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GENETIC AND ENVIRONMENTAL INFLUENCES ON MANDIBULAR MORPHOLOGY AND RELATIONSHIP TO CRANIAL BASE AND MAXILLA

CEPHALOMETRIC TWIN STUDY

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ABBREVIATIONS

2D	–	Two dimensional
3D	–	Three dimensional
3dMD	–	Three dimensional stereophotogrammetric device
CBCT	–	Cone beam computed tomography
CVM	–	Cervical Vertebrae Maturation
CS	–	Cervical stage
c^2	–	Coefficient of cultural inheritance
DC	–	Dichorionic
DNA	–	Deoxyribonucleic acid
DZ	–	Dizygotic
h^2	–	Heritability estimate
LAFH	–	Lower anterior facial high
LOOCV	–	Leave-one-out cross-validation
ME	–	Measurement error
MZ	–	Monozygotic
NS	–	Not significant
rDZ	–	Correlation coefficient of dizygotic twins
rMZ	–	Correlation coefficient of monozygotic twins
QTLs	–	Quantitative trait loci
SD	–	Standard deviation
STR	–	Short tandem repeat
TAFH	–	Total anterior facial high
TPFH	–	Total posterior facial high
UAFH	–	Upper anterior facial high

INTRODUCTION

The craniofacial morphology has two major sources of variability, genetic and environmental. The success of orthodontic and dento-facial orthopedic correction is determined by the extent to which a particular malocclusion can be influenced by therapeutic environmental intervention. But the separation of genetic determination and environmental influences in development of malocclusion is one of the most controversial and important problems in orthodontics. The precise estimation of the role of genetic influence on phenotype variance of craniofacial structures can help to answer this question.

The etiology of malocclusion is polygenic and in addition quantitative characteristics are influenced by environmental factors. The polygenetic variance, effecting phenotype, can be subdivided into components consisting of additive genetic variance, dominance genetic variance and genic interaction variance. The additive genetic influence means a mechanism of quantitative inheritance when combined effects of genetic alleles at two or more gene loci are equal to the sum of their individual effects.The proportion of the phenotypic variation that is due to genetic differences is known as the heritability, and proportion of phenotypic variance that is due to additive genetic variance is called narrow-sense heritability. For the more clarity and simplicity in this study the term heritability is used to define narrow-sense heritability. One of the methods to separate environmental and genetic influences is to compare resemblances between twins. Monozygotic (MZ) twins share the same genes, whereas dizygotic (DZ) twins on average share only half of their genes. Therefore, by assuming that similar environmental factors act upon them we can estimate relative contribution of genetic and environmental influences to observed phenotypic variation. The advantage of twin studies, is that the total phenotypic variance can be split up into genetic, shared or common environmental, and unique environmental components, enabling an accurate estimation of heritability. Building on the pioneering work of Sir Francis Galton in the 19th century, studies of twins became more common and sophisticated throughout the 20th century, providing important insights into the relative contributions of “nature” and “nurture” to variation in many behavioural and physical features, including dento-facial skeletal and dental traits.

The biological structures formed uder the dominant genetic control has small room for environmental intervention and this should be kept in mind when new approaches for the management of developing malocclusion are proposed. The evidences indicating inherited pattern of the mandibular

morphology and its relationship to cranial base and maxilla with twin method has been accumulated, but the data on heritability of cephalometric variables are inconsistent and controversial. In some genetic studies measuring craniofacial variables, it has been suggested that vertical dimensions of the face are more under genetic influence, but the others found low heritability for whole craniofacial complex. This could be due to methodological shortcomings related to zygosity determination and study sample collection. The genetic and environmental influences on phenotypic variance of mandibular morphology and its relation to other craniofacial structures needs to be re-assessed with the use of new technological advances and elimination of the previous methodological shortcomings.

Aim of the study

The aim of this study was to assess additive genetic and environment influences on the variance of cephalometric variables of mandibular morphology and its relationship to cranial base and maxilla of the same sex twins with completed mandibular growth and DNA based zygosity determination.

Objectives of the study

1. To compare accuracy of morphometric 3D facial analysis with DNA testing of 15 highly variable genetic loci in twin zygosity determination.
2. To assess additive genetic influence on the variance of cephalometric variables of mandibular morphology and its relationship to cranial base and maxilla.
3. To assess influence of environment on the variance of cephalometric variables of mandibular morphology and its relationship to cranial base and maxilla.

Novelty and practical significance of the study

The combination of advanced technologies of 3D facial imaging and DNA based zygosity determination of twins allowed to gather new and original data.

A basic problem with the previous twin research was the reliability of the twin zygosity diagnostics. The novelty of the present study is twin zygosity determination based exceptionally on DNA test using 15 specific DNA markers (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TROX, D18S51, D5S818, FGA) and Amel fragment of amelogenin gene. The accuracy of zygosity diagnostics

reached level of 99.99% on a remarkable number of twins (141 pairs). Our results provide reliable data, that zygosity determination by morphometric methods (even with advanced 3D high precision imaging) is not acceptable for genetic studies. The bias in twin zygosity determination with the facial morphometric methodology runs between 10–25%.

The previous twin studies analysed data from the growing subjects or used age adjusted measurements to simulate completed growth of the mandible. The results of such studies of the influence of additive genetic variance on mandibular morphology and its relationship to craniofacial structures should be interpreted with caution, because the full gene expression can be detected only, if mandibular growth completely finished. This study is the first study of the influence of additive genetic variance on mandibular skeletal and dental cephalometric variables of twins with completed dento-facial growth. The study of subjects with full expression of genetic regulation discovered the existence of the area in craniofacial complex with high heritability, which possibly determines heritability of our dento-facial skeleton. This was not possible in previous twin studies with growing subjects.

The results of this study indicate that there is a significant component of additive genetic variance effecting a number of well-known cephalometric variables in twins. The form of mandible is determined by angular variables with high genetic heritability estimates and its modification could be problematic. The presence of genetic component in the mandibular growth control encourage further genome-wide association studies to identify which particular genes are related with mandibular phenotypic variations.

1. LITERATURE REVIEW

1.1. Mandibular growth and malocclusion

1.1.1. Role of the mandible to normal occlusion and malocclusion

Malocclusion may be defined as a significant deviation from what is defined as normal or 'ideal' occlusion [1]. Many components are involved in normal occlusion. The most important are: (a) the size of the maxilla; (b) the size of the mandible, both ramus and body; (c) the factors which determine the relationship between the two skeletal bases, such as cranial base; (d) the arch form; (e) the size and morphology of the teeth; (f) the number of teeth present; (g) soft tissue morphology and behaviour, lips, tongue, and peri-oral musculature. The term "normal occlusion" is arbitrary, but is generally accepted to be Class I molar relationship with good alignment of all teeth and represents a situation that occurs in only 30–40% of the population [2].

There is dental anthropological evidence that population groups that are genetically homogeneous tend to have normal occlusion. In pure racial stocks, such as the Melanesians of the Philippine islands, malocclusion is almost non-existent. However, in heterogeneous populations, the incidence of jaw discrepancies and occlusal disharmonies is significantly greater. Scientific research shows that orthodontic anomalies are one of the most common dental pathologies with a prevalence ranging from 39% to 93% among children [3–7].The prevalence of orthodontic anomalies in Lithuania is high and among 7–15 year old children may reach 85% [8]. Recent studies confirmed that malocclusion is highly prevalent in Lithuania: among 10–11 years old schoolchildren it was stated in 77% cases, and among 14–15 years old – 61.7% [9]. Angle class II occlusal relationship of the first permanent molars was found in 27.9% of cases, and Angle class III – in 1.6% of cases in Lithuanian children aged 7–15 years [10]. Considerable variation in epidemiologic data across studies can be explained by different diagnostic criteria.

At present, researchers recognize that the etiological factors of malocclusion can be differentiated into two groups – the genetically determined factors and the environmental factors [11–14]. The interaction between these two kinds of factors during the development of a child is highly individual. There are a number of publications that prove the influence of genes on the development of dento-facial system [15]. Class III malocclusions show a relatively low prevalence in European-American populations (4% according to Van Vuuren) [16], whereas the prevalence is twice as high in

black Nigerians (8%; Isiekwe) [17] and even higher in Asian populations. As some Asian ethnic groups demonstrate an increased prevalence of Class III malocclusions, it is likely that the skeletal components and soft tissues matrices are genetically determined [18]. Genes also determine dental size and shape, supernumerary teeth and adentia, the time of eruption, etc. [19]. The environmental factors include forces and pressures from soft tissues and muscles surrounding the dental arches, various habits (thumb sucking, nail-biting, etc.), forces resulting from mastication and the effect of orthodontic appliances[12, 13, 20]. Therefore, although the majority of the etiological factors are clear, malocclusion remains one of the most urgent oral health problems. Their treatment lasts for 2–3 years, is expensive and not always effective. The prediction of the mandible growth modification limits in every patient and identification of areas in maxillofacial skeleton that could be influenced by environmental factors is the key to successful orthodontic treatment.

The controversies surrounding the use of dento-facial orthopedics to correct a developing maxilla – mandibular discrepancy – i.e., growth modification – have been based to a large extent on evolving concepts concerning the biological mechanisms of craniofacial development and growth. At the start, these concepts were based on naive assumptions about the perceived competing roles of heredity and the environment, often framed within the context of the age-old “nature-nurture” controversy [21]. Moreover, orthodontists and craniofacial biologists alike tended to believe that there was a single, overriding mechanism governing the growth of the craniofacial skeleton. As a result, much of the orthodontic research on the growth of the face and jaws tended to focus on a search for a single theory of craniofacial growth that is both biologically accurate and clinically effective.

The last major theory of craniofacial growth to emerge, the servosystem theory, was developed by Alexandre Petrovic, a physician-scientist interested in the extrinsic and intrinsic hormonal factors that affect cartilage growth. As a result of influences by many orthodontists throughout Europe and the North America, Petrovic’s research came to focus on the nature of cartilage growth in the craniofacial complex, and especially of the growth of the secondary cartilage of the mandibular condyle [22–26]. Through a comprehensive series of in vitro and in vivo experiments using research approaches that were then state-of-the-art, Petrovic and colleagues demonstrated that the growth of the mandibular condyle is highly adaptive and responsive to both extrinsic systemic factors and local biomechanical and functional factors [27–34]. It was demonstrated that the growth of the primary cartilages of the craniofacial complex, such as the cranial base and

nasal septum, was influenced significantly less by local epigenetic factors [35, 36].

The servosystem theory relies on the vocabulary of cybernetics to describe the growth of the craniofacial complex. Most simply, the servosystem theory is characterized by the following two principal factors: (1) the hormonally regulated growth of the midface and anterior cranial base, which provides a constantly changing reference input via the occlusion, and (2) the rate-limiting effect of this midfacial growth on the growth of the mandible. While growth of the mandibular condyle and of the sutures may be affected directly and indirectly by systemic hormones, growth of these structures is clearly more compensatory and adaptive to the action of extrinsic factors, including local function as well as the growth of other areas of the craniofacial complex.

First, as the midface grows downward and forward under the primary influence of the cartilaginous cranial base and nasal septum, influenced principally by the intrinsic cell-tissue related properties common to all primary cartilages and mediated by the endocrine system, the maxillary dental arch is carried into a slightly more anterior position. This causes a minute discrepancy between the upper and lower dental arches, which Petrovic referred to as the "comparator," that is, the constantly changing reference point between the positions of the upper and lower jaws [34]. Second, proprioceptors within the periodontal regions and temporomandibular joint perceive even a very small occlusal discrepancy and tonically activate the muscles responsible for mandibular protrusion. Third, activation of jaw protruding muscles acts directly on the cartilage of the mandibular condyle and indirectly through the vascular supply to the temporomandibular joint, stimulating the condyle to grow. Finally, the effect of the muscle function and responsiveness of the condylar cartilage is influenced both directly and indirectly by hormonal factors acting principally on the condylar cartilage and on the musculature. This entire cycle is continuously activated as a servomotor as long as the midface-upper dental arch continues to grow and mature and appropriate extrinsic, hormonal, and functional factors remain supportive.

Now that we are in the postgenomic era, of the development of competing concepts and theories of craniofacial development and growth, at least three issues would seem to be clear as they pertain to craniofacial development and growth and to the possibility of modification of craniofacial growth. First, there are a number of genetically encoded regulatory factors that have profound effects on the morphogenesis and prenatal development of the craniofacial complex. Second, it is clear that all of these factors operate within an epigenetic milieu, from the level of the position of

genes on the chromosome to the interaction of cells and entire organisms with the external environment. Genes are turned on and off by factors both within and outside the genome to produce specific traits as well as to influence susceptibility to variations of development and growth. Third, there is a plethora of evidence from experimental embryology, teratology, and functional morphology to support the conclusion that morphogenesis, prenatal development, and postnatal growth of the craniofacial complex can be modified. However, this does not necessarily mean that craniofacial growth can be modified in a predictable, controlled, and clinically effective way. The following are major issues that must be considered regarding clinical efforts at craniofacial growth modification: (1) what are the biological targets of treatment in the attempt to modify craniofacial growth, that is, where is the growth-related problem located? (2) what is the amount of desired growth effect, that is, how much modification of craniofacial growth is reasonable to consider? (3) what are the most appropriate treatment approaches that may be used to bring about the desired growth effect? Only by understanding in detail the biological factors that influence the development and growth of craniofacial tissues, can their growth be predictably modified and controlled especially in mandible.

1.1.2. The orthodontic treatment of skeletal malocclusion: mandibular growth modification

The potential impact of advances in developmental biology for prevention and treatment of craniofacial deformities through the use of dento-facial orthopedics can be found in the essential question put forward initially by Thomas H. Morgan nearly 100 years ago concerning the timing of gene action [37]. Morgan asked whether all genes were always active, or whether genes were active only at certain time periods during development. A similar question can be raised now as it relates to the potential for refinement of orthodontic-dento-facial orthopedic treatment. We now are becoming increasing aware that a number of genes and gene products regulate craniofacial morphogenesis, and that these genes are turned on and off at critical times during development. These gene products do not determine growth and certainly do not determine specific form. Rather, they provide factors that may affect the receptivity and responsiveness of cells to intrinsic and extrinsic stimuli. Is it possible to activate these genes and produce growth factors that may have positive, targeted, and predictable effects on postnatal craniofacial growth? There are ample data from studies of wound healing, skeletal growth associated with distraction ostoegenesis and orthopedic forces, and alteration of neuromuscular function, just to

name a few, to indicate that trauma, mechanical forces, and “function” may be the types of epigenetic factors that activate expression of regulatory genes influencing postnatal growth. Thus, the issue is not the fact that intrinsic factors within the genome regulate morphogenesis, but that the complex interaction of cells and tissues with remote extrinsic factors within both the body and the environment are triggers, or switches for gene expression that influences postnatal growth and responsiveness to clinical treatment [38].

The overall summarizing principles and regulatory mechanisms of craniofacial morphogenesis fundamental conclusions can be drawn. First, there is no question that it is theoretically and practically possible to modify the growth of the craniofacial skeleton, just as it is possible to modify skeletal growth elsewhere throughout the body. Second, there is no “Holy Grail” of craniofacial biology – no single theory of craniofacial growth. The development of the craniofacial region is complex and unique in some respects with regard to the origin of certain tissues and cell-tissue interactions [39]. However, like all areas of the body, the craniofacial complex develops, grows, and adapts within an epigenetic milieu that includes both the genes themselves all the way up to broad environmental and functional factors, but within the parameters that are regulated and permitted by the genome. What remains now is to understand the genomic and epigenetic factors influencing the morphogenesis and growth of the craniofacial complex sufficiently that they can be engineered biologically and environmentally, and subsequently introduced into the treatment of individual patients at the appropriate times and in the appropriate measure to produce a biologically meaningful effect and a predictable and clinically efficacious result.

Jaw discrepancies and malocclusion can be treated by orthodontics except in extreme cases where surgical intervention is required [40]. This is because it is possible to modify the direction of dento-facial growth using orthodontic appliances and therefore change or forestall morphogenetic abnormalities [41–43]. Orthodontic correction of a malocclusion is in effect altering the phenotypic expression of a particular morphogenetic pattern. The degree to which this can be successfully achieved depends on (a) the relative contribution of each factor to the existing problem, and (b) the extent to which skeletal pattern can be influenced by orthodontic and orthopaedic appliances.

In clinical orthodontics it must be appreciated that each malocclusion occupies its own distinctive slot in the genetic/ environmental spectrum and, therefore, the diagnostic goal is to determine the relative contribution of genetics and the environment. The greater the genetic component, the worse

the prognosis for a successful outcome by means of orthodontic intervention. The difficulty, of course, is that it is seldom possible to determine the precise contribution from hereditary and environmental factors in a particular case. For example, the simultaneous appearance of proclined maxillary incisors and digit sucking may lead to the assumption that the digit was the sole causative factor, but the effect of the digit may very well be either potentiated or mitigated by other morphological or behavioural features in that particular individual. A similar argument may apply in cases of mouth breathing where the influence of the habit and associated posture is very much dependent on the genetically determined craniofacial morphology on which it is superimposed, and the reason for the habit developing may well be dependent on the morphology in the first place. These scenarios are classical examples of the interaction of genotype and environment, and ultimately success of treatment will depend on the ability to ascertain the relative contribution of each [40].

Orthodontists believe that it is possible to influence the dento-alveolar regions of the jaws within certain parameters using environmental forces—otherwise orthodontic therapy would be futile. The division in orthodontic opinion arises from the doubt as to whether the skeletal bases can be influenced to any significant effect beyond their genetically-predetermined potential. There is still considerable debate about this, as conclusive evidence is lacking in both camps, but what evidence is available from human studies to date tends to support the genetic determination of craniofacial form with a lack of evidence to show any significant long term influence on mandibular or maxillary skeletal bases using orthopaedic appliances. The search for evidence to support the environmental influence on craniofacial growth is not easy and will require genetic modelling and statistical techniques to family and twin data [44].

If dento-facial structure and malocclusion are primarily genetic, e.g. severe mandibular prognathism or endogenous tongue thrust, then treatment will either be palliative or surgical. The search for a solution would ultimately focus on delineating the responsible genes. Conversely, if components of dento-facial structure and malocclusion have trivial heritability, then the search needs to be directed at environmental factors inducing malocclusion during growth and development. The goal would be to identify causes and formulate means of intercepting their negative influences. Such is the case with much of the interceptive orthodontic treatment presently carried out, in which the long-range goal is to permit the face to grow according to its fundamental genetic pattern with minimal obstruction from environmental influences, habit and adverse functional factors. An appropriate dental analogy in environmental manipulation is the reduced

caries incidence over the past few decades by introduction of fluoride supplements and public water fluoridation programmes. Twin studies, which include heritability estimation is usually a first step in genetic studies because it provides an estimate of how much phenotypic variation is attributable to genetic effects [45].

1.2. Studies of genetic and environmental influences on etiopathogenesis of malocclusion

The bulk of the evidence for the heritability of various types of malocclusion arises from family and twin studies [40].

*Class II Division 1 malocclusion***.** Extensive cephalometric studies have been carried out to determine the heritability of certain craniofacial parameters in Class II division 1 malocclusions [46–48]. These investigations have shown that, in the Class II patient, the mandible is significantly more retruded than in Class I patients, with the body of the mandible smaller and overall mandibular length reduced. These studies also showed a higher correlation between the patient and his immediate family than data from random pairings of unrelated siblings, thus supporting the concept of polygenic inheritance for Class II division 1 malocclusions. Polygenic inheritance, by definition, implies that there is scope for environmental modification but certain degree of genetic determination also exists [40, 49]. Concerning the environmental factors, the soft tissue capsule is of ultimate importance. Soft tissues can exert an influence on the position or inclination of upper and lower incisors and the need to achieve lip/tongue contact for an anterior oral seal during swallowing can encourage the lower lip to retrocline the lower incisors and the protruding tongue to procline the uppers, influencing the severity of the overjet. Likewise, digit sucking habits can produce a Class II division 1 incisal relationship, even if the underlying skeletal base relationship is Class I. Lip incompetence also encourages upper incisor proclination by virtue of the imbalance in labial and lingual pressures on the teeth.

Class II division 2 malocclusion. It is a distinct clinical entity with consistent collection of definable morpho-metric features occurring simultaneously, what is typical for a syndrome. Class II division 2 malocclusion comprises the unique combination of deep overbite, retroclined incisors, Class II skeletal discrepancy, high lip line with strap-like activity of the lower lip, and active mentalis muscle. This is often accompanied by particular morphometric dental features also, such as a poorly developed cingulum on the upper incisors and a characteristic crown root angulation. Peck et

al. [50] also describes characteristic smaller than average teeth when measured mesio-distally, reinforcing a similar observation made by Beresford [51] and a study by Roberston and Hilton [52], which found these teeth to be significantly “thinner” in the labial/lingual dimension. A further feature of the Class II division 2 “syndrome” is a tendency to a forwardly rotating mandibular development, which contributes to the deep bite, chin prominence, and reduced lower face height. This last feature, in turn, has an influence in the position of the lower lip relative to the upper incisors, and an increase in masticatory muscle forces has been reported by Quinn and Yoshikawa [53]. Familial occurrence of Class II division 2 has been documented in several published reports including twin and triplet studies [54, 55] and in family pedigrees from Peck et al. [56]. Markovic [55] carried out a clinical and cephalometric study of 114 Class II division 2 malocclusions, 48 twin pairs and six sets of triplets. Intra- and inter-pair comparisons were made to determine concordance/ discordance rates for monozygotic and dizygotic twins. The monozygotic twins demonstrated, 100% concordance for the Class II division 2 malocclusion, whilst almost 90% of the dizygotic twin pairs were discordant. This is strong evidence for genetics as the main aetiological factor in the development of Class II division 2 malocclusions.

