A Study Model Comparison of Monozygotic Twins, Dizygotic Twins and Sibling Pairs

M.S. Candidate: Laura O. Nichols, DDS

A Thesis submitted to the Department of Orthodontics and
The Advanced Education Committee of the
Oregon Health and Science University School of Dentistry
In partial fulfillment of the requirements
For the degree of Master of Science

December 2006

A Study Model Comparison of Monozygotic Twins,
Dizygotic Twins and Sibling Pairs

A thesis presented by Laura Nichols
In partial fulfillment for the degree of Master of Science in Orthodontics

December 2006

Master of Science in Orthodontics Research Committee:

[redacted]
Larry M. Doyle, D.D.S.
Assistant Professor and Graduate Program Director
Department of Orthodontics

[redacted]
David A. Covell Jr., Ph.D., D.D.S.
Associate Professor and Chair
Department of Orthodontics

[redacted]
Robert W. T. Myall, BDS, FDSRCS, FRCD(C), MD
Professor of OMFS and Surgery
Department of Oral and Maxillofacial Surgery

ACKNOWLEDGEMENTS

I would like to acknowledge and thank and express my sincere appreciation to my committee members: Dr. Larry Doyle, Dr. David Covell and Dr. Bob Myall for their guidance and time throughout this project.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

This cross-sectional, retrospective study was conducted to assess the extent to which genetic factors affect dental morphologic variation. The sample consisted of 96 adolescents with an age range of 12.0-16.3 years (13.6 years). There were 24 monozygotic twin pairs, 6 same-sex dizygotic twin pairs, and 18 same-sex sibling pairs. Records analyzed included study models of the maxillary and mandibular dental arches. The intrapair differences of the monozygotic twin pairs and the dizygotic twin and sibling pairs were analyzed by use of the t-test for overjet, overbite, buccal segment relationship, intercanine width, intermolar width and dental irregularity. Co-pair correlation coefficients and heritability estimates were calculated for all parameters studied.

Results showed a trend in monozygotic twins toward smaller mean intra-pair difference for all parameters studied. Mean intrapair differences for overbite, mandibular intermolar width and mandibular anterior dental irregularity were significantly less than those of dizygotic twins and sibling pairs. Co-pair correlation coefficients for monozygotic twins were generally higher than those for dizygotic twins and sibling pairs – specifically for overbite, overjet, mandibular intermolar width and mandibular anterior dental irregularity, which suggests a genetic influence. The results from this study suggest the etiology of malocclusion to be multifactorial. Significant heritability estimates were calculated for overbite, overjet, mandibular intermolar width and mandibular anterior dental irregularity. Further study with a larger sample is recommended to confirm these findings.

INTRODUCTION

It has been estimated that in the United States some 40% of adolescents have malocclusion (Kelley and Harvey 1977). In fact, an entire field has been built around the correction of malocclusion, yet little conclusive evidence exists as to the underlying cause of this occlusal variation. The great variability in occlusal factors can be broadly ascribed either to genetic factors, environmental factors or to an interaction between genes and environment.

The question of the etiology of malocclusion – whether environmental or genetic – has been debated among orthodontists as far back as 1891 when Kingsley described inheritance as a major factor in the development of malocclusion. This question is of importance to the orthodontist because of the relevance to the possibilities and limitations of treatment, or the degree to which environmental influences (such as orthodontic treatment) can affect the development of occlusion. During the early part of the 1900's, largely due to Edward H. Angle's adamant belief that malocclusions arise from local factors, the generally accepted view was that environmental influences were responsible for the determination of occlusion. Thus, orthodontic treatment as an environmental influence, was assumed to have a major impact on the correction of malocclusion. Therefore, treatment goals included correction of abnormal growth of the upper and lower jaws and treatment of crowding was done primarily with arch expansion.

By the mid-1900's, due in part to a failure of earlier treatment philosophies and also to an increased knowledge of genetics, orthodontists began to accept the opposite view – that malocclusion was the result of an inherited trait and that orthodontic treatment was limited to "making the best of the situation". This theory led to the rejection of arch

expansion or growth modification as viable treatment modalities (Proffit 1986). Currently, the relative importance of environmental versus genetic influences on the development of occlusion remains unclear.

The extent to which variation between individuals is attributable to genetics is known as heritability and is an expression of the relationship between an individual's genotype and phenotype. Genotype is the genetic constitution of the individual. An individual's phenotype is the final product of the combination of genetic and environmental influences. Heritability is then the proportion of phenotypic variance attributable to genotype (Mossey 1999). Heritability can be determined at two levels; by the study of difference between populations or by the study of the variation found within families (Smith and Bailit 1977).

Genetic variation may be described in one of two ways, as either discrete or continuous. Traits which are described as discrete are easily identifiable, such as the ABO blood antigen system, and follow Mendelian type of inheritance. Traits which are discrete can either be dominant, recessive or X-linked recessive in their inheritance pattern. In contrast, continuous traits, such as height, weight, tooth size or position, are more difficult to study because they are not determined by a single, specific allele and are additionally subject to modification by environmental influences. If genetic expression of a trait is dependent upon simultaneous segregation of many genes as well as being affected by the environment, it is said to be subject to multifactorial or polygenic inheritance. Malocclusion is not to be regarded as a disease, or as an abnormality, but as a variation of occlusion described as a multifactorial and polygenic trait (Mossey 1999).

Twin Method

The use of twins to study the relative effect of genetics versus environmental influences was first introduced by Sir Francis Galton in 1875 (Kempthorne and Osborne 1961). He observed a fundamental difference between two types of twins in his studies of twinships, strikingly similar twins (one-egg or identical twins) and those who were no more alike than ordinary brothers and sisters (two-egg or fraternal twins). Galton concluded that it should be possible to separate the effect of environment from the effect of heredity through comparison of co-twin differences between the two types of twins (Lundstrom 1984). Since Galton, the use of twins in the determination of etiology has been well established (Lauweyns et al. 1993). The twin method is used to quantify the magnitude of the contribution of genetic and environmental factors to individual differences in a trait or behavior. The twin method entails the collection of data on both types of twin pairs (monozygotic and dizygotic) with statistical comparison of the data between the pairs in order to assess the relative contribution of genetic versus environmental factors to the trait or behavior studied. Given that monozygotic twins share twice the amount of genetic material as dizygotic twins, greater similarity between monozygotic versus dizygotic twins would indicate a genetic influence (Markovic 1992). However, if dizygotic twins are as similar as monozygotic twins for a trait or behavior, then a shared environmental influence would be indicated. This model is dependant upon two assumptions: (1) that zygosity be accurately determined, and (2) that environmental effects are equal in both twin categories. Since dizygotic twins and siblings pairs are more genetically similar or dissimilar, sibling pairs can be utilized in the analysis along with

dizygotic twins for comparison to monozygotic twins (Hughes et al. 2001, Harris and Johnson 1991, Cassidy et al. 1998, Harris and Smith 1980, Manfredi et al. 1997).

