Dentofacial morphology in Turner syndrome karyotypes

Sara Rizell

Departments of Orthodontics

Institute of Odontology

Sahlgrenska Academy

UNIVERSITY OF GOTHENBURG

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Cover illustration: “Mosaic” with four tesserae representing each study I-IV.

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sara.rizell@vgregion.se

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To Magnus, Simon and Josef

CONTENTS

ABSTRACT

Dentofacial morphology in Turner syndrome karyotypes

Sara Rizell

Department of Orthodontics, Institute of Odontology, The Sahlgrenska Academy at University of Gothenburg, Göteborg, Sweden

The overall aim of this thesis was to study dentofacial morphology in Turner syndrome (TS) versus controls and the influence hereupon from karyotype.

One hundred thirty two TS females (5-66 years of age), from Göteborg, Uppsala and Umeå were participating. Cephalometric analysis, cast model analysis concerning palatal height, dental arch morphology and dental crown width were performed. Eighteen primary teeth were analysed in polarized light microscopy, scanning electron microscopy, microradiography and X-ray microanalysis were performed. The TS females were divided according to karyotype into: 1 45,X; 2 45,X/46,XX; 3 isochromosome; 4 other.

Compared to healthy females, TS were found to have a flattened cranial base as well as small and retrognathic jaws with a posterior inclination. The maxillary dentoalveolar arch was narrower and longer, while the mandibular dental arch was wider and longer in TS compared to controls. The palatal height did not differ comparing TS and healthy females. The dental crown width was smaller in TS for both permanent and primary teeth. Aberrant elemental composition, prism pattern and lower mineral density were found in TS primary enamel compared to enamel in primary teeth from healthy girls.

Turner syndrome karyotype was found having an impact on craniofacial morphology, with the mosaic 45,X/46,XX exhibiting a milder mandibular retrognathism as well as fewer cephalometric variables differing from controls compared to other karyotypes. Also for the dentoalveolar arch morphology the 45,X/46,XX group had fewer variables differing from healthy females. The isochromosome TS group exhibited the smallest dental crown width for several teeth, while 45,X/46,XX hade the largest dental crown with for some teeth and fewer teeth than both 45,X and isochromosomes that differed from controls. Thus, the mosaic 45,X/46,XX seemed to exhibit a milder phenotype, possibly due to presence of healthy 46,XX cell lines.

Keywords: Orthodontics, genetics, Turner syndrome, karyotype, geno-phenotype correlation, anthropometrics, craniofacial morphology, dental arch, dental crown width, enamel, primary teeth, elemental composition

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PREFACE

This thesis is based on the following studies, referred to in the text by roman numerals I-IV.

I. Rizell S, Barrenäs ML, Andlin-Sobocki A, Stecksén-Blicks C, Kjellberg H. 45,X/46,XX karyotype mitigates the aberrant craniofacial morphology in Turner syndrome. European Journal of Orthodontics 2012 Apr 24. (Epub ahead of print)

II. Rizell S, Barrenäs ML, Andlin-Sobocki A, Stecksén-Blicks C, Kjellberg H. Palatal height and dental arch dimensions in Turner syndrome karyotypes. Submitted for publication

III. Rizell S, Barrenäs ML, Andlin-Sobocki A, Stecksén-Blicks C, Kjellberg H. Turner syndrome isochromosome karyotype correlates with decreased dental crown width. European Journal of Orthodontics 2012 Apr:34(2): 213-8.

IV. Rizell S, Kjellberg H, Dietz W, Norén JG, Lundgren T. Altered inorganic composition of dental enamel and dentin in primary teeth from girls with Turner syndrome. European Journal of Oral Sciences 2010 Apr:118(2): 183-90.

GLOSSARY

Allele	any of the alternative forms of a gene that may occur at a given locus.
Aneuploidy	having or being a chromosome number that is not an exact multiple of the usual haploid number.
Deletion	the absence of a section of genetic material from a gene or chromosome.
Genotype	all or a part of the genetic constitution of an individual or a group.
Gonad	a gamete-producing reproductive gland (as an ovary or testis).
Haploinsufficiency	a condition that arises when the normal phenotype requires the protein product of both alleles, and reduction of 50% of gene function results in an abnormal phenotype.
Isochromosome	a chromosome produced by transverse splitting of the centromeres so that both arms are from the same side of the centromere, are of equal length, and possesses identical genes arranged in the same order counting away from the centromere.
Karyotype	the chromosomal characteristics of a cell.
Monosomy	having one less than the diploid number of chromosomes.
Mosaicism	the condition of possessing cells of two or more different genetic constitutions.
Phenotype	the observable properties of an organism that are produced by the interaction of the genotype and the environ.
Trisomy	the condition (as in Down syndrome) of having one or a few chromosomes triploid in an otherwise diploid set.

http://www.merriam-webster.com

http://www.medterms.com

ABBREVIATIONS

AMELX	X chromosomal amelogenin gene
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
BGN	Biglycan gene
FISH	Fluorescence in situ hybridization
GH	Growth hormone
HRT	Hormone replacement treatment
PAR	Pseudoautosomal region
POLMI	Polarized light microscopy
SD	Standard deviation
SDS	Standard deviation score
SEM	Scanning electron microscopy
SHOX	Short stature homeobox gene
SNK	Student-Newman-Keuls post hoc test
SSL	Subsurface lesion
TS	Turner syndrome
XAR	X-added region
XRMA	X-ray microanalysis

INTRODUCTION

History - one syndrome but several names

In 1938 the American endocrinologist Henry Turner described seven young girls with the triad; sexual infantilism, webbing of the skin of the neck and elbow deformity (1). Additional features also found by Turner were retarded growth and low hairline. He believed the underlying reason for the clinical symptoms being a result from a defect in the anterior pituitary gland. In most Euro-American publications this condition was named Turner Syndrome (TS). Although Henry Turner was the first one to describe a group of females with similar symptoms, single patients had been described earlier. It is believed that the Italian anatomist Giovanni Battista Morgnani was the first to report the syndrome, when he in 1768 described an autopsy of a short woman with renal malformations, small uterus and lack of gonadal tissue (2). The Russian endocrinologist N.A. Šereševskij reported in 1925 on a woman with short stature, sexual underdevelopment and features as low hairline, short neck, pterygium colli, micrognathia and high arched palate, why in Russian literature the nomination Šereševskij syndrome is seen (3). Five years later the German pediatrician Otto Ullrich described an eight year old girl with short stature, webbed neck, cubitus valgus and aberrant appearance, why the condition sometimes is called Ullrich-Turner syndrome in European literature (4). The geneticist Paolo Polani discovered in 1954, as the first one, the absence of an X-chromosome and later the British geneticist Charles Ford with co workers confirmed Polani's findings of the underlying genetic aberration behind the clinical features earlier described (5, 6). Forty years after Henry Turner published his article about the seven females with TS characteristics, the circle was closed, when one of the patients described in 1938, was re-examined by Males et al. who were able to confirm her 45,X karyotype (7).

1938 Henry Turner described girls with sexual infantilism, webbed neck and elbow deformity.

Genetics in Turner syndrome

The X-chromosome

The X chromosome contains about 2000 genes out of the estimated 20.000 - 25.000 genes in the human genome. Examples of functions regulated from X chromosomal genes are blood coagulation, skeletal formation, fertility and mental functioning (8-11). In the sixties Mary Lyon introduced the hypothesis of "Lyonisation", which implicated a random "turn off" (silencing) of one of the two sex chromosomes, to compensate for an unequal dosage from X chromosomal gene products in human 46,XX female compared to 46,XY male (12, 13). The inactivation is initiated early in embryogenesis by package of DNA and proteins as dense and inactive heterochromatin instead of as the more active and loosely packed euchromatin and thus, the majority of the genes on either the maternal or the paternal X chromosomes are silenced as a result of X chromosome inactivation (14, 15). However more than 15% of the genes escape silencing and another 10% show a heterogeneous pattern, with escaping inactivation in some of the analysed cells but not all (16). The majority of the escaping genes are located in the so called pseudoautosomal regions, the larger (PAR1) located at the tip of the short p-arm and the smaller (PAR2) at the tip of the long q-arm (PAR2) on the X chromosome (17). There is also a smaller proportion of escapees in the X added region (XAR), located on the p-arm, which is believed to have developed from a translocation of an ancestral autosome to the X chromosome, millions of years ago (17, 18). A loss of a gene in either of these regions might thereby affect the gene expression (16).

When the silencing of a specific gene occurs in a parent-of-origin specific manner, i.e. when the inactivated gene copy is inherited more often from either the mother or the father, it is called genomic imprinting. Imprinting is also discussed for the pattern of inheritance for the unaffected X chromosome in TS, since in 74% of the TS females with total loss of one X chromosome (monosomy), the single remaining chromosome is of maternal origin, while instead in TS with structural aberrations the paternal origin of the unaffected X chromosome dominates (19-21). Among the isochromosome karyotype,

several authors claim the intact X chromosome being more often of a paternal origin than among the monosomies (20, 22), while others claim that the isochromosome karyotype is equally likely to involve either a maternal or paternal X chromosome (19). The parental origin of the X chromosome is believed to influence the phenotype concerning several features and conditions, such as neck webbing, social cognition, GH response, hearing, cardiovascular, renal and ocular abnormalities (20, 22-24).

The presence of healthy (46,XX) cells without haploinsufficiency of certain genes, in TS females with several diagnosed cell lines, has been reported to have a favourable effect on the phenotype e.g. for spontaneous pregnancies, fine motor function, body balance, hearing and number of stigmata (25-28). Moreover, the proportion of 46,XX cells is reported to relate to the severity of the phenotype, so that an increased amount of 46,XX cells results in fewer TS stigmata (27).

Studies on individuals with sex-chromosome aneuploidy reveal that also the number of sex chromosomes affects the phenotype. With one additional X chromosome the general body height increases, (29) but also dentoalveolar characteristica are affected, as root morphology, occlusion and dentoalveolar width (30-34). Additionally, females with an extra X chromosome (47, XXX) have an increased tooth crown width, due to thicker enamel (35-37). For the cranial base morphology the results are more incoherent, since an increasing number of sex chromosomes cause a more acute cranial base angle in males (38-40) while an additional X chromosome in females caused the opposite (41). Findings on how the number of X chromosomes influence dentofacial morphology support that genes involved in both craniofacial growth and tooth formation are located on the X chromosome.

A portion of the X chromosomal genes escape silencing.

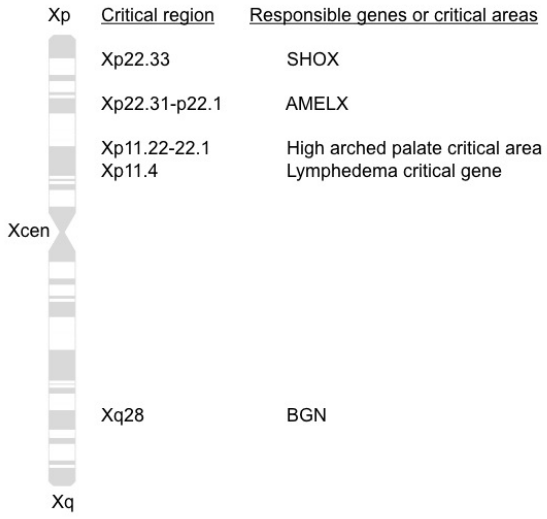


Figure 1. Map of X chromosome showing locations of different genes, discussed in this thesis, of possible importance for the TS dentofacial features.

The Short Stature Homeobox-containing gene

A gene often studied in TS is the Short Stature Homeobox (SHOX) gene. This gene is located in the pseudoautosomal region on the tip of the p-arm of the X chromosome, where a haploinsufficiency is believed to cause both growth retardation and several skeletal TS characteristics (Figure 1) (11, 42, 43). Examples of skeletal deformations that frequently are mentioned being caused by a haploinsufficiency of the SHOX gene are Madelung deformity, short metacarpals, cubitus valgus, high arched palate and scoliosis (11, 44). It is claimed that this gene is coding for limb development but also for structures originating from the first and second pharyngeal arches, which is the origin of maxilla and mandible (except for the condylar cartilage) as well as middle ear structures (11).

SHOX gene deficiency is suggested to cause short stature and skeletal malformations as Madelung deformity, osteoporosis, high arched palate and micrognathia.

The amelogenin gene

Amelogenin is the major protein involved in the enamel formation (45). Both amelogenin and the amelogenin gene (AMELX) are counted as highly preserved in most chordates during evolution, and considered as stable since million of years (46). AMELX is located in the X-added region on the short arm of the X chromosome, Xp22.3-p22.1 and a haploinsufficiency is suggested to reduce the amount of its gene product (Figure 1) (17, 47-49). A reduction of amelogenin, which constitutes the majority of the total amount of the enamel matrix proteins (e.g. enamelin, ameloblastin), causes both qualitative and quantitative disturbances in the amelogenesis (49, 50) and several mutations affecting AMELX have been shown to be part of the cause the generalized hypoplastic enamel seen in X-linked amelogenesis imperfecta (51). A crucial factor for the final thickness of the enamel layer is the duration and timing of the secretory stage of amelogenesis (52), where amelogenin is secreted from the ameloblasts to form the enamel matrix, since this determines the length of the enamel crystals (45, 53). Formed enamel matrix is gradually replaced by mineral components such as calcium and phosphate, being major components of the forming hydroxyapatite. In addition to calcium and phosphate, hydroxy ions are incorporated into the crystals (45, 54).

Haploinsufficiency of AMELX is suggested to affect the amelogenesis.

The biglycan gene

There are reports on both abnormal structure and histochemistry of the cartilage in TS (55). It is found that biglycan, which is a component of cartilage, is dependent on the number of X chromosomes and that the amount of biglycan is decreased in TS and increased in individuals with supernumerary sex chromosomes (56, 57). The gene for biglycan (BGN) is mapped on the q-arm of the X chromosome and the gene expression is decreased in TS, why BGN is suggested to escape X inactivation (Figure 1) (56, 58-60). However, there

are question marks for BGN, since no Y homolog is found in cultivations of human-hamster hybrid cell lines and BGN thus seem to be subjected to X inactivation (56). It is suggested that genes escaping inactivation regulates the transcription of BGN since the gene does not show the conventional correlation between gene dosage and expression rate seen in other X chromosomal genes.