These studies point to incontestable genetic influence, probably autosomal dominant with incomplete penetrance and variable expressivity. It could also possibly be explained by a polygenic model with a simultaneous expression of a number of genetically determined morphological traits (acting additively), rather than being the effect of a single controlling gene for the entire occlusal malformation. The controversy regarding the aetiology of the Class II division 2 malocclusion arises from a failure to appreciate the synergistic effects of genetics and environment on facial morphology. Ballard [57], Houston [58], Mills [59], and others considered that a high lip line, and a particular lip morphology and behaviour were the main aetiological factors. Graber [60], Hotz [61] and Markovic [55] stressed the predominant role of genetic factors in the aetiology of Class II division 2 malocclusions. These views are of course not incompatible if the lower lip morphology, behaviour, and position relative to the upper incisors is considered to be genetically determined or influenced. The skeletal and muscle morphology also may be genetically determined and there is some recent experimental evidence from a twin study [62] indicating strong genetic factors in certain aspects of masticatory muscle behaviour.

Class III malocclusion. The morphology and pathogenesis of Class III is multifactorial. A Class III malocclusion may be resultant from a skeletal imbalance between the maxillary and mandibular bases due to deficiency in

maxillary growth, excessive mandibular growth, or a combination of both. Various studies have also highlighted the influence of a distinctive cranial base morphology with a more acute cranial base angle and shortened posterior cranial base resulting in a more anterior position of the glenoid fossa, thus contributing to the mandibular prognathism [63, 64].

Probably the most famous example of a genetic trait in humans passing through several generations is the pedigree of the so-called Hapsburg jaw. This was the famous mandibular prognathism demonstrated by several generations of the Hungarian/Austrian dual monarchy. Strohmayer [65] concluded from his detailed pedigree analysis of the Hapsburg family line that the mandibular prognathism was transmitted as an autosomal dominant trait. This could be regarded as an exception and, in itself, does not provide sufficient information to predict the mode of inheritance of mandibular prognathism. Suzuki [66] studied 1362 persons from 243 Japanese families and noted that, while the index cases had mandibular prognathism, there was a significantly higher incidence of this trait in other members of his family (34.3%) in comparison to families of individuals with normal occlusion (7.5%). Schulze and Weise [67] also studied mandibular prognathism in monozygotic and dizygotic twins. They reported that concordance in monozygotic twins was six times higher than among dizygotic twins. Both of the above studies report a genetic hypothesis as the primary cause for mandibular prognathism [68].

Familial studies of mandibular prognathism are suggestive of heredity in the aetiology of this condition [69–72]. Various models have been suggested, such as autosomal dominant with incomplete penetrance [73], simple recessive [74], variable both in expressivity and penetrance with differences in different racial populations [75].

A wide range of environmental factors have also been suggested as contributory to the development of mandibular prognathism. Among these are enlarged tonsils [76], nasal blockage [77], congenital anatomic defects [78], hormonal disturbances[79], endocrine imbalances [74], posture and trauma/disease including premature loss of the first permanent molars [80]. Litton et al. [68] examined a group of probands, siblings and parents with Class III malocclusion, and analyzed the results in an effort to determine a possible mode of transmission. Both autosomal dominant and autosomal recessive transmission were ruled out and there was no association with gender since there were equal numbers of males and females. The polygenic multifactorial threshold model put forward by Edwards [81], however, did fit the data that these authors presented and, accordingly, they proposed a polygenic model with a threshold for expression to explain familial distribution, and the prevalence both within the general population and in

siblings of affected persons. They also made the sensible suggestion that different modes of transmission might be operating in different families or different populations.

Soft tissues do not generally play a part in the aetiology of Class III malocclusion, and in fact there is a tendency for lip and tongue pressure to compensate for a skeletal Class III discrepancy by retroclining lower incisors and proclining uppers.

1.3. The role of advanced technologies and twin studies for the craniofacial genetics

1.3.1. From two dimensional to three dimensional craniofacial morphometric studies

In the 20th century, radiographic cephalometry was a pioneering advance that led to many fundamental insights into the behavior of the face and neurocranium during growth. Three-dimensional (3D) soft-tissue surface imaging technologies and cone beam computed tomography (CBCT) imaging promises similar advances in the 21st century. However, to take advantage of this opportunity, clinicians will need to interpret 3D anatomic changes in the context of the underlying growth process, genetic and environmental influences [82].

Although the cephalometrics in orthodontics remains powerful research tool, the projection of the 3D anatomy of the face and skull onto a 2D film did have two disadvantage: firstly all parasagital information was either distorted or lost and secondly radiation possibly harmful for the patient. Three-dimensional (3-D) soft-tissue surface imaging technologies have been developed to overcome the limitations of 2-dimensional clinical and/or research records. These imaging modalities allow the capture of the human face in the 3 dimensions of space, thus collecting enough information to analyze all the facial components. Among the many existing surface imaging systems, photogrammetry are the most widely used [83]. The system is noninvasive and radiation free. The images they produce are valuable for diagnostic and research purposes [84].Many methods have been described to examine 3-D images. Some depend on landmark placement and subsequent analysis of distances, angles, surfaces, and volumes [85]. Other approaches use facial averages (also termed average shells) to examine changes owing to natural development or intervention treatment [86].

In shape observing methods: laser-scanning and three-dimensional (3D) analyses revealed differences in the facial shapes of MZ and DZ twins [87, 88]. The relative contributions of genetic and environmental factors were

different for the upper, middle, and lower facial thirds, but zygosity did not seem to influence the amount of facial symmetry [89–91]. Facial shape analyses showed greater similarity in MZ twins, with the lower third being the least similar. The average faces of MZ and DZ males were coincident in the forehead, the supraorbital and infraorbital ridges, the bridge of the nose, and the lower lip. In MZ and DZ females, the eyes, supraorbital and infraorbital ridges, philtrum and lower part of the cheeks were coincident. The lower facial third was the most asymmetric. In other words, the relative contribution of genetic and environmental factors are different for the upper, middle, and lower facial thirds and in age and gender groups, not only in twins, but in all individuals [92, 93].

There is no doubt that 3D technology will replace two dimensional pictures not only in research, but in clinical orthodontics as well. This technic is more precise, comprehensive and valuable in all means.

1.3.2. The twin study model

The major sources of variability in the skull and dentition combine genetic and environmental influences. The respective importance of the roles of these factors in determining the shape and size of the craniofacial complex is one of the most controversial and important problems in orthodontics. The major problems which delayed progress in the area is the complex nature of multifactorial inheritance. The first step in this type of studies is separation of additive and non-additive genetic factors. The classical twin studies may be used for this purpose with great success. The classical twin study is based on the fact, that MZ co-twins share the same genes, whereas DZ co-twins on average share only half of their genes. Therefore, by assuming that both types of twins have been sampled from the same gene pool and that similar environmental factors act upon them, one can estimate the relative contributions of genetic and environmental influences to observed variation in different features or traits.

There are several assumptions that underlie the classical twin approach and these were not tested fully in many of the early studies. Kang et al. [94] and Christian [95] have outlined that mean values for the trait under investigation should not differ between zygosity groups. Total variance and environmental co-variance within zygosities should also be equal for the model to hold, as heterogeneity of total variance suggests that environmental factors are not equal for MZ and DZ twins. All of these assumptions should be tested statistically prior to calculating genetic and environmental contributions to phenotypic variance. Furthermore, it has often been overlooked that heritability is a population concept, referring to the propor-

tion of genetic variation within a given population at a particular time. The concept should not be applied to a single individual but, rather, to a group of individuals [96].

The classical twin study design with the samples of monozygotic and dizygotic twin pairs has been used extensively to estimate variance components for a wide range of phenotypes in human populations. The primary statistics from these studies are the correlations between MZ pairs (rMZ) and between DZ pairs (rDZ). If twin resemblance due to common environmental factors is the same for MZ and DZ twins, then rMZ>rDZ implies that part of the resemblance is due to genetic factors and rMZ> 2rDZ implies the importance of non-additive genetic effects. Conversely, *rMZ*< 2rDZ implies that common environmental factors cause some of the observed twin resemblance. The heritability estimate is based on these implications and use the formula ($h^2 = 2(rMZ – rDZ)$ to calculate a heritability coefficient (h^2) to quantifying the extent of the genetic contribution to phenotypic variation, with proportions ranging theoretically from 0 to 1. However, since the difference between correlations theoretically can exceed 0.5, the coefficient can be greater than 1. This can lead to misinterpretation of the whole concept of heritability coefficient which conventionally is regarded as percentage estimate of genetic component versus environmental. However, as in majority of genetic research, the estimates of heritability show not the percentage of genetic factors within certain phenotype, but the variance of feature that can be explained by genetic factors.

Two types of heritability can be distinguished: “narrow-sense” heritability refers to the contribution of additive genetic variance to observed phenotypic variance, whereas “broad-sense” heritability refers to the total contribution of genetic factors (additive and non-additive) to the observed variation. Additive effects represent the sum of parental genes influencing the offspring’s trait, whereas non-additive effects encompass the effects of genetic dominance and gene–gene interaction. A long standing controversy has existed concerning the role and importance of additive and non-additive effects in craniofacial development. Twin studies demonstrated that the most important is the additive genetic variance because it determines most of the correlation of relatives and the opportunities for genetic change by natural or artificial selection. From reviews of the literature and presentation of a summary analysis of human twin data, it is made conclusion that a high proportion, typically over half, of the total genetic variance in craniofacial complex is additive [97]. This is the most important result of twin studies relevant to clinical orthodontics and creates background for more advanced genetic studies. Whole-genome linkage analysis, association analysis of putative candidate genes, and whole genome association approaches, now

offer exciting opportunities to locate key genes involved in human dental development and are based on the results from classical twin studies.

1.3.3. Critical evaluation of studies involving twins

One of the main criticisms of the classical twin model has been based on the assertion that MZ co-twins are likely to share more similar environments postnatally than DZ co-twins, so greater similarities between them compared with DZ co-twins may partly reflect more similar environments rather than more similar genetic constitutions. While this can be an important issue with some behavioural phenotypes, it is less likely to be a major factor in studies of dental morphology, although nutritional similarities could possibly affect dental development.

Another consideration is the possibility of an interaction between genetic and environmental influences. The classical twin model tends to assume that these two influences operate independently, hence the often-used phrase “nature versus nurture”. This is seldom the situation and there is frequent interaction between genetic and environmental factors.

A further criticism of the classical twin model has been whether it is reasonable to extrapolate the findings from twin studies to a general population containing many singletons, given the special nature of the twinning event, twin pregnancies and births, and the upbringing of twins. The nature of the phenotype under investigation is important when attempting to assess the importance of these factors s applied to behavior or psychological characteristics. However, there are some who question whether this is an appropriate assumption, even for dental variables [98, 99].

1.3.4. Limitations of the previous twin studies in dentistry: zygosity determination and maturity of study sample

Because of the entrenched misconception that MZ twins are necessarily identical, many MZ twin pairs are mistakenly designated as dizygotic (DZ) [100]. Many parents and professionals continue to use “MZ” and “identical” synonymously. As a result, the majority of parents of MZ twins think of their twins as DZ, because, on close and repeated inspection (and to preserve parental sanity), the twins reveal the ways that they differ in every respect. An alternative scenario commonly occurs in families whose MZ twins have been falsely labeled as DZ twins: naturally, the twins appear too uncomfortably alike to be DZ twins. Potentially harmful consequences of incorrect zygosity determination include failure to provide accurate genetic counselling to families; to initiate preventive medicine strategies (when one of an MZ pair presents phenotypically with disease before the other); and to

offer optimal organ transplantation (the correct match and potential avoidance of immune suppression in MZ twin recipients).

MZ twin pairs are living human laboratories, and when they begin to diverge phenotypically, we can discover new ways in which the genome/epigenome profoundly changes post-zygotically. Some of these divergences occur in embryonic stem cells soon after zygosis [101], perhaps in concert with events that underlie determination and differentiation of axes, organs, and tissues during embryogenesis. It is not clear whether any or most of these divergences actually stimulate the MZ twinning process. Other genetic/epigenetic divergences probably accumulate more slowly over the full lifetime of any MZ twin pair [102]. In addition to genetic/epigenetic events within MZ twins that drive a wedge in their common zygotic phenotype, there are also significant prenatal, intra-uterine environmental considerations that especially apply to the majority of MZ twin pairs (about two-thirds) who are monochorionic (MC), that is, twins who are both connected to a truly single (not fused) placenta. The remaining one-third of MZ twins are dichorionic (DC), developing in separate sacs. Parents of like-sexed DC twins are frequently misinformed that their twins are necessarily dizygotic (DZ). That is why methods of reliable zygosity diagnosis are fundamental in twin research field.

A basic problem with the previous twin research is the reliability of the twin zygosity diagnostics. Zygosity determinations for many years was based on assessment of anthropological similarity including tooth anatomy [92]**.** Although comparison of physical appearance can provide a reasonably reliable means of determining zygosity, errors can occur up to 10–25% with this methodology. The use of blood groups determination, as well as serum and enzyme polymorphisms, improved the ability to assign zygosities to twins [103]**.** More recently, the use of highly polymorphic regions of DNA derived from blood or buccal cells has proved to be accurate up to 90–95% of cases [104]. The more precise determination, for example at the level of 99.99%, requires increase number of highly polymorphic regions of DNA into analysis.

The second problem with previous twin studies relevant to mandibular morphology is the maturity of study sample. The vast majority of studies analyzed data from the growing subjects twins [87, 105–109] who have just passed their peak of pubertal growth spurts [45, 92] or used age adjusted measurements to simulate completed growth of the mandible [110]. The results of such studies on mandibular morphology heritability estimates should be interpreted with caution, because the full gene expression can be detected only, if mandibular growth completely finished.

Final conclusion of the literature review. The literature analysis shows, that there are no consensus on the understanding of the genetic and environmental contribution to malocclusion development and even more importantly our knowledge about morphogenetic aspects, specially form and size of the mandible, is insufficient and needs to be updated. To obtain new data of genetic impact on craniofacial morphology using classical twin study model, it is essential to use precise zygosity determination and study twins with full gene expression on the phenotype, e.g. with completed craniofacial growth.

2. MATERIALS AND METHODS

2.1. Study sample

The twins participated in this study were from the Twin centre of Lithuanian university of health sciences. This ongoing register already covers more than 600 twin pairs voluntary registered and willing participate in different medical and genetic studies. All twins of this register were offered free of charge DNA based zygosity determination and medical consultations including dental and orthodontic consultations. As part of dental and orthodontic examination orthopantomograms or standardized lateral head cephalograms were taken.

The inclusion criteria to this study were as follow: (1) twins of Caucasian descent, (2) lateral head cephalograms or 3D facial images of both twins of the pair available, (3) completed craniofacial growth of the twins.

The exclusion criteria: (1) previous orthodontic treatment, (2) permanent teeth extractions, (3) any facial trauma that could have resulted in bony fracture or soft tissue scarring.

The total number of twins selected for the study was 141 twin pairs. The 90 pairs of twins by DNA method were identified and allocated to MZ group and 51 to DZ group.

The sample's age and gender characteristics are shown in Table 2.1.1.

Table 2.1.1. *Study sample: age and gender of twin pairs*

Twins	**N**	**Age**				
		Mean	**SD**	**SE**	**Min**	**Max**
Study sample	141	21.73	5.24	0.44	15.3	39.6
MZ	90	22.45	5.81	0.61	15.3	39.6
Male	29	22.1	4.82	0.89	15.8	36.4
Female	61	22.62	6.25	0.8	15.3	39.6
DZ	51	20.47	3.78	0.53	15.4	37.8
Male	20	21.20	3.36	0.75	15.4	29.3
Female	31	20.0	4.01	0.72	15.5	37.8

The study protocol has been approved by the Kaunas Regional Ethical Committee (permissions: 2005-04-11 Nr. BE-2-21, 2010-12-10 Nr. P1-52/2005 and 2015-02-09 Nr. BE-2-12) and State Data Protection Inspectorate (Decision Nr. 2R-1840 (2.6-1). The written informed consent was obtained from all twins or their parents.

2.2. Methods

2.2.1. Assessment of the craniofacial growth and skeletal maturity

The cervical vertebrae maturation (CVM) method was used as a method of choice to assess stage of the mandibular growth. The CVM method was introduced by Lamparski [111] for growth assessment, allowing skeletal age evaluation and eliminating the need for additional radiographic exposure, since the vertebrae are already recorded in the lateral cephalogram taken as a pre-treatment record [112–116].

The CVM method modified by Baccetti [117] was used in the current study. Modification is based on visual assessment of the size and shape of the reduced number of cervical vertebrae. The morphology of the bodies of the second (C2), third (C3), and fourth (C4) cervical vertebrae was analyzed and one of six CVM stages was established (Fig. 2.2.1.1).

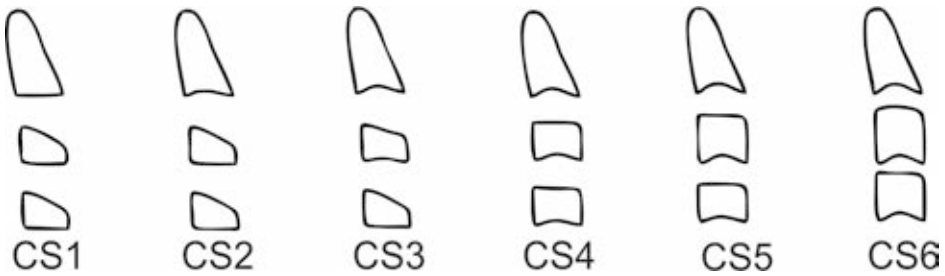

Fig. 2.2.1.1. *The stages of cervical vertebrae maturation*

The six stages are defined as follows:

Cervical stage 1 (CS1). The lower borders of all the three vertebrae (C2-C4) are flat. The bodies of both C3 and C4 are trapezoid in shape (the superior border of the vertebral body is tapered from posterior to anterior). The peak in mandibular growth will occur on average 2 years after this stage.

Cervical stage 2 (CS2). A concavity is present at the lower border of C2 (in four of five cases, with the remaining subjects still showing a cervical stage 1). The bodies of both C3 and C4 are still trapezoid in shape. The peak in mandibular growth will occur on average 1 year after this stage.

Cervical stage 3 (CS3). Concavities at the lower border of both C2 and C3 are present. The bodies of C3 and C4 may be either trapezoid or rectangular horizontal in shape. The peak in mandibular growth will occur during the year after this stage.

Cervical stage 4 (CS4). Concavities at the lower border of C2, C3, and C4 now are present. The bodies of both C3 and C4 are rectangular horizontal in shape. The peak in mandibular growth has occurred within 1 or 2 years before this stage.

Cervical stage 5 (CS5). The concavities at the lower borders of C2, C3, and C4 still are present. At least one of the bodies of C3 and C4 is squared in shape. If not squared, the body of the other cervical vertebra still is rectangular horizontal. The peak in mandibular growth has ended at least 1 year before this stage.

*Cervical stage 6 (CS6).*The concavities at the lower borders of C2, C3, and C4 still are evident. At least one of the bodies of C3 and C4 is rectangular vertical in shape. If not rectangular vertical, the body of the other cervical vertebra is squared. The peak in mandibular growth has ended at least 2 years before this stage.

The example of clinical radiograph at the cervical maturation stage 6 (CS6) presented in the Fig. 2.2.1.2.

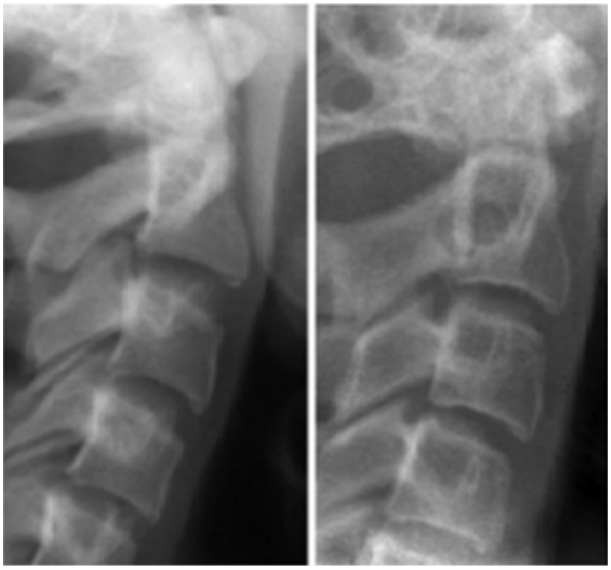

Fig 2.2.1.2. *Cervical maturation stage 6 (CS6)*

All radiographs were analyzed using commercially available software (Dolphin Imaging 11.7 Premium, Patterson Dental Supply, Chatsworth, USA). The assessment of radiographic images for CVM stage was done by the author of dissertation.

2.2.2. Zygosity determination of the twins

Zygosity determination was carried out at the certified laboratory of the enterprise "UAB Synlab Lietuva". The DNA based tests performed using venous blood. The procedure was started with DNA isolation from blood and purification. Then polymerase chain reaction set AmpFℓSTR®Identifiler® (Applied biosystems, USA) was used to amplify short tandem repeats (STR) and 15 specific DNA markers (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TROX, D18S51, D5S818, FGA), and Amel fragment of amelogenin gene were used for comparison of genetic profiles (Fig. 2.2.2.1). Although 99.9% of human DNA sequences are the same in every person, enough of the DNA is different that it is possible to distinguish one individual from another, unless they are monozygotic twins. The true power of STR analysis is in its statistical power of discrimination. Because the 15 loci and Amel fragment of amelogenin gene used for discrimination are independently assorted, the product rule for probabilities was applied. This has resulted in the ability to generate match probabilities of 1 in a quintillion (1×10^{18}) or more. Therefore, the accuracy for zygosity determination was at the level of 99.99%.

