30th CONGRESS OF THE INTERNATIONAL SOCIETY FOR FORENSIC GENETICS
Universidade de Santiago de Compostela, 2025, pp. 175-182
DOI: https://dx.doi.org/10.15304/cc.2025.1869

Integrating physical anthropological techniques and emerging methodologies using a quasi-landmark mesh to decode the upper facial region from DNA

Belén Navarro López[1,2,3*], Victoria Suárez-Ulloa[4], Franziska Wilke[5], Miriam Baeta[1,2,3], Rubén Martos-Fernández[6], Susan Walsh[5], Begoña Martínez-Jarreta[7], Susana Jiménez[8], Marian M. de Pancorbo[1,7]

[1]BIOMICs Research Group, Lascaray Research Center, University of the Basque Country (UPV/EHU), Vitoria-Gasteiz, Spain; [2]Department of Zoology and Animal Cellular Biology, Faculty of Pharmacy, UPV/EHU, Vitoria-Gasteiz, Spain; [3]Bioaraba Health Research Institute, Vitoria-Gasteiz, Spain; [4]Panacea Cooperative Research S. Coop, Castilla y León, Spain; [5]Indiana University Indianapolis, Indianapolis, United States; [6]Department of Legal Medicine, Toxicology, and Physical Anthropology University of Granada, Spain; [7]Faculty of Medicine, University of Zaragoza, Zaragoza, Spain; [8]Department of Pathology and Surgery, University of Miguel Hernández, Alicante, Spain

**Corresponding author at: belennavarrolopez1997@gmail.com*

Abstract

Genetic identification currently relies on comparing unknown DNA samples against reference profiles in forensic databases or indubitable individuals. However, when no match is available, these cases may remain unsolved. In such circumstances, advances in Forensic DNA Phenotyping (FDP), that seeks to determine externally visible characteristics (EVCs) from DNA, could assist in identification efforts. Among EVCs, facial shape is of particular interest. While genome-wide association studies (GWAS) have identified potential SNPs linked to facial traits, these vary between populations and methodology. Addressing this issue, the present study has selected a set of candidate SNPs and assessed their correlation with facial phenotype in a Spanish population, focusing on the upper facial region, which includes the highly informative ocular area. From a collection of 412 individuals, two strategies were performed: one based on traditional anthropometric measurements and indexes with Pearson/Spearman and Chi-squared analyses, and another using a mesh of quasi-landmarks with canonical correlation analyses. Results revealed significant associations between several SNPs and upper facial metrics, although the SNPs and regions identified differed between the two methods and in part, to previous published results. These findings underline the importance of methodology and validation in population groups in

facial description when conducting genetic studies, and most notably, when considering forensic applications.

Keywords

Forensic DNA Phenotyping, facial morphology, correlation study, candidate SNPs.

Introduction

Genetic identification is a key tool in forensic biology, allowing the identification of individuals through genetic profiles comparison. However, unresolved cases may arise when no match is found in genetic databases or among persons involved in the investigation. Recent advances have led to the development of FDP, which aims to predict EVCs from DNA, providing valuable information about an individual's physical appearance for identification purposes [1].

The prediction of facial shape is particularly important due to its recognizability, making it a highly desirable goal for FDP. Despite the complexity of identifying genes involved in facial morphology, recent GWAS have identified potential SNPs associated with facial features [2–18]. Nevertheless, findings from these studies often show inconsistencies, largely due to differences in population samples and analytical strategies. However, advancements in 3D imaging systems have facilitated comprehensive evaluation of global and local variation, enabling different approaches to analyze facial morphology and perform association studies between genetic markers and facial traits [4, 19, 20].

Given the observed variability in results among previous investigations, our research focuses on analyzing candidate SNPs within a Spanish population to evaluate their correlation with facial traits, particularly in the upper facial region, including the highly informative ocular area. We employed two approaches to assess the correlation: a traditional physical anthropology method and an innovative quasi-landmark mesh technique. By comparing these methods, our research seeks to identify the optimal approach for comprehensive analysis, and validate previously published SNPs in a Spanish population.

Material studied, methods, techniques

A total of 412 individuals from a Spanish population were studied, with ethical approval (M10_2021_143) from the Ethics Committee for Research on Human Subjects of the University of the Basque Country. All participants provided written informed consent and completed a form detailing variables potentially influencing facial shape (sex, age, weight, height, surgery, and pathologies/traumas).

Additionally, saliva samples were collected in triplicate using sterile swabs, and 3D facial images were obtained using a 3D scanner (Academia 3D/20, Creaform) following established protocols [21]. Post-acquisition, 3D images were processed using the ACADEMIA Software Bundle.