Genetic aberrations in Turner syndrome

The genetic background for TS is highly heterogeneous and can be described as a partial or complete absence of one of the X chromosomes, in many cases accompanied by cell mocaism (61, 62). The most commonly described is the 45,X karyotype, with complete absence of one of the X-chromosomes in all analyzed cells, occurring in approximately 50% of the TS females (Figure 2) (63-67). Structural aberrations, as isochromosomes, deletions, duplications, transpositions, inversions, ring chromosomes, marker chromosomes or incorporation of an Y chromosome fragment occur in around one third of TS (Figure 2) (64, 65, 67-72). Depending on which genes are affected from e.g. a duplication or deletion, the severity of the damage is determined. The TS isochromosome karyotype has one normal X chromosome and one X chromosome displaying a duplication of the long q-arm and loss of the short p-arm (i.e. long arm trisomy and short arm monosomy), making about half of the TS displaying structural aberrations (64, 65, 68-73) (Figure 2). Both 45,X and other cell-lines with structural aberrations can be accompanied by one or several additional cell-lines and is called cellular mosaicism. In 8-24% of TS females presence from healthy 46,XX cell-lines are displayed (Figure 2) (64, 65, 68-73).

45,X - missing one X chromosome.
Iso - p-arm replacing q-arm.
45,X/46,XX mosaicm - healthy cells accompanying 45,X.

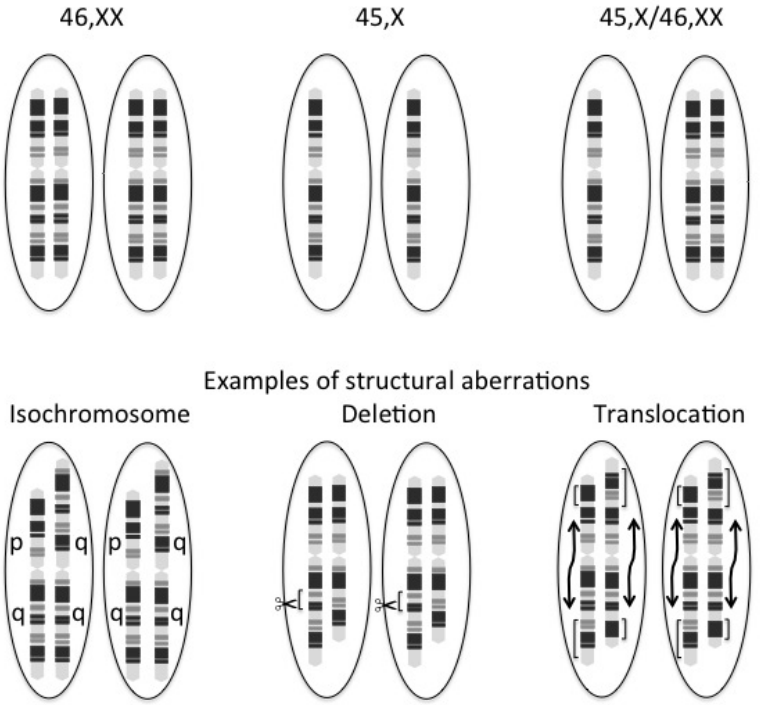


Figure 2. Chromosomal constitution of healthy females (46,XX), monosomy (45,X), mosaicism (45,X/46,XX) together with examples of X-chromosomal aberrations as isochromosomes, deletions and translocations.

Genetic damage affects the cell cycle

A mechanism discussed by several authors as an explanation for the growth retardation described in TS is the cell cycle delay hypothesis. The genetic course of the TS syndrome is reported to affect the cell cycle, since the cell generation time for fibroblasts in 45,X cells is prolonged, due to an extended S-phase (74-77). The prolonged cell cycle causes a selection disadvantage in 45,X cells versus healthy 46,XX cells, which is in line with the decreased severity of the mosaicism by age in TS, i.e. the proportion of 45,X cells is declining due to the slower growth rate (75, 78). Also an increase of the cellular genetic material, as in trisomic cells, seems to slow down the cell division (74, 77, 79). A prolonged cell cycle gives rise to a slower growth rate and might not only affect the quantity but also the quality of tissues formed. It is also discussed whether the effect from the prolonged cell cycle time can cause an irreversible growth disturbance, from fewer cell divisions taking place during a short

developmental time window available for differentiation of an organ or structure in a susceptible area. Structures in the head and neck region are suggested being such susceptible areas where either a reduction of cell number or delayed timing from signalling of growth factors can cause an irreversible growth disturbance (80, 81). A prolonged cell generation time is discussed being responsible for foetal lethality and growth retardation, short stature and somatic anomalies described in TS (75).

45,X fibroblasts have a prolonged cell cycle.

Medical aspects of Turner syndrome

Developmental problems and morbidity

A geno-phenotype correlation means studying of how the genetic constitution of an individual affects the expression of a specific trait, based on genetic or environmental influences. The most consistent feature in TS is short stature, which affects 95-99% of the individuals (68, 82). Due to lack of pubertal growth spurt, the height (without growth promoting treatment) at age of 14 is around -4 SD compared to females of the same age and if puberty is not induced the growth period is prolonged until a final height approximately 20 cm below normal height (83). Without any growth hormone (GH) treatment the final adult height is 141-147 cm while the gain of height by GH therapy around 6 cm (84-86). The growth retardation starts already during the prenatal phase and becomes more pronounced during the failure of pubertal growth spurt (87, 88).

It has been debated whether TS females have a normal or a reduced spontaneous production of GH. There are contradicting reports, both of a lower secretion of GH in TS compared to normal growing girls (89-91) while others found no differences (92, 93). Karyotype seems to affect the GH deficiency so that the GH deficiency occurs more commonly among TS cases with an isochromosome

genotype than the monosomic 45,X group (94). Regardless, if TS females have lower levels of spontaneous GH or not, it seems as they have a less effective form of GH. It is found that, from the two isoforms of circulating GH (22 kDa and non-22 kDa), an increased portion of the less effective (non-22-kDa) isoform is present, which partly might explain the growth deficiency found in TS (95, 96).

Approximately, ninety percent of girls with TS have no pubertal development, due to ovarian insufficiency, which results in absence of secondary sexual characteristics and infertility (97). The infertility is caused by an accelerated loss of oocytes from the ovaries. A few percentages of TS females have spontaneous periods and may become pregnant without intervention and 30% have some spontaneous pubertal development (98, 99). It is reported that pregnancies occur in 12% of swedish TS females, from which 40% had a spontaneous pregnancy and the remaining females had oocyte donation or in vitro fertilization (25). Many genes have been discussed as candidates causing the premature ovarian failure in X chromosome abnormalities, but still the cause remains unknown (100).

Multiple diseases and anatomical malformations are associated with TS. Cardiac abnormalities are present in up to 50 % of TS females and is assumed to be part of the explanation for the increased mortality (64, 101, 102). Cardiovascular malformations seen in TS as bicuspid aortic valve, coarctatio aorta, aortic dilatation/aneurysm together with hypertension seem to be associated with the 45,X karyotype (72, 103). Skeletal malformations as cubitus valgus, short fourth metacarpal bone, madelung deformity and scoliosis are overrepresented in TS women (97, 104). Several conditions and diseases with metabolic origin as hypothyroidism, diabetes, osteoporosis, obesitas and celiac disease are described (27, 105-107). In TS lymphatic disorders and lymphedemas are frequently described, not only of hands and feet but also in the cervical region (108, 109). Lymphatic hypoplasia cause lymph fluid stasis resulting in distended lymphatic vessels and general lymphedema (110). Nuchal translucency is reported being a translucent lymphatic area in the neck region, visible on ultrasound in the first trimester and proposed to indicate

chromosomal aneuploidy (108, 111, 112). In Turner syndrome nuchal translucency often is referred to as nuchal cystic hygroma and of larger extent than in other chromosomal aneuploidies (112). A majority of TS girls have frequent otitis media with temporary hearing loss as a consequence, while sensorineural hearing loss, correlated to karyotype is described among elder TS females (80, 113). Additionally, ophthalmic problems, as strabismus and ptosis are reported in TS females (114). Typical TS stigmata as webbed neck, low hairline, epichantus fold, peripheral oedemas and hypoplastic nails are described (27, 115).

The main features in TS are short stature, infertility, cardiovascular and skeletal malformations, metabolic diseases and lymphedemas.

Incidence, diagnostic and medical treatment issues

The incidence differs depending on whether measured pre- or postnatally. The prevalence of TS is reported to be 1/2000-1/5000 live born females (116-119). However, Gravholt et al (117) found the prevalence among female foetuses being ten times higher than among live born girls, indicating a high rate of TS foetal deaths in the first trimester, possibly together with a high estimated number of unknown cases. The rate of those with 45,X karyotype was markedly higher among the embryonic and foetal deaths in contrast to the live born TS, indicating a lower viability of TS cell monosomy foetuses due to impaired prenatal growth (63, 119, 120). There are suggestions of that some degree of mosaicism is necessary for survival and this statement is supported by the fact that if combining chromosomal analysis of more than one tissue, the rate of apparent 45,X karyotype decreased (121).

Chromosomal analysis on blood lymphocytes is usually undertaken for cytogenetic analysis to diagnose TS, and to be able to exclude 10% mosaicism with 0.95 confidence at least 29 cells are recommended to be analysed (122). To increase the detection of mosaic cell-lines of low percentage or e.g. Y chromosomal material, the molecular technique fluorescence in situ hybridisation (FISH) analysis is supplemented (123). The level of mosaicism

and detection of Y-fragment are applicable for prediction of prognosis and risk assessment for development of gonadoblastoma (124). An increasing number of TS girls are now diagnosed prenatally since TS is suggested to be suspected, if ultrasound findings as nuchal translucency, coarctation of aorta, left sided heart malformations or renal defects are present (125, 126). Moreover, TS is recommended to be anticipated if: presence of lymphedemas or heart failure in a new-born (127-129), general height deficiency of more than two SD below mean (130), lack of pubertal development or amenorrhea (98, 127). However, the estimated number of unknown cases is high since the TS diagnosis often is delayed (mean delay 7 years), although many TS exhibit the described characteristic features and therefore should have been possible to diagnose earlier (130, 131).

In the 1960's, girls with Turner's syndrome started to be given hormone-replacement therapy, first as anabolic steroids. To promote overall growth, women with TS have since 1986 been treated with biosynthetic growth hormone (GH), with doses higher than in the replacement treatment applied in GH deficiency (73, 132). It has been found that factors affecting the outcome of the growth promotion are: age at treatment start, age at onset of puberty and dose of GH (61, 133). The best result is achieved with an early treatment start, puberty onset not before 13-14 years of age and a high dose of GH (61, 134). However, the body height effect of GH treatment does not seem to be influenced by karyotype (73).

Puberty has to be induced in most TS cases by treatment with sex hormone replacement treatment (HRT) and at the time for normal puberty, estrogen is given (135). It is recommended to start treatment with still no signs of puberty, to mimic normal pubertal development (136). Sex hormone treatment is recommended during the entire life since sex hormone insufficiency is involved in increased cardiovascular risk, physical fitness, insulin resistance, bone mineral density and body composition (137, 138).

Prevalence: 1/2.000-5.000 females.
Treatment with GH and sex hormone to promote growth and induce puberty.

Dentofacial features in Turner syndrome

Craniofacial morphology

Concerning craniofacial morphology several studies have revealed that women with the diagnosis TS have a smaller calvarium both concerning height, width, length and diameter measured on lateral radiographs (139-141). However, the results are not unambiguous. Contradictory studies, both antropometric and cephalometric, state no difference in size of the calvarium comparing TS and controls (142-144). However, the study by Rzymski et al. (144) was conducted on a majority of individuals without cytological confirmation of TS diagnosis. Jensen (140) found no significant difference in cranial dimensions comparing 45,X and mosaics. There is a clear unanimity in the literature concerning the cranial base in TS, which is stated to have a shorter posterior length than healthy females (38, 139-141, 143, 145-150). A strong association is found for the length of the posterior cranial base between TS females and their mothers in addition to the magnitude of mandibular retrognathism (148). Filipsson et al. (139) also found the anterior cranial base to be smaller in TS females but is not supported by others (141, 145). Several studies published show that TS females have an increased cranial base angle compared with controls (139-141, 143, 144, 146, 148-152). These changes are seen already prenatally and are present both in young girls and adult TS females (140, 141, 146, 151). From these observations the conclusion is drawn that the growth disturbances occur already prenatally and the pattern of craniofacial morphology is maintained during the growth period. As a consequence from the large cranial base angle TS females develop a retrognathic face with a retrognathic maxilla as well as a retrognathic mandible (38, 140, 141, 143, 145-150, 152-154). The mandible has been found to be shorter in TS individuals (38, 140, 141, 143, 145, 146, 148, 149, 152) with very few exceptions (139). Concerning the maxillary length the results are not as univocal. The literature present both studies stating that the TS females have a shorter maxilla (38, 140, 141, 146) and the opposite, where there is no significant difference in maxillary length between TS and controls or even an increased maxillary length (143, 145, 149, 152). Several studies indicate that TS females have a posterior rotation of the mandible (38, 141, 146). Babic et al. (153) found that both posterior and anterior rotation

Table 1. Published studies on the impact of TS karyotype on craniofacial and palatal morphology as well as dental crown width. No studies on impact from TS karyotype on dentoalveol arch morphology were found.
*A subgroup from Rongen-Westerlaken *et al.,*1992

Reference	Age years	Karyotypes (n)	45,X vs other karyotypes	N of variables differing from controls
Craniofacial morphology (cephalometric analysis)				
Jensen, 1985	Adults	45,X (21) other (20)	45,X < other (s-n-ss)	
Rongen-Westerlaken et al. 1992	4-17	45,X (50) other (19)	45,X = other	
Rongen-Westerlaken et al. 1993*	9-16	45,X (9) 45,X/46,XX (7) other (3)	45,X = 45,X/46,XX	
Midtbø et al. 1996	7-17	45,X (24) other (9)	45,X = other	45,X > others
Dumancic et al. 2010	10-33	45,X (19) other (17)	45,X = other	
Palatal morphology estimated by visual assessment				
Makashima et al. 2009	7-61	45,X (75) others (16)	Dysmorphic palate 45,X > other	
El-Mansoury et al. 2007	16-71	45,X (55) 45,X/46,XX (34) others (37)	High arched palate 45,X > 45,X/46XX	
Permanent dental crown width assessed from cast models				
Varrela et al. 1988		45,X (89) 45,X/46,XX (15)	45,X < 45,X/46,XX (mandibular M_2)	
Mayhall et al. 1991		45,X (89) Iso (6)	Iso < 45,X (maxillary I_1, M_1, mandibular I_1, P_2)	
Midtbø et al. 1994		45,X (23) other (9)	45,X = other	45,X 16/24 other 14/24

were present among TS females but only anterior rotation in controls. The maxilla was found to have posterior inclination as well (140, 141, 146).