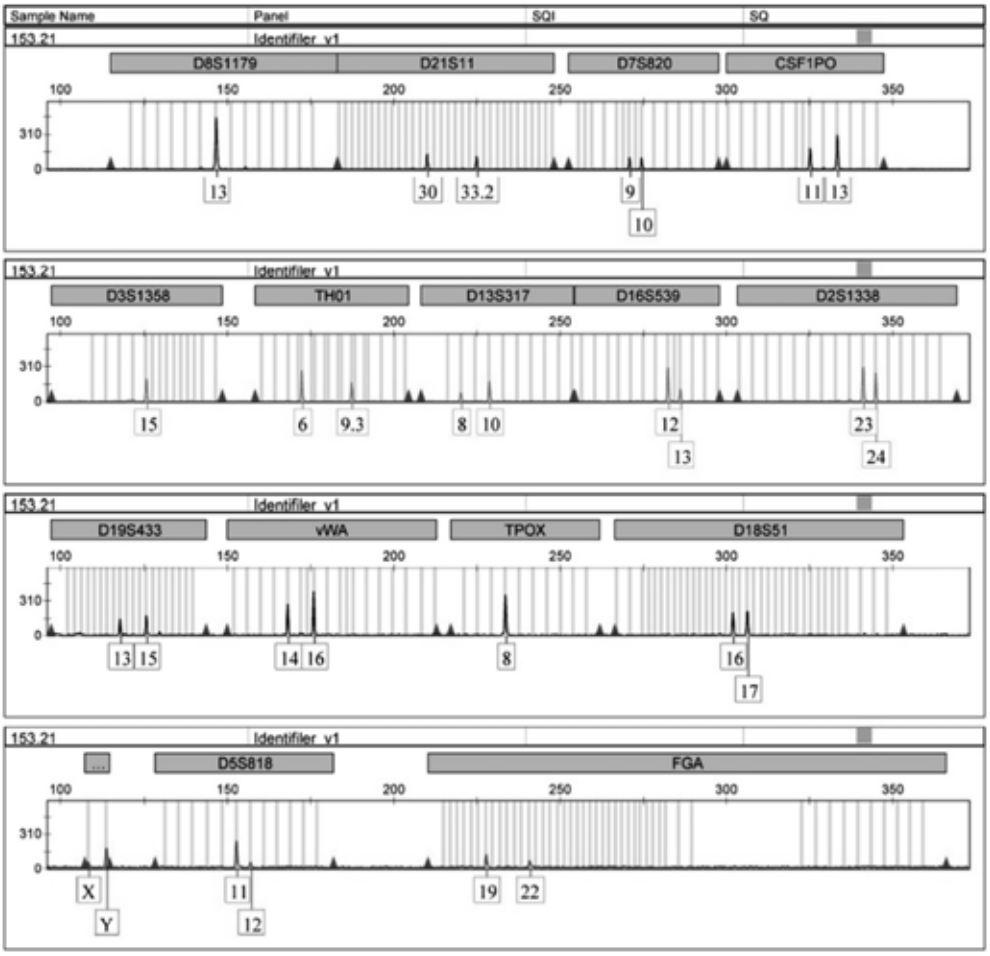

Fig. 2.2.2.1. *Graphic representation of DNA markers used for zygosity determination*

2.2.3. Comparison of morphometric 3D facial analysis with DNA testing for twin zygosity determinations.

2.2.3.1. Morphometric 3D facial analysis

Morphometric analysis of the twin faces was performed using 3D photogrammetry. It is a technique that uses two or more cameras configured as a stereo-pair to obtain 3D coordinates of facial morphology. The structured light system projects a radial grid onto face. This facilitate registration of two photographs, create a perception of depth and gives numerous points of intersection for measurements and correspondence.

3D facial imaging. The portable 3D surface imaging system (3dMD, Atlanta, USA) was used in this study (Fig. 2.2.3.1.1). This system uses multiple cameras, three on each side (one color and two infra-red) to capture the photo-realistic quality pictures as shown in Fig. 2.2.3.1.2.

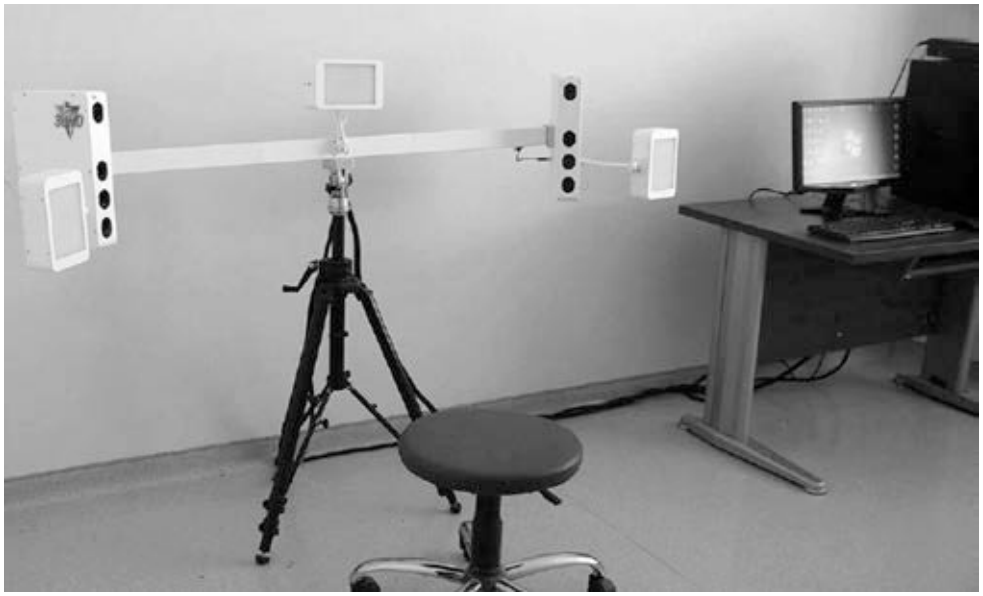

Fig. 2.2.3.1.1. *The 3dMD imaging device*

The system works by projecting a random light pattern onto a subject and an image captured with multiple precisely synchronized digital cameras set as various angles in an optimum configuration. The capture time of the system is 1.5 ms at the highest resolution. It has a manufacturer's quoted accuracy of <0.5 mm (root mean square) and a clinical accuracy of 1.5% of the total observed variance [118].

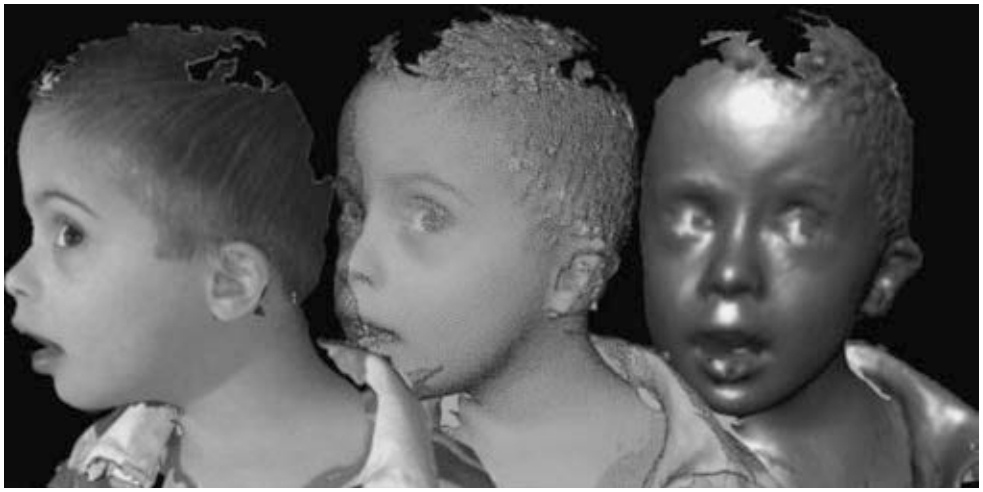

Fig. 2.2.3.1.2. *Surfaces acquired by the 3dMDsystem. All surfaces were produced from the same image. Surfaces are shown at different orientations to illustrate 3D nature of the data. Left: smooth 3D geometry with texture map applied; middle: 3D wireframe geometry data (polygonal mesh surface); Right: smooth 3D geometry data.*

During imaging, the participants were asked to sit on a chair and look at the eyes of their reflection in a mirror ahead to maintain a neutral facial expression. Any hair overlying the face was kept away by a hair band and a black scarf was placed on the shoulders to cover colourful clothes.

Each image was processed so that distinct parts like hair were removed. The edges of the facial shell were removed so that the size of the shell was almost equal between twins. Still, the size of the shell varied between pairs. The position of the faces was standardized [119] by fitting them into the same reference frame, with the point halfway between the inner canthi of the eyes as the origin. A mirror shell of the original shell was created and the two shells were superimposed. The coronal plane (XY plane) was determined by the axis of the cylinder that fit all the data points of the original-mirror face structure. The sagittal plane (YZ plane) was chosen to pass through the middle of the face. It was defined as the symmetry plane of the original-mirror face structure. The transverse plane (XZ plane) was set to pass through the inner canthi of the eyes and was perpendicular to the two previous planes. Twenty-one facial landmarks [120] were manually identified on each facial shell (Fig. 2.2.3.1.3). Pose was standardized to facilitate landmark identification. The images were processed using 3dMD Patient and Rapidform 2006 (INUS Technology, Inc., Seoul, Korea) software.

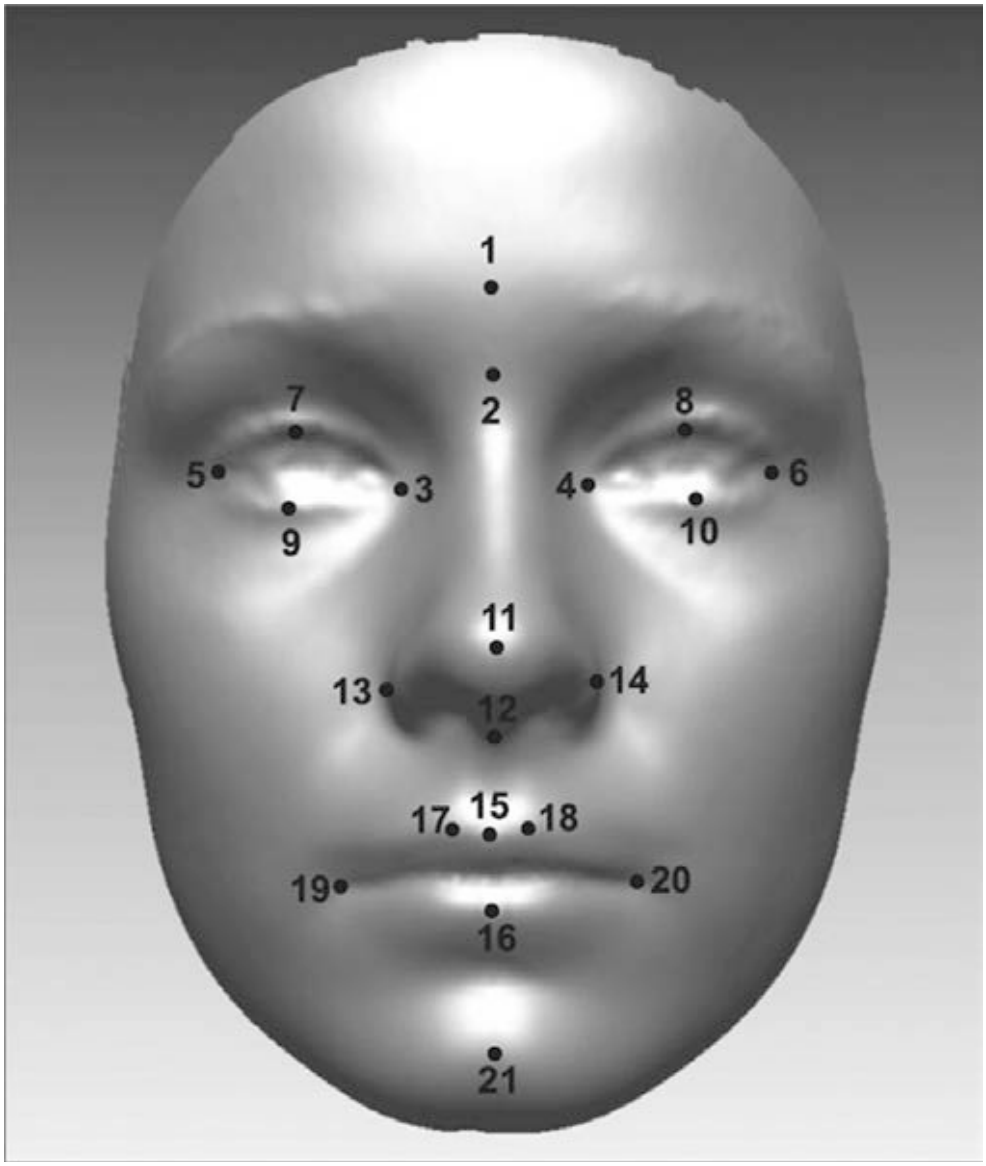

Fig. 2.2.3.1.3. *Twenty-one landmarks which were manually identified for each twin: (1) Glabella; (2) Nasion; (3) Endocanthion right; (4) Endocanthion left; (5) Exocanthion right; (6) Exocanthion left; (7) Palpebrale superius right; (8) Palpebrale superius left; (9) Palpebrale inferius right; (10) Palpebrale inferius left; (11) Pronasale; (12) Subnasale; (13) Alare right; (14) Alare left; (15) Labiale superius; (16) Labiale inferius; (17) Crista philtri right; (18) Crista philtri left; (19) Cheilion right; (20) Cheilion left; and (21) Pogonion*

2.2.3.2. Comparison of morphometric 3D facial analysis with DNA testing in twin zygosity determinations

The analyses described below were carried out for three groups—all twin pairs as well as the male and female pairs separately. Two different scaling methods were used for each group. Landmark coordinates were translated so that their centroid lied at the origin. The Frobenius norm was used as a shape size metric [121]:

$$\|A\|_F = \sqrt{\sum_{i=1}^{n}[(x_i - \bar{x})^2 + (y_i - \bar{y})^2 + (z_i - \bar{z})^2]}$$

where the $n \times 3$ matrix A is composed of the translated landmark coordinates, so that its first row contains the coordinates of the first translated landmark, etc. Means $\bar{x}$, $\bar{y}$, and $\bar{z}$ are the averages of the coordinates and n is the number of coordinates, which is 21 in this study. The Frobenius norm of the translated coordinates was calculated for every face and then the average of the norms was computed for each group. In the first scaling method each face was scaled by its own scaling parameter. The scaling parameter was obtained by dividing the group's average Frobenius norm by the individual's Frobenius norm. In the second scaling method each face was also scaled, but for each pair, the same scaling parameter based on the Frobenius norm of the other twin, was used. The shape size metric Frobenius norm is often called the centroid size in literature dealing with scaling [121]. Analyses were also done without any scaling.

After scaling, the faces of a twin pair were superimposed using best-fit registration based on an iterative closest point algorithm. Then three feature statistics were computed to characterize the differences between the twins. These were the average distance (mm), the standard deviation of distances (mm), and the percentage coincidence of distances within 0.5 mm tolerance. *Rapidform* software provides ready-made tools for computation of these statistics [91]. The software first examines the overlap region between the two shells and then one of the shells is chosen as a source shell and the other is a target shell. Then, for each point in the source shell (vertex in a dense triangulation of the shell surface), the closest point in the target shell is computed. The average, standard deviation, and 0.5 mm coincidence statistics are computed from these distances between the closest points in the two shells. Scaling, registration, and computation of the statistics have been automated with a set of in-house VBA (Visual Basic for Applications) sub-

routines developed for *Rapidform*, so there is no need to do every part of the analysis separately for every twin pair.

Next, the pairs were classified into MZ and DZ twins in each of the three groups separately. Statistical pattern recognition methodology was used for the classification [122, 123]. For good results, the training set for a pattern classifier should preferably be as large as possible. However, one should not use all available data for both training and testing the classifier, because the training and testing data should be independent to avoid overly optimistic estimates of classifier performance. Leave-one-out cross-validation (LOOCV) was used to avoid this problem [122]. In LOOCV a single observation is used as testing data while the rest of the available observations serve as the training data. Thus, if the whole group considered consists of n twin pairs, the training set for recognition of the zygosity of a pair includes all other twin pairs, and its size therefore is n–1.

Two pattern recognition methods were tested. One was a quadratic classifier that assumes Gaussian class distributions [122, 123]. It separates the objects into two classes with a quadratic surface determined by the estimated class probability density functions of the object features considered. Multiple features can be used simultaneously for classification. To construct a quadratic classifier one must estimate class-specific mean vectors and covariance matrices and also define prior probabilities for the two classes. Sample mean vectors and sample covariance matrices of the training data were used as estimates. The value 0.5 was chosen as the prior probability for both classes. It turned out that including more than one feature simultaneously did not give better recognition results than using only one feature at a time. The probable reason for this is that the features correlated strongly with each other.

The other method tested is quite simple. It assigns objects to one of the two classes simply based on the central tendency of the difference feature statistics considered. In the end such a simple method turned out to perform as well as the quadratic classifier in recognizing zygosity. The simple method is easy to describe and computationally less expensive than the quadratic classifier and we therefore use it here as an example of a classification technique. Thus, for each statistic, its mean and median were first computed and then used as thresholds in classification. Based on the DNA test there were 35 MZ and 25 DZ female twins, while for males the number of MZ and DZ twins was 23 and 22, respectively. For each statistic, ordered training data were divided into two groups in the proportions suggested by the DNA test, and the value separating the two groups was considered as the third classification threshold along with the mean and the median. Twins with average distance or standard deviation below a threshold were

classified as MZ, while twins with a coincidence feature below the value were classified as DZ. Statistical classification was done using Matlab R2013b (MathWorks, Natick, Massachusetts).

2.2.4. Cephalometric analysis

The cephalograms were taken in centric occlusion under standard conditions using digital x-ray equipment. For standardized positioning, a cephalostat was used to maintain the subject's head in constant relationship to the sensor (sensor-focus distance of 1.50 m, object-sensor distance 0.15 m). This in turn standardized the distance of the subject to the sensor, the x-ray exposure and magnification exposure. All subjects were asked to stand looking straight forward, with a lead apron on their chest. Ear rods were placed into the ear canals in a comfortable position and orbital pointer was accurately positioned. All radiographs were analized by the author using commercially available software Dolphin Imaging 11.7 Premium, Patterson Dental Supply, Chatsworth, USA (Fig. 2.2.4.1). Cepahlometric landmarks used in the study presented in the Fig. 2.2.4.2.

Thirty nine angular and linear measurements used in the study and their definitions presented in the Table 2.2.4.1. The templates of all cepahlometric measurements presented at the *Appendix 1*. Landmark identification was carried out by manual dot tracing on the digital image using a mouse-driven cursor in a predetermined sequence. To avoid inter-examiner error author of the dissertation (M. Š.) digitized all radiographs. Cephalometric measurements were automatically calculated by computer.

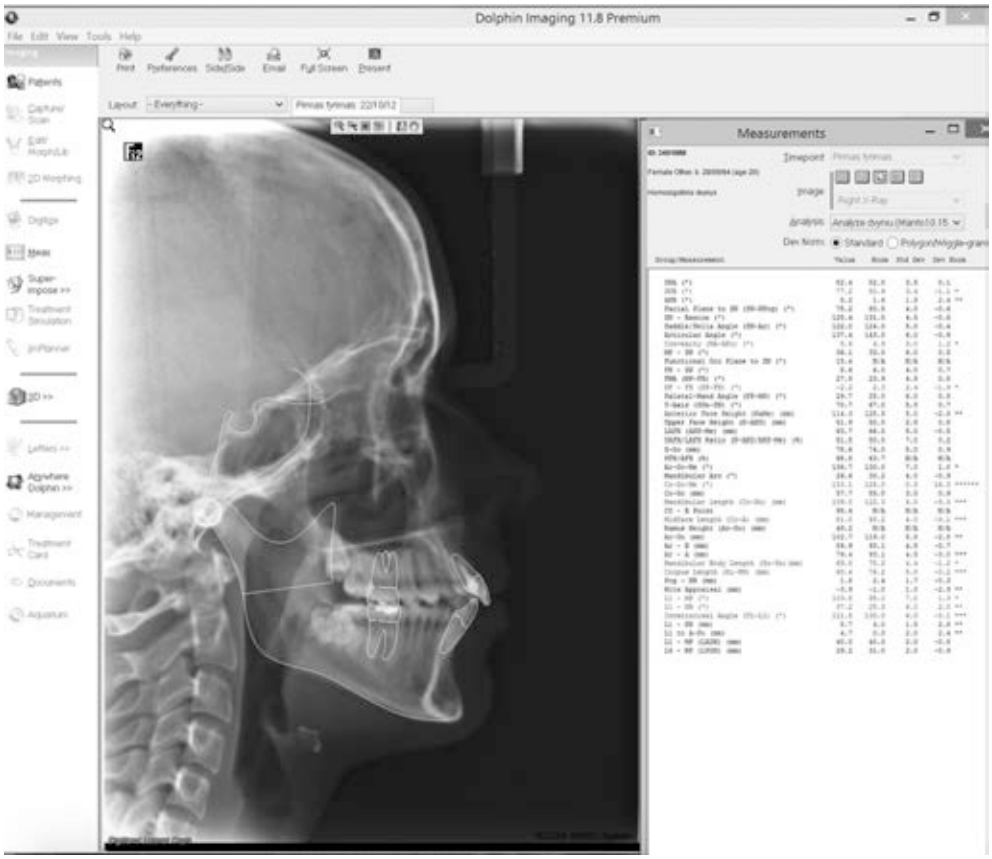

Fig.2.2.4.1. *Dolphin 11.8. Software for cephalometric analysis*

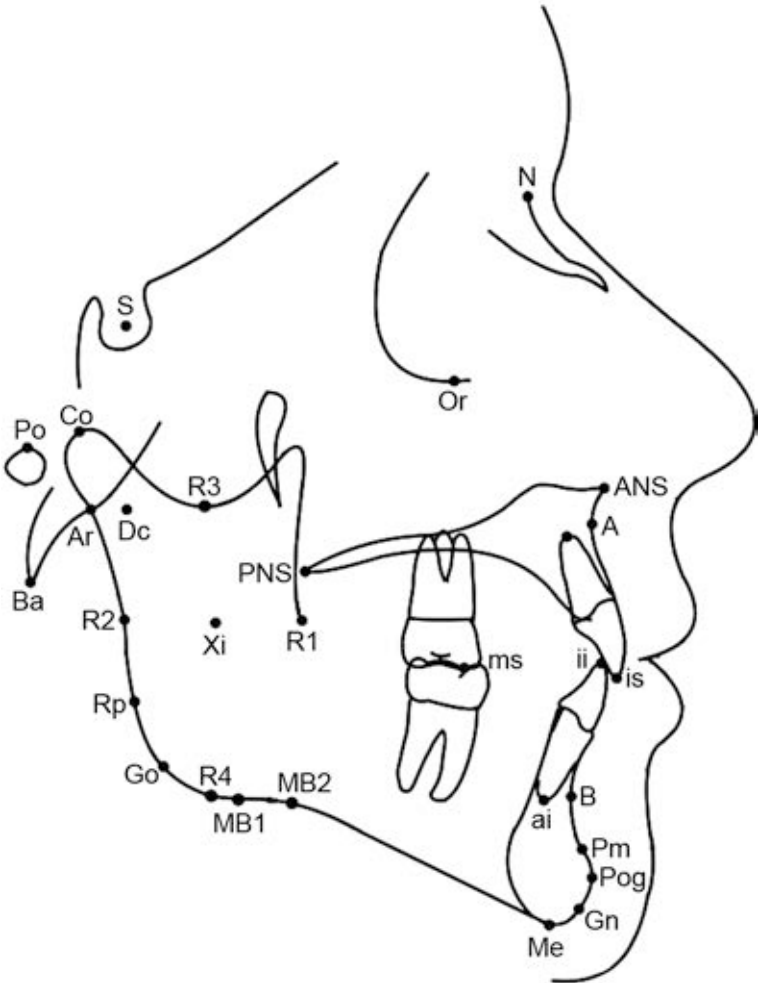

Fig. 2.2.4.2. *Cephalometric landmarks used in the study*

S – Sella: the midpoint of sella turcica.
N – Nasion: the extreme anterior point of the frontonasal suture.
Ba – Basion: the most anterior-inferior point on the margin of the foramen magnum.
A – Point A: the deepest point in the curvature of the maxillary alveolar process.
B – Point B: the deepest point in the curvature of the mandibular alveolar process.
ANS – Point ANS: the tip of the anterior nasal spine.
PNS – Point PNS: the tip of the posterior nasal spine.
Co – Condylion: the most posterior superior point of the condyle.
Ar – Articulare: the point at the junction of the posterior border of the ramus and the inferior border of the posterior cranial base.
Rp – Ramus posterior point: the most prominent postero-superior point at the angle of the mandible on the posterior ramus.
R1 – Ramus point 1: the most concave point on the interior of the ramus.

