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## Genetic and environmental impact on variation in the palatal dimensions in permanent dentition: a twin study

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The objective of this study was to assess the relative contributions of genetic and environmental factors to variation in palatal parameters in twins with completed maxillary growth. The subjects of this study comprised digital dental casts of 50 monozygotic and 35 dizygotic twin pairs. The subjects' average age was  $17.95 \pm 2.83$  years. Zygosity determination was carried out using 15 specific DNA markers and an amel fragment of the amelogenin gene. The interdental distances were measured between selected dental landmarks at the occlusal and gingival planes. The palatal height, surface area and volume were measured between the gingival plane and the midpalate suture. High heritability estimates were observed for all transverse intra-arch measurements. The palate height ( $a^2 = 0.8$ ), dental arch width in the molar area ( $a^2 = 0.86$ ), palatal surface area ( $a^2 = 0.61$ ) and palate volume ( $a^2 = 0.69$ ) were under strong additive genetic control. Moderate genetic dominance was observed for dental arch widths at the gingival line in the canine ( $d^2 = 0.5$ ) and premolar regions ( $d^2 = 0.78-0.81$ ). Sexual dimorphism was shown, with males exhibiting a greater arch width, palatal surface area and volume than females ( $p < 0.01$ ). The majority of palatal parameters variation in twins was controlled by genetic effects, and most were highly heritable.

**Keywords** Twin study, Palatal parameters, Heritability, Orthodontics

The size, shape and transverse dimension of the upper jaw are among the most important factors determining orthodontic treatment options for malocclusions, such as crossbite, dental crowding, lower anterior dentition irregularities and distal lower jaw position<sup>1,2</sup>. The palate form and volume are closely related to the width of the maxillary dental arch and have an impact on the position of the tongue and breathing function. Understanding facial skeletal and functional pattern changes throughout life and their control mechanisms is crucial for orthodontic treatment planning and subsequent stability<sup>3,4</sup>. There is ongoing discussion about the importance of genetic and environmental factors on maxillary dental arch and palatal morphology<sup>5</sup>.

A recent systematic literature review and meta-analysis demonstrated that maxillary arch dimensions have high heritability estimates<sup>6</sup>. For the maxillary arch length heritability estimates were above moderate ranging from 0.42 to 0.92<sup>7-9</sup>. Heritability for the palatal depth was estimated at 0.56 (95% CI range 0.22–0.90)<sup>6</sup>. The heritability of maxillary transversal dimensions such as intercanine and intermolar widths also have high estimates. Eugushi et al.<sup>7</sup> found these estimates equal 0.86 and 0.82 respectively. Similar estimates reported by Hughes et al.<sup>9</sup> (0.84 and 0.87), Lapter et al.<sup>10</sup> (0.69 and 0.58).

In contrast, there are studies showing that environmental factors have a greater influence on the formation of dental arches than previously thought<sup>11,12</sup>. Moreover, there is no doubt that soft tissue imbalance, including mouth breathing, irregular tongue position, irregular posture and other parafunctions, has a major impact on the upper dental arch and palatal formation. Studies have shown that mouth breathers have significantly smaller intermolar widths and palatal volumes and greater palatal heights<sup>13</sup>. A narrower and longer palate forms because of the short lingual frenulum, and these individuals have narrower arches in transverse dimensions and triangular arch shapes because of frontal tooth proclination<sup>14</sup>. Tongue posture is also related to palatal width.

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Fatima and Fida reported significant differences in maxillary intercanine and intermolar widths at the cusp and gingival levels in patients with different resting tongue postures<sup>15</sup>. The combination of direct pressure on the teeth and an alteration in the pattern of resting cheek and lip pressures can change the tooth position and dental arch shape<sup>16</sup>. Mouth breathing allows the tongue to rest on the lower part of the oral cavity. This changes the equilibrium of the forces between the cheeks and the tongue, leading to the development of a narrow maxilla and increased palatal height<sup>17</sup>.

The similarity of twins within pair sources from shared genes and shared family environment. MZ twins share genetic effects and family environment to the full extent. DZ twins share 50% of additive genetic effects, 25% of non-additive genetic effects and 100% of family environment. MZ twins differ because of person-specific environment, DZ twins—because of unique environment and genes. Due to the underlying genetic and environmental similarities in related individuals, twin studies play a crucial role in understanding the aetiology of malocclusion by enabling the separation of genetic and environmental influences on dental arches and occlusion<sup>18</sup>.

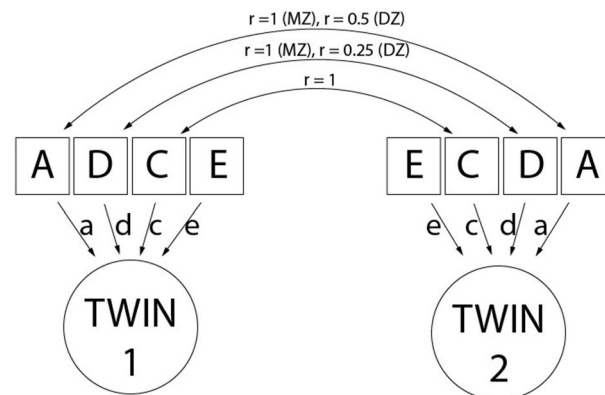
Structural equation modelling (SEM) is a set of methods which allows checking the hypothesis about the structure of relationship between observed and unobserved (latent variables). The model is usually represented by path diagram which include variables and linear relationship between them (Fig. 1). The combination of twin method and SEM allows precise estimation of the role of genes and environment on the phenotype trait. Even in the postgenomic era, estimation of heritability from twin and sibling studies is foundational for investigating the genes involved in complex traits<sup>19</sup>.

