ANNALS OF HUMAN BIOLOGY
https://doi.org/10.1080/03014460.2019.1687752

RESEARCH PAPER

Is the "Habsburg jaw" related to inbreeding?

Román Vilas[a], Francisco C. Ceballos[b], Laila Al-Soufi[a], Raúl González-García[c], Carlos Moreno[c], Manuel Moreno[c], Laura Villanueva[c], Luis Ruiz[c], Jesús Mateos[c], David González[c], Jennifer Ruiz[c], Aitor Cinza[c], Florencio Monje[c] and Gonzalo Álvarez[a]

[a]Department of Zoology, Genetics and Physical Anthropology, University of Santiago de Compostela, Santiago de Compostela, Spain; [b]Sydney Brenner Institute for Molecular Bioscience, University of the Witwatersrand, Johannesburg, South Africa; [c]Department of Oral and Maxillofacial Surgery, University Hospital Infanta Cristina, Badajoz, Spain

ABSTRACT

Background: The "Habsburg jaw" has long been associated with inbreeding due to the high prevalence of consanguineous marriages in the Habsburg dynasty. However, it is thought that mandibular prognathism (MP) is under the influence of a dominant major gene.

Aim: To investigate the relationship between the "Habsburg jaw" and the pedigree-based inbreeding coefficient (*F*) as a relative measure of genome homozygosity.

Subjects and methods: The degree of MP and maxillary deficiency (MD) of 15 members of the Habsburg dynasty was quantified through the clinical analysis of 18 dysmorphic features diagnosed from 66 portraits.

Results: A statistically significant correlation ($r = 0.711$, $p = 0.003$) between MP and MD was observed among individuals. Only MP showed a statistically significant positive regression on *F* as evidenced from univariate analysis ($b = 6.36 \pm 3.34$, $p = 0.040$) and multivariate analysis (PCA) performed from single dysmorphic features ($b = 14.10 \pm 6.62$, $p = 0.027$, for the first PC).

Conclusion: Both MP and MD are generally involved in the "Habsburg jaw." The results showed a greater sensitivity to inbreeding for the lower third of the face and suggest a positive association between the "Habsburg jaw" and homozygosity and therefore a basically recessive inheritance pattern.

ARTICLE HISTORY
Received 4 July 2019
Revised 30 July 2019
Accepted 16 September 2019

KEYWORDS
Inbreeding; human face; Habsburg jaw

Introduction

The "Habsburg jaw," traditionally considered an example of mandibular prognathism (MP), owes its name to its high prevalence in the Habsburgs. The members of this dynasty are characterised by other signs of facial deformity, including an everted lower lip, also known as the "Habsburg lip," and noses with a dorsal hump and overhanging nasal tip, also known as the "Habsburg nose," which are often indicative of maxillary deficiency (MD) (Peacock et al. 2014). Although MP is one of the best-known examples of an inherited facial genetic trait in humans, its genetic basis remains largely unclear. Some of the first studies on the inheritance of the "Habsburg jaw" suggest either autosomal-recessive (Downs 1928; Iwaki 1938) or autosomal-dominant inheritance (Rubbrecht 1939; Stiles and Luke 1953; Kraus et al. 1959). The influence of a dominant major gene is often concluded from extensive segregation studies in which prognathism is treated as a discrete trait (Wolff et al. 1993; El-Gheriani et al. 2003; Cruz et al. 2008). These studies include both simple segregation analysis of the trait in the offspring of European noble families, including the Habsburgs, from the examination of pictures (Wolff et al. 1993) and complex segregation analysis (CSA) of MP diagnosed from cephalometric radiographs, dental models, and photographs in contemporary families (El-Gheriani et al. 2003; Cruz et al. 2008).

Presumably because of extremely high levels of inbreeding (Álvarez et al. 2009; Álvarez and Ceballos 2015), members of the Habsburg dynasty had extreme facial phenotypes, including the "Habsburg jaw" (Grabb et al. 1968; Hodge 1977; Wolff et al. 1993; Richmond et al. 2018). However, the available information does not allow a conclusion about the causal relationship between inbreeding and facial deformity. On the one hand, the high prevalence of the trait in the Habsburg family could be simply a consequence of low effective population size, so that it is at low frequency outside the Habsburg lineage, but is rapidly increased within the lineage due to genetic drift. On the other hand, an autosomal dominant pattern of inheritance does not support that the "Habsburg jaw" is an effect of inbreeding. According to the classic model of inbreeding, a positive association between facial deformity and the inbreeding coefficient (*F*) would suggest that the gene/genes involved in its development are recessive because the main effect of mating between relatives is increasing the homozygosity and therefore the expression of recessive phenotypes (Cavalli-Sforza and Bodmer 1971; Falconer and Mackay 1996; Charlesworth

CONTACT Román Vilas roman.vilas@usc.es Department of Zoology, Genetics and Physical Anthropology, University of Santiago de Compostela, Campus Vida, Santiago de Compostela, 15782, Spain

Supplementary data for this article is available online at 10.1080/03014460.2019.1687752.

and Willis 2009). In fact, empirical evidence shows that the pedigree-based *F* is highly correlated with both wide-genome homozygosity estimated by DNA sequencing (Hoffman et al. 2014) and the proportion of the autosomal genome identical by descendent in runs of homozygosity (ROHs) (McQuillan et al. 2008; Ceballos et al. 2018). In contrast, a negative association would support the widely extended hypothesis of a dominant major gene influencing the trait. The association, whether positive or negative, is not expected in the cases of purely additive gene action or dominance effects occurring in opposite directions (i.e. no directional dominance). Therefore, the study of the relationship between the magnitude of MP and MD and *F* among individuals of the dynasty could provide the first indication of a direct relationship between inbreeding and facial morphology, as well as useful information on its genetic basis. Because the variance of inbreeding in the Habsburg dynasty is remarkably high (*F* ranging from nearly zero to more than 0.25) in comparison to most outbred human populations (Álvarez et al. 2009; Álvarez and Ceballos 2015), the dynasty serves as a "human laboratory" in which to investigate inbreeding effects (Ceballos and Álvarez 2013). Here, we study in the "Habsburg laboratory" a total of 18 dysmorphic features of MP and MD, whose usefulness for diagnosis from portraits has been previously tested (Peacock et al. 2014), in order to determine if the "Habsburg jaw" is related to inbreeding and to investigate its genetic basis.

