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XXIX CYCLE

CLEFT LIP AND PALATE: EVALUATION OF GENETICS, DENTAL ARCH FORM AND MASTICATORY FUNCTION

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1. INTRODUCTION

Cleft of the lip and/or palate (CL/P) represents a heterogeneous group of disorders affecting the lips and oral cavity, being the most common human congenital malformation involving the facial region. Individuals with CL/P may experience problems with speech, hearing, feeding, facial appearance and cognition that can lead to long-lasting adverse outcomes for health and social integration. Although not a major cause of mortality in developed countries, CL/P is associated to considerable morbidity in affected children, needing multidisciplinary care from birth until adulthood. Rehabilitation includes varying degree of surgery, dental treatment, speech therapy and psychosocial intervention, imposing a substantial financial risk for families with a concomitant societal burden.

1.1 Developmental pathogenesis

CL/P occurs at the time of early embryogenesis from a failure in fusion of medial nasal and maxillary processes that results in orofacial clefting involving the upper lip, alveolus and/or primary palate (Johnston et al. 2012).

Development of the lip and palate is outlined in Figure 1.1: the developing frontonasal prominence, paired maxillary processes and paired mandibular processes surround the primitive oral cavity by the fourth week of embryonic development (a). By the fifth week, the nasal pits have formed, which leads to the formation of the paired medial and lateral nasal processes (b). The medial nasal processes have merged with the maxillary processes to form the upper lip and primary palate by the end of the sixth week. The lateral nasal processes form the nasal alae. Similarly, the mandibular processes fuse to form the lower jaw (c). During the sixth week of embryogenesis, the secondary palate develops as bilateral outgrowths from the maxillary processes, which grow vertically down the side of the tongue (d). Subsequently, the palatal shelves elevate to a horizontal position above the tongue, contact one another and commence fusion (e). Fusion of the palatal shelves ultimately divides the oronasal space into separate oral and nasal cavities (f).

The most severe type of defect is the complete cleft of the lip, alveolar process and palate, which can be either unilateral (UCLP) or bilateral (BCLP) (Figure 1.2).

Figure 1.1 Embryological developmental processes of the lip and palate (Dixon et al. 2011).

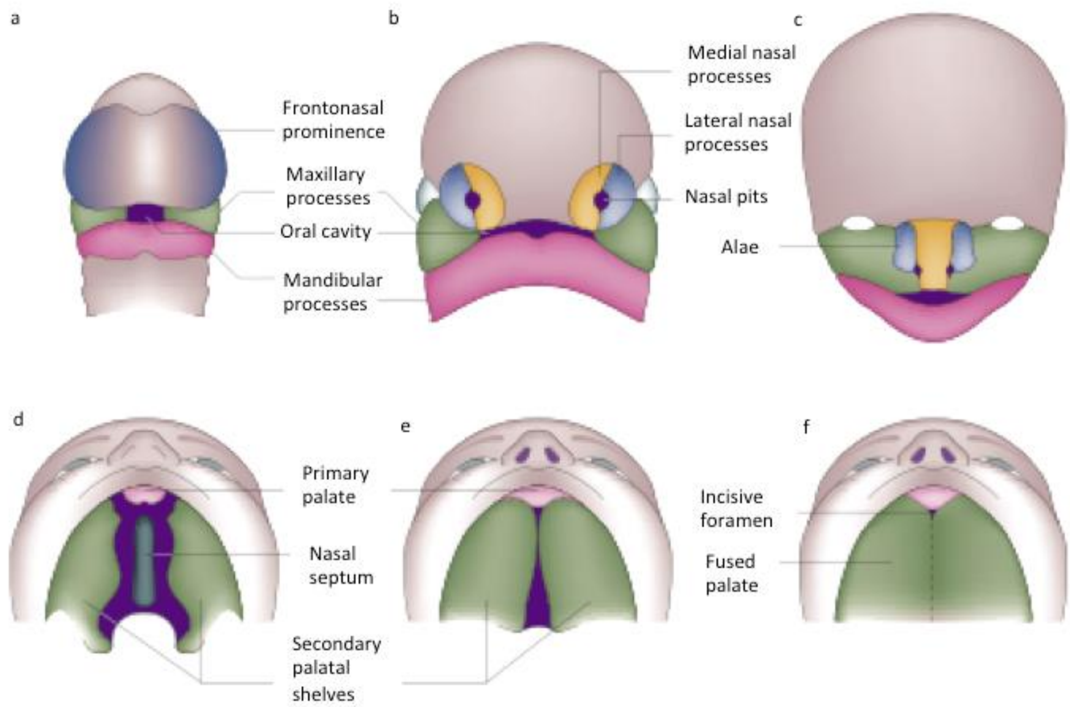
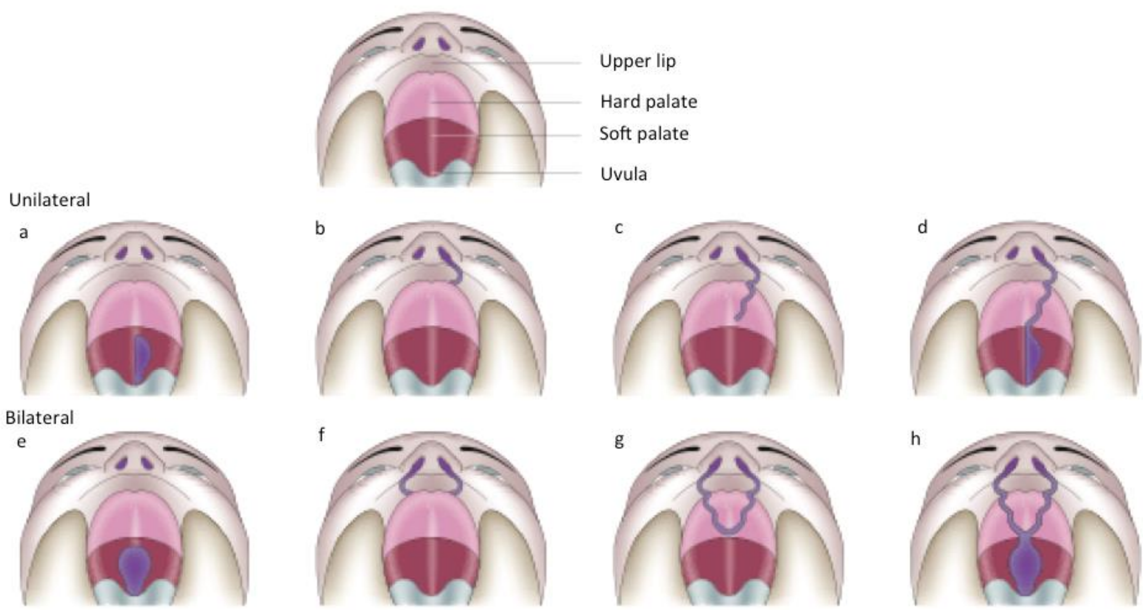


Figure 1.2. Types of clefts (Dixon et al. 2011).



1.2 Aetiology

Aetiology of CL/P is multifactorial, with both genetics and environmental factors interacting (Hartsfield 2015).

Orofacial clefts can be further classified as non-syndromic (NSCL/P – also known as "isolated") or syndromic, depending on whether other structural and/or cognitive abnormalities occur with the cleft. Approximately 70% of all cases of CL/P and 50% of cases of cleft palate lack additional apparent features and are thus considered being non-syndromic (Jugessur et al. 2009). According to some Authors, the non-syndromic designation is therefore arbitrary and to some extent reflects our current lack of certainty about the etiologies of this common congenital anomaly (Marazita 2012).

Compared with other birth defects, orofacial clefts have a high rate of family recurrence (Lie et al. 1994).

Although non-syndromic CL/P can also be inherited as a single-gene disorder, most cases appear to be sporadic and demonstrate some degree of familial aggregation without an obvious mendelian inheritance pattern.

The familiarity has long been noted and reported in pre-1900 publications reporting descriptive or observational family studies as well as in folklore explanations (Marazita 2012).

Fogh-Andersen first supported the genetic component of clefting (Fogh-Andersen 1942), which have been successively confirmed by segregation analysis (Marazita et al. 1986). Studies of twins have been particularly informative regarding the genetics of non-syndromic CL/P: the concordance rate of 40–60% in monozygotic (MZ) twins is higher than the 3–5% rate in dizygotic (DZ) twins, suggesting a strong, but not purely, genetic aetiology (Little & Bryan 1986). The importance of genetic susceptibility is also supported by the predominance of left-sided clefting and the male excess of CL/P.

Epidemiological and experimental evidence suggests that also environmental risk factors such as maternal exposure to tobacco smoke, alcohol, poor nutrition, viral infection, medications and teratogens might be involved in aetiology (Mossey et al. 2009).

Consequently, NSCL/P is considered a genetically complex trait supporting a multifactorial model of inheritance in which genetic risk factor of small individual impact may interact with environmental contributions (Dixon et al. 2011).

1.3 Epidemiology

Isolated or combined cleft of the lip and/or palate have an overall prevalence of approximately 1.7 cases per 1000 liveborn babes (Mossey et al. 2009). Such a prevalence displays important variation worldwide, depending on geographic areas. According to international data collected on the basis of 57 registries during a 5-year period, isolate cleft of lip with or without cleft palate affects in Europe 1.3 to 25.3 babes per 10.000 births (IPDTOC Working Group 2011). Cleft palate is less noticeable externally and such a feature may well have an impact on methods of ascertainment and it may in part explain differences in prevalence, as recorded in different registries.

Higher rates of prevalence of cleft of the lip with or without cleft palate are recorded in Latin America, China, Japan. Lower rates have reported in Israel, South Africa and Southern Europe (Mossey et al. 2009).

It is interesting to note that migrant groups have rates of disease close to those of the area from which they originated than those in the area into which they have moved (Croen et al. 1998).

Cleft of the lip with or without cleft of the palate is more frequent among males (M:F ratio in white ethnic group = 2:1), while isolated cleft palate is typical in females (Mossey & Little 2002).

Additional anomalies are frequently seen in patients with cleft lip with or without cleft palate. Further defects seem to be more common in people with isolated cleft palate(Mossey & Little 2002).

According to a study performed on 4000 patients in Europe, 55% of patients with isolate cleft palate did not show further anomalies, 18% were affected by other defects and in the remaining 27% the cleft was part of a recognised syndrome (Calzolari et al. 2004).

1.4 Clinical management

The most widely adopted management strategy includes the surgical reconnection of the cleft anatomical structures followed by their development to gain proper appearance, occlusion and speech (Mossey et al. 2009).

Management strategies for patients with CLP differ within and between countries. However, there is a general agreement on the goals of treatment: improve the child's ability to eat, speak and hear normally and achieve a normal facial appearance (https://www.mayoclinic.org).

Therapy of CLP consists of a multidisciplinary approach and it involves a number of specialists: otolaryngologists, pediatricians, plastic surgeons, oral surgeons, pediatric dentists, orthodontists, nurses, hearing specialists, speech therapists, genetic counselors and psychologists.

Even though European and WHO recommendations have been issued in the late 1990's, there are data showing that such guidelines are not routinely applied in clinical practice (Neiswanger et al. 2009; Shaw et al. 2001). As a matter of fact, 194 different protocols were recorded in a survey from 201 teams treating unilateral CL/P (Shaw et al. 2001).

In 43% of the teams interviewed, surgeons closed the lip at the first operation and the hard and soft palate together at the second (Shaw et al. 2001). Number of operations needed to close the defect range between 1 and 4 within the protocols evaluated. Half of the teams used presurgical orthopaedic plates (Shaw et al. 2001).

The heterogeneity of surgical approaches reflects the almost complete lack of randomized clinical trials.

As an example, the surgical protocol adopted at the Mayo Clinic follows a timeline which includes:

- Cleft lip repair — within the first 12 months of age;
- Cleft palate repair — by the age of 18 months, or earlier if possible;
- Follow-up surgeries — between age 2 and late teen years.

1.5 Maxillary arch dimensions and occlusion

Maxillary growth in operated CL/P patients is often decreased in the three dimensions. The most important cause of growth inhibition seems to be the iatrogenic effect of surgical intervention and the subsequent constriction induced by scar tissue (Shaw et al. 1992); however, some authors attribute such a deficiency to the developmental hypoplasia of both the alveolar and palatal soft and hard tissues, as well as to functional factors (Kozelj 2000). The maxillary growth deficiency affects the dental arches relationship on the vertical, sagittal and transverse planes, frequently resulting in anterior and/or posterior crossbite occurring in the early dentition (Figure 1.3) (Mars et al. 1992).

Figure 1.3. Examples of crossbite patterns in deciduous dentition of patients with various types of clefts: unilateral posterior and anterior crossbite in UCLP (a); bilateral posterior and anterior crossbite in UCLP (b); bilateral posterior crossbite in BCLP (c).

Crossbite usually affects the cleft side. In UCLP there are a “major segment”, consisting in the praemaxilla fused with the half-maxilla on the healthy side, and the maxillary alveolar segment on the affected side or “smaller segment”. The maxillary constriction mainly involves the “smaller” segment, which usually exhibits an inward deflection on the canine region, resulting in unilateral crossbite (Figure 1.4). The “major segment” may have a normal occlusal scheme.

In BCLP cases, there are an anterior and two posterior displaced alveolar segments. The transverse deficiency is due to the medial shift of both posterior segments, resulting in bilateral posterior crossbite (Figure 1.5).

Figure 1.4. Rotation of the smaller segment in left UCLP and unilateral crossbite on the same side.

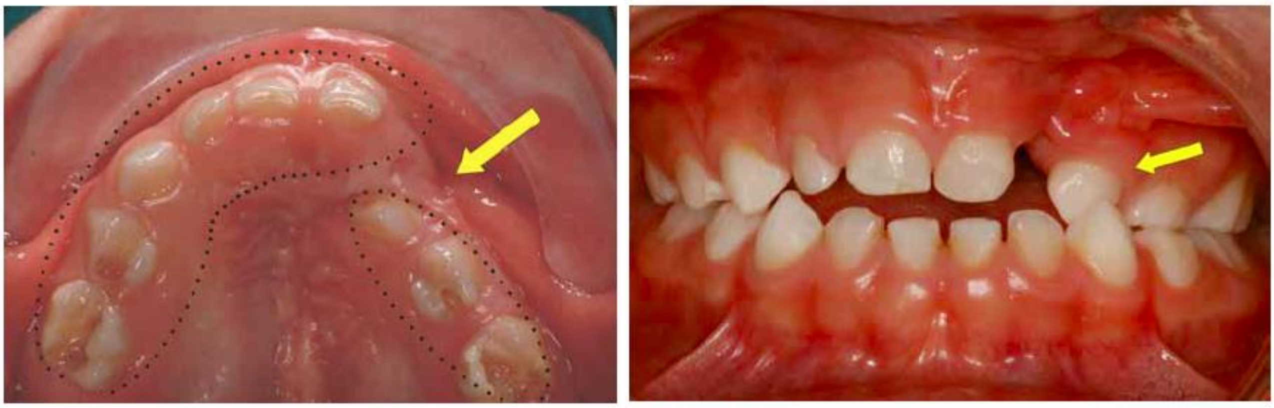
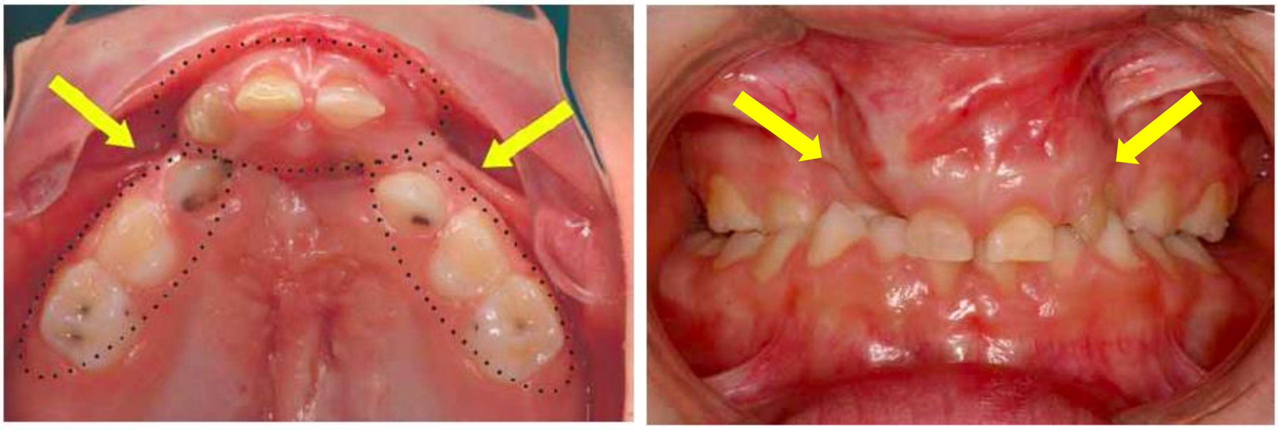


Figure 1.5. Rotation of both posterior segments in BCLP and bilateral posterior crossbite.



Crossbite is a complex, asymmetric and worsening malocclusion, which involves the teeth and affects all the components of the masticatory system together with its functions. It results from dental and/or skeletal discrepancy between the opposing arches and may lead to displacement or malposition of the mandible.

Bjork *et al.* (1964) defined crossbite as a malocclusion that may affect the canine, premolar and molar region of the dental arches, with the buccal cusps of the maxillary teeth occluding lingual to the buccal cusps of the corresponding mandibular teeth. Importantly, this definition subdivides the teeth regions according to different functions. More recently, in 2002, the "Glossary of Orthodontic Terms" defines crossbite as an anomalous relationship of one or more teeth with one or more elements of the opposite dental arch, in the buccal-lingual or labial-lingual direction.

1.6 Alterations of masticatory function in unilateral posterior crossbite

It has long been demonstrated that children with unilateral posterior crossbite display modified chewing patterns during mastication on the crossbite side (Lewin 1985; Ben-Bassat et al. 1993; Throckmorton et al. 2001; Piancino et al. 2006; Sever et al. 2011). Such an alteration consists in a significant increase in the frequency of reverse-sequence chewing pattern, which refers to the movement of the mandible during the closing phase of chewing.

The definition of "reverse chewing cycle" was provided by Lewin (1985) and then adopted by the literature (Throckmorton et al. 2001; Piancino et al. 2006; Sever et al. 2011) and it refers to the inversion of the closing direction of the chewing cycle.

To understand masticatory function from a clinical point of view, it is important to compare chewing patterns between the two sides of the dental arches. In presence of a unilateral posterior crossbite, the number of reverse chewing cycles increases significantly during mastication on the crossbite side in comparison with normal physiological occlusion.

Reverse patterns may be present in small numbers also in physiologically normal conditions, representing a form of abnormal cycle, which may be due, for example, to reuptake of the bolus. These chewing cycles cannot be considered part of the regular pattern. Thus, a reverse sequence chewing cycle is not pathognomonic of crossbite. When such a reverse pattern emerges more often and in significant percentages, it constitutes an unequivocal clinical indicator of crossbite.

The closing direction is the vector of the closing pattern in the last stage of the chewing cycle. The direction of closure in physiological occlusion is linked to the side of mastication, which is the bolus side, and displays a clockwise direction during mastication on the right side, and an anti-clockwise direction during mastication on the left side. This means that in cases of a right unilateral posterior crossbite, during mastication on the right side the chewing cycle displays an anticlockwise closing direction. On the contrary, in cases of a left unilateral posterior crossbite, during mastication on the left side, the closing direction will be clockwise instead of anticlockwise. In normal healthy conditions of occlusion and mastication, the mandible shifts laterally from the bolus side; then, during closure, it shifts medially via the trans-cuspal and inter-cuspal stages of mastication. During a reverse sequence chewing cycle, the mandible shifts first medially and then laterally, in order to deal with the opposite occluding surfaces. The reverse sequence chewing cycle is set and maintained by the automatisms of the central nervous system's motor control based on peripheral input arriving from the periodontal mechanoreceptors (Lund & Kolta 2006; Morquette et al. 2012).