Types of Twins

Monozygotic or identical twins result from an early division of a fertilized egg so that each individual possesses the same chromosomal DNA and thus are genetically identical (they share 100% of their genotype). Dizygotic twins, or fraternal twins, occur when two eggs are released at the same time and fertilized by two separate spermatozoa. Dizygotic twins are therefore no more similar than ordinary siblings except that they share the same intrauterine environment. Dizygotic twins and sibling pairs share on average 50% of their genotype. The comparison of twins is based upon the principle that any observed difference between monozygotic twins is due to environmental influences whereas differences observed between dizygotic twins (or sibling pairs) is due to the combination of environmental and genetic differences.

The absolute frequency with which monozygotic twinning occurs is the same for all races and at all maternal ages. The frequency for monozygotic twins is 3.5 – 4 per thousand maternities. In comparison, the absolute frequency of dizygotic twins is dependant upon several factors, increasing with maternal age and varying among races. Dizygotic twinning, and not monozygotic twinning, is genetically determined by the female line. The frequency of dizygotic twinning varies between 3.5-18 per thousand (Lauweryns et al. 1993).

Determination of Zygosity

Since the twin method is based upon comparison of observed differences and similarities between monozygotic and dizygotic twins, it is important that zygosity be accurately determined. Determination of zygosity can be performed by one of several methods (Ohm Kyvik and Derom 2006).

Gender: Unlike-sexed twins are by definition dizygotic.

Fetal Membranes: Monochorionicity (MC; or the presence of a single chorion or a single outer membrane surrounding the fetus) is strong proof of monozygocity. MC twins represent approximately two-thirds of all monozygotic twins. MC can be determined by ultrasound with nearly 100% accuracy if performed within the first trimester. Additionally, MC can be assessed at the time of birth by careful examination of the fetal membranes.

Blood groups and DNA-fingerprinting: In the case of like-sexed twins and in the absence of MC, genetic markers must be utilized for the determination of zygosity diagnosis. These include the common blood groups ABO, Rh and MNS as they are most efficient in identification of differences between dizygotic twins. A difference in blood group is evidence of dizygosity. DNA-fingerprinting analyzes the genes themselves rather than the protein products. In this type of analysis, several unlinked genetic loci are tested at the same time. Monozygotic twins share the identical genetic pattern, whereas the pattern of the dizygotic twins are different.

Zygosity Questionnaires: If biologically based classification of zygosity is not feasible, questionnaire-based zygosity assessment is an option. Parents, or the twins themselves, respond to a questionnaire with items related to twin similarity in physical characteristics and the frequency of one twin being mistaken for another. The accuracy using this method

has been shown to be high, with around 95% of twins correctly classified.

RATIONALE

The goal of orthodontic treatment is correction of malocclusion by alteration of the phenotypic expression of the dentofacial structures. The greater the genetic influence on the development of the dentofacial structures, the less likely orthodontic treatment is to have an effect (Mossey 1999). It is therefore important to understand the role of both genetic and non-genetic, or environmental factors, in the development of occlusion in order to appropriately establish the goals and limitations of orthodontic treatment. The aim of this study is to gain a better understanding of these effects on the development of occlusion in adolescent subjects.

This study will focus on five specific aspects of occlusion which are of common concern to the orthodontist, and commonly altered with orthodontic treatment: overjet, overbite, buccal segment relationship, arch width and anterior dental irregularity.

Overall Aim:

To determine the extent that dental morphology is affected by genetic and environmental factors by examining occlusions and dental alignment of monozygotic twins, dizygotic twins and sibling pairs.

Hypothesis: There is greater similarity between the occlusions of monozygotic twins in comparison to dizygotic twins and sibling pairs.

Specific Aim 1:

To determine if there is greater similarity in amount of overjet between monozygotic twins or dizygotic twins and sibling pairs.

Hypothesis: The mean intrapair difference in overjet for monozygotic twins is significantly less than that of dizygotic twins and sibling pairs.

Specific Aim 2:

To determine if there is greater similarity in amount of overbite between monozygotic twins or dizygotic twins and sibling pairs.

Hypothesis: The mean intrapair difference in overbite between monozygotic twins is significantly less than between dizygotic twins and sibling pairs.

Specific Aim 3:

To determine if there is greater similarity in buccal segment relationship between monozygotic twins or dizygotic twins and sibling pairs.

Hypothesis: The mean intrapair difference in buccal segment relation between monozygotic twins is significantly less than between dizygotic twins and sibling pairs.

Specific Aim 4:

To determine if there is a greater similarity in arch width (intercanine and intermolar width) between monozygotic twins or dizygotic twins and sibling pairs.

Hypothesis: The mean intrapair difference in arch width of monozygotic twins is significantly less than that of dizygotic twins and sibling pairs.

Specific Aim 5:

To determine if there is greater similarity in dental irregularity as defined by displacement of contacts of anterior teeth between monozygotic twins or dizygotic twins and sibling pairs.

Hypothesis: There is significantly greater similarity in dental irregularity between monozygotic twins than between dizygotic twins and sibling pairs.

LITERATURE REVIEW

In craniometric and cephalometric studies of familial similarities, a good deal of evidence supports the contention that facial form is largely a product of a person's genotype. While the size and morphology of teeth as well as facial form is strongly influenced by hereditary factors (Horowitz and Hixon 1966), it does not necessarily follow that tooth-based malocclusion is also an inherited trait. Conflicting evidence supports both the view that malocclusion is largely an inherited trait and that the development of malocclusion is largely due to environment. The studies reviewed here conclude the various aspects of malocclusion are of multifactorial and polygenic inheritance and are therefore under a combination of genetic and environmental controls.

Many of the early studies on the heritability of malocclusion suggested a strong genetic link. Detlefsen (1928), from a study of 35 pairs of identical twins, concluded that arch shape and size were inherited characteristics. Bachrach and Young (1928) simply looked at the concordance and discordance in the *type* of occlusion and noted that there was a greater incidence of similarity between monozygotic versus dizygotic twins of the same sex. Lundstrom, in his 1949 study of 100 monozygotic and 102 dizygotic twins, found greater similarity between the occlusions of the monozygotic than the dizygotic twins with regard to malocclusion as well as number and type of teeth lost, suggesting a congenital link. Specifically, he found heredity played a significant role in determination of width and length of the dental arch, crowding and spacing of the teeth as well as degree of overbite. A potential problem with the early twin studies of malocclusion is the accuracy of determination of zygosity. Many of the studies fail to mention the method of

determination or state that they accepted the apparent similarity of the twins as the basis for classification of mono/dizygosity.

