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CRANIOFACIAL SHAPE AND DIMENSIONS AS INDICATORS OF OROFACIAL CLEFTING AND PALATAL FORM

A STUDY ON CLEFT LIP AND PALATE AND TURNER SYNDROME FAMILIES

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A study on cleft lip and palate and Turner syndrome families

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Abstract

The aim of this study was to define distinct craniofacial features in subjects with nonsyndromic cleft lip and palate (CLP) and in subjects with Turner syndrome (TS), and to evaluate the resemblance of these features among their family members. This might help in elucidating if there is a parental contribution to possible predisposing craniofacial features in cleft subjects and to the severity of certain distinct craniofacial features in subjects with X chromosome monosomy.

The study population consisted of 29 Costa Rican CLP families including unaffected parents and siblings, and of 71 TS (45,X) subjects and members of their families. Based on lateral and frontal cephalometric analyses, cleft family members were characterized by reduced cranial height and head width, greater interorbital and nasal cavity widths, shorter anterior cranial base and palatal lengths, and shorter total face height compared to control values. With respect to these distinct craniofacial features, there were statistically significant associations in anterior cranial base and palatal length, and head, forehead and outer interorbital width measurements between parents and their children with CLP. The sidedness of the cleft in affected children was related to the asymmetry of the nasal cavity width in their parents. The distinct craniofacial features of the TS subjects, such as short clivus, retrognathic position of mandible, and narrow maxilla at the level of first premolars were related to their mothers' corresponding features. The presence of lateral palatine ridges, which were detected in one third of the TS subjects, was related to the narrowness of the posterior palate rather than to the variation in the tongue position.

Distinct craniofacial features segregate in cleft family members. The several significant associations in distinct craniofacial dimensions between parents and children with CLP emphasize the importance of genetic factors in the genesis of nonsyndromic orofacial clefting. The present results support the concept that maternal factors contribute to the degree of deficiency in the growth of the cranial base and to the magnitude of mandibular retrognathism of their daughters with TS. Maternal influences may also modify the width of the palate in TS.

Keywords: cleft lip, cleft palate, craniofacial abnormalities, Turner syndrome
The most beautiful thing we can experience is the mysterious. It is the source of all true art and all science. He to whom this emotion is a stranger, who can no longer pause to wonder and stand rapt in awe, is as good as dead: his eyes are closed.

–Albert Einstein–

To Ilmi, Nelli and Vilma
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Abbreviations

BMP  bone morphogenetic protein
CL   cleft lip
CL/P cleft lip with or without cleft palate
CLP  cleft lip and palate
CNC  cranial neural crest
CP   cleft palate
FGF  fibroblast growth factor
GH   growth hormone
IRF6 interferon regulatory factor 6
MFT  multifactorial threshold
MSX1 muscle segment homeobox 1
MTR  methylenetetrahydrofolate-homocysteine S-methyltransferase
MTHFR methylenetetrahydrofolate reductase
OFC  orofacial cleft
PC   post conception
PVRL1 polio virus receptor-like 1
RA   retinoic acid
SHH  sonic hedgehog
SHOX short stature homeobox
SUMO1 small ubiquitin-like modifier 1
TGFα transforming growth factor alpha
TGFβ transforming growth factor beta
TS   Turner syndrome
List of original articles


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1 Introduction

Cleft of the lip with or without cleft palate (CL/P) and isolated cleft palate (CP) are considered as threshold traits, in which the accumulation of liable genetic and environmental factors exceeds a threshold and causes the malformation. Clefting does not follow a clear Mendelian inheritance pattern but it is conceivable that susceptibility for clefting is influenced by genetic factors. Craniofacial morphology has been postulated as being a possible predisposing factor for orofacial clefts. During embryogenesis, a subtle deviation in the extent or direction of growth in a critical timeframe can disrupt the fusion of facial prominences. In several studies, the parents of cleft children have been reported to differ from controls in their craniofacial characteristics. However, the set of craniofacial features that best describe the parents of cleft children has not been conclusive.

Subjects with Turner syndrome (TS), 45,X, are characterized by distinct craniofacial morphology including short clivus and mandible, and a relatively narrow palate. CP is suggested to occur with a somewhat higher than normal frequency in TS. TS subjects have a short stature, and haploinsufficiency of the X-chromosomal Short stature homeobox (SHOX) gene has been postulated to contribute to the height reduction in TS. A high correlation with stature has been observed between parents and their TS daughters. However, the association of distinct craniofacial features between parents and their TS daughters has not been examined previously.

A relatively high heritability has been suggested on craniofacial variables based on resemblance of craniofacial dimensions between twins, siblings and between parents and offspring in the general population. The present studies are aimed at assessing the relationship of distinct craniofacial variables between unaffected family members and subjects with nonsyndromic cleft lip and palate (CLP) in cleft families, and between parents and their daughters with X chromosome monosomy in TS families.
2 Review of the literature

2.1 Development and growth of the human palate

2.1.1 Embryogenesis and early foetal development of the palate

The facial primordium is composed of five facial processes: the single median frontonasal process and paired maxillary and the mandibular processes laterally. The frontonasal process is composed of mesenchyme derived from the cranial neural crest (CNC) and covered with epithelia from the neuroectoderm of the forebrain and facial ectoderm. The maxillary and mandibular processes consist of mesenchyme of CNC and mesodermal (paraxial) origin encased by epithelia from facial ectoderm and pharyngeal endoderm (Tapadia et al. 2005). The specific feature of craniofacial development is the crucial role of CNC cells, which contribute extensively to the mesenchyme of the facial structures. During embryogenesis, the CNC cells, unlike the trunk crest cells, give rise to many tissues, such as bone, cartilage, tooth, and cranial nerve ganglia (Le Douarin et al. 2004, Noden 1978, Noden 1983, Noden 1988, O'Rahilly & Muller 2007). Therefore, the specification, proliferation, migration, and survival of CNC cells play an important role in craniofacial development. The frontonasal process is populated by crest cells from fore- and midbrain regions. Crest cells from rhombomeres 1 and 2 of the hindbrain along with crest cells from caudal midbrain region migrate to the first pharyngeal arch (Sadler 2006).

Embryonic morphogenesis occurs under the control of complex reciprocal interactions between embryonic tissues (Le Douarin et al. 2004). The fate of CNC cells is regulated by signals from ectoderm and endoderm including Fibroblast growth factor (FGF), Bone morphogenetic protein (BMP) and Sonic hedgehog (SHH) signalling pathways (Chai & Maxson 2006, Cox 2004, Gritli-Linde 2007, Jiang et al. 2006, Tapadia et al. 2005). These and other signalling molecules target transcription factors, e.g., homeobox-containing genes, in facial mesenchyme (Francis-West et al. 1998), and the response of the mesenchyme depends on which transcription factors are expressed in the tissue (Sadler 2006). The same signalling pathways are expressed during various stages of development, and the timing of aberrant signalling will determine the nature of a possible malformation (Tapadia et al. 2005). The dosage of important regulatory molecules also has a critical role during the development of the facial structures.
Furthermore, different signalling molecules are expressed in a region-specific manner (Hilliard \textit{et al}. 2005). Molecular heterogeneity during craniofacial development contributes not only to craniofacial malformations but also to the morphological differences between individual faces (Coussens \& van Daal 2005).

Human facial development begins with the growth of embryonic facial prominences around the primitive mouth to achieve a typical human facial appearance by approximately ten weeks post conception (PC) (Diewert 1985). The mandibular processes of the first pharyngeal arch are the first facial processes to fuse in the midline. Paired Meckel’s cartilage is later established on each side of the mandibular primordia (Avery 2002). The formation of the upper lip and primary palate occurs during the 5\textsuperscript{th} and 6\textsuperscript{th} weeks PC when the medial nasal prominences of the frontonasal process fuse with maxillary processes in the frontal portions of nasal grooves (Diewert 1985). The nasal fin, which is formed at the epithelial contact area of the medial nasal and maxillary processes, is later replaced by mesenchymal tissue (Diewert \& Shiota 1990). Maxillary mesenchyme grows into the primary palate and there is a concomitant ingrowth of the neurovascular bundle (Diewert \& Wang 1992, Johnston \& Bronsky 1995). This and the subsequent cell proliferation contribute to the increase in maxillary prominence in embryonic facial appearance (Barteczko \& Jacob 2004). Other major changes at the time of primary palate closure are narrowing and elongation of the frontonasal region and withdrawal of brain from between the facial structures before the development of chondrocranium (Diewert \textit{et al}. 1993, Wang \& Diewert 1992).

Cleft of the lip and alveolus is a failure to make contact and fuse between the medial nasal and maxillary prominences. This may result from altered positioning of the nasal placodes or deviations in the quantity and direction of growth of the medial nasal and maxillary prominences. Sometimes the cause of a cleft can be a rupture of the already fused facial prominences. In case of a postfusion rupture, the lateral incisor might locate in the lateral maxillary segment instead of premaxilla, or the rupture line can affect the development of dental lamina, leading to missing or supernumery teeth in the cleft area (Arnold \textit{et al}. 1998, Hovorakova \textit{et al}. 2006, Sperber \textit{et al}. 1989). It is noteworthy that children with oral clefts more often have missing teeth also outside the cleft area than children without a cleft and the number of missing teeth increases with the severity of clefting (Menezes \& Vieira 2008, Ranta 1982, Ranta 1986).
The formation of the secondary palate occurs during the 7th and 10th weeks PC, being one of the last major morphogenetic events occurring during embryonic development (Diewert 1985). At the time of palatal closure, the head grows rapidly with different components of the face exhibiting different rates and directions of growth. The human embryonic facial structures increase four-fold in sagittal length and two-fold in height, whereas, only marginal change can be recognized in the width between the 7th and 10th weeks PC. (Diewert 1985). When the head becomes upright and positioned above the heart, this facilitates jaw opening and the withdrawal of the tongue from between palatal shelves, which originally grow vertically on each side of the tongue (Humphrey 1969). The anterior growth of the Meckel’s cartilage exceeds that of the maxilla, and the tongue attached to the anterior region of Meckel’s cartilage becomes displaced forward (Diewert 1985). During the vertical growth of the oronasal cavity, the developing nasal septum increases rapidly in size (Diewert 1978, Diewert 1985). The palatal shelves of the maxilla become lifted to a position above the tongue to fuse with each other and the nasal septum and with the primary palate in anterior part of the mouth (Ferguson 1987). Shelf elevation starts with a swinging “flip-up” mechanism in the anterior third of the palate (Ferguson 1988). The initial fusion point is about one third from the anterior border of the hard palate and a gradual closure of the rest of the palate proceeds in an anterior and posterior direction (Proffit & Fields 2000). The intrinsic shelf elevation force is attributable to many factors, perhaps most importantly by accumulation and hydration of hyaluronic acid in the palatal shelves (Ferguson 1987, Ferguson 1988). The adequate epithelial contact, adhesion and fusion, as well as mesenchymal integration, are required for intact palate formation (Dudas et al. 2007, Kaartinen et al. 1997, Miettinen et al. 1999, Shuler 1995). The soft palate develops with the posterior displacement of epithelia by growth, migration and merging of subepithelial mesenchyme.

The etiology of CP may arise from primary factors related to the palate such as deficient growth of the palatal shelves, delayed timing of shelf elevation or a failure in fusion of the apposed shelves. The secondary factors associated with the formation of CP relate to growth disturbances in the surrounding craniofacial structures including cranial base and mandible, as well as to problems in withdrawal of the tongue from between the palatal shelves. The cleft of the hard palate often causes a failure in fusion of the soft palate.

At the time of palatal shelf elevation during the 8th week PC, paired ossification centers appear in the mesenchyme of the primary palate, palatal...
shelves and palatine bones (Barteczko & Jacob 2004, Lee et al. 1992). The muscles of the palate subsequently appear as mesenchymal subfields with the related bone structures. Masticatory muscles and tensor veli palatine, which originate from the first pharyngeal arch, emerge and mature earlier than the muscles of the soft palate (Cohen et al. 1993, Cohen et al. 1994). The muscles of the soft palate, levator veli palatini, palatophanryngeus, palatoglossus and uvular muscles, arise from the fourth pharyngeal arch (Cohen et al. 1993, Cohen et al. 1994). Levator veli palatini is responsible of the soft palate elevation and is, therefore, an essential muscle in swallowing and speech production. Tensor veli palatini is implicated in the opening of the eustachian tube.

In cleft of the soft palate, there is an imperfect union of the muscles of the soft palate in the midline of the velum, with a clefting of the uvula being its least severe form. Bifid uvula can also accompany a submucous cleft, in which the separation of the muscles of the soft palate is not seen clinically because of the presence of an intact mucous membrane. (Gorlin et al. 2001a).

### 2.1.2 Pre- and postnatal growth of the palate

During ossification of the palate, the midpalatal suture is formed in the midline and the transverse palatomaxillary suture between the palatal shelves and the palatine bones. These sutures are later responsible for the transverse and anterior-posterior growth of the palate (Njio & Kjaer 1993). The incisive fissure is formed laterally from the incisive canal to the space between the lateral incisors and canines bilaterally and this demarcates the line of fusion between the primary and secondary palates. The incisive fissure fuses during the first postnatal year. (Šperber et al. 1989). The growth in the midpalatal suture ceases between one and two years of age, however, the timing of its bony fusion may be as late as in adulthood (Persson & Thilander 1977, Wehrbein & Yildizhan 2001). The postnatal growth in the transverse palatomaxillary suture has been suggested to be lop-sided so that the maxillary component contributes more than the palatine component to the total increase in the total palatal length (Silau et al. 1994). In addition to sutural growth, the palate grows by bone remodelling. The width and length of the maxillary arch are increased by bone deposition in the lateral direction and on the posterior surface of tuberosity. During the growth, the palatal vault moves in an inferior direction as bone is removed from the floor of the nasal cavity and added to the roof of the mouth. This increases the height and width of the palate. An additional gain of both height and width of the palate appears with
the growth of alveolar ridges and the eruption of teeth. (Bjork & Skieller 1977, Enlow & Hans 1996a). The pre- and postnatal anterior growth of the maxilla is influenced by the enlargement of the nasal septum via the septopremaxillary ligament, which matures at 15 weeks of gestation (Lee et al. 1992).

Palatal abnormalities are often seen in congenital syndromes such as Down (Shapiro et al. 1967), Apert and Crouzon (Kreiborg & Cohen 1992, Peterson & Pruzansky 1974), and cleidocranial dysostosis (Sperber 2002). The palate has been described as being highly arched in Noonan (Horowitz & Morishima 1974), Turner (Laine et al. 1985) and Marfan (Westling et al. 1998) syndromes. Moreover, a high and narrow palate is sometimes seen in nonsyndromic orthodontic patients (Westling & Mohlin 1996). When measured, the high-arched palate is often found to be narrow, but normal in height (Shapiro et al. 1967). Genetic factors (Westling & Mohlin 1996) and facial muscular balance (Gorlin et al. 2001c) have a significant impact on palatal shape. Prominent lateral palatine ridges are a common nonspecific feature associated with various conditions due to a long-standing lack of tongue thrust into the palatal vault (Hanson et al. 1976). This may be related either to a neuromotor dysfunction of the tongue or to a primary malformation of the palate (Gorlin et al. 2001c).