The percentage of reverse sequence chewing cycles during mastication on the crossbite side is extremely high, being around 60-70% on average, depending on the severity of the malocclusion and bolus type. Interestingly, the number of reverse sequence cycles is significantly higher during mastication of hard boluses compared to soft boluses and this increases according to the number of teeth involved in the crossbite condition in the posterior regions (Lewin 1985; Piancino et al. 2006).

It is remarkable that the reverse sequence chewing cycle occurs only on the crossbite side whilst, on the healthy side, the chewing cycle displays normal physiological closing direction. According to the literature (Throckmorton et al. 2001; Piancino et al. 2006; Sever et al. 2011), the chewing pattern during mastication on the healthy side maintains the same

characteristics as physiological mastication: a normal closing direction and normal morphological and positional features (height, width and spatial position display no significant differences from those of mastication in a patient with normal physiological occlusion). Such a finding is extremely important from a clinical point of view and it indicates that dental asymmetry in unilateral posterior crossbite results in a functional asymmetry.
Given that the unilateral posterior crossbite usually occurs at a very early stage, functional asymmetry may disturb the craniofacial development, leading to an asymmetric growth of anatomical structures, that can no longer be corrected by the orthodontic therapy alone (Pirttiniemi et al. 1990; Poikela et al. 1997; Throckmorton et al. 2001; Sonnesen et al. 2001; Thilander & Bjerklin 2012). Such an irreversibility is due to the asymmetric development of complex skeletal and joint structures, which have lower adjustment capacity at the end of the growing process.

1.7 Orthodontic treatment

The role of the orthodontist is crucial in the interdisciplinary management of orofacial clefts. Therapeutic intervention usually starts during the neonatal period with treatment of displaced alveolar segments, and it follows throughout the deciduous and mixed dentition phases with the management of the skeletal and dental components of the developing dentition. Most of the patients will receive orthodontic therapy during adolescence, and sometimes into adulthood (Vig & Mercado 2015).
The continuous and often progressive nature of cleft-related orthodontic problems over the stages of growth and dental eruption makes it difficult to use routine orthodontic approaches. Treatment recommendations can be found for nearly every age; however, no clear-cut guidelines for optimal timing or method of intervention have been developed.
In the following table (Table 1.1) is reported a timeline for dental and orthodontic treatment of CL/P patients; however this treatment timeline varies from child to child, depending on individual characteristics of the cleft, the developing dentition, the facial growth pattern, and the child’s other health care needs (Mercado 2015).

Table 1.1. Timeline for dental and orthodontic treatment of CL/P patients

Age Range	**Intervention**
Birth to 3 months	Evaluation and start of neonatal infant orthopaedics
3 to 6 months	Continued infant orthopaedics as needed
6 to 12 months	Start of paediatric dental care with the first dental visit, prevention of tooth decay, and restoration
4 to 6 years	Orthodontic evaluation for the need of palatal expansion and/or a protraction face mask (see Fig. 2.6)
7 to 11 years	Preparation for bone grafting, including palatal expansion and/or limited incisor alignment
12 to 15 years	After bone grafting, limited orthodontics with further palatal expansion and dental alignment Monitoring of jaw growth
16 to 18 years	Comprehensive orthodontics with or without jaw surgery Preparation of spaces for future replacement of missing teeth
18 to 21 years	Definitive restoration of missing teeth with dental implants and/or a fixed removable prothesis

Particularly, orthodontic treatment of CL/P patients during the deciduous and mixed dentition period has been recommended in order to create more favourable conditions for midfacial growth, normalize the intermaxillary basal relationship and prevent or eliminate functional disturbances (Long R.E. et al. 2000). The most common orthodontic procedures include maxillary expansion to correct the reduced transverse dimension, incisor alignment and proclination to resolve crowding, rotations and anterior crossbites; and maxillary protraction to reduce maxillary retrusion.

Despite the agreement on the need of orthodontic treatment in the multidisciplinary management of CL/P patients, controversy still exists on the best timing to start such a therapy.

AIMS OF THE STUDY

The main aims of the study regard the subject of CL/P:

I. A critical review of the current relevant literature regarding genetics of non-syndromic cleft lip and palate (NSCL/P) has been performed, focusing on multiple methods of genetic investigation, genes and genetic loci best-supported as involved in NSCL/P.

II. The second aim of the research was to report a clinical and epidemiological evaluation of 76 patients affected by different types of CL/P, with regard to maxillary arch width and inter-arch relationship.
In this section, we investigated both the effect of timing and procedure of early orthodontic treatment on development of the dental arches in growing patients with CL/P. Particularly, we compared occlusal changes in children starting orthodontic therapy before six years of age with those in subjects starting treatment later. The null hypothesis was that there was no significant difference between the two groups with regard to treatment effects on widening of the maxillary dentition and on the correction of inter-arch discrepancy. The results have been published in 2017 on The Journal of Craniofacial Surgery (Publication attached to the present Thesis – pages 53 to 58).

III. As third scope, we evaluated the masticatory pattern, through the investigation of the prevalence of reverse sequencing chewing cycles in CL/P children, during chewing on the cross bite and non-cross bite sides before orthodontic treatment.

As additional research, a comprehensive review on the different techniques to reproduce and record head orientation has been performed. The results have been published in 2016 on British Journal of Oral and Maxillofacial Surgery (Publication attached to the present Thesis – pages 59 to 66).

2. MATERIAL AND METHODS

2.1 SEARCH STRATEGY, INCLUSION AND EXCLUSION CRITERIA OF CRITICAL REVIEW

A Medline and Scopus search was conducted in order to identify publications related to the topic, with no limitations of language or time period. Entry words included: “orofacial cleft”, “cleft lip”, “cleft palate”, “genetics”, “nonsyndromic”.
A periodic screening of the databases has been performed, beginning from August 2016. Endpoint of research has been set at October 2016.
Only studies published after 1999 were included in the present critical review.

INCLUSION CRITERIA

Both experimental studies and reviews were considered for the research.
Particularly, among experiental studies, only papers reporting details on patients, animal models and/or tissue samples deriving from non-syndromic CL/P-affected and genetic analysis were selected. Papers included were primarily focused on the relationship between genetic influence on developmentof non-syndromic CL/P. We took in consideration studies performed on humans, animal models, human craniofacial tissues and developing human embryos detailing the disease and providing exact information on methodology of genetic analysis.

EXCLUSION CRITERIA

Case reports, conference proceedings and personal communication were excluded. Studies dealing with genetic analysis of syndromic CLP were not included and, when appropriate, were only cited in order to explain the rationale of selection of candidates genes for non-syndromic CL/P.

2.2 SUBJECTS

Dentoalveolar effects of early orthodontic treatment.

The principles outlined in the Declaration of Helsinki were followed throughout this study and all data were obtained in a clinical context as part as a standardized treatment regime with full acceptance from the parents.

Data of 76 patients (54 males, 22 females; mean age 7,2 years), with various types of orofacial cleft, consecutively referred to the Orthodontic Section of the Academic Hospital of Parma, Italy, between 2004 and 2015, were retrieved and analysed. Variables evaluated included: gender, type of cleft, type of orthodontic treatment and age at different times of follow-up. According to the type of cleft, patients were subclassified as follows: 1) unilateral CLP (UCLP); 2) bilateral CLP (BCLP); 3) Cleft Palate (CP) and 4) Cleft soft palate (CSP).

All patients had dental casts taken before the orthodontic treatment (T0). For 28 patients (17 males, 11 females) dental casts taken at the end of the interceptive orthodontic treatment (T1) were also available. To evaluate the influence of age on treatment response, children of such a group were subclassified according to the age at the beginning of the orthodontic treatment: Group A (age < 6 years) and Group B (age ≥ 6 years). The characteristics of the samples are reported in Table 2.1.

Table 2.1. Demographics of the groups.

	T0				T1			
		Gender		Age (y,m)		Gender		Age (y,m)
Cleft Type	n	M	F	Mean±SD	n	M	F	Mean±SD
All Clefts	76	54	22	7,2±3,6	28	17	11	9,2±2,1
UCLP	53(70%)	38	16	7,3±3,6	20 (72%)	13	7	9,3±2
BCLP	13 (17%)	12	1	7,3±3,7	4 (14%)	3	1	9,1±1,9
CP	5 (6,5%)	1	4	7,2±3,6	2 (7%)	0	2	10,2±1,8
CSP	5 (6,5%)	3	2	7,3±3,9	2 (7%)	1	1	9,9±1,6

n, number of patients; M, male; F, female; y, years; m, months; UCLP, unilateral cleft lip and palate; BCLP, bilateral cleft lip and palate; CP, cleft palate; CSP, cleft soft palate.

Masticatory function and chewing pattern.

Eleven patients (6 males, 5 females; mean age 7,3 years), with various types of orofacial cleft, and consecutively referred to the Orthodontic Section of the Academic Hospital of Parma, Italy, from December 2014 through May 2016, were included in this observational study. The study was approved by the Institutional Review Board of the University Hospital Company – Turin – Italy, n° 764/2014, 23rd July 2014.

The inclusion criteria were as follows: (1) presence of a bilateral posterior, bilateral anterior, unilateral posterior crossbite or physiological occlusion; (2) presence of deciduous, mixed or permanent dentition; the exclusion criteria were: (1) signs or symptoms of dental or myofacial disorders; (2) previous orthodontic therapy; (3) presence of erupting teeth; (4) presence of caries or pain; (5) presence of any prosthesis; (6) presence of diabetes and/or celiac disease.

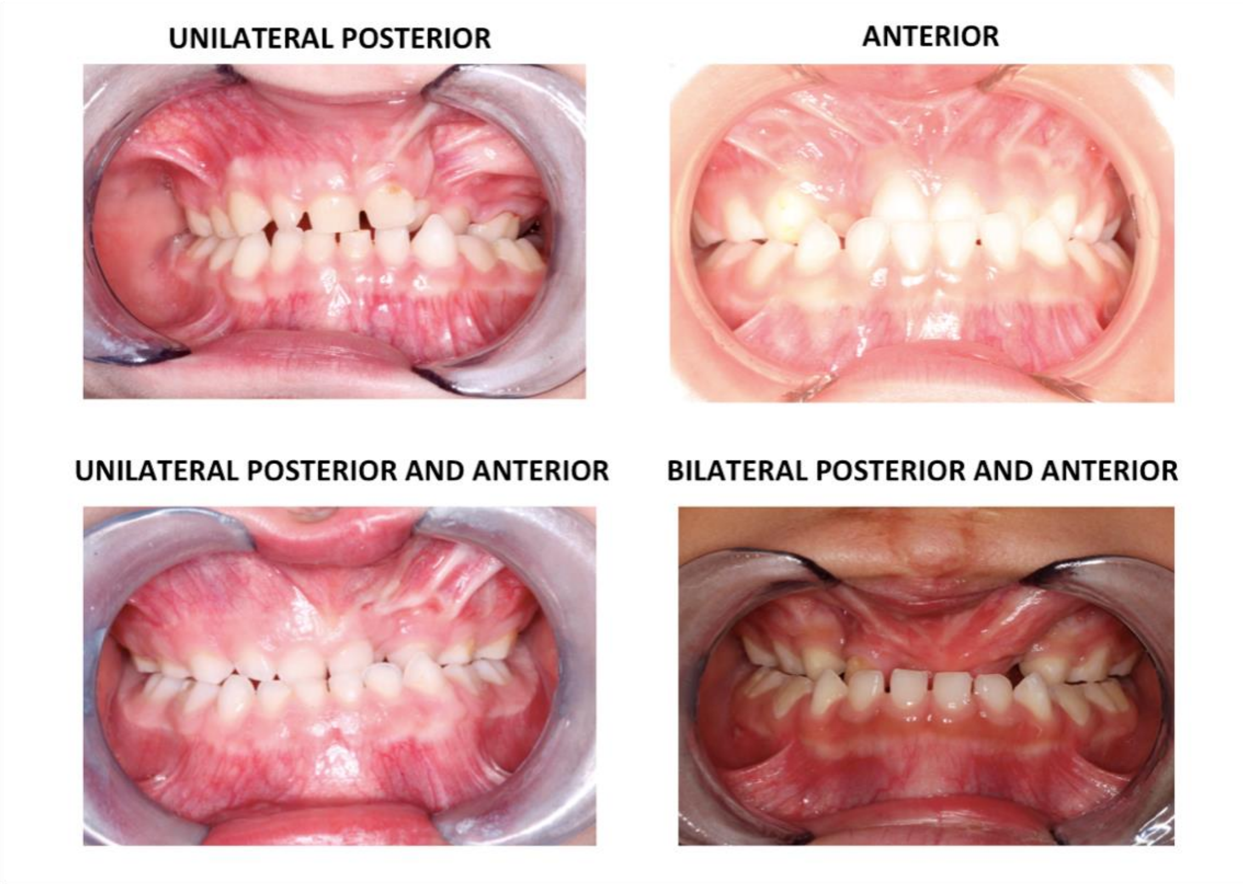
All participants underwent the following sequence of investigations: 1. clinical and orthodontic examination; 2. intra- and extra-oral photos; 3. model casts from alginate impressions for occlusal diagnosis; 4. radiographic evaluation (panoramic, teleradiography in lateral projection) and subsequent cephalometry; 5. Registration of chewing cycles.

The occlusal diagnosis for the selection of the patients was made from the model casts by two skilled operators.

Patients were classified according to the following types of cleft: 1) unilateral CLP (UCLP); 2) bilateral CLP (BCLP); 3) Cleft Palate (CP) and 4) Cleft Lip (CL).

With regard to the occlusion, patients were classified according to the presence and type of crossbite as follows: 1) Unilateral posterior; 2) Anterior; 3) Unilateral posterior and anterior; 4) Bilateral posterior and anterior (Figure 2.1)

Figure 2.1. Crossbite classification in CL/P patients.



2.3 INSTRUMENTS

Dentoalveolar effects of early orthodontic treatment.

DENTAL STUDY MODELS ANALYSIS

All models were cast in white plaster and in centric occlusion and labelled with identification numbers attached to the base of the models.

Dental cast analysis, performed at T0 and T1, took into account the following variables: 1) maxillary arch widths measured with a Beerendonk sliding calliper (measuring size 0-80 mm in tenths of mm). Particularly, inter-molar width was measured as the distance between the mesiopalatal cusp tips of the first molars; inter-canine width was measured as the distance between the cusp tips (Figure 2.2); and 2) dental arch relationships, categorized according to the modified Huddart/Bodenham system (MHB) (Huddart & Bodenham 1972; Mossey et al. 2003). This numerical scoring system requires all maxillary teeth to be scored according to their buccolingual relationship to the corresponding mandibular tooth, except for the lateral incisors, which may be missing or in an abnormal position in CL/P subjects (Figure 2.3). The MHB system is used for the deciduous, mixed, and permanent dentition. The number of teeth scored changes, depending on age: before 6 years, the first permanent molars are not scored, even if erupted and therefore the maximum range of scores is between –24 to +8. After the age of 6, first permanent molars are scored if present; otherwise the midpoint of the maxillary alveolar ridge is used. In this case, the maximum range of scores is –30 to +10.

Figure 2.2. Transversal linear measurements on the study casts.

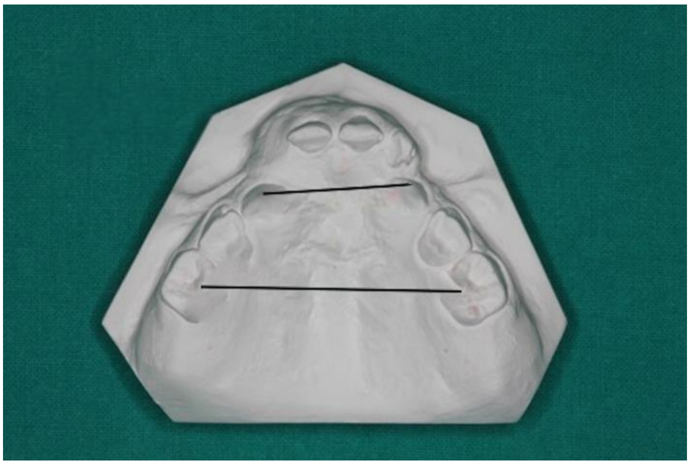
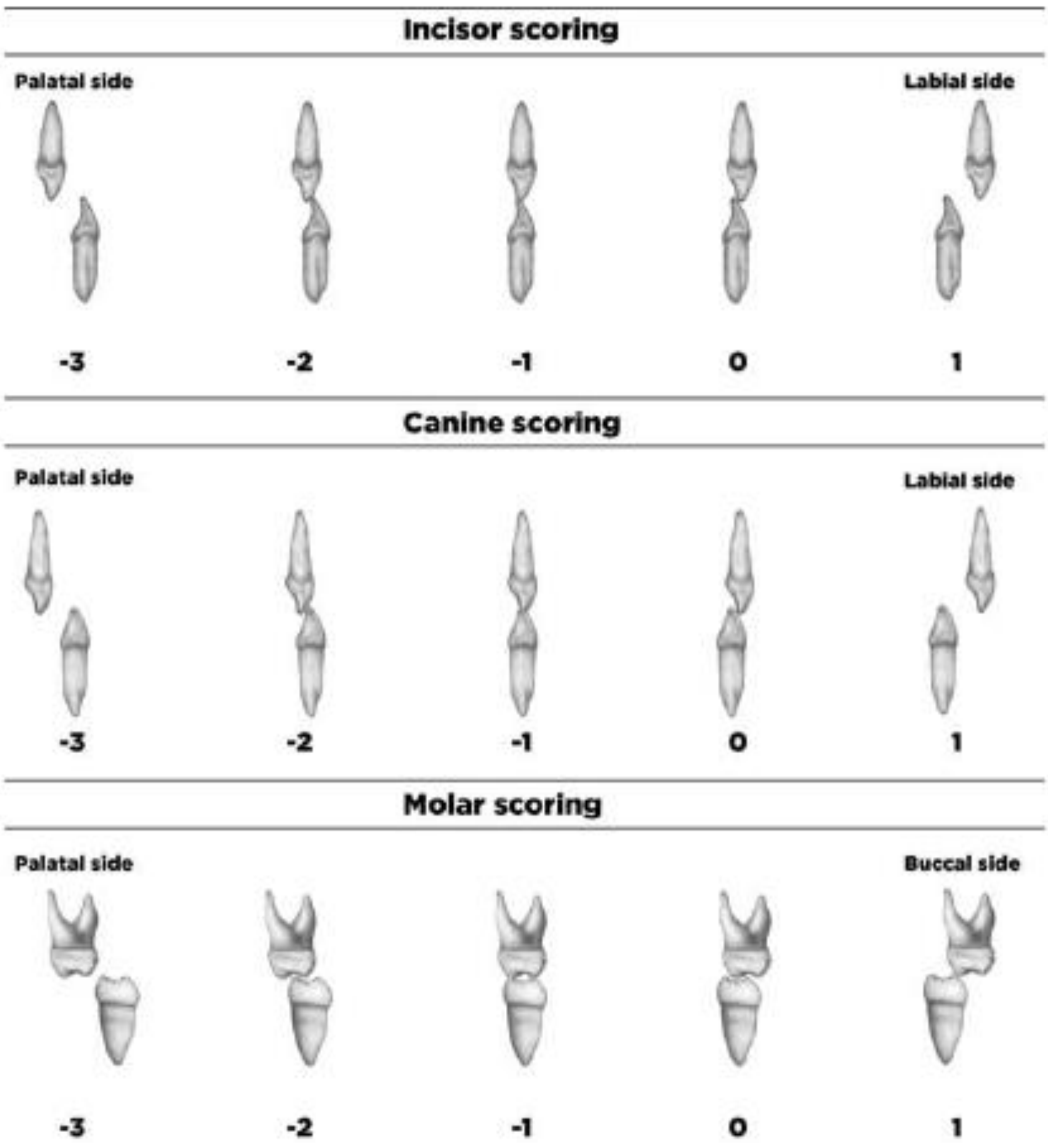


Figure 2.3. Diagram representing the modified Huddart and Bodenham scoring system. Redrawn from Tothill and Mossey (2007) (Tothill & Mossey 2007). The following modifications were taken into account: premolars were scored as for primary molars; if a central incisor was missing, the other central incisor was used to score the missing incisor; where canines were unerupted, the canine score was determined by the midpoint of the maxillary alveolar ridge; if a premolar was absent (for example, due to non-eruption or hypodontia), then a score was allocated equivalent to the adjacent premolar, if erupted. Where no premolars were erupted, the score was determined by the midpoint of the maxillary ridge, in a similar way as previously described. The sum of the scores (the total score) reflected the inter-arch discrepancy.