Subjects and methods

Selection of paintings

A total of 66 publicly available online portraits of members of the Habsburg dynasty, preserved by some of the most important art museums in the world (Supplementary Table S1), were used to carry out the diagnosis of 11 dysmorphic features of MD and 7 of MP. The successive Spanish Habsburg kings and their wives were studied: Philip I (1478–1506), Joana of Spain (1479–1555), Charles I (1500–1558), Isabella of Portugal (1503–1539), Philip II (1527–1598), Elisabeth of Valois (1546–1568), Anna of Austria (1549–1580), Philip III (1578–1621), Margaret of Austria (1584–1611), Philip IV (1605–1665), Elisabeth of France (1602–1644), Mariana of Austria (1634–1696) and Charles II (1661–1700), as well as the parents of Philip I, the Holy Roman Emperor Maximilian I (1459–1519) and Mary of Burgundy (1457–1482). The first two wives of Philip II, his double first cousin Mary of Portugal (1527–1545), and Mary I of England (1516–1558), the cousin of his father, were not included in the study because the number of reliable portraits available was insufficient. The paintings were selected according to two basic criteria: (1) the availability of high quality images from important art museums (almost 70% are photographs of paintings currently preserved in the Prado Museum in Madrid and the Kunsthistorisches Museum in Vienna), which has allowed access to reliable information regarding each of the paintings and their attribution to a particular painter (Supplementary Table S1); (2) in most cases it has been historically confirmed that the painter personally saw the individual portrayed.

Clinical analysis

Diagnosis of MD and MP from portraits was performed from the detection of 11 dysmorphic features for MD and 7 for MP as previously described (Peacock et al. 2014). The features of maxillary hypoplasia were as follows: scleral show (MD1), exorbitism (MD2), peri-alar hollowing (MD3), prominent nasolabial folds (MD4), narrow nasal base (MD5), convex nasal ridge (MD6), overhanging nasal tip (MD7), obtuse nasolabial angle (MD8), thin upper lip vermilion (MD9), overclosed mandible (MD10), and everted or prominent lower lip (MD11); the features of mandibular hyperplasia were as follows: increased thyromental distance (MP1), taut submental soft tissue (MP2), obtuse gonial angle (MP3), shallow labiomental fold (MP4), acute chin-throat angle (MP5), increased depth of lower facial third (MP6), and soft tissue pogonion >5 mm anterior to zero meridian of González-Ulloa (MP7). These features can be observed in portraits of members of different families of the Habsburg lineage and some of them have been popularly identified as "Habsburg nose" (particularly, MD6 and MD7) and "Habsburg lip" (MD11).

Images were examined independently by 10 maxillofacial surgeons for the presence of the 18 dysmorphic features. Each feature was given a score of 1 if present and 0 if not present or indeterminate. The information on the dysmorphic features was additively combined into indices of MD and MP, so that the maximum total score for MD would be 11 and 7 for MP. The number of paintings examined for each member of the Habsburgs ranged from 2 to 6 with a mean of 4.4. The investigators examined each painting independently and each investigator gave a score for each Habsburg by averaging the scores corresponding to the different paintings of that individual (Supplementary Table S2). The total score for each king/queen was obtained as an average of the scores of the investigators.

Statistical analysis

In order to evaluate the impact of inbreeding on MD and MP, the inbreeding coefficient (*F*) computed from a large-scale family tree of the Habsburgs, which included more than 6000 individuals belonging to more than 20 parent–offspring generations, was used (Álvarez and Ceballos 2015). The relation of the facial deformity, whether defined as MP or MD, with *F* was studied by treating both traits as quantitative characters. Considering a single diallelic locus (B) involved in facial deformity where B_2 allele increases facial deformity with respect to the B_1 allele, and using the scale of genotypic values: 0 for B_1B_1; $(1+k)a$ for B_1B_2 and $2a$ for B_2B_2, where a is the additive effect and k provides a measure of dominance, the change in mean value in an inbred population relative to a panmictic population is: $M_F = M_O - (2pqak)F$, where p and q are the frequencies of the alleles B_1 and B_2, respectively (Lynch and Walsh 1998). If there is no dominance and B_1 and B_2 alleles are completely additive ($k=0$), the change of mean value due to inbreeding is not expected. If the B_2 allele, which increases facial deformity, is completely dominant ($k=1$), a reduction in facial deformity

is expected under inbreeding. On the contrary, if the B_2 allele is completely recessive ($k = -1$), an increase in facial deformity (i.e. inbreeding depression) should be found. Considering all the loci that affect the trait, the total inbreeding effect is $2F\sum p_i q_i a_i k_i$. Since the sign of k_i may vary from locus to locus, the occurrence of significant inbreeding depression requires directional dominance (Falconer and Mackay 1996; Lynch and Walsh 1998). Note that $k = d/a$, where d is the measure of dominance in the Falconer's scale.

The magnitude of MD and MP as a function of F was expressed in terms of the linear regression coefficient (b) and two rank correlation coefficients: the Kendall's Tau coefficient and the Spearman's coefficient (r_s). The nonparametric tests based on the rank correlation coefficients are useful for detecting the association between variables in the case of a non-linear relationship, a possibility that is important to consider (Ceballos and Álvarez 2013). Both coefficients of rank correlation were used because they are sensitive to different types of departure from independence. Thus, Tau coefficient weights each discordance in rank equally, while r_s gives greater weight to pairs of ranks that are more different (Sokal and Rohlf 1995). It is known that men of the Habsburg family were more severely affected by the "Habsburg jaw" than women (Chang et al. 2006). In the present study, differences between sexes were statistically significant for MP ($t = 3.38$; $p = 0.008$), but not for MD ($t = 1.70$; $p = 0.113$). Therefore, the total scores for both deformities were adjusted for the effects of sex using linear regression, which resulted in adjusted phenotypes (i.e. residuals) for kings and queens. In the same way, the analyses of the 18 single dysmorphic features for MD and MP were performed with scores adjusted by sex.

