



## **Evaluation of Dento-craniofacial Characteristics in Monozygotic Twins - Case Study**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. Authors TFS and SGMF designed the study. Authors GRG, NAS and FRG collected the data. Authors TFS and RPO analyzed the data. Authors TFS, GRG and BCA drafted the article. All authors read and approved the final manuscript.*

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## **ABSTRACT**

**Introduction:** Monozygotic twins are a valuable sample for the study of human craniofacial growth and development.

**Aims:** The aim of the present study was to analyze the dento-craniofacial characteristics in monozygotic twins and to discuss the genetic, epigenetic and environmental influences in the twin's phenotypic features.

**Material and Methods:** Two 21-year-old female twins attended the Dental Service of Universidade Federal dos Vales do Jequitinhonha e Mucuri were analyzed. For validation of this study, a genetic test of zygosity was performed to confirm the individuals' monozygosity. Intraoral buccal examination revealed occlusal differences as well as disagreement in the eruption of the third molars.

**Results:** The analysis of models showed differences in the mesio-distal diameter of the teeth of both arches, as well as disagreement in the perimeter of the mandibular arches between the two

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individuals. The cephalometric analysis showed remarkable differences in the vertical and horizontal parameters of the craniofacial structure. The SNA, SNB and LAFH showed differences of 10°, 9° and 3mm respectively. Additionally, the components related to the dentition also showed discrepant results.

**Conclusion:** It can be suggested that the dento-craniofacial characteristics present a genetic component; however, the interaction of epigenetic and environmental factors can lead to different phenotypes in univiteline twins.

*Keywords: Monozygotic twins; teeth; development; craniofacial; orthodontic.*

## 1. INTRODUCTION

The craniofacial development is ruled by interactions between genetic and environmental factors that act in the determination of morphological and functional characteristics [1]. In this way, the malocclusion etiology is based on genetic and epigenetic aspects and environmental interactions [2], [3]. Non-nutritive sucking habits, lingual interposition, tooth loss, extensive caries, dento-facial injuries, mouth breathing, among others, are examples of conditions that can alter the dento-craniofacial development and change the genetic stimulation pattern [2], [4].

Studies suggest that craniofacial growth and development depend on both genetic and environmental influences and are indirectly linked to the functional requirements of involved surrounding tissues [5], [6].

Research addressing dento-craniofacial structures in monozygotic twins may be an important way to analyze and evaluate the impact of genetic and environmental factors on the individuals' phenotypic development [7], [8], [1]. Monozygotic twins originate from the fertilization of a single egg, which in the early embryogenesis stages, splits into two identical embryos [9], [10]. The resulting embryos have identical genotypes and are prone to manifest the same phenotypic characteristics. However, epigenetic influences and environmental interactions can cause changes in the individuals' phenotypic pattern [11], [12].

In this context, the studies of twins may help understanding the development of dento-craniofacial characteristics and determining the inheritance of malocclusions. The aim of this study was to analyze the dento-craniofacial characteristics in monozygotic twins and to discuss the genetic, epigenetic and environmental influences in the twin's phenotypic features.

## 2. MATERIALS AND METHODS

Two 21-year-old monozygotic female twins attended to the dental clinic of the Universidade Federal dos Vales do Jequitinhonha e Mucuri – UFVJM. The patients presented for de dentistry service for routine orthodontic consult. Previously to this study and in order to confirm the monozygotic nature of the twins, a zygosity genetic determination test was performed in both individuals. The analysis comprised genotyping 15 microsatellite DNA markers. The DNA samples were obtained from the patients' blood. The genetic analysis was done by a private laboratory. The test result confirmed that the twins were monozygotic and had the same genetic load. All data of twins were acquired from medical and dental records.

### 2.1 Dental Plaster Models and Cephalometric Analysis

The plaster models were obtained from the previous molding of the dental arches made with alginate (Algi-Gel®) and posteriorly casted with type 4 plaster (Asfer®). Both products were prepared according to the manufacturers' instructions. The teeth were measured with a dry point compass and assessed with a flexible ruler calibrated in millimeters. The plaster models were analyzed in triplicates by a single examiner, specialist in Orthodontics [13].

Lateral cephalometric radiographs (telerradiographs) of the twins were used to perform the cephalometric analyzes. All radiographs were acquired using the Rx Eagle Dabi Atlante® device by a single technician specialized in dental radiology. The cephalometric analyzes were performed in triplicates by a single examiner, specialist in Orthodontics, as it was done in previously published studies [14], [15], [16].