ORTHODONTIC TREATMENTS

Orthodontic treatment includes maxillary expansion to correct the reduced transverse dimension, maxillary protraction to reduce maxillary retrusion, and incisor alignment and proclination to resolve crowding, rotations and anterior crossbites.

Transverse expansion of the maxilla was obtained through the quad-helix appliance (.038 inches / 0,965 mm Blue Elgiloy), soldered to bands on the maxillary primary second molars or permanent first molars (Figure 2.4). The appliance was initially activated to provide a force of 200 g per side; subsequent reactivations were done extraorally at 6-week intervals.

In order to achieve maxillary protraction, a posteroanterior orthopedic force carried out by a Delaire facial mask connected to an intraoral double arch appliance was applied (Figure 2.5). Two heavy (700 g, 350 g on each side) elastics were attached from the soldered intraoral hooks in the cuspid area to the support bar of the facemask (Figure 2.6). The direction of the forward force was 15 degrees downward in relation to the occlusal plane. Patients were instructed to wear the facemask for 12 hours per day, including at night.

Maxillary incisor rotation, lingual inclination and anterior cross-bite were variously corrected by using partial fixed (Figure 2.7) and removable orthodontic appliances (Figure 2.8).

Figure 2.4. Intraoral view of a quad helix appliance.

Figure 2.5. Intraoral double arch appliance.

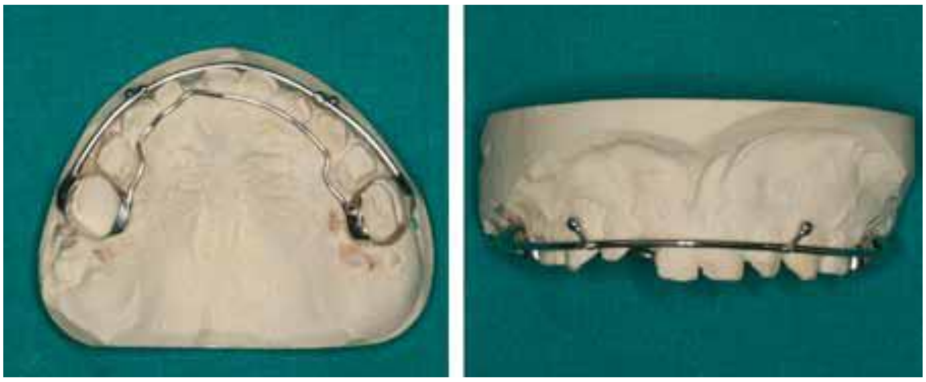


Figure 2.6. Delaire facial mask.

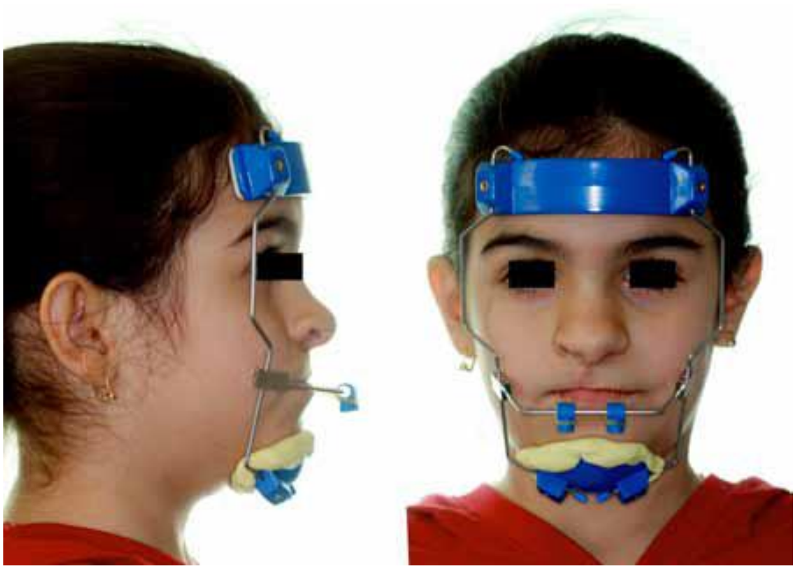


Figure 2.7. Crossbite correction of rotated upper right central incisor by fixed appliance.

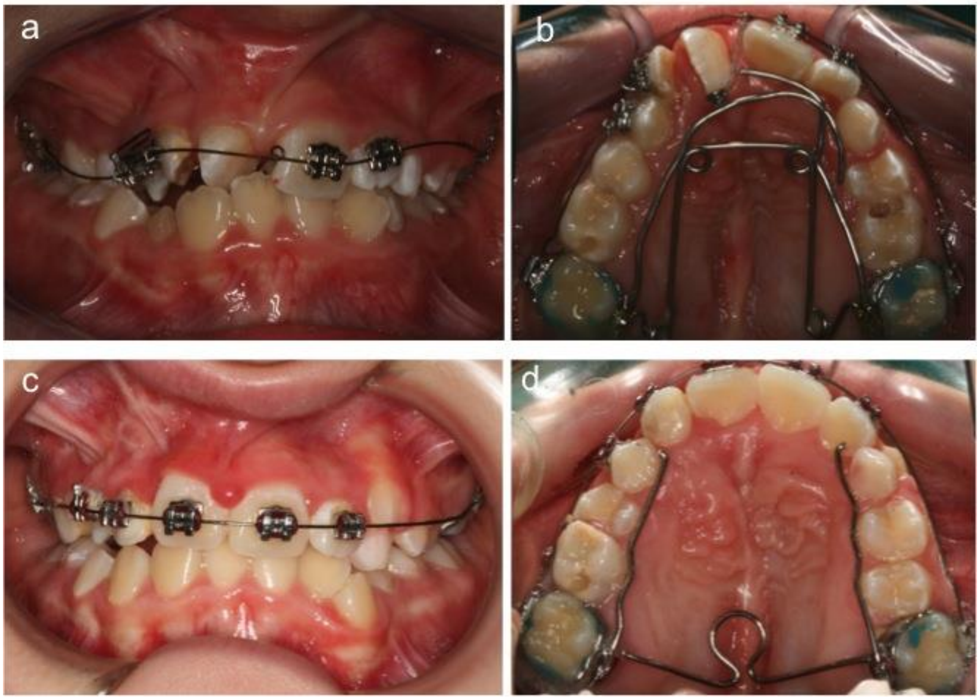
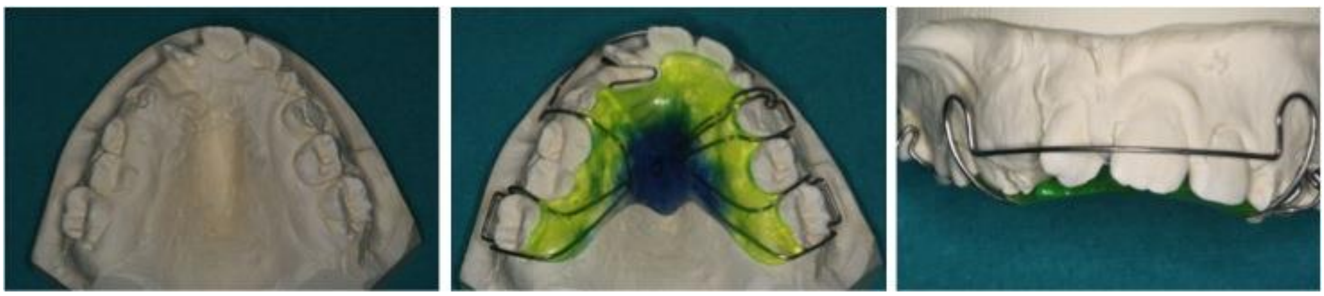


Figure 2.8. Correction of rotated upper right central incisor by upper removable appliance.

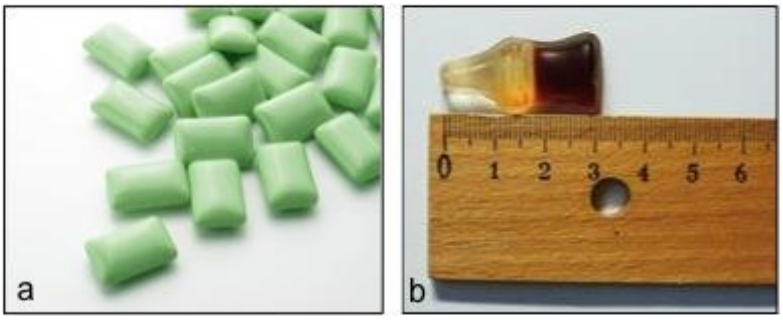


Masticatory function and chewing pattern.

REGISTRATION OF CHEWING CYCLES

Recordings of the chewing cycles were carried out for all subjects with the same protocol, by the same skilled operator. The patients were comfortably seated on a chair and were asked to fix their eyes on a target on the wall, 90 cm directly in front of their seating position, avoiding movements of the head. The recordings were performed in a silent and comfortable environment. Each recording began in a position of maximal intercuspation. The patients were asked to find this starting position by lightly tapping their teeth together and clenching. They were asked to hold this position with a test bolus on the tongue, prior to starting the recording. The participants were then instructed to chew a soft bolus and then a hard bolus, deliberately on the right and left side. The duration of each test was 10 s and each was repeated three times. The side of mastication was visually checked by an operator. The soft bolus was a chewing gum and the hard bolus was a wine gum, with the same size (20 mm in length, 1.2 mm in height and 0.5 mm in width) but with different weights (2 g for the soft bolus and 3 g for the hard bolus). The wine gum was chosen to provide a rubber-like resistance without sticking the teeth (Figure 2.9).

Figure 2.9. The soft (a) and hard (b) bolus.



Mandibular movements were measured with a kinesiograph (K7, Myotronics Inc. Tukwila, Washington, USA) (Figures 2.10 and 2.11), which measures jaw movements within an accuracy of 0.1 mm. Multiple sensors (Hall effect) in a light-weight array (113 g) tracked the motion of a magnet attached to the midpoint of the lower incisors (Jankelson 1980). The kinesiograph was interfaced with a computer for data storage and subsequent analysis. The kinematic signals were analyzed using a custom-made software (Department of Orthognatodontics, University of Turin, Italy). The first cycle, during which the bolus was transferred from the tongue to the dental arches, was excluded from the analysis. The chewing cycles were divided into non-reverse and reverse, based on the vector direction of closure. From each cycle, the following variables were extracted: (i) cycle duration; (ii) opening duration; (iii) closing duration; (iv) maximum closing velocity; (iv) maximum opening velocity and (v) closure angle. The values computed for each variable were averaged over all cycles recorded for the same side of mastication and the same bolus.

Surface EMG signals were recorded from the masseter muscles of both sides using a multichannel EMG amplifier modified with a bandwidth of 45–430 Hz per channel. The EMG amplifier is part of the K7 Diagnostic System. The relatively large high-pass frequency in EMG recordings was selected to reduce low-frequency movement artifacts during chewing. Two electrodes (Duotrode silver/ silver chloride EMG electrodes; Myotronics) were positioned over the masseter muscles bilaterally with an interelectrode distance of 20 mm. Before electrode placement, the skin was cleaned with light abrasive paste and ethanol and the electrodes were positioned along the mandibular angle – cantus straight line to ensure consistency of electrode placement between sessions (Castroflorio et al. 2005). Kinematic and EMG data were recorded concurrently. The surface EMG was rectified and low-pass filtered with a 10 Hz cut-off frequency (signal envelope). During each cycle, the maximum values of the EMG envelope of both sides were computed. The percent difference between ipsilateral (deliberate chewing side) and contralateral masseter peak EMG was computed. The percent difference between ipsilateral and contralateral masseter peak EMG was calculated as an indication of the coordination between the bilateral masseter muscles. Such normalization overcomes the known limitations in the use of the EMG amplitude and allows pooling data from different subjects and computing ensemble averages (Piancino et al. 2008). Muscle onset periods were computed by a wavelet-based method for muscle on–off detection, which provides accuracy suitable for clinical applications and is completely automatic without any intervention required by the operator. Next, the occlusal pause was calculated as the time difference between the end of the EMG activity of the masseter and the beginning of the next opening phase.

Figure 2.10. The K7 Kinesiograph system.

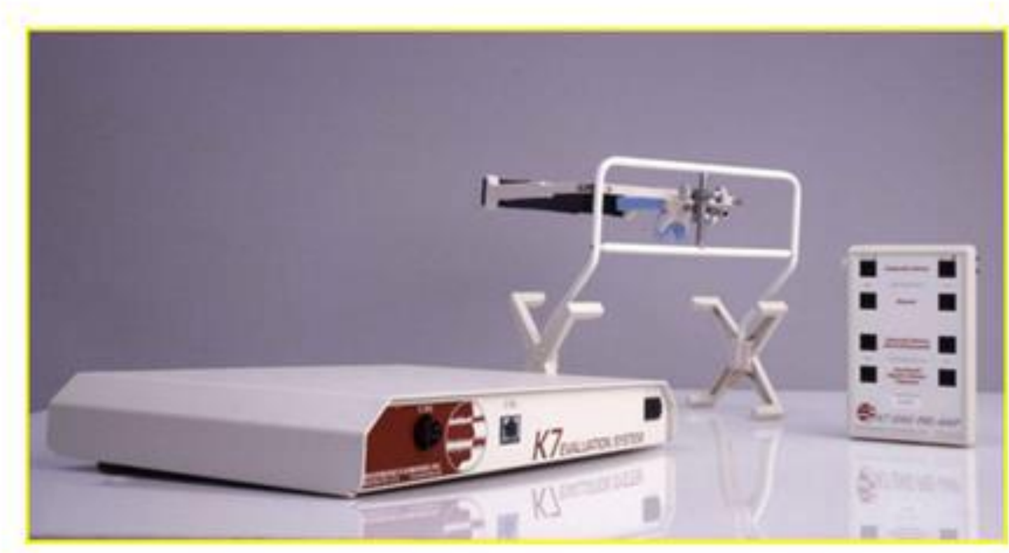
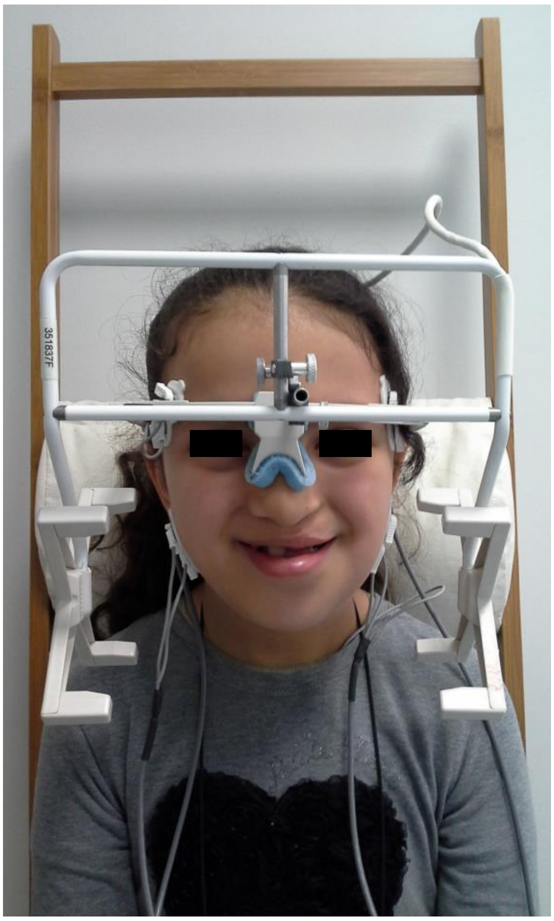


Figure 2.11. The multiple-sensor lightweight array of the K7 Kinesiograph.



2.4 STATISTICAL ANALYSIS

STATISTICAL METHODS

The data was evaluated for normality using the Shapiro-Wilk test. Descriptive statistics were calculated for all variables at T0, and for variations from T0 to T1 either in the overall group of patients and in subgroups A and B. Differences between types of cleft at T0 were established through the Analysis of Variance (ANOVA) and Tukey *post-hoc* tests.

Paired *t*-tests were used to investigate the overall treatment effect by comparing the longitudinal changes in T0 and T1.

The following statistical comparisons were carried out with the independent *t*-test: (1) starting form: differences between the subgroup A and the subgroup B at T0; and (2) treatment effects: T0-T1 changes in the subgroup A *vs* T0-T1 changes in the subgroup B.

Data were analysed with the IBM-SPSS version 20 statistical software. Statistical significance was tested at $P < 0.05$. The power of the study was calculated on the basis of the difference between T0 and T1 in the treated group for a relevant variable (inter-canine distance) as reported in a previous longitudinal investigation of similar nature and on the basis of the standard deviation of this difference.[10] The power exceeded 0.80 at an α level of 0.05.

METHOD ERROR

To test the precision of the measurements, 25 dental casts were randomly selected and were remeasured by the same operator after a 1-month interval. No systematic error was detected.

Random errors were estimated with Dahlberg's formula. The errors for linear measurements ranged from 0.1 mm for inter-canine distance, to 0.2 mm for inter-molar distance. The intra-observer agreement for MHB score, analyzed by the weighted kappa statistic, was good (kappa=0.62).

3. RESULTS

3.1 CRITICAL REVIEW

Analytic results of search strategy

On the basis of title screening 70 papers were selected for undergoing the phase of abstract and/or full text examination. We were able to retrieve 39 full text papers. Of the remaining 31 publications, relevant data were in all cases available within the abstract.

On the basis of the typology of study, we were able to identify 16 reviews (narrative, critical), 46 studies on humans or human derived tissues and 8 studies on animal model (mouse, zebrafish).

Studies were subclassified into 4 groups, according to the genetic approach used: 1) linkage analysis; 2) association studies; 3) identification of chromosomal anomalies or micro-deletions in cases and 4) direct sequencing of affected individuals. These methods can be applied to candidate genes or genome-wide strategies can be used. Results of the present research show that there have been 8 independent genomic wide association studies (GWASs) for CL/P, one genome-wide meta-analysis of two CL/P GWASs, and 2 GWASs for isolated left palate (CP).

Critical review of the selected studies

Orofacial clefts are a heterogeneous group of disorder, showing a decreased penetrance and a wide range of expressivity.

Expressivity, which describes the severity of the disease, can vary considerably among affected individuals, ranging from cleft lip alone, to cleft lip plus cleft palate, to cleft palate alone. Despite the wide spectrum of phenotypic presentations, NSCL/P are generally defined as qualitative traits (affected *versus* unaffected).

Recent evidence, however, suggests that minor defects, including microforms or sub-clinical physical features, are also part of the spectrum of NSCL/P (Weinberg et al. 2009). These sub-clinical phenotypes are observed in either individual with CL/P and/or their “unaffected” relatives and include craniofacial measures (Weinberg et al. 2006), dental anomalies such as tooth agenesis, microdontia and supplementary teeth (Vieira et al. 2008), brain structural differences (Nopoulos et al. 2002; Weinberg et al. 2013) and dermatoglyphic lip print whorls (Neiswanger et al. 2009). Visible microforms observed for lip and palate are also defects of the *orbicularis oris* muscle (Neiswanger et al. 2007; Rogers et al. 2008; Weinberg et al. 2008), bifid uvula, submucous CP, and velopharingeal insufficiency.

