Characterization of lip prints in a Portuguese twins' population

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ABSTRACT

Lip print patterns are referred to as unique to each individual, but controversy exists surrounding twins. In this study, the lip prints of 19 pairs of monozygotic and 47 pairs of dizygotic twins were studied. The left lower lip was photographed and the furrows were classified using Renaud's classification. Results showed the same lip pattern was found only in one monozygotic pair (5.3%) and in 4 dizygotic pairs (8.5%), and no significant statistical differences were found between groups ($p>0.05$). In monozygotic twins only type C furrows presence displayed statistical significant differences ($p=0.034$). As for dizygotic twins, there were statistical significant differences in the frequency of type A ($p=0.005$) and type G furrows ($p=0.018$). As for the most common types, both groups displayed a higher prevalence of vertical furrows (type B: 97.4% and 96.8%, type A: 86.8% and 87.2%, in monozygotic and dizygotic, respectively). The least frequent furrow type was type I and type E in monozygotic (2.6% and 5.3%, respectively) and types E, F and I, in dizygotic (6.4%, 7.4%. and 7.4%, respectively). Our results seem to point out that lip print patterns should be useful carefully in twins' identification.

INTRODUCTION

Lip prints usefulness in human identification has been widely explored. Their ability to distinguish among individuals is largely responsible for their use in forensic matters [1, 2]. Yet, some issues regarding singularity and inheritance have been raised, as some authors pointed out a positive resemblance in lip print patterns among family members [3] and others report no similarity between twins [4, 5] or with their parents.

Suzuki and Tsuchihashi, in 1971, [6] carried out the first twin study on lip prints, and analysed 18 pairs of monozygotic twins. Their results indicated lip prints of the twins were extremely similar and that their characteristics were inherited from either the father or the mother. Mc Donnel, in 1972, reported lip print patterns between two identical twins were quite different from each other [7]. In 1974, Tsuchihashi extended the original research and reported that lip print patterns are unique to each individual. This researcher studied 1364 Japanese natives, including 49 pairs of identical twins, and concluded that it is possible to distinguish different persons through cheiloscopic examination. Actually, the Japanese researcher stated that a detailed comparison of twin pairs proved not to be identical despite the great similarity between the lip grooves. In this study, which occurred during a period of three years,

Tsuchihashi also demonstrated the unchangeability of lip print patterns[2]. The same conclusions were reached by Hirthe et al. [8], analysing 76 families, including 22 pairs of monozygotic and 17 pairs of dizygotic twins. Similarly, Thakur et al. [9], referred to a comparative study performed by Schnuth and Marry Lee on 150 individuals including five pairs of identical twins; in this investigation the authors found that lip prints were not identical in the case of identical twins, but similarities of lip prints between parents and children were found. Further studies involving twins also revealed that lip prints were not exactly identical [10-12] although some of the characteristics were inherited from the parents [10]. However, other more recent studies concluded that lip print patterns were unique in monozygotic twins [13], and that no similarity was found with their parents.[4]

The aim of the present study was to perform a comparative study of lip print patterns in the monozygotic and dizygotic twins in a Portuguese population. It was intended to assess the intra-pair differences and variations of lip prints in monozygotic and dizygotic twins, and therefore contribute to the theory of uniqueness of lip print patterns.

MATERIAL AND METHODS

The studied sample had 19 pairs of monozygotic twins and 47 pairs of dizygotic twins. The sample's distribution by sex is depicted in table 1.

Table 1. Sex distribution of the participants, n (%)

Sex	Monozygotic	Dizygotic
Male	24 (63.2)	50 (53.2)
Female	14 (36.8)	44 (46.8)

The 66 pairs of twins were part of the *Geração XXI* ("Generation XXI") cohort, from the Public Health Institute of the University of Porto. *Geração XXI* consists in the first cohort in Portugal, whose objective is prenatal characterization and the post-natal development, identifying determinants in Health with interest in childhood, adolescence and adulthood. The selected twins were of European ancestry and aged between 11 and 13 years old. This study included monozygotic and dizygotic twins, whose zigotia was proven before its inclusion in the study. All participants who have congenital or acquired disorders, medically relevant conditions were excluded, as were children with a history of orofacial trauma.