Torus palatinus is a fairly common, localized bony protuberance in the palatal midline that is observed more commonly in adults than in children and more commonly in females than in males. Torus palatinus has a genetic background, but its mode of inheritance is not known. The incidence of torus palatinus varies in different populations. (Kari & Alvesalo 1973).

2.2 Nonsyndromic cleft lip with and without cleft palate and isolated cleft palate

2.2.1 Classification of orofacial clefts

Based on embryological and anatomic considerations, orofacial clefts have been classified into two groups; clefts of the lip with or without cleft of the palate (CL/P) and isolated clefts of the palate (CP) (Fogh-Andersen 1942). In CL/P, clefting of the palate is thought to be secondary to a failure in lip closure. Anatomically, the severity of the malformation varies from incomplete cleft lip (CL) to complete CLP and from unilateral to bilateral clefts. The left side unilateral: right side unilateral: bilateral CL/P ratio is in order of 6:3:1 (Saal 2002).
It is very uncommon that CL is associated with intact primary palate and clefting of the hard palate, those cases are often syndromic (Saal 2002). Robin sequence (CP, micrognathia, glossoptosis), in its nonsyndromic form, can be viewed as an extreme form of isolated CP in terms of mandibular retrognathia and upper airway constriction (Hermann et al. 2003).

Clefts of the hard palate almost invariably include a cleft of the soft palate, even though cleft of the hard palate with intact soft palate has been described in the literature (Fara 1971, Mitts et al. 1981, Yu et al. 2005). It has been reported, that overt clefts of the hard palate and soft palate have a different gender ratio and they do not occur in the same families (Christensen & Fogh-Andersen 1994, Christensen & Mitchell 1996) and should, therefore, be considered as two etiologically distinct subgroups of CP (Mossey & Little 2002). Submucous palatal clefts are characterized by bifid uvula, incomplete union of the muscles of the soft palate and notching of the posterior border of the hard palate (Calnan 1954) or in its occult form only by intact mucosa with abnormal insertion of levator muscle into the posterior border of the hard palate (Kaplan 1975). A short palatal length has been described in approximately 60 percent of the occult submucous cleft palate cases (Lewin et al. 1980). Sometimes, the submucous cleft palate can occur in combination with cleft lip (Heliovaara & Rautio 2007, Kono et al. 1981). In these cases, the palatal length is greater compared to the palatal length in isolated submucous cleft palate cases (Heliovaara & Rautio 2007).

Microforms of oral clefts

It has been suggested that inclusion of microforms of cleft lip in the cleft phenotype would increase the power of statistics in mapping of genes which lead to susceptibility to clefting (Bixler 1991, Melnick 1992, Weinberg et al. 2006b). Grooves in the philtrum as a sign of disruption of orbicularis oris muscle and lateral fistulas of the upper lip (Gorlin et al. 2001a) are considered as minimal involvements of CL. An increased number of subclinical orbicularis oris muscle defects among noncleft relatives of cleft subjects (10.3 percent) compared to controls (5.8 percent) has recently been reported (Marazita 2007, Neiswanger et al. 2007). Tolarova, 1969, suggested that a bend in the alveolar arch in the region of the upper lateral incisor and canine, in combination with an atypical shape and position of teeth, may be considered a microform of CL/P. In cleft subjects, agenesis of the lateral incisor on the noncleft side has been suggested to represent an incomplete form of bilateral clefts of the lip (Letra et al. 2007). However,
lateral incisor anomalies themselves (Woolf 1965) or other differences in tooth number or morphology (Anderson & Moss 1996, Haria et al. 2000) were not present as microforms of cleft in unaffected family members of cleft subjects. Other clinical features, such as handedness and dermatoglyphic pattern types, that could be a sign of liability for developmental disturbances of lip and palate, have also been sought (Scott et al. 2005a, Scott et al. 2005b, Scott et al. 2005c). It has been suggested that a tapered and high-arched palatal form (Erickson 1974) or dysmorphic craniofacial features (Weinberg et al. 2006b) in family members without cleft could be viewed as microforms, or subphenotypes, of oral clefts.

2.2.2 Epidemiology

Clefts of the lip and palate are the most common craniofacial malformations. They are called nonsyndromic in the absence of any other major anomalies or recognized maternal teratogenic exposures. Approximately 70 to 80 percent of CL/P and 50 percent of CP cases are nonsyndromic (Milerad et al. 1997, Stoll et al. 2000, Tolarova & Cervenka 1998).

The prevalence of CL/P varies according to gender, ethnicity and socioeconomic factors (Murray 1995, Vanderas 1987). CL/P is more common in males (male to female ratio 2:1) (Tolarova 1987). It is commonly reported that American Indians (3.6/1000) and Asian populations (Japanese 2.1/1000, Chinese 1.4/1000) have the highest prevalence for CL/P, Caucasians (0.7–1.3/1000) intermediate and black populations (0.3/1000) the lowest (Gorlin et al. 2001a). However, there are many exceptions to this, for example some specific areas have a higher incidence for clefting, presumably due to a founder effect or an environmental trigger (Spritz 2001). Individuals of lower socioeconomic status tend to have a higher risk for CL/P (Bender 2000, Croen et al. 1998).

In contrast, CP is more common in females (female to male ratio 2:1) (Gorlin et al. 2001a). In general, the incidence for CP in white and black populations is in order of 0.4/1000 live births (Gorlin et al. 2001a). However, a higher prevalence is reported in certain countries or regions, e.g., in Finland (0.97/1000), Western Scotland (0.81/1000) and in the New Zealand Maori population (1.87/1000) (Mossey & Little 2002). The sex ratio of the cleft of the soft palate and uvula approaches 1:1 (Murray 1995).

CL/P and isolated CP tend to cluster in different families (Saal 2002). Curtis, 1961, reported a recurrence risk of 4 percent for CL/P when one child or one of the parents is affected, 9 percent when two children are affected and 17 percent if
one parent and one child are affected. With respect to CP, the genetic component is somewhat less important, the recurrence risk being 2 percent when one child is affected, 6 percent when one parent is affected and 15 percent when one child and one parent are affected (Curtis et al. 1961).

### 2.2.3 Genetics

**Mode of inheritance**

Orofacial clefts occur as part of a syndrome in 30 percent of CL/P cases and in 50 percent of CP cases. These include syndromes caused by chromosomal aberrations and single gene defects as well as syndromes of unknown etiology. This group also includes anomalies that are caused by teratogenic exposure (Schutte & Murray 1999). However, the majority of orofacial clefts are nonsyndromic and their specific cause usually remains unknown.

The most consistent identified risk factor for nonsyndromic orofacial clefts is a positive family history (Khoury et al. 1988). Twin studies have provided evidence for a major genetic component in the etiology of oral clefts (Christensen & Fogh-Andersen 1993, Natsume et al. 2000, Nordstrom et al. 1996) and a possible influence of maternal factors on the outcome of oral clefting (Gorlin et al. 2001a, Mitchell 2002).

Fogh-Andersen, 1942, postulated the segregation of alleles in a single genetic locus with variable penetrance as a mode of inheritance for CL/P and autosomal dominant inheritance with greatly reduced penetrance for CP. In the 1960s and 1970s, the inheritance of oral clefts was explained with a multifactorial threshold (MFT) model (Carter 1976, Fraser 1976). In the MFT model, several genes, each with equal, minor and additive effects in conjunction with environmental factors contribute to the formation of a cleft (Carter et al. 1982, Hu et al. 1982). However, the epidemiologic data was not consistent with the predictions that could be drawn from the MFT model (Marazita et al. 1986, Melnick et al. 1980). In the 1970s and 1980s, based on segregation analysis models, mixed models (a major locus with multifactorial background) (Chung et al. 1986, Marazita et al. 1984) or a major locus alone (Hecht et al. 1991, Marazita et al. 1992, Nemana et al. 1992) were suggested as the mode of inheritance for CL/P. For isolated CP, the inheritance was explained as being due to a single recessive major locus with reduced penetrance (Clementi et al. 1997). Analysis of recurrence patterns clearly
excluded single-locus inheritance for CL/P (Farrall & Holder 1992, Mitchell & Christensen 1996, Mitchell & Risch 1992) and CP (Christensen & Mitchell 1996, FitzPatrick & Farrall 1993). Statistical analyses of recurrence risk patterns were consistent with oligogenic models for orofacial clefting in which no single locus could account for more than a threefold to sixfold increase in the risk to first degree relatives (Farrall & Holder 1992, Mitchell & Christensen 1996, Mitchell & Risch 1992). Still today, the mode of inheritance of oral clefts has not been elucidated (Schliekelman & Slatkin 2002a). Understanding the mode of inheritance for nonsyndromic CL/P and CP would help in genetic counselling of the cleft individuals and their family members. It would also benefit in the design of studies aimed at identification of cleft susceptible loci.

**Candidate genes**

Nonsyndromic CL/P and CP are complex traits, the etiologies of which are multifactorial including both genetic and environmental factors. Characteristic of nonsyndromic orofacial clefting is that the pattern of inheritance is not well understood, the penetrance of the trait is incomplete and that there is heterogeneity in the etiological factors both within and among populations (Maestri et al. 1997). Candidate genes for CL/P and CP have been suggested in many human linkage and association studies and genome-wide scans as well as in studies with mouse mutants. Chromosomal aberrations have provided clues to cleft susceptible loci. Mapping and identifying genes in mice have benefited in understanding which gene-regulatory pathways are important to clefting. (Lidral & Murray 2004).

Ten chromosomal loci, orofacial cleft (OFC) 1-10 in OMIM (Online Mendelian Inheritance in Man), have been claimed to contribute to the onset of nonsyndromic CL/P. In some of them, the specific gene mutations have been identified. These include mutations in a muscle segment homeobox, MSX1, gene at 4q (OFC5) (Jezewski et al. 2003, Otero et al. 2007, Park et al. 2007, Suzuki et al. 2004, Tongkobpetch et al. 2006), an interferon regulatory factor 6, IRF6, at 1q (OFC6) (Birnbaum et al. 2008, Blanton et al. 2005, Houdayer et al. 2001, Scapoli et al. 2005, Vieira et al. 2007a, Zucchero et al. 2004), a poliovirus receptor-like 1, PVRL1, at 11q (OFC7) (Avila et al. 2006, Scapoli et al. 2004, Scapoli et al. 2006, Sozen et al. 2001) and a tumor protein p73-like, TP73L, at 3q (OFC8) (Leoyklang et al. 2006) as well as disruption of a small ubiquitin-like modifier 1, SUMO1, gene at 2q (OFC10) (Alkuraya et al. 2006). The other chromosomal loci are
6p24-p23 (OFC1), 2p13 (OFC2), 19q13 (OFC3), 4q21-q31 (OFC4), and 13q33.1-q34 (OFC9). A novel genomic region on 18q21.1 has recently been suggested as a new locus, OFC11, for nonsyndromic CL/P (Beiraghi et al. 2007). Other genes that are associated with the susceptibility for CL/P include retinoic acid receptor alpha, RARα, at 17q (Chenevix-Trench et al. 1992, Peanchitlertkajorn et al. 2003, Shaw et al. 1993) and transforming growth factor beta 3, TGFβ3, at 14q (Ichikawa et al. 2006, Reutter et al. 2008, Vieira et al. 2003). Genes belonging to the folate pathway such as methyltetrahydrofolate-homocysteine S-methyltransferase, MTR, at 1q and methylenetetrahydrofolate reductase, MTHFR, at 1p are suggested to be involved in the onset of orofacial clefts (Mostowska et al. 2006, Zhu et al. 2006). There is some evidence that CL/P is associated with gastric cancer in individuals with E-cadherin, ECAD, mutations (Frebourg et al. 2006).

With respect to CP, an X-linked T-box 22, TBX22, gene has been identified (Braybrook et al. 2001, Braybrook et al. 2002, Marcano et al. 2004, Suphapeetiporn et al. 2007) and, recently, TBX22 was shown to be a target gene for SUMO1 (Andreou et al. 2007). A locus at 2q32-33 including a special at-rich sequence-binding protein 2, SATB2, has also been proposed to play a role in the etiology of isolated CP (Brewer et al. 1999, FitzPatrick et al. 2003, Leoyklang et al. 2007). An association between transforming growth factor alpha, TGFα, at 2p13, and nonsyndromic CL/P and CP has been reported in several studies (Ardinger et al. 1989, Chenevix-Trench et al. 1991, Holder et al. 1992, Sassani et al. 1993, Shiang et al. 1993), however, other studies have failed to confirm the linkage (Hecht et al. 1991, Vintiner et al. 1992, Wyszynski et al. 1997). TGFα has been suggested to be a modifier rather than having a major gene role in the genesis of orofacial clefting (Murray 1995). Mixed type of clefting and dental anomalies are found at least in mutations of IRF6 (Kondo et al. 2002, Vieira et al. 2007a, Vieira et al. 2007b, Vieira et al. 2008c), MSX1 (Lidral et al. 1998, Park et al. 2007), and FGFR1 (Dode et al. 2003, Riley et al. 2007, Vieira et al. 2007b) genes. Additional chromosomal loci, which might be associated with dental anomalies and clefting, have recently been postulated (Vieira et al. 2008b).

Several genes that are implicated in syndromic CL/P or CP have also been shown to be involved in the nonsyndromic forms of orofacial clefts (Rice 2005, Stanier & Moore 2004). Examples of such genes include MSX1 gene and CL/P with hypodontia (Jezewski et al. 2003, Suzuki et al. 2004), PVRL1 gene and CLPED1 (Margarita Island Ectodermal Dysplasia) (Avila et al. 2006, Sozen et al. 2001), TP73L and EEC3 (Ectrodactyly Ectodermal dysplasia and Cleft lip/palate
syndrome), AEC (Ankyloplepharon Ectodermal dysplasia-Clefting, Hay-Wells) and RHS (Rapp-Hodgkin Ectodermal Dysplasia) (Leoyklang et al. 2006) syndromes, IRF6 gene and Van der Woude and popliteal pterygium syndromes (Kondo et al. 2002, Zucchero et al. 2004), FGFR1 gene and Kallmann syndrome 2 (Dode et al. 2003, Riley et al. 2007, Trarbach et al. 2006), and TBX22 gene and CP with ankyloglossia (Marcano et al. 2004). It has been suggested that IRF6 could account for as much as 12 percent (Zucchero et al. 2004), MSX1 for approximately 2 percent (Jezevsky et al. 2003, Suzuki et al. 2004, Tongkobpetch et al. 2006) and the FGF signalling pathway (Riley et al. 2007) for 3 to 5 percent of nonsyndromic CL/P (Vieira 2008a).