Such minor structural variants may represent the mildest physical expression of risk genes for orofacial clefting. Thus, the increased presence of subclinical phenotypes in unaffected relatives may explain incomplete penetrance observed in families with overt clefts as well as the discordance in monozygotic twins. Interestingly, according to a recent study on Danish twins, the recurrence risks for offspring of the affected and unaffected twin in discordant monozygotic pairs is essentially identical (Grosen et al. 2011).

Furthermore, incorporation of such phenotypic distinction should be considered in the design of genetic studies, since power is reduced when diverse phenotypes of different etiologies are merged (Leslie & Marizita 2013).

Inheritance patterns, genetic heterogeneity, penetrance and expressivity can significantly impact the ability to identify causative genes (Lidral et al. 2008).

GENETIC APPROACHES TO NON-SYNDROMIC CL/P

After the advent of the genomic era, many of the genetic variants or mutations underlying syndromic forms of CL/P have been identified (Dixon et al. 2011). By contrast, less definitive progress has been made in identifying putative causal associations with the more common non-syndromic CL/P, owing to its complex etiology and genetic heterogeneity.

To date, genetic approaches to non-syndromic CL/P have included: linkage analysis, association studies, identification of chromosomal anomalies or micro-deletions in cases and direct sequencing of affected individuals. These methods can be applied to candidate genes or genome-wide strategies can be used. Each approach has its own advantages and disadvantages, some of which will depend on the underlying genetic architecture of the disease, as well as the realities of economics and technology (Dixon et al. 2011).

CANDIDATES GENES

Initial efforts to identify genes for non-syndromic CL/P were based on candidate gene approaches, since Ardinger and colleagues first found a positive association between CL/P and variants in TGFA (transforming growth factor, alpha) (Ardinger et al. 1989). Such approach relies on the selection of genes or genetic regions that are known *a priori* to be involved in the biological processes of the trait.

Candidate genes have been chosen from a variety of sources, including cleft phenotype in transgenic or knockout mouse models, studies on syndromic forms, role in nutritional or detoxification pathways, and cytogenetic location adjacent to chromosomal anomalies associated with orofacial cleft phenotypes (Lidral & Murray 2004).

Analysis of gene expression during facial development is another powerful tool for identifying lists of candidate genes and providing biological plausibility for the association. The Craniofacial and Oral Gene Expression Network database (COGENE, now available through the FaceBase online resource, www.FaceBase.org) provides human craniofacial tissue expression data from early stage, while the EMAGE database catalogs extensive gene expression information for the developing human embryo (Richardson et al. 2014).

Although positive results are found in many genes/regions, a consistently replication is not common across the studies, primarily owing to a lack of adequate sample size.

In addition to the previously mentioned TGFA on chromosome 2p13, the following loci have the most supporting data in terms of published significant results and consistent replication: 1q32.2 (interferon regulatory factor 6, IRF6), 4p16 (homeobox 1, MSX1), 4q31 (anonymous markers), 14q24 (trasforming growth factor beta-3, TGFB3), 17q21 (retinoic acid receptor alpha, RARA), and 19q13 (proto-oncogene BCL3) (Murray 2002).

GENOME WIDE LINKAGE STUDIES

Linkage analysis studies are based on the co-segregation of genetic loci with disease and can be performed in large, multiplex families or in smaller but inbred families, or in pairs of affected relatives.

Multiple genome-wide linkage scans have been performed for NSCL/P. Although each study noted a number of positive signals, none had LOD scores reaching genome-wide significance because of limited sample size. Therefore, a consortium of research groups pooled their studies and identified the first genome-wide significant results for CL/P on regions 1q32, 2p13, 3q27-28, 9q21, 14q21-24, and 16q24 (Marazita et al. 2004). Subsequent fine mapping of these regions showed significant results for single-nucleotide polymorphisms (SNPs) in IRF6, previously associated in candidate gene studies, and in FOXE1 (forkhead box E1, on chromosome 9q21) (Marazita et al. 2009; Moreno et al. 2009; Letra et al. 2010; Ludwig et al. 2014).

GENOME WIDE ASSOCIATION STUDIES

Among the new genomic tools, GWASs are considered ideal for dissecting common, complex (non-single-gene) traits and NSCL/P is one of the few birth-onset disorders to have been investigated with this powerful method.

To date, there have been eight independent GWASs for CL/P (Birnbaum, Ludwig, Reutter, Herms, Steffens, et al. 2009; Grant et al. 2009; Mangold et al. 2010; Beaty et al. 2010; Camargo et al. 2012; Sun et al. 2015; Wolf et al. 2015; Leslie, Carlson, et al. 2016), a genome-wide meta-analysis of two CL/P GWASs (Ludwig et al. 2012), and two GWASs for CP (Beaty et al. 2011; Leslie, Liu, et al. 2016).

Collectively, these studies have demonstrated that orofacial clefts exhibit significant genetic heterogeneity, having successfully identified multiple genome-wide significant associations with CL/P as well as potential gene-environment interactions for CP. Notably, for CL/P at least 20 different loci have been confirmed with statistical and biological supporting data.

Below, we provide a brief description of each study, with emphasis for the loci that had reached genome-wide significance and population-specific associations. Positive results are summarized in Table 1.

The first successful GWAS for CL/P was performed by Birnbaum et al. using a European case-control sample and confirmed the causative role of IRF6, which had previously been identified in candidate gene studies (Zucchero et al. 2004; Rahimov et al. 2008; Jugessur et al. 2008; Birnbaum, Ludwig, Reutter, Herms, de Assis, et al. 2009) and linkage analysis (Marazita et al. 2009). Additionally, the authors discovered an extremely strong association on a "gene desert" region on chromosome 8q24 (Birnbaum, Ludwig, Reutter, Herms, Steffens, et al. 2009).

Grant et al. independently replicated such results in an increased population from the United States (Grant et al. 2009). In another study, Mangold et al. confirmed previous findings and identified additional significant signals on chromosome 10q25 near VAX1 and on chromosome 17q22 near NOG (Mangold et al. 2010).

The GENEVA Cleft Consortium study used for the first time case-parent trios, including families of European, Asian, and mixed ancestry (Beaty et al. 2010). In the combined analysis for all populations, this consortium study reconfirmed the previous associations with 1q32 (IRF6), 8q24 and 10q25 (VAX1), and identified novel loci on 1p22 (ABCA4) and 20q12 (MAFB). Interestingly, differences between the strength of association were noted according to ethnicity: IRF6, MAFB and ABCA4 reached genome-wide significance with stronger signals in Asian compared to European population, whereas the statistical evidence for 8q24 region was strongest among European ancestry.

To identify additional susceptibility loci and explore population-specific association, Ludwig et al. conducted the first meta-analysis combining data from the GENEVA Cleft Consortium and Mangold et al. studies (Ludwig et al. 2012). They confirmed associations with all previously identified loci and identified six additional susceptibility regions (1p36, 2p21, 3p11.1, 8q21.3, 13q31.1 and 15q22), five of which (excluding 15q22) seemed to be involved in both European and Asian populations. Furthermore, performing an analysis of phenotypic variability, they demonstrated that the genetic locus 13q31 was exclusively associated with cleft palate in the presence of a cleft lip.

In order to trace potential recessive loci that confer a risk of susceptibility for NSCL/P, Camargo et al. performed a GWAS using extended and multigenerational pedigrees of known consanguinity from the Paisa community (a genetic isolate in Colombia, South America) (Camargo et al. 2012). They found new recessive loci overcoming the threshold for GWAS significance in the region 8p23.2, 11q25 and 19p12. In the 8p23.2 region, the CUB and Sushi Multiple Domains 1 (CSMD1) gene is contained; in 11q25 the beta-1,3-glucuronyltransferase 1 (B3GAT1) and beta-galactosidase-1-like protein 2 (GLB1L2) genes, and in 19p12, the Homo Sapiens Zinc Finger Protein 431 (ZNF431) and Homo Sapiens Zinc Finger Protein 714 (ZNF714) genes. However, the functional association between these genes and the genesis of NSCL/P remains to be elucidated.

Wolf et al. performed two parallel GWASs on two species, domestic dogs and humans (Wolf et al. 2015). Both studies provided evidence for a role of the same gene, ADAMTS20 chromosome location 12q12, in CL/P development in dogs and as a candidate gene for CL/P development in humans.

To further elucidate the genetic architecture of NSCL/P in Chinese individuals, Sun et al. conducted a case–control-based GWAS followed by two rounds of replication in a Chinese population (Sun et al. 2015). They identified a new susceptibility locus at 16p13.3 (between CREBBP and ADCY9) associated with NSCL/P. They also confirmed that the reported loci at 1q32.2, 10q25.3, 17p13.1 and 20q12 are involved in NSCL/P development in Chinese populations.

Although all these studies were well designed, they mainly focused on susceptibility loci in people of European ancestry and some of them involved individuals from Asia. Thus, Leslie et al. conducted a GWAS on multiethnic sample including European, Asian, African and Central and South American ancestry (Leslie, Carlson, et al. 2016).

This GWAS revealed novel associations on 2p24 near FAM49A, a gene of unknown function, and 19q13 near RHPN2, a gene involved in organizing the actin cytoskeleton. Other regions reaching genome-wide significance were 1p36, 1p22, 1q32, 8q24 and 17p13, all reported in previous GWASs. Stratification by ancestry group confirmed the population specificity of some risk loci (e.g. 8q24 in European) and revealed a novel association with a region on 17q23 near TANC2, among individuals with European ancestry. Interestingly, the Central/South American group showed evidence of association with risk loci previously identified with either European or Asian ancestry, reflecting the admixture of their population history.

Therefore, all these studies targeted multiple regions associated with the risk of NSCL/P confirming the multifactorial nature of this disease and suggested that European and Asian populations may have different causal variants in the same locus as a result of different genetic backgrounds.

Interestingly, with the important exception of IRF6, the significant risk loci from GWAS of CL/P are diverse than the significant risk loci from genome-wide linkage analyses, this highlighting the different strengths of the two approaches (Marazita 2012). Association studies are more sensitive in detecting common variants of small effect size, while linkage studies are more effective in detecting etiologic genes displaying allelic heterogeneity. Thus, if several different variants (especially rare variants) within a gene can cause orofacial clefts, linkage is much more likely to detect such genes. Further, it is remarkable that the study samples differ for the two approaches: for linkage analysis, multiplex families are necessary (either extended kindreds or affected relative pairs); for association approaches, the most common study samples are either case/control series or nuclear trios. Consequently, the linkage studies were implemented in familial cases, which make up approximately 20%–30% of CL/P samples, and the association studies were implemented in sporadic cases.

In Table 2 we summarize the genomic regions that reached genome-wide significant results from either linkage or association genome scans. For each region we also list those candidate genes, which have been confirmed by supportive evidence such as replication, sequencing, functional, expression studies, and/or association with syndromic forms.

Unlike the multiple studies of NSCL/P, there are few genetic studies of non-syndromic CP. This has been influenced perhaps by the smaller number of cases and more confusion from confounding syndromes.

In the European CL/P GWAS, SNPs from the four best-supported loci (1q32, 8q24, 10q25, and 17q22) were also tested in CP trios but showed no statistically significant results, implying little or no overlap in the findings for CL/P *versus* CP (Mangold et al. 2010).
The first GWAS for CP was performed by Beaty e t al., who found no genome-wide significant signals until gene-environment interaction models were applied (Beaty et al. 2011). Specifically, the authors included three common exposures during pregnancy, such as maternal smoking, alcohol consumption and multivitamin supplementation. The fallowing significant gene-environment interaction were detected: MLLT3 and SMC2 on chromosome 9 with alcohol consumption, TBK1 and on chromosome 12 and ZNF236 on chromosome 18 with maternal smoking, and BAALC on chromosome 8 with multivitamin supplementation.
Recently, Leslie et al. reported the results of a GWAS of non-syndromic CP, with a two-stage study design consisting of case and control subjects and case-parent trios. Significant results were limited to the case-control arm of the study and identified an association between nonsyndromic CP and GRHL3 locus in case subjects of European ancestry, independently replicated. The authors further examined this variant by *in vivo* zebrafish experiments and *in vitro* cell-based transactivation assays and concluded that it is likely to be an etiologic variant for nonsyndromic CP. It is noteworthy that these findings are in agreement with the recent discovery of GRHL3 mutations as a second cause of van der Woude Syndrome (Peyrard-Janvid et al. 2014). By contrast, this study did not replicate a previously reported association with FAF1 (Ghassibe-Sabbagh et al. 2011), probably owing to the insufficient sample size.

In Figure 3.1 are presented the chromosomal locations of candidate genes for NSCL/P.

Figure 3.1. Chromosomal location of candidate genes for NSCL/P.

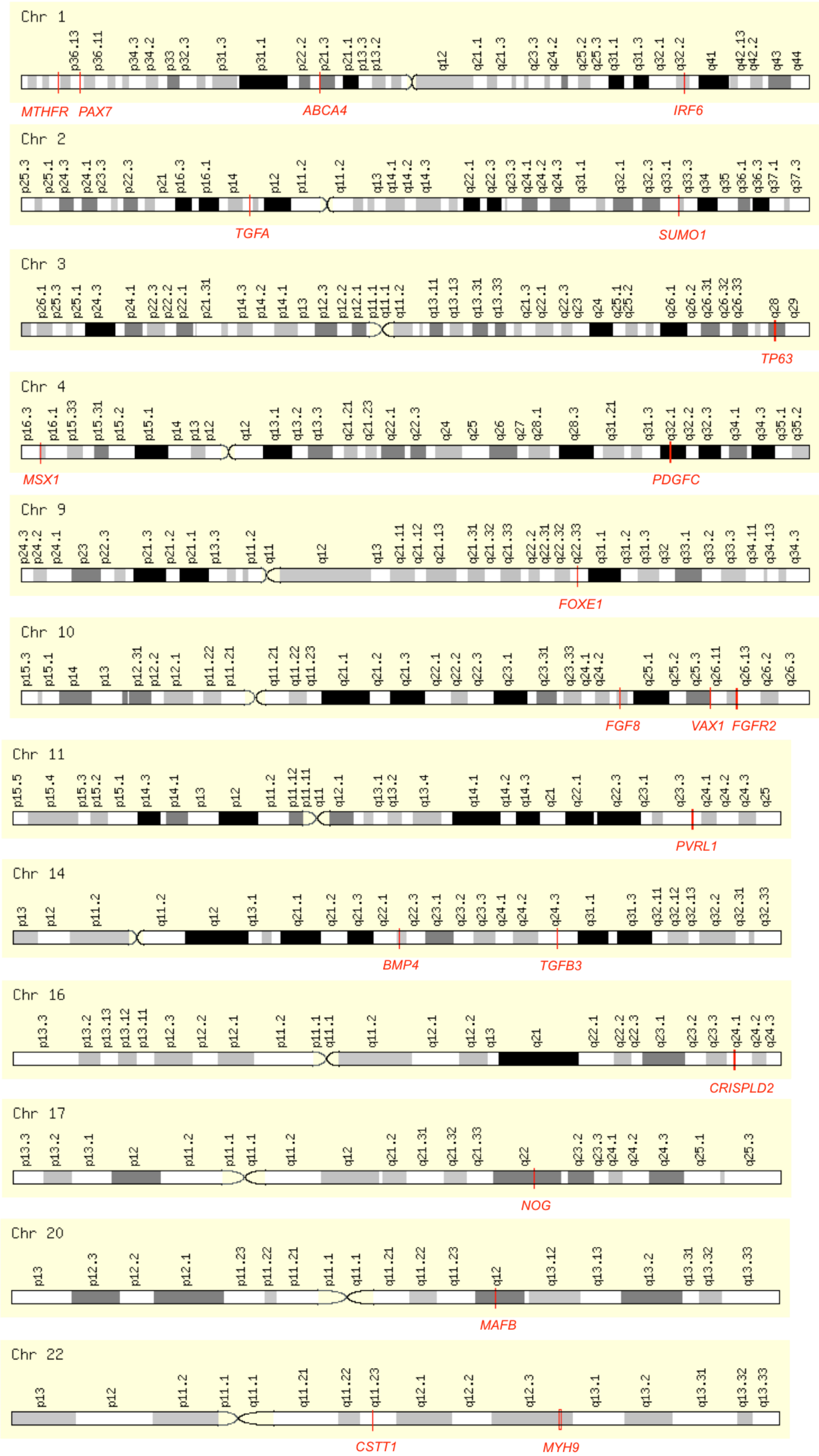


Table 3.1. Summary of GWAS DATA for NSCL/P

		Independent GWAS								Met-analysis
Locus	Candidate gene	Birnbaum (2009)	Grant (2009)	Mangold (2010)	Beaty (2010)	Camargo (2012)	Sun (2015)	Wolf (2015)	Leslie (2016)	Ludwig (2012)
1p22	*ARHGAP29* *ABCA4*				X				+	+
1p36	*PAX7*				*				+	X
1q32	*IRF6*	X		+	+		+		+	+
2p21	*THADA*			*						X
2p24	*FAM49A* *MYCN*								X	
3p11	*EPHA3*									X
8q21	*MMP16*								*	X
8p23.2	*CSMD1*					X				
8q24		X	+	+	+				+	+
10q25	*VAX1*			X	*		+		*	+
11q25	*B3GAT1* *GLB1L2*					X				
12q12	*ADAMTS20*							X		
13q31	*SPRY2*			*					*	X

15q22	*TPM1*	*					X
16p13.3	*CREBBP* *ADCY9*				X		
17q22	*NOG*	X				*	+
17p13	*NTN1*				+	+	
19p12	*ZNF431* *ZNF714*			X			
19q13	*RHPN2*					X	
20q12	*MAFB*		X		+	*	+

X, discovered genome-wide significance
+, confirmed genome-wide significance
*, suggestive of association

Table 3.2. Overview of relevant genes in non-syndromic CL/P genesis.

