ABSTRACT
Objective: To resolve how the preferred chewing side (PCS) affects facial asymmetry in twins, whether there are differences between monozygotic (MZ) and dizygotic (DZ) twins, and whether the twins with PCS have more asymmetric faces compared to symmetrically chewing twins.

Material and methods: The study included 106 Lithuanian twin pairs of the same sex, 59 MZ and 47 DZ pairs. The data were analysed from facial 3D images and manually added landmarks. 3D images were analysed by Rapidform2006 software and statistical analyses were done by using the R software environment version 4.1.0.

Results: The contralateral effect of PCS and larger chin side was dominant among right and non-right side chewing twins. Being female increased the whole face symmetry.

Conclusion: The volume of the chin becomes larger on the side opposite to the twins’ habitual chewing side. As the results are quite similar in both twin types, functional factors are more prominent than heredity.

Introduction
Chewing is a complex sum of many combined horizontal and vertical movements of the jaw. These are called chewing cycles [1,2]. Chewing activity is controlled by a central pattern generator which is located in the brainstem. Peripheral stimuli, such as chewing forces, as well as feedback loops supplement the precise function of the masticatory system [1–5]. Most people have a preferred chewing side (PCS) so that chewing occurs mainly on one side, left or right [6]. However, there is no universal definition of PCS so there are countless methods to study the subject [3,7,8].

Facial asymmetry may have various aetiologies and it may appear in bone, dental or soft tissue, which can be analysed clinically or from documents and anamnestic history of the patient [9]. The halves of the face differ in size, shape and placement of facial landmarks [10]. Facial asymmetry can be divided into fluctuating and directional type. In the former, the asymmetric facial features occur randomly between the left and right side, whereas in the latter, a specific anatomical feature emerges as systematically bigger in one half of the face compared to the other [9,11,12]. In addition, functional laterality disturbance and very uncommon mirror-image type of asymmetry appear among identical twins [13–15]. The origin of the facial asymmetry can be congenital, indiscernible formation of the structures during growth, or acquired asymmetry due to displacements of the jaw, accident or disease. Also, other factors, such as prone sleep position and tooth extractions, contribute to facial asymmetry [16]. In any case, regardless of the origin of the asymmetry, both genetic and environmental factors, such as chewing, interact in forming the face. Consequently, perfectly symmetrical faces do not exist [9,10,17,18]. PCS affects facial asymmetry in many ways: bones and masticatory muscles are strengthened on one side, the condyles on the jaw move asymmetrically, and the apex of the chin twists. At the same time, the possibility of temporomandibular disorders (TMD) increases [19–23].

Chewing can take place on one side permanently when all chewing cycles occur on the same side. Predominant unilateral chewing is thought to occur when over 70% of chewing cycles appear on the same side [6]. Many researchers have thought that the first chewing cycle reveals a patient’s PCS. This is because the neuromuscular system is responsible for the shifting of food from one side to another. When chewing continues, local variables and other factors begin to affect chewing, in which case determination of the PCS becomes more difficult [6,7,24].

The prevalence of PCS ranges from 45% to 98%, and using the right side instead of the left is much more common among people [5,6,19,24]. However, there are numerous factors affecting PCS. The central nervous system controls chewing sidedness if a person does not have relevant peripheral asymmetries [3,5]. Genes may also affect the forming of PCS [13]. Gender and race also affect PCS; women have been found to chew more asymmetrically compared to men, and Caucasian children to have more right-side wear than...
African-American children [19,25]. Among twins, the second-born twin has PCS on the right side more frequently than the first-born twin [26]. Young children usually chew quite asymmetrically because they are incapable of moving food from one side to the other [6]. In addition, adults aged 40–69 years have been found to chew more asymmetrically than younger and older adults [19]. Chewing sidedness is also partly determined by dental status and food texture [5,7,24].