R2 – Ramus point 2: the most concave point on the exterior border of the ramus along the vertical.
R3 – Ramus point 3: a point located at the center and most inferior aspect of the sigmoid notch of the ramus of the mandible.
R4 – Ramus point 4: a point on the lower border of the mandible, directly inferior to the center of the sigmoid notch of the ramus
Pog – Pogonion: the most anterior point of the chin.
Me – Menton: the most inferior point of the chin.
Go – Gonion: the midpoint of the mandibular angle between ramus and the mandibular corpus.
MB1 – Inferior border point: the most convex point along the inferior border of the ramus.
MB2 – Antegonial notch: the highest point of the notch of the lower border of the body of the mandible.
Gn – Gnathion: the midpoint between Pog and Me on the bony chin.
Xi – Xi point: the point located at the geometrical center of the ramus.
Dc – Dc point: the point representing the center of the neck of the condyle on the Ba-N line.
Pm – Protuberance menti: the point at which the shape of symphysis mentalis changes from convex to concave.
ai – Apex inferior: the root apex of the most anterior mandibular central incisor
is – Incision superior: the incisal tip of the most anterior maxillary central incisor
ii – Incision inferior: the incisal tip of the most anterior mandibular central incisor
ms – Molar superior: tip of the mesial buccal cusp of the mandibular first molar

Table 2.2.4.1. *Cepahlometric variables and definitions used in the study*

Variables	Definitions
SNA	Angle determined by points S, N and A.
SNB	Angle determined by points S, N and B.
SNPog	Angle determined by points S, N and Pog.
NSBa	Angle determined by points N, S and Ba.
NSAr	Angle determined by points N, S and Ar.
NAPog	Angle determined by points N, A and Pog.
SN-GoMe	Angle formed by S-N and Go-Me lines.
ANS.PNS-GoMe	Angle formed by ANS-PNS and Go-Me lines.
SN-ArRp	Angle formed by S-N and Ar-Rp lines.
PoOr-GoMe	Angle formed by Po-Or and Go-Me lines.
NGnGo	Angle determined by points N, Gn and Go.
DcXiPm	Angle formed by Dc, Xi and Pm points.
ArRp-MB1Me	Angle formed by Ar-Rp and MB1-Me lines.
CoGoMe	Angle determined by points Co, Go and Me.
ArGoMe	Angle determined by points Ar, Go and Me.

Table 2.2.4.1. *Continued*

Variables	Definitions
ai-ii -NB	Angle formed by line ai-ii and N-B lines
ai-ii -GoMe	Angle formed by line ai-ii and Go-Me lines
Co-A	Distance between points Co and A in mm
Co-Go	Distance between points Co and Go in mm
Co-Pog	Distance between points Co and Pog in mm
Co-B	Distance between points Co and B in mm
Ar-B	Distance between points Ar and B in mm
Ar-A	Distance between points Ar and A in mm
Pog⊥NB	Perpendicular distance from the point Pog to N-B line in mm
Go-Gn	Distance between points Go and Gn in mm
Go-Pog	Distance between points Go and Pog in mm
Xi-Pm	Distance between points Xi and Pm in mm
R1-R2	Ramal width at Xi, distance between points R1 and R2 in mm
MB2⊥ MB1Me	Perpendicular distance from MB1Me line to point MB2
N-Me	Total anterior facial height, distance from N to Me in mm
N-ANS	Upper anterior facial height, distance from N toANS in mm
ANS-Me	Lower anterior facial height, distance from ANS to Me in mm
S-Go	Total posterior facial height, distance from S to Go in mm
ii⊥NB	Perpendicular distance from point ii to N-B line in mm
ii⊥APog	Perpendicular distance from point ii to A-Pog line in mm
ii⊥GoMe	Perpendicular distance from point ii to Go-Me line in mm
ms⊥GoMe	Perpendicular distance from point ms to Go-Me line in mm
OB	Overbite: distance from point ii to point is vertically in mm
OJ	Overjet distance from point ii to point is horizontally in mm

2.2.5. Statistical analysis

Data were analysed using the Statistical Package for Social Sciences (SPSS/PC for Windows, version 19.0, Chicago, Illinois, USA). The results obtained for quantitative variables were described by mean (M), standard deviation (SD) and range.

The Kolmagorov-Smirnov test was used for the investigation of hypothesis about the normality of the parameter distribution.

Parametric Student's t-test and nonparametric Mann-Whitney test were applied for comparison of quantitative variables in two independent groups.

Measurement error. Intra-observer measurement error (ME) was assessed by measuring 20 randomly selected cephalograms twice with a 1-month time interval by the method suggested by Bland and Altman [124] The estimated error between the measurements was calculated using the formula:

$$SDd = \sqrt{\sum (d_1 - d_2)^2 / (2N)},$$

where: SDd – standard deviation of difference between repeated measurements; ± 2 SDd are the limits within which 95% of the differences between the repeated measurements are expected to lie; d1 = first measurement; d2 = second measurement; N=number of study samples.

The Pearson's intra-pair correlation coefficients for cephalometric skeletal and dental variables were calculated for monozygotic twins (rMZ) and dizygotic twins (rDZ).

The influence of additive genetic variance and influence of environment on dento-facial cephalometric variables was assessed using Lundstrom's approach [125] and the Path analysis model [126]. This model enables to calculate the proportion of the phenotype total variance explained by additive genes (heritability), and by environment (common and specific). The heritability (h^2) was calculated using formula $h^2 = 2(rMZ - rDZ)$. The common and specific environment was not separated in this study and influence of environment was calculated as, so called "coefficient of cultural inheritance" (c^2), using formula $c^2 = 2rDZ - rMZ$. The calculation of heritability estimates provides a means of quantifying the extent of the genetic contribution to phenotypic variation, with proportion ranging theoretically from 0 (no genetic contribution) to 1 (variation entirely attributed to genetic influence). The coefficient of cultural inheritance provides the same meaning in regard to environment contribution to phenotype variance.

A p value of less than 0.05 denoted statistical significance.

3. RESULTS

3.1. Measurement error of the cephalometric variables

The measurement error values for angular and linear measurements presented in the Table 3.1.1, 3.1.2. There were no significant differences between the first and the second measurements of the cephalometric variables ($p<0,5$), except for the distances Xi-Pm and OB.

Table 3.1.1. *The ME of cephalometric variables and its statistical significance (mandibular relationship to cranial base and maxillary structures)*

Cephalometric variables	ME	p
SNA (°)	0.60	NS
SNB (°)	0.40	NS
SNPog (°)	0.40	NS
NSBa (°)	1.14	NS
NSAr (°)	1.14	NS
NAPog (°)	0.89	NS
SN-GoMe (°)	0.88	NS
ANS.PNS-GoMe (°)	0.91	NS
N-Me (mm)	0.76	NS
N-ANS (mm)	0.68	NS
ANS-Me (mm)	0.78	NS
Co-A (mm)	1.24	NS
Ar-A (mm)	0.56	NS
SN-ArRp (°)	0.93	NS
PoOr-GoMe (°)	1.20	NS
NGnGo (°)	0.54	NS
S-Go (mm)	0.83	NS

Note: ME – measurement error, calculated as a variance between two measurements; p – probability of means of first and second measurement to be different NS – not significant.

Table 3.1.2. *The ME of cephalometric variables and its statistical significance (mandibular skeletal and dento-alveolar structures)*

Cephalometric variables		ME	p
Mandibular skeletal	DcXiPm (°)	1.93	NS
	CoGoMe (°)	1.49	NS
	Co-B (mm)	1.73	NS
	Ar-B (mm)	0.99	NS
	Go-Gn (mm)	0.84	NS
	Xi-Pm (mm)	0.66	0.01
	R1-R2 (mm)	0.68	NS
	MB2 ⊥ MB1Me (mm)	0.32	NS
	Co-Pog (mm)	1.41	NS
	ArRp-MB1Me (°)	1.47	NS
	ArGoMe (°)	1.30	NS
	Co-Go (mm)	1.55	NS
	Go-Pog (mm)	0.78	NS
	Pog ⊥ NB (mm)	0.38	NS
Dento-alveolar	ai-ii-NB (°)	1.64	NS
	ii ⊥ NB (mm)	0.38	NS
	ii ⊥ APog (mm)	0.31	NS
	ii ⊥ GoMe (mm)	0.38	NS
	ms ⊥ GoMe (mm)	0.69	NS
	OB (mm)	0.32	0.01
	OJ (mm)	0.27	NS
	ai-ii-GoMe (°)	1.71	NS

Note: ME – measurement error, calculated as a variance between two measurements; p – probability of means of first and second measurement to be different NS – not significant.

3.2. Morphometric analysis of 3D facial scans as zygosity determination tool

There were three twin groups, three scaling methods, three features to measure the difference between faces, and three types of thresholds to assign pairs to two classes. Consequently, there were 81 different classification scenarios altogether. The percentage of classification success was computed for every scenario with the results of the DNA test regarded as giving the correct assignment. The success rates for classification are displayed in Table 3.2.1. Clearly, as far as scaling is considered, the first

method performs worse than the two other approaches. The only exception is the female group with the standard deviation feature and the mean, or the DNA zygosity ratio, as the classification threshold. In the first scaling method the level of size adjustment was determined separately for each face. This type of scaling probably changes the relative size of faces within a pair too much, and therefore makes classification more difficult. The results reported in Table 3.2.1 in fact suggest that no scaling generally is better than either type of scaling considered. In fact, the coincidence feature of unscaled faces performed best for nearly all the group-threshold combinations. One exception was the male group; using the average distance statistic and the DNA zygosity ratio threshold, it performed best with the second scaling method.

Table 3.2.1. *Success rates for 3D zygosi classification compared to DNA testing*

%	**Scaling 1**			**Scaling 2**			**No scaling**		
	Av. Dist.	**SD**	**Coinc. 0.5 mm**	**Av. Dist.**	**SD**	**Coinc. 0.5 mm**	**Av. Dist.**	**SD**	**Coinc. 0.5 mm**
	Mean as threshold			Mean as threshold			Mean as threshold		
M	82.22	80.00	82.22	86.67	82.22	88.89	84.44	84.44	88.89
F	81.67	85.00	75.00	83.33	76.67	86.67	85.00	80.00	86.67
All	84.76	80.00	78.10	86.67	80.00	86.67	88.57	81.90	88.57
	Median as threshold			Median as threshold			Median as threshold		
M	86.67	86.67	77.78	88.89	86.67	88.89	84.44	88.89	88.89
F	78.33	78.33	75.00	85.00	81.67	85.00	85.00	81.67	85.00
All	82.86	81.90	77.14	85.71	83.81	86.67	86.67	84.76	89.52
	DNA zygosity ratio as threshold			DNA zygosity ratio as threshold			DNA zygosity ratio as threshold		
M	86.67	86.67	77.78	86.67	86.67	84.44	84.44	86.67	84.44
F	81.67	81.67	76.67	83.33	76.67	86.67	83.33	80.00	86.67
All	84.76	82.86	78.10	85.71	84.76	86.67	84.76	82.86	87.62

Note: M – male, F – female, Av. Dist. – average distance, Coinc. – coincidence.

As for the three statistics tested, it appears that coincidence with 0.5 mm tolerance is a more suitable feature for classification than the average distance or the standard deviation. Also, as noted above, leaving out scaling improves the results in most cases. One might assume that, on average, larger faces have larger differences than smaller faces. Scaling was expected to equalize the magnitude of differences and therefore lead to better recognition performance. However, the two scaling techniques impaired twin

recognition and it remains open, whether some other scaling method would work better. Another observation was that no particular classification threshold appeared to work better than the others.

There was one situation where the quadratic classifier outperformed all the other methods. This was the case for the female group with no scaling and the coincidence with 0.5 mm tolerance statistic. The success rate was 90%, which is over three percentage units greater than the best rates for the female group with any simple classifier.

3.3. The gender differences in intra-pair correlation coefficients of the cephalometric variables

The gender differences in intra-pair correlation coefficients of cephalometric variables of MZ twins shown in Table 3.3.1, 3.3.2.

Table 3.3.1. *The intra-pair correlation coefficients of cephalometric variables in MZ group and gender differences (mandibular relationship to cranial base and maxillary structures)*

Cephalometric variables	Male (n=29)	Female (n=61)	p
SNA (°)	0.83	0.73	0.11
SNB (°)	0.86	0.77	0.51
SNPog (°)	0.85	0.76	0.79
NSBa (°)	0.77	0.86	0.25
NSAr (°)	0.79	0.81	0.69
NAPog (°)	0.87	0.72	0.50
SN-GoMe (°)	0.78	0,76	0.53
ANS.PNS-GoMe (°)	0.89	0.75	0.21
N-Me (mm)	0.87	0.94	0.20
N-ANS (mm)	0.72	0.84	0.41
ANS-Me (mm)	0.93	0.90	0.85
Co-A (mm)	0.91	0.89	0.74
Ar-A (mm)	0.93	0.90	0.24
SN-ArRp (°)	0.78	0.79	0.42
PoOr-GoMe (°)	0.86	0.74	0.58
NGnGo (°)	0.84	0.78	0.09
S-Go (mm)	0.81	0.84	0.57

Note: p – statistical significance of the differences between genders.

Table 3.3.2. *The intra-pair correlation coefficients of cephalometric variables in MZ group and gender differences (mandibular skeletal and dento-alveolar structures)*

Cephalometric variables		Male (n=29)	Female (n=61)	p
Mandibular skeletal	DcXiPm (°)	0.72	0.74	0.80
	CoGoMe (°)	0.79	0.71	0.65
	Co-B (mm)	0.92	0.90	0.09
	Ar-B (mm)	0.95	0.91	0.07
	Go-Gn (mm)	0.91	0.91	0.57
	Xi-Pm (mm)	0.92	0.93	0.82
	R1-R2 (mm)	0.87	0.74	0.27
	MB2 ⊥ MB1Me (mm)	0.73	0.65	0.73
	Co-Pog (mm)	0.93	0.89	0.06
	ArRp-MB1Me (°)	0.82	0.82	0.42
	ArGoMe (°)	0.79	0.79	0.69
	Co-Go (mm)	0.76	0.76	0.16
	Go-Pog (mm)	0.92	0.90	0.99
	Pog ⊥ NB (mm)	0.86	0.85	0.33
Dento-alveolar	ai-ii-NB (°)	0.75	0.68	0.77
	ii ⊥ NB (mm)	0.89	0.85	0.72
	ii ⊥ APog (mm)	0.88	0.86	0.74
	ii ⊥ GoMe (mm)	0.89	0.93	0.55
	ms ⊥ GoMe (mm)	0.60	0.75	0,01
	OB (mm)	0.74	0.68	0.55
	OJ (mm)	0.62	0.47	0.40
	ai-ii-GoMe (°)	0.62	0.73	0.91

Note: p – statistical significance of the differences between genders.

The gender differences in intra-pair correlation coefficients of cephalometric variables of DZ twins shown in Table 3.3.3, 3.3.4.

Table 3.3.3. *The intra-pair correlation coefficients of cephalometric variables in DZ group and gender differences (mandibular relationship to cranial base and maxillary structures)*

Cephalometric variables	Male (n=20)	Female (n=31)	p
SNA (°)	0.33	0.19	0.95
SNB (°)	0.59	0.40	0.05
SNPog (°)	0.57	0.48	0.11
NSBa (°)	0.33	0.44	0.14
NSAr (°)	0.42	0.17	0.19
NAPog (°)	0.46	0.37	0.77
SN-GoMe (°)	0.61	0.55	0.15
ANS.PNS-GoMe (°)	0.48	0.56	0.33
N-Me (mm)	0.63	0.84	0.90
N-ANS (mm)	0.76	0.68	0.06
ANS-Me (mm)	0.53	0.81	0.09
Co-A (mm)	0.26	0.76	0.95
Ar-A (mm)	0.35	0.76	0.51
SN-ArRp (°)	0.46	0.21	0.24
PoOr-GoMe (°)	0.65	0.54	0.34
NGnGo (°)	0.57	0.45	0.07
S-Go (mm)	0.62	0.78	0.64

Note: p – statistical significance of the differences between genders.

Table 3.3.4. *The intra-pair correlation coefficients of cephalometric variables in DZ group and gender differences (mandibular skeletal and dento-alveolar structures)*

Cephalometric variables		Male (n=20)	Female (n=31)	p
Mandibular skeletal	DcXiPm (°)	0.60	0.37	0.52
	CoGoMe (°)	0.56	0.37	0.30
	Co-B (mm)	0.73	0.83	0.99.
	Ar-B (mm)	0.78	0.80	0.83
	Go-Gn (mm)	0.59	0.77	0.40
	Xi-Pm (mm)	0.73	0.81	0.68
	R1-R2 (mm)	0.72	0.53	0.07
	MB2 ⊥ MB1Me (mm)	0.63	0.26	0.49
	Co-Pog (mm)	0.75	0.83	0.87
	ArRp-MB1Me (°)	0.66	0.11	0.05
	ArGoMe (°)	0.60	0.20	0.08
	Co-Go (mm)	0.63	0.71	0.50
	Go-Pog (mm)	0.65	0.80	0.73
	Pog ⊥ NB (mm)	0.45	0.50	0.47
Dento-alveolar	ai-ii-NB (°)	0.26	0.32	0.08
	ii ⊥ NB (mm)	0.56	0.35	0.03
	ii ⊥ APog (mm)	0.65	0.07	0.09
	ii ⊥ GoMe (mm)	0.44	0.80	0.53
	ms ⊥ GoMe (mm)	0.54	0.77	0.93
	OB (mm)	0.05	0.46	0.05
	OJ (mm)	0.55	0.38	0.11
	ai-ii-GoMe (°)	0.29	0.53	0.93

Note: p – statistical significance of the differences between genders.

The intra-pair correlation coefficient in MZ twin pairs were considerably higher than in DZ twin pairs for almost all cephalometric variables.

There were no statistically significant differences in correlation coefficients of cephalometric measurements between genders in both, MZ and DZ groups. The only exceptions were for the vertical position of lower molars in the alveolar bone (variable ms ⊥ GoMe) in MZ group and lower incisor position in relationship to NB line (variable *ii ⊥ NB*) in DZ group. The difference between genders in these two, out of thirty nine cephalometric parameters, we found not critical and for the calculation of heritability estimates we used pooled the data of both genders. This increased statistical power for heritability estimates and cultural inheritance calculations.

3.4. The heritability estimates and cultural inheritance of the cephalometric variables

The estimates of heritability (additive genetic variance) and cultural inheritance (influence of environment) of cephalometric variables presented in Table 3.4.1, 3.4.2. As already mentioned, term “heritability” in this study was used to define “narrow-sense heritability” (h^2).

The mandiblular skeletal cephalometric variables demonstrated high heritability estimates with angular measurements being considerably higher than linear, except chin thickness (distance Pog ⊥ NB). The comparative distribution of heritability coefficient (h^2) of mandibular skeletal angular and linear cephalometric measurements shown in Fig. 3.4.1.

The comparative distribution of heritability estimates (h^2) and cultural inheritance (c^2) presented in the Fig. 3.4.2–3.4.4. The heritability estimates of angular variables describing horizontal mandibular position in relationship to cranial base and maxilla were considerably higher (NAPog, $h^2 = 0.79$; SNB, $h^2 = 0.68$; SNPog, $h^2 = 0.57$) than defining its vertical position (SN-GoMe, $h^2 = 0.37$; PoOr-GoMe, $h^2 = 0.37$). The highest heritability estimate we found for angles describing sagittal position of the posterior ramus of the mandible and skeletal part of maxilla to cranial base (respectively, NSAr, $h^2 = 1.16$ and SNA, $h^2 = 1.10$).