Given the high correlation observed among dysmorphic features, principal component analysis (PCA) was performed on the 11 features of MD and 7 of MP in order to reduce the data set to a few linearly uncorrelated variables or principal components (PCs). The raw data of the multivariate analyses were averages of scores from the maxillofacial surgeons for each single dysmorphic feature; therefore, PCA was carried out from variables that are approximately normally distributed. PCA was performed using singular value decomposition (SVD) of the data matrix (Manly and Navarro 2017). In addition, it was performed from the sample correlation matrix instead of the covariance matrix because the standardisation of the original variables ensures that they all have equal weight. Although variables all share a common measurement scale, they showed important differences in variance, which could be partially explained by differences in the degree of uncertainty of the diagnosis that is based on the subjective, although expert, determination of the presence or absence of features of different nature. The PCs values for MD and MP were used in further analyses to detect variation in the data that could be related to inbreeding. Most statistical analyses were performed with SPSS software (IBM SPSS Statistics v. 20) and the PCA was carried out through the statistical software R (R Foundation for Statistical Computing 2011, http://www.R-project.org).

Results

Quantification of facial deformity and association between deformities

The clinical analysis of portraits of members of the Habsburg dynasty was carried out in order to quantify the degree of MD and MP and investigate its relation with consanguinity. Scores of MD and MP, as well as F previously computed from a large-scale family tree (Álvarez and Ceballos 2015), are given in Table 1. Mean values of MD and MP were 3.59 ± 0.42 and 2.39 ± 0.38, respectively. MD ranged from 1.41 (Mary of Burgundy) to 6.41 (Margaret of Austria) and MP ranged from 0.84 (Mary of Burgundy) to 5.06 (Philip IV). In general, the lowest values of MD and MP were observed in non-Habsburg queens, such as Mary of Burgundy, Isabella of Portugal, and Elisabeth of Valois. MD was particularly pronounced in Maximilian I, Charles I, Margaret of Austria, Philip IV and Charles II, but the last four individuals also showed strong MP. In fact, a statistically significant correlation between MD and MP among individuals was detected ($r = 0.758$, $p = 0.001$ and $r = 0.711$, $p = 0.003$ for unadjusted and adjusted data by sex, respectively). The quantification of the facial deformity was highly correlated with previously published information (Peacock et al. 2014) for both MD ($r = 0.881$; $p = 0.009$) and MP ($r = 0.842$; $p = 0.017$). Since the clinical diagnosis from different portraits of the same members of the Habsburg dynasty was performed independently by different authors, the high correlation detected between diagnoses from very different studies suggests that the methodology is capturing individual variation objectively. This result supports the hypothesis that fundamental properties of natural faces are preserved in paintings of the face, particularly if such representations belong to the Baroque, a cultural movement characterised by a realistic approach to the human face (Brown and Garrido 1998), despite differences between artistic representations and natural faces (Graham et al. 2014).

Table 1. Scores of maxillary deficiency (MD) and mandibular prognathism (MP) and inbreeding coefficient (F) for the Habsburg kings and their wives.

King/Queen	F	MD	MP
Maximilian I	0.0003	5.54	2.20
Mary of Burgundy	0.0766	1.41	0.84
Philip I	0.0253	2.25	1.35
Joana of Castile	0.0394	3.25	1.02
Charles I	0.0375	5.02	4.61
Isabella of Portugal	0.1006	1.97	1.42
Philip II	0.1234	3.22	3.03
Elisabeth of Valois	0.0013	1.59	1.15
Anna of Austria	0.1064	2.73	1.34
Philip III	0.2177	3.10	3.17
Margaret of Austria	0.1391	6.41	3.22
Philip IV	0.1145	5.85	5.06
Elisabeth of France	0.0076	2.88	1.27
Mariana of Austria	0.1559	3.56	1.37
Charles II	0.2539	5.13	4.72
Mean ± SE	0.0933 ± 0.0200	3.59 ± 0.42	2.39 ± 0.38
Kings	0.1104 ± 0.0368	4.30 ± 0.53	3.45 ± 0.53
Queens	0.0784 ± 0.0205	2.98 ± 0.56	1.45 ± 0.26

Association between facial deformity and inbreeding

The relationship between facial deformity (scores of MD and MP adjusted by sex) and inbreeding for the Habsburg kings and queens was evaluated in terms of the linear regression coefficient and two non-parametric rank correlations, which were used because that relationship could be non-linear. A statistically significant positive association between the degree of MP and *F* was detected through both linear regression ($b = 6.36 \pm 3.34$, $p = 0.040$) and the non-parametric tests (Table 2; Figure 1). A positive association between MD and *F* was also observed, although it was not statistically significant (Table 2; Figure 1). Similar results were obtained with scores from Peacock et al. (2014) (Supplementary Figure S1). Differences in *F* among individuals explained 22% of the variation of MP and only 4% of the variation of MD ($r^2 = 0.218$ and 0.036 for MP and MD, respectively). The effect of inbreeding on single dysmorphic features of MD and MP was also investigated (Figure 2). All dysmorphic features of MP except MP2 showed a significant positive association with *F* through the Tau nonparametric test. The regression coefficients for MP3, MP5, and MP7 were statistically significant, while MP1, MP4, and MP6 were close to statistical significance. Only two dysmorphic features of MD (MD1 and MD11) were statistically significant (Figure 2).

Multivariate analysis of facial deformity and inbreeding

Most pairs of dysmorphic features of MP showed statistically significant positive correlations (14/21; 66.7%) while only 30.9% (17/55) were significant in the case of MD. 28.6% of the correlations (22/77) between MD and MP features were significant, although most of them involved MD1, MD10, and MD11. In general, those features that correlated with *F* were highly correlated with each other (Figure 3). Principal component analysis (PCA) of the 11 dysmorphic features of MD and 7 of MP was performed in order to reduce the original variables to a small number of indices or principal components (PCs). These PCs could be useful to capture effects of inbreeding on facial deformity in a different way to a previously proposed index (Peacock et al. 2014), which equally weighs each dysmorphic feature. The first 3 PCs of MD explained 76.7% of the variation and the first 2 PCs of MP explained 87.6% of the variation. The first PC of MP, which explained 66.1% of the variation, basically corresponds to the index mentioned above after excluding MP2. Thus, all coefficients of the variables of MP are positive and nearly equal in magnitude excepting the coefficient of MP2 (Table 3; Figure 4). The values for the first PC as a function of *F* showed a strong and statistically significant regression ($b = 14.10 \pm 6.62$; $p = 0.027$). According to this, individuals with high *F* (over the mean value, $F > 0.0933$) presented high values for the first PC while individuals with *F* below the mean had lower values for this PC (Figure 4). On the other hand, the first PC for MD, which accounted for the 48.8% of variation, did not show a statistically significant association with *F*, but the second and third PCs, which explained 14.5 and 14.0% of variation, respectively, showed a significant association with *F* (Table 3; Figure 4). The variables MD11 for PC2 and MD1 and MD8 for PC3 had a great influence on these PCs due to their large negative coefficients, which explains the negative association between both PCs and *F* (Table 3; Figure 4). However, it is important to realise that the signs of the coefficients of a given PC could be reversed and that PC is still measuring exactly the same aspect of the data, although in the opposite direction (Manly and Navarro 2017). Therefore, the results suggest some effect of inbreeding on MD. In fact, MD1 and MD11 were the two features of MD that presented the strongest association with *F*.