Stein (1956) conducted a study of 275 subjects and examined family pedigrees as well as lateral cephalograms for similarities and differences in both the presence and type of malocclusion. Her results supported the conclusion that heredity does play an important role in the determination of malocclusion. Similar types of occlusion were found to occur within families when family pedigrees were examined, and significantly positive correlations between same-siblings for the measurement of facial angles were found. In 1984, Lundstrom summarized the data from the heritability studies and concluded that approximately 40% of occlusal variation could be accounted for on the basis of heredity and that the remaining 60% of variation was due to environmental considerations.

Study of non-modernized populations reveals a relative absence of malocclusion among non-developed populations. Corrucini and Pacciani (1989) found good occlusions with minimal crowding among Etruscan skulls. This was supported by Begg (1977), in his study of Australian aboriginal skulls, which demonstrated a small prevalence of malocclusion. The increasing prevalence of malocclusion accompanying the process of modernization supports the importance of environmental influences. Corruccini (1984) compared the occlusions of various groups ranging from primates to aboriginal humans to modernized civilizations. He compared five aspects of occlusion: overjet, overbite, presence of crossbite, buccal segment relationship and tooth displacement. All aspects of occlusion studied showed significantly greater variation among modernized subjects with respect to the non-industrialized cultures. Tooth displacement scores (as measured by the Treatment Priority Index) specifically demonstrated significantly higher values, with only

0.2% of aboriginals scoring greater than a 10 on the treatment priority index (the definition of malocclusion) versus 16.5% of the industrialized population. Corruccini concluded that the relative absence of malocclusion in comparison to the industrialized population "throws the weight of suspicion toward environmental, not genetic, etiologic factors." He sites as specific environmental influences related to urbanization which may contribute to the relative increase as caries, respiratory infection, nutritional and growth differences, premature deciduous tooth loss and dietary consistency.

In Corruccini and Whitley's 1981 article, they hypothesized that environmental influences were responsible for the increase in occlusal variation among modernized communities, specifically that the reduction of masticatory stress resulting from modern urbanization and the resulting alteration of dietary habits contributed to the increase in occlusal variation. To test their hypothesis, they collected a cross-sectional sample of the occlusions of the population of a rural community in Kentucky. The found a significant rise in the predominance of occlusal variation within a single generation that correlated to the movement of industry and mechanized farming which had occurred in the area within the last 25 years. This significant increase in occlusal variability within a single generation in the absence of genetic influx supports the evidence that malocclusion is a "disease of civilization" and under predominantly environmental control.

More recent studies have concluded that the cause of occlusal variation is more complex than previously thought and is due neither to a purely genetic nor to a purely environmental influence. Shapiro (1969) examined the palatal dimensions of 102 twin pairs (63 monozygotic, 39 dizygotic). He found in his sample a significant genetic contribution to palatal height and palatal width, but no stronger correlation between

monozygotic twins than dizygotic twins with respect to palatal length. Heritability estimates were considerably lower than those of Lundstrom in 1948, which Shapiro attributed to the significant difference in age between the two samples. The subjects in Shapiro's study (>21 years) were significantly older than Lundstrom's (12-15 years).

Harris and Smith (1980), in their study of occlusal and arch size variables among families in a Melanesian population, concluded that the genetic contribution to occlusal variation was quite low. They found that only about 10 percent of the total variation in overjet, overbite, crowding, tooth rotations and molar rotation was due to genetic factors. The findings emphasized that variations in tooth position as assessed by crowding, rotations and occlusal relationships, were almost entirely due to non-genetic causes. They suggested that the results implied that occlusal similarities within families may be due more to the common environmental effect than to heredity. The investigators did find a significant genetic component to arch size and shape, with a heritability estimate of approximately 60 percent.

Everett and Matthews analyzed the arch form and dimension of the Oregon Child Growth Study sample in their 1978 thesis. They found mandibular arch form as well as mandibular arch width (as defined by intercanine and intermolar width) to be under significant genetic control. Conversely, they found that the maxillary arch was not under strong genetic control, supporting the clinical impression that the maxillary dental arch form and width can be modified with greater stability by orthodontic treatment than can the mandibular arch form and width.

Corruccini and Potter (1980) found the environmental determination of occlusion to be roughly twice as important as previously thought in their study of American twins.

The authors contended that identical twins tend to be raised in similar environments and that this could contribute to the extreme similarity in dental and facial development. They examined dental casts of 60 twin pairs (32 monozygotic, 28 dizygotic) and analyzed the occlusal variation by using the Treatment Priority Index (TPI). After applying corrections for the similarity of environments between the twin pairs, they concluded that there was a strong genetic component to the inheritance of arch size, tooth displacement and posterior crossbite, but did not find a significant heritability of overjet, buccal segment relationship, overbite or rotations.

Boraas and associates (1988) examined the occlusions of twins reared apart, analyzing the heritability of occlusal characteristics. They found significant similarity between arch width for monozygotic and dizygotic twins. They also demonstrated significant heritability of malalignment. They did not find significant intraclass correlation between twins for overbite and overjet, and accordingly the heritability estimates for these variables were small. The sample of twins reared apart allows for the optimal comparison of the occlusions of twins. However, further evidence is required to support these conclusions due to a small sample size.

Corruccini and colleagues (1990) compared 10 occlusal traits in 358 monozygotic and dizygotic twin pairs from four different ethnic samples and generated estimates of genetic variance and heritability for those features. The genetic variance was variable across the samples for overbite, overjet, sagittal molar relationship, posterior crossbite and the rotations and displacement of anterior teeth. Heritability estimates were generally low and varied greatly between the four populations, emphasizing the importance of

environmental influences on occlusal variation as well as the variability of apparent genetic determinants with respect to the environment or population in which they are measured.

Harris and Johnson (1991) looked specifically at tooth-based occlusal variables between siblings. They found that there were very low heritability estimates for the occlusal variables and that with growth and transition into the early permanent dentition, these heritability estimates decreased. They concluded that malocclusion, as defined as tooth malpositions, was an acquired condition, indicating the greater importance of environmental influences in the development of occlusion rather than genetic influences.

