

## Facial morphology differences in monozygotic twins: a retrospective stereophotogrammetric study

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### ABSTRACT

**Objective:** To assess soft tissue differences between monozygotic twins (MZ) for the total face and between facial regions using three-dimensional (3D) stereophotogrammetry and quantitative surface-based 3D deviation analyses.

**Materials and Methods:** The study sample consisted of 14 untreated MZ twins (6 males, 8 females, mean age: 14.75 years) from the archive of Marmara University, Department of Orthodontics. The images were taken by the 3dMDface system, and 3dMDvultus software was used for removal of undesired areas and approximation of the images. Then, stereolithography (.stl) format images were superimposed using the best-fit algorithm using 3-matic software. The face was divided into facial thirds, and upper lip and lower lip + chin regions were created. For the comparison, 3D deviation analyses were performed, and a color map and histogram were created. The data were presented as mean deviation, root mean square (RMS), median, and interquartile range.

**Results:** Between the facial thirds, there was no significant difference in soft tissue differences for mean deviation. A statistically significant difference was found between the upper and lower face for the RMS value. For the comparison of upper lip and lower lip + chin region, the only significant difference was for the RMS. When the data were presented as median and interquartile range, there were no statistically significant differences between any facial regions.

**Conclusions:** Lower facial third and lower lip + chin regions had the greatest differences within MZ twin pairs. The genetic and environmental influences might not be the same for different parts of the face. (*Angle Orthod.* 2023;93:706–711.)

**KEY WORDS:** Monozygotic; Twins; Stereophotogrammetry; 3D deviation analyses

### INTRODUCTION

Face shape and attractiveness have a great influence on both professional and personal life in the modern era. Therefore, the capability to influence the development and morphology of the face has become interesting and gains importance day by day. From

the very beginning of its development, the craniofacial region goes through a complicated process that is affected by genes and molecular interactions in embryonic life and also by environmental factors in later stages.<sup>1,2</sup> Therefore, the success of changing facial morphology either by orthodontic or orthopedic treatment depends on the capacity of changing these factors.

In the literature, twin<sup>3–6</sup> and family studies<sup>7,8</sup> have been used to evaluate the interactions between genes and environmental factors. Specifically, studies in monozygotic (MZ) twins have shed some light on those interactions, as MZ twins share the same genes but not the same environmental factors, which might include hormones, nutrition, trauma, diseases, treatments, lifestyle (alcohol, smoking, exercise), and oral functions (respiration, mastication).<sup>9,10</sup>

To analyze facial morphology, previous studies have used two-dimensional (2D) or three-dimensional (3D) imaging techniques.<sup>5</sup> However, when the complex morphology of the face is considered, 2D studies ignore

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most information about the face.<sup>11</sup> Advances in 3D imaging technology have overcome the limitations related to 2D imaging techniques, and 3D acquisition systems (cone-beam computed tomography, laser scanners, 3D stereophotogrammetry, etc) have become the primary tools for accurately analyzing facial morphology.<sup>6,11–13</sup>

In 3D imaging techniques, landmark-based (linear or angular measurements between corresponding points) or surface-based (point-to-point distance on the entire point cloud of the 3D images of the two structures) comparisons can be performed. However, because of the complex morphology of the facial structures, it is suggested that a surface-based comparison be used to evaluate deviations within the entire face three-dimensionally rather than only a comparison between specific points.<sup>14</sup>

Studies in the literature have conducted 3D comparisons of the facial morphology of MZ twins.<sup>3–6,15–17</sup> Most of the studies used a landmark-based comparison for twins,<sup>3,15–17</sup> and only three studies compared the entire face using a surface-based comparison.<sup>4–6</sup> According to the landmark-based studies, the strongest genetic determination was found for the midfacial region (orbital rims, intercanthal area, nose), and heritability was also reported for the upper lip projection in MZ twins.<sup>3,4,15,16</sup> On the other hand, Djordjevic et al.<sup>5</sup> performed a surface-based comparison and calculated the absolute average distance between the superimposed images of MZ twin pairs as 0.82 mm. In addition, the authors compared the upper, middle, and lower facial thirds and found that although the lower facial third was the least similar area for male MZ twins, no significant difference was reported for females. In their study, Gibelli et al.<sup>6</sup> calculated the root mean square (RMS) value for the entire face with a surface-based comparison after the superimposition of 3D images of MZ twin pairs and reported the value as 1.90 mm. Naini and Moss<sup>4</sup> also compared the surface shape of MZ twin faces with the entire facial shape comparison. Although it was not a quantitative comparison, they found the greatest similarity in the region of the forehead, in a triangular form with its base near the eyes and its apex just below the nose.