Table 2. Relationship between both deformities, maxillary deficiency (MD) and mandibular prognathism (MP), and the inbreeding coefficient (*F*) expressed in terms of two rank correlation coefficients, the Kendall's Tau coefficient and the Spearman's coefficient (r_s), and the linear regression coefficient (*b*).

	MD	*p*	MP	*p*
Tau	0.143	0.229	0.390	0.021
r_s	0.221	0.214	0.550	0.017
$b \pm$ SE	3.56 ± 5.11	0.250	6.36 ± 3.34	0.040

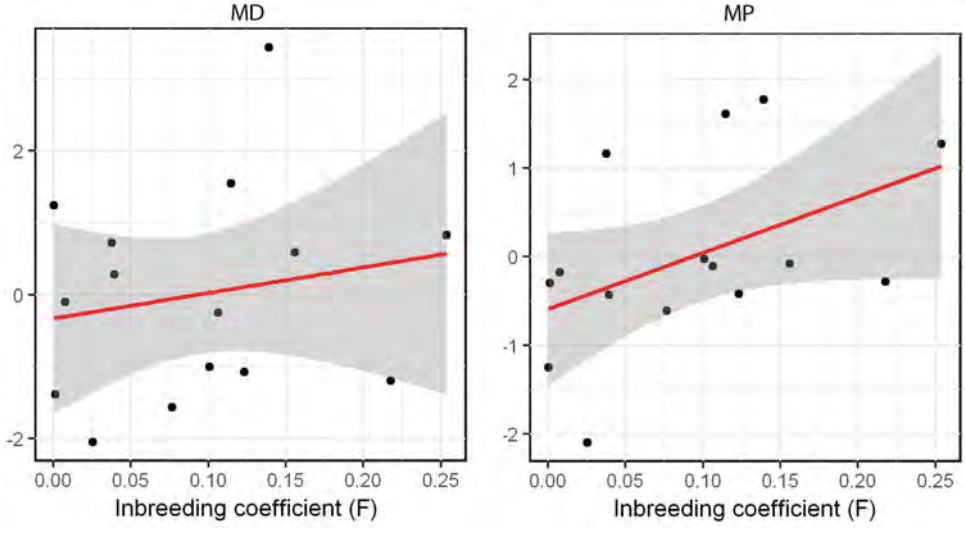

Figure 1. Scores of maxillary deficiency (MD) and mandibular prognathism (MP) as a function of the inbreeding coefficient (*F*). The regression lines with 95% confidence interval (shaded areas) are shown.

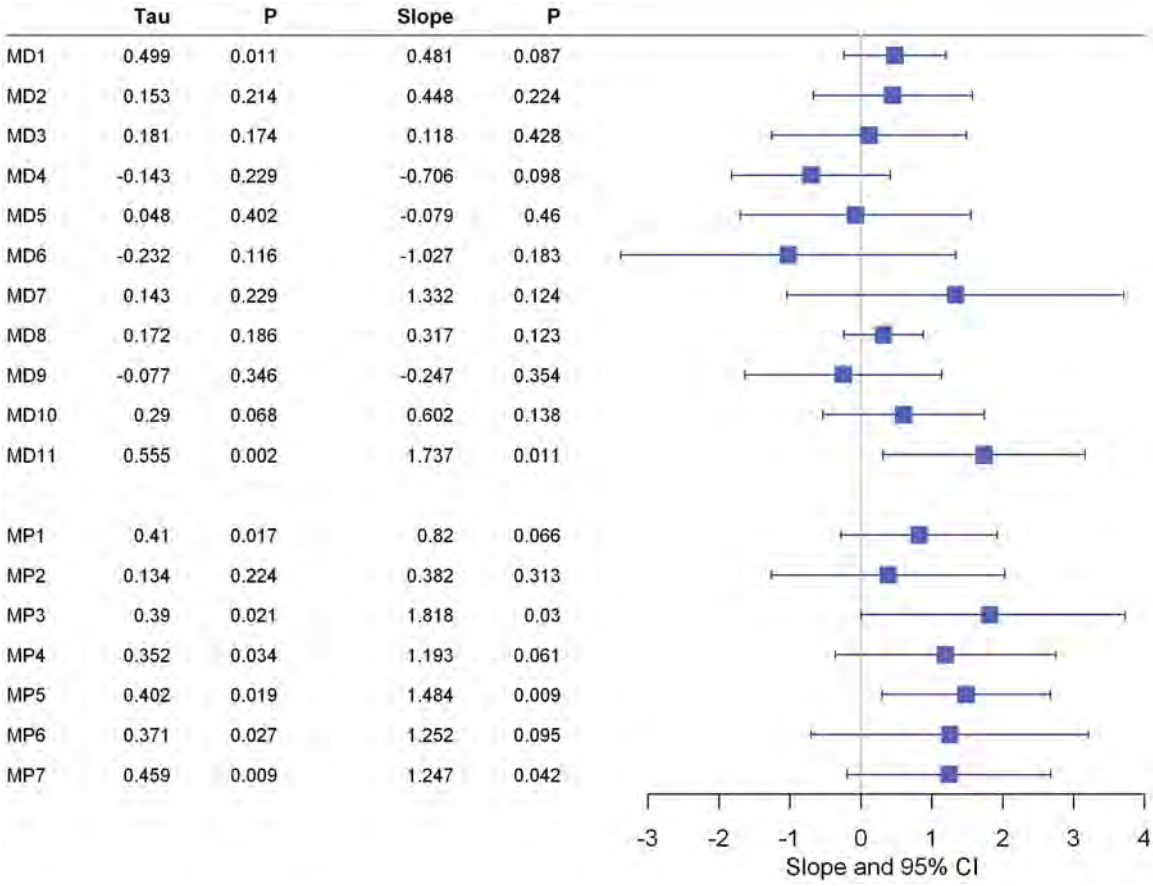

Figure 2. Forest plot of the relationship between *F* and dysmorphic features of MD (MD1–MD11) and MP (MP1–MP7). Linear regression coefficient and Kendall's Tau coefficient of rank correlations are shown.