Studies of primary dentitions further support the finding of stronger heritability estimates at earlier developmental stages. Hughes and associates (2001) studied twins and singletons, or single born children, in the primary dentition and found heritability estimates ranging from 0.28-0.89, indicating moderate to relatively high genetic contribution to the observed variations in the primary dentition. They reported high correlations between twins for arch breadth. Monozygotic twins had moderate to low correlations for overbite and overjet, but dizygotic twins did not show a correlation significantly different from zero for these variables.

Cassidy et al, in a 1998 study of 320 dental casts of 155 siblings, found a strongly significant heritability estimate for buccal segment relationship (defined as maxillary to mandibular relationship at the first permanent molar) with about one-half of the total variation due to the genetic influence. They found similar correlations for arch width in accordance with several other studies. Conversely, they found tooth angulation (rotations and crowding) to have a low heritability estimate. For their sample, they concluded that

buccal segment relationship and arch width were under genetic influence, but that tooth angulation was predominately due to environmental factors.

Bishara (1996) conducted a longitudinal study of the changes in the dental arches and dentition that occur in mid-adulthood in an untreated, normal sample. He followed adults from the ages of 25-45 years. Findings indicated that over the span of the study, significant changes occur in the dental arches and dentition for both males and females. Specifically, he noted an increase in dental crowding with age, as well as a decrease in intercanine width and arch length. Harris (1997) found similar changes in a study of 60 adults, with measurements recorded at 20 and again at 55 years of age. In agreement with Bishara, Harris found no significant changes in overbite, overjet or buccal segment relationship among adults. As in Bishara's sample there was a significant decrease in arch length. In contrast to Bishara's findings, mandibular intercanine width was invariant among Harris' sample. Those occlusal variables which continue to change after the completion of maturations can be concluded to be affected by environmental influences to a greater extent than those occlusal variables which remain stable over time.

Generally, conflicting conclusions have been drawn from previous studies of the heritability of occlusion and the question remains as to the extent environment and genetics play in the development of occlusion. The majority of evidence suggests that individual positions of teeth as well as interarch relationships is under predominantly environmental control, while arch form and arch width are under greater genetic influences.

MATERIALS AND METHODS

Case Selection

The subjects for use in this sample were collected from the Child Growth Study at the School of Dentistry, Oregon Health and Science University (1950's-1970's).

Inclusion criteria for use in the analysis for this study included: evidence of zygosity for twin pairs, dizygotic twins and sibling pairs of like-sex, intact dental casts available for each member of the pair in the early permanent dentition with no orthodontic appliances. In order to control for age, each twin and sibling pair were matched for age.

Same-sex twin and sibling pairs were utilized for analysis in this study to reduce variability due to sex difference. Ten pairs of dizygotic twin subjects were excluded from analysis due to unlike sex.

Determination of Zygosity

Zygosity of the sample was determined based on blood group systems. Blood samples were collected from the subjects and from both of the parents. The serological study was carried out by the University of Oregon Medical School. Blood group systems tested were: ABO, MNS, Rh, P, Kell, Duffy and Kidd. The serum antibodies tested were: A, Ai, B; M, N, S; C, D, E; c, e, C^w; P; K, k, Kp^b; Fy^a; jk^a, jk^b. Discordance for any one of these antisera was regarded as sufficient evidence for dizygosity. In addition to blood groupings, the diagnosis of zygosity was supplemented by dermatoglyphics (or fingerprints), phenylthiocarbamide testing and concordance of physical characteristics such as sex, color of the eyes and hair, ear form and facial configuration. All subjects were of Caucasian origin (Arya et al 1973).

Records for 16 pairs of same-sex twins of unconfirmed zygosity were present in the Child Growth Study. Being of unknown zygosity, these twins were excluded from this study.

Subjects

Data from 96 subjects (48 twin and sibling pairs) was collected from the Child Growth Study at the Oregon Health and Science University. The mean age of the total sample was 13.6 years with a range of 12.0-16.3 years. Table 1 shows the number of twin and sibling pairs, including zygosity, sex, average age and range of ages for each group.

Methods

All data (inter- and intraarch) was collected as continuous variables. Measurements were taken directly on the dental casts for the following interarch parameters: overjet, overbite and buccal segment relationship. Digital calipers (Mitutoyo Corporation, Japan, model CD-6'P) calibrated to the nearest 0.01 mm were used to record the measurement in millimeter increments. All recorded measurements of interarch relationship were taken with the dental casts positioned in maximum intercuspation. Measurements were taken for both right and left sides and recorded as an average of the two to eliminate any bias due to possible mirror imagery between monozygotic twins.

Overjet (OJ)

Overjet was measured as the horizontal distance recorded in millimeters from the labial surface of the maxillary central incisors to the labial surface of the mandibular

central incisors (Figure 1). Positive overjet was recorded as a positive value, an end-to-end relationship as a zero and an underbite was recorded as a negative score. Overjet was calculated as the average of the scores from the right and left central incisors.

Overbite (OB)

Overbite was recorded from the study models of subjects as the millimeter measurement of the lower central incisor crown height that is overlapped by the upper central incisor (Figure 2). An open bite was recorded as a negative value and overbite recorded as a positive value. Overbite was recorded for both the right and left central incisors and an average score was calculated.

Buccal Segment Relationship (BSR)

Buccal segment relationship was scored according to the sagittal relationship of the maxillary first molar to the mandibular first molar (Figure 3). Interdigitation of the mesial-buccal cusp of the maxillary first molar with the buccal groove of the mandibular first molar (Class I) received a score of zero. The position of the mandibular first molar mesial to this relationship (Class III) received a negative score recorded in millimeters between the mesio-buccal cusp of the maxillary molar to the buccal groove of the mandibular molar. Mandibular first molars positioned distal to this relationship (Class II) received a positive score. Values were recorded for both right and left first molar relationships. An average of the right and left scores was calculated.

Intraarch measurements were recorded from digitized images of the dental casts. Maxillary and mandibular dental casts were scanned perpendicular to the occlusal plane

(Epson Expression 1680, Seiko Epson Corporation, Nagano, Japan). A millimeter ruler was scanned at the base of each image to allow for calibration of the image (Figure 4). Images were scanned at a resolution of 600 dpi. Dolphin Imaging Software (Version 10.0, Build 52 Premium) was utilized to calibrate and digitize the study models for analysis. Each image was magnified to five times the original size to aid in the identification of landmarks.

Arch Width (Intercanine width (Mx/Md 3-3) and intermolar width (Mx/Md 6-6))

Width of the arch was measured for both upper and lower casts as intermolar and intercanine distance. Intercanine width was measured from cusp tip to cusp tip of the permanent canines. Intermolar width was measured from central fossa to central fossa of the permanent first molars. Intermolar width was measured from central fossa rather than from cusp tip to eliminate discrepancies due to molar rotation (Figure 5).