The difference in craniofacial morphology between karyotypes of TS is not sufficiently studied. To our knowledge only five studies intended to investigate the influence of karyotype on craniofacial morphology, several with limited patient materials and with only one exception subdividing into 45,X and "others" (Table 1). Jensen (140) found that 45,X had a more retrognathic

maxilla than the group of mixed mosaics, while the other four authors presented in Table 1 found no significant differences between 45,X and the group of various kinds of mosaics and isochromosomes. Rongen-Vesterlaken et al. (143) found no difference comparing 45,X and 45,X/46,XX. Only Midtbø et al. (141) compared the karyotypes (45,X and a group consisting from mosaics and isochromosomes) one by one versus controls and found more variables with significant differences in the 45,X group, why they concluded that mosaics and isochromosomes follow the pattern of 45, X but less pronounced. Grön et al. (155) studied a group of fourteen 45,X/46,XX females but compared only with healthy females and relatives and found results corresponding to the entire TS group.

The question about how the relatively high doses of GH affects craniofacial growth is only addressed by three studies and only little or no effect, comparing pre and post GH treatment, has been registered (143, 147, 149). Rongen-Westerlaken et al. (143) found, in addition to an increased diameter of the calvarium, a positive minor effect on mandibular growth, with an increased mandibular length mainly due to vertical growth, after 2 years of GH treatment. The findings by Simmons (147), that one year of GH treatment increased one maxillary and seven mandibular linear measures, together with four measures associated with facial height and for the mandibular measures these with a vertical component dominated, seem to agree quite closely with previous mentioned study. One contradicting study found no significant difference at all between GH treated TS females and TS females without GH treatment and they conclude that GH treatment should be initiated earlier to affect craniofacial growth (149). The effects from GH treatment on craniofacial growth are difficult to evaluate since small materials, limited observation time an inhomogeneous GH distribution. The craniofacial effect of GH treatment on different TS karyotypes is not described in the literature.

TS display flattened cranial base and retrognathic face. Few and limited studies on impact from karyotype hereupon are published.

Palatal height

Females with TS are in several studies reported to have high arched palate (27, 44, 131, 156-167). The literature is univocal about that high arched palate is one of the principal features for TS that indicates for karyotype analysis, if combined with one additional feature. However, the majority of the published studies on palatal height are based on visual assessment solely and there are only few references that have quantified the palatal height from measurements on plaster casts (156-159, 168). Laine et al. (159) reported that the palate of 45,X females was equal to healthy females, with exception for the anterior part, at the canine level, where a statistically significantly increase in palatal height was found in TS. Johnson et al. (158) (measuring palatal height in the molar area) had a limited material and statistical analysis was not possible. The remaining studies have compared palatal index (palatal height/palatal width) between TS females with controls and found that TS females have higher values than controls, and with a tendency of lower values comparing to individuals with additional number of X chromosomes present (i.e. Klinefelter syndrome) (156, 157, 168). A higher palatal index reflects either a higher palatal vault or a narrower palatal width and estimation of palatal height is unfeasible from these results.

The majority of studies on palatal height are performed on either a mixture of different TS karyotypes or 45,X karyotype solely. A couple of studies are published about influence from karyotype on palatal height and found that a high arched or dysmorphic palate is more common in the 45, X karyotype (Table 1) (27, 162). However, these two studies are based on visual assessment of the palatal height only. To date no studies are found on impact from karyotype on palatal height measured on cast models.

Palatal vault is reported high in TS, but principally studied by visual assessment.

Dentoalveolar arch morphology and malocclusions

TS females are reported on having a narrower maxilla than controls while the mandible being broader in comparison with controls (139, 159, 164, 166, 169). Additionally, the transversal dimensions gave 45,X females the lowest values for maxillary/mandibular width ratios compared to relatives and controls (169). For the maxillary length, the results are not as unanimous as for the transversal measures, since Laine et al. (169) found an increase of the maxillary length while the results from others are contradicting (164, 166). The mandibular length is less controversial and is reported to be decreased compared to controls (164, 166, 169). To our knowledge no studies are published on impact from karyotype on dental arch morphology.

An increased prevalence of occlusal anomalies compared to healthy females is described in TS (164, 170-172). Only one author reported no difference between the groups (166). Distal molar relation was found more frequently in TS females than controls, in addition to large overjet, lateral crossbite and a tendency to frontal open bite (Figure 3) (164, 170-172). Lateral crossbite occurred in 17-50% (164, 170-172) compared to 11% in Swedish schoolchildren (173). Additionally, lateral open bites are described, often in association with submerged maxillary premolars (164, 172). Only few studies have investigated the space conditions, but with opposing results. An increased frequency of mandibular crowding was found (164) but contradicted by a report of less mandibular crowding in TS vs controls (139).

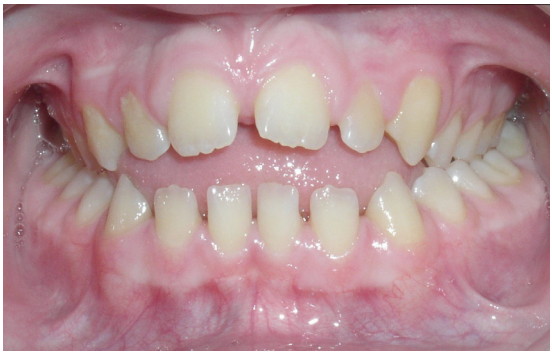


Figure 3. Clinical example of lateral crossbite and frontal open bite.

Midtbø et al. (172) investigated the influence of TS karyotype on frequency of malocclusions but found no significant differences comparing monosomies with a group consisting of mosaics and isochromosomes. On the other hand, comparing each karyotype group versus controls an increased number of statistically significant differences between 45,X patients and controls was found, while the group consisting of mosaics and isochromosomes showed a similar pattern of malocclusions but more varying (172). Harju et al. (171) concluded that a group of 45,X/46,XX and isochromosomes showed milder expression of malocclusions compared to 45,X, while 45,X/46,XX was more affected than 46,Xi(Xq) women. However, the figures are difficult to judge since the patient materials are relatively small. Even if females with TS have an increased frequency of malocclusions, no controlled studies are found on orthodontic treatment of TS females, only a few case reports exist, emphasising the root resorption risks (174, 175).

TS display narrow maxilla, broad mandible and increased frequency of malocclusions. No studies on karyotype and dental arch are found.

Dental morphology

The literature is convincingly unanimous about females with TS having smaller crown width of permanent teeth than healthy controls (28, 139, 161, 164, 166, 176-180). This is true for both mesio-distal and bucco-lingual width but the results are not as consistent concerning the bucco-lingual dimension, due to fewer teeth exhibiting statistical significant differences versus controls. Also the crown height seem to be affected, with a decreased height in TS (181). The results for deciduous mesio-distal dental crown width are parallel with the results for permanent teeth, but only differences for primary molars were statistically significant smaller compared to normal individuals and no differences for the bucco-lingual dimensions were proven (176, 177). The mentioned results of dental crown width measurements emanate from measurements on plaster cast models, except for a recent study on tooth width measured by a 3D system,

that has confirmed the previous findings of tooth size discrepancy in TS (182). Moreover, an increased frequency of contralateral tooth pairs with asymmetric crown width is reported, where incisors were dominating (179).

There are indications about differences in tooth size between different karyotypes (Table 1). Females with isochromosome of the X chromosomal q-arm seem to have smaller teeth than 45,X females, while 45,X/46,XX females seem to have larger tooth crown size than 45,X females (iso < 45,X < 46,XX) (28, 178, 183). However, Midtbø et al. (179) found no significant difference between different karyotypes, but including 45,X/46,XX in the same group as isochromosomes might have biased the results. The reason behind the smaller teeth is mainly a significantly thinner enamel (both in height and width), a result originating from radiographic measurements of enamel and dentine thickness in maxillary first incisors and canines, as well as mandibular first and second molars (184, 185). The dentin thickness did not differ from female controls except for the second molar occlusal surface (184).

Not only tooth size is affected but also dental crown morphology (161, 179, 186, 187). Cervicoincisal convergence of approximal surfaces, wedge shaped incisors, altered cusp form, reduced cusp volume, reduced number of cusps and triangular molar occlusal surface are examples of traits (161, 179, 186, 187).

Females with TS are reported on having shorter dental roots in comparison with healthy females (161, 166, 181, 188). Findings as molarization of premolars, bifurcated roots, atypical root canal pattern and altered crown-root proportions of some teeth are described (181, 189, 190). Concerning karyotype influence on root length and morphology, two rooted premolars were described also for 45,X/46,XX karyotype but with a more simplified root morphology than 45,X (191). No significant difference in anatomy of dental roots was found comparing 45,X, structural aberrations and mosaic karyotype (190). Midtbø et al. (181) found no difference in root length comparing 45,X versus other karyotypes. Isochromosomes had remarkably decreased tooth length for several teeth, but no statistical analysis was performed on this group solely (181). Besides case reports, there is one single study describing significantly

higher frequency of root resorptions of idiopathic type in TS, but there was no difference found concerning root resorptions of inflammatory type between TS females and controls (139).

TS exhibit small dental crowns and roots with atypical morphology. Impact from karyotype on tooth size is anticipated.

Dental age and eruption

Several studies report markedly advanced dental age in TS compared to either healthy or healthy short children (149, 166, 192, 193). Midtbø et al. (192) found that TS girls exhibited a mean advanced dental age of 1 year. No difference in dental maturation was found comparing TS individuals treated with GH with untreated (149). A shorter duration of tooth formation due to the smaller teeth with thinner enamel and shorter roots are discussed as reason for the advanced dental maturation. In addition to dental maturity Midtbø et al. (192) investigated the timing of tooth eruption and found an acceleration of eruption on average 3,7 months for TS girls, although the acceleration was not statistically significant. Their results are supported by Filipsson et al. (139) who found a tendency to early eruption among TS girls. After the age of ten, tooth eruption is reported to be delayed in TS and coincidence with a decrease of GH and estrogen at the same age is discussed (192). It seems as the eruption might be early among younger TS females and then delayed among older, but only few studies are published on this topic. Some authors suggest that the prolonged cell cycle affect the eruption time in TS, but also hormonal factors as thyroid function and GH activity together with the dysplastic skeletal structure are discussed (139, 192).

Enamel defects and oral health

It is reported in a few clinical studies that females with TS express more macroscopic enamel defects than healthy females (161, 180, 194). Kusiak et al. (194) found increased occurrence of enamel hypoplasias and opacities in

TS in comparision with controls and the frequency of both was less in patients with mosaics in comparison with 45,X. The defects were observed in all types of teeth and both lingualy and labially (194).

A lower prevalence of caries is described for TS females compared to controls (161, 164, 195) with one exception (180). Also the results concerning periodontal health are deviating. The three publications found on periodontal health are completely dissentient, but the largest and best conducted study by Väisänen et al. (196) describes a better periodontal health in TS females compared to controls (161, 164).

Normal enamel formation

Tooth formation occurs from interactions between epithelial and mesenchymal cells. The inner layer of the enamel organ (formed by epithelial condensation) deposits and forms the enamel and at the future enamel-dentine border the process of hard tissue formation is started. The complex process of amelogenesis occurs during an extended time period, where each separate stage is initiated by interactions between the different tissues, i.e. ecto-mesenchymal interactions. After initiation of dentin mineralization by the odontoblasts, the ameloblasts (originating from ectoderm) secrete enamel matrix proteins (secretion stage), a cocktail consisting of approximately 90% of amelogenin, which decides the length of the enamel prisms and subsequently the enamel thickness (45). Thus, the volume of the matrix decides the final volume of enamel. Each enamel prism is developing from one single ameloblast (197). When the full amount of the enamel matrix is established the number of ameloblasts is radically reduced by apoptosis and the remaining ameloblasts transform from secretory cells to resorbing cells (transition stage) (52). During the maturation stage the process of resorption of matrix runs simultaneously with the process of formation of calcium phosphate salts within the gradually disappearing matrix. The ameloblasts are subsequently responsible for the proteolytic degradation of the organic matrix (198, 199). The result is an almost complete resorption of enamel matrix, which prevents an incomplete mineralization (200). The mature enamel consists of approximately 96% of inorganic material i.e. crystals of

calcium hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$, in a form containing carbonate (52). The organic portion of mature enamel consists of water and matrix proteins, where in regular prism pattern the enamel proteins may constitute only 0.05% whereas in an irregular prism pattern as much as 3% (52). As enamel, dentin consists of calcium hydroxyapatite, but to an extent of only 70%, while the organic material (to major extent consisting from fibrils of collagen), together with water constitutes for 30% (52). The dentin is formed by two simultaneous processes, formation of both collagenous matrix and mineral crystals in this matrix (201). Neither studies on elemental composition pattern, nor histological studies on morphology in TS enamel or dentin are to our knowledge published.

Amelogenin secreted from ameloblasts decide the enamel volume, which mineralize by calcium phosphate salts replacing the matrix. No previous studies on enamel formation in TS are found.

AIMS

The overall aim of this thesis was to study dentofacial morphology in Turner syndrome versus controls and the influence hereupon from karyotype.

Specific aims were

- to study impact from TS karyotype on craniofacial morphology, palatal height, dental arch morphology as well as dental crown width and to compare with normative reference values from healthy females.

- to study impact from age on craniofacial morphology, palatal height and dental arch morphology in TS.

- to study impact from presence of one or two intact X-chromosomal p-arms on craniofacial morphology and dental crown width in TS.

- to compare histological findings and elemental composition pattern in dental enamel in TS with controls.

PATIENTS AND METHODS

Patients, normative reference data and controls

The patients in these studies are individuals participating in a longitudinal and multidisciplinary ongoing study of females with TS, where the overall aims are to investigate the influence of genetic factors on phenotype, health aspects related to risk factors and the effects of growth promoting treatment. One hundred thirty-two females with a diagnosis of TS living in the regions of Göteborg (n=50), Uppsala (n=50) and Umeå (n=32) in Sweden approved to participate in this study. The collection of patient material lasted from 1998 until 2010. From these 132 patients in study I-III, some individuals were excluded due to missing or poor records, extensive dental restorations, tooth loss or unilateral cleft lip and palate. The included participants were 5-66 years of age (presented in table 2 together with mean age and range as well as in figure 4) and 78% had been treated with GH. Out of the TS females, 31% in

Table 2. TS females included in study I-IV, presenting the distribution of TS karyotype (study I-IV), number of intact X chromosomal p-arms (study I, III), age (study I-III) and previous orthodontic treatment (study II).