Although MZ twins share the same genetic background, the similarity between different regions of the face may differ due to other factors such as environment. This motivates the clinician to examine each patient individually, even if they are twins, and create a personalized treatment plan. The aims of the present study were (1) to assess the soft tissue difference numerically between the MZ twin pairs for the total face and (2) to compare soft tissue differences between facial regions using 3D stereophotogrammetry.

## MATERIALS AND METHODS

### Study Sample

The sample of the present retrospective study consisted of 14 MZ twin pairs (6 male pairs, 8 female pairs; mean age:  $14.75 \pm 1.58$  years) recruited from the archive of Marmara University, Department of Orthodontics. The inclusion criteria for the study were (1) Class I or mild Class II dental and skeletal relationship, (2) no previous orthodontic treatment, (3) no previous trauma or surgery, (4) no craniofacial syndrome, and (5) high-quality 3D stereophotogrammetry images before any orthodontic treatment. Zygosity was determined by genetic testing for previous studies not related to the present study.

The present study was approved by the Ethical Committee of Marmara University, Faculty of Medicine (2021/783, Istanbul, Turkey) and conducted in accordance with the Declaration of Helsinki of 1975 as revised in 2013. Informed consent was taken from parents/legal guardians of all individuals.

### Data Collection and Measurements

The 3dMDface system (3dMD Inc, Atlanta, Ga) was used for the soft tissue comparison of the twin pairs in 3D stereophotogrammetric images. In the 3dMDface system, six cameras of two modular units capture images simultaneously from predetermined distances and angles. Each individual was seated on a height-adjustable stool and asked to look at the eyes of their reflection in the mirror on the wall to ensure a natural head position for standardization. The system was calibrated before each acquisition. The 3dMDvultus version 2.1 software (3dMD Inc) was used for the initial image process. The ears, hair, and neck regions were removed, and the 3D images of twin pairs were approximated using global registration for the first superimposition (3D image of the second-born twin was superimposed to the 3D image of first-born twin). For further evaluation, the 3D images were converted to stereolithography (.stl) format and transferred into 3-matic software (Materialise Europe, Leuven, Belgium).