Genetic and environmental factors influence the shape of the face with different proportions, structures of the middle facial third being the most heritable. The differences can be seen between age and gender groups, as well as individually born children beside twins. The effect of environmental factors is largest for the mandibular ramus height and horizontal facial asymmetry. Also, national differences have been found among some genetic traits [17,27]. The development of the face is symmetrical for healthy individuals in the youth, so environmental factors might play greater role in facial asymmetry [10]. Genes might have an effect to craniofacial disorders and development, which can be manifested as asymmetry in the face [28,29]. For example, hemifacial microsomia is linked to multiple maternities, which suggest that genes are important part in the development of the condition [30,31]. Also, Beckwith-Wiedemann Syndrome, Russell-Silver Syndrome and oculo-auriculo-vertebral spectrum abnormalities are associated with twinning [32].

Compared to other twin types, identical twins have the most similar facial features [33–35]. Among all twin types, the biggest differences in the amount of facial asymmetry can be seen in the area of the lower facial third [35]. Facial asymmetry is the most obvious in the area of the jaw [9,35,36]. Pogonion, the protruding landmark in the middle of the jaw, is the most asymmetric landmark on the face [37]. By comparing different twin types, it might be possible to determine whether larger volume of the chin in relation to facial midsagittal plane is caused by environmental or genetic factors.

The aim of this study was to solve how chewing side preference affects facial asymmetry in twins. We identified the larger side of the chin and compared it to the subjects’ chewing side preference. We also compared the results between monozygotic (MZ) and dizygotic (DZ) twins and deduced the magnitude of genetic and environmental factors. We were also interested in whether the twins with PCS have more asymmetric faces compared to symmetrically chewing twins. The hypothesis is that the larger side of the chin is opposite to the PCS in most of the individuals. The additional hypothesis is that if the asymmetry has genetic background rather than environmental, there should be the difference between the MZ twins and DZ twins. It is worth doing this research, because specially orthodontists consider patient’s occlusion and face a lot, thinking if the facial asymmetry is genetical or not and how much function and habits affect to that.

**Materials and methods**

This study included 106 Lithuanian twin pairs of the same sex where 59 pairs were MZ and the rest were DZ twins. The twins were selected to the study on the basis of their willingness to participate in the research conducted by the Lithuanian University of Health Sciences Twin Centre. The exclusion criteria were facial trauma, permanent dental extraction, congenital disorders as well as pregnancy. The mean age of the twins was 20.2 years, ranging from 8.6 to 45.7. The number of male and female pairs was 46 and 60, respectively. For each twin pair, a blinded DNA test (AmpFlSTR® Identifiler® PCR) was done to determine true zygosity. The test compared genetic profiles, which ensures quick and authentic determination of zygosity by utilizing specific DNA markers (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TROX, D18S51, D5S818, FGA) and Amel fragment STRs (short tandem repeats). In the asymmetry analyses, one male pair was excluded because of facial hair which caused erroneous roughness on the 3D surface. Furthermore, one female pair was excluded because chewing side preference had not been registered. Thus, the number of twin pairs with both 3D asymmetry analysis and PCS is 104.

The faces of the twins were recorded by a 3dMDFace system (3dMD, Atlanta) based on stereophotogrammetry technology in Kaunas, Lithuania in 2011–2013. During imaging, the twins were asked to sit on a chair and look ahead to a mirror to maintain as neutral a facial expression as possible. The subjects’ colourful clothes were covered by a black scarf and hair was kept out of the way by a hairband. Rapidform2006 (INUS Technology, Inc., Seoul, South Korea) software was used to process and analyse the images. At first, distinct parts on the facial surface such as hair and clothes were removed. The facial position was standardized by setting the twins in the same reference frame, and the origin was set as a point halfway between endocanthion of the left and right eye [38]. Twenty-one facial soft tissue landmarks [39] had been identified manually in a previous study [40]. This study utilizes eight of those landmarks (Figure 1).

Next two asymmetry scores were measured as in Djordjevic et al. [10]. Each original 3D face was mirrored across the sagittal plane (YZ plane). The original surface and the mirrored one were then superimposed with the best-fit technique on the facial area above the subnasal. The average distance (AD) between the original and mirrored 3D model was calculated for the whole face and separately for the lower face (below the mid-lip line). Furthermore, the matching of the original and mirrored face was measured by symmetry percentage (SP) of the facial area where the distance from the mirrored surface did not exceed 0.5 mm. In addition, SP was analysed separately for the whole face and lower face. The greater the AD value, the more asymmetry the face has, whereas greater SP value means a more symmetrical face.