TS karyotype		Study I		Study II		Study III		Study IV
		One p-arm (n)	Two p-arms (n)	Non-ortho group (n)	Ortho group (n)	One p-arm (n)	Two p-arms (n)	Total (n)
1. 45,X		40	0	29	13	46	0	2
2. 45,X/46,XX		0	12	9	1	0	12	0
3. Isochromosomes		28	0	19	7	26	0	2
4. Other	Deletion	2	5	3	4	3	5	1
	Translocation	1	2	1	1	1	1	0
	Inversion	3	1	2	2	2	0	1
	Marker	0	5	4	2	0	6	0
	Ring	4	0	4	1	5	0	0
	Y material	4	0	3	0	3	0	0
	45,X/47,XXX	0	1	2	0	0	2	2
Total		108		107		112		8
Mean age, years (range)		18.7 (5-62)	17.5 (5-60)	15.2 (6-50)	22.7 (6-52)	18.2 (6-64)	20-0 (6-66)	

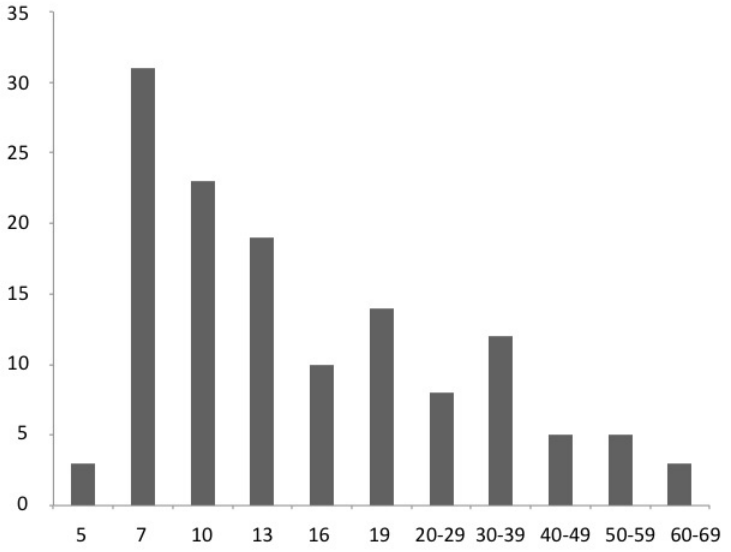


Figure 4. Age distribution among the participating females with TS.

study I and 29% in study II, had a history of orthodontic treatment (TS ortho group).

The TS diagnoses were obtained from the Swedish Genetic Turner Register including TS females diagnosed postnatally from 1967. Chromosomal analysis was performed on peripheral blood lymphocytes in the majority of cases. Before 1995 analysis was undertaken on 10-25 cells, but from 1995 the analyses were performed on ≥30 cells. The TS females were sugrouped into four karyotype categories:

1. monosomy (45,X)
2. mosaic (45,X/46,XX)
3. isochromosomes 46,X,i(X) and (45,X/46,X,i(X)
4. others

Additionally, the TS females were subgrouped according to the presence of

one or two unaffected X chromosomal p-arms (study I, III) and the distribution of karyotypes and number of unaffected p-arms is presented in table 2. The mosaic karyotype 45,X/46,XX was counted as having two unaffected X chromosomal p-arms even though this was not true for all cells lines. Also 45,X/47,XXX were included in that group even if the number of intact p-arms exceeded two in a portion of the cell lines.

As reference for study I, we used published data from the Thilander longitudinal study of healthy Swedish females, between 5 and 31 years of age, with normal occlusion and profile with no history of orthodontic treatment (202). For the variable s-ba, data from Bolton standards (203) were used. For study II and III, published data from the Thilander longitudinal study of healthy Swedish females, 5 to 31 years of age, with the criteria described above, were used (204).

Participants from study I-III, who were in the stage of primary or mixed dentition, were invited to donate exfoliating primary teeth to study IV. The material consisted of 18 exfoliated primary teeth (13 molars, 4 canines and 1 incisor) from 8 TS girls, with the karyotype distribution presented in table 2. Eleven primary teeth (7 molars and 4 canines) from 9 healthy girls were collected as controls from The Public Dental Clinic at Odontologen, Gothenburg. All TS and control teeth were registered in Västra Götaland biobank, ID-nr VGR 830.

Cephalometric analysis

Lateral cephalometric radiographs were taken of the participants. One investigator, who was blinded for the TS karyotype, performed all the cephalometric measurements, using landmarks and lines displayed in figure 5. For linear variables the values were corrected for radiographic enlargement. The cephalometric variables were converted into age and gender-specific standard deviation scores (SDS) using a reference group of healthy females (202), except for the line s-ba where the Bolton standards were used (203). The difference between the actual value from the studied individual (X) and the mean of the age specific population (Mean) was divided by the standard deviation (SD) for the age specific population.

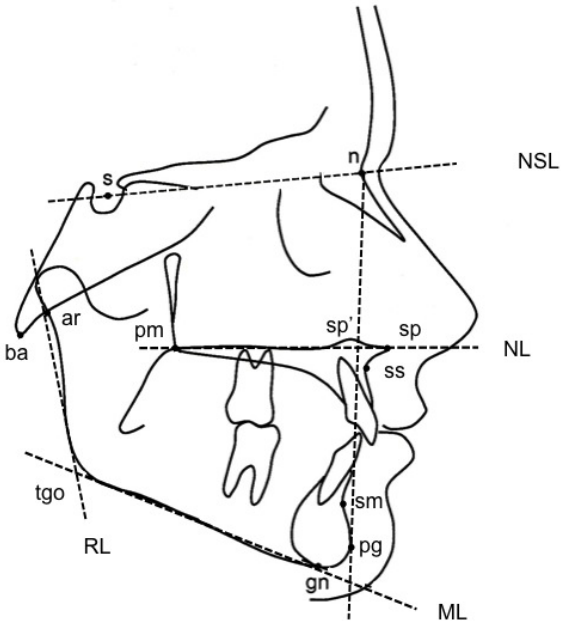


Figure 5. Landmarks and lines used in study I.

$$SDS = \frac{X\text{-}Mean}{SD}$$

SDS thus displays how many SD the actual value differs from the mean of the age specific reference group. An SDS close to zero reflects similarity with the reference group. Calibration to locate the landmarks was made together with one of the investigators in the previously mentioned reference study (202). The inter-individual error, calculated from measurements on 16 randomly chosen radiographs, did not exceed 0.7 degrees for the angular measurements and 0,8 mm for the linear measurements, with exception for the variables n-s-ba and sp´- pm which displayed the inter-individual error of 1.4 degrees and 1.5 mm respectively (205). The intra-individual error for the cephalometric measurements were calculated on repeated measurements of 16 randomly chosen radiographs and did not exceed 0.8 degrees for the angular measurements and 0.3 mm for the linear measurements, except for the variable sp´- pm, which displayed 1.0 mm of intra-individual measurement error (205). From patient files at the orthodontic clinics in Göteborg, Uppsala and Umeå, data about possible history of orthodontic treatment was obtained.

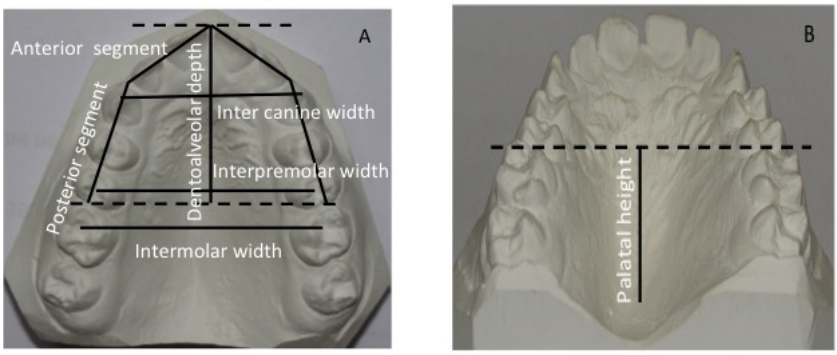


Figure 6. A) Schematic illustration of dentoalveolar arch measurements according to Thilander (204). B) Schematic illustration of palatal height measurement according to Thilander (204).

Cast model analysis

Plaster casts models were made from impressions of the upper and lower dentition from the TS individuals. All measurements were made, using a sharp-edged digital calliper, by one investigator, blinded for the karyotype of the females. The measurements of dental arch width, depth, length of anterior and posterior segments, total circumference (Figure 6A) as well as palatal height (Figure 6B) were performed according to Thilander (204). In case of spacing in the location of the landmarks for the anterior segment or the mesial landmark for the posterior segment the landmarks were set in the midpoint of the approximal diastema. The measurements of dental arch and palatal height were converted into age and gender-specific standard deviation scores (SDS) using a reference group of healthy Swedish females with normal occlusion but no history of orthodontic treatment (204). The measurement of mesio-distal tooth width was assessed according to Moorrees (206) excluding teeth that were partially erupted, restored approximally or damaged by trauma, caries or severe occlusal wear. The mean of the measurements from right and left contralaterals was used for statistical calculation. The intra-individual error of measurement were calculated according to Dahlberg (205) which did not exceed 0.59 mm for dental arch morphology as well as palatal height and 0.10 mm for dental crown width.

Histological and biochemical analyses

Sample preparation

Directly after exfoliation, all teeth were stored in sampling tubes filled with 5% buffered formaldehyde that were enclosed with the participants. Prior to embedding, the teeth were washed several times in 70% ethanol, with a final wash in absolute ethanol. After pre-embedding in methylmethacrylate/ absolute ethanol the teeth were embedded in benzoylperoxid catalyzed methylmetacrylate and undecalcified sections with a thickness of 110 µm were prepared in a Leitz Low Speed Microtome (Leitz, Wetzlar, Germany).

Polarized light and scanning electron microscopy

The most central sections of 18 teeth, from 8 TS individuals were analyzed in an Olympus polarizing microscope (Olympus, Tokyo, Japan) equipped with a Nikon Coolpix 990 digital camera. Morphologic variations in degree of mineralization in enamel were recorded both dry in air and after a 10 minutes imbibition in water to reveal variations in mineral content. Subsurface lesions (SSL) i.e. superficial zones of lower mineralized enamel covered by a thin layer of intact enamel surface, the neonatal line (NNL), enamel irregularities, enamel structure aberrations located superficially excluding SSL or enamel structure aberrations centrally were noted. Scanning electron microscopy (SEM) was performed with a Philips SEM 515 microscope to illustrate the enamel prism pattern.

X-ray microanalysis and rule induction analysis

Sixteen undecalcified central sections of primary teeth from 8 TS females were prepared for X-ray micro analysis (XRMA) for the composition of calcium, phosphorus, oxygen (constituents of hydroxyapatite), and carbon. In addition, 10 sections of primary teeth from 8 healthy females were analyzed. After etching with 30% phosphoric acid and rinsing with de-ionized water the sections were coated with carbon by vapour deposition to avoid build-up of surface charges. Analyses were made in a Philips SEM 515 with an EDAX DX4 ECON-detector (Philips, Eindhoven, Holland). Measurements were made in 6.1x4.3 µm measuring areas, with a depth of 1-1,5 µm, at 10 locations on the surface

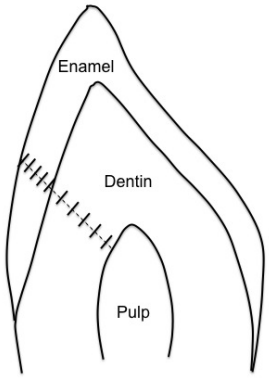


Figure 7. Enamel and dentin locations for measurements with XRMA.

of the section along the enamel and dentin (Figure 7). Additionally, measurements of Ca and P were performed with XRMA in pure hydroxyapatite for calibration. The relative amounts of the four elements were calculated, in addition to the Ca/P ratio.

In order to elucidate possible patterns of element composition in TS an inductive analysis was performed on data from XRMA, using the inductive analysis program XpertRule Analyser® (Attar Software Ltd., Lancashire, UK) (207, 208). The diagnosis of the patient, either TS or control, was used as Outcome. The results are presented as a hierarchic diagram (knowledge tree) in which the importance of every variable (attribute) is specified by its position in the tree (Figure 8). The higher position an attribute has in the tree, the more important for the outcome. The knowledge tree is generated by repeatedly splitting data until terminal points (leaves) are reached. The inductive analysis was carried out in all ten locations in enamel and in dentin.

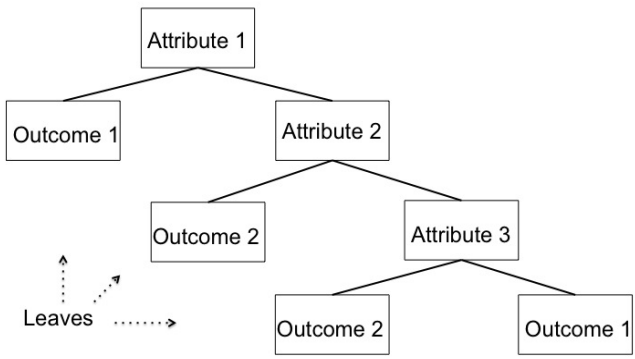


Figure 8. Schematic illustration of a knowledge tree generated from rule induction analysis, with attributes, outcome and leaves. The higher position an attribute has in the knowledge tree the more important it is for reaching a correct classification of the outcome. In this knowledge tree attribute 1 has a more important role than attribute 3 to reach the correct outcome.

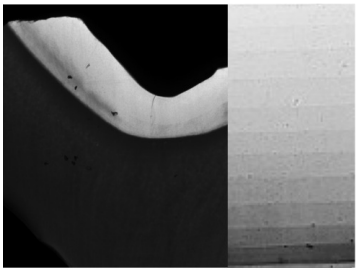


Figure 9. Example of microrddiography of primary tooth together with aluminium step wedge for gray-scale reference.

Microradiography

Contact microradiographs of 6 undecalcified sections from 6 TS individuals, together with 6 sections from 6 controls, were made on AGHD high definition photoplates (HTA Enterprises, San Jose, CA, USA) with an X-ray tube set to low voltage (20 kV). The time of exposure was 45 minutes. For calibration a reference aluminum step wedge was included (Figure 9). The micrographs were photographed in an Olympus photo microscope equipped with a Leica DFC420C digital camera. Images were taken of the cervical and occlusal enamel, as well as of dentin. The mineral density in the sections was determined by comparisons with an aluminum wedge gray-scale step.

Statistical methods

- *Descriptive statistics* were calculated and presented as mean values, standard deviation and range.

- *One sample t-test* was used to test for differences between SDS for the cephalometric, palatal height, dental arch and dental crown width measurements of the entire TS group as well as for each karyotype separately versus the reference groups of healthy females.