**Environmental factors**

As with genes, there is no consensus that any particular environmental factor is critical in most orofacial clefts. Gene-environment interactions and increased risk for orofacial clefts have been suggested between maternal smoking and an infant with TGFα allele (Hwang et al. 1995, Maestri et al. 1997, Shaw et al. 1996) and between maternal smoking and foetal inheritance of a glutathione S-transferase theta 1, GSTT1, null deletion (Shi et al. 2007). An increased risk is observed between maternal smoking and a CP infant with allelic variants at the MSX1 or TGFβ3 gene, and between maternal alcohol consumption and a CL/P infant with an allelic variant at the MSX1 site (Romitti et al. 1999). Other environmental risks include the environmental pollutants, dioxins, the effect of which is mediated through the aryl hydrocarbon receptor (AHR) and AHR nuclear translocator (ARNT) genes (Swanson & Bradfield 1993). The dioxins alter the gene expression in the AHR-ARNT pathway that is important in palate development (Abbott & Birnbaum 1990, Kayano et al. 2004, Mimura et al. 1997). Many nutrients including vitamins are important in influencing embryonic development (Schutte & Murray 1999). Folate-metabolizing enzymes have been examined, based on the data that suggest that vitamin supplementation (water soluble B-complex vitamin) might reduce the incidence of neural tube defects and oral clefts (Czeizel & Hirschberg 1997, Eichholzer et al. 2006, Kjaer et al. 2008, Shaw et al. 1995, Tolarova & Harris 1995). There are several studies on whether MTHFR polymorphism is related to these anomalies (Davis et al. 2005, Gaspar et al. 1999, Martinelli et al. 2001, Martinelli et al. 2006, Mills et al. 1999, Rampersaud et al. 2003, Zhu et al. 2006) and, recently, the maternal MTR genotype was associated with an increased risk of having a child with CL/P.
(Mostowska et al. 2006). Many anti-epileptic drugs are folate antagonists and, therefore, might interfere with normal craniofacial development (Finnell et al. 2004). The teratogenic effects of vitamin A are mediated through its conversion to retinoic acid (RA). RA receptors have highly specified expression patterns during embryogenesis, and both reduced and excessive amounts of vitamin A can have teratogenic effects in the developing embryo (Cammas et al. 2007, Dolle et al. 1992, Dolle et al. 1994, Halilagic et al. 2007, Ribes et al. 2006, Serpente et al. 2005, Vermot et al. 2005). In studies with mice, dioxin and RA have been shown to alter TGFβ expression (Degitz et al. 1998, Nugent et al. 1998). Cholesterol is known to be an essential part of SHH signalling, thus, alterations in cholesterol metabolism, e.g., through pharmacological intervention, such as statin therapy, may be a risk factor for CL/P or CP (Edison & Muenke 2004a, Edison & Muenke 2004b, Edison et al. 2007). The use of corticosteroids has been claimed to increase the risk for clefts (Carmichael et al. 2007, Kallen 2003, Rodriguez-Pinilla & Martinez-Frias 1998). The effect of infection as an environmental trigger for orofacial clefting has not been clarified (Saxen 1975). It is notable that two genes (PVRL1, IRF6) that are essential for palate development, are members of gene families that modulate the immune response to infection (Murray & Schutte 2004, Rice 2005).

### 2.2.4 Craniofacial features

**Craniofacial features in subjects with CL/P and CP**

Orofacial clefts have a major impact on craniofacial morphology. Some of the disturbances are directly caused by the primary anomaly and other features are related to the surgical interventions for cheilo- and palatoplasty and subsequent growth (Semb & Shaw 1998). Functional adaptation to impaired nasal breathing may further modify the facial structures (Hocevar-Boltezar et al. 2006, Warren et al. 1991). Intrinsic craniofacial features related to a cleft phenotype can be studied on infants with a cleft before any surgery is performed, and there is also some information available about older unoperated cleft subjects. Nonetheless, most of the studies describe craniofacial morphology and craniofacial growth in children and adults with operated clefts.

In comparison to controls without cleft, the following features have been reported: wider cranial base dimensions in unoperated CL/P infants (Hermann et
al. 1999, Molsted et al. 1995), shorter cranial base length in unoperated CP infants (Dahl et al. 1982, Hermann et al. 2002), wider sphen-occipital synchondrosis and its closer position to sella in unoperated CL/P infants (Molsted et al. 1993), as well as increased cranial base angle in bilateral CLP infants (Hermann et al. 2004). In older unoperated (Bishara 1973, Capelozza Junior et al. 1993, Mars & Houston 1990, da Silva Filho et al. 1998) and operated (Dahl 1970, Horswell & Gallup 1992, Smahel & Brejcha 1983) cleft subjects, cranial base dimensions have commonly been reported to be either reduced or unchanged as compared to controls.

Increased interorbital width dimensions are characteristic to unoperated infants with clefting of the primary palate (Figalova et al. 1974, Han et al. 1995, Hermann et al. 1999, Smahel et al. 1985). Furthermore, a tendency for hypertelorism has been reported in unoperated (Motohashi et al. 1994) and operated (Dahl 1970, Han et al. 1995, Smahel & Brejcha 1983) adults with CLP. The wider transversal nasal cavity is related to clefting of the secondary palate in unoperated cleft infants (Hermann et al. 1999, Hermann et al. 2000, Hermann et al. 2002, Hermann et al. 2003, Hermann et al. 2004, Smahel et al. 1987). This feature has also been reported in unoperated (Motohashi et al. 1994) and operated (Dahl 1970, Smahel & Brejcha 1983) adults with a cleft of the secondary palate. Both hypertelorism and increased nasal cavity width are features that tend to reduce after cleft surgery (Han et al. 1995, Ishiguro et al. 1976).

have been published in operated cleft individuals of various ages (Dahl 1970, Han et al. 1995, Normando et al. 1992, Smahel & Brejcha 1983, Smahel & Mullerova 1986). Surgical intervention (Capelozza Filho et al. 1996) or cleft type (Jain & Krogman 1983, Krogman et al. 1982, Smahel & Brejcha 1983) have no significant effect on mandibular shape or growth pattern in cleft individuals.

Asymmetry in CL/P subjects is expressed in the anterior nasal spine and dental midline of the upper arch which both deviate to the noncleft side (Bishara et al. 1985, Dahl 1970, Hermann et al. 1999, Hermann et al. 2000). The size of the nasal cavity is reduced in the cleft side, because of the deviation in the nasal septum (Motohashi et al. 1994). Otherwise, the superior and posterior aspects of the septum are located in the midline (Hermann et al. 1999, Hermann et al. 2000). Asymmetries of the lower face are thought to be compensatory for the vertical asymmetries of the mandibular fossae and dentoalveolar structures (Kyrkanides et al. 2000).

**Craniofacial features in unaffected parents of cleft children**

Fraser and Pashayan, 1970, were the first to describe the relationship of facial shape to susceptibility to congenital CL in the human population. Subsequently, a number of papers have been published about craniofacial features of the parents (AlEmran et al. 1999, Coccaro et al. 1972, Kurisu et al. 1974, Maulina et al. 2006, McIntyre & Mossey 2002a, McIntyre & Mossey 2002b, McIntyre & Mossey 2003, McIntyre & Mossey 2004, Mossey et al. 1997, Mossey et al. 1998a, Mossey et al. 1998b, Nakasima & Ichinose 1983, Prochazkova & Tolarova 1986, Prochazkova & Vinsova 1995, Raghavan et al. 1994, Sato 1989, Shibasaki & Ohtsuka 1978, Suzuki et al. 1999, Ward et al. 1989, Ward et al. 1994, Weinberg et al. 2006a, Zandi & Miresmaeili 2007), siblings (Chatzistavrou et al. 2004, Erickson 1974, Laatikainen et al. 1996), and both parents and siblings (Blanco et al. 1992, Nakasima & Ichinose 1984, Weinberg et al. 2008) of subjects with CL/P or CP. One meta-analysis (Weinberg et al. 2006a) and two review articles (Maulina et al. 2006, McIntyre & Mossey 2002b) have been published about the distinct craniofacial features in parents of cleft children. The premise in these studies was that unaffected family members in cleft families would display subtle deviations in their craniofacial morphology that would distinguish them from the general population. These distinct features are considered to increase the liability to clefting when transmitted from the parents to their offspring.
Fraser and Pashayan, 1970, reported that parents of children with CL/P had a straighter facial profile with an underdeveloped maxillary anterior region, an increased face height and wider dizzymatic, albeit normal inter- and intraocular measurements compared to the control sample. They used both direct measurements and photographs in their study. Coccaro et al., 1972, used lateral cephalograms and confirmed the result of a straighter facial profile of Fraser and Pashayan, but found it to refer to a tendency towards mandibular prognathism. In their study, the parents also showed decreased horizontal and vertical dimensions of the upper face. Kurisu et al., 1974, used both lateral and frontal cephalograms to study parents of children with different cleft types. They also analyzed mothers and fathers separately. Similar to Coccaro et al., 1972, they reported straight facial profiles with a tendency towards mandibular prognathism and decreased vertical dimensions of the upper face in the parents of cleft children. The total face height was shortened in both fathers and mothers. They also described an increased interorbital distance, though this finding was statistically significant in fathers only. Ward et al., 1989, clustered the parents of cleft children according to their facial pattern profile. One of the three major clusters did not differ greatly from the normal values; however, the two other clusters had remarkably flatter facial profiles and increased face height. The latter two groups differed from each other mainly in their mandibular dimensions. In their later study with a single multiplex family, the obligate gene carriers showed increased midfacial and nasal widths in the absence of decreased facial convexity or increased lower face height (Ward et al. 1994). In 2008, Weinberg et al., used a combination of 3D photogrammetry and direct anthropometric measurements for evaluation of craniofacial features in unaffected female and male relatives of CL/P and CP subjects. They reported that female relatives had an increased soft tissue nose width, a wider upper face and retrognathic midface compared to control females without orofacial clefts in their family. The unaffected male relatives of cleft subjects were characterized by increased lower face height, decreased upper face height and increased upper face and cranial base width (Weinberg et al. 2008).

In early studies, as a consequence of MFT model for orofacial clefting (Carter 1969a, Carter 1969b), parental data were often pooled or mid-parental values for mothers and fathers were used, assuming that both parents contribute to the predisposing factors of their affected child. In 1974, Kurisu et al., criticized the MFT model since in their study sample, the cleft severity in affected offspring did not correlate with the facial anomalies in their parents. In 1989, Ward et al., clustered the parents into subgroups according to their craniofacial pattern profile.
and reported considerable variation in the phenotypes of the parents. In 51 percent of the parental pairs, only one parent exhibited an unusual facial pattern. In 31 percent of parental pairs neither one, and only in 15 percent of the cases both parents displayed unusual facial features. Thus, etiologic heterogeneity was evident also in craniofacial morphology in cleft families.

Mossey et al., 1998a, studied craniofacial characteristics in parents of children with orofacial clefting in West Scotland. The Scots are known to have a higher prevalence of CP to CL/P, approximately 1:1 (FitzPatrick et al. 1994, Gregg et al. 1994) compared to the 1:2 ratio in most European populations (Jensen et al. 1988). Another characteristic feature of Scottish population is the tendency towards Class III malocclusion (Hill 1992, Luffingham & Campbell 1974). In this population, the fathers of cleft children had reduced mandibular, symphyseal and maxillary areas, and a shorter palatal length (Mossey et al. 1998b). The cranial base angle was more acute and the cross-sectional area of the cranium was small, although, the occipital subtenue was large. Mothers had longer than normal mandibles, increased anterior facial height, facial length, and cranial base length dimensions, and, similar to fathers, reduced cranial area with a large occipital subtenue. By using these parameters, the fathers of cleft children could be correctly classified in 83 percent and mothers in 95 percent using stepwise discriminant analysis (Mossey et al. 1998a). In a frontal view, the Scottish parents of cleft children showed increased superolateral width dimensions in their faces, while the central midface width dimensions were decreased. According to these results, the nasomaxillary complex was small in the parents of cleft children (McIntyre & Mossey 2003). In the frontal view, no significant difference in craniofacial shape was detected between parents of CL/P and CP parents (McIntyre & Mossey 2004). In a separate report, directional size asymmetry of the face with a wider left side of the face and a shorter vertical dimension on the right side were reported in the Scottish parents (McIntyre & Mossey 2002a).

Japanese craniofacial morphology differs from that of the Caucasian population in the wider face (Chung & Kau 1985, Engel & Spolter 1981) and smaller anteroposterior dimensions of the head (Chung & Kau 1985, Miyajima et al. 1996). Posteriorly rotated faces and protrusive lower incisors are characteristic of the Japanese population (Miyajima et al. 1996). The Japanese are known to have a higher population incidence of CL/P but a lower recurrence risk among relatives compared to Caucasians (Chung et al. 1986). It has been speculated that the differences in the susceptibility for orofacial clefting between Caucasians and
Asians might be related to differences in craniofacial morphology and genetic contributions (Diewert & Shiota 1990, Kayano et al. 2004, Kondo et al. 2002). In 1978, Shibasaki et al., reported that Japanese parents of cleft children have more convex faces with posteriorly rotating mandibles compared to the general population in Japan. Their maxillary height and length were reduced and their upper lip was thin, especially in the fathers of children with CL/P. In 1989, Sato, described the Japanese parents of children with a cleft to have decreased maximum head width, although their interocular, zygomatic and maxillary widths were increased compared to controls. The dimensions of upper face height and mandibular growth pattern were similar to those reported by Shibasaki, 1978. A narrow head with increased midfacial and nasal width dimensions, and decreased maxillary height and open-rotated mandible in the parents of cleft children have been reported by Nakasima and Ichinose, 1983, and Sato, 1989. They also reported asymmetry in alveolar width and nasal shelf height in the parents (Nakasima & Ichinose 1983). Nakasima and Ichinose, 1983, were able to distinguish parents grouped according to their child’s cleft type from the general population with a high level of statistical significance using only frontal cephalometric variables; probabilities of misclassification ranged from 14 to 17 percent. The brain case was reported to be smaller both in parents and their children with CL/P compared to controls, and the correlation coefficients in brain case measurements between parents and their CL/P children were lower than correlation coefficients between parents and their children in families without cleft malformation (Nakasima & Ichinose 1984). In 1999, Suzuki et al., examined parents of children with CL/P, who also had another relative with CL/P within three generations. The parents had similar facial width patterns as that described by Sato et al., 1989, and Nakasima and Ichinose, 1983. The cranial base dimensions were, however, increased and Suzuki et al. were unable to detect any difference in maxillary height or mandibular growth pattern in parents of CL/P children. A discriminant function analysis revealed that a larger ratio of interocular, intercondylar and nasal cavity widths to maximum head width and a larger ratio of mandibular length to anterior cranial base length discriminated best the parents that were likely to have a child with a cleft. However, the accuracy of the discrimination was less than 70 percent (Suzuki et al. 1999).