Informed consent was obtained from all individual participants included in the study, and the investigation was submitted and approved by the Ethics Commission of the Faculty of Dental Medicine of Porto University (reference number 000030 – 10/01/2017) and by the Portuguese Data Protection Authority (reference number 64.567.634 – 13/10/2017).

The furrows on the left lower lip were photographed and analysed using Renaud's classification[14] (Figure 1 and Table 2). Each furrow was classified for its presence or absence. The analysis of the left lower lip alone was applied, as it simplifies the classification process, and has been applied previously. [15, 16]

Reproducibility or intra-observer error was evaluated assessing agreement between 20 randomly selected photographs examined twice by the same examiner 1 month apart. Cohen's kappa (k) was used to evaluate the quality of the agreement, as suggested by Landis and Koch.[17] The agreement was almost perfect with k =0.856. Repeatability or inter-observer error was evaluated by measuring the agreement between 20 randomly selected photographs examined by two different examiners. Again, the agreement was almost perfect with k =0.802.

Figure 1. Lip print types according Renaud's Classification

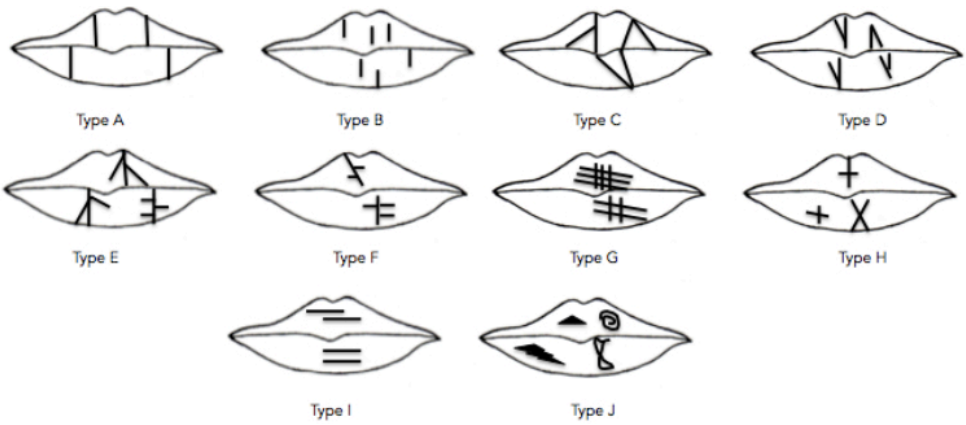

Table 2. Renaud's classification

Classification	Furrow type
A	Complete vertical
B	Incomplete vertical
C	Complete bifurcated
D	Incomplete bifurcated
E	Complete branched
F	Incomplete branched
G	Reticular pattern
H	X or coma form
I	Horizontal
J	Other forms (ellipse, triangle)

Statistical analysis was performed using SPSS 25.0 software (SPSS Inc., Chicago, IL). Pearsons' Chi-square test was used to compare qualitative data and determine statistical significance. The level of statistical significance was set at $p \leq 0.05$.

RESULTS

Lip furrows distribution in the left lower lip is depicted in table 3.

The furrows' frequency was quite similar in both groups. As a matter of fact, in both groups, the most prevalent furrow type was the vertical incomplete (type B), with percentages of frequency over 95% in both groups (97.4% and 96.8%, in monozygotic and dizygotic, respectively). The second most frequent furrow type was, in both groups, the type A (86.8% and 87.2%, in monozygotic and dizygotic, respectively). The least frequent furrow type was the horizontal type (type I) in monozygotic (2.6%) and type E in the dizygotic group (6.4%), although both types depicted low frequencies in both groups. Overall, vertical furrows were present in 46.7% of the total sample.