- *Analysis of covariance (ANCOVA)* was used to test for impact from age, karyotype and number of intact X chromosomal p-arms on the cephalometric variables converted into age specific SDS. Additionally, ANCOVA was used analysing impact from karyotype and age on dental arch morphology and palatal height converted into age specific SDS.

- *Analysis of variance (ANOVA)* was used to analyse possible differences in dental crown width both between the four karyotype groups and between the groups with one or two intact chromosomal p-arms. Additionally, ANOVA

was used testing for differences between TS and controls for the XRMA.

- *Student-Newman-Keuls post hoc test (SNK)* was used to indicate which groups were divergent in both the ANOVA and ANCOVA tests above mentioned.

- *Student's t-test* was used to test for significant differences between individuals with or without previous orthodontic treatment for the cephalometric analysis in addition to testing for differences between morphological parameters found in polarization light microscopy, comparing TS and controls.

- *Wilcoxons signed rank test* was used for the analysis of the grey scale values between TS and controls in the microradiography.

P-values less than 0.05 were considered as statistically significant.

Ethical considerations

The studies I-III were approved by the University of Gothenburg ethics committee, Dnr R 595-98, as well as from the ethics committees of the Universities in Umeå and Uppsala. Study IV was approved by the University of Gothenburg ethics committee, Dnr 210-08. The children in the studies were referred from respective paediatric endocrinologist after the diagnosis of TS and examined according to the National Turner syndrome care program. The parents and children received written information about the project and were given the choice to participate or not. The adult women received a letter with information about the study and an offer to voluntarily participate. The exfoliated teeth in study IV were donated to the project of free will, a written consent was received and the teeth were registered in Västra Götaland biobank, ID-nr VGR 830. The studies in this thesis contribute to the knowledge of how the genetic background of TS influences dentofacial morphology, which is of benefit for the entire community of females with the diagnosis of TS.

RESULTS

Craniofacial morphology (study I)

The TS ortho group were unitized with the TS non-ortho group analysing the cephalometric variables, since no statistically significant differences between these two groups were found. The comparison versus controls displayed that TS females had a more obtuse cranial base angle, a shorter posterior portion and a longer anterior portion of the cranial base (Table 3A). Both the maxilla and mandible were retrognathic and exhibited more posterior inclination in TS. Additionally, both the maxilla and mandible in TS females were shorter while ramus instead was longer. Both the ratios upper/total anterior facial height and anterior/posterior facial height were increased in TS.

Age had a negative correlation with anterior cranial base length as well as mandibular length and retrognathism and a positive correlation with the skeletal sagittal relation (Table 4). This means that with increased age the relative length of the anterior cranial base and mandible decrease versus controls while the mandibular retrognathism and sagittal skeletal relation discrepancy increase.

Impact from karyotype was found only on the mandibular retrognathism, but Student–Newman–Keuls post hoc test could not detect between which karyotypes the differences were found (Table 4). However, the most divergent group concerning mandibular retrognathism was 45,X while 45,X/46,XX differed the least versus controls (Table 4). Comparing group by group versus controls displayed that the 45,X/46,XX group had fewer statistically significant differences from controls compared to both isochromosomes and 45,X karyotype (Table 3A). Impact was also found from the number of X chromosomes with intact p-arms on both maxillary and mandibular length (sp'-pm, ar-pg) and from age on intermaxillary relation, anterior cranial base length and mandibular prognathism and length (ss-n-sm, s-n, s-n-sm, s-n-pg, tgo-gn and ar-pg) (Table 4).

Table 3a. Results from comparisons of craniofacial morphology, palatal height and dental arch dimensions in the entire TS group as well as the karyotypes versus controls in study I-III (202-204).

		All TS	45,X	45,X/46,XX	Iso	Other
Craniofacial morphology						
	s-n-ss	-2.14 ***	-2.30 ***	-1.41 **	-2.33 ***	-2.03 ***
	s-n-sm	-2.37 ***	-2.87 ***	-1.76 **	-2.16 ***	-2.12 ***
	s-n-pg	-2.45 ***	-2.91 ***	-2.12 **	-2.12 ***	-2.25 ***
	ss-n-sm	-0.02	0.52	0.10	-0.58	-0.29
	n-s-ba	1.13 ***	1.14 ***	0.89 *	0.99 ***	1.34 **
	n-s-ar	0.91 ***	0.90 ***	0.51	0.87 ***	1.12 **
	s-n	0.82 ***	1.10 ***	0.03	0.79 *	0.80 **
	s-ar	-0.61 ***	-0.45 *	-0.87	-0.77 *	-0.57
	s-ba	-1.65 ***	-1.54 ***	-1.80 *	-1.45 ***	-1.93 ***
	NSL/NL	1.57 ***	1.66 ***	1.55 **	1.59 ***	1.43 ***
	NSL/ML	1.17 ***	1.01 ***	1.58 **	1.14 **	1.25 ***
	NL/ML	0.18	-0.12	0.67	0.23	0.34
	ML/RL	0.08	-0.28	0.51	0.27	0.24
	n-sp'/n-gn	0.25 *	0.36 *	0.15	0.37	0.03
	sp'-gn/n-gn	-0.02	-0.13	0.04	-0.14	0.20
	sp'-pm	-0.71 ***	-0.74 ***	-1.18 *	-0.48	-0.70
	n-gn/s-tgo	2.17 ***	1.66 ***	2.93 **	2.05 **	2.68 ***
	ar-tgo	0.51 ***	0.75 ***	-0.04	0.66 *	0.28
	tgo-gn	-2.05 ***	-2.28 ***	-2.06 *	-1.91 ***	-1.88 ***
	ar-pg	-0.59 ***	-0.92 ***	-0.71	-0.30	-0.37
Palatal height and dental arch morphology						
Maxilla	Palatal height	-0.15	0.32	-0.59	-0.62	-0.14
	Arch depth	2.06 ***	2.30 ***	1.71 *	1.77 ***	2.15 ***
	Inter M_1 width	-1.03 ***	-0.87 *	-0.27	-1.44 **	-1.25 **
	Inter P_2 width	-0.5 **	-0.56	-0.01	-1.14 **	-0.24
	Inter C width	-0.05	-0.03	0.46	-0.66	0.13
	Ant segment	-0.01	0.09	-0.29	-0.30	0.28
	Post segment	-0.40 *	-0.48	0.61	-0.88 *	-0.29
	Circumference	-0.29	-0.33	0.40	-0.81	-0.04
Mandible	Arch depth	1.05 ***	0.96 *	0.51	0.68	1.79 ***
	Inter M_1 width	0.71 ***	1.01 **	0.82	0.26	0.65
	Inter P_2 width	0.58 ***	0.82 **	0.33	0.38	0.56
	Inter C width	0.03	0.24	-0.02	-0.52 *	0.32
	Ant segment	-0.25	-0.24	-0.32	-0.70 *	0.23
	Post segment	-0.41 **	-0.44 *	-0.15	-0.68 *	-0.20
	Circumference	-0.48 **	-0.52 *	-0.18	-0.96 *	-0.08

Table 3b. Results from comparisons of dental crown width in the entire TS group as well as the karyotypes versus healthy females (204) in study III.
Central (I1), lateral (I2), canine (C), 1st premolar (P1), 2nd premolar (P2), 1st molar (M1), 2nd molar (M2).

		Tooth width				
Maxilla	I1	8.0***	8.0***	8.3	7.8***	8.1***
	I2	6.2***	6.3**	6.5	5.9***	6.3*
	C	7.4***	7.4*	7.5	7.2***	7.4
	P1	6.4***	6.5***	6.5***	6.3***	6.5***
	P2	6.1***	6.1***	6.3**	5.9***	6.1***
	M1	9.4***	9.4***	9.6***	9.2***	9.4***
	M2	8.9***	8.9***	9.0*	8.8***	8.8***
Mandible	I1	4.9***	4.9***	5.0***	4.8***	5.0***
	I2	5.5***	5.5***	5.6**	5.3***	5.5***
	C	6.3***	6.4**	6.4	6.1***	6.4***
	P1	6.7***	6.7***	6.7*	6.5***	6.9
	P2	6.6***	6.6***	6.6***	6.5***	6.7***
	M1	9.8***	9.8***	9.9***	9.6***	9.9***
	M2	9.5***	9.6**	9.9	9.3**	9.4***
	Ns variables (n)	10	12	29	14	23

Palatal height and dental arch morphology (study II)

The TS females (excluding the ortho group) exhibited an decreased maxillary but increased mandibular transversal width, except for at the canine level and the dentoalveolar depths were increased for TS in both the maxilla and the mandible compared to controls (Table 3A). The posterior segments were shorter in both jaws, while only the mandibular circumference was decreased in TS compared to healthy females (Table 3A). No difference was found in palatal height comparing TS versus healthy females. In the comparison of each karyotype separately versus controls (ortho group excluded) the 45,X/46,XX karyotype displayed fewer variables with statistically significant differences from healthy females, than the other karyotypes (Table 3A). Impact from karyotype could not be proven on any of the dentoalveolar arch or palatal height variables while age had an impact on nine of the variables (Table 4). From these variables only the mandibular intermolar width was deteriorating more versus controls among older TS individuals (Table 4). The TS ortho group

Table 4. Cephalometric and dental arch variables converted into age specific SDS, showing statistically significant impact from age, karyotype or number of p-arms (only for cephalometric variables). A positive regression coefficient indicates an increased value of the studied variable with increased age and conversely, a negative regression coefficient indicates a decreased value of the variable with increased age. The dentoalveolar depth (arch depth) was measured at the level of the second premolar (P_2), the intermolar width at the level of the first molar (M_1) and the interpremolar width at the level of the second premolar (P_2).

Variable		ANCOVA	Impacting variable	Regression coefficient	SDS (mean)	
Craniofacial morphology						
s-n-sm		0.006	Age	−0.033	−2.37	
		0.041	Karyotype		45,X/46XX	−1.76
					Others	−2.12
					Iso	−2.16
					45,X	−2.87
s-n-pg		0.001	Age	−0.042	−2.45	
ss-n-sm		0.002	Age	0.037	−0.02	
s-n		0.002	Age	−0.031	0.82	
sp'-pm		0.028	p-arm		1 p-arm	−0.51
					2 p-arms	−1.32
tgo-gn		0.001	Age	−0.040	−2.05	
ar-pg		<0.000	Age	−0.049	−0.59	
		0.048	p-arm		1 p-arm	−0.54
					2 p-arms	−0.74
Dentofacial morphology						
Mx	Arch depth	<0.001	Age	-0.070	2.060	
	Inter M_1 width	<0.001	Age	0.070	-1.032	
	Inter P_2 width	0.005	Age	0.047	-0.562	
	Post segment	<0.001	Age	0.050	-0.403	
	Circumference	0.023	Age	0.034	-0.289	
Md	Arch depth	0.003	Age	-0.064	0.581	
	Inter M_1 width	0.006	Age	0.046	0.706	
	Post segment	<0.001	Age	0.048	-0.406	
	Circumference	0.011	Age	0.036	-0.481	

was found to have statistically significant smaller maxillary and mandibular dentoalveolar depths (p=0.001 and p=0.026 respectively) compared to the non ortho group while the remaining tested variables showed no differences between these two groups. The karyotype distribution was equally distributed between the ortho and the non ortho groups.

Dental crown width (study III)

All permanent teeth together with primary canines and molars proved to

Table 5. Mesio-distal crown width of primary teeth in TS (study III). Asterisks indicate level of statistical significance of comparison with female reference data (204). No statistical analysis was performed on primary incisors due to low number.
Primary central (i_1), lateral (i_2), canine (c), 1st molar (m_1), 2nd molar (m_2).

	Primary tooth	Number	Mean (mm)	SD
Maxilla	i_1	2	5.7	0.48
	i_2	3	4.8	0.55
	c	28	6.4***	0.43
	m_1	30	6.6***	0.40
	m_2	33	8.3***	0.64
Mandible	i_1	0		
	i_2	1	4.3	
	c	23	5.4**	0.35
	m_1	28	7.0***	0.45
	m_2	32	9.0***	0.55

have a statistically significant smaller mesio-distal crown width (Tables 3B and 5). Comparisons of dental crown width between the karyotypes showed statistically significant differences for maxillary laterals, canines, 2nd premolars, mandibular canines as well as 1st premolars, and Newman-Keuls post hoc test revealed that the isochromosome karyotype had the most reduced dental crown width for these 5 teeth (Table 6). Among females with 45,X/46,XX mosaicism, the maxillary lateral, canine and 2nd premolar dental crown width were statistically significant wider (Table 6) and in the comparison of each karyotype group versus controls 45,X/46,XX displayed fewer differences from controls compared to the other groups. No significant differences in dental crown width were found between the groups with either one or two intact X chromosomal p-arms. Testing these two groups one by one versus healthy females, all teeth in both groups with one or two intact p-arms were significantly smaller than controls, except for mandibular second molars in the group with two intact p-arms that showed no difference.

Histological and biochemical analyses (study IV)

Polarized light microscopy and scanning electron microscopy

All mineralization features studied were found in both TS and controls but with different frequencies. Subsurface lesions (SSL), seen as a positively birefringent zone under a normal mineralized surface (Figure 10A), together

Table 6. Comparison of mesio-distal crown width between the four karyotypes: 1. monosomy (45,X), 2. mosaic (45,X/46,XX), 3. isochromosome and 4. other with ANOVA (study III). Student-Newman-Keuls post hoc test indicating which karyotype groups were divergent. Remaining permanent teeth showed no significant differences between the karyotype groups. I_2=lateral, C=canine, P_1=first premolar, P_2=second premolar

	Permanent tooth	ANOVA (p)	Newman-Keuls
Maxilla	I_2	0.02	Isochromosomes < 45,X/46,XX
	C	0.04	Isochromosomes< 45,X/46,XX
	P_2	0.03	Isochromosomes < 45,X/46,XX
Mandible	C	0.01	Isochromosomes < remaining groups
	P_1	<0.01	Isochromosomes < other

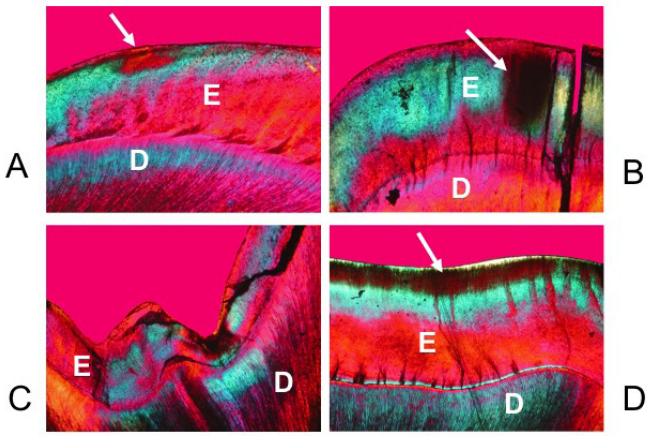


Figure 10. Examples of features seen utilizing polarized light microscopy in TS. (A) SSL, i.e. zone of lower mineralized enamel covered by an intact enamel surface. (B) Mineralization aberration located centrally, i.e. a zone of poor refraction of light located in the central parts of enamel. (C) Areas with irregular enamel prism pattern. (D) Superficial mineralization aberration, i.e. a zone of poor refraction of light located in the superficial part of enamel.