Reports of the distinct craniofacial morphology of the parents of cleft children have also been published in Indian, Saudi and Iranian populations. According to Raghavan et al., 1994, the Indian parents of cleft children have the anterior nasal spine located more anteriorly and, thus, longer palates compared to
controls. The palatal plane was also inclined in an anterior-superior direction. The nasal cavity width and the ratios of inner interorbital, frontal and gonial widths to head width were increased (Ward et al. 2002). In a Saudi population, an increased nasal width, but decreased maxillary width in fathers of cleft children has been reported. Mothers had all facial dimensions reduced and both parents displayed facial asymmetry. (AlEmran et al. 1999). In an Iranian study, mothers of cleft children were characterized by increased mandibular body length and fathers by decreased posterior cranial base length (Zandi & Miresmaeili 2007).

The craniofacial morphology of the parents of CP children has been described to be somewhere between the craniofacial morphology of parents of CL/P children and controls in terms of facial height, width and convexity (Kurisu et al. 1974, Nakasima & Ichinose 1983, Sato 1989). Prochazkova and Tolarova, 1986, described parents of children with CP in Czech population to have longer anterior cranial base and palatal length and shorter mandibular body length, thus, characterizing these parents as displaying a skeletal Class II tendency. When measured from dental casts, the mothers of CP children had an increased intercanine distance. Later, in a larger study sample, Prochazkova and Vinsova, 1994, reported anthropometric and cephalometric threshold values for identification of the “at risk” parents. They reported normal face width and increased mandibular length for fathers of CP children and decreased face width, but normal mandibular length for mothers of CP children (Prochazkova & Vinsova 1995). In 1997, Mossey et al., reported that the parents of children with CL/P and CP differed in their mandibular and cranial measurements. The parents of children with CP could be best discriminated by their longer ramus lengths (Mossey et al. 1998a).

**Craniofacial features in unaffected siblings of cleft children**

There are only a few studies which have examined these so-called predisposing craniofacial features in unaffected siblings of cleft subjects. Erickson, 1974, reported measurable differences in profile, palatal shape and dental arch form in siblings of CL/P children. The profile was straighter, the palatal vault was more s-shaped as in cases of so-called high-arched palates, and dental arch was more tapered (narrow) than in the general population. Blanco et al., 1992, reported distinct craniofacial features only in unaffected female relatives in CL/P families. The unaffected females’ measurements were midway between the measurements of affected family members and controls (Blanco et al. 1992). There are two twin
studies (Chatzistavrou et al. 2004, Laatikainen et al. 1996) and one abstract (Johnston & Hunter 1989) in which the craniofacial measurements of the unaffected co-twins have been compared to controls. A slight tendency to mandibular posterior rotation was noted in the non-cleft twins of CP co-twins (Chatzistavrou et al. 2004, Laatikainen et al. 1996). The non-cleft co-twins of pairs discordant for CL/P displayed reduced facial width dimensions and increased sagittal dimensions including a narrower nasal cavity and a longer cranial base (Chatzistavrou et al. 2004). Johnston & Hunter, 1989 reported that the nasal cavity was narrow in two thirds and normal or only moderately increased in one third of non-cleft co-twins of pairs discordant for CL/P. The authors suggested that there would be a different pathogenesis for these groups: small embryonic medial nasal prominences for the first group and small embryonic maxillary prominences for the second group (Johnston & Hunter 1989). The non-cleft co-twins of pairs discordant for CP had steep mandibular plane angles and large bigonial widths. The authors suggested that these features reflect a large embryonic tongue size in non-cleft affected siblings of CP twin pairs (Johnston & Hunter 1989).

Combining the morphometric craniofacial data with genetic markers

Mossey et al., 1998a, examined the polymorphism in the TGFα locus. They reported that C2 allele of the TGFα Taq1 polymorphism predicted the likelihood of an individual in the population having a child with a cleft per se. They were also able to identify parents who would be likely to have a child with CL/P and CP according to TGFα polymorphism using digestion with BamHI. Combining the genetic and morphometric data improved the prediction of cleft risk in parents in comparison with using either type of information alone (Mossey et al. 1998a).

Ward et al., 1994, analyzed craniofacial morphology of a large family with five generations of affected individuals. The researchers reported that by using a discriminant function analysis with four cephalometric variables, they were able to find minimally affected gene carriers within a multiplex family. The authors suggested that identifying the minimally affected individuals in a family would provide critical information in the search for molecular markers that segregate in a cleft family with a genetic risk for clefting. The four identified individuals with distinct craniofacial phenotypes were considered as the likely gene carriers by pedigree analysis (i.e., individuals that had a parent or a sib and a child with a cleft, but did not have a cleft themselves). These morphologically susceptible
individuals were confirmed as candidate gene carriers by Beirahgi (Ward et al. 2002).

2.3 Turner syndrome

2.3.1 Etiology

Turner syndrome (TS) is a sex chromosome disorder, which is related to the loss of X-chromosomal material. Approximately 50 percent of TS subjects have only one X chromosome (karyotype 45,X) and approximately 15 percent of subjects have two X chromosomes but with only one of them being structurally normal. The remainder of the TS subjects are mosaics (Gelehter et al. 1998a, Therman & Susman 1993a). The phenotype in TS is always female. The incidence of TS is 1/2500 live-born females. However, only a small minority of the foetuses with the abnormality survive till birth; it has been estimated that 99 percent of embryos or foetuses with TS are spontaneously aborted (Kelly et al. 1992). The X chromosome monosomy is in most cases caused by nondisjunction during paternal meiosis. Thus, the TS females’ sole X chromosome is of maternal origin in approximately 70 to 80 percent of the cases (Hassold et al. 1990, Sagi et al. 2007).

The severity of the phenotype among TS subjects varies and the characteristic developmental defects of TS are more pronounced in X chromosome monosomy than in mosaicism (Sarkar & Marimuthu 1983). In normal females with two X chromosomes, one of the X chromosomes is randomly inactivated several weeks after conception (Lyon 1963). The distal end of the short arm of the X chromosome escapes the inactivation, and the genes located on this so-called pseudoautosomal region have functional homologs on the Y chromosome (Therman & Susman 1993b). Some of the clinical features of TS have been suggested to arise from the haploinsufficiency of the genes that escape X inactivation. The haploinsufficiency of the SHOX gene is at least partly responsible for the height reduction in X chromosome monosomy (Clement-Jones et al. 2000, Ellison et al. 1997). In spite of the reduction in the statural height, there is a similar correlation of height between parents and their daughters with TS as there is between parents and their children in the general population (Bondy et al. 2007, Kochi et al. 2007, Lenko et al. 1988). This kind of relationship of stature has not been observed, e.g., between subjects with Down’s syndrome and
their parents (Brook et al. 1977). The SHOX involvement has also been suggested in the expression of other TS skeletal stigmata such as high-arched palate, abnormal auricular development, cubitus valgus, and short metacarpals (Clement-Jones et al. 2000). The effect of X-linked lymphogenic gene and foetal lymphatic oedema might be involved in the soft tissue, visceral and skeletal abnormalities encountered in TS. Other etiologic factors suggested to be involved in the TS stigmata include the nonspecific effect of aneuploidy on cell proliferation (Haverkamp et al. 1999, Ogata & Matsuo 1995, Ogata et al. 2002).

2.3.2 Clinical characteristics

The two most characteristic features of TS are short stature and gonadal dysgenesis (Gelehter et al. 1998a). The growth retardation in TS starts during the intrauterine period and becomes most evident by lack of the growth spurt during puberty (Andersen et al. 2000, Davenport et al. 1999). There is almost a constant reduction in the height (Batch 2002, Davenport et al. 1999). The average adult height is below 150cm. Even though TS females have normal growth hormone (GH) levels, high dose GH substitution for several years prior to oestrogenization has been reported to increase the final adult height of TS females (Batch 2002, Ranke & Saenger 2001). In contrast, oestrogen treatment alone does not affect the final height of TS subjects (Park et al. 1983). It has been observed that TS foetuses originally have normal gonads but the oocytes are rapidly destroyed. By the age of two years, virtually all oocytes have disappeared, causing streak gonads and infertility in adult TS females (Weiss 1971).

Other skeletal features in TS include broad chest (66 percent), cubitus valgus (56 percent), short fourth metacarpals (48 percent), high-arched palate (36 percent) and micrognathia. It is thought that in TS, CP might occur in a somewhat higher than normal frequency (Gorlin et al. 2001b). A short (74 percent) and webbed (46 percent) neck with a low hair line is commonly observed (Therman & Susman 1993a). Subjects with TS are often described as having a triangular face, low set ears, and eyes with epicanthal folds (25 percent) and ptosis of the lids (14 percent). Because of the peripheral lymphoedema at birth, the hands and feet of TS girls might appear puffy (38 percent), nails are often hypoplastic and multiple pigmented nevi (63 percent) are commonly present (Gorlin et al. 2001b, Therman & Susman 1993a).

Distal molar occlusion (60 percent), narrow upper arch and wide lower arch with concomitant lateral crossbite (39 percent) and anterior open bite (17 percent)

The median survival age is somewhat reduced in TS. Cardiovascular anomalies such as bicuspid aortic valve and coarctation of the aorta are common. Diabetes mellitus, hypothyroidism and kidney malformations are more frequent in TS females than in the general population. (Gravholt 2004, Morgan 2007).

**2.3.3 Craniofacial features**

TS females are characterized by increased cranial base flexion and reduced length of the clivus, which result in retrognathic maxilla, this being even more pronounced in the retrognathic mandible (Babic et al. 1993, Jensen 1985, Midtbo et al. 1996, Peltomäki et al. 1989, Rongen-Westerlaken et al. 1992). A reduced posterior upper face height has been repeatedly reported in cephalometric studies (Jensen 1985, Midtbo et al. 1996, Peltomäki et al. 1989). The length of the maxilla is either slightly reduced or normal (Babic et al. 1993, Jensen 1985, Midtbo et al. 1996, Peltomäki et al. 1989, Rongen-Westerlaken et al. 1992). The reduced total length of the mandible is caused by the short body of the mandible, while the ramus height is not affected in TS (Babic et al. 1993, Midtbo et al. 1996, Peltomäki et al. 1989). The angle between the sella-nasion and mandibular planes is increased, whereas the palatomandibular angle does not differ between TS subjects and controls (Babic et al. 1993, Jensen 1985, Midtbo et al. 1996, Peltomäki et al. 1989). There have been conflicting reports about the calvarial dimensions which have been claimed to be either reduced (Jensen 1985, Midtbo et al. 1996) or normal (Peltomäki et al. 1989, Varrela et al. 1984).

The deviations in craniofacial morphology of TS females are largely influenced by changes in the cranial base structure. These changes have been suggested to arise from retardation in cartilage growth during early development (Peltomäki et al. 1989). The abnormal form of the cranial base can be observed in
TS foetuses already at the time of ossification (Andersen et al. 2000). In 1985, Jensen, suggested that the craniocervical area is sometimes affected in TS and that the occurrence of malformations such as basilar impression and anterior displacement of the odontoid process might be related to the webbing of the neck in TS. Generally, a flat anterior cranial base, short clivus and craniocervical anomalies tend to coincide as shown for instance in patients with osteogenesis imperfecta (Kovero et al. 2006).

2.4 Parent-offspring correlations

Quantitative traits, which commonly are polygenic, differ between individuals in degree rather than in kind. The study of quantitative traits is based on measurements and analysis of variance of the specific trait in a population. The estimation of the heritability of the trait is based on the degree of resemblance between relatives. (Falconer & Mackay 1996).

Monozygotic twins have identical genes. First-degree relatives share 50 percent and the second-degree relatives 25 percent of their genes with the proband. In case of a “pure” polygenic trait, the degree of correlation of the measurements corresponds closely to the number of shared genes between the relatives (Gelehter et al. 1998b). However, in most cases, complex genetic factors and environmental influences are involved and modify the correlation between family members. Since they are quantitative traits, craniofacial dimensions are distributed continuously in the population in a more or less normal, Gaussian, frequency distribution. Several previous association studies between parents and offspring, as well as between siblings and twins have reported a relatively high heritability of craniofacial variables with some of the craniofacial structures being under more rigid genetic control than the others (Byard et al. 1984, Byard et al. 1985, Lobb 1987, Lundstrom & McWilliam 1987, Manfredi et al. 1997, Nakata et al. 1974a, Nakata et al. 1974b, Ramsey et al. 1992, Saunders et al. 1980, Suzuki & Takahama 1988). In clinical cephalometric studies, the usual correlation coefficient of craniofacial measurements between parents and offspring has been found to be in the order of 0.30 (Hunter 1990).
3 Aims of the study

The aim of this study was to define distinct craniofacial features in subjects with nonsyndromic CLP and in subjects with TS, and to evaluate the resemblance of these features among family members. The study also aimed to assess whether facial asymmetry in parents is related to the sidedness of the cleft in their children. To evaluate the palatal form in TS, the relationship of the occurrence of lateral palatine ridges, palatal width and height dimensions, as well as tongue position in TS subjects was assessed.
4 Cleft lip and palate family study

4.1 Subjects

The cleft lip and palate family study was performed at the Craniofacial Research Core, Eastman Department of Dentistry, University of Rochester, Rochester, NY, USA.

The study was based on the Costa Rican Study sample of 44 families collected between the years 1989 and 1999 in Cleft Lip and Palate Clinic at National Children’s Hospital of San Jose, San Jose, Costa Rica. The study subjects were examined in the Cleft Lip and Palate Clinic by a postgraduate in orthodontic postgraduate program (IB) at the University of Rochester. The data included lateral and frontal cephalograms, and medical and family history of the cleft lip and palate family members. The study protocol was reviewed and approved by the University of Rochester Research Subject Review Board and Costa Rican National Children’s Hospital Ethical Scientific Board.

The present study subjects consisted of members of 29 families from the Costa Rican Study sample. Each family included a mother, a father and at least one offspring with nonsyndromic unilateral CLP. In 25 families, there was also an unaffected sibling. Three families had two children with complete unilateral CLP. The affected children included 15 boys and 17 girls and unaffected children 15 boys and 10 girls. All cleft subjects had a complete unilateral CLP. Syndromic cases were excluded. Approximately two thirds of the clefts were on the left side and one third on the right side.

The cleft lip and palate repair program at the National Children’s Hospital in San Jose includes a lip repair at the age of three months using the Millard technique, complete palatal closure at the age of 15 months using the Wardill-Killmer technique, and bone grafting at approximately six years of age. Orthognathic surgery is performed after 17 to 18 years of age when necessary.