Overall, the same lip pattern was found only in one monozygotic pair (5.3%) and in 4 dizygotic pairs (8.5%), and no significant statistical differences were found between groups ($p>0.05$).

As to dissimilarities among twins in the different groups, in monozygotic twins only type C furrows presence displayed statistically significant differences ($p=0.034$) (table 4).

As for dizygotic twins, there were statistically significant differences in the frequency of type A ($p=0.005$) and type G furrows ($p=0.018$) (table 5).

Table 3. Type of lip furrows in monozygotic and dizygotic twins, n(%)

Type	Monozygotic, n=38	Dizygotic, n=94	TOTAL, n=520
A	33 (86.8)	82(87.2)	115(22.1)
B	37(97.4)	91(96.8)	128(24.6)
C	16(42.1)	30(31.9)	46(8.8)
D	20(52.6)	54(57.45)	74(14.2)
E	2(5.3)	6(6.4)	8(1.5)
F	3(7.9)	7(7.4)	10(1.9)
G	8(21.1)	31(33.0)	39(7.5)
H	8(21.1)	30(32.9)	38(7.3)
I	1(2.6)	7(7.4)	8(1.5)
J	14(36.8)	40(42.5)	54(10.4)

Table 4. Differences in lip furrows frequency in monozygotic twins, n(%); p value (significant values in bold) (n.c. – not calculated)

Type	Pair 1, n=19	Pair 2, n=19	p
A	17(89.5)	16(84.2)	0.110
B	19(100)	18(94.7)	n.c.
C	9(47.4)	5(26.3)	**0.034**
D	12(63.2)	7(36.8)	0.585
E	1(5.3)	1(5.3)	1
F	3(15.8)	14(73.7)	n.c
G	5(26.3)	3(15.8)	1
H	3(15.8)	4(21.1)	1
I	1(5.3)	0(0)	n.c
J	7(36.8)	7(36.8)	1

Table 5. Differences in lip furrows frequency in dizygotic twins, n(%); p value (significant values in bold)

Type	Pair 1, n=47	Pair 2, n=47	p
A	38(80.9)	44(93.6)	**0.005**
B	46(97.9)	45(95.7)	1.000
C	17(36.2)	12(25.5)	0.306
D	28(59.6)	25(53.2)	1.000
E	2(4.3)	4(8.5)	1.000
F	1(2.1)	6(12.8)	1.000
G	18(38.3)	13(27.7)	**0.018**
H	14(29.8)	15(31.9)	1.000
I	1(2.1)	5(10.6)	1.000
J	21(44.7)	19(40.4)	0.361

DISCUSSION

To the best of our knowledge, this is the first characterization of lip print patterns in a Portuguese twins' population.

Lip prints have been used as a genetic marker in many congenital and clinical diseases [18], and some lip print patterns have been related with cleft lip and palate deformities.[19, 20] Cardoso Fernandes and co-workers[21] have linked a specific type of lip furrow (type I) with Down Syndrome individuals, referring that statistical significant differences occurred between these individuals and their siblings ($p<0.001$). According to the authors this link between type I furrows and Down Syndrome individuals may imply a lower potential of cheiloscopic identification due to the poor divergence of labial phenotypes among these individuals.

Other patterns, however, have been considered as a genetic marker for the health of the offspring if

it appears in parents' lips.[20, 22] Furthermore, lip print patterns have been associated with hypertension, as the frequencies of the branched and undifferentiated patterns were higher in the hypertensive than in the normotensive [23]. It has also been referred to that the association of lip print patterns with smoking habits, previous labial trauma with no residual scars, parafunctional oral habits, or being a professional brass player showed no statistical significance[24], suggesting environmental factors play little of a role in lip print patterns' morphology.