However, the basic problems with the majority of previous twin studies are the reliability of the twin zygosity determination, the statistical methods used to analyse the twin data and the growth stage of the study sample. Regarding the first problem, for many years, zygosity determination was based on assessments of anthropological similarity, including tooth anatomy<sup>20</sup>. Although a comparison of physical appearance can provide a reasonably reliable means of determining zygosity, errors can occur in up to 15–20% of cases with this methodology<sup>21</sup>. The use of blood group determination, as well as serum and enzyme polymorphism analysis, improved the ability to assign zygosity to twins<sup>22</sup>. More recently, the use of highly polymorphic regions of DNA derived from blood or buccal cells has been shown to accurately measure zygosity in up to 90–95% of cases<sup>23</sup>. A more precise determination requires an increased number of highly polymorphic regions of DNA<sup>24</sup>.

The second problem lies in the statistical methods used to analyse the twin data. In twin studies performed 20–30 years ago, the heritability coefficient was calculated using the classical correlation approach. The essential limitation of the heritability coefficient is that it does not estimate the influence of the shared environment, and consequently, the calculated heritability coefficient could be inflated<sup>25</sup>. The path analysis and Dahlberg quotient used in the 1980s are also not appropriate for today's studies, and model-fitting methods should be used to obtain more accurate data<sup>6</sup>.

The third problem with twin studies relevant to maxillary dental arch and palate morphology is the maturity of the study sample. Many studies have assessed the maxilla in the intensive growth process of growing children<sup>11,26,27</sup>. The results of such studies on the heritability of maxillary dental arch and palate final parameters should be interpreted with caution because complete genetic predisposition to maxillary morphology can be detected only if growth is complete.

The aim of this study was to determine the genetic and environmental impacts on the maxillary arch and palatal morphology of twins with completed maxillary growth using structural equation modelling (SEM) and precise zygosity determination.



**Fig. 1.** Path diagram for the univariate twin model. Squares are latent variables (A—additive genetic factors, D—non-additive genetic factors, C—common environmental factors and E—unique environmental factors) shown with their respective path coefficients (a, d, c, e) indicating the relative importance of each of the contributing influences. Circles are observed variables, single-headed arrows are one-way (causal) relationships, and double-headed arrows are two way relationships (covariance).

## Results

### Descriptive statistics

The results of the descriptive statistics are shown in Table 1. Compared with females, males had slightly greater increases in all parameters of dental arch width at the occlusal plane. The most notable difference at the occlusal plane was registered for 1IPW ( $p < 0.01$ ), while at the canine and molar regions males had wider dental arches, but with lower statistical significance ( $p < 0.05$ ). Dental arch widths of males at the gingival line also demonstrated higher values, but the differences had no statistical significance. The palate height at the second premolar and molar region, the palatal surface area and the palate volume in males were significantly greater than those in females ( $p < 0.01$ ).

### Measurement reliability analysis

The results of the measurement error analysis revealed no significant differences between the first and second measurements on the models. The ICCs showed high intrarater reliability for all measurements (0.90–0.96,  $p < 0.01$ ). Dahlberg's formula showed a random error of less than 1.0 mm for all linear measurements, 15 mm<sup>2</sup> for the palate surface area and 40 mm<sup>3</sup> for the palate volume.

### Genetic analysis

The AIC values for each model were calculated (Table 2). The most parsimonious model and the lowest values were chosen. The AE and DE models were found to be the most parsimonious for variables. Variables with the best-fitting model of the contribution of factors ( $a^2$ ,  $c^2$ ,  $d^2$ ,  $e^2$ ) were counted. The results of the model-fitting analysis are summarized in Table 3. Variables representing interdental distances between cusp tips of teeth 1IPW, 2IPW and IMW showed high genetic determination (AE model), with  $a^2 = 0.76$ , 0.72 and 0.86, respectively, and ICW had a strong dominant determination (DE), with  $d^2 = 0.59$ .

The variables representing interdental distances at the palatal gum lines ICWG, 1IPWG, and 2IPWG had strongly dominant values of  $d^2 = 0.5$ , 0.78, and 0.81, respectively, while IMWGL had an additive genetic factor of  $a^2 = 0.78$ .

Variables showing interdental height 2IPH and IMH were affected by additive genetics ( $a^2 = 0.7$  and 0.8, respectively), while 1IPH and ICH were determined by dominant genetic factors.

The parameters representing the maxillary depth, palatal area and palatal volume were affected by additive genetic factors.

A model with specific environmental factors ( $e^2$ ) and common environmental factors ( $c^2$ ) was rejected. The AE and DE models were adequate for all variables. Heritability estimates were high for all widths, maxillary depths, palatal surface areas and palatal volumes, ranging from 0.48 to 0.8.

Variables	Males (n = 38)	Females (n = 47)	MZ (n = 50)	DZ (n = 35)	P Males vs. females	P MZ vs DZ
Dental arch widths at occlusal line (mm)						
ICW	34.54 ± 1.93	33.83 ± 2.3	34.27 ± 2.16	33.98 ± 2.17	0.034	0.399
1IPW	41.40 ± 2.47	40.40 ± 2.25	40.98 ± 2.58	40.66 ± 2.15	0.006	0.391
2IPW	46.78 ± 2.81	45.85 ± 2.37	46.3 ± 2.74	46.22 ± 2.43	0.02	0.851
IMW	51.31 ± 3.68	50.35 ± 2.63	50.94 ± 3.36	50.56 ± 2.89	0.049	0.442
Dental arch widths at gingival line (mm)						
ICWG	24.68 ± 1.55	24.97 ± 1.83	25.08 ± 1.78	24.49 ± 1.56	0.276	0.028
1IPWG	27.62 ± 2.39	26.88 ± 2.09	27.19 ± 2.53	27.24 ± 1.79	0.032	0.869
2IPWG	32.62 ± 2.75	32.08 ± 2.7	32.19 ± 2.79	32.51 ± 2.66	0.201	0.454
IMWG	34.75 ± 3.23	34.16 ± 2.47	34.49 ± 3.22	34.33 ± 2.19	0.186	0.709
Palatal heights (mm)						
ICH	5.01 ± 1.39	5.21 ± 1.57	5.22 ± 1.43	4.98 ± 1.33	0.343	0.269
1IPH	11.25 ± 1.83	11.08 ± 1.73	11.22 ± 1.9	11.06 ± 1.56	0.529	0.545
2IPH	15.83 ± 1.81	14.86 ± 1.77	15.24 ± 1.87	15.37 ± 1.84	0.001	0.651
IMH	16.61 ± 2.03	15.17 ± 1.85	15.76 ± 2.02	15.89 ± 2.13	0.001	0.668
Maxillary arch depth (mm), MD	28.12 ± 1.7	27.58 ± 2.22	28.25 ± 1.61	27.2 ± 2.36	0.078	0.124
Palate surface area (mm <sup>2</sup> ), PSA	1385.81 ± 144.85	1304.72 ± 123.04	1346.38 ± 141.78	1333.24 ± 135.17	0.001	0.545