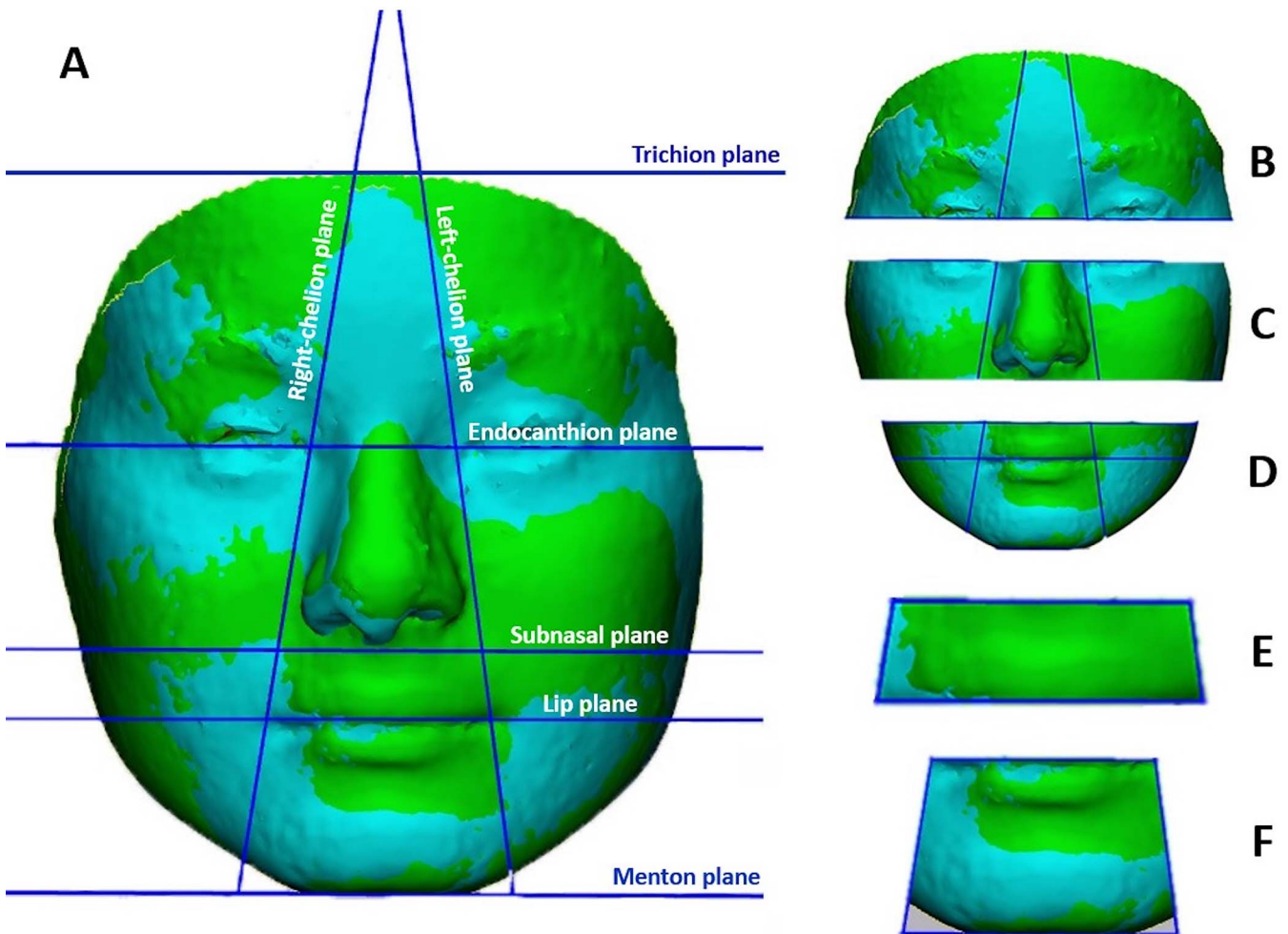
In 3-matic software, initially, the approximated 3D images of twin pairs were again superimposed using the best-fit algorithm based on the iterative closest point method.<sup>5</sup> Following superimposition, the face was divided into facial thirds, and upper lip and lower lip + chin regions were also created as in Table 1 and Figure 1.<sup>14,18</sup> For the comparison of superimposed and segmented regions with each other, the “part comparison” tool in the software was used, and 3D deviation analyses were performed. Following this step, a color map and histogram were created by the software automatically (Figure 2). In the histogram, the

**Table 1.** Definition of the planes and the morphological regions.

Plane	Definition
Trichion	A plane perpendicular to the image and passing through the trichion point
Endocanthion	A plane perpendicular to the image and passing through the right and left endocanthion points
Lip	A plane perpendicular to the image and passing through the right and left chelion points
Subnasal	A plane perpendicular to the image and passing through the soft tissue subnasal point
Menton	A plane perpendicular to the image and passing through the soft tissue menton point
Right-chelion	A plane perpendicular to the image and passing through right chelion and endocanthion points
Left-chelion	A plane perpendicular to the image and passing through left chelion and endocanthion points
Morphological Region	Definition
Upper face	The region between the trichion plane and the endocanthion plane
Midface	The region between the endocanthion plane and the subnasal plane
Lower face	The region between the subnasal plane and the menton plane
Upper lip	The region between the subnasal plane, lip plane, and right and left chelion planes
Lower lip + chin	The region between the lip plane, menton plane, and right and left chelion planes

mean deviation and RMS values were given for meshes at the 95th percentile. The RMS value was calculated automatically by the software by taking the square root of the mean of the squares of all values. The data

were also presented as median, and the interquartile range (25th percentile = Q1 and 75th percentile = Q3) was calculated.<sup>5,14</sup> The data were edited, measured, and analyzed by the same author Dr. Onem Ozbilen.