The linear vertical measurements related to facial heights showed low genetic determination: total posterior facial height (S-Go, $h^2 = 0.10$), total anterior facial height (N-Me, $h^2 = 0.23$), upper anterior facial height (N-ANS, $h^2 = 0.19$) and lower anterior facial height (ANS-Me, $h^2 = 0.31$) and comparatively high cultural inheritance. Both variables representing anterior (ii ⊥ GoMe) and posterior (ms ⊥ GoMe) dento-alveolar heights together with incisor inclination (ai-ii-GoMe) showed high cultural inheritance and low heritability values.

It is worth to note, that highest cultural inheritance was found for the cephalometric variables representing “mandibular size“ dimensions: Co-B, $c^2 = 0.74$; Co-Pog, $c^2 = 0.74$; Co-Go, $c^2 = 0.70$.

Table 3.4.1. *The intra-pair correlation coefficients (r), estimates of genetic heritability (h^2)and cultural inheritance (c^2) according to the path Analysis model (mandibular relationship to cranial base and maxillary structures)*

Cephalometric variables	rMZ	rDZ	h^2	c^2
SNA (°)	0.79	0.24	1.10	–0.31
SNB (°)	0.82	0.48	0.68	0.14
SNPog (°)	0.81	0.53	0.57	0.24
NSBa (°),	0.81	0.38	0.86	–0.05
NSAr (°)	0.79	0.21	1.16	–0.37
NAPog (°)	0.80	0.40	0.79	0.00
SN-GoMe (°)	0.77	0.58	0.37	0.40
ANS.PNS-GoMe (°)	0.79	0.53	0.52	0.28
N-Me (mm)	0.93	0.81	0.23	0.70
N-ANS (mm)	0.83	0.73	0.19	0.64
ANS-Me (mm)	0.91	0.76	0.31	0.60
Co-A (mm)	0.90	0.70	0.39	0.50
Ar-A (mm)	0.91	0.73	0.35	0.55
SN-ArRp (°)	0.78	0.30	0.97	–0.19
PoOr-GoMe (°)	0.77	0.59	0.37	0.40
NGnGo (°)	0.79	0.49	0.61	0.18
S-Go (mm)	0.85	0.81	0.10	0.76

Table 3.4.2. *The intra-pair correlation coefficients (r), estimates of genetic heritability (h^2), cultural inheritance (c^2) according to the path Analysis model (mandibular skeletal and dento-alveolar cephalometric variables)*

Cephalometric variables	rMZ	rDZ	h^2	c^2
DcXiPm (°)	0.73	0.47	0.52	0.21
CoGoMe (°)	0.74	0.45	0.59	0.15
Co-B (mm)	0.91	0.83	0.17	0.74
Ar-B (mm)	0.93	0.83	0.21	0.72
Go-Gn (mm)	0.91	0.74	0.34	0.57
Xi-Pm (mm)	0.94	0.81	0.25	0.68
R1-R2 (mm)	0.78	0.61	0.34	0.44
MB2 ⊥ MB1Me (mm)	0.68	0.54	0.29	0.40
Co-Pog (mm)	0.92	0.83	0.18	0.74
ArRp-MB1Me (°)	0.82	0.29	1.06	–0.24
ArGoMe (°)	0.79	0.35	0.89	–0.10

Table 3.4.2. *Continued*

Cephalometric variables	**rMZ**	**rDZ**	**h^2**	**c^2**
Co-Go (mm)	0.78	0.74	0.08	0.70
Go-Pog (mm)	0.91	0.77	0.28	0.63
Pog ⊥ NB (mm)	0.87	0.46	0.81	0.06
ai-ii-NB (°)	0.70	0.30	0.81	–0.10
ii ⊥ NB (mm)	0.86	0.41	0.90	–0.04
ii ⊥ APog (mm)	0.86	0.27	1.17	–0.31
ii ⊥ GoMe (mm)	0.93	0.74	0.37	0.55
ms ⊥ GoMe (mm)	0.75	0.77	–0.03	0.79
OB (mm)	0.72	0.26	0.92	–0.20
OJ (mm)	0.55	0.50	0.10	0.46
ai-ii-GoMe (°)	0.69	0.47	0.44	0.25

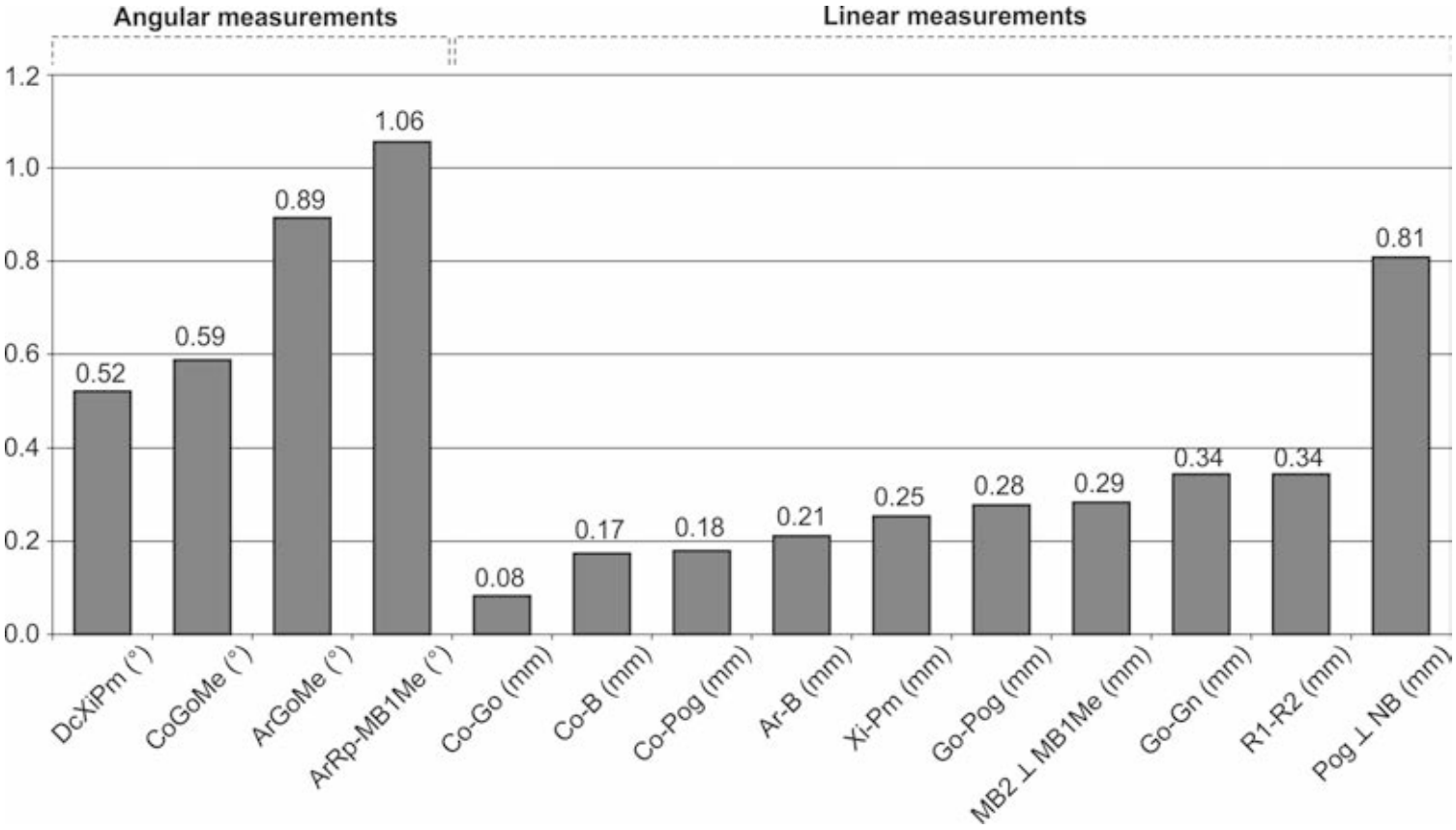

Fig. 3.4.1. *The comparative distribution of heritability estimates ($h^{2)}$ of mandibular skeletal linear and angular cephalometric variables*

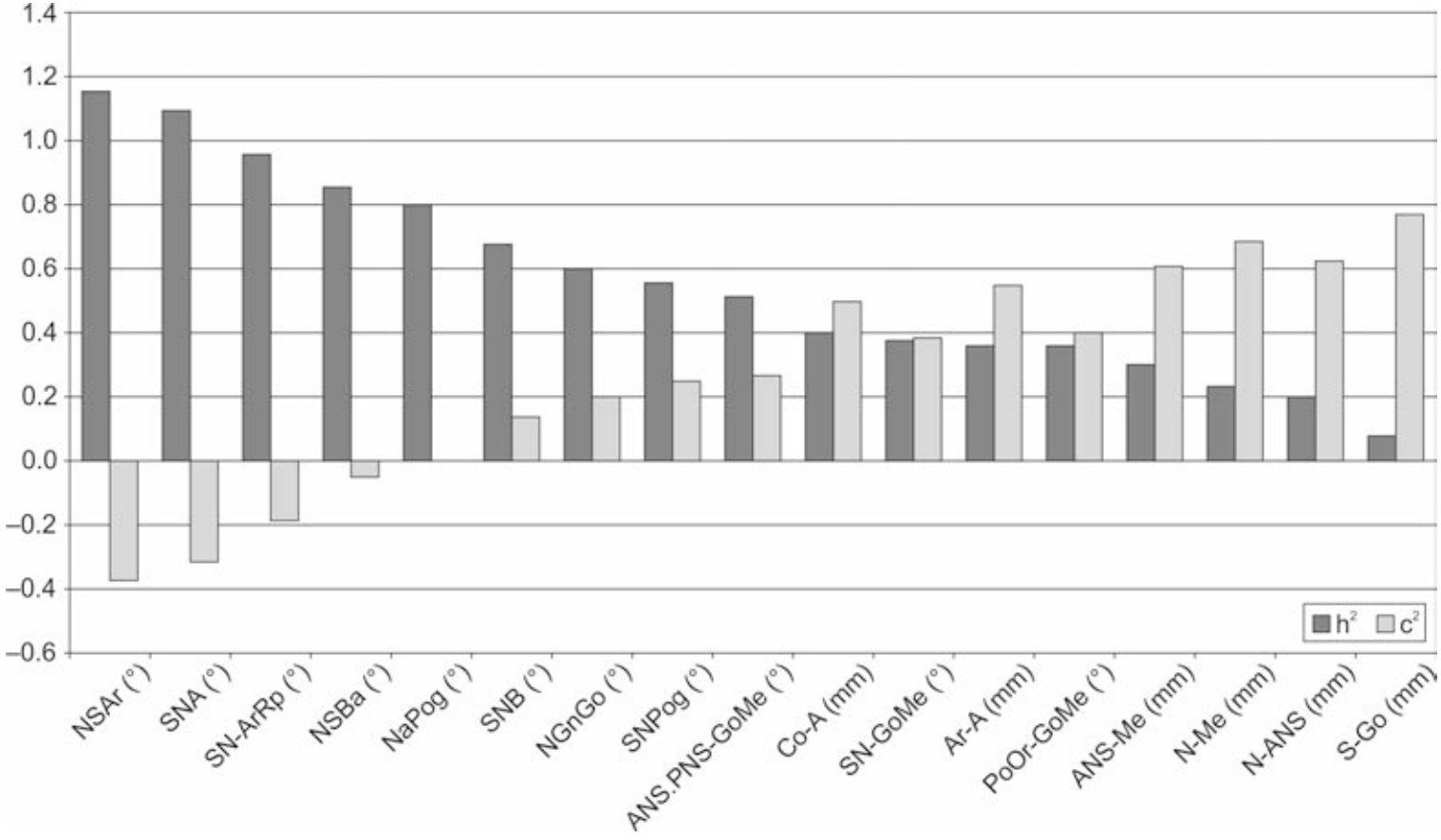

Fig. 3.4.2. *The comparative distribution of heritability estimates ($h^{2)}$ and cultural inheritance coefficients (c^2) of mandibular relationship to cranial base and maxilla cephalometric variables*

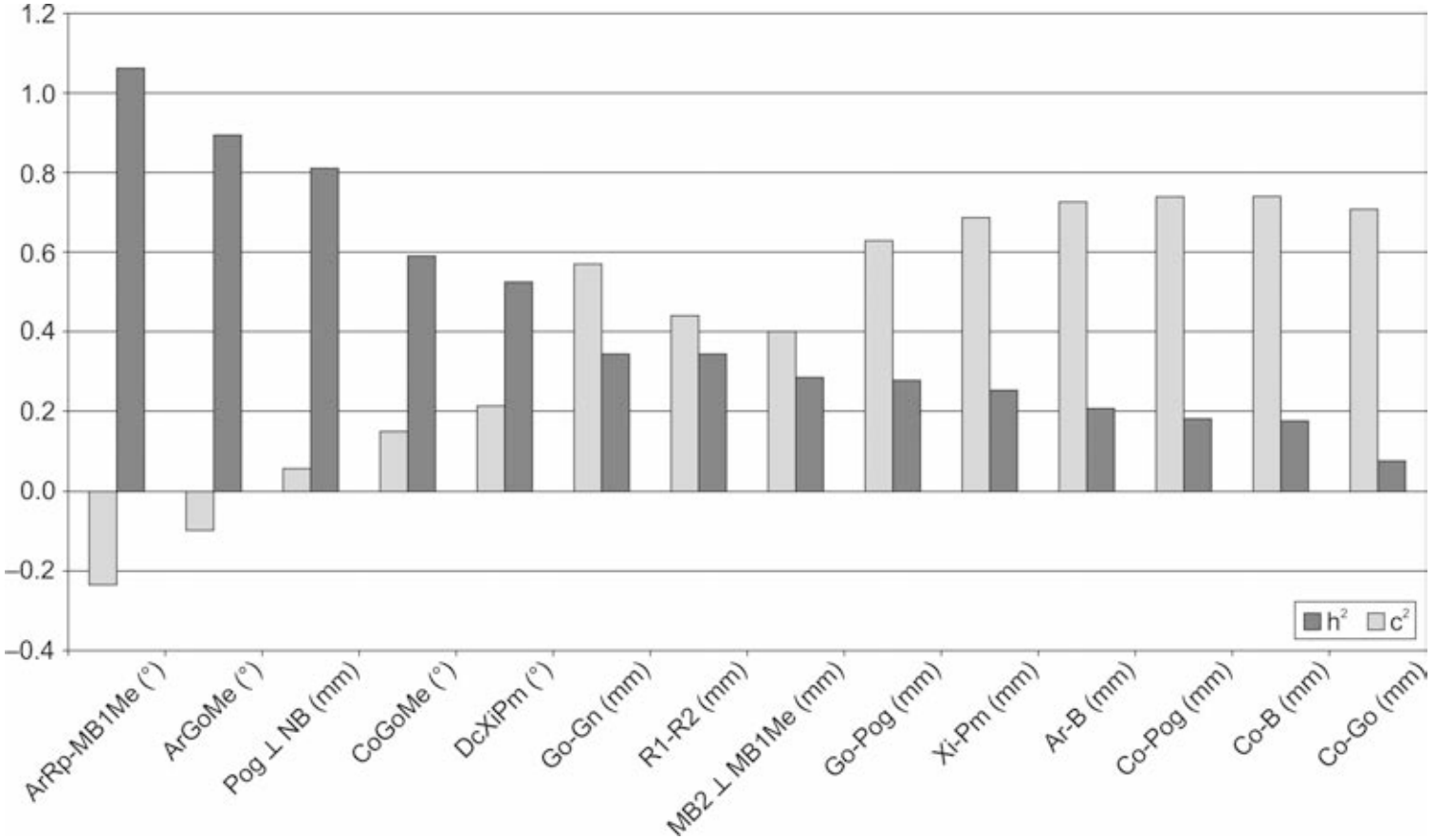

Fig. 3.4.3. *The comparative distribution of heritability estimates (h^2) and cultural inheritance coefficients (c^2) of mandibular skeletal cephalometric variables*

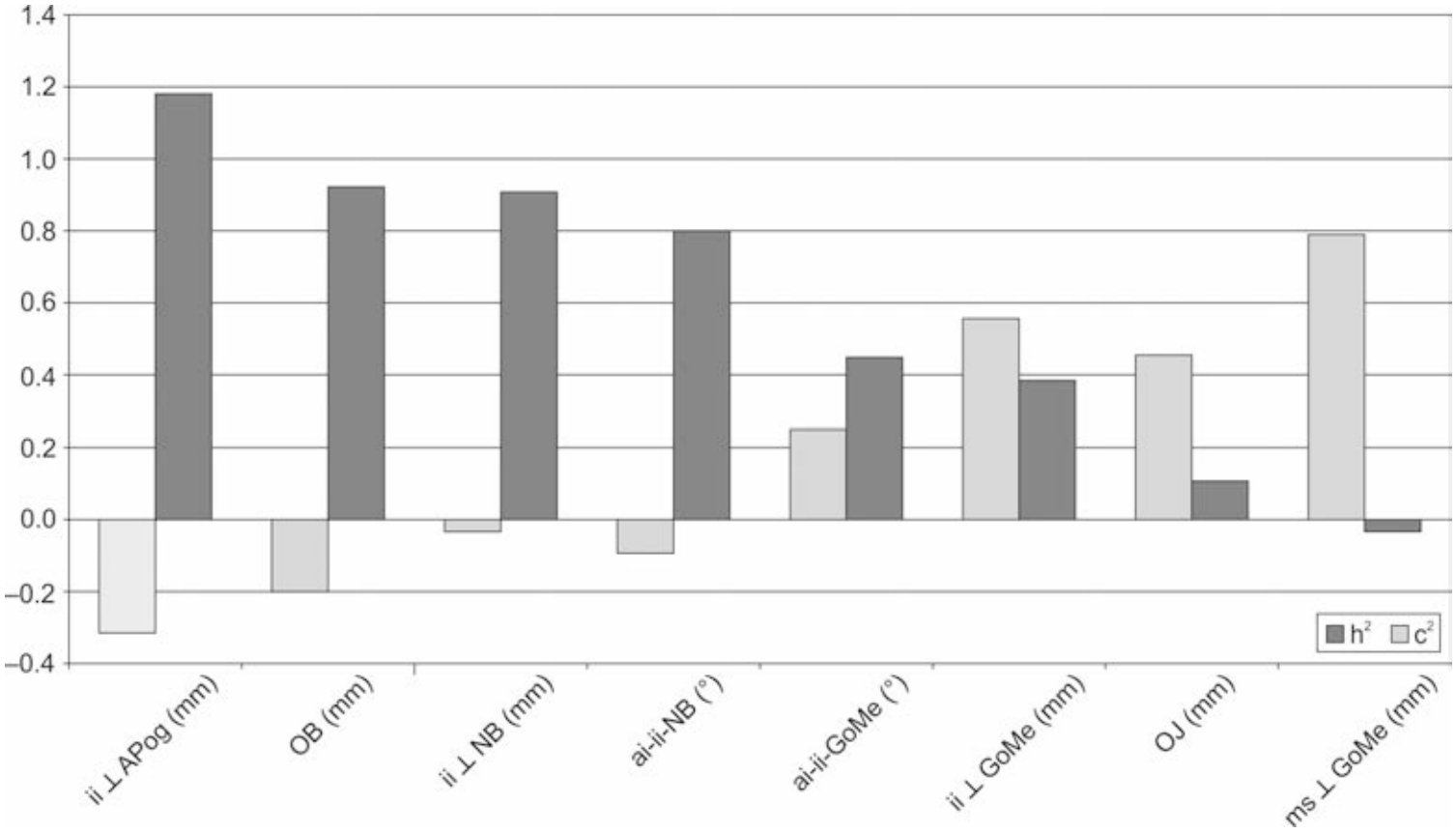

Fig. 3.4.4. *The comparative distribution of genetic heritability estimates (h^2) and coefficients of cultural inheritance (c^2) of mandibular dento-alveolar cephalometric variables*

4. DISCUSSION

4.1. Measurement error

The overall reproducibility of the measurements was good except for the Xi-Pm and OB. These two of 39 variables showed small, but significant differences between the first and second measurement. The inaccuracy of determining the anatomical landmarks for some variables is a well-known problem in the clinical cephalometrics. It is difficult and often nearly impossible to distinguish between left-right sides and it complicates landmark definitions due to over-projecting structures in lateral head radiograms. This problem is especially true for the deepest point of the mandible and maxilla concavity [127]. The inaccuracy for distance Xi-Pm could be explained by the fact, that Xi point represents the geometric centre of the ramus, determined by the intersection of the diagonals of the rectangle drawn to fit the width and height of the ramus at its narrowest dimensions (Fig. 4.1.1). Such sophisticated way of locating this point may cause the problem. But Xi-Pm value was larger than measurement error by 1.14 mm in MZ group and 2.2 mm in DZ group. The OB was larger than ME, respectively by 0.78 mm and 1.62 mm. This is acceptable and do not cause significant bias for final results of the study.