Discussion

Our species is characterised by an extraordinary facial diversity and an impressive ability to recognise, memorise and portray faces. However, in addition to the large variation within and among populations, facial morphology is characterised by a remarkably similarity within families. This resemblance suggests the importance of genetic factors in the development of the human face and is confirmed by the recent identification of patterns of global-to-local genetic effects on facial shape highlighted by several loci (Claes et al. 2018). One of the best examples of an inherited facial genetic trait in humans is the protrusion of the mandible (Wolff et al. 1993), a deformity that can be due to true mandibular overgrowth (i.e. absolute MP) and poor development of the maxilla with retrusion of the midface (i.e. relative MP) (Jacobson et al. 1974). A recent analysis of dysmorphic features of MD and MP identified in portraits of the Spanish Habsburgs kings suggests that the prognathic appearance of the Habsburg family is largely due to MD (Peacock et al. 2014). This hypothesis is consistent with results from the cephalometric analysis of Joanna of Austria (1547–1578), a member of the Habsburg lineage (Lippi et al. 2012; Giuffra et al. 2014). In the present study, we found that MD and MP are highly correlated ($r = 0.711$, $p = 0.003$), which suggests that the "Habsburg jaw" generally is a condition characterised by both deformities, although the degree of each one may be very different in some individuals (Table 1). This result is consistent with the hypothesis that the "Habsburg jaw" is primarily indicating a Class III skeletical pattern due to MD and therefore relative MP (Peacock et al. 2014).

Since the phenotypic correlation between characters is often an approximate estimate of its genetic correlation (i.e. correlation of breeding values or additive genetic correlation) (Cheverud 1988; Roff 1995, 1996), the high correlation observed between MD and MP suggests a common genetic basis for these traits, either because both are under the influence of the same genes (i.e. pleiotropy) or different genes at linkage disequilibrium. This is consistent with recent evidence of high phenotypic and genetic correlations for many features of the human face (Cole et al. 2017). However, the phenotypic correlation could also be due to correlation between genotypes of different loci caused by the combination of inbreeding and linkage (Crow and Kimura 1970; Hedrick 2011). In the case of the Habsburg lineage, phenotypic correlations due to inbreeding are even possible with unlinked loci because individuals vary greatly in their *F* value (i.e. non-uniform inbreeding) (Crow and Kimura 1970).

A clear statistically significant positive association between MP and *F* among members of the Habsburg dynasty was detected for the first time ($b = 6.36 \pm 3.34$, $p = 0.040$) (Figure 1). In addition, all single dysmorphic features of MP showed a positive association with *F*, being statistically significant in six out of seven (Figure 2). The same trend was observed in MD, although it is not statistically significant. Two features of

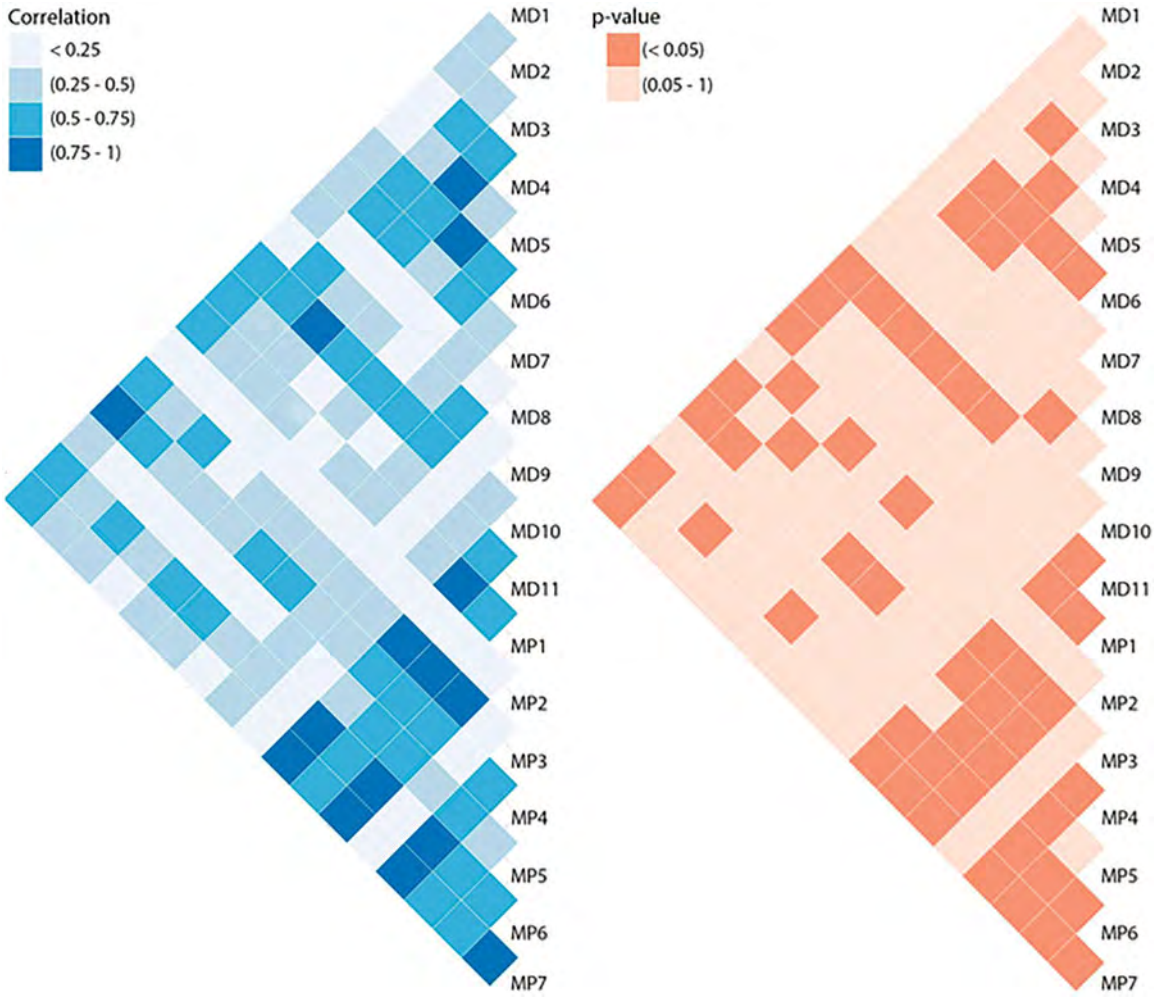

Figure 3. Pairwise correlation coefficients diagram for dysmorphic features of MD and MP. Correlation coefficients (left) and their corresponding probabilities (right) are shown.