**Figure 1.** Morphological regions. (A) Total face. (B) Upper face. (C) Midface. (D) Lower face. (E) Upper lip. (F) Lower lip + chin.

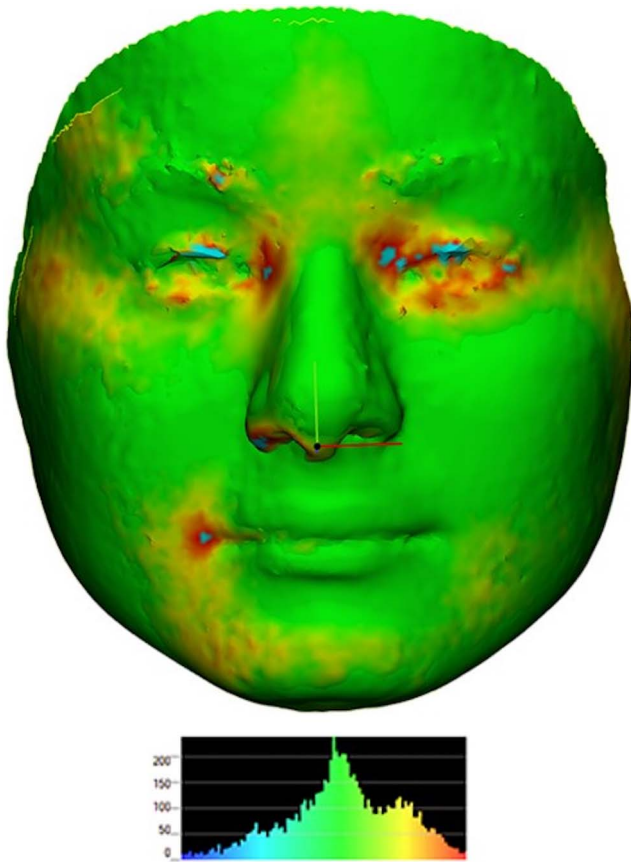


Figure 2. Color map and histogram.

**Statistical Analysis**

The data were analyzed using IBM SPSS Statistics (version 25.0; IBM Corp, Armonk, NY). The conformity of the parameters to the normal distribution was evaluated with the Shapiro-Wilk test. One-way analysis of variance and Kruskal-Wallis test with the Bonferroni adjustments were performed for the comparison of facial thirds. For the comparison of upper lip and lower lip + chin regions, Student’s *t*-test and Mann-Whitney *U*-test were used. Statistical significance was set at  $P < .05$ .

**RESULTS**

All measurements were repeated 10 days apart by the same author (Dr Ozbilen). The intraclass correlation coefficient was used to assess intraexaminer reliability and ranged from 0.908–0.984, showing a high level of agreement.

The descriptive statistics of the soft tissue differences within the MZ twin pairs for the total face are provided in Table 2. The mean deviation for the total face was  $0.08 \pm 0.25$  mm, and THE RMS value was  $1.01 \pm 0.22$  mm for meshes at the 95th percentile. When the data were presented as median and interquartile

**Table 2.** Descriptive Statistics of the Soft Tissue Differences Between Monozygotic Twin Pairs for Total Face

Descriptive Statistic	Min	Max	Mean $\pm$ SD
Mean deviation	-0.25	0.53	$0.08 \pm 0.25$
Root mean square	0.66	1.46	$1.01 \pm 0.22$
Q1	-1.09	-0.17	$-0.53 \pm 0.27$
Median	-0.29	0.44	$0.08 \pm 0.2$
Q3	0.37	1.29	$0.71 \pm 0.29$

range, the Q1, median, and Q3 values were  $-0.53 \pm 0.27$  mm,  $0.08 \pm 0.2$  mm, and  $0.71 \pm 0.29$  mm, respectively (Table 2).