### 3. RESULTS

#### 3.1 Individuals' Characterization

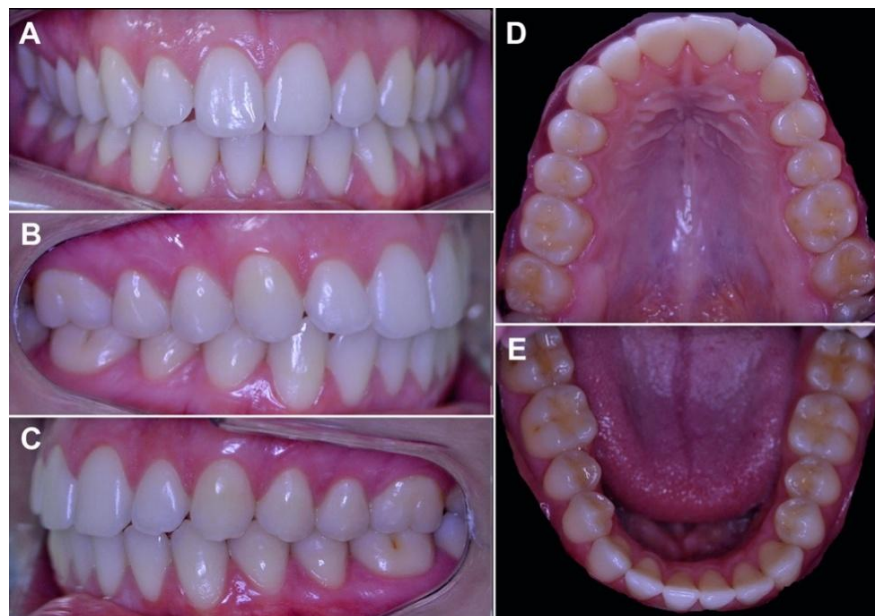
The individual 01 was the first to be born at 39 weeks of gestation, healthy, weighing 3.050 kg and measuring 49 cm in length, in the city of Diamantina, Brazil. In the first months of life she was fed with artificial feeding. The previous dental history revealed that the individual was a mouth breather and had the digital sucking habit until approximately five years old. The etiology of mouth breathe was considered unclear because the etiology information was not recovery. In addition, at the age of 11, the individual received orthodontic treatment to treat open bite. The orthodontic treatment was made with fixed appliance, for two years, and use of Hawley retainer on superior arch and fixed retainer on inferior incisors and canines. When adult, the individual was diagnosed with mitral valve prolapse.

The individual 02 was born last, healthy, weighing 2.170 kg and measuring 45 cm in length. Like her sister, in the first months of life, she was fed artificially. The previous dental history revealed nasal breathing, digital sucking habit until 5 years old and no orthodontic

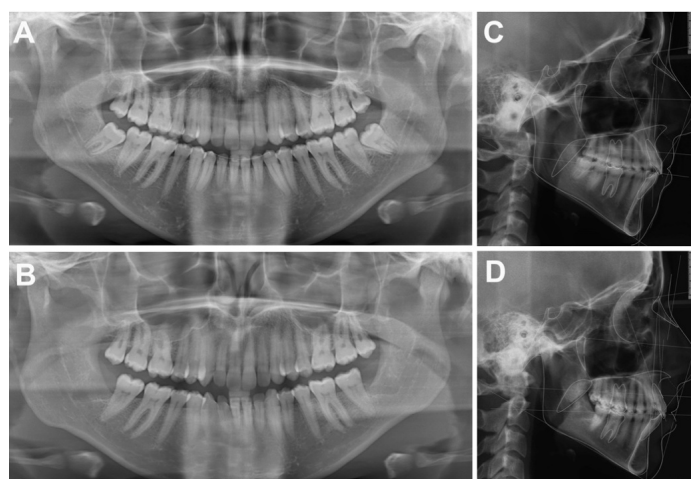
intervention. When adult, no systemic alterations were found.

#### 3.2 Clinical and Radiographic Characterization

The individual 1 clinical examination showed: presence of *linea alba* and Fordyce granules, unsatisfactory composite resin restorations in the occlusal and buccal surface of the right and left inferior first and second molars, and sealants in the grooves of the remaining posterior teeth. Superior third molars were erupted and the inferior third molars, partially erupted. Occlusal analysis showed the presence of Class I molar and canine relationships and slight misalignment/ unevenness of some teeth (Fig. 1). Panoramic radiograph showed the presence of radiolucent area on the occlusal surface of the left lower second molar and right lower first and second molars. The presence of a metallic wire, compatible with an orthodontic retainer, was also noticed in the lower anterior teeth. The superior third molars showed complete rhizogenesis and were in vertical position [17]. The lower third molars presented in Nolla's stage 8 of rhizogenesis [18], in mesioangular position and class IB [17], [19] (Fig. 2).



