch Turner Oxandrolone Study

In 1991 the Dutch study "*a placebo controlled study on the effect of oxandrolone in combination with authentic biosynthetic human growth hormone and low-dose*

estrogens on growth and metabolic parameters in girls with Turner syndrome" (further referred to as the Dutch Turner Ox Study; Current Controlled Trials number, ISRCTN54336338; Trialregister.nl number, NTR365) commenced in ten centers in the Netherlands. In this double-blind, randomized, multi-center study, the growth promoting effect of Ox at a low (0.03 mg/kg/day) and previously conventional (0.06 mg/kg/day) dosage was assessed in GH-treated girls with TS. Hundred thirty-three patients were included in age group 1 (2-7.99 years), 2 (8-11.99 years), or 3 (12 -15.99 years), and treated with GH from baseline, combined with placebo (Pl) or Ox 0.03 or 0.06 mg/kg/day from the age of eight, and estrogens from the age of twelve. After reaching adult height and finishing GH+Ox/Pl therapy, the girls were followed for another 1.5 years.

Besides the effect on growth, the study included evaluation of potential side effects. These included virilizing side effects (such as hirsutism, clitoromegaly, and voice deepening), and possible changes in liver enzymes, insulin-like growth factor-I (IGF-I), metabolic parameters, and blood pressure. In addition, possible effects on pubertal maturation, body proportions and composition, and psychological and behavioral characteristics were assessed. The effects on objective voice deepening and psychological and behavioral characteristics were studied by means of two accompanying study protocols. The first was called "*A study on the psychological effects of treatment with oxandrolone in girls with Turner syndrome*". Psychological questionnaires were filled out yearly by all girls to assess the effect of Ox on behavior, aggression, romantic and sexual interest, mood, and gender role in GH-treated girls with TS. The latter protocol was called "*A study on the effect of oxandrolone treatment on characteristics of the voice of girls with Turner syndrome*". The aim of this study was to describe the effect of Ox on subjective and objective voice frequency by analyzing the yearly voice recordings and questionnaires obtained from the girls. Lowering of the fundamental voice frequency (FVF) was used as an indicator of possible virilizing effects of the anabolic steroid Ox.

Outline of this thesis

Chapter 2 addresses the benefit to risk ratio of Ox at a low (0.03 mg/kg/day) and previously conventional dosage (0.06 mg/kg/day) in GH-treated girls with TS. The chapter describes the effect on height gain, bone maturation and duration of GH therapy. It furthermore assesses the effect on pubertal development, blood pressure, IGF-I, and liver enzymes, as well as the occurrence of subjective virilizing side effects (including hirsutism, clitoromegaly, and voice deepening). The following chapters address the effect of GH+Ox on subjective and objective speaking voice frequency (chapter 3), body proportions and body composition (chapter 4), glucose metabolism (chapter 5), and behavior, aggression, romantic and sexual interest, mood, and gender role (chapter 6). Chapter 7 gives a brief overview of the major findings, limitations, and implications of the work presented in this thesis. This chapter furthermore briefly introduces a follow-up study that has recently started to investigate long-term effects of GH+Ox therapy as well as genetic characteristics of TS.

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