Gene	Name	Cytogenetic location	OMIM	Cleft Syndrome	Protein		Evidence
					Category	Function (*)	
ABCA4	ATP-binding cassette, subfamily A, member 4	1p22.1	601691		Transport carrier	Acts in the visual cycle.	GWAS
BMP4	Bone morphogenetic protein 4	14q22.2	112262		Structural protein/ Signaling cytokine growth factor	Induces cartilage and bone formation. Also act in mesoderm induction, tooth development, limb formation and fracture repair.	M
CRISPLD2	Cysteine-rich secretory protein LCCL domain-containing 2	16q24.1	612434		Transcription factor	Promotes matrix assembly.	CGA
FGF8	Fibroblast growth factor 8	10q24.32	600483		Signaling growth factor	Plays an important role in the regulation of embryonic development, proliferation, differentiation and migration.	M
FGFR2	Fibroblast growth factor receptor 2	10q26.13	176943		Enzyme, signaling growth factor, receptor	Tyrosine-protein kinase that acts as cell-surface receptor for fibroblast growth factors and plays an essential role in the regulation of cell proliferation, differentiation, migration and apoptosis, and in the regulation of embryonic development.	M
FOXE1	Forkhead box E1	9q22.33	602617	Bamforth-Lazarus	Transcription factor	Transcription factor that binds consensus sites on a variety of gene promoters and activate their transcription. Involved in proper palate formation, most probably through the expression of MSX1 and TGFB3 genes, which are direct targets of this transcription factor.	L, CGA, M
GSTT1	Glutathione S-transferase theta 1	22q11.2	600436		Enzyme	Conjugation of reduced glutathione to a wide number of exogenous and endogenous hydrophobic electrophiles.	CGA

IRF6	Interferon regulatory factor-6	1q32.2	607199	Van der Woude	Regulatory, transcription factor, signaling cytokine	Probable DNA-binding transcriptional activator. Key determinant of the keratinocyte proliferation-differentiation switch involved in appropriate epidermal development. Plays a role in regulating mammary epithelial cell proliferation.	GWAS, CGA, L, M
MAFB	v-maf musculoaponeurotic fibrosarcoma oncogene homolog B	20q12	608968		Transcription factor	Acts as a transcriptional activator or repressor, involved in development and differentiation of keratinocytes.	GWAS
MSX1	Muscle segment homeobox 1	4p16.2	142983		DNA associated, transcription factor	Acts as a transcriptional repressor. May play a role in limb-pattern formation. Acts in cranofacial development and specifically in odontogenesis.	CGA, M
MTHFR	Methylenetetrahydrofolate reductase	1p36.22	607093		Enzyme	Catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, a co-substrate for homocysteine remethylation to methionine.	CGA
MYH9	Myosin heavy chain 9	22q12.3	100775		Motor/contractile	Cellular myosin that appears to play a role in cytokinesis, cell shape, and specialized functions such as secretion and capping.	CGA
NOG	Noggin	17q22	602991		Regulatory	Inhibitor of bone morphogenetic proteins (BMP) signaling which is required for growth and patterning of the neural tube and somite. Essential for cartilage morphogenesis and joint formation.	GWAS
PAX7	Paired box 7	1p36.13	167410		Transcription factor, tumor suppressor	Transcription factor playing a role in myogenesis through regulation of muscle precursor cells proliferation.	GWAS, CGA
PDGFC	Platelet derived growth factor C	4q32.1	608452		Signaling growth factor	Growth factor that plays an essential role in the regulation of embryonic development, cell proliferation, cell migration, survival and chemotaxis. Potent mitogen and chemoattractant for	CGA, M

						cells of mesenchymal origin. Required for normal skeleton formation during embryonic development, especially for normal development of the craniofacial skeleton and for normal development of the palate.	
PVRL1	Poliovirus receptor-related 1	11q23.3	600644	cleft lip/palate-ectodermal dysplasia syndrome (CLPED1)	Adhesion	Promotes cell-cell contacts by forming homophilic or heterophilic trans-dimers.	M, CGA
SUMO1	Small ubiquitin-like modifier 1	2q33.1	601912		Regulatory , transport	Regulate a network of genes involved in palate development	M
TGFA	Transforming growth factor alpha	2p13.3	190170		Signaling cytokine growth factor	TGF alpha is a mitogenic polypeptide that is able to bind to the EGF receptor and to act synergistically with TGF beta to promote anchorage-independent cell proliferation in soft agar.	CGA
TGFB3	Transforming Growth Factor Beta 3	14q24	190230	Loeys-Dietz syndrome-5	Signaling cytokine growth factor	Involved in embryogenesis and cell differentiation.	CGA, M
TP63	Tumor protein p63	3q28	603273	Ankyloblepharon-ectodermal dysplasia-clefting, ectrodactyly-ectodermaldysplsia-clefting, Hay-Wells	Transcription factor	Acts as a sequence specific DNA binding transcriptional activator or repressor. Plays a role in the regulation of epithelial morphogenesis.	CGA
VAX1	Ventral anterior homeobox 1	10q25.3	604294	Microphthalmia, syndromic 11	Transcription factor	May function in dorsoventral specification of the forebrain.	GWA, CGA

(*) Data collected from the UniProtKB database (www.uniprot.org).
GWA= Genome-wide association; CGA= Candidate Gene Association; L=Linkage; M= Mutation detection

3.2 Dentoalveolar effects of early orthodontic treatment.

MAXILLARY ARCH WIDTH AND DENTAL ARCH RELATIONSHIP AT T0

There were no significant differences in the measurements of inter-canine and inter-molar distances of the patients in the different cleft groups (Table 3.3).

A statistically significant difference for MHB score was found between UCLP *vs* CP (P<0.01); BCLP *vs* CP (P<0.01) and BCLP *vs* CSP (P<0.05).

TREATMENT OUTCOME

Table 3.4 reports the effects of treatment by comparing changes observed after the T0-T1 period. Significant differences were highlighted for all the variables: the mean inter-canine widening was 4.7 mm (P <0.001) and the mean inter-molar widening was 5.3 mm (P <0.05); a mean MHB score of 4.8 was gained (P <0.05).

COMPARISON OF TREATMENT OUTCOME BETWEEN GROUP A (AGE < 6 YEARS AT T0) AND GROUP B (AGE ≥ 6 YEARS AT T0)

Analysis of the starting forms showed that Group A (age <6 years) and Group B (age ≥ 6 years) had no statistically significant differences in maxillary arch width and dental arch relationship at T0 (Table 3.5).

Statistical comparison of T0-T1 changes (Table 3.6) showed a significant difference between Group A and Group B for the anterior maxillary expansion and inter-arch relationship: Group A exhibited a greater increase of inter-canine distance (mean value: 8 mm *vs* 2.7 mm; P<0.001) and MHB score (mean value: 7.1 *vs* 3; P < 0.05) than group B. Regarding inter-molar distance, patients in Group A gained a mean widening of 7.2 mm compared to 5 mm in Group B (P=0.06, close to significance).

Table 3.3. Mean values of measurements at T0 and statistical comparisons between the types of cleft.

	UCLP (n=53) Mean ± SD	BCLP (n=13) Mean ± SD	CP (n=5) Mean ± SD	CSP (n=5) Mean ± SD	UCLP *vs* BCLP	UCLP *vs* CP	UCLP *vs* CSP	BCLP *vs* CP	BCLP *vs* CSP	CP *vs* CSP
Maxillary arch width										
Inter-canine distance (mm)	25.7±4.2	25.7±4.5	25.6±4.3	25.4±4.4	NS	NS	NS	NS	NS	NS
Inter-molar distance (mm)	35.5±4.8	35.5±4.8	35.4±5	35.4±5.1	NS	NS	NS	NS	NS	NS
Dental arch relationship										
HB total score	-7.5±6.3	-10.7±5.3	2±3.8	-2±3.4	NS	**	NS	**	*	NS

Statistical comparisons were performed with ANOVA and Tukey *post-hoc* tests (P< 0.05).

n, number of patients; UCLP, unilateral cleft lip and palate; BCLP, bilateral cleft lip and palate; CP, cleft palate; CSP, cleft soft palate

*P<0.05; ** P<0.01

Table 3.4. Comparisons of changes after treatment (T0-T1) within the study group (n=28).

	T0		T1			
	Mean	SD	Mean	SD	Difference	P
Maxillary arch width						
Inter-canine distance (mm)	24.7	4.3	29.4	4.3	4.7	0.0003***
Inter-molar distance (mm)	34	4.9	39.3	4.6	5.3	0.01*
Dental arch relationship						
HB total score	-6	0.2	-1.4	5.1	4.6	0.002**

Statistical comparisons were performed with Paired *t*-test (P< 0.05).

*P< 0.05; ** P< 0.01; ***P< 0.001

Table 3.5. Comparison of starting forms (T0) between groups of different ages.

	< 6 years (n=12)		≥ 6 years (n=16)			
	Mean	SD	Mean	SD	Difference	P
Maxillary arch width						
Inter-canine distance (mm)	23	3.4	25.8	4.4	-2.8	0.41
Inter-molar distance (mm)	31.1	3.2	35.6	5	-4.5	0.31
Dental arch relationship						
HB total score	-7.2	7.0	-5	5.5	-2.2	0.17

Statistical comparisons were performed with Independent *t*-test ($P < 0.05$).

Table 3.6. Comparison of changes after treatment (T0-T1) between groups of different ages.

	< 6 years (n= 12)		≥ 6 years (n= 16)			
	Mean	SD	Mean	SD	Difference	P
Maxillary arch width						
Inter-canine distance (mm)	8	4.4	2.7	3.3	5.3	0.0005***
Inter-molar distance (mm)	7.2	4.9	5	3.9	2.2	0.06
Dental arch relationship						
HB total score	7.1	6.0	3	6.8	4.1	0.04*

Statistical comparisons were performed with Independent *t*-test ($P < 0.05$).

*$P < 0.05$; ** $P < 0.01$; ***$P < 0.001$

3.3 Masticatory function and chewing pattern.

In the group of patients evaluated here, BCLP was the most frequently diagnosed type of cleft (6 out of 11 patients – 55%). Three (27%) patients had UCLP and 1 (9%) and 1 (9%) were diagnosed with CL and CP, respectively.

When considering occlusion, 6 (55%) patients had unilateral posterior and anterior crossbite, 1 (9%) bilateral posterior and anterior crossbite and 2 (18%) and 1 (9%) patients were diagnosed with unilateral posterior and anterior crossbite, respectively. One (9%) out 11 patients did not show anomalies of occlusion with regard to presence of crossbite.

Demographic details and masticatory function data of the 11 patients are reported in Table 3.7 .

Particularly, we detailed for each patient the following values:

1) overall number of masticatory cycles, subclassified into physiological and reverse cycles;

2) percentage of reverse cycles.

All values were reported for each bolus (soft and hard) and for both chewing sides (left and right).

Masticatory function and chewing pattern analysis showed that all but 3 patients (73%) had reverse cycles. In all of these cases, reverse cycles occurred on the crossbite side.

Particularly, of the 3 (27%) patients not presenting reverse cycles, patient #4, affected by CP, had no crossbite and patient #1, diagnosed with BCLP, had crossbite only in anterior inter-canine region. The remaining patient #6 with UCLP and unilateral posterior and anterior crossbite, unexpectedly did not present reverse cycles.

Both patients with unilateral posterior crossbite dysplaied reverse cycles on the side of crossbite. Specifically, patient #2 had left UCLP and crossbite of 6.3, 6.4, 6.5, while patient #3 was affected by CL with a single tooth crossbite (2.7).

Among 6 patients with unilateral posterior and anterior crossbite, 4 with UCLP (patients #5, #7, #8 and #11) and 1 with BCLP (patient #10) had reverse cycles on the crossbite side.

Noticeably, patient with bilateral posterior and anterior crossbite also had bilateral reverse cycles. Such a patient was affected by BCLP (patient #9).

Examples of physiological and pathological chewing pattern on the frontal plane are reported respectively in Figure 3.2 and Figure 3.3.

Table 3.7. Demographics and masticatory function data of the study group. Percentage of reverse masticatory cycles is highlighted in yellow.

					CHEWING ON THE RIGHT SIDE								CHEWING ON THE LEFT SIDE							
					SOFT BOLUS				HARD BOLUS				SOFT BOLUS				HARD BOLUS			
Patient, gender, age			Cleft	Crossbite	**TOT**	**FISIO**	**INV**	%	**TOT**	**FISIO**	**INV**	%	**TOT**	**FISIO**	**INV**	%	**TOT**	**FISIO**	**INV**	%
1. Angelo M.	M	5,5	BCLP	ANT	37	35	2	5	38	38	0	0	37	27	10	27	41	40	1	2
2. Michele O.	M	5	UCLP	UNILATERAL POST	28	24	4	14	42	42	0	0	30	1	29	97	40	0	40	100
3. Serena L.	F	12,9	CL	UNILATERAL POST	35	31	4	11	46	25	21	46	30	28	2	7	46	25	21	46
4. Alessandro F.	M	9,8	CP	NO CRB	39	30	9	23	39	29	10	26	38	37	1	3	42	41	1	2
5. Cristian G.	M	5	UCLP	UNILAERAL POST/ANT	24	23	1	4	34	21	13	38	40	38	2	5	20	20	0	0
6. Siham S.	F	8,9	UCLP	UNILATERAL POST/ANT	41	37	4	10	50	48	2	4	38	36	2	5	46	42	4	9
7. Giada M.	F	6,10	UCLP	UNILATERAL POST/ANT	30	0	30	100	22	0	22	100	31	31	0	0	23	22	1	4
8. Sara P.	F	7,7	UCLP	UNILATERAL POST/ANT	17	14	3	18	20	19	1	5	25	10	15	60	24	1	23	96
9. Alessio E.	M	7,1	BCLP	BILATERAL POST/ANT	33	18	15	45	41	7	34	83	36	21	15	42	40	29	11	28
10. Emma F.	F	5,7	BCLP	UNILATERAL POST/ANT	22	21	1	5	30	30	0	0	22	8	14	64	27	2	25	93
11. Cristian D.	M	6,4	UCLP	UNILATERAL POST/ANT	25	25	0	0	27	21	6	22	24	20	4	17	28	14	14	50

Figure 3.2. Bilateral chewing patterns of a patient #1 with anterior crossbite. The closure direction is not altered on both sides: chewing cycles exhibit a clockwise closure direction during mastication on the right side and an anticlockwise closure direction during mastication on the left side. Green tracings: opening; Red tracings: closing.

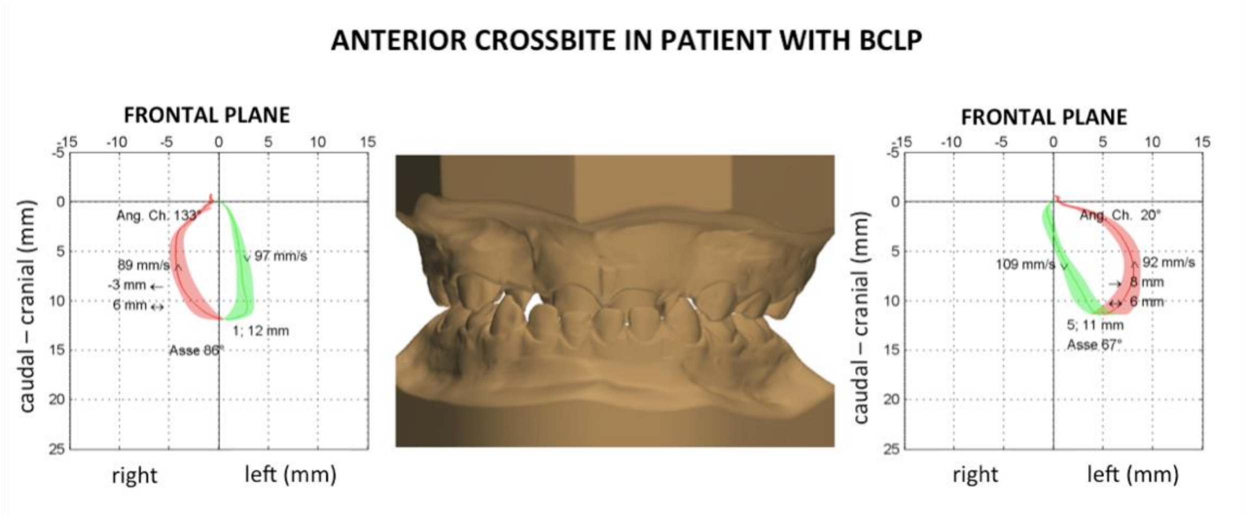
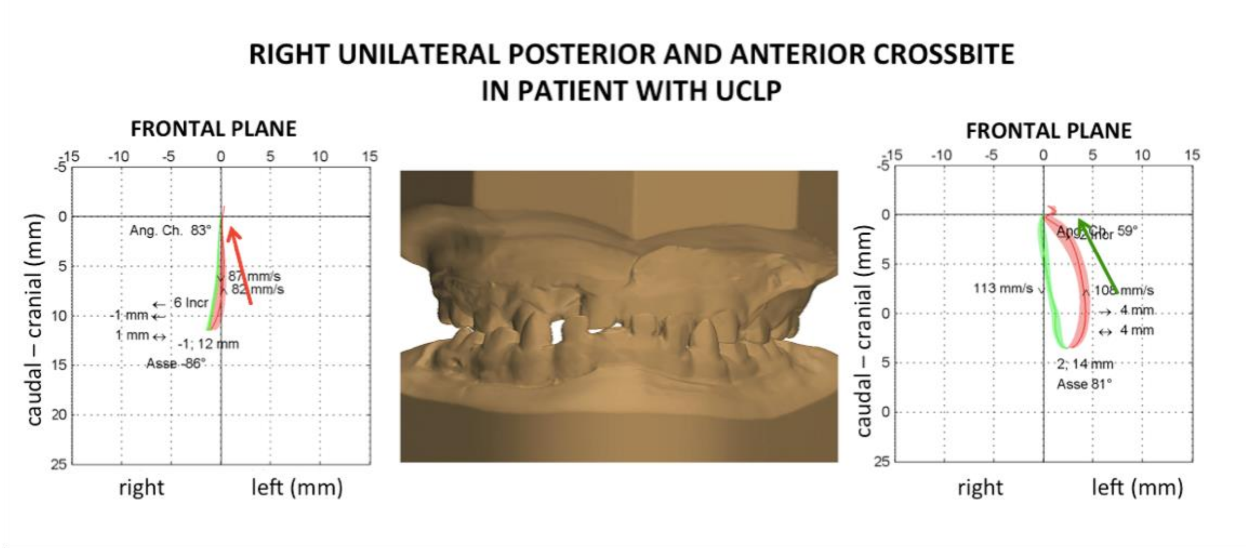


Figure 3.3. Bilateral chewing patterns of patient #11 with unilateral posterior and anterior crossbite, during chewing on the right crossbite side and on the left non-crossbite side. The reverse sequence chewing cycle occurs only on the crossbite side (right) whilst, on the healthy side, the chewing cycle displays normal physiological closing direction (left).



5. DISCUSSION

Many indices have been proposed to measure clinical outcomes related to different aspects of anatomical form and function in parts affected by the clefting process, usually reflecting specific interests of different disciplines (Jones et al. 2014). The primary purpose of this study was to compare the effectiveness of orthodontic treatment for maxillary hypoplasia on cleft patients starting the therapy at different ages. The sample of children was sub-classified according to the age the interceptive orthodontic treatment was initiated.

The modified HB (MHB) system was selected from systems for assessing the severity of malocclusions since: 1) it can be applied to any cleft type at any age, making its use easy in many different study samples (Mossey et al. 2003); 2) while the rest of indices utilize ordinal or categorical scales, the MHB follows a continuous 32-points scale before the age of 6, and a 40-points scale after that age. The large scoring range improves the level of sensitivity in the differentiation of the severity between the categories, and it tends to make the data more likely to be normally distributed, allowing the powerful, parametric statistical analysis (Dobbyn et al. 2012).

In addition, MHB is the sole index that does not require calibration; it is simple and objective, showing similar rates of reliability among trained and non-trained operators (Gray & Mossey 2005; Tothill & Mossey 2007). Moreover, a recent systematic review evaluating indices to assess malocclusion in CL/P patients based on the WHO criteria, recommended the MHB system as the index of choice (Altalibi et al. 2013).

The disadvantage of the MHB system is that it does not consider the skeletal component of the malocclusion, not differentiating dental cross-bites from skeletal discrepancies leading to cross-bites. Also, it cannot differentiate between a generalized mild and a localized severe malocclusion, and it cannot assess the vertical discrepancies.

In this, the study group includes various subtypes of orofacial clefts that may exhibit different anatomical characteristics and maxillary growth patterns. Nevertheless, no statistically significant differences were found between UCLP and BCLP in terms of maxillary constriction and inter-arch discrepancy.

Notably, initial casts measurements of inter-canine and inter-molar distance were similar among the various subtypes of cleft. A possible explanation of such a finding may be that the sample is quite homogeneous with regard to the technique and timing of the preceding surgical approach. The maxillary growth patterns of CL/P patients are affected by the iatrogenic effect of surgical repair, which has been demonstrated to be strongly related to the experience of the surgeon and the organization of the multidisciplinary team-work.[17] The lack of statistical significance between groups at T0 demonstrates that patients had no differences in the initial maxillary dental arch dimensions. Such findings confirm the sample homogeneity and ensure the effectiveness of statistical comparisons of treatment outcomes.

The mean MHB score for the BCLP group was -10.7, and for UCLP -7.5. This indicates constriction of the maxillary dental arch respective to the mandibular dental arch. Although such a difference is not statistically significant, the characteristics of the initial dentoskeletal disharmony seem to be more severe in the BCLP group.