**Fig. 1. Individual 1 occlusal aspect: (A) frontal occlusal view; (B) right lateral occlusal view; (C) left lateral occlusal view; (D) superior dental arch occlusal view; (E) lower dental arch occlusal view**



**Fig. 2. Radiographic aspects of individuals 1 and 2: (A) Individual 1 panoramic radiograph; (B) Individual 2 panoramic radiograph; (C) cephalometric tracing on lateral aspect of individual 1; (D) cephalometric tracing on lateral aspect of individual 2**

The individual 2 clinical examination showed: presence of *linea alba* and Fordyce granules, 0.5mm gingival retraction on the right superior central incisor, satisfactory composite resin restoration on the left lower second molar occlusal surface, unsatisfactory composite resin restorations on the occlusal and buccal surfaces of the left lower first molar and right lower first and second molars, and sealants in the grooves of the remaining posterior teeth. The maxillary third molars were erupted and the mandibular third molars were impacted. Occlusal analysis showed Class II malocclusion, division 1, with slight misalignment/unevenness of some teeth and diastemas (Fig. 3). Panoramic radiograph revealed the presence of radiolucent area on the occlusal surface of the left lower first molar and right lower first and second molars. The superior third molars showed complete rhizogenesis and were in the vertical position [17]; the left lower third molar was in the Nolla stage 7 of rhizogenesis and the right one in the Nolla's stage 8 [18], both in the mesioangular position and class IB [17], [19] (Fig. 2).

### 3.3 Dental Plaster Models and Cephalometric Analysis

Table 1 and Table 2 presented data of analysis of mesio-distal width of teeth and the arch length discrepancy model analysis of individuals 1 and 2. The analysis of models showed differences in the mesio-distal diameter of the teeth of both arches, as well as disagreement in the perimeter

of the mandibular arches between the two individuals.

Table 3 exhibit the linear and angular cephalometric measurements of individuals 1 and 2. The cephalometric analysis showed remarkable differences in the vertical and horizontal parameters of the craniofacial structure, as well as in the components related to the dentition.

## 4. DISCUSSION

Although monozygotic twins are genetically identical, they can express phenotypic differences resulting from genetic interactions with epigenetic and/or environmental factors throughout life [9], [10], [20].

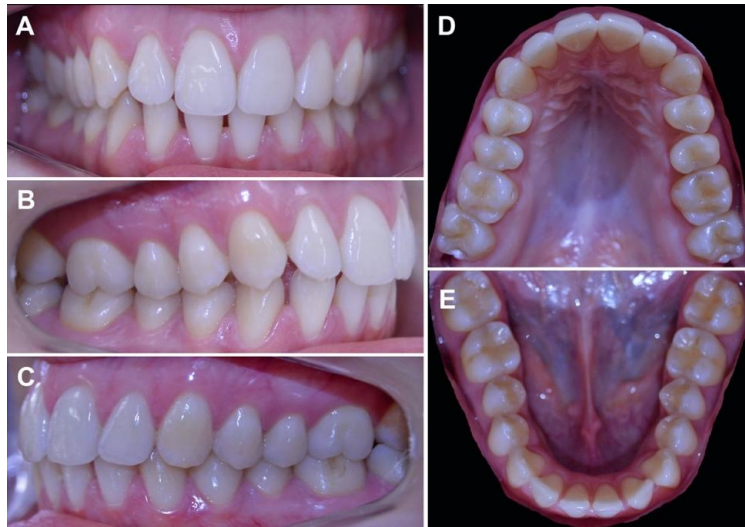
The present study highlights dental and craniofacial differences between homozygous twins who shared the same environment from conception to postnatal life. Therefore, monozygotic twins may exhibit different developmental and growth patterns even in similar conditions [21]. The literature shows that craniofacial development can be influenced by external stimulus such as nutrition, habits, light exposure, temperature variations, mechanical stimulus, among many others [2], [4]. Intrinsic events can also occur, such as biophysical and chemical stimuli among cells, which can result in changes in the epigenetic pattern and significantly influence the shape and arrangement of craniofacial structures from the prenatal period to adulthood [21].