Dental Irregularity (Mx/Md DI)

Dental irregularity was measured as the millimeter displacement between anatomic contacts of the maxillary and mandibular incisors in a method adapted from the Irregularity Index (Little 1975). The millimeter displacement was measured from a digitized image of the models utilizing Dolphin Imaging Software model analysis (Figures 6 & 7).

Statistical Analysis

SPSS (Version 13.0 for Windows) statistical software was utilized for analysis of the data. The mean of the intra-pair differences between monozygotic and dizygotic twins

and sibling pairs were tested for statistical significance by the use of the unpaired t-test for means. An alpha value of less than 0.05 was considered statistically significant. The distribution of the absolute value of the differences approached the normal distribution for all variables studied. Clinical significance was defined as a critical difference of 1 mm or greater.

Buccal segment relationship was transformed into categorical data for analysis for concordance for Class I, Class II and Class III within twin or sibling pairs. For buccal segment relationship, a measurement of greater than 1mm (Class II) was categorized as 1, between -1mm and 1mm (Class I) was categorized as a 0 and a score of less than -1mm (Class III) was categorized as a -1. Concordance and discordance of twin and sibling pairs was computed with the use of the Wilcoxon-signed rank test.

Correlation coefficients were calculated between twin and sibling pairs for all variables studied. Under strictly polygenic mode of inheritance, we would expect a maximum correlation coefficient value of 1.0 for the monozygotic twin pairs and of 0.5 for dizygotic twin and sibling pairs. A correlation coefficient significantly less than the theoretical maximum would indicate possible measurement errors and/or significant modifications of the occlusal traits by environmental factors. Correlations were tested to be significantly different from zero. Weak, moderate, strong and very strong correlations where arbitrarily defined as follows: weak 0.1-0.3, moderate 0.3-0.6, strong 0.6-0.9, and very strong 0.9-1.

The path analysis model heritability estimate was determined for each variable (Lundstrom 1984). Heritability estimates (h^2) were calculated from the correlation coefficients utilizing the formula

$$h^2 = 2(R_{MZ}-R_{DZ/Sib})$$

where R_{MZ} is the correlation coefficient for monozygotic twin pairs and $R_{DZ/Sib}$ is the correlation coefficient for dizygotic twins or sibling pairs (h^2 is defined as two times the intraclass correlation; Falconer 1960). Each of these estimates can theoretically range from 0-1 (or 0-100%), reflecting the proportion of the observed phenotypic variation resulting from genetic factors. Negative values or values greater than one are mathematically possible which raises interpretive problems (Corruccini 1990).

Error of the Method

Three pairs of twins/siblings (six sets of casts), one pair from each group studied, were randomly selected for determination of the error of the method. All measurements were duplicated on the six sets of casts. Interarch measurements recorded with the digital calipers directly from the dental casts were repeated. Dental casts were rescanned for the intraarch measurements and the measurements were repeated on the digitized image. The error of the method was determined by the Dahlberg statistic (Dahlberg 1948) using the formula

$$s = \sqrt{(\sum d^2/2n)}$$

where d is the difference between the first and second measurements and n is the number of determinations (in this case, six). The precision for all variables was small and less than 1mm for all variables with a range of 0.09-0.69 (Table 2).

RESULTS

Descriptive statistics were utilized to summarize the data and are presented in Table 3. The mean intrapair differences in monozygotic and dizygotic twins and sibling pairs for each variable are shown in Table 4.

Overjet

The mean intrapair difference for overjet for monozygotic twins (1.12 mm) was less than that of dizygotic twins and sibling pairs (1.82 mm) although the difference did not approach statistical significance (diff = 0.70 mm, $p = 0.228$).

The intrapair correlation coefficient for overjet between monozygotic twins was 0.62 ($p = 0.001$) – demonstrating a strong correlation between monozygotic twin pairs. The dizygotic twin and sibling pair correlation was weak ($R = 0.02$), indicating that overjet was not well correlated within dizygotic and sibling pairs.

Overbite

The mean intrapair difference for overbite for monozygotic twins (0.68 mm) was less than that of dizygotic twins and sibling pairs (1.39 mm) and was statistically significant at the $p<0.05$ level ($p = 0.014$). The difference between the two groups however, approached, but did not meet clinical significance at a value of 0.71 mm.

Overbite was strongly correlated between monozygotic twins ($R = 0.80$) and was statistically different from zero ($p = 0.000$). Overbite was weakly correlated within dizygotic twins and sibling pairs ($R = 0.25$).

Buccal Segment Relationship

The mean intrapair difference for buccal segment relationship for monozygotic twins (1.39 mm) was less than that of dizygotic twins and sibling pairs (1.58 mm) but did not approach statistical or clinical significance (diff = 0.23, p = 0.615).

Categorization of the buccal segment relationship revealed that approximately one-half of both monozygotic twins and dizygotic twins and sibling pairs were concordant for buccal segment relationship as either Class I, Class II or Class III, with the remaining one-half discordant for buccal segment relationship.

Buccal segment relationship was weakly correlated both for monozygotic twins (R = 0.292) and for dizygotic twins and sibling pairs (R = 0.193), neither correlation was significantly different from zero.

Arch Width

Intermolar width

Maxillary

The mean intrapair difference in intermolar width for monozygotic twins (1.32 mm) was less than that of dizygotic twins and sibling pairs (1.81 mm) but the difference (0.48 mm) did not reach clinical or statistical significance (p = 0.173).

There was a strong intraclass correlation for maxillary intermolar width for both monozygotic twins (R = 0.83) and dizygotic twins and sibling pairs (R = 0.68). Both correlations were significantly different from zero (p = 0.000).

Mandibular

The mean intrapair difference in intermolar width for monozygotic twins (1.22 mm) was statistically significantly less than that of dizygotic twins and sibling pairs (2.16 mm) ($p = 0.005$). The absolute difference between the two groups was 0.933 mm, which approached, but did not reach clinical significance.

Intraclass correlation between monozygotic twins for mandibular intermolar width was strong ($R = 0.89$, $p = 0.000$) in contrast to the mandibular intermolar width correlation for dizygotic twins and sibling pairs, which was moderate ($R = 0.42$ $p = 0.042$).

Intercanine width

Maxillary

The mean intrapair difference in intercanine width for monozygotic twins (1.19 mm) was less than that of dizygotic twins and sibling pairs (1.52 mm). However, the difference between the groups (0.33 mm) did not reach clinical or statistical significance ($p = 0.256$).