DNA extraction was performed using the DNA Purification System Puregen™ (Gentra System, Inc.). A set of 116 candidate SNPs previously related to facial morphology [2–18] was analyzed using Fluidigm (Fluidigm Corp.) and SNaPshot minisequencing (Applied Biosystems) technologies. SNPs were coded according to the additive genetic model (AA = 0, Aa = 1, aa = 2).

Physical anthropology approach

3D images were imported into Skeleton-ID software [22] to place five cephalometric landmarks (en', ex', ft', fz', and zy'), used to calculate six linear measurements and three proportionality indices (face width, superior facial width, minimum frontal width, intercanthal width, biocular width, eye fissure width, forehead-face width index, intercanthal index, and palpebral fissure length index). These metrics were analyzed as both continuous and categorical variables. Associations with continuous variables were evaluated using Pearson or Spearman correlation tests, depending on data normality. For categorical variables, Chi-square tests ($\chi 2$) were employed. Variables were classified into three categories (smaller, normal, increased) based on their mean ± 0.5x standard deviation. All analyses were conducted in RStudio [23].

Quasi-landmark mesh approach

A quasi-landmark mesh was aligned to all 3d images using the MeshMonk pipeline [19, 24]. Facial scans were symmetrized by averaging each image with its reflection, and aligned in space using Procrustes superimposition. Covariate effects (sex, age, height, and weight) were removed using partial least-squares regression. Faces were then divided into 63 segments following the labels of Sero et al. [25], with 15 regions corresponding to the upper face. Each segment underwent generalized Procrustes Superimposition, followed by principal component (PCA) and Horn's parallel analysis to capture key variance and reduce dimensionality. Associations between SNPs and phenotypic variation in each of the 15 facial segments, represented as PCs, were evaluated through canonical correlation analysis (CCA). Additionally, a false discovery rate according to Benjamin-Hochberg (FDR) threshold of 0.05 was applied. Normal displacement maps were plotted for significant SNPs. All code was adapted from prior publications using Matlab 2023b [16, 26].

Results

Our results identified statistically significant associations for eight out of 116 SNPs with the metrics analyzed using traditional physical anthropology methods (Table 1). These SNPs were selected for their robustness, consistently showing *P-values* < 0.05 when analyzed as both continuous and categorical variables. Meanwhile, five out of the 116 SNPs showed significant associations (*P-value* < 0.05) after the FDR adjustment with facial segments in the upper face region using the quasi-landmark mesh approach (Figure 1).

Table 1. SNPs showing significant association with the measurements and indices.

Variable	SNP	*P-value*		Variable	SNP	*P-value*	
Intercanthal width (en'- en')	rs10175706[a]	Continuous	0.009	Superior facial width (fz'- fz')	rs10962767[a]	Continuous	0.007
		2 Categories	0.002			2 Categories	0.001
		3 Categories	0.015			3 Categories	0.008
Biocular width (ex'- ex')	rs10868138[b]	Continuous	0.030	Face width (zy'- zy')	-		
		2 Categories	0.032	Intercanthal index (en'- en' / ex'- ex' x 100)	rs12495832[a]	Continuous	0.012
		3 Categories	0.023			2 Categories	0.001
	rs1454072[c]	Continuous	0.003			3 Categories	0.003
		2 Categories	0.020		rs13097965[b]	Continuous	0.008
		3 Categories	0.015			2 Categories	0.016
Eye fissure width (ex'- en')	rs6129564[c]	Continuous	0.007			3 Categories	0.033
		2 Categories	0.038		rs62578082[a]	Continuous	0.024
		3 Categories	0.038			2 Categories	0.017
	rs62578082[a]	Continuous	0.001			3 Categories	0.038
		2 Categories	0.006	Palpebral fissure length index (en'- ex' / fz'-fz' x 100)	rs12495832[a]	Continuous	0.000
		3 Categories	0.020			2 Categories	0.000
Minimum frontal width (ft'- ft')	rs10175706[a]	Continuous	0.033			3 Categories	0.001
		2 Categories	0.004	Forehead-face width index (ft'- ft' / zy'- zy' x 100)	-		
		3 Categories	0.024				

[a] SNPs revealing previously unreported associations with the upper face, previously linked to the chin [13].

[b] SNPs revealing previously unreported associations with the upper face, previously linked to the nose [13, 17].

[c] SNPs previously associated with the upper face [7, 15].