Between the facial thirds, for the mean deviation, there was no statistically significant difference with regard to soft tissue differences (Table 3;  $P > .05$ ). However, for the RMS value, a statistically significant difference was found between the upper face and lower face ( $0.86 \pm 0.22$  mm and  $1.16 \pm 0.35$  mm, respectively;  $P < .05$ ) within MZ twin pairs (Table 3).

For the comparison of soft tissue differences between the upper lip and lower lip + chin region, a significant difference was found for the RMS value ( $0.88 \pm 0.29$  mm and  $1.44 \pm 0.77$  mm, respectively,  $P < .05$ ; Table 3).

When the data were presented as median and interquartile range (Q1 and Q3), there were no statistically significant differences between the facial thirds or between the upper lip and lower lip + chin region within twin pairs, as presented in Table 4 ( $P > .05$ ).

**DISCUSSION**

Twin studies in the literature provide the opportunity to investigate the effects of genetic and other related factors in terms of evaluating the different morphologies of the facial structures. The aim of the present retrospective study was to quantitatively compare the facial soft tissue differences of MZ twins using stereophotogrammetry and surface-based 3D deviation analyses, since there have been few studies showing the similarity of MZ twin faces numerically. Although there are studies comparing twin faces for the evaluation of interactions between the genes and environment, most used a landmark-based comparison, which does not compare the entire face and thus may provide incomplete information.<sup>3,15–17</sup> On the other hand, using a surface-based comparison, the whole face can be compared quantitatively using the average distances of thousands of points in 3D images.<sup>14</sup>

Among the 3D acquisition systems, stereophotogrammetry, which was preferred in the present study, has become popular since it is a noninvasive, fast, reliable, accurate, and valid method without any radiation.<sup>19,20</sup> In addition, 3D stereophotogrammetry is advantageous due to standardized image-capturing conditions (lighting, camera distances, and angulations)

**Table 3.** Comparison of the Soft Tissue Differences Between Morphological Regions Within Twin Pairs for 95th Percentile of the Meshes

	Region 1 Upper Face Mean $\pm$ SD	Region 2 Midface Mean $\pm$ SD	Region 3 Lower Face Mean $\pm$ SD	<i>P</i>	Post Hoc Test	Region 4 Upper Lip Mean $\pm$ SD	Region 5 Lower Lip + Chin Mean $\pm$ SD	<i>P</i>
Mean deviation	0.11 $\pm$ 0.23	0.13 $\pm$ 0.44	-0.2 $\pm$ 0.42	.62 <sup>a</sup>	NS	-0.17 $\pm$ 0.62	-0.13 $\pm$ 1.24	.77 <sup>b</sup>
Root mean square	0.86 $\pm$ 0.22	0.92 $\pm$ 0.22	1.16 $\pm$ 0.35	.02 <sup>*a</sup>	R1-R3	0.88 $\pm$ 0.29	1.44 $\pm$ 0.77	.03 <sup>*b</sup>

<sup>a</sup> Kruskal-Wallis test.

<sup>b</sup> Mann-Whitney *U*-test.

\* *P* < .05.

and is able to reproduce 1:1 surface imaging. In the present study, 3D images were recorded of MZ twins with a natural head posture, which was reported to be highly reproducible.<sup>21</sup> Also, the anatomical points used in the present study to divide the face into facial thirds have been reported in previous studies to be reliable and reproducible.<sup>20,22</sup>

In the present study, the RMS value for the total face was 1.01  $\pm$  0.22 mm within MZ twin pairs. There is only one study in the literature that calculated the RMS value for facial soft tissues within MZ twin pairs, and the authors reported this value as 1.90  $\pm$  0.50 mm.<sup>6</sup> Different methodology, age groups, and ethnic groups could be reasons for the different values obtained in the two studies. While the ages of the twins in the current study sample were similar, the twins used in the other study were selected from among individuals with a large age range.<sup>6</sup>