Maxillary intercanine width correlation was strong for both monozygotic twins ($R = 0.71$, $p = 0.000$) as well as dizygotic twins and sibling pairs ($R = 0.80$, $p = 0.000$).

Mandibular

The mean intrapair difference in intercanine width for monozygotic twins (0.95 mm) was less than that of dizygotic twins and sibling pairs (1.55 mm). The difference between the groups approached statistical significance ($p = 0.076$) but did not obtain clinical significance (0.604 mm).

Mandibular intercanine width correlation was strong for both monozygotic twins ($R = 0.67$, $p = 0.000$) and for dizygotic twins and sibling pairs ($R = 0.62$, $p = 0.001$).

Dental Irregularity

Maxillary

The mean intrapair difference in maxillary dental irregularity for monozygotic twins (1.72 mm) was less than that of dizygotic twins and sibling pairs (2.33 mm) but the difference did not meet statistical or clinical significance (diff = 0.60, p = 0.143).

There was a strong intraclass correlation for maxillary dental irregularity for both monozygotic twins (R = 0.636, p = 0.001)) as well as dizygotic twins and sibling pairs (R = 0.601, p = 0.002).

Mandibular

The mean intrapair difference in mandibular dental irregularity for monozygotic twins (1.30mm) was significantly less than that of dizygotic twins and sibling pairs both statistically and clinically (2.43 mm), (diff = 1.13 mm, p = 0.016).

Mandibular dental irregularity had a moderate intraclass correlation for monozygotic twins (R = 0.428, p = 0.037) and a weak correlation which was not statistically significant between dizygotic twins and sibling pairs (R = 0.152).

DISCUSSION

The findings of the twin analysis in this study provides evidence to support the view that occlusal variation results from a combination of genetic and environmental factors – that the inheritance of malocclusion is polygenic. Specifically, this investigation examined five parameters of occlusal variation, including overjet, overbite, buccal segment relationship, arch width (as defined by intermolar and intercanine width) and anterior dental irregularity. The mean intrapair difference between monozygotic twins and dizygotic twins and sibling pairs was analyzed and showed that the mean intrapair difference for all parameters was smaller for monozygotic twins than for dizygotic twins and sibling pairs. Significant differences in the mean intrapair difference were found for overbite, mandibular intermolar width and mandibular anterior dental irregularity. The mean intrapair difference in mandibular intercanine width approached statistical significance. The only parameter to obtain clinical significance was mandibular anterior discrepancy index, with the mean intrapair difference between dizygotic twins and sibling pairs 1.13 mm greater than that of monozygotic twins. These results suggest that there exists a genetic component to determination of overbite, mandibular dental arch width and anterior dental irregularity in adolescent patients. A less significant genetic contribution is suspected for the determination of overjet, buccal segment relationship, maxillary arch width and maxillary anterior dental irregularity.

Co-pair correlation coefficients as well as calculated heritability estimates are shown in Table 5. Co-pair correlation coefficients were greater between monozygotic twins than for dizygotic twins for all parameters with the exception of maxillary intercanine width (Figure 9). Co-twin correlations for overbite and overjet were strong

between monozygotic twin pairs. Conversely, co-pair correlation coefficients for dizygotic twins and sibling pairs were weak for these parameters. Heritability estimates calculated from the correlation coefficients were high for both parameters, suggesting that overbite and overjet are predominantly under genetic control. This is in conflict with findings from previous studies, which have found overbite and overjet to be under, at most, moderate genetic control, and to be predominately determined by environmental influences (Corruccini and Potter 1980, Corruccini et al. 1990, Harris and Johnson 1991, Cassidy et al. 1998). Low to moderate heritability estimates for overbite and overjet has led investigators to conclude that incisor relationship is largely influenced by local pressures exerted by the lips and tongue as well as differential growth of the upper and lower jaws. Heritability estimates for overbite and overjet in the present study were high (greater than one) which may be due to the small sample size (Booras 1999).

Buccal segment relationship was only weakly correlated for both monozygotic and dizygotic twins and sibling pairs. Heritability estimates calculated were small and within the range of heritability estimates calculated for buccal segment relationship in previous studies of adolescents (Corruccini and Potter 1980, Corruccini et al. 1990, Harris and Johnson 1991, Cassidy et al. 1998). In agreement with previous studies (Corruccini and Potter 1980, Corruccini et al. 1990, Harris and Johnson 1991, Cassidy et al. 1998), buccal segment relationship was found to be neither significantly heritable nor highly correlated between twin and sibling pairs in this sample.

Arch width and form have been found to be significantly heritable (Shapiro 1969, Corruccini and Potter 1980, Harris and Smith 1980, Harris and Johnson 1991, Cassidy et al 1998). In this study, intermolar width, but not intercanine width was found to be

moderately to strongly heritable. The correlation for intercanine width was found to be only negligibly higher between monozygotic twins than between dizygotic twins and sibling pairs. The high heritability estimate for mandibular intermolar width supports the finding of a significantly smaller mean intrapair difference between monozygotic twins than between dizygotic twins and sibling pairs.

Mandibular dental irregularity was found to be moderately correlated between monozygotic twins, but only weakly correlated between dizygotic twins and sibling pairs. This finding is supported by the significantly smaller mean intrapair difference in mandibular dental irregularity between monozygotic twins than between dizygotic twins and sibling pairs. Few studies have examined the heritability or genetic influence in terms of dental irregularity. The majority of studies have examined anterior rotations and displacement as an index of genetic determination of displacement of individual teeth. While the measure of dental irregularity provides a continuous variable for comparison, neither method may be sensitive enough to detect the true level of similarity or difference between the two groups.

Twin and sibling correlations tend to overestimate the additive genetic component because they incorporate any dominance effect and all acquired similarities that result from a common environment, this has been called the "co-habitational effect" (Harris and Johnson 1991). Parameters for which both monozygotic twins and dizygotic twins and sibling pairs demonstrated a strong correlation may suggest a shared environmental influence between pairs for these traits. In this study, those parameters which were strongly correlated for both groups included: mandibular intercanine width, maxillary intermolar and intercanine width and maxillary anterior dental irregularity. These results

support the general observation that occlusal relationships between siblings and twins are similar, although not necessarily for genetic reasons (Harris and Smith 1980).