Table 3. Principal component analysis of 11 dysmorphic features for maxillary deficiency (MD1–MD11) and 7 for mandibular prognathism (MP1–MP7). Proportion of the variance (Φ) explained by each principal component (PC) and coefficients for the first three and two PCs for MD and MP, respectively, are given.

PC	Φ	MD1	MD2	MD3	MD4	MD5	MD6	MD7	MD8	MD9	MD10	MD11	Tau	*p*	*b* ± SE	*p*
1	48.2	0.265	0.333	0.362	0.302	0.321	0.346	0.296	0.083	0.238	0.385	0.273	0.143	0.229	4.59 ± 8.13	0.291
2	14.5	−0.204	0.150	−0.071	0.505	−0.048	0.430	−0.247	0.368	−0.095	−0.132	−0.515	−0.486	0.006	−8.10 ± 3.93	0.030
3	14.0	−0.483	0.097	0.031	0.150	0.069	0.005	0.193	−0.601	0.513	−0.140	−0.221	−0.467	0.008	−7.35 ± 3.95	0.043
		MP1	MP2	MP3	MP4	MP5	MP6	MP7								
1	66.1	0.436	−0.038	0.432	0.357	0.333	0.435	0.441					0.543	0.002	14.10 ± 6.62	0.027
2	21.5	0.025	−0.780	0.015	0.395	−0.451	0.076	−0.159					−0.067	0.365	−3.11 ± 4.30	0.242

The relationship between each PC and the inbreeding coefficient (*F*) is expressed by means of the Kendall's Tau coefficient of rank correlation and the regression coefficient (*b* ± SE).

MD (MD1 and MD11) showed a significant association with *F*, as well as with most dysmorphic features of MP, which suggests its usefulness in diagnosing relative prognathism. Multivariate analysis (PCA) confirmed these results. The values for the first PC of MP as a function of *F* showed a statistically significant regression ($b = 14.10 \pm 6.62$, $p = 0.027$). Regression was also significant for the second and third PCs of MD, although the meaning of this result is not clear due to the high heterogeneity of the effects of each dysmorphic feature on these PCs (Table 3). Our findings show a strong association between the degree of MP and *F* and suggest that the "Habsburg jaw" is enhanced by inbreeding. In addition, they suggest a greater sensitivity to inbreeding for the lower third of the face, which is consistent with the modular nature of the human face and differences in heritability between the upper and lower facial parts (Claes et al. 2018; Hoskens et al. 2018). In comparison with midface structures, the lower facial parts show low to moderate levels of heritability (Hoskens et al. 2018). This result could be due to a higher value of the dominance of the genetic component of the variation for the lower face, which could explain the strong observed effect of *F* on MP. However, it must be considered that the royal lineage of the Habsburg family is constituted by a small number of individuals. Therefore, it is