Instead of calculating the difference within twin pairs for only the total face, in the present study, the face was also divided into facial thirds, which were compared with each other to determine the differences between different parts of the face. The mean deviations were 0.11  $\pm$  0.23 mm for the upper face, 0.13  $\pm$  0.44 mm for the midface, and -0.2  $\pm$  0.42 mm for the lower face. In addition, the median data were 0.13  $\pm$  0.23 mm, 0.11  $\pm$  0.43 mm, and 0.03  $\pm$  0.3 mm for the upper face, midface, and lower face, respectively, with no significant difference between them. In addition, no significant differences were found for interquartile range. The only study in the literature comparing the facial thirds within MZ twins was performed by Djordjevic et al.,<sup>5</sup> who also found no significant differences

for both median and interquartile range. They reported the median data as 0.78 mm, 0.79 mm, and 0.93 mm for the upper face, midface, and lower face, respectively. The different values between the two studies might be attributed to methodological differences. In the study conducted by Djordjevic et al.,<sup>5</sup> a laser scanning device was used, the images were initially scaled to an average Procrustes size, and the boundaries of the facial thirds were different from the present study. In addition, while those authors took the absolute differences between all pairs of points while calculating the average distances, they were calculated in the current study by averaging the positive and negative entire data set.

In the present study, a significant difference was found in the RMS value between the upper and lower facial thirds. Djordjevic et al.<sup>5</sup> also reported that the lower third was the least similar area in MZ male twins when it was compared with other facial thirds, and they suggested that this finding might show that the contribution of genetic and environmental factors is not the same for different facial thirds. Landmark-based studies also reported that the lower part of the face was quite different and that the environmental contribution was higher in the lower face,<sup>4,12,15,16</sup> which may cause significant differences for the lower facial third. However, the methodological differences between the studies should be considered when interpreting the data.

For the comparison of upper lip and lower lip + chin regions, only the RMS value showed a significant difference between the two regions, which was 0.88  $\pm$  0.29 mm and 1.44  $\pm$  0.77 mm, respectively. No

**Table 4.** Comparison of the Soft Tissue Differences Between Morphological Regions Within Twin Pairs for the Median and Interquartile Range Data

	Region 1 Upper Face Mean $\pm$ SD	Region 2 Midface Mean $\pm$ SD	Region 3 Lower Face Mean $\pm$ SD	<i>P</i>	Post Hoc Test	Region 4 Upper Lip Mean $\pm$ SD	Region 5 Lower Lip + Chin Mean $\pm$ SD	<i>P</i>
Q1	-0.46 $\pm$ 0.18	-0.55 $\pm$ 0.63	-0.72 $\pm$ 0.52	.34 <sup>a</sup>	NS	-0.63 $\pm$ 0.64	-0.97 $\pm$ 1.27	.31 <sup>b</sup>
Median	0.13 $\pm$ 0.23	0.11 $\pm$ 0.43	0.03 $\pm$ 0.3	.85 <sup>a</sup>	NS	-0.18 $\pm$ 0.64	-0.28 $\pm$ 1.31	.78 <sup>b</sup>
Q3	0.69 $\pm$ 0.34	0.67 $\pm$ 0.5	0.7 $\pm$ 0.41	.97 <sup>a</sup>	NS	0.38 $\pm$ 0.62	0.44 $\pm$ 1.34	.82 <sup>b</sup>

<sup>a</sup> One-way analysis of variance.

<sup>b</sup> Mann-Whitney *U*-test.

\* *P* < .05.

previous study in the literature compared the upper lip and lower lip + chin regions using a surface-based comparison. However, most of the landmark-based studies showed a significant genetic contribution for the upper lip<sup>15,16</sup> and stronger environmental influence for the lower face, such as the chin and lips,<sup>4,23,24</sup> which may assist in validating the results of the present study, although the methodology was not the same. In the literature, only one study was contrary to the current result and stated specifically that the upper lip was influenced by environmental problems.<sup>25</sup>

Including MZ twin pairs with the same age and from the same geographical region was the strength of the present study. However, there were also some limitations such as the small sample size of a group consisting of growing individuals, since the facial similarity between twins increases after growth cessation.<sup>25</sup> In addition, factors that may affect soft tissue such as body mass index, medications, and medical conditions were not taken into consideration in the present study.

## CONCLUSIONS

- Lower facial third and lower lip + chin regions had the greatest soft tissue differences within MZ twin pairs, which may indicate that genetic and environmental influences might not be the same for different parts of the face.

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