**Table 1.** Descriptive statistics of the dental arch and palate variables. 1IPH interfirst premolar palate height; 1IPWG interfirst premolar width at the gum line, 2IPH intersecond premolar palate height, 2IPW intersecond premolar width, 2IPWG intersecond premolar width at the gum line, DZ dizygotic twin, ICH intercanine palate height, ICW intercanine width, ICWG intercanine width at the gum line, IPW interfirst premolar width, IMH interfirst molar height, IMW interfirst molar width, IMWG interfirst molar distance at the gum line, MD maxillary depth, MZ monozygotic twin, PSA palate surface area, PV palate volume. All values are provided in mean ± standard deviation.

Variables	ACE	ADE	DE	AE	CE	E
Dental arch widths at occlusal line						
ICW	-3.12	-5.53	<b>-7.54</b>	-5.12	9.34	30.13
1IPW	-6.53	-6.81	-8.35	<b>-8.53</b>	13.26	64.47
2IPW	-7.83	-7.31	-5.98	<b>-9.31</b>	3.24	63.78
IMW	-6.41	-6.41	-6.47	<b>-8.41</b>	25.47	93.16
Dental arch widths at gingival line						
ICWG	5.97	3.41	<b>1.41</b>	3.97	14.7	32.32
1IPWG	2.35	0.48	<b>-1.52</b>	0.35	27.37	77.6
2IPWG	1.24	-2.72	<b>-4.72</b>	-0.76	36.8	71.27
IMWG	0.2	1.14	2.04	<b>-0.86</b>	10.65	86.54
Palatal heights						
ICH	7.25	2.96	<b>0.96</b>	5.25	17.13	28.33
1IPH	-5.02	-5.43	<b>-7.34</b>	-7.02	2.01	33.3
2IPH	-10.93	-9.42	-6.82	<b>-11.42</b>	-5.83	50.04
IMH	-8.65	8.33	-6.45	<b>-10.33</b>	9.46	68.81
Maxillary arch depth						
MD	8.32	8.35	8.88	<b>6.35</b>	13.27	37.46
Palate surface area and volume						
PSA	-4.14	-3.16	43.84	<b>-5.26</b>	-1.57	41.84
PV	-7.44	-6.27	54.52	<b>-8.28</b>	-3.11	52.52
Principal component						
PC1	-3.42	-4.01	<b>-5.83</b>	-5.42	25.42	84.8
PC2	-9.77	-9.77	-10.52	<b>-11.77</b>	-2.44	35.34
PC3	1.67	-1.87	<b>-3.88</b>	-0.33	27.92	49.37

**Table 2.** AIC values of all the models. ACE additive genetic factors, common environmental factors, and specific environmental factors; ADE additive genetic factors, dominant genetic factors, and specific environment; AE additive genetic factors and specific environmental factors; CE common and specific environmental factors; DE dominant genetic factors and specific environmental factors; E specific environmental factors; ICH intercanine palate height; ICW intercanine width; ICWG interfirst premolar width; ICWG intercanine width at the gum line; IMH interfirst molar height; IMW interfirst molar width; IMWG interfirst molar distance at the gum line; 1IPH interfirst premolar palate height; 2IPH intersecond premolar palate height; 2IPW intersecond premolar width; 1IPWG interfirst premolar width at the gum line; 2IPWG intersecond premolar width at the gum line; MD maxillary depth; PSA palate surface area; PV palate volume. Best-fitting models (lowest AIC values) are indicated in bold.

### Principal components

Principal component analysis revealed that three principal components explained 69.3% of the total variance. The first component consisted of 1IPW, 1IPWG, 2IPW, 2IPWG, ICWG, IMW, and IMWG and explained 46.2% of the total variance, and the best-fitting model was DE. The palatal surface area and volume (IMW) were determined for the PC2 group, which showed an additive genetic determination of  $a^2 = 0.62$  with the best-fitting model AE. PC2 explained 23.2% of the total variance. The third component showed a correlation between two variables, ICW and maxillary depth, and these components showed strong genetic dominance (Table 4, Figs. 2 and 3).

### Discussion

There are many twin studies assessing genetic and environmental contributions to the upper arch form and palate parameters. However, it is challenging to compare different twin studies due to differences in the sample size, population, zygosity, and statistical methods used. The use of a model-fitting analysis allows the most accurate differentiation of sources of variation affecting the dental arch and palate form and size. This statistical method was used in our study.

Our results showed sexual dimorphism in palatal parameters. Compared with females, males exhibited slightly greater dental arch widths, whereas the palatal surface area and palate volume in males were significantly greater ( $p < 0.01$ ).

According to our findings, the AE and DE models best explained the variance in the palatal parameters. Inter-dental distances at the gingival plane are mostly affected by the DE model, except for the IMWG. This means that palatal variances for patients with complete maxillary growth were due to additive genetic factors and specific non shared environmental factors. Distances at the gingival planes are mainly defined by dominant genetic factors.