4.2 Methods

4.2.1 Cephalometric and anthropometric measurements

Lateral and frontal (posterior-anterior, PA) cephalograms were obtained of each study subject. Lateral cephalometric landmarks and measurements (Figure 1)
were digitally traced by the present author (MRP) using Dolphin Imaging\textsuperscript{®} program (Dolphin Imaging Systems, Woodland Hills, CA, USA). The frontal landmarks and reference lines (Figure 2a,b) were traced by hand by the other researcher (Y-JY). Two anthropometric measurements, head circumference and head length, were taken by one investigator (IB) in Costa Rica.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig1.png}
\caption{The lateral cephalometric landmarks, lines and measurements of CLP family members. \textit{Abbreviations of the cephalometric landmarks:} A = A-point (subspinale), ANS = anterior nasal spine, B = B-point (supramentale), Ba = basion, Gn = gnathion, Me = menton, N = nasion, Or = orbitale, PNS = posterior nasal spine, Po = porion, Pog = pogonion, S = sella. \textit{Abbreviations of the cephalometric lines:} SNL = S-N -line, FH = Frankfort horizontal (Po-Or -line), PL = palatal line (ANS-PNS -line), ML = mandibular line (tangent to the lower border of mandible through Me). \textit{Abbreviations of the cephalometric measurements:} S-N = anterior cranial base length, ANS-PNS = palatal length (basal), A-PNS = palatal length (dentoalveolar), N-Ne = total anterior face height, N-ANS = upper anterior face height, ANS-Me = lower anterior face height, S-Gn = Y-axis, <N-S-Ba = cranial base angle, <SNA = sagittal location of the maxilla in relation to cranial base, <SNB = sagittal location of the mandible in relation to cranial base, <ANB = facial convexity angle, <FH-NA = the angle of FH to N-A -line, <FH-NPog = the angle of FH to N-Pog -line <ML-SNL = the angle of ML to SNL, <PL-SNL = the angle of PL to SNL, <ML-PL = the angle of ML to PL, <FH-SNL = the angle of FH to SNL, <ML-FH = the angle of ML to FH.}
\end{figure}
Fig. 2. a) The frontal cephalometric landmarks and measurements of CLP family members. *Abbreviations of cephalometric landmarks:* AP = apex (the highest point on the cranial vault), EU and EU' = euryon (the most lateral point of the parietal surface), ro = supraorbitale (the highest point on the superior margin of the orbit), CRO = center of line drawn between the left and right ro, FT and FT' = frontotemporale (the most medial point on the incurve of the frontotemporal ridge), lo and lo' = intersection of linea innominata and lateral orbital margin, LO and LO' = latero-ornbitale (the most lateral point on the lateral orbital margin), MO and MO' = medio-orbitale (the most medial point on the medial orbital margin), ZY and ZY' = zygoma (the most lateral point on the zygomatic arch), NC and NC' = nasal cavity (the most lateral point in the nasal cavity), ns and ns' = nasal shelf (the lowest point on the outline on ns), CNS = center of the line drawn between the left and right ns, MX and MX' = maxillary notch (the most medial point on the maxilloalveolar surface), MS and MS' = mastoideale (the lowest point of the mastoid process), GO and GO' = gonion (the most lateral point on the convex margin of the angle of the mandible), ME = menton (the most inferior point on the midline border of the mandible). *Abbreviations of the cephalometric measurements:* EU-EU' = head width, FT-FT' = forehead width, LO-LO' = outer interorbital width, MO-MO' = inner interorbital width, ZY-ZY' = facial width, NC-NC' = nasal cavity width, MX-MX' = maxillary width, MS-MS' = bimastoid width, GO-GO' = mandibular width, AP-ME = total face height, AP-CRO = calvarial height, CRO-CNS = midface height, CNS-ME = lower face height, ZY-ZY'/AP-ME = ratio of the face width to face height. b) The frontal reference lines and measurements of asymmetry. *Cephalometric reference lines:* LOL = the line connecting lo and lo', LOM = the line perpendicular to LOL drawn at the midpoint of lo and lo' (lom). *Cephalometric measurements of asymmetry:* Head asymmetry = the difference of the perpendicular distances of EU and EU' from LOM, Orbital asymmetry = the difference of the perpendicular distances of MO and MO' from LOM, Zygomatic asymmetry = the difference of the perpendicular distances of ZY and ZY' from LOM, Nasal asymmetry = the difference of the perpendicular distances of NC and NC' from LOM, Maxillary asymmetry = the difference of the perpendicular distances of MX and MX' from LOM.
4.2.2 Statistical analyses

All lateral and frontal cephalometric measurements as well as the anthropometric measurements were expressed as z-scores to enable comparison of craniofacial measurements of subjects of different age and sex (Garn et al. 1984, Garn et al. 1987). Each measurement was compared to age- and sex-matched normative values using a formula $z = \frac{(m - x)}{SD}$, where $m =$ measurement, $x =$ the age- and sex-matched normative value of the measurement, and $SD =$ the standard deviation of the normative value. The normative values published by Riolo et al. 1974, for lateral cephalometric measurements, by Saksena et al. 1990, for frontal cephalometric measurements, and by Dekaban 1977, for anthropometric measurements were used. Control values for the measurements had a z-score of zero.

The z-scores of the craniofacial measurements were compared with normative values ($z = 0$) using Student’s $t$ test. The craniofacial measurements, which in both male and female subjects with CLP were statistically significantly different from normative values, were defined as distinct craniofacial features. The significance of the difference of these distinct craniofacial features was also assessed between unaffected family members and normative values. The association of the distinct craniofacial features between parents and their children with and without CLP was assessed using linear regression analysis for lateral cephalometric and anthropometric measurements and Spearman’s correlation coefficient for PA cephalometric measurements. Chi-square test was performed to assess if a wider or narrower side of the facial transverse dimension in the parents was related to the side of cleft in their children.

Intraobserver reliability was assessed between repeated lateral (MRP) and frontal (Y-JY) measurements in 10 subjects with a 1-week interval between measurements. The correlation coefficient $> 0.9$ for all measurements indicated a satisfactory level of intra-investigator reliability.

4.3 Results

4.3.1 Distinct craniofacial features of subjects with CLP

In the lateral view, both male and female subjects with CLP had significantly shortened head length, anterior cranial base (S-N) and palatal length (ANS-PNS, A-PNS) measurements as well as a significantly increased angle of PL to SNL.
These five features were defined as distinct lateral craniofacial features in the subjects with CLP and they were used in the subsequent association analyses. The females with CLP also had significantly shortened upper (N-ANS) and total (N-Me) anterior face heights. Moreover, the males with CLP had increased angles of FH to SNL (<FH-SNL) and FH to N-Pog line (<FH-NPog) (Table 1b).

### Table 1. a) Anthropometric measurements in subjects with CLP.

<table>
<thead>
<tr>
<th>Anthropometric measurements</th>
<th>Males with CLP (n = 13)</th>
<th>Females with CLP (n = 16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head length</td>
<td>−1.04 (0.88)**</td>
<td>−1.24 (1.18)***</td>
</tr>
<tr>
<td>Head circumference</td>
<td>−0.18 (1.05)</td>
<td>−0.76 (2.19)</td>
</tr>
</tbody>
</table>

The values are z-scores. Asterisks indicate the significance of the deviation of z-scores from the normative values. * p < 0.05; ** p < 0.01; *** p < 0.001.

### Table 1. b) Lateral cephalometric measurements in subjects with CLP.

<table>
<thead>
<tr>
<th>Lateral cephalometric measurements</th>
<th>Males with CLP (n = 13)</th>
<th>Females with CLP (n = 16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-N</td>
<td>−2.73 (1.07)***</td>
<td>−3.01 (1.53)***</td>
</tr>
<tr>
<td>ANS-PNS</td>
<td>−2.72 (1.5)**</td>
<td>−2.76 (1.92)***</td>
</tr>
<tr>
<td>A-PNS</td>
<td>−1.40 (1.56)**</td>
<td>−1.35 (2.07) *</td>
</tr>
<tr>
<td>N-Me</td>
<td>−0.40 (1.28)</td>
<td>−0.60 (0.73) **</td>
</tr>
<tr>
<td>N-ANS</td>
<td>−0.36 (1.55)</td>
<td>−1.02 (0.97) ***</td>
</tr>
<tr>
<td>S-Gn</td>
<td>−0.42 (1.38)</td>
<td>−0.03 (1.40)</td>
</tr>
<tr>
<td>&lt;N-S-Ba</td>
<td>0.60 (1.10)</td>
<td>−0.44 (1.43)</td>
</tr>
<tr>
<td>&lt;SNA</td>
<td>−0.78 (1.54)</td>
<td>0.24 (1.91)</td>
</tr>
<tr>
<td>&lt;SNB</td>
<td>−0.28 (1.31)</td>
<td>0.52 (1.66)</td>
</tr>
<tr>
<td>&lt;ANB</td>
<td>−0.85 (1.83)</td>
<td>−0.38 (2.21)</td>
</tr>
<tr>
<td>&lt;FH-NA</td>
<td>0.42 (1.53)</td>
<td>0.05 (1.75)</td>
</tr>
<tr>
<td>&lt;FH-NPog</td>
<td>0.73 (0.97) *</td>
<td>0.37 (1.20)</td>
</tr>
<tr>
<td>&lt;ML-SNL</td>
<td>−0.02 (0.97)</td>
<td>0.66 (1.51)</td>
</tr>
<tr>
<td>&lt;ML-PL</td>
<td>−0.02 (0.97)</td>
<td>−0.01 (1.36)</td>
</tr>
<tr>
<td>&lt;PL-SNL</td>
<td>1.80 (1.62)**</td>
<td>1.20 (1.83) *</td>
</tr>
<tr>
<td>&lt;ML-FH</td>
<td>0.25 (0.83)</td>
<td>0.65 (1.32)</td>
</tr>
<tr>
<td>&lt;FH-SNL</td>
<td>1.10 (0.80)***</td>
<td>0.23 (1.41)</td>
</tr>
</tbody>
</table>

The values are z-scores. Asterisks indicate the significance of the deviation of z-scores from the normative values. * p < 0.05; ** p < 0.01; *** p < 0.001. The abbreviations are shown in Figure 1.

In the frontal view, the CLP subjects’ facial structures, such as the width of the forehead (FH-FH’), outer and inner interorbital distances (LO-LO’ and MO-MO’), and the width of the nasal cavity (NC-NC’) were increased, while the head width (EU-EU’) and calvarial height (AP-CRO) were decreased. The subjects with CLP
were characterized by a disproportionately wide face (ZY-ZY' / AP-ME) as their face height (AP-ME) was decreased and width of the face at the level of zygoma (ZY-ZY') was normal (Table 1c). These eight measurements were defined as distinct frontal craniofacial features in subjects with CLP and they were used in further association analysis.

Table 1. c) Frontal cephalometric measurements in subjects with CLP.

<table>
<thead>
<tr>
<th>Frontal cephalometric measurements</th>
<th>Males with CLP (n = 14)</th>
<th>Females with CLP (n = 17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EU-EU'</td>
<td>−1.79 (0.48)**</td>
<td>−1.97 (0.49)**</td>
</tr>
<tr>
<td>FT-FT'</td>
<td>1.03 (0.33)**</td>
<td>1.66 (0.31)**</td>
</tr>
<tr>
<td>LO-LO'</td>
<td>1.59 (0.36)*****</td>
<td>2.36 (0.48)*****</td>
</tr>
<tr>
<td>MO-MO'</td>
<td>1.37 (0.30)*****</td>
<td>1.65 (0.27)*****</td>
</tr>
<tr>
<td>ZY-ZY'</td>
<td>0.40 (0.40)</td>
<td>0.32 (0.35)</td>
</tr>
<tr>
<td>NC-NC'</td>
<td>1.22 (0.48)*</td>
<td>1.69 (0.32)**</td>
</tr>
<tr>
<td>MX-MX'</td>
<td>0.88 (0.53)</td>
<td>0.21 (0.41)</td>
</tr>
<tr>
<td>MS-MS'</td>
<td>−1.26 (0.35)</td>
<td>−0.68 (0.32)</td>
</tr>
<tr>
<td>GO-GO'</td>
<td>−1.16 (0.40)*</td>
<td>0.14 (0.38)</td>
</tr>
<tr>
<td>AP-ME</td>
<td>−2.18 (0.43)*****</td>
<td>−2.64 (0.51)*****</td>
</tr>
<tr>
<td>AP-CRO</td>
<td>−2.52 (0.31)*****</td>
<td>−2.94 (0.57)*****</td>
</tr>
<tr>
<td>CRO-CNS</td>
<td>−0.01 (0.41)</td>
<td>0.52 (0.49)</td>
</tr>
<tr>
<td>CNS-ME</td>
<td>−0.49 (0.40)</td>
<td>−0.59 (0.23)*</td>
</tr>
<tr>
<td>ZY-ZY' / AP-ME</td>
<td>2.12 (0.17)*****</td>
<td>2.47 (0.41)*****</td>
</tr>
</tbody>
</table>

The values are z-scores. Asterisks indicate the significance of the deviation of z-scores from the normative values. * p < 0.05; ** p < 0.01; *** p < 0.001. The abbreviations are shown in Figure 2a.

4.3.2 Distinct craniofacial features of unaffected family members

All unaffected family member groups had significantly shortened S-N and ANS-PNS measurements in terms of the five distinct craniofacial features in the lateral view compared to normative values (Table 2b). In addition, the mothers had significantly shortened head lengths (Table 2a).

Table 2. a) Distinct anthropometric measurements in unaffected family members of subjects with CLP.

<table>
<thead>
<tr>
<th>Anthropometric measurements</th>
<th>Mothers (n = 28) mean (SD)</th>
<th>Fathers (n = 28) mean (SD)</th>
<th>Unaffected brothers (n = 11) mean (SD)</th>
<th>Unaffected sisters (n = 7) mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head length</td>
<td>−0.44 (0.80)**</td>
<td>−0.33 (1.28)</td>
<td>−0.59 (1.31)</td>
<td>−0.36 (0.48)</td>
</tr>
</tbody>
</table>

Values are z-scores. Asterisks indicate the significance of the deviation of z-scores from the normative values. * p < 0.05; ** p < 0.01; *** p < 0.001.
Table 2. b) Distinct lateral cephalometric measurements in unaffected family members of subjects with CLP.

<table>
<thead>
<tr>
<th>Lateral cephalometric measurements</th>
<th>Mothers (n = 28)</th>
<th>Fathers (n = 28)</th>
<th>Unaffected brothers (n = 11)</th>
<th>Unaffected sisters (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-N</td>
<td>−1.53 (0.65) ***</td>
<td>−1.75 (0.71) ***</td>
<td>−2.51 (1.43) **</td>
<td>−2.06 (0.78) **</td>
</tr>
<tr>
<td>ANS-PNS</td>
<td>−1.16 (0.57) ***</td>
<td>−1.35 (0.97) ***</td>
<td>−2.19 (1.42) **</td>
<td>−0.97 (0.61) *</td>
</tr>
<tr>
<td>A-PNS</td>
<td>−0.06 (0.71)</td>
<td>0.03 (1.02)</td>
<td>−0.61 (1.40)</td>
<td>0.18 (0.59)</td>
</tr>
<tr>
<td>&lt;PL-SNL</td>
<td>−0.16 (1.10)</td>
<td>−0.43 (1.28)</td>
<td>0.00 (1.52)</td>
<td>0.37 (0.95)</td>
</tr>
</tbody>
</table>

Values are z-scores. Asterisks indicate the significance of the deviation of z-scores from the normative values. * p < 0.05; ** p < 0.01; *** p < 0.001. The abbreviations are shown in Figure 1.