The mean MHB scores for the CP and CSP groups were 2 and -2, respectively. This also indicates constriction of the maxillary dental arch respective to the mandibular, even if to a lesser extent than in BCLP and UCLP. Maxillary arch constriction was therefore present in both the CP and the BCLP groups, which is in agreement with previous studies.[18,19] Arch constriction may be related to palatal closure, which often includes incisions along the dental arches. The scars produced may induce inward deflection of the dentoalveolar processes, resulting in anterior and transverse crossbites. Unexpectedly, the pre-orthodontic occlusal scheme is worse in CSP patients compared to CP patients. However, such a finding may be biased because of the very small number of patients in the CSP and CP groups.

As reported by Tindlund (Tindlund et al. 1993) and Vasant (Vasant et al. 2009), in the present study outcomes of orthodontic treatment have been considered regardless of the sub-type of clefting. Results of the T0-T1 interval showed a significant effect of therapy in terms of improvement of maxillary arch width as well as of dental arch relationship. The maxillary canine and molar width were increased of 4.7 mm and 5.3 mm, respectively, resulting in a mean increase of 4.8 on the MHB index. Consequently, maxillary changes have contributed to the favourable inter-maxillary outcomes.

The orthodontic treatment induced a significantly greater improvement in the inter-canine region when compared to the inter-molar region. A differential expansion in the anterior region is frequently necessary in patients with CL/P that usually exhibit a greater constriction of the inter-canine width compared to the inter-molar because of the medial shift of the smaller segment (Heidbuchel & Kuijpers-Jagtman 1997; Ayub et al. 2016). Such a result can be obtained with common appliances such as the quad helix. This flexible device delivers light forces, and induces anteriorly divergent expansion by increasing the distance between the cleft segments. The transversal changes of our sample are in accordance with those in other studies performed on CL/P patients, reporting similar arch width increases after maxillary expansion with the quad-helix appliance (Tindlund et al. 1993; Li & Lin 2007; Vasant et al. 2009; de Medeiros Alves et al. 2015).

Although early orthodontic therapy was effective for the improvement of maxillary arch dimensions and dental arch relationship in both the deciduous and mixed dentition groups, children starting the therapy before the age of 6 showed a more favourable change in maxillary expansion, especially in the canine region. In fact, the mean inter-canine widening was 8 mm in Group A and 2.7 mm in Group B; the mean inter-molar widening was 7.2 mm and 5 mm in groups A and B, respectively.

The optimal timing of orthodontic interventions on CL/P patients is still matter of great controversy.

The aims and supposed advantages of an early phase of treatment include improvement of alveolar development in the cleft site by “unlocking” overlapped maxillary segments; improvement in masticatory function by eliminating crossbites; improvement in permanent tooth eruption and alignment; improvement in speech development and in nasal breathing by expanding the maxilla, and providing more space for the tongue (Long R.E. et al. 2000). In fact, maxillary transverse and sagittal deficiency can be associated to functional problems as narrowing of the pharyngeal airway, increased nasal resistance and alterations in tongue posture, resulting in upper airway constriction and mouth breathing (McNamara et al. 2015).

Children with CL/P have structural and functional changes of the upper airway, which may play a role in the pathophysiology of respiratory disorders (Maclean et al. 2009; Smith et al. 2014). Accordingly, several studies have reported an increased risk of sleeping disordered breathing (SDB) and obstructive sleep apnea (OSA) in cleft population (Muntz et al. 2008; Robison & Otteson 2011; MacLean et al. 2012).

The management of OSA may require various craniofacial procedures both in growing subjects and adults (Alexander & Schroeder 2013; Villa et al. 2011); notably, early orthopaedic maxillary expansion has been reported to enhance respiratory function and reduce symptoms of OSA in children (Villa et al. 2011; Marino et al. 2012; Pirelli et al. 2015).

The orthopaedic response seems to be more favourable in younger patients, and closely connected with sutural growth of the upper jaw. Thus, Delaire (Delaire et al. 1972) reported favourable skeletal maxillary effects in deciduous and mixed dentition, showing that after 12 years of age the response is mainly dentoalveolar.

Early interceptive orthodontic treatment reduces some of the typical CL/P patient stigmata, and creates a more favourable basis for subsequent conventional orthodontic treatment.

Moreover, an improvement of soft-tissue profile is of obvious psychosocial importance (Tindlund & Rygh 1993; Di Blasio et al. 2009).

On the other hand, the main argument against primary dentition treatment is that it does not pass the ‘‘burden *versus* benefit of treatment’’ test. There is currently no evidence that the additional treatment provided at an early stage either eliminates the need for mixed dentition intervention, or can provide results not achievable through a single phase of treatment in the mixed dentition. The effectiveness of age-related orthodontic approaches to CL/P patients have not been evaluated through randomized control trials. Therefore, it is not possible at the moment to state that one management strategy is better than another.

The greater increase of the inter-canine width observed in the present evaluation is in agreement with findings from other studies (Li & Lin 2007), and it may be associated with the severe constriction of the anterior region in patients with CL/P, which is commonly more pronounced compared to noncleft individuals. It is the opinion of the Authors that this outcome should be preferred, as early management of transverse deficiencies in CL/P patients usually require a greater amount of anterior maxillary expansion with segment rotation, secondary to the collapse of the buccal segment on the cleft side. Consequently, these findings demonstrate that a practical advantage of expanding maxillary segments in the primary dentition is the ease of skeletal movement and segment rotation. In addition, early correction of anterior crossbite can give the additional benefit of maximizing anterior development of the maxillary dentoalveolar process. Importantly, interceptive treatment of functional crossbite is recommended because it eliminates the lateral functional mandibular shift, preventing the development of skeletal asymmetry and of muscle function disturbances (Piancino & Kyrkanides 2016).

It is noteworthy that according to the masticatory function analysis performed on our sample before orthodontic treatment, functional asymmetry and anomalous chewing pattern occurred in all subjects (except one) with posterior cross bite. By contrast, the patient with anterior crossbite displayed a physiological chewing pattern without an increased percentage of reverse sequence chewing cycles. These findings are in accordance with those observed in non-cleft patients, in which functional asymmetry is typical of unilateral posterior crossbite, being not present in anterior crossbite (Piancino et al. 2012). Thus, crossbite has different results on masticatory function according to the dental region involved. Ostensibly, the impact of malocclusion on masticatory function might depend on the functional role of teeth involved, having anterior teeth different effects on the chewing cycle on the frontal plane compared to posterior teeth.

Management of maxillary width at earlier periods does not necessarily preclude the need for additional expansion later, raising again the question of the "benefit *versus* burden" of these additional phases of treatment. Future directions of research should be focused on monitoring long-term outcomes with longer longitudinal follow-up of patients.

6. CONCLUSIONS

Studies selected for the critical review demonstrated that orofacial clefts exhibit significant genetic heterogeneity, having successfully identified multiple genome-wide significant associations with CL/P as well as potential gene-environment interactions for CP.
However, with the important exception of IRF6, the significant risk loci from GWAS of NSCL/P are diverse than the significant risk loci from genome-wide linkage analyses, this highlighting the different strengths of the two approaches.

No agreement exists on the most appropriate timing of treatment in CL/P patients. The present study indicates that CL/P subjects may benefit from interceptive early treatment to correct posterior and anterior crossbites; children starting therapy before the age of 6 showed a better response in terms of anterior maxillary expansion and improvement of dental arch relationship.
The quad helix seems to be a convenient and efficient appliance for expanding the maxillary arch in patients with CL/P, providing a controlled and differential movement of the segment on the cleft side.

With regard to the masticatory function, our preliminary results show that the chewing pattern of CL/P patients is similar to that of non-CLP patients with the same malocclusion, regardless the morphology of birth defects.

As future perspective of the present study, we will test a photogrammetric 3D scanning system for acquiring, analysing and measuring some variables of the facial soft tissues, thus checking the morphological modifications after orthodontic treatment as well as investigating genotype/phenotype correlations.

7. PUBLISHED PAPERS AND PRESENTATIONS

Papers published during the PhD course:

1. Dentoalveolar Effects of Early Orthodontic Treatment in Patients With Cleft Lip and Palate
   D. CASSI, A. Di BLASIO, M. GANDOLFINI, M. MAGNIFICO, F. PELLEGRINO, M.G. PIANCINO
   J Craniofac Surg 2017;28(8):2021-26.
   Doi: https://doi.org/10.1097/SCS.0000000000003854
   (Database: Medline; www.pubmed.com)

2. Natural position of the head: review of two-dimensional and three-dimensional methods of recording
   D. CASSI, C. DE BIASE, I. TONNI, M. GANDOLFINI, A. DI BLASIO, M.G. PIANCINO
   Br J Oral Maxillofac Surg 2016;54(3):233–40
   Doi: 10.1016/j.bjoms.2016.01.025
   (Database: Medline; www.pubmed.com)

Oral presentations:

1. Influence of Bolus Hardness on Masticatory Function and Nutrition
   M.G. PIANCINO, C. DE BIASE, I. TONNI, R. CANNAVALE, D. CASSI, U. GARAGIOLA
   Poster Session- Nutrition Research
   IADR General Session, San Francisco, California, US, March 23 2017.

2. Genetics of non-syndromic cleft lip and palate: a critical review
   D. CASSI, M. GANDOLFINI, A. DI BLASIO, M. MAGNIFICO, M.G. PIANCINO
   24th National Congress of the Collegio dei Docenti Universitari di discipline Odontostomatologiche.
   Milano (Italy), April 7th-8th, 2017
   J Osseointegr 2017;9(1):117

3. Early orthodontic treatment in patients with cleft-lip and palate: e retrospective study
   F. PELLEGRINO, D. CASSI, A. DI BLASIO, M.G. PIANCINO, M. GANDOLFINI
   Scientific Poster 347; 92nd EOS Congress, Stockholm, Sweden, June 11-16 2016
   Eur J Orthod 2016 Oct;38(5):e173-e174
   DOI: http://dx.doi.org/10.1093/ejo/cjw053

4. Early orthodontic treatment in patients with cleft-lip and palate
   D. CASSI, M. GANDOLFINI, A. DI BLASIO, F. PELLEGRINO, M.G. PIANCINO
   XXIII Congresso Nazionale, Collegio dei Docenti Universitari di Discipline Odontostomatologiche
   ROMA, 14-16 Aprile 2016
   Minerva Stomatologica Vol. 65- Suppl. 1 al No. 3

5. Natural head position: a review on two- and three-dimensional recording methods
   D. CASSI, C. DE BIASE, M. GANDOLFINI, A. DI BLASIO, M.G. PIANCINO
   XXIII Congresso Nazionale, Collegio dei Docenti Universitari di Discipline Odontostomatologiche
   ROMA, 14-16 Aprile 2016
   Minerva Stomatologica Vol. 65- Suppl. 1 al No. 3

6. Clinical and epidemiological evaluation of 84 patients with cleft lip and palate.
   D. CASSI, M. GANDOLFINI, A. DI BLASIO, M.G. PIANCINO
   XXII Congresso Nazionale, Collegio dei Docenti Universitari di Discipline Odontostomatologiche
   Milano, 9-11 Aprile 2015
   Minerva Stomatologica Vol. 64- Suppl. 1 al No. 2

OPEN

Dentoalveolar Effects of Early Orthodontic Treatment in Patients With Cleft Lip and Palate

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Abstract: No agreement exists on the most appropriate timing of orthodontic treatment in patients with cleft lip and palate. The aim of this study is to investigate the effect of early orthodontic treatment on development of the dental arches and alveolar bone.

A dental casts analysis was performed on 28 children with cleft lip and palate before orthodontic treatment (T0; mean age, 6.5 ± 1.7) and at the end of active treatment (T1; mean age, 9.2 ± 2.1 years). The considered variables were: intercanine and intermolar distances; dental arch relationships, evaluated according to the modified Huddart/Bodenham system.

The study group was divided into 2 samples according to the age at T0: Group A (age < 6 years) and Group B (age ≥ 6 years). A statistical comparison of the treatment effects between the 2 samples was performed.

Patients in Group A exhibited a greater increase of intercanine distance (8 mm versus 2.7 mm; $P<0.001$), intermolar distance (7.2 mm versus 5 mm; $P=0.06$), and Huddart/Bodenham score (7.1 versus 3; $P<0.05$) when compared with patients in Group B.

Early orthodontic treatment strongly improved the dental arch relationship, since subjects starting the therapy before the age of 6 had a better response in terms of anterior maxillary expansion.

Key Words: Cleft lip and palate, early orthodontic treatment, maxillary growth deficiency

(*J Craniofac Surg* 2017;28: 2021–2026)

Cleft of the lip and palate (CLP) is the most common human congenital malformation affecting the facial region. Cleft lip and palate occurs at the time of early embryogenesis from a failure in fusion of medial nasal and maxillary processes that results in orofacial clefting involving the upper lip, alveolus and/or primary palate.[1] The development of CLP is associated with genetic and environmental factors.[2]

The most severe type of defect is the complete cleft of the lip, alveolar process, and palate, which can be either unilateral CLP (UCLP) or bilateral CLP (BCLP). The most widely adopted management strategy includes the surgical reconnection of the cleft anatomical structures followed by their development to gain proper appearance, occlusion, and speech.[3]

Maxillary growth in operated CLP patients is often decreased in the 3 dimensions. The most important cause of growth inhibition seems to be the iatrogenic effect of surgical intervention and the subsequent constriction induced by scar tissue.[4] However, some authors attribute such a deficiency to the developmental hypoplasia of both the alveolar and palatal soft and hard tissues, as well as to functional factors.[5] The maxillary growth deficiency affects the dental arches relationship on the vertical, sagittal, and transverse planes, frequently resulting in anterior and/or posterior crossbite occurring in the early dentition.[6]

Orthodontic treatment of CLP patients during the deciduous and mixed dentition period has been recommended to create more favorable conditions for midfacial growth, normalize the intermaxillary basal relationship, and prevent or eliminate functional disturbances.[7] The most common orthodontic procedures include maxillary expansion to correct the reduced transverse dimension, incisor alignment and proclination to resolve crowding, rotations and anterior crossbites; and maxillary protraction to reduce maxillary retrusion.

Despite the agreement on the need of orthodontic treatment in the multidisciplinary management of CLP patients, controversy still exists on the best timing to start such a therapy.

The aim of this retrospective study is to investigate the effect of timing and method of early orthodontic treatment on development of the dental arches in growing subjects with various types of orofacial cleft. Particularly, we compared occlusal changes in children starting orthodontic therapy before 6 years of age with those in subjects starting treatment later.

The null hypothesis was that there was no significant difference between the 2 groups with regard to treatment effects on widening of the maxillary dentition and on the correction of interarch discrepancy.

METHODS

The principles outlined in the Declaration of Helsinki were followed throughout this study and all data were obtained in a clinical context as part as a standardized treatment regime with full acceptance from the parents.

Data of 76 patients (54 males, 22 females; mean age 7.2 years), with various types of orofacial cleft, consecutively referred to the Orthodontic Section of the Academic Hospital of Parma, Italy, between 2004 and 2015, were retrieved and analyzed. Variables evaluated included: gender, type of cleft, type of orthodontic treatment, and age at different times of follow-up. According to the type of cleft, patients were subclassified as follows: UCLP; BCLP; cleft palate (CP), and cleft soft palate (CSP).

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TABLE 1. Demographics of the Groups

	T0				T1			
		Gender		Age (Y, Mo)		Gender		Age (Y, Mo)
Cleft Type	n	M	F	Mean ± SD	n	M	F	Mean ± SD
All clefts	76	54	22	7.2 ± 3.6	28	17	11	9.2 ± 2.1
UCLP	53 (70%)	38	16	7.3 ± 3.6	20 (72%)	13	7	9.3 ± 2
BCLP	13 (17%)	12	1	7.3 ± 3.7	4 (14%)	3	1	9.1 ± 1.9
CP	5 (6.5%)	1	4	7.2 ± 3.6	2 (7%)	0	2	10.2 ± 1.8
CSP	5 (6.5%)	3	2	7.3 ± 3.9	2 (7%)	1	1	9.9 ± 1.6

BCLP, bilateral cleft lip and palate; CP, cleft palate; CSP, cleft soft palate, F, female; M, male; Mo, months; n, number of patients; UCLP, unilateral cleft lip and palate; Y, years.

All patients had dental casts taken before the orthodontic treatment (T0). For 28 patients (17 males, 11 females) dental casts taken at the end of the interceptive orthodontic treatment (T1) were also available. To evaluate the influence of age on treatment response, children of such a group were subclassified according to the age at the beginning of the orthodontic treatment: Group A (age < 6 years) and Group B (age ≥ 6 years). The characteristics of the samples are reported in Table 1.

Dental Study Models Analysis

All models were cast in white plaster and in centric occlusion and labeled with identification numbers attached to the base of the models.

Dental cast analysis, performed at T0 and T1, took into account the following variables: a) maxillary arch widths measured with a Beerendonk sliding calliper (measuring size 0–80 mm in tenths of mm). Particularly, intermolar width was measured as the distance between the mesiopalatal cusp tips of the first molars; intercanine width was measured as the distance between the cusp tips (Fig. 1); b) dental arch relationships, categorized according to the modified Huddart/Bodenham system (MHB).[8,9] This numerical scoring system requires all maxillary teeth to be scored according to their buccolingual relationship to the corresponding mandibular tooth, except for the lateral incisors, which may be missing or in an abnormal position in CLP subjects (Fig. 2). The MHB system is used for the deciduous, mixed, and permanent dentition. The number of teeth scored changes, depending on age: before 6 years, the first permanent molars are not scored, even if erupted and therefore the maximum range of scores is between −24 and +8. After the age of 6, first permanent molars are scored if present; otherwise, the midpoint of the maxillary alveolar ridge is used. In this case, the maximum range of scores is −30 to +10.

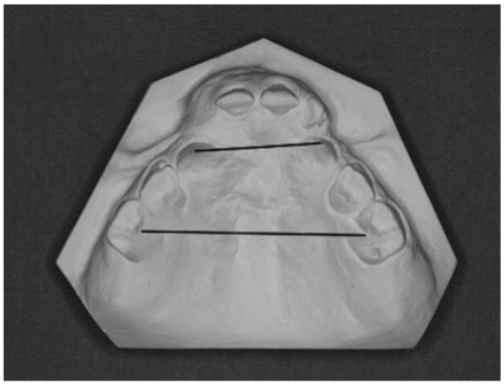

FIGURE 1. Transversal linear measurements on the study casts.

Orthodontic Treatments

Orthodontic treatment includes maxillary expansion to correct the reduced transverse dimension, maxillary protraction to reduce

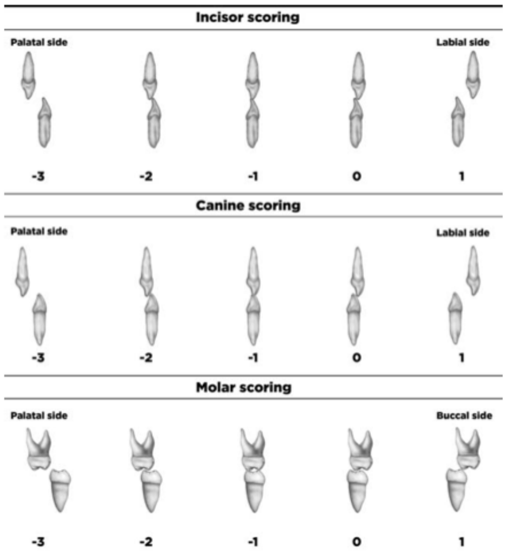

FIGURE 2. Diagram representing the modified Huddart and Bodenham scoring system. Redrawn from Tothill and Mossey (2007).[15] The following modifications were taken into account: premolars were scored as for primary molars; if a central incisor was missing, the other central incisor was used to score the missing incisor; where canines were unerupted, the canine score was determined by the midpoint of the maxillary alveolar ridge; if a premolar was absent (for example, due to noneruption or hypodontia), then a score was allocated equivalent to the adjacent premolar, if erupted. Where no premolars were erupted, the score was determined by the midpoint of the maxillary ridge, in a similar way as previously described. The sum of the scores (the total score) reflected the interarch discrepancy.