The correlations for all parameters in the MZ twin analysis were greater than those in the DZ, which is likely due to genetic influences. The variance in the dental arch width and palatal morphology (depth, height, and volume area) had a high genetic contribution. Recent research in twin studies of palatal parameters confirms

Variables	a <sup>2</sup>	SE (a <sup>2</sup> )	d <sup>2</sup>	SE (d <sup>2</sup> )	c <sup>2</sup>	SE (c <sup>2</sup> )	e <sup>2</sup>	SE (e <sup>2</sup> )
Dental arch widths at occlusal line								
ICW (DE)			0.59	0.06			0.17	0.06
1IPW (AE)	0.76	0.04					0.1	0.04
2IPW (AE)	0.72	0.04					0.1	0.04
IMW (AE)	0.86	0.02					0.06	0.02
Dental arch widths at gingival line								
ICWG (DE)			0.5	0.07			0.22	0.07
1IPWG (DE)			0.78	0.03			0.09	0.03
2IPWG (DE)			0.81	0.03			0.08	0.03
IMWG (AE)	0.78	0.03					0.09	0.03
Palatal heights								
ICH (DE)			0.48	0.08			0.22	0.8
1IPH (DE)			0.56	0.06			0.19	0.06
2IPH (AE)	0.7	0.04					0.13	0.04
IMH (AE)	0.8	0.03					0.08	0.03
Maxillary arch depth								
MD (AE)	0.56	0.07					0.18	0.07
Palate surface area and volume								
PA (AE)	0.61	0.05					0.18	0.05
PV (AE)	0.69	0.04					0.15	0.04
Principal components								
PC1 (DE)			0.82	0.03			0.07	0.03
PC2 (AE)	0.62	0.06					0.16	0.06
PC3 (DE)			0.76	0.04			0.09	0.036

**Table 3.** Best-fitting models for each variable. *1IPH* interfirst premolar palate height, *1IPWG* interfirst premolar width at the gum line, *2IPH* intersecond premolar palate height, *2IPW* intersecond premolar width, *2IPWG* intersecond premolar width at the gum line, *a<sup>2</sup>* additive genetic factors, *d<sup>2</sup>* dominant genetic factors, *c<sup>2</sup>* common environmental factors, *e<sup>2</sup>* specific environmental factors, *ICH* intercanine palate height, *ICW* intercanine width, *ICWG* intercanine width at the gum line, *ICWG* interfirst premolar width, *IMH* interfirst molar height, *IMW* interfirst molar width, *IMWG* interfirst molar distance at the gum line, *MD* maxillary depth, *PSA* palate surface area, *PV* palate volume, *SE* standard error.

this finding<sup>18,26</sup>. In contrast, a longitudinal study with identical and fraternal twins performed by Chaaban et al.<sup>11</sup> showed that heritability had a weak influence on palatal transverse variables and was more strongly affected by environmental factors. However, in the Chaaban et al.<sup>11</sup> study, heritability patterns were retrospectively obtained from the Pearson correlation coefficient and Falconer's heritability test.

Lione et al.<sup>27</sup> reported that the maxillary arch form is determined by tongue pressure. The dental arch usually has an impact on palate form, but we did not find a remarkable environmental influence on the palatal area or volume variability. We did not examine whether the twins in our study were mouth breathers, so it was difficult to evaluate the tongue position and possible impact on the dental arch palate. Our results showed that genetic contributions have a remarkable impact on the variability of palatal parameters.

For patients with complete maxillary growth, the suture of the maxilla is ossified after approximately 13 years<sup>28</sup>, and treatment with conventional RME possibly causes only buccal inclination of the teeth, which can lead to relapse. To expand the palate, surgery and miniscrew-assisted RME may be needed. Palatal suture ossification may occur even at eleven years of age, and chronological age is not reliable for determining suture development<sup>29</sup>. According to our findings, the palatal surface area (*a<sup>2</sup>* = 0.61), palate volume (*a<sup>2</sup>* = 0.69), and maxillary arch depth (*a<sup>2</sup>* = 0.56) are mostly affected by additive genetic factors. These findings suggest that miniscrew-assisted RME should be considered a better choice than conventional RME for maxillary expansion in patients older than 11 years for expansion stability. Although it has been proven that even with MARPE treatment, long-term stability is not reliable, dental and skeletal relapse are still observed over time<sup>30</sup>. The heritability estimates in the area of the first molars (*a<sup>2</sup>* = 0.86) were the highest of all the parameters. These are teeth where the RME appliance is bonded, and due to the strong genetic influence on the width of the dental arch between the first molars, relapse is more likely to occur. The high heritability estimates maxillary intermolar width was reported by Eguchi et al.<sup>7</sup> (0.82) and Hughes et al.<sup>9</sup> (0.87).

The genetic influence on the dental arch width in the canine region is lower (*a<sup>2</sup>* = 0.59), and possibly, environmental factors are more responsible for this transverse dimension. The genetic influence on the dental arch and palatal variables in the canine region showed reduced genetic dominance (ICW *d<sup>2</sup>* = 0.59, ICWG *d<sup>2</sup>* = 0.5, ICH *d<sup>2</sup>* = 0.48). This is in agreement with findings of King et al.<sup>31</sup> and Cassidy et al.<sup>32</sup> reporting estimates of ICW at 0.53 and 0.56 respectively. This can be explained by the stronger environmental influence in this area, such as the position of the tongue, swallowing parafunction and mouth breathing. These abnormal functions can lead to a flatter and narrower palate and maxillary anterior tooth protrusion<sup>33</sup>.