The unaffected family members also had a tendency for increased midfacial dimensions and decreased cranial dimensions in frontal view. All unaffected family members had increased LO-LO’ and fathers and their unaffected sons also increased MO-MO’. The nasal cavity (NC-NC’) was wider than normal in all family members, but not to a statistically significant degree in the unaffected daughters. The ratio ZY-ZY’ / AP-CRO was increased, and AP-CRO and EU-EU’ were decreased in all unaffected family members, but the difference in EU-EU’ did not reach statistical significance in the unaffected daughters (Table 2c).

Table 2. c) Distinct frontal cephalometric measurements in unaffected family members of subjects with CLP.

<table>
<thead>
<tr>
<th>Frontal cephalometric measurements</th>
<th>Mothers (n = 29)</th>
<th>Fathers (n = 29)</th>
<th>Unaffected brothers (n = 15)</th>
<th>Unaffected sisters (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EU-EU</td>
<td>−2.03 (0.4) ***</td>
<td>−0.80 (0.28) **</td>
<td>−1.63 (0.46)**</td>
<td>−0.78 (0.50)</td>
</tr>
<tr>
<td>FT-FT’</td>
<td>−0.29 (0.37)</td>
<td>0.23 (0.32)</td>
<td>1.35 (0.39) **</td>
<td>0.87 (0.54)</td>
</tr>
<tr>
<td>LO-LO’</td>
<td>0.83 (0.31) *</td>
<td>1.29 (0.21) ***</td>
<td>1.79 (0.39) ***</td>
<td>2.56 (0.53) **</td>
</tr>
<tr>
<td>MO-MO’</td>
<td>0.33 (0.25)</td>
<td>1.97 (0.22) ***</td>
<td>1.05 (0.32) **</td>
<td>0.99 (0.44)</td>
</tr>
<tr>
<td>NC-NC’</td>
<td>0.82 (0.36) *</td>
<td>1.36 (0.36) ***</td>
<td>1.07 (0.29) **</td>
<td>0.91 (0.82)</td>
</tr>
<tr>
<td>AP-ME</td>
<td>−2.74 (0.21) ***</td>
<td>−1.32 (0.28) ***</td>
<td>−1.71 (0.32) ***</td>
<td>−1.54 (0.47) **</td>
</tr>
<tr>
<td>AP-CRO</td>
<td>−2.18 (0.21) ***</td>
<td>−0.82 (0.20) ***</td>
<td>−2.25 (0.26) ***</td>
<td>−1.73 (0.54) *</td>
</tr>
<tr>
<td>ZY-ZY’/AP-ME</td>
<td>1.86 (0.27) ***</td>
<td>2.09 (0.31) ***</td>
<td>2.10 (0.31) ***</td>
<td>2.07 (0.52) **</td>
</tr>
</tbody>
</table>

Values are z-scores. Asterisks indicate the significance of the deviation of z-scores from the normative values. * p < 0.05; ** p < 0.01; *** p < 0.001. The abbreviations are shown in Figure 2a.
4.3.3 Association of distinct craniofacial measurements between parents and their children with and without CLP

Of the five distinct lateral cephalometric measurements, S-N, ANS-PNS and <PL-SNL measurements showed significant associations between mothers and their CLP daughters and A-PNS measurement between fathers and their CLP sons (Table 3a). There were no significant associations of the distinct craniofacial measurements between mothers and their unaffected daughters or between fathers and their unaffected sons (Table 3b). However, mothers did display a significant association in <PL-SNL with their affected and unaffected sons (Tables 3a,b).

Table 3. a) Association of distinct lateral craniofacial measurements between parents and their children with CLP

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Son (n = 13)</th>
<th>Daughter (n = 16)</th>
<th>Son (n = 13)</th>
<th>Daughter (n = 16)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R (p)</td>
<td>R (p)</td>
<td>R (p)</td>
<td>R (p)</td>
</tr>
<tr>
<td>Anthropometric</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Head length</td>
<td>0.31 (0.27)</td>
<td>0.38 (0.41)</td>
<td>0.55 (0.06)</td>
<td>0.22 (0.30)</td>
</tr>
<tr>
<td>Lateral cephalometric</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-N</td>
<td>0.61 (0.11)</td>
<td>1.61 (0.04)</td>
<td>−0.21 (0.71)</td>
<td>0.89 (0.07)</td>
</tr>
<tr>
<td>ANS-PNS</td>
<td>−0.46 (0.65)</td>
<td>1.54 (0.04)</td>
<td>0.10 (0.05)</td>
<td>0.86 (0.10)</td>
</tr>
<tr>
<td>A-PNS</td>
<td>−0.26 (0.78)</td>
<td>1.18 (0.07)</td>
<td>1.17 (0.02)</td>
<td>0.66 (0.24)</td>
</tr>
<tr>
<td>&lt;PL-SNL</td>
<td>0.87 (0.04)</td>
<td>0.63 (0.047)</td>
<td>−0.52 (0.26)</td>
<td>0.50 (0.13)</td>
</tr>
</tbody>
</table>

The values are regression coefficients obtained by linear regression analysis (p values in parentheses).
Abbreviations are shown in Figure 1.

Table 3. b) Association of distinct lateral craniofacial measurements between parents and their unaffected children.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Son (n = 13)</th>
<th>Daughter (n = 16)</th>
<th>Son (n = 13)</th>
<th>Daughter (n = 16)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R (p)</td>
<td>R (p)</td>
<td>R (p)</td>
<td>R (p)</td>
</tr>
<tr>
<td>Anthropometric</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Head length</td>
<td>1.54 (0.16)</td>
<td>0.06 (0.79)</td>
<td>0.18 (0.65)</td>
<td>−0.00 (0.97)</td>
</tr>
<tr>
<td>Lateral cephalometric</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-N</td>
<td>0.82 (0.25)</td>
<td>0.36 (0.33)</td>
<td>0.12 (0.92)</td>
<td>0.04 (0.91)</td>
</tr>
<tr>
<td>ANS-PNS</td>
<td>−0.35 (0.72)</td>
<td>−0.15 (0.70)</td>
<td>0.24 (0.53)</td>
<td>0.16 (0.91)</td>
</tr>
<tr>
<td>A-PNS</td>
<td>−0.53 (0.48)</td>
<td>0.18 (0.54)</td>
<td>0.07 (0.85)</td>
<td>0.31 (0.06)</td>
</tr>
<tr>
<td>&lt;PL-SNL</td>
<td>0.92 (0.02)</td>
<td>0.23 (0.64)</td>
<td>0.52 (0.06)</td>
<td>0.31 (0.32)</td>
</tr>
</tbody>
</table>

The values are regression coefficients obtained by linear regression analysis (p values in parentheses).
Abbreviations are shown in Figure 1.
Of the eight distinct frontal cephalometric measurements, FT-FT' and LO-LO’ measurements displayed a statistically significant association between mothers and affected sons and between fathers and affected daughters. Furthermore, mothers had a statistically significant association with their affected daughters in the values of EU-EU’ and with their affected sons in AP-ME. In general, the correlation coefficients tended to be greater between mothers and their affected sons and between fathers and their affected daughters than between mothers and their affected daughters and between fathers and their affected sons (Table 4).

Table 4. Association of frontal cephalometric measurements between parents and their children with CLP.

<table>
<thead>
<tr>
<th>Frontal cephalometric measurement</th>
<th>Son (n = 14)</th>
<th>Daughter (n = 17)</th>
<th>Son (n = 14)</th>
<th>Daughter (n = 17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EU-EU’</td>
<td>0.30 (0.34)</td>
<td>0.56 (0.02)</td>
<td>0.09 (0.76)</td>
<td>0.24 (0.35)</td>
</tr>
<tr>
<td>FT-FT’</td>
<td>0.72 (0.00)</td>
<td>0.45 (0.07)</td>
<td>0.34 (0.23)</td>
<td>0.51 (0.04)</td>
</tr>
<tr>
<td>LO-LO’</td>
<td>0.62 (0.02)</td>
<td>0.47 (0.06)</td>
<td>0.35 (0.22)</td>
<td>0.77 (0.00)</td>
</tr>
<tr>
<td>MO-MO’</td>
<td>0.46 (0.10)</td>
<td>0.12 (0.64)</td>
<td>0.20 (0.50)</td>
<td>0.36 (0.16)</td>
</tr>
<tr>
<td>NC-NC’</td>
<td>0.46 (0.10)</td>
<td>0.45 (0.07)</td>
<td>0.37 (0.19)</td>
<td>0.18 (0.95)</td>
</tr>
<tr>
<td>AP-ME</td>
<td>0.58 (0.03)</td>
<td>0.32 (0.22)</td>
<td>0.15 (0.61)</td>
<td>0.21 (0.42)</td>
</tr>
<tr>
<td>AP-CRO</td>
<td>0.41 (0.15)</td>
<td>0.27 (0.29)</td>
<td>0.16 (0.60)</td>
<td>0.32 (0.22)</td>
</tr>
<tr>
<td>ZY-ZY’/AP-ME</td>
<td>0.14 (0.63)</td>
<td>0.27 (0.29)</td>
<td>0.43 (0.13)</td>
<td>0.15 (0.56)</td>
</tr>
</tbody>
</table>

The values are Spearman’s correlation coefficients (p values in parenthesis). Abbreviations are shown in Figure 2a.

4.3.4 Association of the parental facial asymmetry with the sidedness of the cleft in the CLP children

Nasal asymmetry was the only parental parameter that was related to the sidedness of the cleft in the offspring. The children with a cleft on the left side (n = 22) had a parent with wider left nasal cavity (n = 33) three times more often than a parent with wider right nasal cavity (n = 11). The children with a cleft on the right side (n = 10) had a parent with a wider right side nasal cavity (n = 13) twice as often than a parent with wider left side nasal cavity (n = 7) (Table 5).
Table 5. Association of the side of parental asymmetry with the side of the cleft in their children.

<table>
<thead>
<tr>
<th>Parents</th>
<th>Asymmetry of</th>
<th>Children</th>
<th>Side of the cleft</th>
<th>Chi-Square value (X)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head (EU)</td>
<td>15 L &gt; R 29 R &gt; L</td>
<td>Cleft (L)</td>
<td>0.045</td>
<td>0.83</td>
<td></td>
</tr>
<tr>
<td>Orbit (MO)</td>
<td>23 L &gt; R 21 R &gt; L</td>
<td>Cleft (L)</td>
<td>0.07</td>
<td>0.79</td>
<td></td>
</tr>
<tr>
<td>Nasal cavity (NC)</td>
<td>33 L &gt; R 11 R &gt; L</td>
<td>Cleft (L)</td>
<td>7.76</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Maxilla (MX)</td>
<td>19 L &gt; R 25 R &gt; L</td>
<td>Cleft (L)</td>
<td>0.00</td>
<td>0.97</td>
<td></td>
</tr>
<tr>
<td>Zygoma (ZY)</td>
<td>13 L &gt; R 21 R &gt; L</td>
<td>Cleft (L)</td>
<td>0.29</td>
<td>0.59</td>
<td></td>
</tr>
</tbody>
</table>

The definitions of the measurements of asymmetry are shown in Figure 2b. L = left; R = right.
5 Turner syndrome family study

5.1 Subjects

The analyses of TS family study were performed in the Department of Oral Development and Orthodontics, Institute of Dentistry, University of Oulu, Oulu, Finland and in the Craniofacial Research Core, Eastman Department of Dentistry, University of Rochester, Rochester, NY, USA.

The study population is part of Professor Lassi Alvesalo’s Kvantti research project which has collected subjects since 1970’s mainly in the University of Turku, Finland. The Kvantti research project consists of more than 300 subjects with various sex chromosome aberrations and a large number of their unaffected family members. The study protocol of the Kvantti research project has been approved by the Ethical Committee of the University of Turku.

The present studies utilized data of 71 TS subjects and 41 of their mothers, 12 fathers and 27 sisters. The karyotype of all TS subjects was 45,X. The karyotype of the TS subjects has been established for medical reasons. About 35 percent of the TS subjects had received oestrogen treatment and low dose GH substitutes, the impact of which on the craniofacial growth of the TS subjects can be considered as insignificant (Hass et al. 2001, Park et al. 1983, Rongen-Westerlaken et al. 1993). It is noteworthy that family members of females with X chromosome monosomy were used as controls in defining the distinct craniofacial features in TS subjects. In this way it was hoped to eliminate the possibility that the distinct craniofacial features characterizing the TS subjects were not simply familial features in these particular families.

5.2 Methods

5.2.1 Cephalometric measurements

Lateral cephalometric landmarks and measurements of the TS study population were obtained by digital (Dolphin Imaging, Dolphin Imaging Systems, Woodland Hills, CA, USA) and hand tracing by the present author. The cephalometric measurements of TS family members, which were used to evaluate the distinct craniofacial features and the position of the tongue, are shown in Figures 3 and 4, respectively.
Fig. 3. Cephalometric landmarks and measurements of TS family members. 
Abbreviations of cephalometric landmarks: A = A-point (subspinale), ANS = anterior nasal spine, Art = articular, B = B-point (supramentale), Ba = basion, Gn = gnathion, Go = gonion, Me = menton, N = nasion, PNS = posterior nasal spine, Pog = pogonion, S = sella. Abbreviations of cephalometric lines: SNL = S-N -line, PL = palatal line (ANS-PNS -line), ML = mandibular line (tangent to the lower border of mandible through Me).
Abbreviations of cephalometric measurements: S-N = anterior cranial base length, N-Ba = total cranial base length, S-Ba = clivus length, S-PNS = distance between sella and posterior nasal spine, S-Go = total posterior face height, S-Art = upper posterior face height, Art-Go = ramus height of the mandible, Go-Pog = corpus length of the mandible, Art-Pog = total length of the mandible, N-ANS = upper anterior face height, ANS-Me = total anterior face height, ANS-PNS = palatal length, <N-S-Ba = cranial base angle, <SNA = sagittal location of the maxilla in relation to cranial base, <SNB = sagittal location of the mandible in relation to cranial base, <ANB = facial convexity angle, <ML-SNL = the angle of ML to SNL, <PL-SNL = the angle of PL to SNL, <ML-PL = the angle of ML to PL, <Art-Go-Me = the gonial angle of the mandible.

Fig. 4. Tongue position. T-PL = the perpendicular distance of the tongue surface from the palatal line at the distal end of the upper first molar.
5.2.2 Palatal width and height measurements and the evaluation of the presence of the lateral palatine ridges

The form of the palate was evaluated by palatal height and width measurements at the level of canines, first and second premolars and first molars. The palatal width was measured as the distance between the most prominent points of each tooth pair (Figure 5a) using a sliding calliper. The palatal height was measured at the same tooth levels (Figure 5b) using a palatometer (Figure 6) which has been manufactured in the Dental Institute of Oulu. The height of the palate was measured perpendicular to the midline of the palate at the level of the most prominent points of each tooth pair separately from the right and left sides. The mean values of the right and left side measurements were used for further calculations.