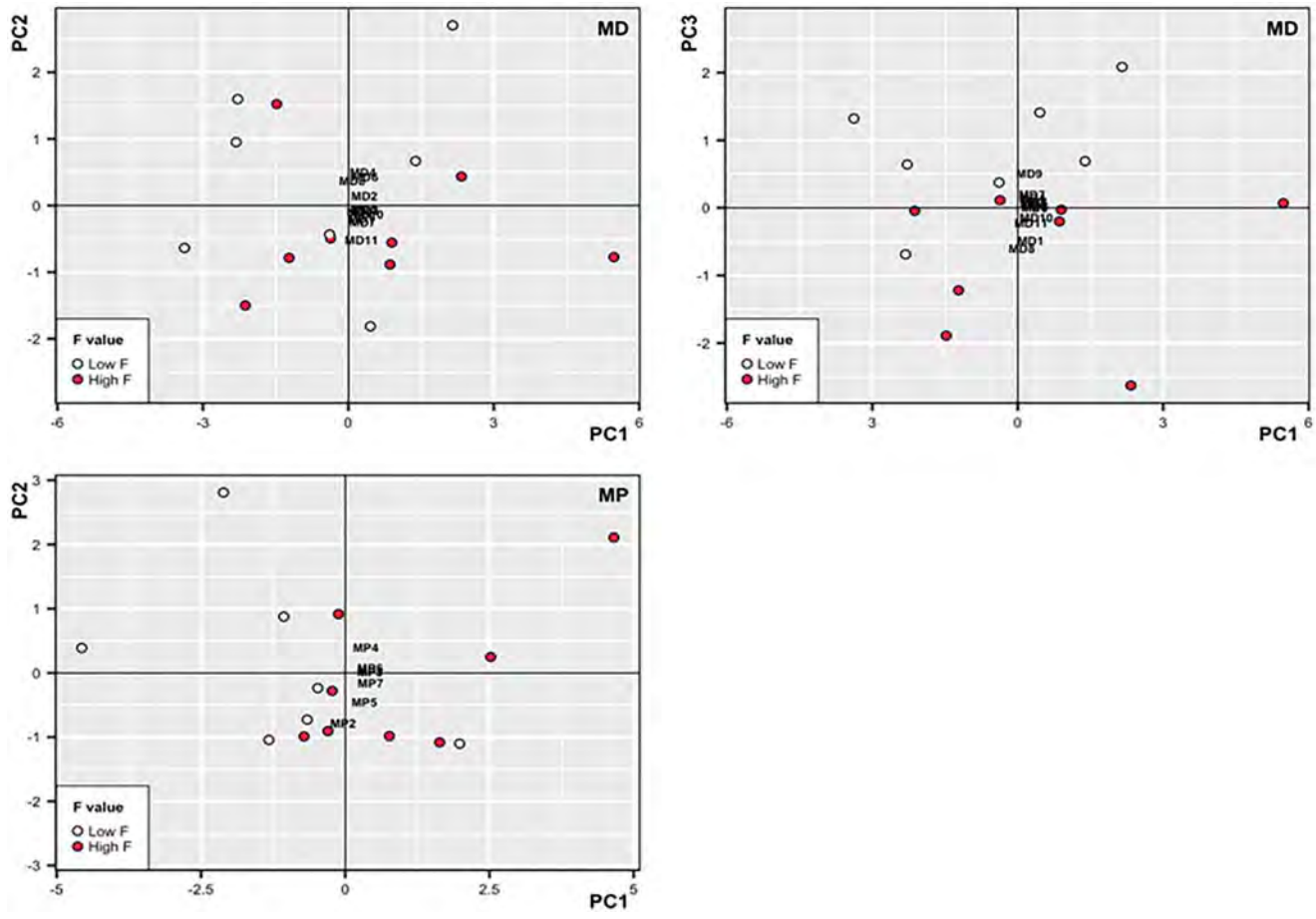

Figure 4. PCA of facial deformity. Plots of 15 individuals of the Habsburg dynasty against values from principal components (PCs). PC1, PC2 and PC3 for MD (top); PC1 and PC2 for MP (bottom). Individuals with *F* over the mean value and those with relatively low values are indicated by red and white dots, respectively. Coefficients of the variables (MD1 to MD11 and MP1 to MP7) are also shown.

theoretically possible that not only the prevalence of the trait but even its magnitude increases as a result of genetic drift in this particular lineage. Thus, the positive association observed between *F* and the degree of MP, as well as in each of the seven dysmorphic features of MP (Figure 2), could be due to a random increase in the frequency of alleles responsible for deformity from one generation to the next. Although this scenario seems unlikely because the random change due to genetic drift should correlate with the systematic increase in *F* caused by a particular policy favouring consanguineous marriages along the six generations studied (Álvarez et al. 2009; Ceballos and Álvarez 2013), we cannot rule out this hypothesis.

If the positive association observed between the degree of MP and *F* is due to an increase in homozygosis caused by the occurrence of consanguineous marriages, our results suggest that MP is a recessive trait. This hypothesis contrasts with segregation analyses, including CSA, which suggest that MP is under the influence of a dominant major gene (Stiles and Luke 1953; Wolff et al. 1993; El-Gheriani et al. 2003; Cruz et al. 2008). It is likely that the reduction of MP to an all-or-none phenotype in segregation analysis could explain its tendency to provide a dominant inheritance pattern. Thus, the possibility that the complexity that underlies the diagnosis of "Habsburg jaw" simulates a dominant disorder due to consanguineous unions has been speculated (Gorlin 1993). CSA methods generally compare different models of inheritance by a likelihood ratio test and the final choice of a particular model between equally likely models is often assessed on the basis of Akaike's information criteria (AIC). In the case of the first CSA of MP (El-Gheriani et al. 2003), the autosomal-dominant and autosomal-recessive models were not rejected by the test. The authors chose the first one because it had the lowest AIC, but the difference observed was small (El-Gheriani et al. 2003). A similar result was obtained by a second study using nuclear families with at least one proband (Cruz et al. 2008). When the whole sample broken into their constituent nuclear families was employed, results were consistent with a multifactorial component in the pattern of inheritance (Cruz et al. 2008). Regardless of the chosen model, the influence of one or a few recessive genes on MP could not be rejected in either study. Only one CSA did not detect evidence compatible with the influence of a major gene, either dominant or recessive (Ko et al. 2013). Other studies, including genome-wide association studies, suggest that MP is a complex trait under the influence of a number of polygenes and environmental factors (Chen et al. 2015; Dorazynska-Kowalik et al. 2017; Lee et al. 2017; Saito et al. 2017). Therefore, the severity of MP may depend on the interaction between multiple genes and environmental factors. It is likely that several factors involved in MP, such as certain congenital anatomic defects and endocrine disorders

(Chang et al. 2006), are related to the increase in homozygosity associated with inbreeding. In the case of a polygenic basis, which seems likely, the hypothesis of an increase of MP as a consequence of inbreeding would be compatible with the influence of a dominant gene of relatively important effect as long as the cumulative effect is recessive (i.e. directional dominance).

Regardless of whether the "Habsburg jaw" is relative and/or absolute MP, the statistically significant positive association between the degree of MP and the pedigree-based *F* among individuals detected in the present study suggests a greater sensitivity to inbreeding for the lower third of the face. Results clearly show that "Habsburg jaw" is related with inbreeding but the causes of this relationship remain uncertain. The increase in the degree of MP with genomic homozygosity seems the most likely explanation, in which case the widely extended hypothesis of a dominant major gene influencing the trait would not be supported. However, further studies in other lineages showing the "Habsburg jaw" are necessary in order to rule out an association between MP and *F* due to genetic drift and provide what, to our knowledge, would be the first evidence of inbreeding depression on the human face.

Acknowledgements

The authors are grateful to Prof. Carlos López-Fanjul for critical reading of the manuscript and Dr. Carlos Martín, Christian Calvo-Henríquez and Dr. Gabriel Martínez-Capoccioni for fruitful discussions about the diagnosis of the "Habsburg jaw."

Disclosure statement

The authors declare no conflict of interest.

References

Álvarez G, Ceballos FC. 2015. Royal inbreeding and the extinction of lineages of the Habsburg dynasty. Hum Hered. 80(2):62–68.

Álvarez G, Ceballos FC, Quinteiro C. 2009. The role of inbreeding in the extinction of a European royal dynasty. PLoS One. 4:e5174.

Brown J, Garrido C. 1998. Velázquez: the technique of genius. New Haven (CT): Yale University Press.

Cavalli-Sforza LL, Bodmer WF. 1971. The genetics of human populations. San Francisco (CA): WH Freeman.

Ceballos FC, Álvarez G. 2013. Royal dynasties as human inbreeding laboratories: the Habsburgs. Heredity. 111(2):114–121.

Ceballos FC, Joshi PK, Clark DW, Ramsay M, Wilson JF. 2018. Runs of homozygosity: windows into population history and trait architecture. Nat Rev Genet. 19(4):220–234.

Chang HP, Tseng YC, Chang HF. 2006. Treatment of mandibular prognathism. J Formos Med Assoc. 105(10):781–790.

Charlesworth D, Willis JH. 2009. The genetics of inbreeding depression. Nat Rev Genet. 10(11):783–796.