Variable	PC1	PC2	PC3
Dental arch widths at occlusal line			
ICW	0.5	-0.01	<b>0.66</b>
1IPW	<b>0.83</b>	0.13	0.36
2IPW	<b>0.91</b>	0.15	0.13
IMW	<b>0.92</b>	0.16	-0.03
Dental arch widths at gingival line			
ICWG	<b>0.65</b>	0.01	0.47
1IPWG	<b>0.91</b>	0.13	0.04
2IPWG	<b>0.91</b>	0.05	-0.11
IMWG	<b>0.91</b>	0.11	-0.17
Palatal heights			
ICH	0.08	<b>0.52</b>	-0.42
1IPH	0.01	<b>0.92</b>	-0.13
2IPH	-0.01	<b>0.93</b>	0.16
IMH	0.01	<b>0.8</b>	0.28
Maxillary arch depth			
MD	-0.13	0.25	<b>0.72</b>
Palate surface area and volume			
PSA	0.36	<b>0.83</b>	0.29
PV	0.45	<b>0.82</b>	0.1

**Table 4.** Factor loadings after varimax rotation. *1IPH* interfirst premolar palate height, *1IPWG* interfirst premolar width at the gum line, *2IPW* intersecond premolar width, *2IPWG* intersecond premolar width at the gum line, *ICH* intercanine palate height, *ICW* intercanine width, *ICWG* intercanine width at the gum line, *ICWG* interfirst premolar width, *IMH* interfirst molar height, *IMW* interfirst molar width, *IMWG* interfirst molar distance at the gum line, *MD* maxillary depth, *PSA* palate surface area, *PV* palate volume. Factor loadings greater than 0.50 are significant and indicated in bold.

The results of our study may have some clinical relevance and may be useful in predicting the response of different parts of the dental arch to various orthodontic interventions, especially maxillary dental arch expansion. The occlusal variables that are more influenced by genetic factors may be less receptive to corrective procedures; in contrast, traits that are more influenced by environmental factors may be more prone to respond positively and demonstrate a greater level of stability after orthodontic treatment. The most important finding from this study regarding orthotreatment planning is that changes in the dimensions of the dental arch and palate should not surpass the biological limits. The balance between bone, dental, and muscular structures should be maintained, as deviations from the original shape of the dental arch may increase the likelihood of treatment relapse to a genetic norm.

The present study has several strengths, including DNA-based zygosity determination and the use of a model-fitting analysis that allowed for a more accurate partitioning of different sources of variation affecting the palate parameters. This study also has some limitations. The complete dental and medical records were not available for all twins, and a questionnaire was used to determine whether previous orthodontic treatment was undertaken, which may have led to bias. Additionally, this study involved a sample of Lithuanian twins, and thus the generalizability of the findings to other populations may be limited.

## Conclusion

Palatal dimensions have high heritability. The majority of dental arch widths at the occlusal line, palate height, palatal surface area, palate volume and maxillary arch depth were found to be under strong-to-moderate additive genetic control. Maxillary dental arch inter-canine width and widths at gingival line in premolar regions demonstrated dominant genetic determination. Sexual dimorphism was shown, with males exhibiting greater arch width, palate surface area and volume than females.

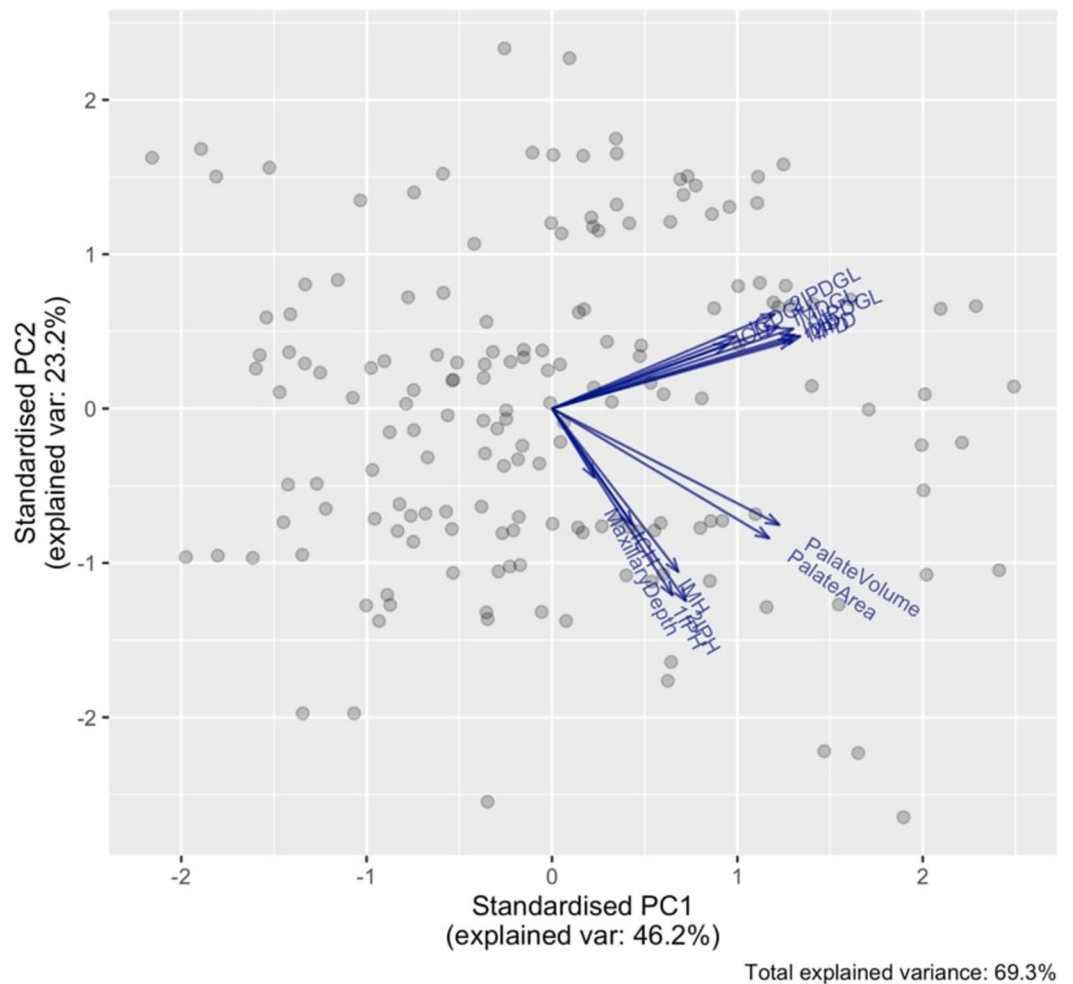
## Methods

### Study sample

The present study sample consisted of dental casts of 50 monozygotic (19 males and 31 females) and 35 dizygotic (19 males and 16 females) twin pairs of the same sex. Twins were selected from the Twin Centre of the Lithuanian University of Health Sciences (LSMU). All twins were of European ancestry. Their mean age was  $17.95 \pm 2.83$  years. The protocol of the study was approved by the Regional Ethical Committee No. BE-2-41, and informed consent was given by the twins and their parents of any participant younger than 18 years. The study was conducted in accordance with relevant guidelines and regulations.