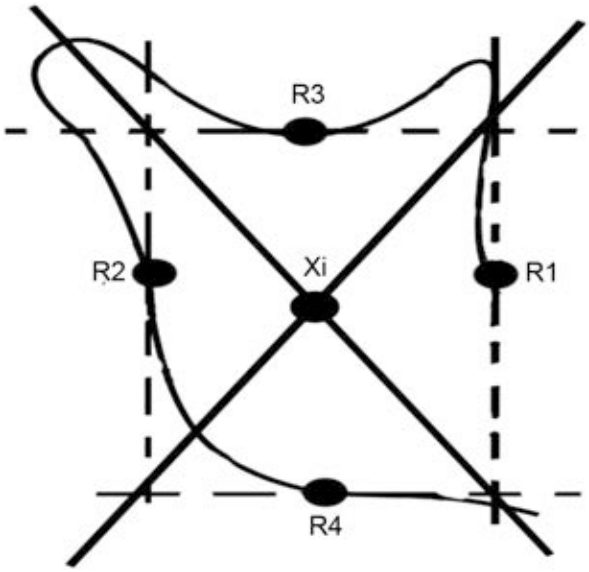

Fig. 4.1.1. *The Xi point is located in the centre of mandible ramus*

4.2. Comparative assessment of 3D facial morphometric and DNA based analyses as zygosity determination tools

DZ twin pairs arise from two fertilization events, while MZ twin pairs most likely arise from the splitting of a single early embryo. Accurate determination of zygosity is important, because has implications for tissue

compatibility in organ transplantation, for the assessment of disease risk in the co-twin of an affected individual, for the personal right to identity, for legal and educational reasons, for estimation of the likelihood of the mother or close relatives giving birth to further sets of twins, and to avoid embarrassment when asked by family, friends, and strangers [128]. All same-sex dichorionic pairs require a genetic test to determine zygosity accurately, of which the 'Gold Standard' is a 15 marker polymorphic minisatellite test. But this test is expensive and available only for the last decade. The majority of previous twin studies and clinical judgments in everyday life are based on morphological phenotype assessment. Our results demonstrated that this is not accurate and researches as well as twins and their parents could be misinformed about twins' zygosity.The accuracy of zygosity recognition by 3D morphometric methods in this study produces only about 80% success rates compare to DNA based determination. Depending on the analysis method the accuracy range was from 77.14 to 89.52%. Examining erroneously classified twin pairs, we concluded that most of them could be regarded as outliers in the sample. Thus, it may be that the faces of DZ twins are so similar that it is very hard to define a feature that would help classify the pair as DZ. In Fig. 4.2.1 A, twins were classified as MZ by 3D analysis but the DNA test indicated that the pair was DZ.

Correspondingly, MZ twins may have faces that differ substantially in shape. Fig. 4.2.1 B shows a misclassified MZ pair. As it can be seen, the twins look the same and the shapes of their faces is the same but one's face is clearly smaller. Another misclassified MZ pair is shown in Fig. 4.2.2. This is an interesting pair because when the mirrored face of the other twin was used in 3D analysis the pair was classified as MZ, the same as in the DNA test. Such divergent twin pairs, as in the examples above, are interesting exceptions but they form a considerable portion in both zygosity groups. There are also figures of correctly classified MZ and DZ twins (Fig. 4.2.1 C–D).

Measurement errors were deemed to be minor. The error in both the 3D device and in repeating neutral expression was below 0.2 mm. Reproducibility in landmarking is weaker, but landmarking was used only for scaling in this study and the best classification result was obtained without scaling. Success rate results themselves indicate the complete error of the method.

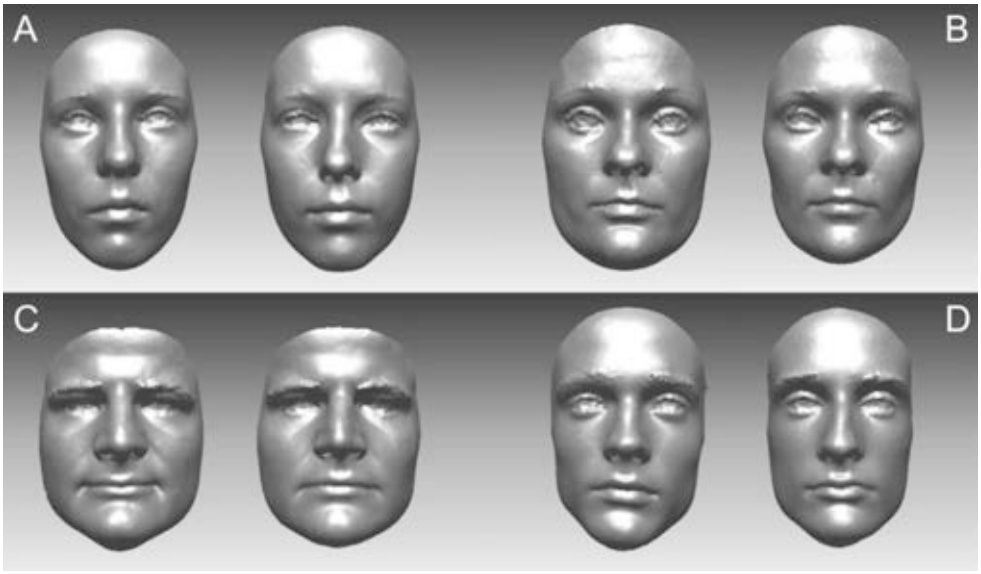

Fig. 4.2.1. *(A) Female twin pair regarded as DZ by DNA, but MZ by 3D analysis. (B) Female twin pair regarded as MZ by DNA, but DZ by 3D analysis. (C) Male twin pair regarded as MZ by DNA and 3D analysis. (D) Male twin pair DZ pair regarded as DZ by DNA and 3D analysis.*

Genetically, twinning has been considered a heritable trait and the genes involved may interfere with many facial traits, which is more common in twins than in singletons. Examples are asymmetry of the face in Beckwith-Wiedemann and Russel-Silver syndromes, and some oculo-vertebral spectrum abnormalities [129]. This spectrum includes conditions with phenotypic overlaps that predominantly are derivatives of first and second brancial arch developmental aberrations. These phenotypes are highly variable and those with most evident facial asymmetry are individuals with the hemifacial microsome phenotype. This varied presentation is indicative of a causal heterogeneity, where a number of environmental, genetic, and multifactorial associations are reported [130]. The incidence of multiple pregnancies is almost 10 times more common in association with oculo-vertebral-syndromes than in controls [131]. There is an excess of such malformations in MZ twins and it has led to the conclusion of a common cause for MZ twinning and these aberrations [132]. Facial asymmetry has been demonstrated in MZ twins with earlier 3D technology by Burke et al. [90].

Facial asymmetry may be of the fluctuating type, with random deviations in the symmetry of traits, or it may be of the directional type, where an anatomical feature is systematically greater on one side than the other [133,

134]. In MZ twins some special types of asymmetry may also appear, such as a rare mirror-image type of asymmetry and functional laterality disturbance, which are of significant scientific interest. Some clinical reports provide evidence of an interwoven genesis of these traits [135–137]. In our twins an example of almost complete face mirroring came up in the MZ (DNA) group, but was recognized as DZ by 3D (Fig. 4.2.2).

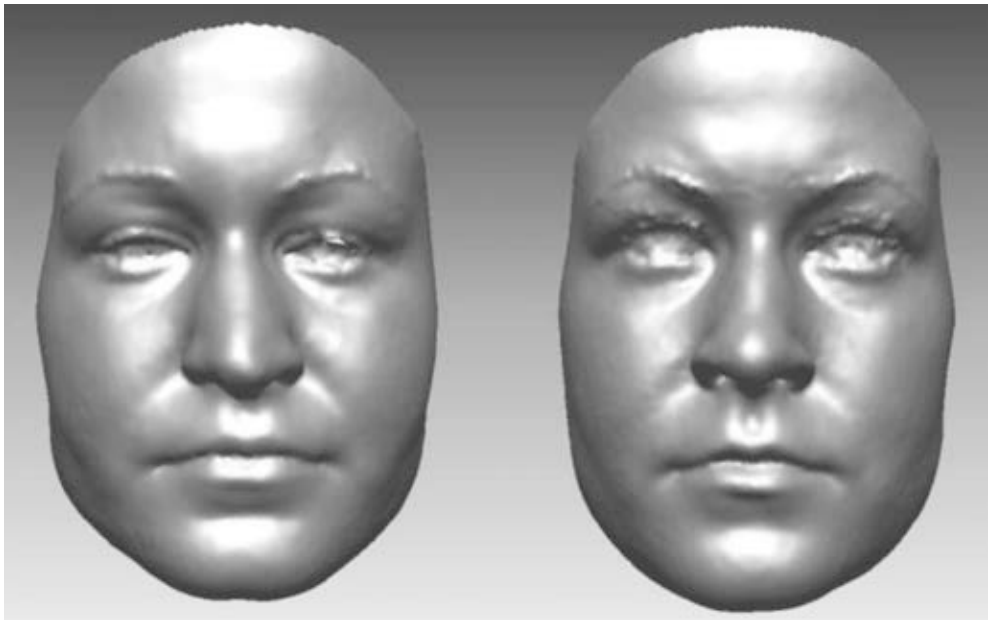

Fig. 4.2.2. *Female twin pair was MZ according to the DNA test, but DZ according to 3D analysis. When the other twin's face was mirrored in the 3D analysis, zygosity was classified as MZ.*

The difference between male and female twin pairs observed in this study is intriguing and likely due to the fact that males have more prominent, rectangular faces, whereas those of females are round with thicker soft tissue. Therefore males have more distinct features than females and this can be seen in the results. In most of the cases (22 out of 27) males got better success rates in classification than females even though the males sample was smaller. This result could be explained by gender differences and opposite sex twins, who may have reduced dimorphism in various traits, due to suspected prenatal hormonal interference between fetuses [138].

General knowledge of facial morphology, its development, and control mechanisms, is very important. There is no doubt about the interaction of genes and environmental factors in the etiology of dento-facial proportions. We expect that the 3D method will provide new, interesting data on genetic

and gestational influences on facial morphology. This research has given birth to new ideas and development of further studies. It would be sensible also to compare different areas of faces separately [91]. The results of examining different facial areas of MZ twins might explicate the effect of specific genes in different parts of the face.

The final result of our 3D morphometric studies on zygosity determination is evidence based conclusion, that this type of zygosity diagnostics may lead to inaccuracy approximately from 20 to 10% of the sample depending on the employed methodology. It is not acceptable for genetic studies and results from previous studies based or including elements of morphological face assessment for zygosity determination should be interpreted with caution.

4.3. The gender effects on genetic variance of mandibular morphology in twins

The gender effect on cranio-facial heritability estimates have been studied by many researches. Majority of the studies stated, there were no, or very little heritability estimate differences related to the gender [139,140]. But it should be understood, that it was anlyzed intra-pair differences or correlation coefficients of cephalometric variables between a pair of male and a pair of female, but not the absolute values of measurements between man and woman. There are no doubt, that males has bigger jaws in absolute values. But intra-pair correlations of cephalometric measurement values between male and female twin pairs in our study do not demonstrated statistically significant differences except for two parameters. The MZ female twin pairs showed higher intra-pair correlation of dento-alveolar height at the molar region (distance ms ⊥ GoMe), when compare to male, (respectively 0.75 and 0.60, $p < 0.01$). And the second exception was in the DZ group. The DZ male twin pairs showed higher intra-pair correlation of lower incisor distance to NB line (variable ii ⊥ NB), respectively 0.56 and 0.35, $p < 0.03$. This interesting fact may indicate a role of hormonal influence on mandible growth and possible greater stress of the twinning process in girls as it is a case with dental crown development and eruption [138]. Therefore, full understanding of the interplay between environmental and genetic patterns involved in the mandible development and growth required more detailed studies.

The main result from this part of the study is that we do not found significant gender influence in intra-pair correlations of cephalometric variables in both, MZ and DZ groups. For the further analysis of heritability

estimates and cultural inheritance we used pooled data of both genders in MZ and DZ groups

4.4. The heritability estimates and cultural inheritance of the mandibular cephalometric parameters

Heritability assessment is usually a first step in genetic studies, because it provides an estimate of how much phenotypic variation is attributable to genetic influence [45]. Path Analysis model calculates the proportion of the total variance explained by additive genes (narrow-sense heritability) and by the common environment [45, 92, 106]. The "common environment" means that twins share a large part of their environment, because they are raised together. The term "cultural inheritance" is used to define environment in a more comprehensive way, including cultural and social factors.

It is acknowledged that facial maturity develops in females between 12 and 15 years and 2 years later for males. The studies of craniofacial growth from 4 to 20 years demonstrated increasing heritability estimates of cephalometric variables with the age [141]. Therefore, comparison of hereditary characteristics is more valid in the post-adolescent period when the growth is completed, as it is the case in our study.

The mandibular skeletal morphology and relationship to cranial base demonstrated significant additive genetic component present. Sagittal position of the mandible in the craniofacial complex depends on many determinants such as: position of mandibular fossa, cranial base length, growth pattern and others. Sagittal localization of mandibular fossa is determined by morphology of cranial base and can be described by saddle angle (NSBa). As few previous studies, the present study also find high heritability ($h^2 = 0.86$) for this angle [45]. But also there are some previous studies reporting only a moderate heritability estimate for the saddle angle [110, 142]. This might be due to fact that our twins were more mature with completed growth. As it was demonstrated in the study of heritability between parents and their offspring, heritability estimate for saddle angle increases with age [143]. The other angular measurements representing sagittal position of the mandible to cranial base (NSAr, SN-ArRp, NSBa, SNB) also were found to be under strong genetic control. The vertical mandibular position to cranial base and maxilla demonstrated moderate to low heritability estimates. The angles representing vertical relationship of mandible to upwards located skeletal structures demonstrated slight tendency of increasing heritability estimates towards downward direction, respectively: SN-GoMe ($h^2=0.37$) and PoOr-GoMe ($h^2=0.37$) compared to

ANS.PNS-GoMe (h^2 = 0.52). Linear measurements, do not demonstrated significant differences for heritability estimates between horizontal and vertical positions of the mandible in relationship to cranial base. The horizontal measurements – Co-B, h^2=0.17, Co-Pog, h^2=0.18 and very similar the vertical – total anterior facial height (N-Me, h^2=0.23) and total posterior facial height (S-Go, h^2=0.10). This is in contrast with some previous studies reporting higher heritability estimates for many vertical linear measurements compared with horizontal ones [87, 106, 140, 144]. This may be due to methodological differences, we used actual lengths, while many other studies used projected lengths [45, 106, 125]. The projected lengths may not reflect actual ratio between horizontal and vertical measurements. The results of our study indicate that genetic control is more attributable to mandible sagittal than vertical position.

The most important characteristics of mandible skeletal morphology are gonial angle, mandibular body length and ramus width. The present study showed high heritability values for the gonial angle (ArGoMe, h^2 = 0.89). This is consistent with the findings of some other studies [45, 106]. The mandibular linear skeletal cephalometric variables, representing lower jaw size, suggest that environment has stronger influence on these parameters of mandibular morphology than heritability and it reflects on the higher values of the cultural inheritance coefficient (mandibular body length, Go-Gn, h^2=0.34 vs. c^2=0.57; ramal width, R1-R2, h^2=0.34 vs. c^2=0.44; depth of antegonial notch, MB2 ⊥ MB1Me, h^2=0.29 vs. c^2= 0.40).This is similar to the results of other twin studies reporting that gonial angle and mandibular arc angle is under stronger genetic control compare to mandibular length and ramus height [45, 106, 140]. But some early twin studies found very strong genetic determination for mandibular length [139]. As it was already mentioned this could be explained by differences in study sample age and accuracy of zygosity determination. The zygosity determination in majority of earlier twin studies was based or included elements of morphological face similarity assessment. This is one of major sources of possible disagreement with more current research.

The results of our study showed that alveolar height ant tooth position depends more on the environmental, than genetic influence. This is in agreement with previous studies demonstrating that heritability estimates for inter-arch variables such as overjet were considerably lower than skeletal variables [145–147]. It is know from clinical research, that environmental factors like lips, tongue and cheeks, oral muscles and certain functions (breathing and mastication) or even body posture play an important part in the development of tooth position and occlusion [40, 148–153]. Our results support this clinical observations. But variables describing sagittal position

of lower incisors (ii ⊥ NB, ii ⊥ APog) showed very high heritability. The resultant position of lower incisors depends on mandibular skeletal position and dento-alveolar adaption to soft tissue capsule [154]. The dento-alveolar compensatory mechanism controls development of the dental and alveolar arches to secure occlusion of the teeth and adaptation to the basal parts of the jaws. The best example is retroclined position of lower incisors to compensate excess growth of the mandible in Class III cases. It seems that inside of these compensatory mechanism, is an integrated balance between morphological units in the dento-facial complex that are under strong genetic control and those units that may more accommodate to environmental factors [45]. The high heritability estimates for the sagittal position of the lower incisors in this study could be dependent on strong genetic control of the mandibular skeletal position. And only in cases with significant deviations from normal skeletal positions compensatory adaption comes into the play with all its power. Majority of twins from our study were within the normal range of mandibular skeletal position in craniofacial complex and compensatory mechanisms possibly were not so expressed. The final sagittal position of the lower incisors was more determined by genetically controlled mandibular skeletal base relationship to the cranium, then environmental influence.

The high heritability estimates of OB (h^2=0.92) should be interpreted with caution, because ME for this measurement was statistically significant. The identification of the lower and upper incisal tips may lack precision due to overlap of incisors and lead to significant measurement errors.

A specific area on the lateral view of the face delimitated by the angles with high heritability estimates (SNA, h^2=1.10; NSAr, h^2=1.16; ArRp-MB1Me, h^2=1.06) attracted our attention (Fig. 4.4.1). This area presumably could be responsible for heritability of the facial bony structures. The skeletal bones forming facial part of the cranium and their relationship has the highest heritability estimates and from this perspective could be considered as a single unit. The cranial base flexure influence position of glenoid fossa and thereby the spatial position of mandible. The vertical position of the maxilla with regard to glenoid fossa as determined by the flatness of the cranial base, can cause rotation of the mandible forward or backward. Thus, mandible is influenced, but not completely controlled, by those bones with which it articulates. And this influence has high heritability estimates. The similar results concerning craniofacial area with high heritability and related to Na, Go and Gn points was found by some other researches. [106].

The very close proximity of mentioned above structures to the point *condylion*, with relatively high influence of environment to linear measure-

ments starting at this point, nicely correlates with the theory of dento-alveolar compensation introduced to clinical orthodontics by Beni Solow [155]. The classical model of dento-alveolar compensatory mechanism implies, that functionally effective and secure occlusion could be achieved only with balance between compensatory growth of alveolar bone, adaptation of teeth position and jaws skeletal growth pattern.

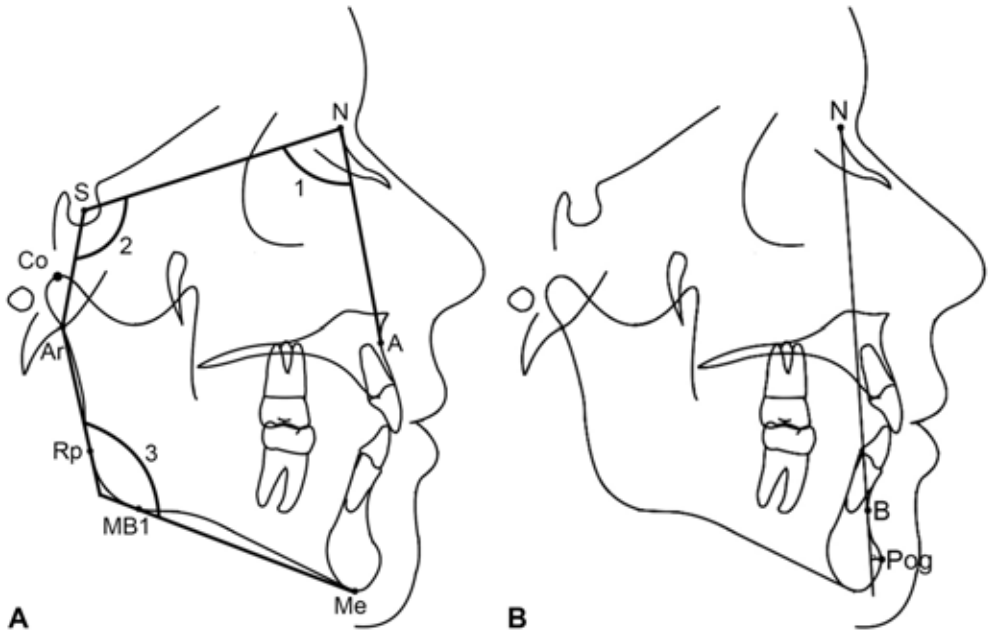

Fig. 4.4.1. *The craniofacial area with high heritability estimates: A)1 – SNA, 2 – NSAr, 3 – ArRp-MB1Me; B) chin thickness*

The discussion could be summarized by pointing two aspects of the new knowledge gained from this twin study.

The first aspect – results of this twin cephalometric study clearly defines areas, structures and relationships being under strong additive genetic influence with low reactivity to environmental changes and possibly to orthodontic treatment. It includes: sagittal relationship (position) of maxillary anterior and mandibular distal skeletal aspects to cranial base, and form (shape) of mandible. The latter is represented by the angulation of mandibular ramus to mandibular body (angle Ar-Rp – MB1-Me).

The dento-alveolar structures and linear measurements related to condyle demonstrated comparatively high values of cultural inheritance and consequently indicate susceptibility to environmental influences including orthodontic treatment. But the clinical relevance of our results should be

regarded with one assumption. The heritability is a population concept and h^2 is irrelevant to the individual as well as cultural inheritance expressed as c^2 in this study. It would be misleading to suggest that structures with low heritability are always more amenable to prevention or treatment at the individual level [45]. Therefore using the present findings it is difficult to predict success of orthodontic treatment procedures with high degree of certainty for every clinical case.

The second aspect – deals with potential to use our data for the future genetic studies. For more than 100 years, twin research has proved invaluable in helping to separate the effects of genes and environment on variation in human physical characteristics, behaviors, and susceptibility to diseases [156]. The recent advances in molecular biology and genome scanning techniques, innovative approaches involving the study of twins have much to offer in complementing molecular studies and helping to unravel how genes ant the environment contribute to both normal and abnormal phenotypic variation [103]. Before starting assessment of quantitative trait loci (QTLs) related to complex traits, it is worthwhile to show that there is a significant component of genetic variation present [157]. Furthermore, when it comes to carrying out linkage and association studies, data of MZ and DZ twins can be analyzed simultaneously to achieve and gain more statistical power to detect QTLs responsible for phenotypic variation. The results of our study indicate that the cephalometric variables with high heritability estimates could be one of potential phenotypic variations suitable for genome – wide association studies in oro-facial region. The identification of causal genetic polymorphism could be next step in our further research.