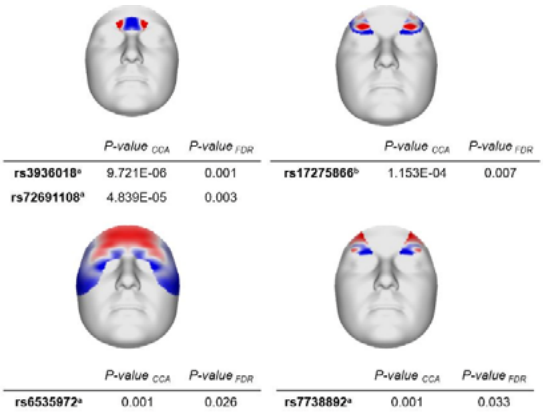

Figure 1. SNPs showing significant association with different facial modules in the upper face. Inward and outward movement are represented in blue and red, respectively. (a)SNPs previously associated with the upper face [4, 7, 16, 18] and (b)SNPs revealing a previously unreported association with the upper face, previously linked to the chin [13].

Discussion

Association analyses in this study revealed significant correlations for several SNPs, although these varied depending on the analytical approach employed.

Using the physical anthropology approach, eight SNPs were found to be significantly correlated with facial measurements and indices in our population (Table 1), with three of these SNPs being associated with more than one facial trait. While some SNPs had previously been linked to the upper face, our results identified novel unreported associations with this region (rs10175706, rs62578082, rs10962767, and rs12495832 were previously related to chin morphology, and rs10868138 and rs13097965 had been related to nasal features in prior studies) [7, 13, 15, 17]. Most of these associations were concentrated in the ocular region, affecting intercanthal, biocular, and eye fissure width, as well as intercanthal and palpebral fissure length indices (Table 1).

In contrast, the quasi-landmark mesh approach identified in our population five SNPs with significant correlations to facial segments in the upper face region (Figure 1). Similar to the physical anthropology method, some SNPs had known associations with the upper face, but our results also uncovered new correlations with this region (rs17275866 was previously related to chin morphology) [4, 7, 13, 16, 18]. The majority of these associations were localized to the forehead area.

The differences in SNPs identified between the two methods likely reflect the distinct facial traits captured by each analytical approach.

Conclusion

This study supports the existence of genetic markers associated with facial morphology in a Spanish population. Of the 13 identified SNPs, six validate correlations with facial regions noted in previous studies, while seven reveal novel associations. In our population, physical anthropology methods demonstrated greater associations with the ocular region, whereas quasi-landmark mesh techniques identified correlations primarily in the forehead region. Therefore, these findings highlight the variability in significant SNPs depending on the analytical approach employed, suggesting that a multifaceted strategy may be necessary for a comprehensive understanding of the genetic basis of facial morphology.

Acknowledgments

Authors acknowledge funding support from Basque Government through predoctoral fellowship PRE_2021_2_0157, as well as project grants IT-1271-19 and IT1633-22.

Conflict of interest statement

Authors declare no competing interest.

References

[1] Kayser M (2015) Forensic DNA Phenotyping: Predicting human appearance from crime scene material for investigative purposes. Forensic Sci Int Genet 18:33–48. https://doi.org/10.1016/j.fsigen.2015.02.003

[2] Adhikari K, Fuentes-Guajardo M, Quinto-Sánchez M, et al (2016) A genome-wide association scan implicates DCHS2, RUNX2, GLI3, PAX1 and EDAR in human facial variation. Nat Commun 7:1–11. https://doi.org/10.1038/ncomms11616

[3] Cha S, Lim JE, Park AY, et al (2018) Identification of five novel genetic loci related to facial morphology by genome-wide association studies. BMC Genomics 19:1–17. https://doi.org/10.1186/s12864-018-4865-9

[4] Claes P, Roosenboom J, White JD, et al (2018) Genome-wide mapping of global-to-local genetic effects on human facial shape. Nat Genet 50:414–423. https://doi.org/10.1038/s41588-018-0057-4

[5] Cole JB, Manyama M, Kimwaga E, et al (2016) Genomewide Association Study of African Children Identifies Association of SCHIP1 and PDE8A with Facial Size and Shape. PLoS Genet 12:1–19. https://doi.org/10.1371/journal.pgen.1006174

[6] Crouch DJM, Winney B, Koppen WP, et al (2018) Genetics of the human face: Identification of large-effect single gene variants. Proc Natl Acad Sci USA 115:E676–E685. https://doi.org/10.1073/pnas.1708207114