The following inclusion criteria were applied: (1) full adult dentition not including the third molars and (2) maxillary growth largely completed (defined as age > 15). The twins were excluded on the basis of the following





**Fig. 2.** Principal component biplot.

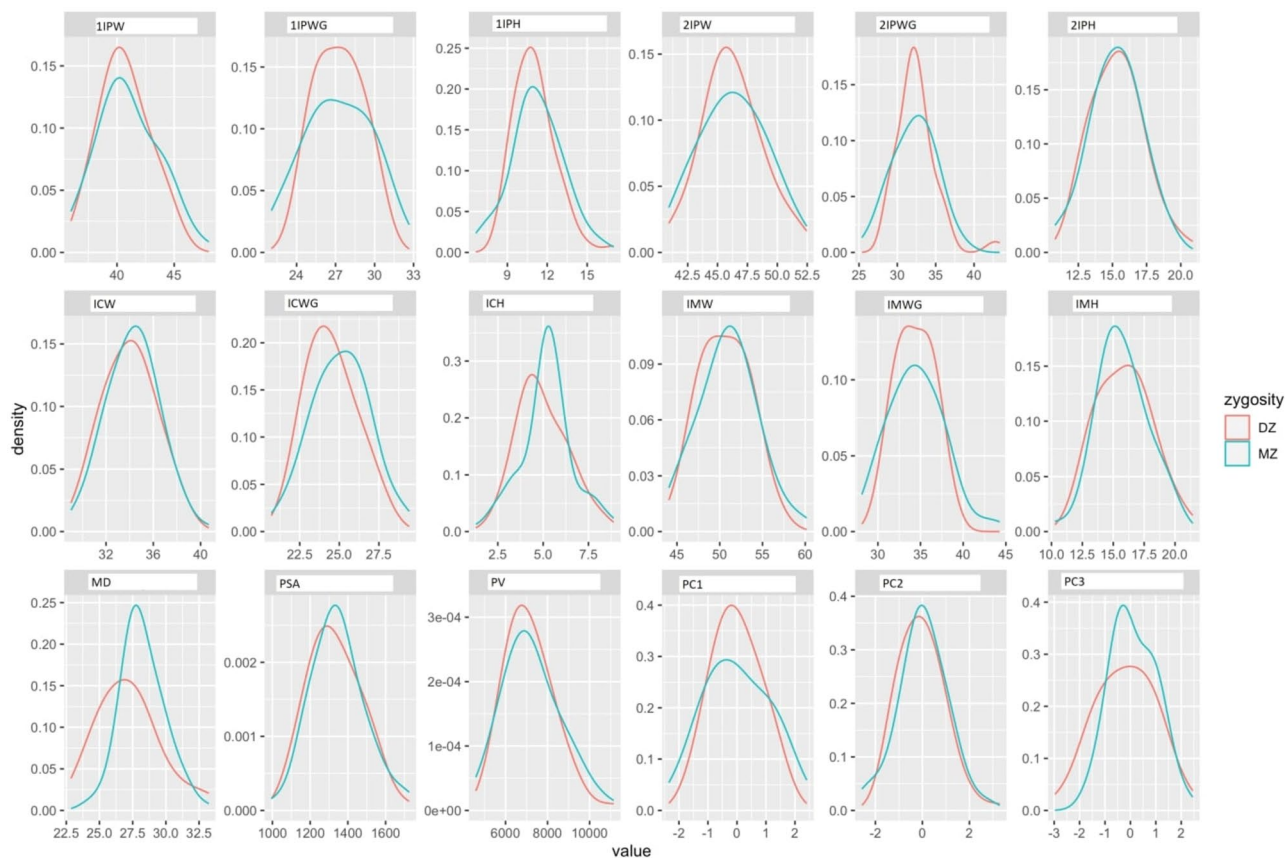
criteria: (1) existing restorations involving landmarks on the cusps and incisal regions, (2) previous orthodontic treatment, (3) poor quality or damaged models, (4) excessive wear of the teeth, or (5) dental anomalies such as supernumerary or missing teeth.

### Dental arch and palate measurements

Alginate dental impressions were obtained from the study participants. A three-dimensional 3Shape scanner (3Shape e3, Copenhagen, Denmark) with a reported accuracy of 7/10  $\mu\text{m}$  was used. (Scan time 18 s, Resolution 2 cameras 5.0 megapixels) was used to obtain 3D data from dental casts (format STL) of maxillary dentition and palate.

The definitions of the measurements used in the study are presented in Table 5. Linear measurements were calculated utilizing the selected dental landmarks as well as the maxillary occlusal plane as a plane of reference (Fig. 4). The maxillary occlusal plane was defined as the midpoint between a line connecting the central point of the incisal edges of the two maxillary central incisors and the mesiobuccal cusp tips of the maxillary first molars (Fig. 4a). The widths at the gingival level were measured connecting the appropriate points at the dentogingival junctions of the teeth on the palatal side (Fig. 4a). The gingival plane and distal plane were used as margins for the palate. The gingival plane was obtained by connecting the midpoints of the dentogingival junction of all permanent teeth on the palatal side (Fig. 4a). The distal plane was created through two points at the distal aspect of the first molars perpendicular to the gingival plane (Fig. 4b). Palate height was measured as the distance between the line connecting the centres of the dentogingival junctions of the canines, first and second premolars, and first molars on the palatal side and the highest point of the palatal vault on the midpalatal rafe (Fig. 4c). The measurements of the palate surface area and palate volume are presented in Fig. 4d and were performed according to the methods proposed by Kecik<sup>34</sup> and Primožič et al.<sup>35</sup>.