**Table 1. Mesio-distal width of the individuals 1 and 2 teeth in millimeters**

<b>Individual 1</b>	<b>Superior teeth</b>	17	16	15	14	13	12	11	21	22	23	24	25	26	27
	Mesio-distal width (mm)	10	11	7	7.5	8	7.5	8.5	8.5	7	8	7.5	7	10	10.5
	<b>Lower teeth</b>	47	46	45	44	43	42	41	31	32	33	34	35	36	37
	Mesio-distal width (mm)	11.5	11.5	7	7.5	7	6	5.5	6	6	6.5	8	7.5	11.5	11
<b>Individual 2</b>	<b>Superior teeth</b>	17	16	15	14	13	12	11	21	22	23	24	25	26	27
	Mesio-distal width (mm)	10	10.5	6.5	7	9	7.5	9	9	7.5	8.5	7.5	7	11	10.5
	<b>Lower teeth</b>	47	46	45	44	43	42	41	31	32	33	34	35	36	37
	Mesio-distal width (mm)	11.5	10.5	7.5	8	7	6.5	5.5	5.5	6	7	7.5	8	11	11

**Table 2. Arch length discrepancy model analysis of individuals 1 and 2**

	<b>Total tooth material (mm)</b>	<b>Available space (mm)</b>	<b>Required space (mm)</b>	<b>Discrepancy (mm)</b>
<b>Individual 1</b>				
Superior	118	76	77	-1
Inferior	112.5	67.5	67	0.5
<b>Individual 2</b>				
Superior	120.5	77	78.5	-1.5
Inferior	112.5	70.5	69.5	1



**Fig. 3. Individual 2 occlusal aspect: (A) frontal occlusal view; (B) right lateral occlusal view; (C) left lateral occlusal view; (D) superior dental arch occlusal view; (E) lower dental arch occlusal view**

**Table 3. Linear and angular cephalometric measurements of individuals 1 and 2**

Parameters	Normal range	Individual 1	Individual 2
SN	73° +/- 4°	83mm	81mm
S-BA	46mm +/- 3°	51mm	48mm
N-S-BA	130° +/- 3°	138°	138°
SNA	82° +/- 2°	71°	81°
SNB	80° +/- 2°	68°	77°
ANB	2° +/- 2°	3°	4°
WITS	0mm	0mm	1mm
CO-A	83mm	93mm	98mm
CO-GN	100mm	126mm	131mm
LAFH	60mm +/- 2	83mm	80mm
TOTAL AXIS	90° +/- 3°	85°	91°
UI-NA	22°	28°	23°
UI-NA	4mm	7mm	5mm
LI-NB	25°	29°	36°
LI-NB	4mm	10mm	8mm
UI-LI	131°	124°	122°
NASOLABIAL ANGLE	95° a 110°	98°	110°

The etiological factors that cause differences in the development between monozygotic twins are still not completely understood. Studies suggest that the placenta membrane may influence the general and dental development of the twins [22],[23]. The intrauterine organization of univithelinous twins can vary in the number of placentas and amniotic sacs: dichorionic and diamniotic (two placentas and two amniotic sacs), mono-chorionic and diamniotic (one placenta and two amniotic sacs), and mono-chorionic and monoamniotic (one placenta and one amniotic sac) [10]. Studies suggest that the

mono-chorionic membrane is directly linked to the difference between the weights and sizes of the twins, which may be related to the inequality of umbilical cord insertion in mono-chorionic pregnancies and to the fetal position more favorable to the food reception and absorption [24], [22], [25]. In this way, even sharing the same environment, each individual would experience unique environmental influences that could interfere the dento-craniofacial development [26]. Some hypotheses support that the hemodynamic imbalance caused by arteriovenous anastomoses could also induce

one twin to be better nourished than the other, which may result in significant difference in the size and morphology of the permanent teeth in addition to alterations in the tooth eruption chronology [27]. In the present study, monozygotic twins showed differences in weight and height at birth, which suggests a possible influence of the chorionicity type and the presence of a single placenta during the gestational period, which was detected in prenatal exams.