[7] Hoskens H, Liu D, Naqvi S, et al (2021) 3D facial phenotyping by biometric sibling matching used in contemporary genomic methodologies. PLoS Genet 17:1–28. https://doi.org/10.1371/journal.pgen.1009528

[8] Jacobs LC, Liu F, Bleyen I, et al (2014) Intrinsic and Extrinsic Risk Factors for Sagging Eyelids. JAMA Dermatol 150:836–843. https://doi.org/10.1001/JAMADERMATOL.2014.27

[9] Lee MK, Shaffer JR, Leslie EJ, et al (2017) Genome-wide association study of facial morphology reveals novel associations with FREM1 and PARK2. PLoS One 12:1–13. https://doi.org/10.1371/journal.pone.0176566

[10] Li Y, Zhao W, Li D, et al (2019) EDAR, LYPLAL1, PRDM16, PAX3, DKK1, TNFSF12, CACNA2D3, and SUPT3H gene variants influence facial morphology in a Eurasian population. Hum Genet 138:681–689. https://doi.org/10.1007/s00439-019-02023-7

[11] Liu F, van der Lijn F, Schurmann C, et al (2012) A Genome-Wide Association Study Identifies Five Loci Influencing Facial Morphology in Europeans. PLoS Genet 8: e1002932. https://doi.org/10.1371/journal.pgen.1002932

[12] Paternoster L, Zhurov AI, Toma AM, et al (2012) Genome-wide association study of three-dimensional facial morphology identifies a variant in PAX3 associated with nasion position. Am J Hum Genet 90:478–485. https://doi.org/10.1016/j.ajhg.2011.12.021

[13] Pickrell JK, Berisa T, Liu JZ, et al (2016) Detection and interpretation of shared genetic influences on 42 human traits. Nat Genet 48:709–717. https://doi.org/10.1038/ng.3570

[14] Qiao L, Yang Y, Fu P, et al (2018) Genome-wide variants of Eurasian facial shape differentiation and a prospective model of DNA based face prediction. Journal of Genetics and Genomics 45:419–432. https://doi.org/10.1016/j.jgg.2018.07.009

[15] Shaffer JR, Orlova E, Lee MK, et al (2016) Genome-Wide Association Study Reveals Multiple Loci Influencing Normal Human Facial Morphology. PLoS Genet 12:1–21. https://doi.org/10.1371/journal.pgen.1006149

[16] White JD, Indencleef K, Naqvi S, et al (2021) Insights into the genetic architecture of the human face. Nat Genet 53:45–53. https://doi.org/10.1038/s41588-020-00741-7

[17] Wu W, Zhai G, Xu Z, et al (2019) Whole-exome sequencing identified four loci influencing craniofacial morphology in northern Han Chinese. Hum Genet 138:601–611. https://doi.org/10.1007/s00439-019-02008-6

[18] Xiong Z, Dankova G, Howe LJ, Lee MK (2019) Novel genetic loci affecting facial shape variation in humans. eLife 8:e49898. https://doi.org/10.7554/eLife.49898

[19] Claes P, Walters M, Clement J (2012) Improved facial outcome assessment using a 3D anthropometric mask. Int J Oral Maxillofac Surg 41:324–330. https://doi.org/10.1016/J.IJOM.2011.10.019

[20] Martos R, Valsecchi A, Ibáñez O, Alemán I (2018) Estimation of 2D to 3D dimensions and proportionality indices for facial examination. Forensic Sci Int 287:142–152. https://doi.org/10.1016/J.FORSCIINT.2018.03.037

[21] Heike CL, Upson K, Stuhaug E, Weinberg SM (2010) 3D digital stereophotogrammetry: A practical guide to facial image acquisition. Head Face Med 6:1–11. https://doi.org/10.1186/1746-160X-6-18/FIGURES/7

[22] Skeleton-id. https://skeleton-id.com/.

[23] R Core Team (2021) R: A language and environment for statistical computing

[24] White JD, Ortega-Castrillón A, Matthews H, et al (2019) MeshMonk: Open-source large-scale intensive 3D phenotyping. Sci Rep 9:6085. https://doi.org/10.1038/s41598-019-42533-y

[25] Sero D, Zaidi A, Li J, et al (2019) Facial recognition from DNA using face-to-DNA classifiers. Nat Commun 10:2557. https://doi.org/10.1038/S41467-019-10617-Y

[6] Wilke F, Herrick N, Matthews H, et al (2023) Exploring regional aspects of 3D facial variation within European individuals. Sci Rep 13:3708. https://doi.org/10.1038/s41598-023-30855-x