All linear landmark-based dimensions were calculated using the open-source universal 3D processing and animation software Blender 3.4.1 with the “3D Print Toolbox”. The digitization of the landmarks was conducted by a single investigator (VP). Prior to data collection, the investigator (VP) was calibrated in the use of the software.



**Fig. 3.** Correlation coefficient densities of palatal variables. *MZ* monozygotic twins, *DZ* dizygotic twins, *ICW* intercanine width, *ICWG* interfirst premolar width, *2IPW* intersecond premolar width, *IMW* interfirst molar width, *ICWG* intercanine width at the gum line, *1IPWG* interfirst premolar width at the gum line, *2IPWG* intersecond premolar width at the gum line, *IMWG* interfirst molar distance at the gum line, *ICH* intercanine palate height, *1IPH* interfirst premolar palate height, *2IPH* intersecond premolar palate height, *IMH* interfirst molar height, *MD* maxillary depth, *PSA* palate surface area, *PV* palate volume.

### Measurement error

Measurements were performed twice on the digital models by the same investigator, with a 1-month time interval on both members of 20 randomly selected twin pairs to determine measurement error. Intraobserver method error was assessed using the intraclass correlation coefficient (ICC) of reliability and the method suggested by Bland and Altman<sup>36</sup>. The estimated random error between the measurements was calculated using the Dahlberg formula:

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

### Zygosity determination

Zygosity determination was carried out using a DNA test. The polymerase chain reaction set AmpFISTR® Identifier® (Applied Biosystems, USA) was used to amplify short tandem repeats, and 15 specific DNA markers (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TROX, D18S51, D5S818, FGA) and an amel fragment of the amelogenin gene were used for comparison of genetic profiles. Zygosity determination using this molecular genetic technique reached 99.9% accuracy<sup>21</sup>.

### Heritability estimation

Heritability was estimated by structural equation modelling (SEM) with the OpenMx software package (<http://openmx.psyc.virginia.edu>) and R code examples provided at <https://github.com/OpenMx/OpenMx>. The variance of a trait was estimated by evaluating the contributions of three factors: the additive genetic factor (A), the shared environment (C), the nonadditive genetic factor (D), and the unique environment (E)<sup>37</sup>. As the C and D components cannot be estimated simultaneously in twins reared together, only the ACE (or ADE) models with two degrees of freedom were tested<sup>38</sup>. Univariate ACE/ADE models were constructed with standardized path coefficients and expected variance and covariance matrices. The goodness of fit of the full and reduced ACE/ADE



Measurements	Definition
Dental arch widths at occlusal line	
ICW—intercanine width	Distance between cusp tips of the canines on the maxillary occlusal plane
1IPW—interfirst premolar width	Distance between buccal cusp tips of the first premolars on the maxillary occlusal plane
2IPW—intersecond premolar width	Distance between buccal cusps tips of the second premolars on the maxillary occlusal plane
IMW—interfirst molar width	Distance between mesiobuccal cusps tips of the first molars on the maxillary occlusal plane
Dental arch widths at gingival line	
ICWG—intercanine width at the gum line	Distance connecting the centres of the dentogingival junctions of canines on the palatal side
1IPWG—interfirst premolar width at the gum line	Distance connecting the centres of the dentogingival junctions of the first premolars on the palatal side
2IPWG—intersecond premolar width at the gum line	Distance connecting the centres of the dentogingival junctions of the second premolars on the palatal side
IMWG—interfirst molar distance at the gum line	Distance connecting the centres of the dentogingival junction of the first molars on the palatal side
Palatal heights	
ICH—intercanine palate height	Distance between the line connecting the centres of the dentogingival junctions of the canines on the palatal side and the highest point of the palatal vault on the midpalatal rafe
1IPH—interfirst premolar palate height	Distance between the line connecting the centres of the dentogingival junctions of the first premolars on the palatal side and the highest point of the palatal vault on the midpalatal rafe
2IPH—intersecond premolar palate height	Distance between the line connecting the centres of the dentogingival junctions of the second premolars on the palatal side and the highest point of the palatal vault on the midpalatal rafe
IMH—interfirst molar palate height	Distance between the line connecting the centres of the dentogingival junctions of the first molars on the palatal side and the highest point of the palatal vault on the midpalatal rafe
Maxillary arch depth, palate surface area and volume	
MD—maxillary depth	Distance between a tangent from the incisal edge of the central incisors and a line connecting the contact point between the first molar mesiobuccal cusps
PSA—palate surface area	Palate surface area below the gingival plane and limited by the distal plane. Gingival plane constructed by connecting the line of the midpoints of the dentogingival junction of all teeth (except second molars). The distal plane constructed perpendicular to the occlusal plane passing from the two most distal points corresponding to the distal surface of the first molars
PV—palate volume	Volume below the gingival plane and limited by the palate surface and distal plane. Gingival plane constructed by connecting the line of the midpoints of the dentogingival junction of all teeth (except second molars). The distal plane constructed perpendicular to the occlusal plane passing from the two most distal points corresponding to the distal surface of the first molars

**Table 5.** Definitions of the measurements.

models were compared with a univariate saturated twin model imposing equal means and variance restriction across twins and zygoty to maximize information.