We also developed a new method for measuring chin asymmetry from the facial 3D model. We divided the chin area into two solid figures and calculated their volumes (Figure 1). Asymmetry is quantified by the ratio of these volumes in a similar way as was done by Meyer-Marcotty et al. [41] for measuring cranial asymmetry in their study. First, the chin region is separated from the face by two planes so that
the planes close the region. The one plane is parallel to the
coronal plane (XY plane) and goes through the posterior
exoncanthion point, and the other goes through labiale infe-
rius and is parallel to the transverse plane (XZ plane). This
closed 3D object is then divided into two parts by the sagit-
tal plane, and the volume for these parts is calculated. Chin
volume asymmetry score (CVAS) is defined as the larger vol-
ume divided by the smaller one. The distance of the pogon-
ion landmark from the sagittal plane was also calculated to
quantify the chin asymmetry.

The chewing side preference was first studied by asking.
If the subject was not able to give an answer, they were
asked to chew gum and their chewing was followed visually
for a minute by the researcher. If the result was unclear, the
test was repeated. If the result did not become clear even
then, it was recorded that the subject used both sides for
chewing. In PCS, left and both side chewers have been com-
bined as being non-right side chewers. This is because there
were a relatively low number of left side chewing twins and
it is a common habit in the twin studies concerning lateral-
ities [13,42].

The 3dMDFace system is reliable and its geometric accu-
rance is < 0.2 mm [40]. The twins were asked to retain a neu-
tral facial expression during imaging, and reproducibility of
neutral expression also has good accuracy, with 0.17 mm
error [40]. Landmarks, such as pogonion, were added to
facial shells manually so minor human errors are inevitable.
The accuracy of landmarks has been reported to range from
0.39 to 1.49 mm [43].

**Statistical analysis**

Statistical analyses were done using R software environment
version 4.1.0. Effect of chewing side preference on larger
chin side was explored by using generalized linear mixed
model (GLMM) with logit link function. In GLMM, depend-
ence within twin pairs can be considered in covariance struc-
tures. GLMM was done separately for MZ, DZ, male, female
and all the twins with larger chin side as dependent and PCS
as independent variable. Zygosity, sex or both were included
in models as confounder variables. Facial asymmetry
between symmetrical and asymmetrical chewers was com-
pared with linear mixed model (LMM). Again, twin pair cor-
relation was noticed by intercept with random effect and
zygosity and sex were confounder variable. Possible genetic
effect on PCS and facial asymmetry was examined by com-
paring pairwise tetrachoric and intraclass correlations
between MZ and DZ twins. Difference of correlations
between MZ and DZ twins was tested by using Fisher's r-to-
Z transformation. Chosen level of significance for p value
was .05.

**Results**

Based on GLMM estimates in Table 1, it seems that all twins
have exp(0.67)=1.95 times higher odds for having bigger
chin volume on the opposite side from PCS. MZ twins have
even higher odds exp(0.86)=2.36, but the model is little dif-
f erent so straight comparison is not that simple. DZ, male
and female twins have odds over 1.68 but estimates are not
statistically significant.

Table 2 shows that chewing symmetrically decreases
asymmetry in each asymmetry score, even though results are
not statistically significant. With SP measured from lower
face, symmetrical chewing has almost significant estimate
(\( p = .057 \)). Note that in SP greater percentage means better
facial symmetry. Confounder variable sex has significant
effect on AD and SP measured from whole face. Being
female decrease asymmetry in both AD and SP.
Table 1. Logistic mixed effect model estimating effect of PCS on larger chin side.  

|                     | All twins          | MZ twins          | DZ twins          |
|---------------------|--------------------|-------------------|-------------------|
| (Intercept)         | 24.23 – 2.89 – 0.45| 2.76 – 2.38 – 1.18| 3.62 – 2.74 |
| PCS opposite        | 0.67 – 0.08 – 1.25 | 0.86 – 0.09 – 1.63| 0.52 – 0.39 – 1.43|
| Zygosity            | 1.91 – 0.39 – 0.76 | 0.42 – 0.13 – 0.01| 1.20 – 0.983 |
| Sex                 | 0.25 – 0.33 – 0.83 | 0.21 – 1.00 – 0.57| 0.79 – 0.13 – 1.71|

More precisely, model shows how the chewing side effects on the risk of having larger chin volume on the opposite side. Twin pair has random effect on intercept and zygosity and sex are confounder variables. Bold p values denote statistical significance. MZ: monozygotic; DZ: dizygotic; CI: confidence interval; PCS: preferred chewing side.