For all parameters studied, greater heritability estimates were found for the mandible in comparison to maxilla. The higher heritability estimates in the mandibular arch supports the commonly held belief among orthodontist that the mandibular arch is affected to a greater extent by heredity than the maxillary arch. Accordingly, if the maxillary arch is under greater environmental control than the mandibular arch, treatment modification of maxillary arch would have greater stability. In this study, heritability estimates were greater for the mandibular arch than for the maxillary arch for the parameters of arch width and anterior dental irregularity. Additionally, the difference in mean intrapair differences between monozygotic twins and dizygotic twins and sibling pairs was statistically significant for mandibular intermolar width as well as mandibular dental irregularity index. There was a trend for the mean intrapair difference for mandibular intercanine width to be more similar between monozygotic twins, but the difference between the groups did not reach statistical significance. These findings suggest greater genetic influence on the dimensions of the mandibular arch than the maxillary arch are supported by several previous studies (Everett and Matthews 1978, Harris and Smith 1980, Corruccini and Potter 1980, Harris and Johnson 1991).

Comparison to previous studies of the heritability of certain traits should be performed cautiously. Shapiro (1969) and Corruccini (1990) have demonstrated that heritability estimates vary between populations, implying that inferences from twin studies should be specific to the sample studied. Additionally, studies have shown both correlations and heritability estimates tend to decrease with age (Harris and Johnson 1991).

Therefore, correlations between adolescent twin and sibling pairs would be expected to be higher than those found between adults (Bishara et al 1996, Hughes et al 2001, Harris and Johnson 1991).

One limitation of this study was the small sample size, especially for the dizygotic twins. Due to the small sample size, differences between the two groups may have been undetected. There was a trend that the intrapair differences of the dizygotic twins and sibling pairs were larger than the intrapair differences of the monozygotic twins for all parameters studied. The trend suggests that this may be more than a random observation, and that for the majority of the parameters studied, the statistical analysis did not have significant power to detect the differences between the groups. Heritability estimates greater than zero, or less than one may reflect errors due to the small sample size (Borraas 1988).

The sample utilized for this study was not purely a random sample. There was an absence of subjects with severe malocclusion in the Oregon Child Growth Study. Subjects were screened as children and were either referred to the graduate orthodontic department at the University of Oregon for early orthodontic treatment, or were referred to the Child Growth Study. Most significantly, this was evident in the absence of subjects with an anterior open bite and few subjects with full-cusp Class II or Class III molar relationships. In the presence of a greater discrepancy, it is suspected that a stronger correlation may have been found for buccal segment relationship.

The difference in mean intrapair differences between the groups were small for all parameters, ranging from 0.2-1.3 mm. It may be asserted that the small differences found

are not of clinical significance. Thus, even if a true difference between the groups does exist, the clinical significance of this finding may be minimal.

Ideally, comparison and calculation of heritability estimates would be performed between monozygotic and dizygotic twins. Same-sex full sibling pairs were utilized in this study due to limited accessibility of same-sex dizygotic twins of confirmed zygosity that were available for comparison. Genetically, dizygotic twins are no more similar or dissimilar than siblings, however, siblings do not share the same intrauterine environment as do dizygotic twins. Additionally, since siblings were controlled for age at the time of data collection, any changes within the household environment (such as a change in dietary habits) between the years that the dental casts were collected for the two siblings could result in a greater difference between the occlusions of the two siblings.

The role of soft tissue and its influences on the dentoalveolar development is not clear. Van der Linden (1966) hypothesized that soft tissue morphology and behavior have a significant genetic component and it is the soft tissues which have a significant influence on the dentoalveolar morphology. He described the interaction between external and internal functional matrices. The external functional matrix is thought to be strongly genetically determined while the internal matrix, determined mainly by tongue posture and behavior, is thought to be influenced by the environment as well as genetic factors. In our study, heritability estimates were found to be higher for all parameters in the mandibular arch than in the maxillary arch. The mandibular dentoalveolar arch width may not be directly determined by genetics, rather the arch width may be determined by the interaction between internal and external soft tissue forces. Lingual pressure from the tongue is balanced by the lingually directed pressure from the cheeks and extraoral musculature.

Some have hypothesized that it is these factors which are genetically determined and thereby influence the dentoalveolar structures (van der Linden 1966, Moss 1981). According to the functional matrix hypothesis (Moss 1981) the origin, growth and maintenance of all skeletal tissues and organs are secondary responses that occur due to the interaction with the related non-skeletal tissues. Under this theory, the morphology which is thought to be genetically determined is the surrounding soft tissues. It is those soft tissue influences which are hypothesized to determine the parameters which were found to be significantly heritable in our sample. For example, overjet was found to be significantly heritable in this sample. Excess overjet, in the case of a Class II, division 1 malocclusion may be the result of a short upper lip and a lower lip level that would favor proclination of the upper incisors rather than strictly the result of tooth bud positions of the maxillary and mandibular teeth.

Further research should include the comparison of correlations for dental parameters and correlations for facial parameters between twins, especially those soft tissue parameters which may influence the dentoalveolar morphology. Many monozygotic twins have a very similar facial appearance, as well as having similar occlusions, while some show a marked divergence in facial appearance (Townsend et al. 1998). The same is true for some dizygotic twins. A finding of high correlations between those twins with similar physical appearance and dental occlusions would indicate that the similarity in physical and dental occlusion is due to a greater extent to environmental influences than to heredity. Additionally, a decrease in heritability with maturation has been demonstrated in several studies (Harris and Johnson 1991). Records are available for our sample of adolescents during the mixed dentition as well as during adulthood. Further research could

include re-calculation of heritability estimates in the mixed dentition and adulthood with comparison to those found during adolescence.

Assessment of the relative importance of genetics in the determination of form, size, alignment and occlusion of the dental arches is important to the success of orthodontic treatment. A greater genetic component to the determination of a trait implies a worse prognosis for a successful outcome by orthodontic intervention (Mossey 1999). If, however, the heritability of arch size and form and tooth position is low, then the environment plays a more important role, and orthodontic intervention as an environmental factor, will likely have a more successful outcome. In the case of trivial heritabilities, then treatment goals should include identification and interception of environmental factors with negative influences on the development of occlusion or the craniofacial complex. Clinically, it is important to remember that each malocclusion is distinct in its etiology and therefore the diagnostic goal is to determine the relative contribution of genetics and environmental factors in each case. The problem is that it is often difficult or impossible to determine the precise contribution.

SUMMARY AND CONCLUSION

This cross-sectional, retrospective study attempted to ascertain the extent to which genetic factors affect the occlusal variation present in adolescents by analysis of the intrapair differences between pairs of monozygotic twins compared to dizygotic twins and sibling pairs. Co-pair correlations coefficients and heritability estimates were calculated for each parameter studied. Twenty-four pairs of monozygotic twins, six pairs of same-sex dizygotic twins and eighteen pairs of same-sex siblings were included in this study. Records analyzed included dental study models in the early permanent dentition. Specific occlusal parameters studied included overjet, overbite, buccal segment relationship, arch width and anterior dental irregularity. The results confirmed that the inheritance of malocclusion is multifactorial. Occlusal variables were generally well correlated between monozygotic twin pairs with the exception of buccal segment relationship. Arch width and maxillary anterior dental irregularity were similarly well correlated between dizygotic twins and sibling pairs. Based on this study, a significant genetic component is suspected to the determination of overbite, mandibular intermolar width and mandibular anterior dental irregularity. The results suggest that the maxillary arch is affected to a greater extent by environmental influences than the mandibular arch.