Table 7. Findings from polarization light microscopy. No difference in occurrence for remaining studied features were found.

POLMI findings in enamel	TS (n=8) %	Controls (n=9) %	p
Subsurface lesion	62	22	< 0,05
Central mineralization aberration	50	22	< 0,05
Irregular enamel rods	75	56	< 0,05

with regions with positive birefringence located centrally in the enamel (Figure 10B) and areas with irregular enamel prism pattern (Figure 10C), occurred more commonly in TS compared to controls (Table 7). Superficial aberrations of the enamel (Figure 10D) were seen in both TS and healthy girls, however no difference in frequency was found.

Comparing to enamel from healthy females (Figure 11A) in scanning electron microscope, TS enamel appeared to have an irregular character with rods of varying sizes, deviating in several directions (Figure 11B). TS enamel appeared to have more rod free regions or areas with sparsely arranged rods both along the enamel-dentin border as well as superficially.

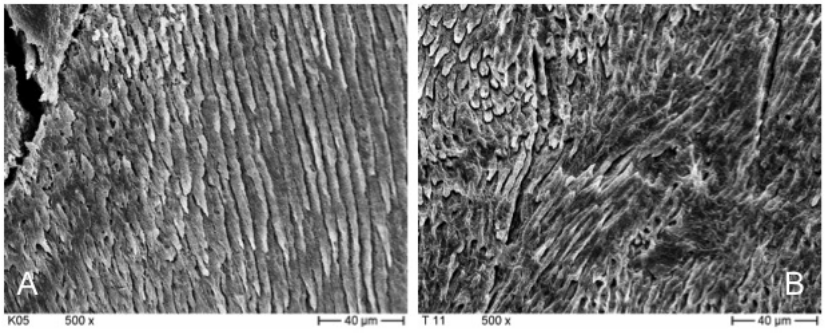


Figure 11. Example of scanning electron features in TS enamel. (A) Enamel from a healthy female showing regular prism pattern (500x). (B) Irregular enamel pattern. Rods of varying sizes deviate in several directions (500x).

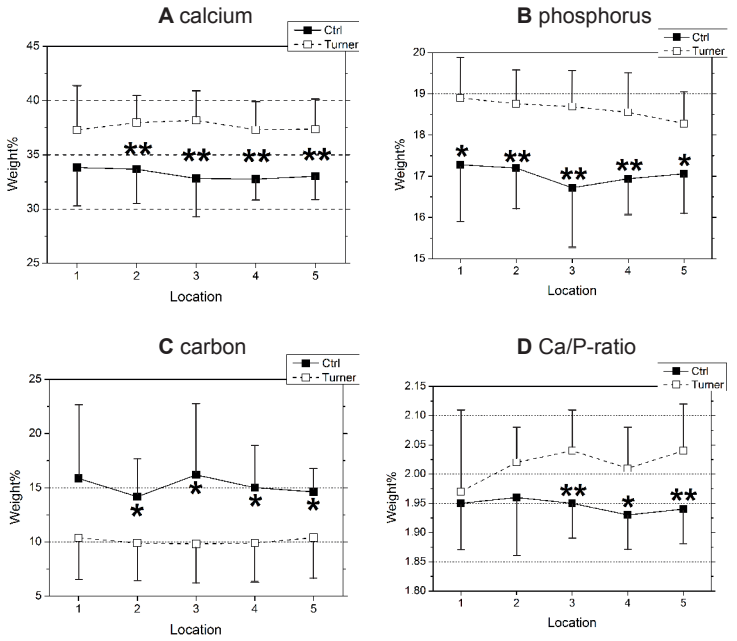


Figure 12. Elemental composition of TS and control enamel measured by XRMA. The levels of (A) calcium, (B) phosphorus, (C) carbon and (D) Ca/P ratio in the five enamel measurement locations. (* $p<0.05$ ** $p<0.01$ *** $p< 0.001$)

X-Ray Micro Analysis

In TS primary teeth enamel the levels of calcium (Ca) and phosphorus (P) were higher than in controls (Figs 12A, B). Except for the enamel surface, the level of Ca was significantly higher in all measured locations. No differences were noted in the levels of oxygen in TS compared to controls. The levels of carbon were significantly lower in TS than in controls, except for the enamel surface location (Figs. 12C). From the surface of TS enamel towards the dentin interface the Ca/P ratio increased gradually and exceeded the level of controls in the central and inner parts of the enamel (Fig. 12D). In TS dentin the levels of calcium and phosphorus were significantly higher in the dentin mid part. The levels of oxygen in TS dentin did not differ from controls, while the levels of carbon were significantly lowered in all locations. In comparison to controls, no differences were observed in TS dentin Ca/P levels.

The attribute carbon (weight % from XRMA) was found in the top position of the “upside down” knowledge tree in the rule induction analysis of enamel,

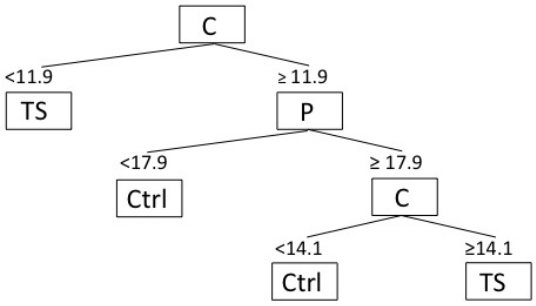


Figure 13. Example of knowledge tree, from location 3 in enamel, interpreted with sentences of: ”if”, ”and”, ”then”:
-If carbon <11.9, then the diagnosis is TS
-If carbon is ≥14.1 and phosphorus ≥17.9, then the diagnosis is TS
-If carbon is ≥11.9 and phosphorus <17.9, then the diagnosis is control
-If carbon is ≥11.9 but <14.1 and phosphorus ≥17.9, then the diagnosis is control

except for two middle locations in the enamel where calcium was located in the highest position in the knowledge tree. An example of a knowledge tree from location 3 in enamel is displayed in figure 13.

Microradiography

Mineral density in undemineralized tooth sections was estimated from microradiographs. The value in TS enamel was significantly lower in comparison to controls (mean 4.0 and 7.0 respectively, p=0.016). Most aberrations in mineral density were found in occlusal enamel. No differences were seen in dentin.

DISCUSSION

Three principle findings

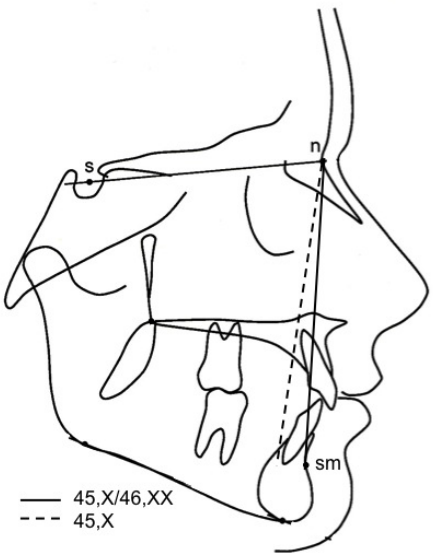


Figure 14. Schematic illustration of the mandibular retrognathism (s-n-sm) in 13 year old 45,X and 45,X/46,XX TS karyotypes.

The three principle findings from this thesis were: 1) the 45,X/46,XX mosaicism mitigated the dentofacial aberrations; 2) the isochromosome karyotype distinguished by having the smallest crown width; 3) the palatal height was equal in TS and healthy females. The studies I-III included in this thesis are, to our knowledge, the first published on dental crown width (measured on cast models), craniofacial and dentoalveolar arch morphology as well as palatal height comparing the karyotypes 45,X, 45,X/46,XX and isochromosomes in three separate groups. Moreover, no previous studies on how karyotype influence dental arch dimensions or palatal height, assessed from cast models, are found. For the first time histology and elemental composition in TS dental hard tissue is studied.

45,X/46,XX mosaicism mitigates the dentofacial aberrations

The 45,X/46,XX karyotype has previously been described as displaying a less aberrant phenotype in several medical aspects (25-27) why a less affected dentofacial phenotype was expected. For the craniofacial morphology, indications were seen for a mitigation of the mandibular retrognathism in 45,X/46,XX (Figure 14) and a lower number of variables deviating from controls were revealed in 45,X/46,XX in comparison with 45,X. Previous results are divergent, with findings of either increased maxillary retrognathism in 45,X compared to other karyotypes or no differences at all comparing craniofacial morphology between different TS karyotypes (Table 1). However,

except for the Dutch study, (the only one distinguishing the 45,X/46,XX group), the cephalometric comparisons were made between 45,X and a group of mixed karyotypes (143). Still, no difference were found between 45,X and 45,X/46,XX, possibly depending on a limited sample of only 16 individuals (143).

The 45,X/46,XX karyotype was the least divergent from controls, not only for craniofacial morphology, but also for the dentoalveolar arch measurements since only one variable was differing. However, comparing the four separate karyotype groups no differences were revealed. No previous reports are found on impact from karyotype on dentoalveolar arch morphology, but studies on distribution of malocclusions between different karyotypes are in line with our results, as the 45,X/46,XX karyotype was found being less affected by malocclusions than 45,X (171).

Females with 45,X/46,XX karyotype also displayed less aberrant dental crown width than isochromosomes for maxillary laterals, canines and second premolars, especially in comparison with the isochromosome karyotype. Additionally, the mosaic group had a higher number of variables that were similar to healthy females in comparison to the other karyotypes. Indications pointing in the same direction are emphasised by Varrela et al. (28) who found 45,X/46,XX having broader mandibular second molars compared with 45,X.

45,X/46,XX displayed a milder mandibular retrognathism, fewer deviating dentoalveolar arch variables and wider dental crowns.

Isochromosomes distinguish with smallest crown width

TS isochromosomes are reported to have a more aberrant phenotype in some general respects i.e. birth size, GH deficiency, bone age delay, hypothyreosis and hearing loss (94, 208-210). Our karyotype comparison revealed that isochromosomes distinguished as having the smallest dental crown width for

Table 8. Variables displaying impact from karyotype. The karyotype comparison revealed that s-n-sm was lower in 45,X (more retrognathic) than 45,X/46,XX, while isochromosomes (iso) were situated in between. No dentoalveolar arch variables with impact from karyotype were found. Maxillary laterals (I_2), canines (C), 2nd premolars (P_2), mandibular canines (C) and 1st premolars (P_1) displayed smaller dental crown width in iso compared with 45,X/46,XX or other karyotypes.

The 45,X/46,XX karyotype displayed a higher number of non significantly different variables from controls for all dentofacial variables while 45,X together with iso displayed a lower number, except for the craniofacial morphology variables were iso were situated in between.

Variable		Karyotype comparison	Number of ns variables vs controls
Craniofacial morphology	s-n-sm	45,X < Iso < 45,X/46,XX	45,X < Iso < 45,X/46,XX
Dental arch morphology		=	Iso < 45,X < 45,X/46,XX
Dental crown width	Maxillary I_2 C P_2	Iso < 45,X/46,XX	Iso = 45,X < 45,X/46,XX
	Mandibular C	Iso < all	
	Mandibular P_1	Iso < other	

five dental crown measurements (maxillary lateral, canine, second premolar, mandibular canine and first premolar). Moreover, together with 45,X, the isochromosome karyotype displayed a higher number of dental crown width variables differing from controls than 45,X/46,XX. Studies on dental crown width, subdividing isochromosomes, as one separate group are rare, but indications in line with our findings have been presented earlier (178). Additionally, together with 45,X the isochromosomes displayed a higher number of statistically significant different variables from controls for the dentoalveolar arch morphology and were situated between 45,X and 45,X/46,XX for the craniofacial morphology (Table 8).

Isochromosomes had smaller dental crown width.

The high arched palate - an illusive illusion

The presence of “high arched palate” in TS is a widely spread truth, why our result of equal palatal height in TS and healthy females was surprising. However, the majority of all studies being the foundation for this verity are

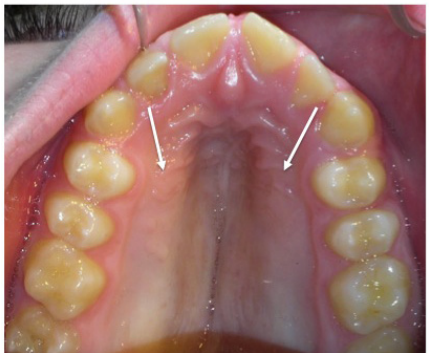


Figure 15. Clinical example of lateral palatal ridges

based on visual assessment (27, 44, 131, 161-167). We were able to measure the palatal height on cast models and in spite of previous reports on high palatal vault being a cardinal sign for TS, we found no significant differences between TS and controls at the molar level. Our finding confirm the results by Laine et al. (159) who assessed the palatal height from cast model analysis and found no difference in palatal height, except for in the canine area. Our interpretation is that the narrower maxillary arch together with the presence of persisting lateral palatal ridges (Figure 15), gives a false illusion of a higher palatal vault (157, 159, 168). The increased distance between the tongue and the palatal plane, indicating a low position of the tongue, is discussed to be of importance for development of the atypical palatal morphology seen in TS (168). Normally, the palatal ridges appear prenatally but are gradually smoothed out by the moulding forces from the tongue (211). There are suggestions of that the dysfunctional tongue position in TS may result from neuromotor dysfunction of facial muscles (168). Also marked hypotonia of cheeks and lips, dysfunctional tongue movements and poor chewing skills are reported in TS, which might support that suggestion (212). Major problems in motor performance in TS are also reported, together with lower movement speed (213). No indications of an abnormal size of the tongue in TS are found (214).

The palatal height was equal in TS and healthy females.