The idea behind studying monozygotic and dizygotic twins was to compare genetic and environmental influences in lip print formation. Monozygotic (or identical) twins are derived from the division of a single zygote, whereas dizygotic (or fraternal) twins are derived from the fertilization of two independently released ova, and are not more genetically alike than ordinary brothers and sisters.[25]

Our results seem to point out that lip print patterns may not be unique even in monozygotic twins. This is because, in spite of only studying the presence or absence of the types of furrows, one pair had the same elements. We cannot argue about the specific location of the furrows in this particular case, and therefore cannot state if singularity exists in this particular case. Moreover, discussion exists about if all the lip print must be studied to study a lip print, particularly for sex estimation[26]. For identification purposes, the methodology we have used is the one adopted by most authors to determine singularity [1, 27-32].

Nevertheless, it can still be stated that in the remaining 94.7%, singularity did exist in monozygotic twins. As for dizygotic, 8.5% (4 pairs) did have the same element in the left lower lip. Once more, the specific location or number of these elements was not evaluated, but we are able to state that in the remaining 91.5% singularity was proven.

As for differences in lip patterns, in monozygotic twins, only type C furrows presence displayed differences, whereas, among dizygotic twins, there were differences in the frequency of type A and type G furrows. So, it is possible that the presence of complete bifurcated grooves can be used for distinguishing monozygotic twins, whereas, in dizygotic, complete vertical and reticular patterns can separate dizygotic twins. In theory, there should be more dissimilarities in dizygotic twins, and distinguishing them by lip print patterns is not needed. Yet, in monozygotic twins, using lip print patterns to distinguish between pairs has been reported [25, 33].

Considering the differences found in lip print patterns, both in monozygotic and dizygotic twins, it is fair to assume that similarities are rare, as most pairs, monozygotic or dizygotic, showed that lip patterns did not resemble each other. So, it may be that the theory of uniqueness can be applied and the potential for identification in twins may exist. It must, however, be underlined that the studied sample, particularly in what concerns monozygotic twins, was small, and thus a more robust hypothesis cannot be achieved.

As for the most common types, both groups displayed a higher prevalence of vertical furrows (type B and A). The least frequent furrow type was type I and type E in monozygotic, and types E, F and I, in dizygotic. These numbers may suggest a connection between lip furrows' morphological features and population affinity, as other authors have suggested [34], since all the sample had Portuguese ancestry. For instance, Moshfeghi et al. [30]reported that furrows displaying other forms (type J) were the most common in both Iranian males and females. This same type was the most prevalent in male and female Goan students [32] . As for Mangaloreans, Jeergal et al. [29] referred that incomplete vertical grooves were the most prevalent in both sexes. Conversely, these furrows were the least common among adults of Sebha, Libya; the most common in this population were the complete vertical furrows [31].

Among Nigerians, Adamu et al. [35] found that the most prevalent furrow was "other type", followed by intersected groove pattern and being the least frequent type incomplete vertical grooves.

All of these studies suggest that a particular type of furrow is more likely in a given population. Nonetheless, among a Portuguese population, Costa and Caldas [27] referred that branched furrows were the most prevalent, which does not agree with our data. As a matter of fact, in this study the incomplete vertical groves, the most prevalent in our sample, were one of the least prevalent, raising doubts if lip print patterns can in fact be used in population affinity estimation. Other authors have described discrepancies in population studies concerning the most prevalent furrow type, as well. Abdel Aziz et al. [36] referred in their study of an Egyptian population that

bifurcated and branched grooves were the predominant patterns of lip prints. Yet, in another study conducted in Dakahlia, in Egypt, the highest recorded lip print among the studied individuals was type A (complete vertical) in both sexes [5]. The authors justify discrepancies with different sample sizes used in the two studies (n=60 vs. n=955). In our case, different size samples was also an issue (n=132 vs. n=66).

Overall, our results seem to point to the lack of lip print pattern singularity, with 94.7%, of monozygotic twins presenting unique lip print patterns, and, in dizygotic, in 91.5% singularity was proven. As for the most common types, both groups displayed a higher prevalence of vertical furrows, which differs from the previous data concerning the Portuguese population, raising doubts if lip print patterns can in fact be used in population affinity estimation. We do realize that the studied sample, particularly concerning the monozygotic twins, was small, and a more robust hypothesis can only be achieved in further studies, with larger samples.

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