Dental plaster models analysis showed significant differences in the mesio-distal diameters among some of the twins' teeth, especially the first molars. According to Dempsey & Townsend (1999), as the first molars are the first permanent teeth to develop in the intrauterine period, they are more susceptible to changes in shape and size in this pregnancy stage [28]. The morphology and dimensions of the teeth crowns are genetically determined, however, the crown characteristics can be influenced by environmental factors and modify the genetic development pattern [29], [30]. Additionally, some authors support that modifications or disturbances in the gene transcription and translation processes may result in developmental differences between the teeth of monozygotic twins during odontogenesis [31], [32].

Many authors report that genetic factors associated with environmental influences, such as bite forces, food consistency, oral habits, mouth breathing, the effect of the muscles of the lips and tongue, and the dental positioning, act in the dentofacial development resulting in phenotypic variations among monozygotic twins [2]; [5]. The results of the present study evidenced that the twins' mandibular arches had different dimensions. Comparisons between twins showed that genetic factors were more strongly linked to the length of the upper arch when compared to the lower arch, given the greater susceptibility of the mandibular bone to environmental influences [30]. In addition, the difference in the lengths of the lower dental arches of the twins reported in the present study may be related to the orthodontic treatment of one of the individuals.

In the present study, radiographic analysis revealed the lack of synchronism of the formation and eruption of the twins' third molars. Evidence points out that monozygotic twins are not completely identical in relation to the teeth

formation and eruption, even under strong genetic influences [27]. The presence of independent epigenetic factors in the twins can act as modifying agents of the interactions among signaling molecules and odontogenesis growth factors, resulting in developmental differences of each individual's teeth [32]. Besides that, local influences such as the space available in the dental arches can interfere in the teeth eruption processes [27].

Many studies have shown that genetic factors are more influential in the determination of skeletal characteristics when compared to dental characteristics [1]. In a study about the occlusion of monozygotic twins, Corruccini et al [33] showed that environmental factors have significant influences in the establishment of the sagittal molar relationship, overbite, overjet, and posterior crossbite [33]. In the present case study, it was observed that individual 1 presented class I molar relationship and anterior open bite, while individual 2 presented class II molar relationship, division 1, right subdivision and diastemas in the lower incisors. These differences in the twins' occlusal patterns may be related to the influence of several particular environmental factors on their stomatognathic system. In other hand, is possible suggest that differences may be due to the orthodontic treatment performed on one of the patients.

Despite the contribution of environmental agents in the facial development, genetic factors seem to play a major role in determining phenotypic characteristics. Cakan et al (2012) described that the facial shape is predominantly a product of the genotype, thus the personal appearance has a hereditary tendency [34]. It is suggested that the vertical dimensions of the face have a stronger genetic component than the sagittal dimensions, as well as the dimensions of the skull base and the mandibular length in relation to the skull [4], [35], [1]. The dolichocephalic pattern was observed in both individuals of the present study, but the lower anterior facial height (LAFH) measurement was higher in one of the twins. This difference may be directly related to the mouth breathing habit present only in this individual. Atypical respiratory functions, such as mouth breathing, contribute to greater antero-inferior vertical growth of the face by changes in muscle balance and head position [36]. Additionally, Chambi-Rocha et al [37] described that muscle changes as a result of erroneous posture of mouth breathers interfere the craniofacial development, with a decrease in the

anteroposterior growth of the mandible and maxilla in relation to the skull, without affecting the maxillomandibular relationship [36].

As in the present study, Brock-Jacobsen et al (2009) showed important differences regarding the skull base length and inclination in homozygous twins [38]. This evidence may suggest that environmental and/or epigenetic factors could play an important role in the anatomical organization of the craniofacial complex. However, it is important to note that, in this case study, one of the patients was treated orthodontically. This point may be also related with the differences in the arches, molar relationships and some cephalometric magnitudes.

The present study limitations are attributed to the design of case studies such as the small sample size and the difficulty in obtaining retrospective information. Additionally, in this case study, the orthodontic treatment in one of the patients may be related with some differences founds. However, it is important to emphasize that further studies on the dento-craniofacial growth and development in homozygous twins are necessary for a better understanding of interactions of genetic, epigenetic, and environmental factors in the stomatognathic system structural formation. Clinically, for orthodontic treatment of twins must be proposed individually considering the malocclusion and cephalometric deviations of each one.

## 5. CONCLUSION

It can be suggested that the dento-craniofacial characteristics present a genetic component; however, the interaction of epigenetic and environmental factors can lead to different phenotypes in univiteline twins.

## CONSENT AND ETHICAL APPROVAL

This study was approved by the Research Ethics Committee (protocol no 2778781). As per international standard or university standard, patient's written consent has been collected and preserved by the authors.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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