CONCLUSIONS

1. The accuracy of morphometric 3D facial analysis in zygosity determination is not acceptable for twin studies. The bias vary from 10 to 25% of the cases when compare with DNA analysis of 15 highly variable genetic loci.

2. The cephalometric parameters of the facial bony structures have high heritability coefficient (h^2). There is a strong additive genetic influence on cephalometric variables defining chin thickness, form and sagittal position of the mandible.

3. The environment has significant contribution to the variance of facial height, mandibular skeletal linear cephalometric variables and vertical position of the lower molars and incisors.

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PUBLICATIONS

1. Vuollo, Ville; Šidlauskas, Mantas; Šidlauskas, Antanas; Harila, Virpi; Šalomskiene, Loreta ; Zhurov, Alexei; Holmström, Lasse; Pirttiniemi, Pertti; Heikkinen, Tuomo. Comparing Facial 3D Analysis with DNA Testing in Recognition of Twin Zygosity // Twin Research And Human Genetics. 2015, April 14, p.1-8. Impact factor: 1.918 (2013)
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1. Heikkinen, Tuomo; Vuollo, Ville; Šidlauskas, Mantas; Harila, Virpi; Šidlauskas, Antanas. Twin Zygosity Determination Using Facial Stereophotogrammetry Compared With the Dna-Method Among Young Adults //EOS 2014 – 90th Congress of the European Orthodontic Society: 18-22 June 2014, Warsaw, Poland: abstracts / (Scientific Posters.). p. 112 / 441, no. SP 112, ID 636.
2. Heikkinen, Tuomo; Forchini, Federico; Harila, Virpi; Perkiömäki, Marja-Riitta; Pirttiniemi, Pertti Mikael; Šidlauskas, Mantas; Zhurov, Alexei. Three Dimensional Method in Facial Soft Tissue Analysis During Growth, Orthodontic, Orthognathic and Cleft Treatments Using 3d-Photography // ACPA 2013 – The 12th International Congress on Cleft Lip/Palate and Related Craniofacial Anomalies: May 5-10 2013, Orlando, Florida, USA / The American Cleft Palate-Craniofacial Association (ACPA). Orlando : ACPA, 2013. no. 966.
3. Heikkinen, Tuomo; Forchini, Federico; Harila, Virpi; Šidlauskas, Mantas; Zhurov, Alexei. Three dimensional method in facial soft tissue analysis during growth, orthodontic treatment and orthognathic surgery using 3D-photography // 22nd Congress of the Nordic Orthodontic Society - NOS 2012 : Oulu, Finland 22-25 August 2012: Program and Abstracts (Oral session V.), ISBN 978-951-42-9887-5. p. 39, no. OP-12.
4. Šidlauskas, Antanas; Vasiliauskas, Arūnas; Šidlauskas, Mantas; Švalkauskienė, Vilma. Early orthodontic treatment with a prefabricated functional appliance // 87th Congress of the European Orthodontic Society: abstract book: 19-23 June, 2011, Istanbul, Turkey. p. 96, SP 044.

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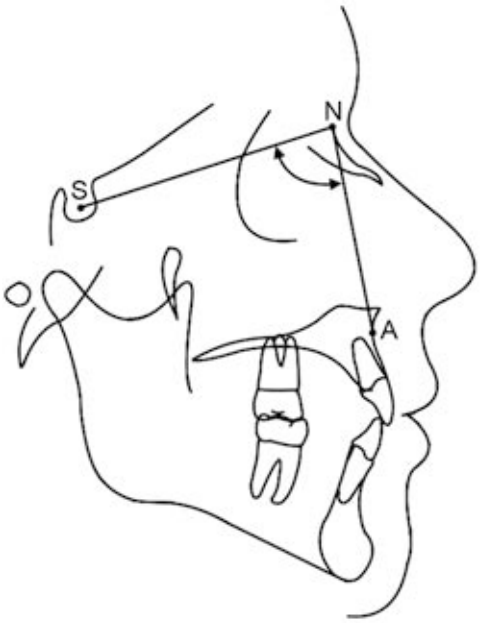
6. Heikkinen, Tuomo; Grön, Mathias; Šidlauskas, Mantas; Harila, Virpi; Alvesalo, Lassi. Dimorphism of the dentition in opposite sex twins // 21st congress of the Nordic orthodontic society (NOS): Aalborg, Denmark 25.09.2010. The Danish Society of Orthodontic Specialists, 2010. (Abstracts.). [1 p.].
7. Heikkinen, Tuomo; Harila, Virpi; Šidlauskas, Mantas; Alvesalo, Lassi. Dental Dimorphism in opposite sex twins // CLEFT 2009 - 11th International Congress on Cleft Lip and Palate and Related Craniofacial anomalies. Cleft and Craniofacial Care: Experience, Evolution and Innovation: Fortaleza/Brazil - September 10th to 13th, 2009 / Instituto de Cirurgia Plástica Crânio Facial - SOBRAPAR. 2009. (Abstracts.). p. 187, no. 24.13.

APPENDICES

Appendix 1 (Templates of cephalometric measurements)



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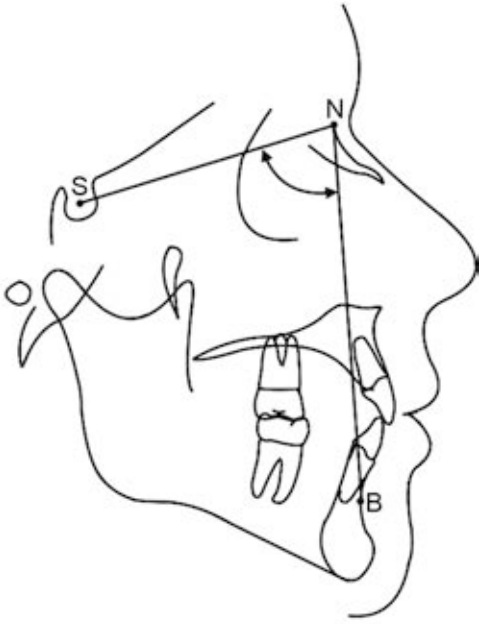

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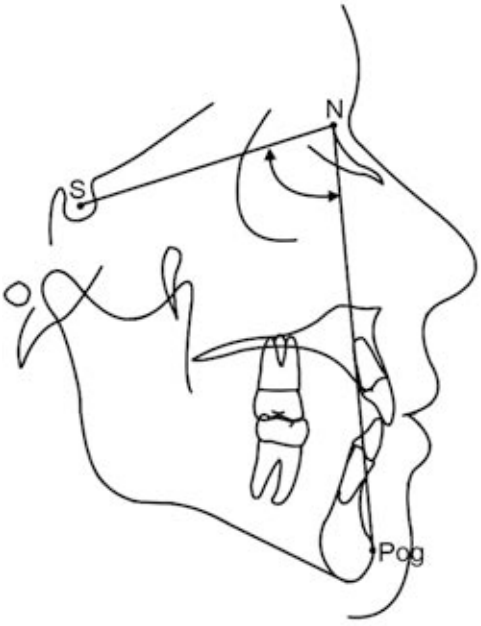

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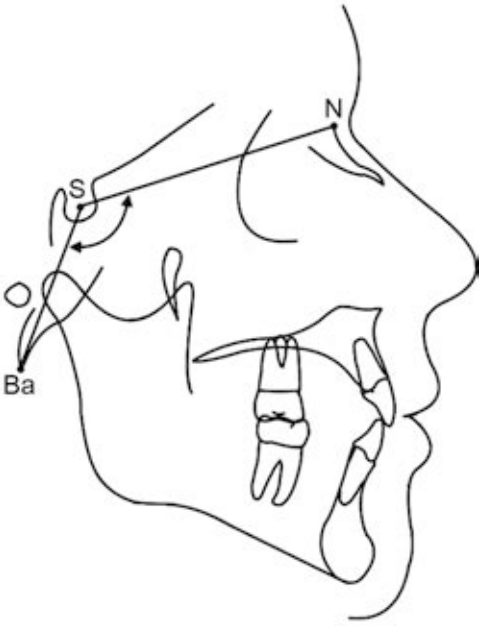

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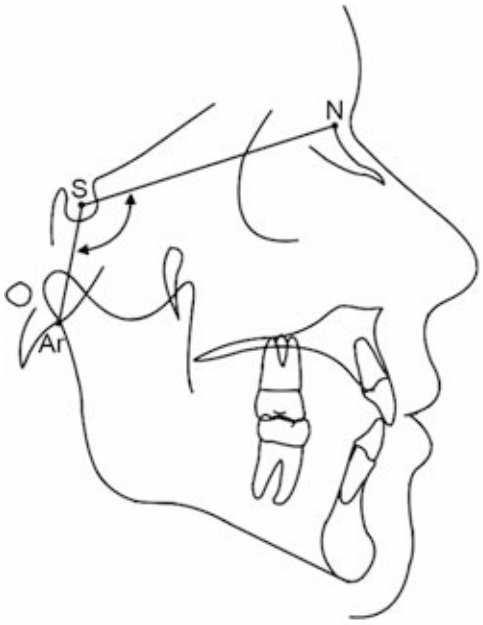

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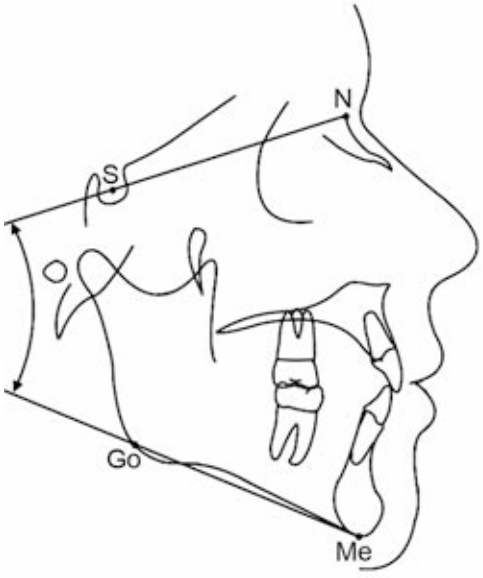

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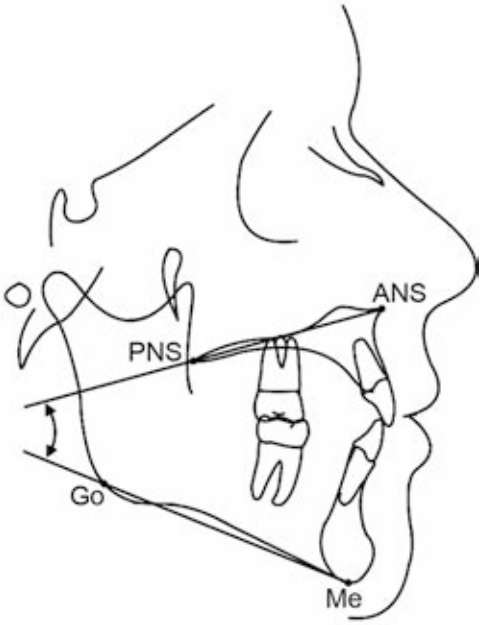
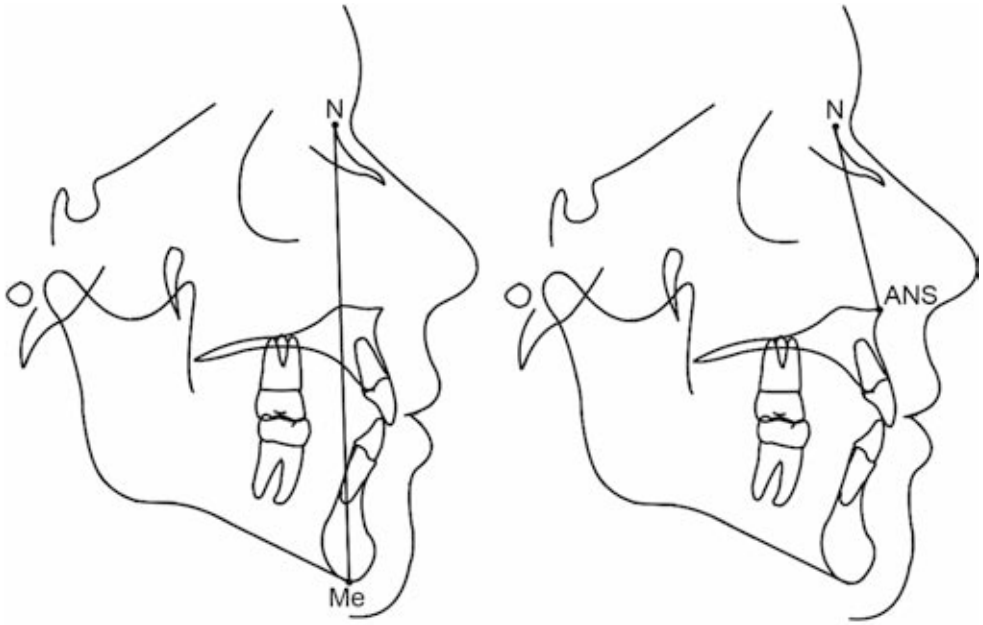
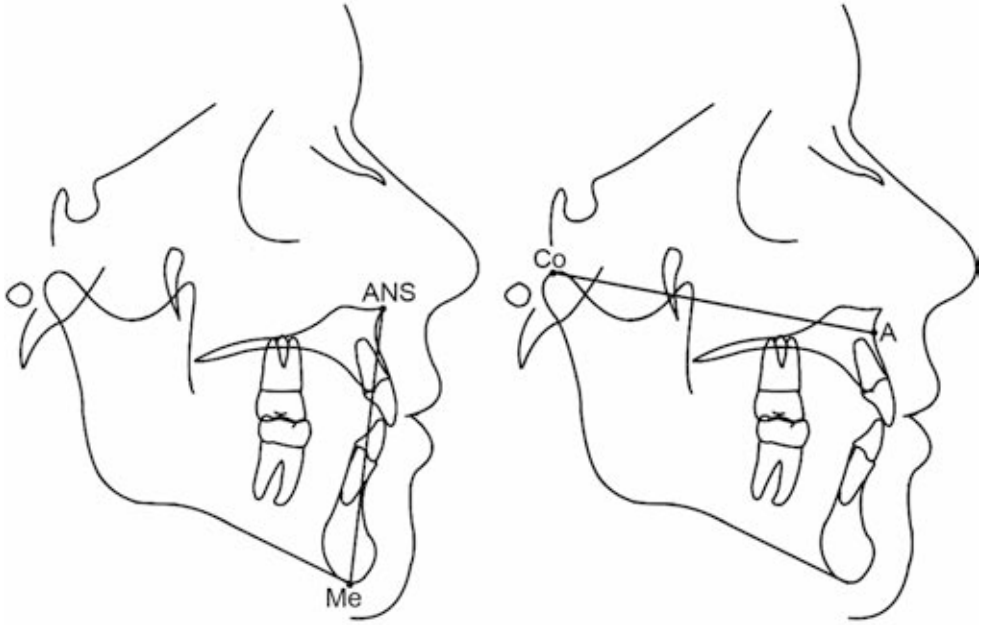


ANS.PNS-GoMe (°)



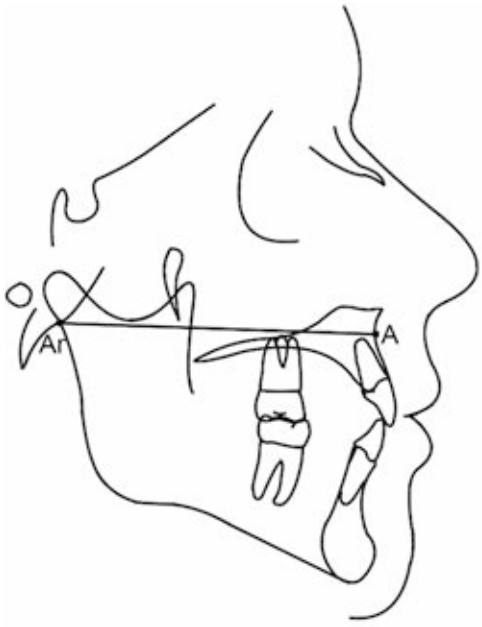
N-Me (mm)

N-ANS (mm)



ANS-ME (mm)

Co-A (mm)



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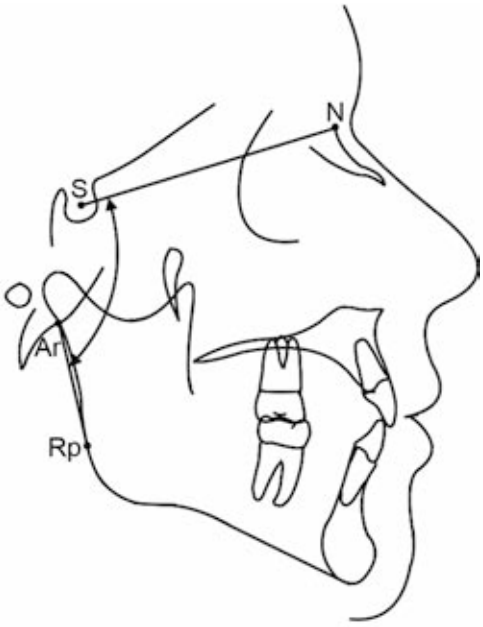

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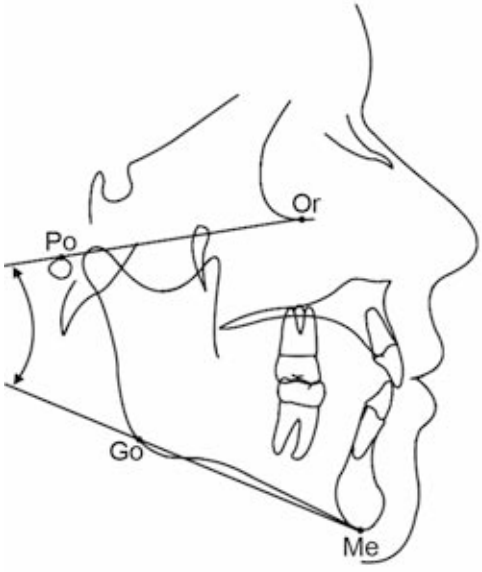

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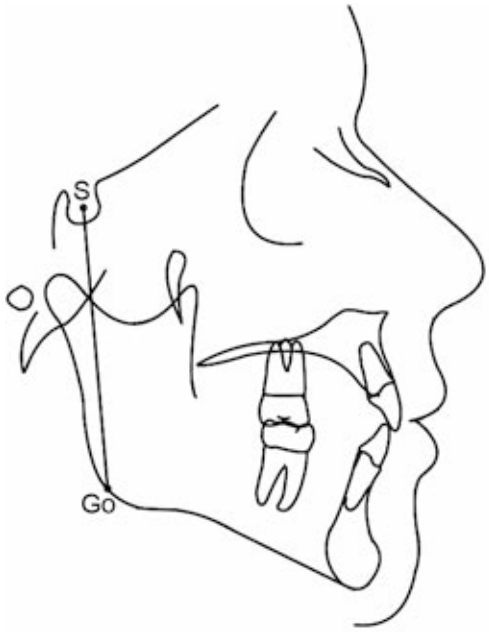
NGNGo (°)



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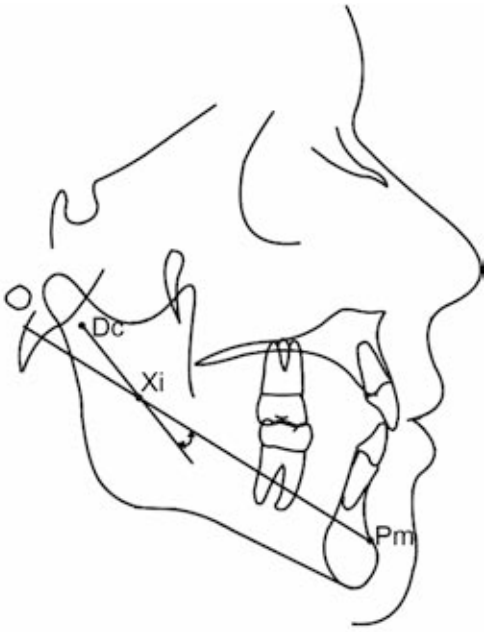

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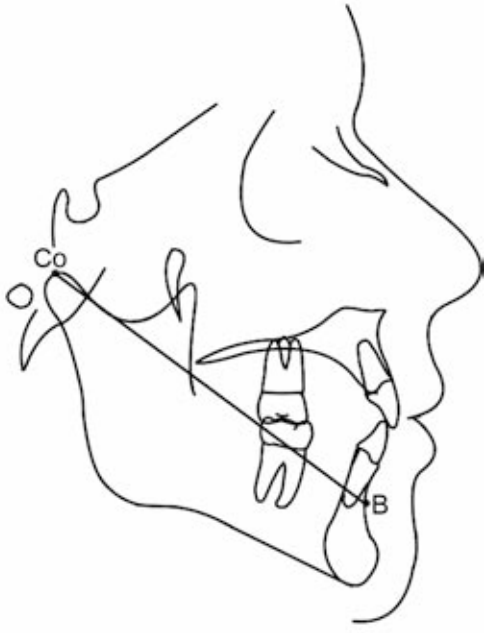
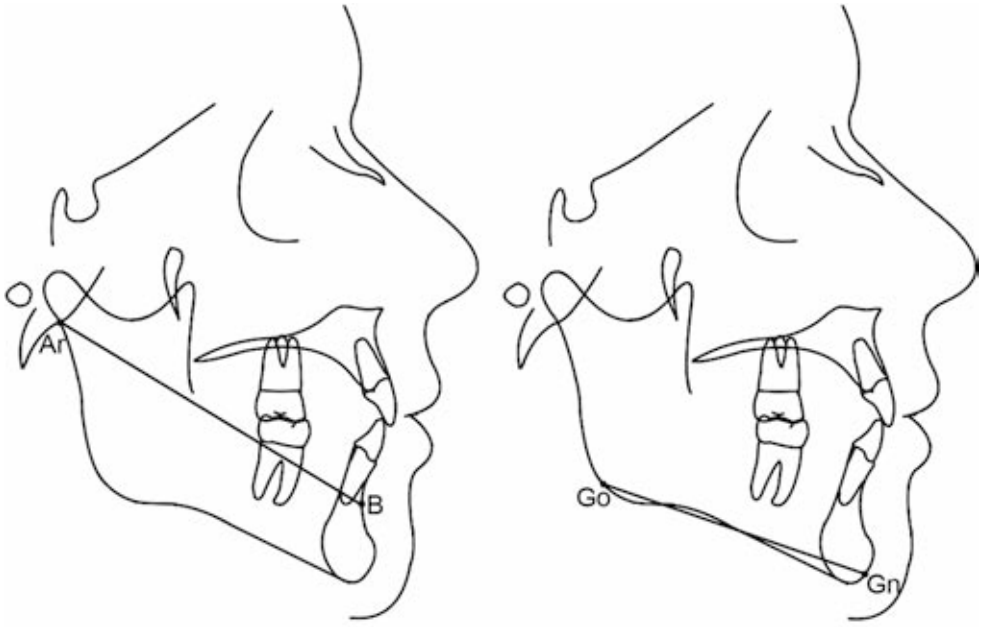
CoGoMe (°)