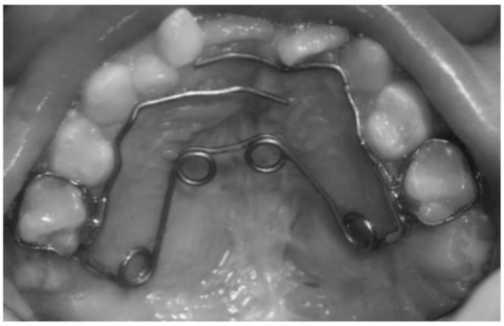

FIGURE 3. Intraoral view of a quad helix appliance.

maxillary retrusion, and incisor alignment and proclination to resolve crowding, rotations, and anterior crossbites.

Transverse expansion of the maxilla was obtained through the quad-helix appliance (0.038 inches/0.965 mm Blue Elgiloy), soldered to bands on the maxillary primary second molars or permanent first molars (Fig. 3). The appliance was initially activated to provide a force of 200 g per side; subsequent reactivations were done extraorally at 6-week intervals.

To achieve maxillary protraction, a posteroanterior orthopedic force carried out by a Delaire facial mask connected to an intraoral double arch appliance was applied. Two heavy (700 and 350 g on each side) elastics were attached from the soldered intraoral hooks in the cuspid area to the support bar of the facemask. The direction of the forward force was 15° downward in relation to the occlusal plane. Patients were instructed to wear the facemask for 12 hours per day, including at night.

Maxillary incisor rotation, lingual inclination, and anterior cross-bite were variously corrected by using partial fixed and removable orthodontic appliances.

Statistical Methods

The data was evaluated for normality using the Shapiro–Wilk test. Descriptive statistics were calculated for all variables at T0, and for variations from T0 to T1 either in the overall group of patients and in subgroups A and B. Differences between types of cleft at T0 were established through the analysis of variance and Tukey post-hoc tests.

Paired *t* tests were used to investigate the overall treatment effect by comparing the longitudinal changes in T0 and T1.

The following statistical comparisons were carried out with the independent *t* test: starting form: differences between the subgroup A and the subgroup B at T0; and treatment effects: T0-T1 changes in the subgroup A versus T0–T1 changes in the subgroup B.

Data were analyzed with the IBM-SPSS version 20 statistical software. Statistical significance was tested at $P < 0.05$. The power of the study was calculated on the basis of the difference between T0 and T1 in the treated group for a relevant variable (intercanine distance) as reported in a previous longitudinal investigation of similar nature and on the basis of the standard deviation of this difference.[10] The power exceeded 0.80 at an α level of 0.05.

Method Error

To test the precision of the measurements, 25 dental casts were randomly selected and were remeasured by the same operator (FP) after a 1-month interval. No systematic error was detected.

Random errors were estimated with the Dahlberg formula. The errors for linear measurements ranged from 0.1 mm for intercanine distance, to 0.2 mm for intermolar distance. The intraobserver agreement for MHB score, analyzed by the weighted kappa statistic, was good (kappa = 0.62).

RESULTS

Maxillary Arch Width and Dental Arch Relationship at T0

There were no significant differences in the measurements of intercanine and intermolar distances of the patients in the different cleft groups (Table 2).

A statistically significant difference for MHB score was found between UCLP versus CP ($P < 0.01$); BCLP versus CP ($P < 0.01$), and BCLP versus CSP ($P < 0.05$).

Treatment Outcome

Table 3 reports the effects of treatment by comparing changes observed after the T0-T1 period. Significant differences were highlighted for all the variables: the mean intercanine widening was 4.7 mm ($P < 0.001$) and the mean intermolar widening was 5.3 mm ($P < 0.05$); a mean MHB score of 4.8 was gained ($P < 0.05$).

Comparison of Treatment Outcome Between Group A (Age < 6 Years at T0) and Group B (Age ≥ 6 Years at T0)

Analysis of the starting forms showed that Group A (age <6 years) and Group B (age ≥ 6 years) had no statistically significant

TABLE 2. Mean Values of Measurements at T0 and Statistical Comparisons Between the Types of Cleft

	UCLP (n = 53) Mean ± SD	BCLP (n = 13) Mean ± SD	CP (n = 5) Mean ± SD	CSP (n = 5) Mean ± SD	UCLP Versus BCLP	UCLP Versus CP	UCLP Versus CSP	BCLP Versus CP	BCLP Versus CSP	CP Versus CSP
Maxillary arch width										
Intercanine distance (mm)	25.7 ± 4.2	25.7 ± 4.5	25.6 ± 4.3	25.4 ± 4.4	NS	NS	NS	NS	NS	NS
Intermolar distance (mm)	35.5 ± 4.8	35.5 ± 4.8	35.4 ± 5	35.4 ± 5.1	NS	NS	NS	NS	NS	NS
Dental arch relationship										
HB total score	−7.5 ± 6.3	−10.7 ± 5.3	2 ± 3.8	−2 ± 3.4	NS	**	NS	**	*	NS

Statistical comparisons were performed with analysis of variance and Tukey post-hoc tests ($P < 0.05$).
BCLP, bilateral cleft lip and palate; CP, cleft palate; CSP, cleft soft palate; n, number of patients; UCLP, unilateral cleft lip and palate.
*$P < 0.05$;
**$P < 0.01$.

TABLE 3. Comparisons of Changes After Treatment (T0-T1) Within the Study Group (n = 28)

	T0		T1			
	Mean	SD	Mean	SD	Difference	P
Maxillary arch width						
Intercanine distance (mm)	24.7	4.3	29.4	4.3	4.7	0.0003‡
Intermolar distance (mm)	34	4.9	39.3	4.6	5.3	0.01*
Dental arch relationship						
HB total score	−6	0.2	−1.4	5.1	4.6	0.002†

Statistical comparisons were performed with paired t test ($P < 0.05$).
HB, Huddart/Bodenham.
*$P < 0.05$.
†$P < 0.01$.
‡$P < 0.001$.

TABLE 5. Comparison of Changes After Treatment (T0–T1) Between Groups of Different Ages

	< 6 Y (n = 12)		≥6 Y (n = 16)			
	Mean	SD	Mean	SD	Difference	P
Maxillary arch width						
Intercanine distance (mm)	8	4.4	2.7	3.3	5.3	0.0005‡
Intermolar distance (mm)	7.2	4.9	5	3.9	2.2	0.06
Dental arch relationship						
HB total score	7.1	6.0	3	6.8	4.1	0.04*

Statistical comparisons were performed with independent t test ($P < 0.05$).
HB, Huddart/Bodenham.
*$P < 0.05$.
‡$P < 0.001$.

differences in maxillary arch width and dental arch relationship at T0 (Table 4).

Statistical comparison of T0–T1 changes (Table 5) showed a significant difference between Group A and Group B for the anterior maxillary expansion and interarch relationship: Group A exhibited a greater increase of inter-canine distance (mean value: 8 versus 2.7 mm; $P < 0.001$) and MHB score (mean value: 7.1 versus 3; $P < 0.05$) than group B. Regarding intermolar distance, patients in Group A gained a mean widening of 7.2 mm compared with 5 mm in Group B ($P = 0.06$, close to significance).

DISCUSSION

The role of the orthodontist is central in the interdisciplinary management of orofacial clefts. Therapeutic intervention usually starts during the neonatal period with treatment of displaced alveolar segments, and follows throughout the deciduous and mixed dentition phases with the management of the skeletal and dental components of the developing dentition. Most of the patients will receive orthodontic therapy during adolescence, and sometimes into adulthood.[11]

The continuous and often progressive nature of cleft-related orthodontic problems over the stages of growth and dental eruption makes it difficult to use routine orthodontic approaches. Treatment recommendations can be found for nearly every age; however, no clear-cut guidelines for optimal timing or method of intervention have been developed.

Many indices have been proposed to measure clinical outcomes related to different aspects of anatomical form and function in parts affected by the clefting process, usually reflecting specific interests of different disciplines.[12] The primary purpose of this study was to compare the effectiveness of orthodontic treatment for maxillary hypoplasia on cleft patients starting the therapy at different ages. The sample of children was subclassified according to the age the interceptive orthodontic treatment was initiated.

The MHB system was selected from systems for assessing the severity of malocclusions since: it can be applied to any cleft type at any age, making its use easy in many different study samples;[9] while the rest of indices utilize ordinal or categorical scales, the MHB follows a continuous 32-point scale before the age of 6, and a 40-point scale after that age. The large scoring range improves the level of sensitivity in the differentiation of the severity between the categories, and it tends to make the data more likely to be normally distributed, allowing the powerful, parametric statistical analysis.[13]

In addition, MHB is the sole index that does not require calibration; it is simple and objective, showing similar rates of reliability among trained and nontrained operators.[14,15] Moreover, a recent systematic review evaluating indices to assess malocclusion in CLP patients based on the WHO criteria, recommended the MHB system as the index of choice.[16]

The disadvantage of the MHB system is that it does not consider the skeletal component of the malocclusion, not differentiating dental cross-bites from skeletal discrepancies leading to cross-bites. Also, it cannot differentiate between a generalized mild and a localized severe malocclusion, and it cannot assess the vertical discrepancies.

In this, the study group includes various subtypes of orofacial clefts that may exhibit different anatomical characteristics and maxillary growth patterns. Nevertheless, no statistically significant differences were found between UCLP and BCLP in terms of maxillary constriction and interarch discrepancy.

Notably, initial casts measurements of intercanine and intermolar distance were similar among the various subtypes of cleft. A possible explanation of such a finding may be that the sample is quite homogeneous with regard to the technique and timing of the preceding surgical approach. The maxillary growth patterns of CLP patients are affected by the iatrogenic effect of surgical repair, which has been demonstrated to be strongly related to the experience of the surgeon and the organization of the multidisciplinary team-work.[17] The lack of statistical significance between groups at T0 demonstrates that patients had no differences in the initial maxillary dental arch dimensions. Such findings confirm the sample homogeneity and ensure the effectiveness of statistical comparisons of treatment outcomes.

TABLE 4. Comparison of Starting Forms (T0) Between Groups of Different Ages

	< 6 Y (n = 12)		≥6 Y (n = 16)			
	Mean	SD	Mean	SD	Difference	P
Maxillary arch width						
Intercanine distance (mm)	23	3.4	25.8	4.4	−2.8	0.41
Intermolar distance (mm)	31.1	3.2	35.6	5	−4.5	0.31
Dental arch relationship						
HB total score	−7.2	7.0	−5	5.5	−2.2	0.17

Statistical comparisons were performed with independent t test ($P < 0.05$).
HB, Huddart/Bodenham.

The mean MHB score for the BCLP group was −10.7, and for UCLP −7.5. This indicates constriction of the maxillary dental arch respective to the mandibular dental arch. Although such a difference is not statistically significant, the characteristics of the initial dentoskeletal disharmony seem to be more severe in the BCLP group.

The mean MHB scores for the CP and CSP groups were 2 and −2, respectively. This also indicates constriction of the maxillary dental arch respective to the mandibular, even if to a lesser extent than in BCLP and UCLP. Maxillary arch constriction was therefore present in both the CP and the BCLP groups, which is in agreement with previous studies.[18,19] Arch constriction may be related to palatal closure, which often includes incisions along the dental arches. The scars produced may induce inward deflection of the dentoalveolar processes, resulting in anterior and transverse crossbites. Unexpectedly, the preorthodontic occlusal scheme is worse in CSP patients compared with CP patients. However, such a finding may be biased because of the very small number of patients in the CSP and CP groups.

As reported by Tindlund et al[20] and Vasant et al,[21] in the present study outcomes of orthodontic treatment have been considered regardless of the subtype of clefting. Results of the T0–T1 interval showed a significant effect of therapy in terms of improvement of maxillary arch width as well as of dental arch relationship. The maxillary canine and molar width were increased of 4.7 and 5.3 mm, respectively, resulting in a mean increase of 4.8 on the MHB index. Consequently, maxillary changes have contributed to the favorable intermaxillary outcomes.

The orthodontic treatment induced a significantly greater improvement in the intercanine region when compared with the intermolar region. A differential expansion in the anterior region is frequently necessary in patients with CLP that usually exhibit a greater constriction of the intercanine width compared with the intermolar because of the medial shift of the smaller segment.[22,23] Such a result can be obtained with common appliances such as the quad helix. This flexible device delivers light forces, and induces anteriorly divergent expansion by increasing the distance between the cleft segments. The transversal changes of our sample are in accordance with those in other studies performed on CLP patients, reporting similar arch width increases after maxillary expansion with the quad-helix appliance.[10,20,21,24]

Although early orthodontic therapy was effective for the improvement of maxillary arch dimensions and dental arch relationship in both the deciduous and mixed dentition groups, children starting the therapy before the age of 6 showed a more favorable change in maxillary expansion, especially in the canine region. In fact, the mean intercanine widening was 8 mm in Group A and 2.7 mm in Group B; the mean intermolar widening was 7.2 and 5 mm in Groups A and B, respectively.

The optimal timing of orthodontic interventions on CLP patients is still matter of great controversy.

The aims and supposed advantages of an early phase of treatment include improvement of alveolar development in the cleft site by "unlocking" overlapped maxillary segments; improvement in masticatory function by eliminating crossbites; improvement in permanent tooth eruption and alignment; improvement in speech development and in nasal breathing by expanding the maxilla, and providing more space for the tongue.[7] In fact, maxillary transverse and sagittal deficiency can be associated with functional problems as narrowing of the pharyngeal airway, increased nasal resistance, and alterations in tongue posture, resulting in upper airway constriction and mouth breathing.[25]

Children with CLP have structural and functional changes of the upper airway, which may play a role in the pathophysiology of respiratory disorders.[26,27] Accordingly, several studies have reported an increased risk of sleeping disordered breathing and obstructive sleep apnea (OSA) in cleft population.[28–30]

The management of OSA may require various craniofacial procedures both in growing subjects and in adults [31,32]; notably, early orthopaedic maxillary expansion has been reported to enhance respiratory function and reduce symptoms of OSA in children.[33–35]

The orthopaedic response seems to be more favorable in younger patients, and closely connected with sutural growth of the upper jaw. Thus, Delaire et al[36] reported favorable skeletal maxillary effects in deciduous and mixed dentition, showing that after 12 years of age the response is mainly dentoalveolar.

Early interceptive orthodontic treatment reduces some of the typical CLP patient stigmata, and creates a more favorable basis for subsequent conventional orthodontic treatments. Moreover, an improvement of soft-tissue profile is of obvious psychosocial importance.[37,38]

On the other hand, the main argument against primary dentition treatment is that it does not pass the "burden versus benefit of treatment" test. There is currently no evidence that the additional treatment provided at an early stage either eliminates the need for mixed dentition intervention, or can provide results not achievable through a single phase of treatment in the mixed dentition. The effectiveness of age-related orthodontic approaches to CLP patients has not been evaluated through randomized control trials. Therefore, it is not possible at the moment to state that one management strategy is better than another.

The greater increase of the intercanine width observed in the present evaluation is in agreement with findings from other studies,[24] and it may be associated with the severe constriction of the anterior region in patients with CLP, which is commonly more pronounced compared with noncleft individuals. It is the opinion of the authors that this outcome should be preferred, as early management of transverse deficiencies in CLP patients usually requires a greater amount of anterior maxillary expansion with segment rotation, secondary to the collapse of the buccal segment on the cleft side. Consequently, these findings demonstrate that a practical advantage of expanding maxillary segments in the primary dentition is the ease of skeletal movement and segment rotation. Importantly, interceptive treatment of functional crossbite is recommended because it eliminates the lateral functional mandibular shift, preventing the development of skeletal asymmetry and of muscle function disturbances.[39] In addition, early correction of anterior crossbite can give the additional benefit of maximizing anterior development of the maxillary dentoalveolar process.

Management of maxillary width at earlier periods does not necessarily preclude the need for additional expansion later, raising again the question of the "benefit versus burden" of these additional phases of treatment. Future directions of research should be focused on monitoring long-term outcomes with longer longitudinal follow-up of patients.

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Review

Natural position of the head: review of two-dimensional and three-dimensional methods of recording

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Abstract

Both the correct position of the patient's head and a standard system for the acquisition of images are essential for objective evaluation of the facial profile and the skull, and for longitudinal superimposition. The natural position of the head was introduced into orthodontics in the late 1950s, and is used as a postural basis for craniocervical and craniofacial morphological analysis. It can also have a role in the planning of the surgical correction of craniomaxillofacial deformities. The relatively recent transition in orthodontics from 2-dimensional to 3-dimensional imaging, and from analogue to digital technology, has renewed attention in finding a versatile method for the establishment of an accurate and reliable head position during the acquisition of serial records. In this review we discuss definition, clinical applications, and procedures to establish the natural head position and their reproducibility. We also consider methods to reproduce and record the position in two and three planes.

Keywords: Head posture; Cephalometry; Orthognathic surgery; Three-dimensional imaging

Introduction

The natural position of the head is the most balanced, natural position of the head when someone views an object at eye level.[1] It is an individual, functional, physiological position that indicates a person's true appearance.[2]

Since its introduction into orthodontics in the late 1950s it has been used as a reference position for the assessment of craniofacial morphology,[3–5] and has been advocated as a better option than intracranial reference lines because it allegedly varies less. The concept is not new: Leonardo da Vinci (1452- 1519) and Albrecht Durer (1471- 1528) used scaffolds of horizontal and vertical lines on drawings of models positioned in a "natural pose" to permit more accurate artistic and scientific replication of the human head. Artists, anatomists, and anthropologists have used it to study the human face throughout the ages, and it has been used routinely for clinical examination in medicine and dentistry by plastic and maxillofacial surgeons, and orthodontists.

Measurement of the natural head position is relevant in orthodontics for cephalometric analysis of dentofacial anomalies, orthognathic surgical planning, and evaluation of the relation between the head and the cervical column (craniocervical angulation).

In this review we have focused on techniques to establish it, and how to transfer it to the cephalostat, together with an

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Table 1
Different definitions describing head position.

Head position	Definition
Natural head position	Position of the head when the subject looks at a distant point at eye level and their visual axis is parallel to the ground.[6]
Natural head posture	The standing or seated patient feels that his or her head is in balance.[7]
Estimated natural head position or natural head orientation	Position of the patient's head while they are looking towards a distant point at eye level, reorientated by the operator; it is considered a better option for research purposes.[2]

overview of the 3-dimensional recording methods recently introduced into clinical practice.

Methods

We searched the databases PubMed and Scopus for all relevant publications, with no limitations of language or time. We used the terms "head posture", "cephalometric analysis", "natural head position", "lateral cephalometric radiographs", "cephalostat", "3-dimensional imaging", "self balance position", and "mirror position". Only original papers (randomised and non-randomised clinical trials, cohort studies, case-control studies, case reports, and reviews) were selected. References were also derived from the lists of those retrieved.

Definition and terminology

Even if all the terms reported in Table 1 describe the spatial relations of the head, they are not synonymous. In particular, the terms "natural head position" and "natural head posture" are not interchangeable: the first indicates a standard procedure applied to all subjects for the analysis of dentofacial morphology, and the second is an individual characteristic physiological posture of the head used to study the relation between posture and morphological features.[6,7]

The concept of "estimated natural head position" or "natural head orientation" was introduced because patients can have a habit of flexing or extending their heads.[2] Natural head orientation is defined as the orientation estimated by a trained clinician while the subject stands with a relaxed body and head, and looks at a distant point at eye-level.[2,8] Because patients with skeletal malocclusions such as mandibular prognathism or retrognathism may assume altered head positions to mask their skeletal patterns, it is better for an experienced clinician to modify it.[9,10]

Reproducibility of the natural head position

Reproducibility refers to how consistently a subject can reproduce the same position of the head on separate occasions. This should not be confused with reliability, which refers to how well one can locate a landmark on a lateral cephalograph.