LITERATURE CITED

1. Arya BS, Savara BS, Clarkson QD, Thomas DR. Genetic variability of craniofacial dimensions. 1973; 43(2): 207-215.

2. Bachrach H, Young M. A Comparison of the degree of resemblance in dental characters shown in pairs of twins of identical and fraternal types. Dental Cosmos. 1928; 70: 465-466.

3. Begg PR, Kesling PC. Malocclusion in Australian aboriginals and civilized man. In: Begg PR, Kesling PC, eds. Begg Orthodontic Theory and Technique. Philadelphia: WB Saunders, 1977.

4. Bishara SE, Treder J, Damon P, Olsen M. Changes in the dental arches and dentition between 25 and 45 years of age. The Angle Orthodontist. 1996; 66(6): 417-422.

5. Boraas JC, Messer LB, Till MJ. A genetic contribution to dental caries, occlusion and morphology as demonstrated by twins reared apart. Journal of Dental Research. 1988; 67(9):115-1155.

6. Cassidy KM, Harris EF, Tolley EA, Keim RG. Genetic influence on dental arch form in orthodontic patients. Angle Orthodontist. 1998; 68(5): 445-454.

7. Corruccini RS, Potter RH. Genetic analysis of occlusal variation in twins. American Journal of Orthodontics. 1980; 78(2): 140-154.

8. Corruccini RS, Whitley LD. Occlusal variation in a rural Kentucky community. American Journal of Orthodontics. 1981; 79(3): 250-262.

9. Corruccini RS. An epidemiologic transition in dental occlusion in world populations. American Journal of Orthodontics. 1984: 86(5): 419-426.

10. Corruccini RS, Pacciani E. "Orthodontistry" and dental occlusion in Etruscans. Angle Orthodontist. 1989; 59(1): 61-64.

11. Corruccini RS. Townsend GC, Richards LC, Brown T. Genetic and environmental determinants of dental occlusal variation in twins of different nationalities. Human Biology. 1990; 62(3): 353-367.

12. Dahlberg G. Environmental inheritance and random variations with special reference to investigations on twins. Acta Genetica et Statistica medica. 1948; 1: 104-114.

13. Detlefsen JA. Intrinsic of hereditary factors versus extrinsic or environmental factors in the determination of tooth and oral peculiarities. Journal of Dental Research. 1928; 8: 419-420.

14. Everett HL, Matthews C. An assessment of genetic control of form and dimension of human dental arches. Thesis, July 1978.

15. Falconer DS. Introduction to Quantitative Genetics. NY 1960 Ronald Press.

16. Harris EF, Smith RJ. A study of occlusion and arch widths in families. American Journal of Orthodontics. 1980; 78(2): 155-163.

17. Harris EF, Johnson MG. Heretability of craniometric and occlusal variables: A longitudinal sib analysis. American Journal of Orthodontics and Dentofacial Orthopedics. 1991; 99(3): 258-268.

18. Harris EF. A longitudinal study of arch size and form in untreated adults. American Journal of Orthodontics and Dentofacial Orthopedics. 1997; 111: 419-427.

19. Horowitz SL, Hixon EH. The Nature of Orthodontic Diagnosis. Saint Louis 1966 The C.V. Mosby Company.

20. Hughes T, Thomas C, Richards L, Townsend G. A study of occlusal variation in the primary dentition of Australian twins and singletons. Archives of Oral Biology. 2001; 46: 857-864.

21. Kelley, Harvey. An assessment of the occlusions of youths 12-17 years, US Public Health Service, Vital and Health Statistics, Series 11, No. 162, 1977.

22. Kempthorne O, Osborne RH. The interpretation of twin data. American Journal of Human Genetics. 1961; 13: 320-339.

23. Lauweryns I, Carels C, Vlietinck R. The use of twins in dentofacial genetic research. American Journal of Orthodontics and Dentofacial Orthopedics. 1993; 103(1): 33-38.

24. Little RM. The irregularity index. American Journal of Orthodontics. 1975; 68(5): 554-563.

25. Lundstrom A. Tooth size and occlusion in twins. American Journal of Orthodontics. 1949; 35: 878-879.

26. Lundstrom A. Nature vs. nurture in dentofacial variation. European Journal of Orthodontics. 1984; 6: 77-91.

27. Manfredi C, Martina R, Battista Grossi G, Giuliani M. Heritability of 39 orthodontic cephalometric parameters on MZ, DZ twins and MN-paired singletons. American Journal of Orthodontics and Dentofacial Orthopedics. 1997; 111(1): 44-51.

28. Markovic MD. At the crossroads of oral facial genetics. European Journal of Orthodontics. 1992; 14: 469-481.

29. Moss ML. Genetics, epigenetics, and causation. American Journal of Orthodontics. 1981; 88(4): 366-375.

30. Mossey PA. The heritability of malocclusion: Part I – Genetics, principles and terminology. British Journal of Orthodontics. 1999; 26: 103-113.

31. Mossey PA. The heritability of malocclusion: Part II – The influence of genetics in malocclusion. British Journal of Orthodontics. 1999; 26: 195-203

32. Ohm Kyvik K, Derom C. Data collection on multiple births – establishing twin registers and determining zygosity. Early Human Development. 2006; 82: 357-363.

33. Proffit WR. On aetiology of malocclusion. British Journal of Orthodontics. 1986: 13:1-11.

34. Shapiro BL. A twin study of palatal dimensions partitioning genetic and environmental contributions to variability. Angle Orthodontist. 1969; 39: 139-151.

35. Smith RJ, Bailit HL. Problems and methods in research on the genetics of dental occlusion. 1977; 47(1): 65-77.

36. Stein KF, Kelley TJ, Wood E. Influence of heredity in the etiology of malocclusion. American Journal of Orthodontics. 1956; 42: 125-141.

37. Townsend GC, Aldred MJ, Bartold PM. Genetic aspects of dental disorders. Australian Dental Journal. 1998; 43(4): 000-000.

38. van der Linden F. Genetic and environmental factors in dentofacial morphology. American Journal of Orthodontics. 1966; 52(8): 576-583.