Fig. 5. a) and b). Palatal width (W) (a) and height (H) (b) measurements. a) W3 = palatal width at the level of the canines, W4 = palatal width at the level of the first premolars, W5 = palatal width at the level of the second premolars, W6 = palatal width at the level of the first molars. b) H3 = palatal height at the level of the canines, H4 = palatal height at the level of the first premolars, H5 = palatal height at the level of the second premolars, H6 = palatal height at the level of the first molars.
Fig. 6. Palatometer. A dental cast is placed on a movable base and positioned so that the pointer of the meter can be set at the gingival margin of the tooth for calibration to zero. Then the dental cast is moved transversely so that the pointer of the meter can be set at the midline of the palate and the palatal height read on the digital display.

The presence of lateral palatine ridges was evaluated visually from dental casts. Three groups were formed: group 0 with no lateral palatine ridges, group 1 with small palatine ridges, and group 2 with prominent lateral palatine ridges (Figure 7).
Fig. 7. Lateral palatine ridges. A = Dental cast demonstrating a normal palate without lateral palatine ridges, group 0. B = Dental cast demonstrating a palatal vault with small lateral palatine ridges, group 1. C = Dental casts demonstrating palatal vaults with prominent lateral palatine ridges of two different degrees, group 2.

5.2.3 Statistical analyses

The distinct craniofacial characteristics of the study subjects were identified by determining the statistical difference of the cephalometric measurements between adult TS subjects and their mothers, and between adult TS subjects and their adult sisters using paired \( t \)-test. Multiple regression models were computed by a statistician (AN) to assess the extent to which the cephalometric measurements of the TS subjects could be predicted from the values of the cephalometric measurements of their parents. Age was included in the regression model to
eliminate the effect of varying age of the TS subjects. The cephalometric measurements of the subjects with TS were assigned as dependent variables. The cephalometric measurements of the mothers and fathers and the age and age squared of the TS subjects were used as independent variables. As the data of the fathers were limited, the fathers’ cephalometric measurements were changed to dummy variables. The fathers were divided into three groups, small (S), missing (M) and large (L), for each cephalometric measurement. The fathers belonged to S and L groups depending on whether the cephalometric measurement was below or above the median value of all fathers. If the data of the father were missing, he was designated to the M group.

The association of palatal dimensions between TS daughters and their mothers was assessed with partial correlation analysis controlling for the effect of age.

The statistical significance of differences in tongue position between TS subjects and their mothers and sisters as a control group was assessed by the independent-samples t-test. The relationship between the presence of lateral palatine ridges, tongue position and palatal dimensions was studied using one-way ANOVA and the Bonferroni post-hoc multiple comparison test.

5.3 Results

5.3.1 Distinct craniofacial features of subjects with TS

Females with TS differed statistically significantly both from their mothers and sisters in six linear and five angular cephalometric measurements (Tables 6 and 7). Females with TS were characterized by shortened clivus (S-Ba) and their palate and gonion were located closer to sella (S-PNS, S-Go) and the upper posterior face height (S-Art) was decreased. The length of the body of the mandible (Go-Pog) and the total length of mandible (Art-Pog) were shortened. TS females’ cranial base angle (<N-S-Ba) was increased and their face was retrognathic, mandible (<SNB) more than maxilla (<SNA). Their facial skeleton was also hyperdivergent (<ML-SNL, <PL-SNL). These 11 measurements were defined as the distinct craniofacial features of the subjects with TS.
Table 6. Cephalometric measurements of adult TS subjects and their mothers.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>TS subjects (n = 23)</th>
<th>Mothers (n = 23)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean (SD)</td>
<td>mean (SD)</td>
<td></td>
</tr>
<tr>
<td>S-N</td>
<td>71.11 (3.29)</td>
<td>71.60 (2.81)</td>
<td>0.355</td>
</tr>
<tr>
<td>N-Ba</td>
<td>105.12 (6.18)</td>
<td>105.82 (4.92)</td>
<td>0.581</td>
</tr>
<tr>
<td>S-Ba</td>
<td>42.70 (3.48)</td>
<td>45.70 (3.28)</td>
<td>0.002</td>
</tr>
<tr>
<td>S-PNS</td>
<td>42.78 (2.67)</td>
<td>49.24 (3.14)</td>
<td>0.000</td>
</tr>
<tr>
<td>S-Go</td>
<td>74.80 (6.54)</td>
<td>79.00 (5.07)</td>
<td>0.012</td>
</tr>
<tr>
<td>N-Me</td>
<td>117.37 (8.29)</td>
<td>117.72 (5.54)</td>
<td>0.855</td>
</tr>
<tr>
<td>N-ANS</td>
<td>52.16 (3.26)</td>
<td>52.90 (2.74)</td>
<td>0.363</td>
</tr>
<tr>
<td>ANS-Me</td>
<td>66.49 (7.68)</td>
<td>65.73 (5.45)</td>
<td>0.662</td>
</tr>
<tr>
<td>ANS-PNS</td>
<td>52.56 (2.85)</td>
<td>52.54 (3.40)</td>
<td>0.980</td>
</tr>
<tr>
<td>S-Art</td>
<td>32.56 (2.57)</td>
<td>34.69 (3.46)</td>
<td>0.016</td>
</tr>
<tr>
<td>Art-Go</td>
<td>47.17 (6.12)</td>
<td>49.61 (4.63)</td>
<td>0.223</td>
</tr>
<tr>
<td>Go-Pog</td>
<td>71.07 (4.81)</td>
<td>77.37 (4.93)</td>
<td>0.000</td>
</tr>
<tr>
<td>Art-Pog</td>
<td>103.24 (6.11)</td>
<td>112.11 (7.37)</td>
<td>0.000</td>
</tr>
<tr>
<td>&lt;N-S-Ba</td>
<td>134.05 (7.38)</td>
<td>130.05 (5.82)</td>
<td>0.014</td>
</tr>
<tr>
<td>&lt;SNA</td>
<td>78.42 (3.57)</td>
<td>81.75 (3.48)</td>
<td>0.001</td>
</tr>
<tr>
<td>&lt;SNB</td>
<td>74.52 (4.12)</td>
<td>80.10 (4.29)</td>
<td>0.000</td>
</tr>
<tr>
<td>&lt;ANB</td>
<td>3.89 (2.66)</td>
<td>1.69 (4.31)</td>
<td>0.022</td>
</tr>
<tr>
<td>&lt;ML-SNL</td>
<td>34.33 (8.49)</td>
<td>29.63 (5.36)</td>
<td>0.026</td>
</tr>
<tr>
<td>&lt;ML-PL</td>
<td>23.80 (8.65)</td>
<td>22.97 (5.47)</td>
<td>0.670</td>
</tr>
<tr>
<td>&lt;PL-SNL</td>
<td>10.60 (3.28)</td>
<td>6.67 (2.47)</td>
<td>0.000</td>
</tr>
<tr>
<td>&lt;FH-SNL</td>
<td>6.33 (4.23)</td>
<td>4.86 (2.91)</td>
<td>0.072</td>
</tr>
<tr>
<td>&lt;Art-Go-Me</td>
<td>123.70 (7.77)</td>
<td>123.95 (6.95)</td>
<td>0.894</td>
</tr>
</tbody>
</table>

The values are mean (SD). The abbreviations are shown in Figure 3.
Table 7. Cephalometric measurements of adult TS subjects and their sisters.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>TS subjects (n = 27)</th>
<th>Sisters (n = 27)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean (SD)</td>
<td>mean (SD)</td>
<td></td>
</tr>
<tr>
<td>S-N</td>
<td>72.33 (3.28)</td>
<td>72.30 (2.90)</td>
<td>0.970</td>
</tr>
<tr>
<td>N-Ba</td>
<td>106.75 (5.12)</td>
<td>108.57 (4.44)</td>
<td>0.148</td>
</tr>
<tr>
<td>S-Ba</td>
<td>42.48 (2.64)</td>
<td>46.23 (2.83)</td>
<td>0.000</td>
</tr>
<tr>
<td>S-PNS</td>
<td>44.21 (2.98)</td>
<td>48.43 (2.78)</td>
<td>0.000</td>
</tr>
<tr>
<td>S-Go</td>
<td>74.86 (4.86)</td>
<td>78.36 (5.70)</td>
<td>0.003</td>
</tr>
<tr>
<td>N-Me</td>
<td>119.16 (5.75)</td>
<td>116.98 (6.35)</td>
<td>0.1651</td>
</tr>
<tr>
<td>N-ANS</td>
<td>53.46 (3.08)</td>
<td>53.03 (2.78)</td>
<td>0.593</td>
</tr>
<tr>
<td>ANS-Me</td>
<td>67.17 (4.72)</td>
<td>65.35 (5.36)</td>
<td>0.115</td>
</tr>
<tr>
<td>ANS-PNS</td>
<td>53.23 (3.03)</td>
<td>53.82 (2.44)</td>
<td>0.337</td>
</tr>
<tr>
<td>S-Art</td>
<td>32.54 (3.96)</td>
<td>34.54 (2.66)</td>
<td>0.010</td>
</tr>
<tr>
<td>Art-Go</td>
<td>47.63 (3.69)</td>
<td>48.38 (4.85)</td>
<td>0.433</td>
</tr>
<tr>
<td>Go-Pog</td>
<td>72.21 (4.82)</td>
<td>76.95 (3.54)</td>
<td>0.000</td>
</tr>
<tr>
<td>Art-Pog</td>
<td>105.80 (4.20)</td>
<td>109.59 (5.26)</td>
<td>0.003</td>
</tr>
<tr>
<td>&lt;N-S-Ba</td>
<td>135.84 (6.07)</td>
<td>131.55 (4.74)</td>
<td>0.000</td>
</tr>
<tr>
<td>&lt;SNA</td>
<td>78.06 (3.68)</td>
<td>82.49 (3.49)</td>
<td>0.000</td>
</tr>
<tr>
<td>&lt;SNB</td>
<td>73.63 (3.96)</td>
<td>78.8 (4.05)</td>
<td>0.000</td>
</tr>
<tr>
<td>&lt;ANB</td>
<td>4.44 (2.61)</td>
<td>3.76 (2.79)</td>
<td>0.174</td>
</tr>
<tr>
<td>&lt;ML-SNL</td>
<td>35.19 (5.93)</td>
<td>29.79 (5.48)</td>
<td>0.000</td>
</tr>
<tr>
<td>&lt;ML-PL</td>
<td>24.95 (5.67)</td>
<td>22.18 (5.19)</td>
<td>0.045</td>
</tr>
<tr>
<td>&lt;PL-SNL</td>
<td>10.02 (3.46)</td>
<td>7.60 (3.03)</td>
<td>0.005</td>
</tr>
<tr>
<td>&lt;FH-SNL</td>
<td>8.02 (3.84)</td>
<td>5.81 (2.87)</td>
<td>0.007</td>
</tr>
<tr>
<td>&lt;Art-Go-Me</td>
<td>124.17 (6.14)</td>
<td>122.05 (6.01)</td>
<td>0.110</td>
</tr>
</tbody>
</table>

The values are mean (SD). The abbreviations are shown in Figure 3.

5.3.2 Association of the distinct craniofacial measurements between TS subjects and their parents

The statistical significances of the regression coefficients of the distinct cephalometric measurements between TS subjects and their parents are shown in Table 8. Mothers’ S-Ba and <SNB measurements predicted well their TS daughters’ distinct S-Ba and <SNB measurements. The cephalometric measurements S-Go and S-Art also revealed a statistically significant association between mothers and their daughters with TS. Fathers and their daughters with TS displayed a significant association only in Art-Pog measurement.
Table 8. Association of distinct cephalometric measurements between females with TS and their parents.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Mothers (n = 41)</th>
<th>Fathers (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>p</td>
</tr>
<tr>
<td>S-Ba</td>
<td>0.59</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-PNS</td>
<td>0.22</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-Go</td>
<td>0.41</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-Art</td>
<td>0.24</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Go-Pog</td>
<td>0.16</td>
<td>0.28</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Art-Pog</td>
<td>0.19</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;SNA</td>
<td>0.33</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;SNB</td>
<td>0.56</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;ML-SNL</td>
<td>0.40</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;N-S-Ba</td>
<td>0.35</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;PL-SNL</td>
<td>0.26</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The regression coefficients (β) with p values for parents’ measurements are shown. The fathers were divided into three groups: small (S), missing (M) or large (L), using a dummy variable for each cephalometric measurement. The intercept, linear and quadratic effect of the TS subjects’ age are not included in the table. The abbreviations for the measurements are shown in Figure 3.

5.3.3 Association of palatal height and width measurements between TS subjects and their mothers

None of the palatal dimensions had a statistically significant association between TS females and their biological mothers. However, a strong trend (p = 0.07) for an association of palatal width at the level of first premolars between mothers and TS daughters was observed (Table 9).
Table 9. The association of palatal height and width measurements between Turner syndrome (TS) subjects and their biological mothers (n = 26).

<table>
<thead>
<tr>
<th>Measurement</th>
<th>TS daughters and mothers</th>
<th>r</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palatal height</td>
<td>Canines (n = 26)</td>
<td>−0.11</td>
<td>(0.83)</td>
</tr>
<tr>
<td></td>
<td>First premolars (n = 22)</td>
<td>0.56</td>
<td>(0.24)</td>
</tr>
<tr>
<td></td>
<td>Second premolars (n = 19)</td>
<td>−0.52</td>
<td>(0.29)</td>
</tr>
<tr>
<td></td>
<td>First molars (n = 21)</td>
<td>−0.29</td>
<td>(0.58)</td>
</tr>
<tr>
<td>Palatal width</td>
<td>Canines (n = 22)</td>
<td>0.46</td>
<td>(0.36)</td>
</tr>
<tr>
<td></td>
<td>First premolars (n = 19)</td>
<td>0.78</td>
<td>(0.07)</td>
</tr>
<tr>
<td></td>
<td>Second premolars (n = 14)</td>
<td>0.67</td>
<td>(0.15)</td>
</tr>
<tr>
<td></td>
<td>First molars (n = 15)</td>
<td>−0.65</td>
<td>(0.16)</td>
</tr>
</tbody>
</table>

The values are correlation coefficients (r). The number in parenthesis following the name of the tooth shows the number of tooth pairs which were compared between TS daughters and their mothers.

5.3.4 Tongue position in TS and control subjects

The distance of the tongue from the palatal plane was significantly larger in TS subjects compared with controls, reflecting a lower tongue position in the TS subjects (Table 10).