Numerous studies have successfully measured the reproducibility and stability of the natural head position, both in short and long time-lapses.[11–14] In the longitudinal serial studies by Cooke et al, the long-term stability was investigated 3-6 months, 5 years, and 15 years after the initial radiography.[11,13,15] In the last study particularly, 20 of the 618 adolescents went through 15 sequential years of observation from the ages of 12-27 years, which showed that the natural head position is a stable reference line. Its reproducibility is commonly reported as a mean square error (ranging from 1.1° -3.2°) or Dahlberg value.[11,13–21]

Dahlberg's coefficient is commonly used to assess the reproducibility of a given method or the agreement between two methods.[18] A coefficient with a value below the cut-off point of roughly 1.5°-2° indicates good reproducibility. However, Bister et al[14] suggested that it has a tendency to camouflage the true variability of the results, and they concluded that reproducibility could be assessed more accurately with a reproducibility coefficient and its corresponding graphical representation.

Although natural head position has less variability than intracranial reference lines, it is also influenced by balance (the vestibular canals of the middle ear), vision (the need to maintain a horizontal visual axis), and proprioception from joints and muscles involved in maintaining erect posture, so it depends on the subject's neuromuscular condition. In addition, it is not a single angular measurement, but a small range of angles oscillating around a mean posture,[16] so is a dynamic concept which should be recorded both dynamically and continuously.[22–24]

According to some authors the protocol for obtaining the natural head position seems to influence reproducibility, and fairly minor changes in the procedure may have appreciable effects on possible discrepancies.[14] There is also some evidence that the success of a certain protocol depends on the operator.[14]

Clinical application: natural head position compared with intracranial reference lines

Natural head position may be a more valid craniofacial reference system than anatomical craniofacial planes. According to some authors the inherent variability of intracranial cephalometric reference structures makes analysis based on them potentially misleading, with serious implications for planning orthodontic and orthognathic treatment.[2] Studies that have evaluated the relation between position of the head and selected cephalometric planes have shown wide variability in the inclination of intracranial reference lines.[16,25] In particular, Madsen et al showed that intersubject variability of the craniofacial reference planes is greater than the intra-subject reproducibility of the natural head position, which supports the use of a true vertical or horizontal reference plane established from recording the natural head position in preference to other planes.[25] However, these findings are expected given the overall variation in craniofacial morphology in an unbiased sample. To compare the variability of intracranial reference lines of different patients to the true horizontal we use cross-sectional data, while the reproducibility of the natural head position depends on longitudinal data.[14]

Additional features that validate the use of natural head position in cephalometric analysis include the fact that it is a true-life appearance, and head positioning can substantially influence the profile and perception of the mandibular and maxillary positions in relation to the calvaria, which influences the objectives of treatment.[26] The common cephalometric landmarks and planes for the surgical correction of craniomaxillofacial deformities cannot be used to orientate the composite skull model of patients with asymmetries of the upper face and skull base, [27] but natural head position obviates the need for internal landmarks by providing a reproducible reference framework.

The execution of radiographs in natural head positioning is also related to the evaluation of posture. The analysis of craniocervical angulation has been a concern for many years because of the supposed relation that exists between the head and cervical posture, and temporomandibular disorders or neck pain and headache.[28–30] The biomechanical relation between the position of the head and cervical spine and dentofacial morphology has been investigated.[31]

The most accurate way to measure the head and cervical posture is to use teleradiographs and cephalometric analysis. To take teleradiographs in clinical practice the subject is positioned in the cephalostat with the Frankfurt plane (line from porion to orbitale) parallel to the floor. The technique is reproducible and provides a clear view of the teleradiographs with few projection errors, but it can modify the natural posture of the subject. On the other hand, lateral cephalograms of the natural head position would reproduce the subject's own postural pattern and allow the exact posture of the head and cervical spine to be evaluated accurately in the sagittal plane. Some authors have questioned whether the teleradiographs using the Frankfurt method and those obtained through the natural head position give different information about craniocervical variables,[32] and found that the head position according to the Frankfurt method has a slight tendency to back-rotation and a diminution of cervical lordosis compared with the natural head position method, even if the results did not differ significantly. Only values of the craniocervical angles differed, but not enough to be clinically relevant.[32]

Ways to obtain natural head position: "self-balance" and "mirror" positions

Most studies use the technique described by Solow and Tallgren.[16] As the position of the head is part of the total posture of the body, they first defined a reproducible and physiologically relevant body posture. Among the standing positions (such as "attention" or "relaxed") the "orthoposition" was selected, which was defined as the "intention position from standing to walking" and this was achieved clinically by letting the subject walk lightly on the spot. It was considered a habitual symmetrical standing position and was reproducible in postural investigations.

Natural head position can be calculated in two ways, the first of which uses the subject's own feeling of a natural head balance without external reference. The head position is the result of proprioceptive information from muscles and ligaments and possibly from the utricular and semicircular canals, and the position is termed the "self-balance" position. The second method is based on visual cues from some external reference, as the subject positions the head so that they can observe either their eyes in the mirror ("mirror" position) or some device placed at a distance, horizontally, in front of the eyes. Positioning according to external reference (for example mirror position) should be done only after the head has been placed in the self-balanced position.[17]

This can be summarised as: the self-balanced position is when the subject is asked to tilt the head forwards and backwards with decreasing amplitude until the natural head balance is reached, and the mirror position is when the subject is asked to look straight in into the eyes reflected on a mirror in front. Both recordings are made with the teeth in occlusion. In adults the head is kept about 3° higher in the mirror position than in the self–balance position.

Two-dimensional recording: procedure to record and transfer the natural head position to the cephalostat

Once the natural head position has been achieved it can be recorded radiographically or photographically, the latter being preferred as it allows most freedom in producing a natural position.[4,5,33]

There are two ways to record the natural head position: first, the head of the patient is orientated to it and then a marker or a plumb line is used as a true vertical reference before radiographs or photographs are taken. This is called the "registered natural head position" and it is pointed out by the marker or the plumb line. Secondly, the patient's conventional cephalograms or lateral facial photographs are taken and then rotated to their natural head position (reorientating).

Cephalometric radiographs taken in the natural head position

The procedures for recording and transferring the natural head position to the cephalostat vary.

Positioning involves instructing and rehearsing the patient outside the cephalometer before actual positioning in it.[17] The cephalostat is adjusted vertically and the head is supported with the ear rods, which should not be inserted into the acoustic meatus - they have to be reversed to support the head from both sides by lightly touching the ears.

Table 2
Description of different methods to reorientate lateral radiographs to the natural head position according to standard photographs made of the natural head position.

First author, year, and reference	Method
Lundstrom, 1992[19]	Natural head position is photographed. A vertical axis is recorded on the photographs with a plumb line and transferred to the patients' lateral head radiographs.
Ferrario, 1994[12]	The angle between the soft tissue nasion-pogonion line and the true vertical is calculated on the photograph of natural head position, and this is used to rotate the standard radiograph around the Bolton point.
Dvorstin, 2011[40]	Three superimposing protocols (the soft tissue N/subnasale line [V-line], the aesthetic line [E-line], and a proposed nasal "best fit" line [N-line]) were compared for the reorientation of the cephalogram according to the photographs made of the natural head position.

Some measuring devices will record the natural head position and transfer it to the cephalostat: a fluid level device, an inclinometer, and a bubble air device.[20,34–38] These should make it possible to measure and reproduce the head position accurately, transfer a prearranged head position to the cephalostat, and take lateral films of the head. Even though some devices could measure both pitch and roll of the head by transferring the natural head position in two planes of the space in lateral cephalometrics, a transverse adjustment is always required. The conventional use of two ear rods to stabilise the head in radiographic cephalometry (lateral or frontal radiographic projections) is therefore based on the assumption that the transmeatal axis of humans is perpendicular to the midsagittal plane. In cases where the relation of the left and right ears in their vertical and horizontal relation to each other is asymmetrical, the insertion of ear rods results in vertical or horizontal rotation of the head, or both, and produces an altered and misleading image.[39] However, the capture of a natural head position while taking a radiograph without the use of a cephalostat will give unsatisfactory pictures. Taking cephalograms of the natural head position is challenging and takes more time than the conventional method.

Reorientating techniques

Reorientation of the cephalogram according to the photographs at the natural head position: the natural head position is captured on a photograph with a plumb line and then transferred or superimposed on to a conventional cephalogram (photographic superimposition) (Table 2).[40]

Placement of markers on subject's face: a wire shadow or red laser level beam projects a true vertical line on to the subject's profile, and two points are marked on the vertical line on the face with a radiopaque material and the cephalometric radiograph is taken in the usual way.[41,42] The metal beads give radiopaque shadows that are connected as a natural vertical axis, and they permit orientation of the cephalogram in the natural head position.

Three-dimensional recording

Since the recent increase in interest in 3-dimensional imaging for orthodontic evaluation, several studies have been published that describe different ways to record natural head position.[43–48]

Assessment of craniofacial morphology is influenced by the experience and perceptions of the examiner, [49] so the standard positioning of the patient and system for acquisition of the measurements are crucial for objective assessment. An accurate 3-dimensional coordinate system is required and Swennen et al describe how to set up a Cartesian, anatomical, 3-dimensional, cephalometric reference system.[49]

Current 3-dimensional imaging software contains tools for rotation and translation of 3-dimensional rendering, volumes, and surfaces, as well as recordings of different acquisitions with methods based on landmarks, volumes, or surfaces. However, to our knowledge there is still no external reference for head positioning, and there seems to be no stable reference structure in 3-dimensional facial photographs for soft-tissue assessments in longitudinal studies.

Positioning of the head in cone-beam computed tomography (CT) is difficult because the scanning time is relatively long (20–40 seconds), which requires the patient's head to be fixed to avoid movement. Because the images derived from cone-beam CT are 3-dimensional, the position of the head must be recorded in all three planes of space, as the pitch, roll, and yaw of the head accurately orientate the 3-dimensional image.[50]

Many studies have dealt with the issue of recording the head position when it is unrestrained, as it is during 3-dimensional photography or cone-beam CT. As for 2-dimensional imaging, there are two approaches: 3-dimensional recording with the patient in the natural head position, and acquisition of the 3-dimensional image and subsequent reorientation of the volume according to the previously chosen natural head position (Fig. 1). The first method is limited to acquisition of 3-dimensional photographs.

Some authors evaluated the use of minisensors for recording unrestrained head position during 3-dimensional stereophotogrammetry by testing a device composed of three orientation sensors with six degrees of freedom placed on a headset during acquisition of a 3-dimensional photograph. They suggested that a digital 3-dimensional tracking system can be promising for reproducibility of the head position, even though several improvements are required before they can be incorporated practically for research or clinical use.[45]

Hsung et al. recently developed a technique to capture information about natural head position using stereophotogrammetry and defined it as "stereophotogrammetric

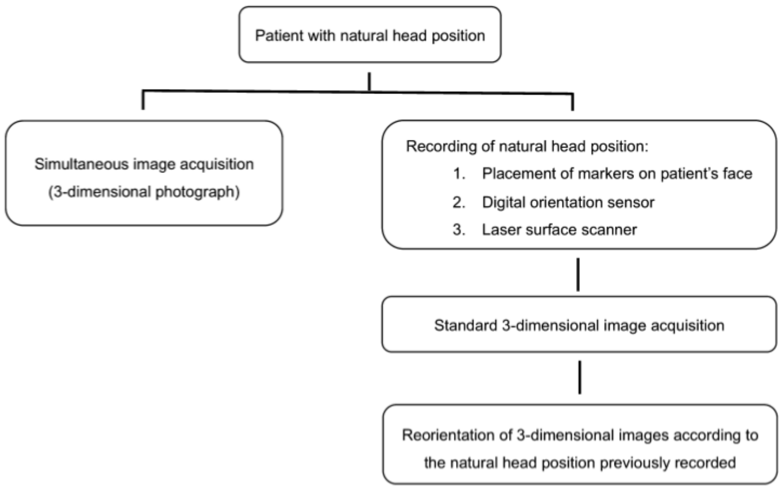

Fig. 1. Flowchart of 3-dimensional methods for recording the natural head position.

natural head position".[48] Stereophotogrammetry machines provide non-invasive, photographic-quality, 3-dimensional, accurate, capture of objects, and because calibration does not usually need any physical reference, the stereophotogrammetry system can capture only the surface morphology irrespective of its anatomical orientation. The resulting facial image is tilted to an unknown orientation, so the authors proposed additional calibration to correct the orientation by using some physical references such as true vertical and mirror orientation.[48]

Their first step is to record a digital mesh model of a hanging reference board placed at the capturing position of the stereophotogrammetry machine. The board is aligned to the true vertical using a plumb bob, and to a laser plane parallel to a hanging mirror located at the centre of the machine. The measurements derived from the digital mesh model of the board are used to adjust the roll, pitch, and yaw of the subsequent facial images, and the physical reference information is valid until the next time that the machine is calibrated. This approach allows the patient to achieve the natural head position without the use of any devices or troublesome markings on the face. More importantly, it is extremely repeatable and accurate, because the placement of the board showed a SD of less than 0.1° for pitch and yaw angles and less than 0.15° for roll angles. Alternatively, the 3-dimensional image can be reorientated according to the position previously recorded.

Methods of recording to orientate 3-dimensional images into the natural head position

Laser-assisted surface marking followed by acquisition of the image: laser lines are used as reference lines to place soft tissue reference markers. Some authors place six glass spheres on the patient's face as soft-tissue reference markers. They then take a cone-beam CT and the image is orientated in the three planes of space by aligning the reference marks to the horizontal references lines.[43] In another study an iCAT™ device was used as a reference to place four ink points on the faces for orientation by using the machine laser-light beams projected to record true horizontal and vertical lines. The placement of dots to record natural head position is followed by facial imaging with the stereophotographic imaging system.[46] However, putting marks on a patient's face could introduce problems of reproducibility and variability, and it also lengthens the operating time for making each of the 3-dimensional or CT scans.

Laser surface scanning: a 3-dimensional laser scanner records the surface geometry and absolute orientation of soft facial tissues while the patient is in the natural head position, and the CT model is orientated to the natural head position based on these results.[27] Although recording the position by laser scanner is accurate, the method is impractical for routine use, because the device is bulky and expensive.[44]

Natural head position recording device: this uses a small and inexpensive device that consists of a digital orientation sensor attached to the patient by a bite jig and a facebow, which is capable of digitally recording natural head position in three dimensions and transferring it precisely to a 3-dimensional model.[44,51] Although the orientation sensor method is inexpensive compared with the laser scanner, it requires the construction of a patient-specific bite jig, and severely displaces the upper and lower lips during the CT imaging. Because the position of the upper lip is the most important landmark for predicting soft tissue in the simulation of 3-dimensional surgery, an undeformed resting lip position is essential in computer-assisted orthognathic surgery.

Table 3
Recent studies on recording head position during 3-dimensional image capture (cone-beam computed tomography (CT) and 3-dimensional photograph).

First author, year, reference	Method	3-dimensional image
Damstra 2010[43]	Laser lines used to place six soft-tissue markers (glass spheres); cone-beam CT taken and patient's image orientated in the three planes of space by aligning the reference marks to the horizontal lines.	Cone-beam CT
Xia 2011[44]	Digital orientation scanner attached to patient by an bite jig and facebow permits recording of natural head position in 3 dimensions and transfers it precisely to a 3-dimensional model.	CT
De Paula 2012[45]	Three-dimensiosnal picture taken of patient with unrestrained head position wearing headset with 3-dimensional live tracking sensors, each of which records six degrees of freedom in head position.	Photograph
Weber 2013[46]	iCAT™ device used as reference to place four orientation ink spots on face using the machine's laser beams projected to record true horizontal and vertical to record natural head position. This is followed by facial imaging with stereophotographic imaging system.	Photograph
Kim 2014[47]	Spherical ceramic markers attached with transparent tape to the face as feature points. Frontal photograph of natural head position taken using ordinary digital camera parallel to the horizon, followed by CT. Positions of ceramic markers calculated on 2-dimensisonal image and correspond to points on the 3-dimensional model through POSIT.	CT
Hsung 2014[48]	Digital mesh model of reference board recorded and superimposed on model of patient's face. Facial images are corrected to natural head position according to pitch, yaw, and roll angle of the reference board model.	Photograph

iCAT™ = cone-beam CT scanner.
POSIT = pose from orthography and scaling with iterations.

Discussion

The volumes can be reorientated into the natural head position on cone-beam CT scans taken vertically or horizontally. This reorientation and the subsequent cephalometric evaluation can reliably be done only when the soft-tissue facial profile has not changed as a consequence of recording in the supine head position. Some research workers claim that the drape of the soft tissues of the face is different when a patient is supine.[52] Hoogeveen et al investigated whether the soft-tissue facial profile, as evaluated by soft-tissue cephalometric analysis, is different for a subject when in the natural or supine head position,[53] and they found significant differences depending on the head position while the recording is being made. However, these differences were not clinically relevant, except for the chin–throat angle, which is over 5° more acute when recorded supine, suggesting a more prominent chin. This can influence the planning of orthognathic surgery and compromise the result of the procedure. To avoid this, the authors advise that the picture of the chin–throat area should be accompanied by a complementary (photographic) recording in the natural head position.[53]

Kim et al developed a new way to record and reproduce the 3-dimensional natural head position from a single frontal photograph of a patient face using a "pose from orthography and scaling with iterations" (POSIT) algorithm.,[47] which was developed by DeMenthon and Davis to calculate the rotation matrix of an object (the patient's head) and to transform its coordinates into a camera coordinate system.[54] It combines two algorithms: the first, pose from orthography and scaling (POS), approximates the perspective projection using a scaled orthographic projection, and calculates the rotation matrix of an object by solving a linear system. The second, POS with iterations (POSIT), uses the approximate POS in an iteration loop to compute better orthographic projections for the featured points.[54] The authors proposed that spherical ceramic markers 4 mm in diameter should be attached with transparent tape to the patient's face as feature points. A frontal photograph of the patient's natural head position is taken with an ordinary digital camera parallel to the global horizon. A CT image is then taken of the patient with the markers. The positions of the ceramic markers are calculated on the 2-dimensional image and correspond to points in the 3-dimensional model. The 3-dimensional rotation matrix calculated from the feature points by the POSIT method is applied to the CT model to reproduce the natural head position with no additional recording or processing. The authors say that the method is easy to use, accurate, and inexpensive. In addition it does not affect the patient's lip position, and it can be applied to various CT images to diagnose and treat orthognathic patients, particularly those with facial asymmetry (Table 3).[47]

We conclude, first, that taking cephalograms of the natural head position is useful to investigate the association between craniocervical posture and dentofacial morphology. However, stabilisation of the head in the cephalostat produces rigid fixation that might prevent the patient from reaching the relaxed natural position.

Secondly, the natural head position has been proposed as a postural basis for assessment of craniofacial morphology. It has therefore been advocated as a better option than intracranial reference lines for cephalometric analyses in orthodontics and orthognathic surgery. It would therefore be appropriate to combine them in clinical decision-making. The aesthetic and anthropometric examination of the profile should be made on the patient's natural head position to provide important supplementary information for the intracranial cephalometric diagnosis.

Finally, we have described various techniques to reproduce and record the natural head position in two and three planes of space. The ideal method should avoid the use of any device attached to the head to achieve the natural head position. It should be simple and easy to do, with no troublesome markings on the patient's face or subjective identification of specific reference points. The success of the protocol should not be operator-dependent, so that an inexperienced practitioner can follow the procedures to record and reproduce the 3-dimensional natural head position without complex training. It is therefore desirable that the method does not produce major artefacts in the CT images and does not affect the patient's lip position or deform the facial soft tissues, and lastly it should be quick and cost-effective.

Conflict of Interest

We have no conflict of interest.

Ethics statement / Confirmation of patients' permission

Not required.

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