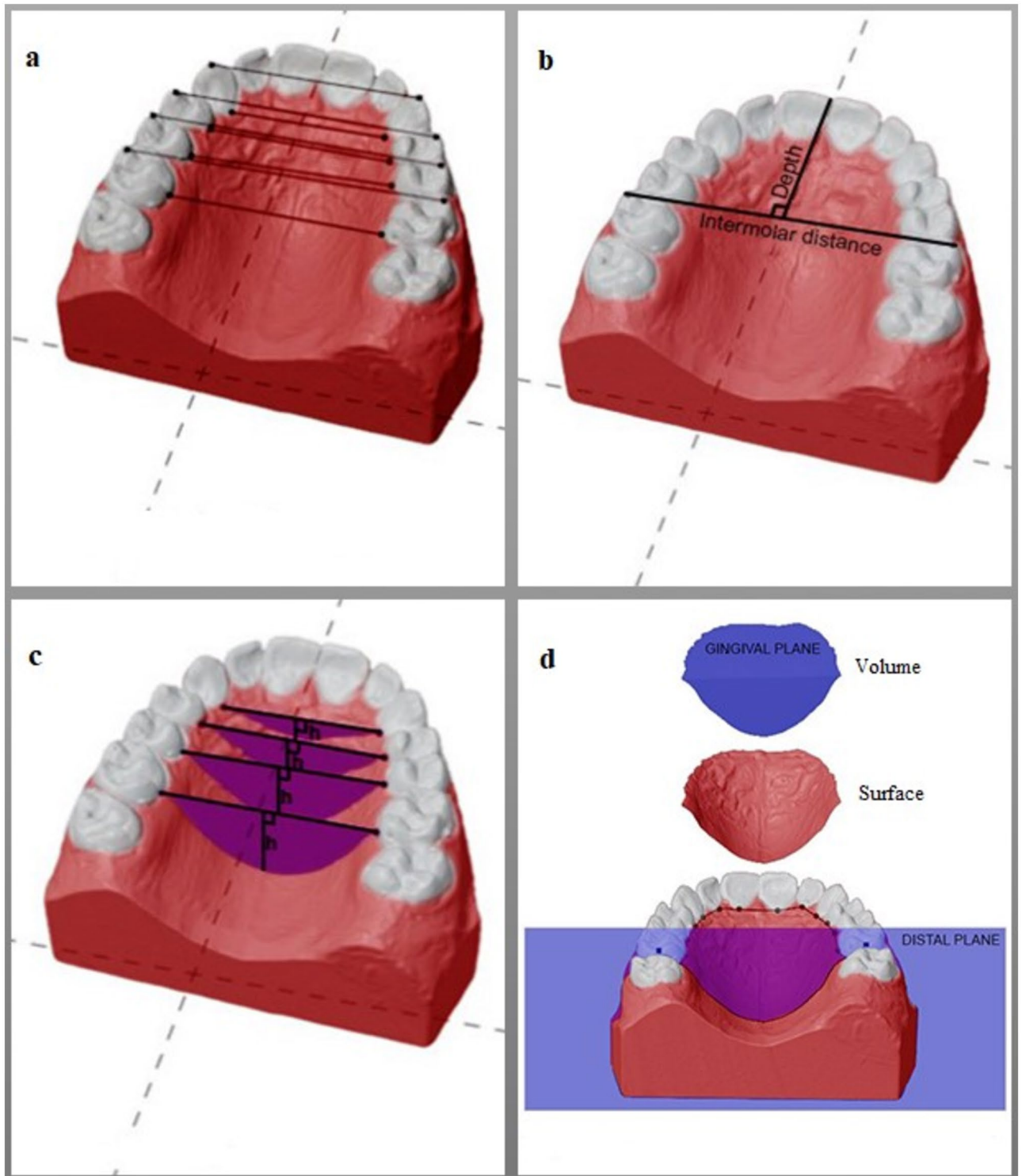
The Akaike information criterion (AIC) statistic and the difference in the chi-square ( $\chi^2$ ) value relative to the chance in degrees of freedom provided an indication of the models' goodness of fit. The most parsimonious model (lowest AIC value) to explain the observed variance was selected<sup>39</sup>.

### Principal component analysis

Principal component analysis (PCA) of the palatal measurements was performed using the “Psych package” (Procedures of Psychological, Psychometric and Personality Research) to reduce dimensionality and to assess correlations between variables. The principal components were rotated using varimax rotation. A variable was considered a component if the absolute value of the component loading was greater than 0.5.

### Statistical analysis

Descriptive statistics included the mean and standard deviation. The normality of the data distribution was tested with the Shapiro–Wilk test. Parametric Student's t tests were applied for comparisons of quantitative variables between two independent groups. Pearson's correlation coefficient was calculated, and the difference between the two correlation coefficients was computed. Statistical analyses were performed in the statistical computing environment R (version 4.3.3). P values less than 0.05 were considered to indicate statistical significance.



**Fig. 4.** The upper dental arch parameters. **(a)** The upper dental arch widths were defined as the distances between the two reference points at the occlusal and dento-gingival junctions. The interdental distances were measured between the cusp tips of the canines, first premolars, second premolars, and first molars at the occlusal plane and between the centers of the dento-gingival junction of the canines, first premolars, second premolars and first molars at the palatal side. **(b)** The upper dental arch depth. Distance between a tangent from the incisal edge of the central incisors and a line connecting the contact point between the first molar mesiobuccal cusps. **(c)** Palate height. Distance between the line connecting the centers of the dento-gingival junctions of second premolars on the palatal side and the highest point of the palatal vault on the midpalatal rafe. **(d)** Palate surface area—area below the gingival plane and limited by the distal plane; palate volume—volume below the gingival plane and limited by the palate surface and distal plane.

## Data availability

The data underlying this article will be shared upon reasonable request to the corresponding author.

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## Author contributions

M.Š.—conceptualization, data curation, formal analysis, investigation, methodology, visualization, writing of the original draft, review and editing. V.P. – data curation, methodology. M.Š. – data collection, formal analysis. A.Š. – supervision, formal analysis, writing, review and editing, project administration, data collection, funding acquisition. S.J. – statistical analysis and formal analysis. K.L. – formal analysis, funding acquisition, supervision, writing, review and editing, project administration. All authors reviewed the manuscript.

## Competing interests

The authors declare no competing interests.

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