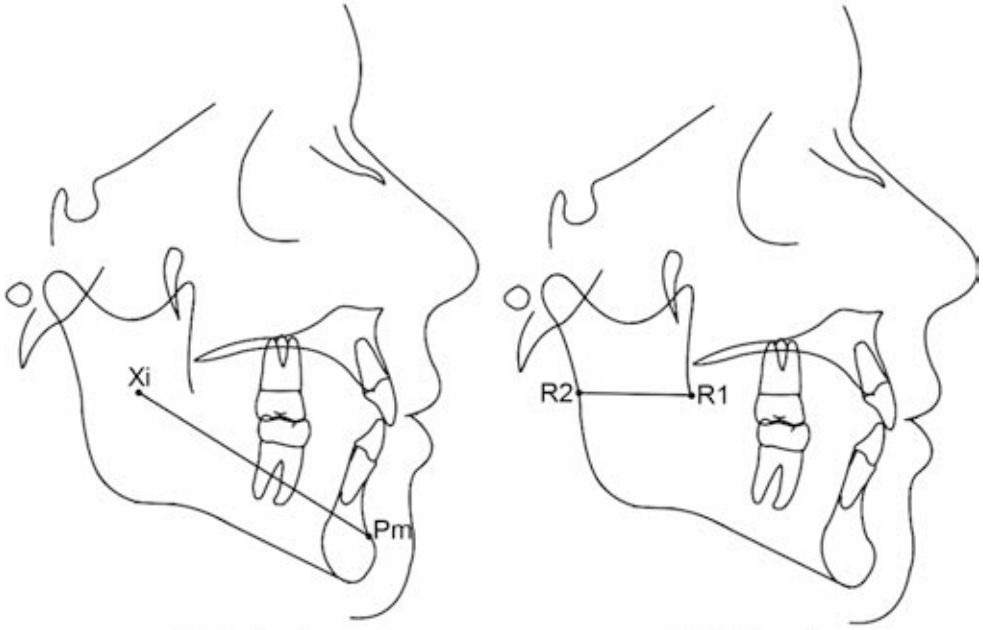


Co-B (mm)



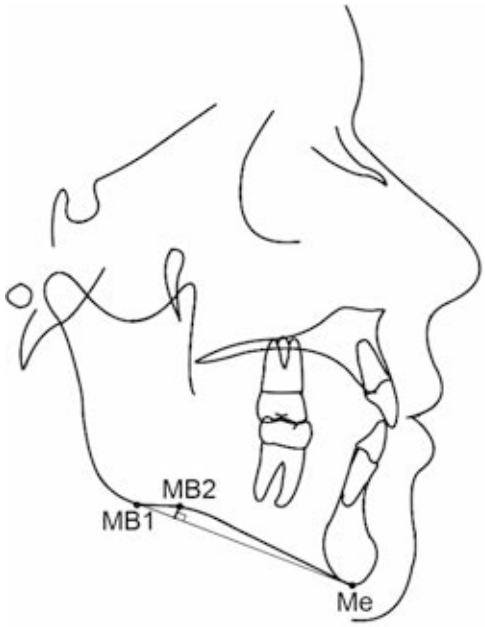
Ar-B (mm)

Go-Gn (mm)

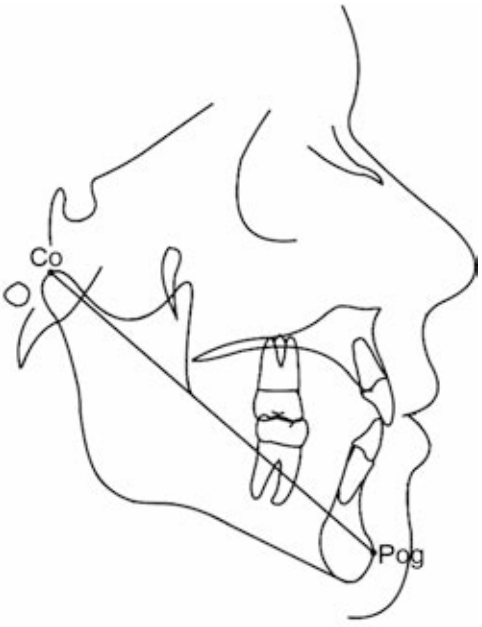


Xi-Pn (mm)

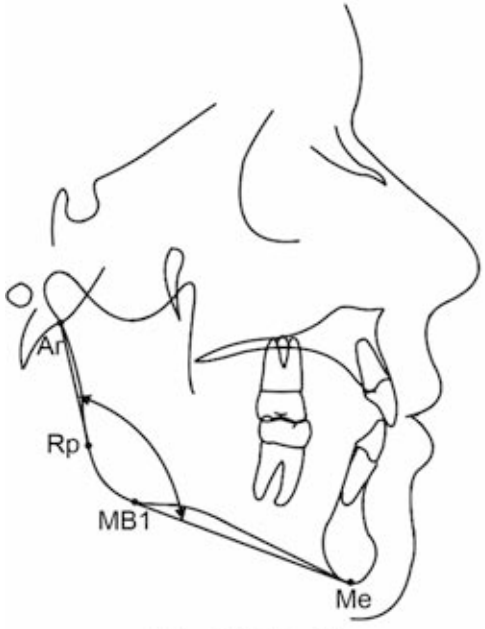
R1-R2 (mm)



MB2 ⊥ MB1Me (mm)

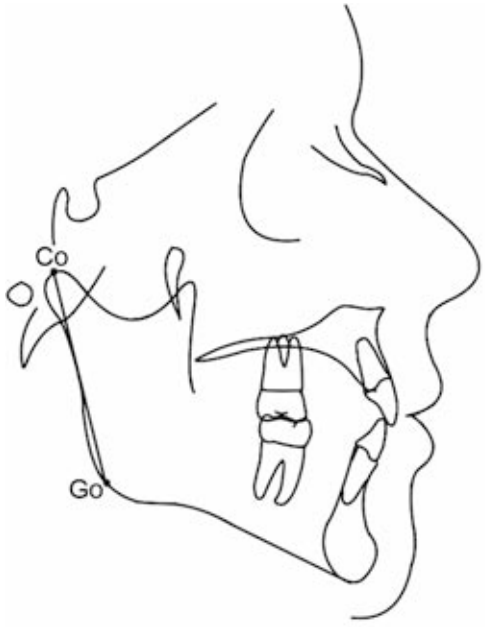


Co-Pog (mm)



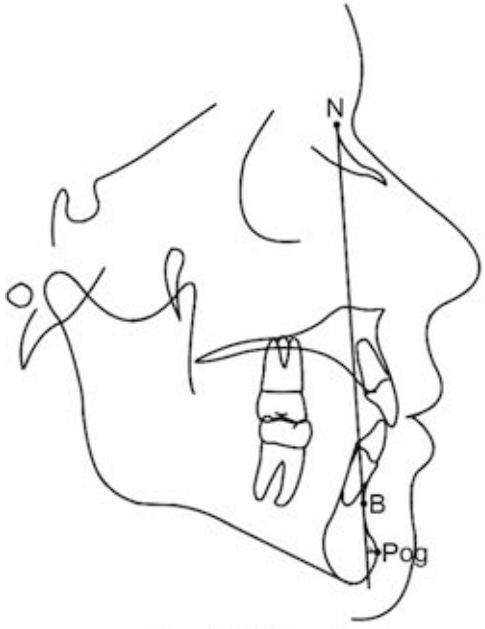
ArRp-MB1Me (°)

ArGoMe (°)



CoGo (mm)

Go-Pog (mm)



Pog ⊥ NB (mm)

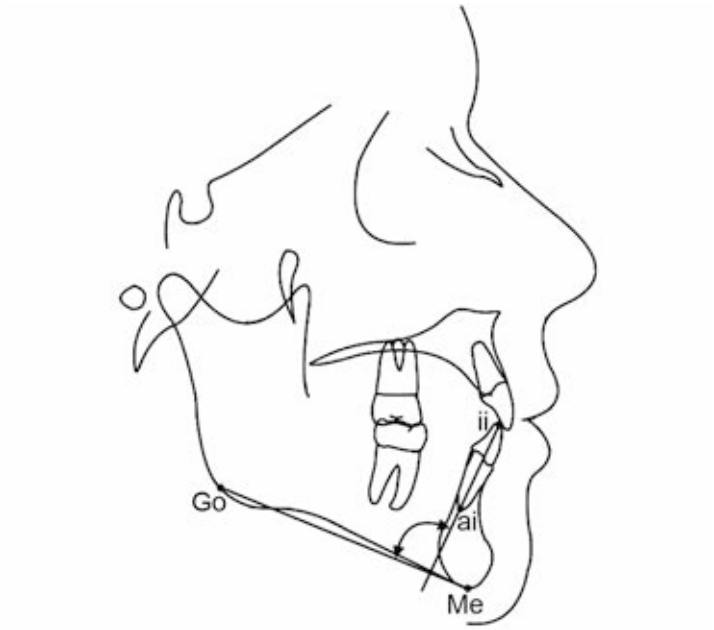
ai-ii-NB (°)

ii ⊥ NB (mm)

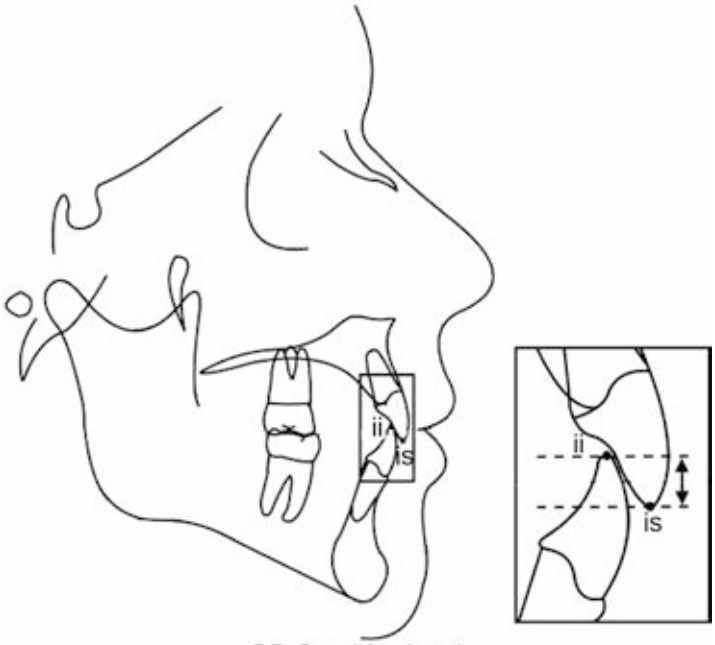
ii ⊥ APog (mm)

ii ⊥ GoMe (mm)

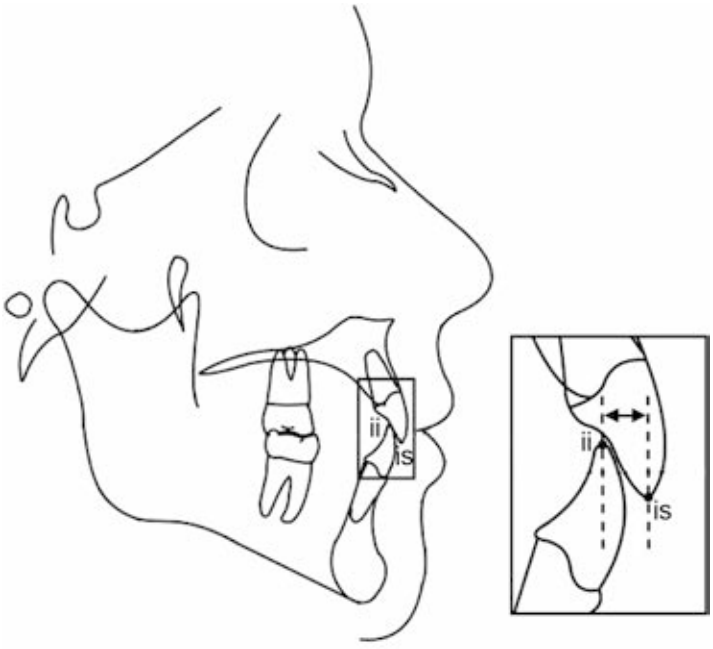
ms ⊥ GoMe (mm)



ai-ii-GoMe (°)



OB Overbite (mm)



OJ Overjet (mm)

Appendix 2

KAUNO REGIONINIS BIOMEDICININIŲ TYRIMŲ ETIKOS KOMITETAS

Lietuvos sveikatos mokslų universitetas, A. Mickevičiaus g. 9, LT 44307 Kaunas, tel. (+370) 37 32 68 89; el.paštas: kaunorbtck@lsmuni.lt

LEIDIMAS ATLIKTI BIOMEDICININĮ TYRIMĄ

2015-02-09 Nr. BE-2-12

Biomedicininio tyrimo pavadinimas:	**"Sąkandžio formavimosi ir ortodontinių anomalijų išsivystymo etiologiniai veiksniai"**
Protokolo Nr.:	**01**
Data:	**2015-01-15**
Versija:	-
Asmens informavimo forma	**Atleista nuo asmens informavimo formos naudojimo**
Pagrindinis tyrėjas:	**Doc. Kristina Lopatienė**
Biomedicininio tyrimo vieta: Įstaigos pavadinimas: Adresas:	**LSMUL Kauno klinikos** Eivenių g. 2, LT-50009, Kaunas **Ortodontijos klinika** J. Lukšos – Daumanto g. 6, LT-50106, Kaunas

Išvada:

Kauno regioninio biomedicininių tyrimų etikos komiteto posėdžio, įvykusio **2015 m. vasario 5 d.** (protokolo Nr. BE-10-1) sprendimu pritarta biomedicininio tyrimo vykdymui.

Mokslinio eksperimento vykdytojai įsipareigoja: (1) nedelsiant informuoti Kauno Regioninį biomedicininių Tyrimų Etikos komitetą apie visus nenumatytus atvejus, susijusius su studijos vykdymu, (2) iki sausio 15 dienos – pateikti metinį studijos vykdymo apibendrinimą bei, (3) per mėnesį po studijos užbaigimo, pateikti galutinį pranešimą apie eksperimentą.

Kauno regioninio biomedicininių tyrimų etikos komiteto nariai			
Nr.	Vardas, Pavardė	Veiklos sritis	Dalyvavo posėdyje
1.	Prof. Romaldas Mačiulaitis	Klinikinė farmakologija	taip
2.	Prof. Edgaras Stankevičius	Fiziologija, farmakologija	taip
3.	Doc. Eimantas Peičius	Filosofija	taip
4.	Dr. Ramunė Kasperavičienė	Kalbotyra	ne
5.	Med. dr. Jonas Andriuškevičius	Chirurgija	taip
6.	Agnė Krušinskaitė	Teisė	taip
7.	Prof. Skaidrius Miliauskas	Pulmonologija, vidaus ligos	taip
8.	Med. dr. Rokas Bagdonas	Chirurgija	ne
9.	Egle Vaižgelienė	Visuomenės sveikata	taip

Kauno regioninis biomedicininių tyrimų etikos komitetas dirba vadovaudamasis etikos principais nustatytais biomedicininių tyrimų Etikos įstatyme, Helsinkio deklaracijoje, vaistų tyrinėjimo Geros klinikinės praktikos taisyklėmis.

Pirmininkas Prof. Romaldas Mačiulaitis

KAUNO REGIONINIS BIOMEDICININIŲ TYRIMŲ ETIKOS KOMITETAS
KMUK Eivenių 2, Centrinis korpusas 71 kab., 50009 Kaunas, tel. +370 37 326243; faks. +370 37 326901, e-mail: **cmeinfo@kmu.lt**

PRITARIMAS
PROTOKOLO PATAISOMS / PAKEITIMAMS

2010-12-10 Nr. P1-52/2005

Biomedicininio tyrimo pavadinimas: **„Daugiaveiksmis patologijos paveldėjimas dvynių metodu".**

Pagrindinis tyrėjas: **Prof. Algimantas Sinkus**

Biomedicininio tyrimo vieta: **LSMU Biologijos katedra**

Peržiūrėti šie [√] su minėtu tyrimu susiję dokumentai:

[√] Prašymas papildyti atliekamo biomedicininio tyrimo tyrėjų sąrašą naujais tyrėjais.
[√] Gydytojų ortodontų Manto Šidlausko, Vilmos Švalkauskienės, Giedrės Trakinienės Curriculum Vitae.

Nutarta:
[√] Tyrėjų sąrašą papildyti tyrėjais: gydytoju Mantu Šidlausku, gydytoja Vilma Švalkauskiene, gydytoja Giedre Trakiniene.

Kauno regioninio biomedicininių tyrimų etikos komiteto nariai		
Nr.	Vardas, Pavardė	Veiklos sritis
1.	Doc. Irena Marchertienė	anesteziologija
2.	Doc. Romaldas Mačiulaitis	klinikinė farmakologija
3.	Prof. Nijolė Dalia Bakšienė	pediatrija
4.	Prof. Irayda Jakušovaitė	filosofija
5.	Dr. Eimantas Peičius	filosofija
6.	Gintaras Česnauskas	chirurgija
7.	Zelmanas Šapiro	terapija
8.	Jurgita Laurinaitytė	bioteisė
9.	Laima Vasiliauskaitė	psichoterapija

Kauno regioninis biomedicininių tyrimų etikos komitetas dirba vadovaudamasis etikos principais nustatytais biomedicininių tyrimų Etikos įstatyme, Helsinkio deklaracijoje, vaistų tyrinėjimo Geros klinikinės praktikos taisyklėmis.

Pirmininkė Doc. Irena Marchertienė

LIETUVOS SVEIKATOS MOKSLŲ UNIVERSITETAS
KAUNO REGIONINIS BIOMEDICININIŲ TYRIMŲ ETIKOS KOMITETAS

VALSTYBINĖ DUOMENŲ APSAUGOS INSPEKCIJA

Lietuvos sveikatos mokslų universiteto
VšĮ Kauno klinikoms
Eivenių g. 2, LT-50009 Kaunas
[illegible]

SPRENDIMAS
DĖL LEIDIMO LIETUVOS SVEIKATOS MOKSLŲ UNIVERSITETO VŠĮ KAUNO KLINIKOMS ATLIKTI ASMENS DUOMENŲ TVARKYMO VEIKSMUS

2015 m. kovo 26 d. Nr. 2R-1840 (2.6-1.)
Vilnius

Valstybinė duomenų apsaugos inspekcija, išnagrinėjusi Lietuvos sveikatos mokslų universiteto VšĮ Kauno klinikų pateiktą 2015-03-10 Pranešimą Nr. 1 dėl išankstinės patikros (toliau – Pranešimas) dėl asmens duomenų tvarkymo mokslinio medicininio tyrimo tikslu ir 2015-03-20 raštu pateiktus patikslinimus (Inspekcijoje gauta 2015-03-12, reg. Nr. 1R-1928 ir 2015-03-24, reg. Nr. 1R-2228),

n u s t a t ė,

kad Lietuvos sveikatos mokslų universiteto VšĮ Kauno klinikų Pranešime ir patikslinimuose nurodyti asmens duomenų tvarkymo veiksmai atitinka Lietuvos Respublikos asmens duomenų teisinės apsaugos įstatyme nustatytus asmens duomenų tvarkymo ir duomenų subjektų teisių įgyvendinimo reikalavimus, bei numatytos tinkamos organizacinės ir techninės duomenų saugumo priemonės.

Valstybinė duomenų apsaugos inspekcija, vadovaudamasi Lietuvos Respublikos asmens duomenų teisinės apsaugos įstatymo 33 straipsniu, Valstybinės duomenų apsaugos inspekcijos direktoriaus 2006 m. vasario 2 d. įsakymu Nr. 1T-6 patvirtintų Išankstinės patikros atlikimo taisyklių 11 ir 18.1 punktais,

n u s p r e n d ž i a

Lietuvos sveikatos mokslų universiteto VšĮ Kauno klinikoms išduoti leidimą atlikti Pranešime ir patikslinimuose nurodytus asmens duomenų apie sveikatą tvarkymo mokslinio medicininio tyrimo „Sąkandžio formavimosi ir ortodontinių anomalijų išsivystymo etiologiniai veiksniai", prot. Nr. 01, tikslu veiksmus.

Direktorius | dr. Algirdas Kunčinas

V. Pozeckaitė, tel. (8 5) 219 7964, el. p. [illegible]
K. Praspaliauskienė, tel. (8 5) 219 7965, el. p. [illegible]

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Duomenys kaupiami ir saugomi
Juridinių asmenų registre
Kodas 188607912

AKNOWLEDGEMENTS

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I wish to express my especial gratitude to my wife, Monika, for all her love and understanding during the years that this work has taken and to my son Vytautas for the joy he had added to my life during this time! I am forever grateful to mother and father for being my guiding stars in life.

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