Aberrant dentofacial morphology in TS

A flattened cranial base and small, retrognathic jaws

The cranial base flexion is unique for human beings and is suggested to be a

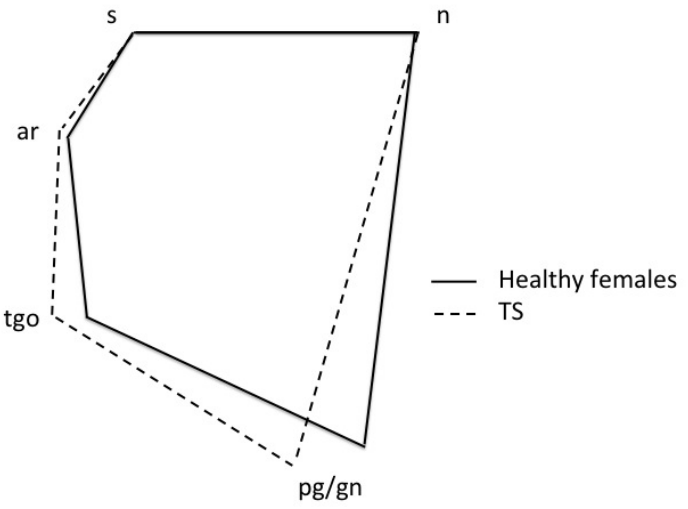


Figure 16. Facial polygon, a schematic figure made from cephalometric measurements of TS individuals ≥19 years of age and normative cephalometric data from healthy females 19 years of age (202).

result from an adaptation to an evolutionary gradual increase of brain volume (215). Our results confirm a flattened cranial base and a shortened posterior cranial base in TS versus the reference group are in line with previously reported results (38, 140, 141, 146-149). We also found the somewhat surprising result of a longer anterior part of the cranial base in TS than in controls (Table 3A). However, with one exception the previously mentioned studies report the anterior cranial base as being longer or of the same size as controls. Both the maxilla and the mandible were found to be retrognathic and posteriorly inclined in the TS group. As long ago suggested by Björk, the increased cranial base angle is associated with a more retrognathic face (216). The mandibular retrognathism and decreased length are the underlying measures for one of the significant clinical features in TS, the mandibular micrognathia (27) and a schematic figure of the "facial polygon" is displayed in figure 16.

TS displayed flattened cranial base and small, retrognathic jaws with posterior inclination.

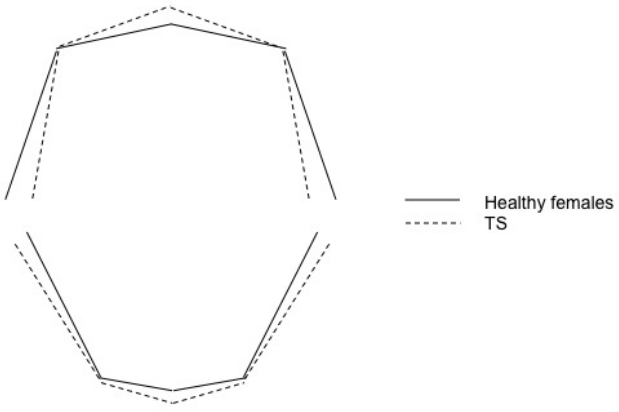


Figure 17. Schematic illustration of dentoalveolar morphology in TS and healthy females

Narrow maxilla and broad mandible

Our results for dental arch morphology are essentially in accordance with previous publications for the transversal measures, with a narrow maxillary arch but a broader mandibular arch in TS compared to controls (Table 3A and Figure 17). However, we found both the maxillary and mandibular arch depths (also named arch lengths) being increased while others have contradicting result, mostly a shortened mandibular arch depth (164, 169). The choice of individuals for control, different landmarks, and karyotype distribution as well as inclusion of both growing and adult individuals without age matching, might of course have affected the results. To our knowledge, the length of the dental arch segments and the circumference was not previously studied in TS. We found the posterior segments being shorter both in the maxilla and mandible together with the mandibular circumference, which we assume to be caused by a mesial migration of posterior teeth due to the decreased tooth width.

It is feasible to assume that the mentioned lowered tongue position in TS, have an effect not only on the palatal morphology but also on the dentoalveolar morphology with a development of the typical narrow maxilla and broad mandible (168). The disturbed equilibrium between pressure from cheeks and tongue increases the relative pressure from the cheeks on the maxillary arch, which is narrowing, while the increased thrust from the tongue on the

mandibular arch is causing a widening (168). The narrow maxilla, the broad mandible and the increased maxillary arch depth found in the present study goes hand in hand with the reports of increased frequency of distal molar relation, large overjet and lateral crossbite (164, 170-172). The increase of the mandibular dentoalveolar depth was lower than the increase in the maxilla, which does not contradict the reports of large overjet (Table 3A).

The TS maxillary dentoalveolar arch was narrower while the mandibular was broader.
Both jaws displayed an increased arch depth vs controls.

Small dental crown width

Our results with a statistically significant smaller mesio-distal dental crown width in all TS permanent teeth were expected, since the earlier published findings are univocal (28, 161, 164, 166, 177-180). Also all primary teeth in TS (canines and molars) that were compared statistically with teeth from healthy females were found to be smaller. Dental crown width of primary teeth is less previously studied but other authors only report a portion of the primary molars being statistically smaller in TS compared with controls (176, 177).

TS had smaller dental crown width vs controls both for permanent and primary teeth

Different age effects in TS: normalisation vs deviation

Several of the mandibular cephalometric variables were deteriorating in an age dependent manner. The mandibular retrognathism became more pronounced and the mandible was shorter relatively to controls, which also

was associated with an enlarged sagittal intermaxillary discrepancy with an increasing age. Only the anterior cranial base length seemed to become more normalized with age versus controls. The deterioration of growth after puberty is in line with several other syndromes and conditions as cleft lip and palate or craniosynostosis syndromes where the craniofacial growth impairment is becoming more manifest after the pubertal growth spurt. The present study included females up to around 60 years of age. The oldest participants consisted likely from a higher portion of females with no previous GH treatment, since this was added to the treatment regimen in the eighties in Sweden and thereby might have had less favourable effect on the mandible. All variables with an impact from age, except for the length of anterior scull base, illustrate that older TS females in our sample seem to have a more discrepant mandibular morphology and retrognathism than younger females in the same sample. If this is an effect from lack of GH treatment among the older females or only that the growth deficiency became more evident after the pubertal growth spurt is not possible to answer with the data we to date have on GH treatment within the present sample.

The dental arch morphology presented an opposite situation where several of the variables were affected by age but only the mandibular intermolar width exhibited an obvious worsening by age relatively to controls. It seems as the local factors affecting dental arch morphology as tongue position or tooth migration are not impairing the dental arch morphology by age. The dental arch depths might have normalised in older TS females due to mesial migration of posterior teeth.

Several craniofacial variables deteriorated with age, while some dentoalveolar arch variables seemed to normalize.

Aberrant histology and biochemical composition in TS enamel

For the first time the clinical reports of enamel defects have been confirmed with histological techniques, by the findings of an aberrant histological morphology and elemental composition in dental hard tissue in TS (161, 180, 194). No previous studies on this topic are to date found, why our result can not be compared with results from others. However, studying syndromes and conditions with a different aetiology than TS, such as Down's syndrome or amelogenesis imperfecta, qualitative and quantitative hard tissue aberrations have been found (217-218). In Down's syndrome, where as in TS a prolonged cell cycle in fibroblasts is documented, enamel aberrations as less mineralised and smaller amount of enamel were found compared to controls (217). In teeth from children with X-linked amelogenesis imperfecta, an increased level of carbon together with obscured prisms and crystals were reported (218).

Morphological divergences, as altered enamel rod and crystal directions, were seen by means of polarized light microscopy and scanning electron microscopy. The appearing dark areas revealed in POLMI are seen due to occurrence of fewer enamel prisms that are refracting light, being overrepresented in TS. Since a lower content of the building block of enamel prisms (the inorganic hydroxyapatite), was found in these areas, consequently a higher proportion of the organic component, consisting from matrix proteins, is assumed i.e. an incomplete enamel mineralization. Our findings of SSL, irregular enamel rods and central mineralization aberrations being overrepresented in TS, reflect disturbed formation of the enamel inorganic phase possibly due to an imperfect or insufficient amelogenin matrix formation during amelogenesis. Interestingly, all morphological aberrations were found in enamel only, while dentin appeared normal from the POLMI images.

An incomplete mineralization was found also generally in the TS enamel, which was revealed by the results from the microradiography analysis. The mineralization starts after secretion of the organic matrix until full enamel thickness. The mineral content increases gradually, by removal of water and

proteins from enamel matrix by smooth-ended ameloblasts and active transport of calcium and phosphate into the matrix by ruffle-ended ameloblasts (52). A decreased expression of amelogenin, causing imperfect enamel matrix and consequently a decreased amount of hydroxyapatite, may explain the lower level of mineral density found in TS enamel, as revealed by microradiography. In a defective enamel matrix precursors of hydroxyapatite with a lower calcium content, as octacalcium phosphate and amorphous calcium phosphate, are deposited, which together with the fully mineralized hydroxyapatite gives a lower calcium content.

The XRMA displayed a higher weight % of calcium and phosphorus, with consequently lower levels of carbon in all enamel locations. Low percentage of carbon may in part reflect low levels of organic matrix, in the case of enamel formation, a matrix dominated in volume by amelogenin. A lower AMELX expression gives a decreased enamel matrix volume, and possibly an aberrant enamel quality. The lower amount of amelogenin is reflected by a lower relative content of the organic marker carbon. The results from XRMA only give indications for the relative elemental content and not the absolute levels. If the weight % of carbon is decreasing, consequently the weight % of calcium and phosphorus will increase, which partially might explain the high figures of the inorganic components calcium and phosphorus.

The importance of carbon for TS enamel, both higher and lower levels, is demonstrated by the rule induction analysis, a technique used to find patterns in complex amounts of data. With this analysis the attribute with the highest importance for distinguishing the outcome (in study IV TS or control) is revealed. In almost all measurement locations in enamel and dentin, carbon was found in the highest positions demonstrating a high impact of carbon as a key attribute, carbon as an organic matrix marker.

TS enamel displayed higher frequencies of SSL, irregular prism pattern, lower mineral density and aberrant elemental composition.

Hypotheses regarding underlying mechanisms

Craniofacial morphology

The aberrations of the cranial base, being the fundament for the craniofacial morphology, are presumably caused by several mechanisms and the role of specific genes, the cell cycle delay hypothesis, developmental time windows and lymphedemas will be discussed. The craniofacial skeleton is formed by the foetal chondrocranium, acting as a template for the later bony cranium. The cranial base is formed from this cartilage model, and is transformed into bone by endochondral ossification. Moreover, the remaining bands of cartilage in the cranial base, the synchondroses, act as growth sites between the ossifying centers. An association between the disordered growth of cartilage and the abnormal shape of the cranial base in TS is suggested and consequently also an influence on the craniofacial growth (55, 141, 146). Both the genes BGN and SHOX are discussed being involved in the aberrations of the craniofacial growth seen in TS, since they are suggested to escape inactivation and SHOX additionally seems to be involved in skeletal deformities in other parts of the body in TS.

Craniofacial growth is a complex mechanism, and if the prolonged cell cycle reported for fibroblasts is applicable also on osteoblasts or chondroblasts, the consequently slower growth rate might be an underlying reason for both the smaller dimension seen in the posterior portion of the cranial base and the shorter maxilla and mandible. Several measures for the dental arch parameters were decreased as well but local factors as tongue position and tooth migration has to be taken into account evaluating these variables. The flattened cranial base angle in TS might also reflect the delayed timing for a developmental window, which is suggested in the delayed cell cycle theory to cause irreversible growth deformities (Figure 18). The human craniofacial morphology is established early during the prenatal period and Diewert found that already 7 weeks post conception the cranial base angle was established around 130 degrees in normal foetuses and seem to be maintained during life (81, 202). This proposes that the developmental window for the cranial base angle is open early prenatally and a delayed timing of signalling due to a prolonged cell cycle might cause irreversible effects on the craniofacial growth.

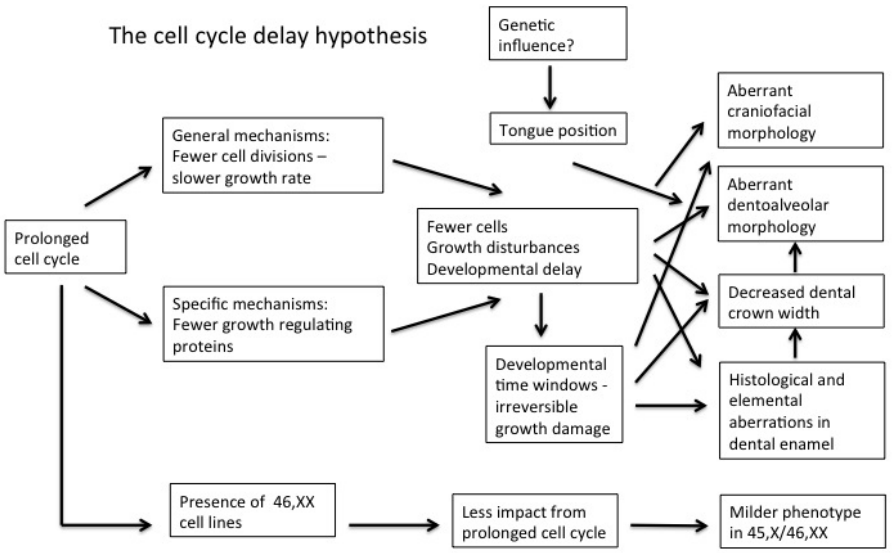


Figure 18. Schematic illustration of the cell cycle delay hypothesis applicated on dentofacial variables, after original by Barrenäs et al. (80).

Also 47,XXX females are reported on having a prolonged cell cycle (77) and display a flattened cranial base as well (41). The preservation of the flattened cranial base in TS from prenatal to adult life is presented in the literature (140, 141, 146, 151) and our results of no impact fro age on the cranial base angle are in line with these reports.

As earlier mentioned lymphedemas are frequently described in TS (108, 109). The regions were lymphedemas appear are coinciding with areas of malformations as neck, hands and thorax and are believed to cause malformations by the mechanical force itself as well as a compressive effect on ossification centers (108, 109). The regional lymphatic changes in the cervical region are suggested by some authors to be associated with the deviant craniofacial morphology as e.g. the increased cranial base angle (59, 60, 151). Ogata et al suggest a compressive effect from these distended lymphatics or lymphoedema on the developing skeletal tissues not only in the limbs but also in the cervical area (109). Studying the phenotype in various X chromosomal deletions in Turner syndrome a lymphoedema critical region in Xp11.4 is proposed (219). Additionally, an increased prevalence of high arched palate is reported among individuals with Xp deletions involving this lymphogenic gene compared to individuals with a preserved lymphogenic gene (109).