Table 2. Effect of symmetrical chewing on facial asymmetry using linear mixed model.  

| AD whole face (mm)            | AD lower face (mm)            | SP whole face (%) |
|--------------------------------|--------------------------------|-------------------|
| Symmetrical chewing            |                                |                   |
| Coefficient CI 95 % p value    | Coefficient CI 95 % p value    | Coefficient CI 95 % p value |
| Intercept                      | 0.78 – 0.66 – 0.90 <.001       | 1.28 – 0.95 – 1.61 <.001 | 45.87 – 39.44 – 52.30 <.001 |
| Symmetrical chewing            |                                |                   |
| Coefficient CI 95 % p value    | Coefficient CI 95 % p value    | Coefficient CI 95 % p value |
| Intercept                      | 24.23 – 12.41 – 36.05 <.001    | 1.10 – 1.06 – 1.14 <.001 | 1.26 – 0.75 – 1.77 <.001 |
| Symmetrical chewing            |                                |                   |
| Coefficient CI 95 % p value    | Coefficient CI 95 % p value    | Coefficient CI 95 % p value |
| Intercept                      | 6.00 – 0.10 – 12.10 .057       | –0.01 – 0.03 – 0.01 .433 | –0.05 – 0.32 – 0.22 .736 |
| Symmetrical chewing            |                                |                   |
| Coefficient CI 95 % p value    | Coefficient CI 95 % p value    | Coefficient CI 95 % p value |
| Intercept                      | 1.37 – 4.06 – 6.80 .632        | –0.01 – 0.03 – 0.01 .147 | –0.12 – 0.36 – 0.12 .314 |
| Symmetrical chewing            |                                |                   |
| Coefficient CI 95 % p value    | Coefficient CI 95 % p value    | Coefficient CI 95 % p value |
| Intercept                      | 2.85 – 2.62 – 8.32 .309        | 0.00 – 0.02 – 0.02 .927 | 0.01 – 0.23 – 0.25 .933 |

Both MZ and DZ twins did not have pairwise correlation within PCS and facial asymmetry scores in means of statistical significance, except MZ twins had weak correlation (0.32) in SP measured from whole face (Table 3). Still, the comparison to DZ twin correlation did not give significant difference (p = .108).

Discusssion

This study found that chewing asymmetrically has an effect on bigger side of the chin. PCS increases the risk that opposite side of the chin becomes bigger. The result is statistically significant among all twins and MZ twins. There can be many reasons that could cause these results. It has been found in previous studies that left-sided weakness of the jaw is an example of well-preserved anatomical asymmetry and for most people, the right side is regnant in both lateral and frontal plane [11,44]. One explaining factor could be the bones of the masticatory system and especially their growth in length. Unilateral chewing burdens the masticatory system unevenly [19]. Chewing on PCS strengthens bones and muscles unilaterally, possibly creating facial asymmetry [9,11,19,22]. The activation of osteoblasts and osteoclasts differs on both sides of the facial midline which may create facial asymmetry.

Another alternative explanation to asymmetrical facial appearance might be found from temporomandibular joint (TMJ) and its asymmetrical function. The load of TMJ is greater on the non-preferred side compared to the preferred side [19]. This is because the movements of the condyles are unequally distributed. Chewing on the same side most of the time leads to asymmetrical trajectory of the condyle, which can predispose to TMD or changes in normal function [19,21]. The function of the condyles on both sides of the facial midline becomes different. One moves a lot at a time while the other can, for example, get stuck and so predispose to asymmetrical function of the masticatory system and facial appearance. If unilateral chewing continues for a long time, the route of the condyles can shift permanently, further intensifying the skeletal effect.