Chen F, Li Q, Gu M, Li X, Yu J, Zhang YB. 2015. Identification of a mutation in FGF23 involved in mandibular prognathism. Sci Rep. 5(1):11250.

Cheverud JM. 1988. A comparison of genetic and phenotypic correlations. Evolution. 42(5):958–968.

Claes P, Roosenboom J, White JD, Swigut T, Sero D, Li J, Lee MK, Zaidi A, Mattern BC, Liebowitz C. 2018. Genome-wide mapping of global-to-local genetic effects on human facial shape. Nat Genet. 50(3):414–423.

Cole JB, Manyama M, Larson JR, Liberton DK, Ferrara TM, Riccardi SL, Li M, Mio W, Klein OD, Santorico SA. 2017. Human facial shape and size heritability and genetic correlations. Genetics. 205(2):967–978.

Crow JF, Kimura M. 1970. An introduction to population genetics theory. Caldwell (NJ): The Blackburn Press.

Cruz RM, Krieger H, Ferreira R, Mah J, Hartsfield J, Oliveira S. 2008. Major gene and multifactorial inheritance of mandibular prognathism. Am J Med Genet A. 146:71–77.

Dorazynska-Kowalik A, Nelke KH, Pawlak W, Sasiadek MM, Gerber H. 2017. Genetic factors involved in mandibular prognathism. J Craniofac Surg. 28:e422–e431.

Downs WG. 1928. Studies in the causes of dental anomalies. J Dent Res. 8(3):367–379.

El-Gheriani AA, Maher BS, El-Gheriani AS, Sciote JJ, Abu-Shahba FA, Al-Azemi R, Marazita ML. 2003. Segregation analysis of mandibular prognathism in Libya. J Dent Res. 82(7):523–527.

Falconer DS, Mackay T. 1996. Introduction to quantitative genetics. 4th ed. Harlow: Pearson.

Giuffra V, Panetta D, Salvadori PA, Fornaciari G. 2014. A historical case of amilogenesis imperfecta: Giovanna of Austria, Grand Duchess of Tuscany (1547 - 1578). Eur J Oral Sci. 122(1):1–6.

Gorlin RJ. 1993. Living history-biography: from oral pathology to craniofacial genetics. Am J Med Genet A. 46(3):317–334.

Grabb WC, Hodge GP, Dingman RO, O'Neal RM. 1968. The Habsburg jaw. Plast Reconstr Surg. 42(5):442–445.

Graham D, Pallett PM, Meng M, Leder H. 2014. Representation and aesthetics of the human face in portraiture. Art Percept. 2(1-2):75–98.

Hedrick PW. 2011. Genetics of populations. 4th ed. Sudbury (ON): Jones and Bartlett.

Hodge GP. 1977. A medical history of the Spanish Habsburgs, as traced in portraits. J Am Med Assoc. 238(11):1169–1174.

Hoffman JI, Simpson F, David P, Rijks JM, Kuiken T, Thorne MA, Lacy RC, Dasmahapatra KK. 2014. High-throughput sequencing reveals inbreeding depression in a natural population. Proc Natl Acad Sci U S A. 111(10):3775–3780.

Hoskens H, Li J, Indencleef K, Gors D, Larmuseau MHD, Richmond S, Zhurov A, Hens G, Peeters H, Claes P. 2018. Spatial dense 3D facial heritability and modules of co-heritability in a father-offspring design. Front Genet. 9:554.

Iwaki H. 1938. Hereditary influence of malocclusion. Am J Orthod Oral Surg. 24:328–336.

Jacobson A, Evans WG, Preston CB, Sadowsky PL. 1974. Mandibular prognathism. Am J Orthod. 66(2):140–171.

Ko JM, Suh YJ, Hong J, Paeng JY, Baek SH, Kim YH. 2013. Segregation analysis of mandibular prognathism in Korean orthognathic surgery patients and their families. Angle Orthod. 83(6):1027–1035.

Kraus BS, Wise WJ, Frei RH. 1959. Heredity and the craniofacial complex. Am J Orthod. 45(3):172–217.

Lee MK, Shaffer JR, Leslie EJ, Orlova E, Carlson JC, Feingold E, Marazita ML, Weinberg SM. 2017. Genome-wide association study of facial morphology reveals novel associations with *FREM1* and *PARK2*. PloS One. 12(4):e0176566.

Lippi D, Pierleoni F, Franchi L. 2012. Retrognathic maxilla in "Habsburg jaw". Skeletofacial analysis of Joanna of Austria (1547 - 1578). Angle Orthod. 82(3):387–395.

Lynch M, Walsh B. 1998. Genetics and analysis of quantitative traits. Sunderland (MA): Sinauer.

Manly BFJ, Navarro JA. 2017. Multivariate statistical methods. A primer. 4th ed. Boca Raton (FL): CRC press.

McQuillan R, Leutenegger AL, Abdel-Rahman R, Franklin CS, Pericic M, Barac-Lauc L, Smolej-Narancic N, et al. 2008. Runs of homozygosity in European populations. Am J Hum Genet. 83(3):359–372.

Peacock ZS, Klein KP, Mulliken JB, Kaban LB. 2014. The Habsburg jaw re-examined. Am J Med Genet A. 164(9):2263–2269.

Richmond S, Howe LJ, Lewis S, Stergiakouli E, Zhurov A. 2018. Facial genetics: a brief overview. Front Genet. 9:462.

Roff DA. 1995. The estimation of genetic correlations from phenotypic correlations: a test of Cheverud's conjecture. Heredity. 74(5):481–490.

Roff DA. 1996. The evolution of genetic correlations: an analysis of patterns. Evolution. 50(4):1392–1403.

Rubbrecht O. 1939. A study of the heredity of the anomalies of the jaws. Am J Orthod Oral Surg. 25(8):751–779.

Saito F, Kajii TS, Oka A, Ikuno K, Iida J. 2017. Genome-wide association study for mandibular prognathism using microsatellite and pooled DNA method. Am J Orthod Dentofacial Orthop. 152(3):382–388.
Sokal RR, Rohlf FJ. 1995. Biometry. New York: WH Freeman.
Stiles KA, Luke JE. 1953. The inheritance of malocclusion due to mandibular prognathism. J Hered. 44(6):241–245.
Wolff G, Wienker TF, Sander H. 1993. On the genetics of mandibular prognathism: analysis of large European noble families. J Med Genet. 30(2):112–116.