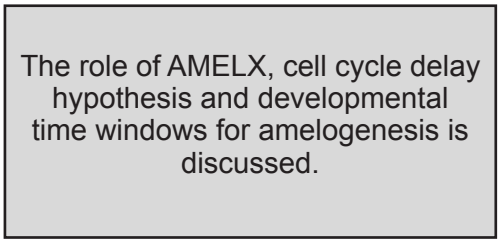
The role of SHOX and BGN, cell cycle delay hypothesis, developmental time windows and lymphedemas for craniofacial development are discussed.

Dental crown width, histology and elemental composition

Several factors have been found affecting tooth size generally. Factors as birth weight, nutrition, mothers health condition, agents as A-vitamin and phosphorus as well as genetic factors are proposed to be of importance (220-222). The causes for the small dental crown width in TS, found in study III, as well as the qualitative changes seen in enamel in study IV cannot be explained by one single mechanism. In 46,XX females, two copies of AMELX are present. Since AMELX is suggested to escape inactivation, the haploinsufficiency seen in the majority of TS is feasible to cause a reduction of the gene product amelogenin, and thereby a decrease in the enamel thickness. Amelogenin null mice display loss of the characteristic enamel prism pattern, since enamel crystals fail to form and elongate, and thus supports the involvement from AMELX as an underlying factor for the present findings from polarized light microscopy (53). The suboptimal formation of amelogenin matrix, influencing incorporation of calcium and phosphate into the forming hydroxyapatite might be caused by the haploinsufficiency of AMELX, but probably not as a sole factor. It has been shown that several amelogenin-related proteins interact during enamel formation (199). Since the enamel thickness is established during the secretion phase, where the ameloblasts secrete the extracellular matrix majorly consisting from amelogenin, and the mineralization come about mainly during the maturation phase, it is feasible to assume that a disturbance has occurred during these both phases.

As in craniofacial growth the prolonged cell cycle seen in TS may account for both the decreased dental crown width and the qualitative changes seen in study III and IV. The fully differentiated ameloblast is no longer capable of undergoing mitosis (the post-mitotic stage), a characteristica in common with

the neurons and a few other cell types. This enables formation of the extreme tissue, i.e. dental enamel with almost entirely inorganic compounds, but on the other hand the ameloblast is vulnerable since a developed damage can not be repaired. The suggested effects from fewer cell divisions during a critical developmental time window is a model of explanation that fits in with the vulnerable ameloblast and the irreversible effects from an early disturbance (Figure 18).



Geno-phenotype correlation

The present studies showed univocal results of a divergent craniofacial morphology both in the cephalometric and dental arch analyses as well as affection of the amelogenesis in TS. These aberrations of craniofacial and dental arch morphology together with dental crown width were generally milder in the 45,X/46,XX karyotype. Several different genes involved in dentofacial development, suggested to escape X inactivation (SHOX, AMELX, BGN), are mentioned in the introduction. Our results with a milder phenotype, seen in the 45,X/46,XX group, support the theory that presence of cell-lines with full gene expression seem to milder the aberrations but not fully normalize the craniofacial growth or amelogenesis. Thus, presence of healthy cell lines without haploinsufficiency of these genes or with a normal cell cycle velocity would most probably be part of the explanation for the milder 45,X/46,XX phenotype (Figure 18). Yet unknown genes are of course probable to be involved in the dentofacial development.

In this thesis the isochromosome karyotype was found to have a more severe phenotype for dental crown width measures and together with 45,X a higher number of significant differences from controls for dental arch and craniofacial morphology than 45,X/46,XX. A haploinsufficiency of AMELX and SHOX,

located on the p-arm, is seen not only in 45,X but also in isochromosomes. However, BGN, located on the q-arm might be triplicated in isochromosomes. Which effect this triplication gives on craniofacial growth is unknown. Not only 45,X fibroblasts were shown to have an increased cell generation time, but also cells with an increased genetic material, as trisomy. Isochromosomes, with the short X chromosomal arm replaced by a long arm, have an increased amount of genetic material as well, but no studies on cell generation time in TS isochromosomes were found. Is it possible that the theory of cell cycle delay gives part of the answer for the severe aberrations seen in the isochromosome group? The question to be asked is why the isochromosome distinguished as being the most aberrant for the dental crown width but not for craniofacial and dentoalveolar arch morphology? Another question to be asked is if there are other factors or genes located on the q-arm that might be of importance for craniofacial growth, that are expressed in the isochromosome karyotype but not in 45,X?

The effect from genomic imprinting is a theme discussed concerning phenotype differences between karyotypes. A paternal origin of the healthy X chromosome in a majority of the isochromosomes is suggested, but debated, while a maternal origin in 45,X karyotype is more common. The genomic imprinting is also suggested as a factor explaining phenotype differences separating isochromosomes from others, as in this thesis a more severe phenotype for dental crown width. However the effect from genomic imprinting is controversial and contradicted by others who claim no parent-of-origin effects on stature, body mass index, cardiac, renal, skeletal, lymphatic, hearing or ocular systems (21).

The role of presence of 46,XX cell lines, the prolonged cell cycle and imprinting for karyotype differences is discussed

Benefits and shortcomings

The major strength in this thesis was that we were able to collect the largest material published hitherto on karyotype correlations for craniofacial morphology and tooth width, but also for measurement of palatal height on cast models. The multicentre study design made it possible to collect an extended material, which enabled separate groups of the mosaic karyotype exhibiting 46,XX cell-lines and the isochromosomes, in addition to the 45,X karyotype. By this design we managed to reduce the limiting effects on the sample size from the low prevalence of the syndrome and escaped the probable bias from portioning various mosaics and isochromosomes in the same group. It would have been highly interesting to study also rare karyotypes as e.g. ring chromosomes and deletions in separate groups, but than a dramatically increased material would have been required. The heterogeneity of the sample in regard to age was both a strength and a disadvantage. By the wide age span we had the possibility to study the impact of age on craniofacial and dentoalveolar arch morphology. The conversion of the craniofacial morphology and dental arch variables into age specific SDS were prerequisites for the possibility of assessing all individuals independent of age in one group and thereby avoiding separation into different age groups.

A major issue to discuss for many clinical studies is the reference material. The most optimal control group might have been a group of mothers and sisters, frequently used in studies on different X chromosome anomalies emanating from Finland, to escape from bias from an underlying familiar hereditary pattern (177, 185). The ethical consideration of exposing healthy individuals to unnecessary radiation doses makes it difficult to collect a contemporary material for cephalometric comparison among healthy individuals. Collecting controls among patients referred to an orthodontic clinic might also bias the comparisons, since this is a selection of the material. However, it might also be discussed if a selected group of individuals with normal occlusion and profile as the one we used is the most optimal reference group, or if a female population group might have been a more optimal choice. With collection of an own control material instead of using published data, the choice of landmarks

and assessments would have been less limited and other statistical methods could have been chosen. For example an age matching of individuals might have been a possibility to avoid converting values into SDS. The reference groups of choice are collected some decades ago and there might be qualms of secular trends, which also might bias the results. Differences are reported in lateral arch length between samples born in the sixties and the eighties due to loss of tooth substance, while no tooth width differences between these groups were found (223). However, the choice of the Swedish reference material (202, 204), made it possible to escape bias from eventual racial differences and by calibration together with one of the measurers of the reference material, the inter individual error was limited, which were predominant advantages with the choice of this reference group.

In this project, we collected a heterogeneous group of participants, in different stages of GH treatment, with different age at GH start, with varying GH doses and length of GH treatment or no previous history of GH treatment at all. No data is presented on which GH regime the participants have experienced. This is of course a highly interesting topic and remains for future projects. However, the indications of a major effect from treatment with GH on craniofacial growth are weak and the probability of affection on tooth development is unlikely since tooth development to a large extent is finished before TS diagnose is set and GH treatment initiated (143, 147, 149).

In our karyotype subgroup of isochromosomes we assembled both isochromosomes with only one diagnosed cell line together with those with a mosaic pattern exhibiting an isochromosome cell line together with e.g. a 45,X cell line. Our isochromosome group consisted from less than 30% of isochromosomes with only one single cell line. An optimal karyotype division would of course have been to separate mosaic isochromosomes from “pure” isochromosomes. It is possible that a comparison between a group of “pure” isochromosome versus 45,X/46,XX might have made the karyotype correlations more evident. Thus, the TS prevalence is obviously a limiting factor.

Testing of how the number of unaffected X-chromosomal p-arms affect craniofacial growth or tooth width is an unassayed way of subgrouping. Influence on tooth width caused by the condition of the p-arms could not be verified, even though the presence of two intact X chromosomal p-arms seemed to normalize the crown width. However, the effect we saw from the condition of the p-arms on craniofacial morphology disaffirmed what we expected, since it seemed as two intact p-arms increased the deviation from controls for both maxillary and mandibular length. The break down into the two groups dependent on number of intact p-arms was an attempt to divide the material into a simpler karyotype subgrouping that allowed inclusion of rare karyotypes, which might have been useful in small materials. It is probable that the categorization into the two groups with either one or two intact X chromosomal p-arm is too unspecific to reveal any differences, since the p-arm can display an aberration but still express the causing gene(s) or other unknown genes or factors with higher impact than the number of intact p-arms are involved. We encountered individuals with mosaic karyotype including 46,XX cell lines in the group with two unaffected X chromosomal p-arms. It might have been a too small portion of 46,XX cells to affect the results positively. The value of this subdivision can thus be questioned for future use.

Almost 30 % of the participants in study II have had orthodontic intervention. Excluding such a major portion from the investigation would have increased the risk of losing important information. On the contrary, even if only two of the dentoalveolar arch variables (maxillary and mandibular dentoalveolar depth) differed comparing non-ortho and ortho group, including the ortho group in the karyotype correlation would possibly have biased the results. We choose the conservative approach and included only the TS non-ortho group, since we anticipated that an eventual subtle influence from karyotype might be obscured from the effects from orthodontic treatment. No statistically differences between the groups with or without orthodontic treatment regarding karyotype distribution could be proven. This tells us that there was not an overrepresentation of a certain karyotype in any of the groups.

In study IV only a hand full of individuals with a broad spectrum of karyotypes, some relatively rare, were participating. Due to the low prevalence of TS we had difficulties receiving additional teeth and thereby ending up with a limited number of subjects, which precluded a desirable karyotype comparison. We utilised the sample to a large extent by performing several different laboratory methods on these collected specimens. The study might be looked upon as a project where we aimed to test as many techniques as possible, since nothing was published on this topic before. Of course it would have been interesting not only studying enamel quality in primary teeth but also permanent teeth. However, since TS exhibit less crowding, the orthodontic extraction rate limits the acquisition of permanent teeth (139, 164).

The first studies separating 45,X, 45,X/46,XX and isochromosome karyotypes into three separate groups studying dentofacial morphology.

Clinical considerations and recommendations

The mandibular retrognathism seen in TS is the underlying cause for a postnormal occlusion with large overjet. A common treatment for this kind of malocclusion is normally a growth stimulating orthodontic treatment with a so called functional appliance. However, the effect of this treatment, aiming to stimulate mandibular growth, among girls with TS, who exhibit a general growth deficiency and increased mandibular retrognathism, is not fully known since no studies addressing this question are published. Is it possible that females with 45,X or isochromosome karyotypes have a less favourable starting position for such a treatment compared to the 45,X/46,XX karyotype?

A substantial portion of TS females display cross bite. The standard treatment for an individual with crossbite is transversal expansion of the maxillary dental arch with for example a Quad-Helix orthodontic appliance. Instead our findings of a narrow maxillary arch together with a broader mandibular dental

arch in TS question the benefit of a maxillary transversal expansion. Since a transversal narrowing of the mandibular arch is difficult, the question if a transversal expansion to fully correct the cross bite will be stable remains. One of the indications for treatment of a cross bite is the space deficiency, which in many TS cases is absent. An alternative to consider might be to accept a remaining cross bite, especially as the small dental crown width cause spacing (our unpublished results) and by expansion even more space is achieved.

Our finding of equal palatal height in TS and healthy females is of importance for a broad group of clinicians as paediatrics, geneticists and endocrinologists. The “high palatal vault” is regarded as a cardinal feature for TS. Since the palatal height did not differ in comparison with healthy females, a change from the established nomenclature “high palatal vault” into the more accurate “narrow palatal vault” is suggested in the clinical practice.

CONCLUSIONS

TS karyotype has an impact on craniofacial morphology, dental arch dimensions and dental crown width.

45,X/46,XX was the least aberrant TS karyotype for craniofacial morphology, dental arch dimensions and dental crown width aberrancy.

The isochromosome karyotype distinguished to have the smallest dental crown width.

The palatal height did not differ comparing TS versus healthy females.

TS females exhibit an aberrant craniofacial growth with a short posterior and flattened cranial base, retrognathic, short and posteriorly rotated maxilla and mandible, increased ramus height and relatively shorter posterior facial height. They also have a longer and narrower maxillary arch as well as a longer and wider mandibular dental arch together with a smaller mesio-distal crown width in both primary and permanent teeth.

A portion of the craniofacial variables, mainly mandibular measures, were deteriorating with increasing age relatively to controls, while several of the dentoalveolar variables normalized.

Impact from number of intact X-chromosomal p-arms was found on maxillary and mandibular length but not on dental crown width.

Morphological findings demonstrate a disturbed enamel rod formation and a lower mineral density in TS enamel. Low relative carbon levels and elevated calcium and phosphorus levels in enamel are striking findings.

FUTURE PERSPECTIVES

A clinical odontological problem for individuals with TS is the idiopathic root resorptions. In the care program for these females, panoramic radiographs are included. Our clinical experience is that following the development, progressing idiopathic root resorptions are noticed in several of the TS females, sometimes to a severe degree. Only one previous study is found, however, not longitudinal, on this important topic (139). A longitudinal study on the prevalence and progression of root resorptions, in correlation to possible previous orthodontic treatment, is planned.

From study II indications of deviating dental arch form raise the question of occurrence of malocclusions among TS and the impact of karyotype on these frequencies. Data on this topic is collected and is under preparation.

In study IV we tested Rule induction analysis on a limited number of variables with convincing results. We plan to analyse a broader group of variables on dentofacial morphology in TS to identify which variables are of most importance to separate TS from healthy females or to distinguish different TS karyotypes.

The hypothesis that the enamel deficiencies depend on imperfect amelogenin expression is indicated but not proven. We plan for analysis of amelogenin expression in TS on both nucleotide and protein synthesis levels, by measuring of the amount of RNA for amelogenin in peripheral lymphocytes from TS females.

The question about the role of AMELX and expression of amelogenin during amelogenesis in TS still remains unsolved. This is an interesting and challenging topic for future studies.

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APPENDIX

Papers I-IV