We found that PCS did not affect significantly on facial asymmetry. However, in all asymmetry scores, facial symmetry increased when a twin chewed symmetrically. This needs a closer look, so we are going to research the subject with a larger study sample in the future. Anyhow, gender affected whole face asymmetry scores, albeit we did not get statistically significant results from the chin region. Being female increases facial symmetry, as found in previous studies. Differences in facial asymmetry could be due to different soft tissue profile between genders. Females have been found to have quite round faces with thicker soft tissue, whereas males have more prominent faces resembling a square [40]. That is why males have on average more asymmetrical faces than females. 3D images capture expressly soft tissue so asymmetry can be more easily seen in male images compared to female images.
We did not find differences in correlation coefficients between MZ and DZ twins, so genetical component in facial asymmetry cannot be seen. Also, this topic should be researched more specifically later on.

One of the exclusion criteria in this study was permanent dental extraction. In some studies, the lack of a tooth has not been observed to influence chewing side preference [24]. However, missing a back tooth has been linked to PCS [5]. As the chipping up of food in the grinding phase is mostly done by the back teeth, the lack of a tooth impairs chewing performance. To compensate the situation, chewing is moved to the other side and chewing becomes unilateral. Ignoring this fact could have led to bias in the results. Because statistical evidence is on quite low level, most of the evidence here indicates to do more research on this topic with larger study sample.

The weakness of this study concerns the method of data collection on PCS and the number of twins and especially the low amount of left side chewers. Even though many of the twins were immediately sure about their chewing side preference, for others it can be difficult to figure out. Being monitored by an unknown person (the researcher) as well as an unfamiliar and unsettling situation can change the normal function of the masticatory system. Furthermore, the subjects’ awareness of the study and the texture of the food may have contributed to the results. We used standardized gum particles, but hard food indicates PCS more powerfully [2,5].

**Conclusions**

The volume of the chin becomes larger on the side opposite to the preferred chewing side. Being female increases the whole face symmetry. The results are quite similar with both twin types, indicating that functional factors are more significant than heredity but genetical factors are not overruled either.

**Disclosure statement**

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**Table 3.** Comparing pairwise correlation in PCS (tetrachoric) and facial asymmetry (ICC) between MZ and DZ twins.

|                     | MZ                      | DZ                      | MZ vs DZ                |
|---------------------|-------------------------|-------------------------|-------------------------|
|                     | Correlation coefficient | CI 95 %                 | p Value                 | Correlation coefficient | CI 95 %                 | p Value | Absolut correlation difference | p Value |
| PCS                 | -0.02                   | -0.43 – 0.39            | .912                    | -0.02                   | -0.48 – 0.43            | .920    | 0.00                         | .500    |
| AD whole face       | 0.08                    | 0.21 – 0.37             | .526                    | -0.06                   | -0.35 – 0.23            | .678    | 0.14                         | .233    |
| AD lower face       | 0.01                    | -0.24 – 0.26            | .942                    | -0.18                   | -0.45 – 0.12            | .244    | 0.19                         | 0.177   |
| SP whole face       | 0.32                    | 0.07–0.53               | .014                    | 0.08                    | -0.21 – 0.36            | .584    | 0.24                         | 0.108   |
| SP lower face       | 0.10                    | -0.15 – 0.34            | .432                    | -0.17                   | -0.44 – 0.12            | .254    | 0.27                         | 0.090   |
| CVAS                | 0.07                    | -0.18 – 0.31            | .582                    | -0.11                   | -0.39 – 0.19            | .482    | 0.18                         | 0.192   |
| Pg distance from midline | -0.14                          | -0.39 – 0.13          | .308                    | 0.06                    | -0.23 – 0.35            | .670    | 0.20                         | 0.157   |

Bold p-values denote statistical significance. MZ: monozygotic; DZ: dizygotic; CI: confidence interval; PCS: preferred chewing side; AD: average distance; SP: symmetry percentage; CVAS: Chin Volume Asymmetry Score; Pg: pogonion; TC: tetrachoric correlation; ICC: intraclass correlation.

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