Table 10. Tongue position (T-PL = the distance of the tongue surface from the palatal plane measured at distal end of the upper first molar) in Turner syndrome (TS) and control subjects.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>TS subjects (n = 71)</th>
<th>Controls (n = 36)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-PL / mm</td>
<td>mean (SD)</td>
<td>mean (SD)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10.9 (4.0)</td>
<td>7.6 (3.4)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

5.3.5 Significance of tongue position and palatal dimensions on the presence of lateral palatine ridges

There was a statistically significant difference (p<0.05) in the palatal width at the level of first molars and a strong trend towards a difference in palatal width at the level of second premolars between the TS subjects with different lateral palatine ridges (Table 11). In the Bonferroni post-hoc analysis, TS subjects with prominent palatine ridges had significantly narrower palates compared with the TS subjects with normal palates. Tongue position was not statistically significantly different.
between the TS subjects classified according to the manifestation of lateral palatine ridges (Table 11).

Table 11. Tongue position (T-PL = the distance of the tongue surface from the palatal plane at the distal end of the upper first molar) and palatal height and width in Turner syndrome (TS) subjects with respect to the occurrence of lateral palatine ridges.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Group 0 (n=26)</th>
<th>Group 1 (n=20)</th>
<th>Group 2 (n=25)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean</td>
<td>SD</td>
<td>mean</td>
<td>SD</td>
</tr>
<tr>
<td>Tongue position</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T-PL</td>
<td>10.3</td>
<td>4.6</td>
<td>10.4</td>
<td>4.5</td>
</tr>
<tr>
<td>Palatal height</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Canine</td>
<td>3.4</td>
<td>1.5</td>
<td>4.1</td>
<td>2.0</td>
</tr>
<tr>
<td>First premolar</td>
<td>9.6</td>
<td>1.8</td>
<td>10.5</td>
<td>2.4</td>
</tr>
<tr>
<td>Second premolar</td>
<td>14.0</td>
<td>2.1</td>
<td>14.5</td>
<td>2.2</td>
</tr>
<tr>
<td>First molar</td>
<td>15.1</td>
<td>2.8</td>
<td>15.2</td>
<td>2.4</td>
</tr>
<tr>
<td>Palatal width</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Canine</td>
<td>22.6</td>
<td>2.2</td>
<td>22.1</td>
<td>1.9</td>
</tr>
<tr>
<td>First premolar</td>
<td>25.4</td>
<td>2.0</td>
<td>24.5</td>
<td>1.4</td>
</tr>
<tr>
<td>Second premolar</td>
<td>29.7</td>
<td>2.4</td>
<td>28.8</td>
<td>1.9</td>
</tr>
<tr>
<td>First molar</td>
<td>31.5</td>
<td>2.2</td>
<td>31.0</td>
<td>2.2c</td>
</tr>
</tbody>
</table>

Group 0, TS subjects with normal palates; Group 1, TS subjects with small lateral palatine ridges; Group 2, TS subjects with prominent lateral palatine ridges. The values are millimetres.
6 Discussion

Embryonic facial morphology and growth pattern of facial processes have been suggested to be related to a predisposition to CL (Fraser & Pashayan 1970, Trasler 1968). Susceptable craniofacial features inherited from the parents might increase the risk for a perturbation of facial prominences during embryogenesis in the offspring. There is a consensus that parents of cleft children exhibit craniofacial features that distinguish them from the general population. However, there is no consensus about the exact characteristics of these features. The aim of the present study was to identify the deviant craniofacial features first in the subjects with complete unilateral CLP and then to evaluate the presence of these features in their unaffected parents and siblings. The contribution of the genetic component to these critical features was assessed by evaluating the association of these features between parents and their children with and without a cleft.

The relationship of the distinct craniofacial features was also studied in families with a daughter with TS (45,X), who commonly are characterized by the so-called high-arched palate and who might have an increased incidence for CP. Daughters with X chromosome monosomy have a nearly constant reduction in statural height compared to the height of their parents. The height reduction in TS is claimed to be caused, at least partly, by a haploinsufficiency of the SHOX gene located on the short arm of the X chromosome. A high correlation in stature is observed between parents and TS daughters. The aim of this study was to evaluate if there was a similar kind of association in distinct craniofacial features and palatal dimensions between parents and their TS daughters as has been demonstrated for stature.

The cleft family members in the present study displayed increased transversal and decreased vertical and sagittal dimensions of the face and head. All cleft family members displayed reduced calvarial height and head width, and shortened anterior cranial base. A small brain case and reduced calvarial height have occasionally been reported in cleft subjects (Dahl 1970, Smahel & Brejcha 1983, Smahel & Mullerova 1986) and in unaffected family members of cleft subjects (Mossey et al. 1998b, Nakasima & Ichinose 1984). These findings are in agreement with the findings of abnormalities in brain structure in children and adults with a cleft (Nopoulos et al. 2001, Nopoulos et al. 2002, Nopoulos et al. 2007). The involvement of shortened anterior cranial base in the cleft phenotype is more controversial. In some studies, the deficient development of the embryonic chondrocranium at the time of cleft formation has been related to oral
clefting (Dahl et al. 1982, Dahl 1970, Molsted et al. 1993, Molsted et al. 1995). In some other studies, cranial base has been claimed to be essentially unaffected by the cleft in individuals with nonsyndromic clefting (Bishara & Iversen 1974, Bishara et al. 1976, Hermann et al. 1999, Ross 1965). Ross (1965) emphasized that because of the smaller average body size of cleft individuals compared to their similar aged non-cleft counterparts, the cranial base differences often disappear when the anterior cranial base length is expressed as a proportion of total cranial length. In the present study, the anterior cranial base length was more severely reduced in CLP subjects compared to their unaffected siblings and parents, supporting the concept that cleft-predisposing factors segregate in cleft families to a different degree in subjects with and without cleft. However, proportional cranial base dimensions were not calculated.

In subjects with CLP, the short and retropositioned maxilla is masked by premaxillary protrusion before surgical invention. The iatrogenetic effect of cheiloplasty increases the retardation of the maxillary growth (Capelozza Filho et al. 1996, Normando et al. 1992). Increased (Raghavan et al. 1994, Ward et al. 1989) and decreased (Coccaro et al. 1972, Mossey et al. 1998b, Nakasima & Ichinose 1983) palatal length values have been reported in parents of children with CL/P. The present observations suggest that in all CLP family members, including unaffected siblings, the palatal length is reduced. The deviation in palatal length from normative values was greatest in children with CLP, intermediate in unaffected children and least divergent in parents. It is interesting that the reduced palatal length values of both affected males and females were related to the palatal length values of their fathers and mothers, respectively. It is possible that these findings reflect an inherited decreased growth potential of the palate in families with a cleft.

In infants with unoperated clefts of the palate, the clefting of the palate is related to the increase in the posterior width and to a reduction in the posterior height of the maxilla. The decreased height of the posterior maxilla influences the inclination of the palatal plane. A hyperdivergent angle of palatal plane to anterior cranial base has been described more often in young subjects with CL/P (Han et al. 1995, Hermann et al. 1999, Smahel & Mullerova 1986) than in adults with CLP (Smahel & Brejcha 1983) or in parents of CLP children (Nakasima & Ichinose 1983). In the present study, although the palatal plane inclination was normal in parents of cleft children, there were associations of this measurement between parents and their offspring, showing that parents contribute to the appearance of this feature in their children.
Hypertelorism and increased nasal cavity width have been associated with CLP. Discontinuity of the circumoral muscular ring in the anterior part of the maxilla also causes a disruption in the musculature ring of oro-bucco-pharyngeus that encircles the maxillary complex in the transversal plane. The unequal and asymmetric forces which are exerted tend to pull the maxillary cleft segments apart and increase the width of the nasal cavity pre- and postnatally before the lip surgery (Subtelny 2000). Increased face widths, interorbital and nasal cavity widths have been reported in parents of cleft children (AlEmran et al. 1999, Coccaro et al. 1972, McIntyre & Mossey 2003, Nakasima & Ichinose 1983, Prochazkova & Vinsova 1995, Sato 1989, Suzuki et al. 1999, Ward et al. 1994, Weinberg et al. 2008), and increased nasal cavity width has been shown to be the most robust finding in a meta-analysis of parental craniofacial morphology in CL/P families (Weinberg et al. 2006a). However, there are some studies reporting decreased nasal cavity width in unaffected cleft family members (Chatzistavrou et al. 2004, McIntyre & Mossey 2003), and different pathogenetic reasons for clefting have been proposed to be involved in the narrow and wide nasal cavity cases (Johnston & Hunter 1989). In the present sample, increased outer interorbital and nasal cavity widths were present in all cleft family members. The statistically significant associations between mothers and affected sons and between fathers and affected daughters in the outer interorbital and forehead widths provide some support for a parental contribution to the facial width dimensions in their offspring. This was further supported by the observation that the asymmetry between right and left side nasal cavity widths of the parents was related to the sidedness of the cleft in their offspring. The children with a cleft on the left side had a parent with wider left nasal cavity three times more often than a parent with wider right nasal cavity. The children with a cleft on the right side had a parent with a wider right nasal cavity two times more often than a parent with wider left nasal cavity.

In most of the previous studies, the craniofacial dimensions of the parents of cleft children have been compared to those of the general population. The offspring with or without cleft have been included in few previous studies (Blanco et al. 1992, Nakasima & Ichinose 1984). The advantage of the present study was in the possibility to evaluate the relationship of distinct craniofacial features between parents and their offspring with and without cleft. Our study population consisted of Costa Ricans of European origin. Since there were no Costa Rican control values available, we employed normative values, which are based on the North American population of European origin (Dekaban AS 1977,
It has also been reported that Hispanic populations have similar upper face heights, anterior cranial base and maxillary lengths as Caucasians. Differences in craniofacial form between Hispanic and Caucasian populations have been found in facial profile, mandibular plane angle, and dentoalveolar dimensions (Evanko et al. 1997, Lee et al. 1997).

The craniofacial features associated with CP are much the same as those reported in X chromosome monosomy. Both conditions are characterized by retrognathic and posteriorly inclined maxilla, and small, retrognathic mandible. In the present study, a similar type of association of the clivus length and antero-posterior position of the mandible between TS daughters and their mothers was observed as previously reported in statureal height. Both long bones and cranial base develop through endochondral ossification and the growth in cranial base synchondroses is basically the same as in the epiphyses of the long bones. The main growth site in the cranial base is the sphenoid synchondrosis, located in the middle cranial fossa, as the intersphenoidal synchondroses cease to grow around the time of birth (Enlow & Hans 1996b). In normal individuals, the lateral parts of the cranial base grow in parallel with the elongation of the clivus (Bjork 1955). In TS, the growth in both middle cranial fossa and lateral parts of the cranial base is retarded. The glenoidal fossa is located more superiorly than normally, thus, resulting in retrognathism of the mandible. The association of cephalometric variables related to the position of the mandible supports the concept that maternal factors influence the degree of mandibular retrognathism of their TS daughters.

A so-called high-arched palate is a characteristic feature in many females with TS. This feature is associated with a variety of congenital anomalies in the lists of syndromic descriptions. High and narrow palatal vaults are also seen in orthodontic patients, where this feature is related more to the inherited or congenital factors than to the local factors such as mouth breathing or finger sucking (Westling & Mohlin 1996). In the present study, a low tongue position in TS females was found. The low rest position of the tongue increases the relative pressure of the cheeks on the upper dental arch, which leads to its narrowing. On the other hand, it is known that the sex chromosomes influence the palatal width, i.e., palatal width increases with the number of sex chromosomes from 45,X to 46,XX and from 46,XY to 47, XYY (Laine & Alvesalo 1993, Laine et al. 1985). There are very few studies where a correlation of palatal dimensions has been made between parents and their children. The common problem when measuring the palatal width between tooth pairs arises from the inaccuracy caused by
forward drifting of posterior teeth due to either malalignment or missing teeth. This causes some fluctuation in the reference points and results in lower correlation. In a previous study with family members, who all had relatively well-aligned teeth, a greater similarity in upper dental arch width measurements was reported between mothers and daughters than between mothers and sons or between fathers and daughters, suggesting that there may be some maternal effect on the upper arch width on daughters (Hu et al. 1991). In the present study, there was a tendency for an association in palatal width at premolar region between mothers and their daughter with TS, in whom the dental arches were more compromised than in subjects included in the above study.

About one third of the present TS females had prominent lateral palatine ridges. Lateral palatine ridges are a non-specific feature of a variety of disorders in which they are related to the lack of tongue thrust into the palatal vault resulting from neuromotor dysfunction, functionally restricted tongue or by the primary palatal malformation itself. In the present study, the palatal width and height dimensions as well as the distance of the tongue from the palatal plane were evaluated in TS subjects classified according to the manifestation of lateral palatine ridges. The prominent lateral palatine ridges were found to be associated with the reduced posterior palatal width rather than with the differences in tongue position. However, the degree to which the narrow palatal anomaly present in some females with X chromosome monosomy is related to the primary growth failure in TS still remains unresolved, nor is it clear to what degree other possible factors such as low tongue position are involved.
7 Conclusions

The results of the present study support the hypothesis that CLP is not a dichotomous trait but is associated with the convergence of distinct craniofacial features in cleft family members. The present CLP family members displayed shortened anterior cranial base and palatal dimensions, decreased head width and calvarial height measurements, and increased interorbital distances and nasal cavity widths. Their faces were disproportionally wide (width/height). In the lateral view, the results support the concept that distinct craniofacial features are expressed in cleft family members to different degrees in affected and unaffected family members. In the frontal view, the cleft subjects were not always the most severely deviant from normative values. The association of distinct craniofacial characteristics between parents and affected children emphasizes the importance of genetic factors in the development of orofacial clefting. This was further supported by the concept, that the sidedness of the cleft in affected children was related to the asymmetry in the nasal cavity width in their parents.

The lack of one X chromosome causes the shortening of clivus, a retrognathic position of the mandible and a narrow palate in females with TS. The present results support the concept that maternal influences contribute to the degree of deficiency of the clivus length and to the magnitude of mandibular retrognathism of their daughters with X chromosome monosomy. There was a tendency for maternal contribution to the palatal width at the premolar level. The tongue position of the TS females was low. However, the prominent lateral palatine ridges, which were present in approximately one-third of the TS subjects, were related to the narrowness of the posterior palate rather than to the differences in tongue position.


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CRANIOFACIAL SHAPE AND DIMENSIONS AS INDICATORS OF OROFACIAL CLEFTING AND PALATAL FORM

A STUDY ON CLEFT LIP AND PALATE AND TURNER SYNDROME FAMILIES

FACULTY OF MEDICINE, INSTITUTE OF DENTISTRY, DEPARTMENT OF ORAL DEVELOPMENT AND ORTHODONTICS, UNIVERSITY OF OULU; CRANIOFACIAL RESEARCH CORE, EASTMAN DEPARTMENT OF DENTISTRY, UNIVERSITY OF ROCHESTER; SCHOOL OF DENTAL SCIENCES, UNIVERSITY OF LIVERPOOL