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ENPP1 and GHR Genes in Determination of Face Morphology Potential Importance in Forensic Science ENPP1 and GHR Genes and Facial Recognition

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Abstract

Human facial morphology is diverse between individuals and communities, and is affected by gene polymorphisms. We investigated the effects of Single Nucleotide Polymorphisms (SNPs) of ENPP1 and GHR genes on facial features. 20 volunteers over the age of 18 without birth defects, facial trauma, reconstructive and plastic surgery, facial and dental implant treatment, orthognathic/maxillofacial surgery story and facial paralysis or neurological problems resulting any facial abnormalities were selected for the study. Two SNP genotypes and a total of 40 samples were studied. 15 anatomic regions were identified on the face and 27 physical measurements were made between these 15 regions. ENPP1 (rs7754561) and biometric measurements between the points were found associated significantly. We believe that the data obtained from this study might be one of the study for the development of the powerful identification methods from DNA in forensic sciences. The information obtained from DNA analysis might be used for drawing 2D or 3D robot images. If the genes affecting the facial morphology and the variants of these genes are well understood, it would be possible to reach the person whose DNA could not be identified in databases.

Keywords

Forensic science • Gene polymorphism • Genotype • Face morphology • SNP

Introduction

Face is a visible feature that plays an important role in human life for social interaction and identification of people in potential forensic cases. It contains enormous diversity that contributes to our physical identities, which vary between individuals and communities. The cause of this diversity is based on genetics and is due to the phenotypic effect of the genotype. The human face is affected by many complex components, in particular the environmental factors as well as genetic components. Current understanding of genes and pathways for normal facial development and morphology originated from research efforts on craniofacial malformations in both humans and animal models. The facial morphological characteristics are influenced by various genes such as BMP, GHR, SHH, FGF, ENPP1 and are closely related to the activity of several signaling pathways including Wnt/b-catenin [1-4]. The genes that influence the formation of the facial shape are partially understood, but the variations that they make and the origin of these variations are not fully known. Ongoing research into the genetics of facial morphology improves the quantitative diagnosis and treatment of craniofacial syndromes, but the possible relationship between gene polymorphisms and facial morphology, especially the variations in facial appearance between individuals is still unrevealed. However, the main genetic pathways that describe craniofacial morphogenesis have been partially elucidated, it is not known at which stage of small genetic variations affects face morphology.

A few genes related to the genetic modification of the face are emerging. When mutation occurs on ENPP1 (ecto-nucleotide pyrophosphatase/phosphodiesterase 1), it affects bone mineralization negatively and causes a change in facial height ENPP1 (ecto-nucleotide pyrophosphatase/phosphodiesterase 1) is important in order to prevent bone abnormalities and abnormal accumulation of calcium (calcification) and other minerals (mineralization) in the body [5]. The GHR (growth hormone receptor) gene affects the normal growth and development of the human body. Two GHR genetic variants, Pro561Thr (rs6184) and I526L (rs6180), have been shown to be effective in the development of mandibles [6].

Our aim in this study is to analyze the effects of specific single nucleotide polymorphisms of genes associated with the development of facial morphology, to make biometric measurements on face and to correlate genotype and phenotype interactions.

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Materials and Methods

Sample collection, DNA extraction and quantification

Sample collection in this study was carried out with the approval of the ethics committee of the Üsküdar University. Our work was carried out at the laboratories of the Institute of Forensic Sciences of the Üsküdar University.

Our study group consists of 20 volunteer individuals over 18 years of age. In the selection of the study group, the following criteria were taken into account: 1) no face trauma; 2) no facial reconstructive and plastic surgery; 3) no facial and dental implant therapy; 4) No orthognathic/jaw surgery; 5) No facial paralysis, stroke or neurological pathology that affects the face; 6) no facial anomalies or birth defects; 7) No any syndrome or congenital (birth, congenital) problems known to affect facial morphology.

DNA isolation from biological specimens taken with buccal swab was carried out in accordance with the protocol of the manufacturer using Purelink Genomic DNA Mini Kit (In vitro gen). Quantitation of the isolated DNA samples was performed using a Thermo Multiskan Go spectrophotometer. 5µl of DNA sample was loaded to 1% agarose gel containing 3 µg/ml of ethidium bromide, and the presence of DNA was confirmed by visualizing the bands formed after electrophoresis on a UV transilluminator.

Imaging of face and biometric measurements

Individuals were photographed by the same photographer (Nikon D7000), from the same distance (30 cm) from the front, and the left and

right side of the profile. Our volunteers were photographed face-to-face with a steady position at the same height as the camera and at a distance of 30 cm. For accurate analysis, the faces of the individuals were made loose and expressionless during the photographing as seen in Figure 1.

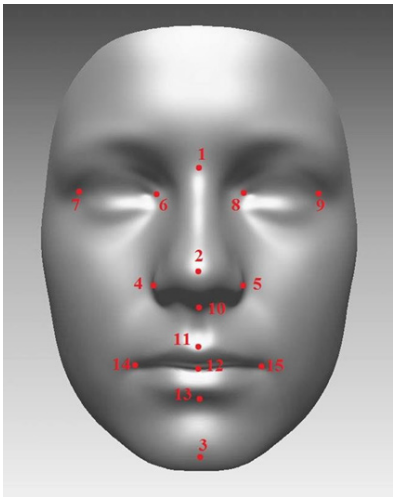

Figure 1. 15 anatomical points on facial features; 1-Nasion Point [N], 2-Pronasale [Prn], 3-Chin Point [ChiP], 4- Left Alare [LAl], 5- Right Alare [RAl], 6-Left İnternal Canthus [LInCant], 7- Left External Canthus [LExCant],8-Right İnternal Canthus [RInCant], 9-Right Extarnal Canthus [RExCant], 10-Subnasale [Sn], 11-Upper Lip Point [ULipP], 12-Stomion [Sto], 13- Lower Lip Point [LLipP], 14-Left Lip Corner [LLipCorn], 15-Right Lip Corner [RLipCorn].

Measurements were made on the photographs taken from 15 anatomical points. Biometric measurements were performed with Image J7 program among these identified points. The 15 anatomical points determined on the facial shape are shown in Figure 1. The measured points are given in Table 1.

Selected dual regions		
RAl–RLipCorn 5-15	RLipCorn–ULipP 15-11	RLipCorn–Sn 15-10
LAl–LLipP 4-13	ULipP–LLipP 11–13	LLipCorn–Sn 14-10
RLipCorn–Prn 15-2	RLipCorn–LLipP 15-13	RLipCorn–ChinP 15-3
ChinP–LAl 3-4	RLipCorn–N 15-1	LLipCorn–N 14-1
ULipP–RInCant 11-8	ULipP–RExCant 11-9	RLipCorn-LLipCorn 15-14
RLipCorn–Sto 15-12	LInCant–LExCant 6-7	RInCant–RExCant 8-9
LExCant–RExCant 7-9	LInCant-RInCant 6-8	Sn–ChinP 10-3
N–LLipP 1-13	N–ChiP 1-3	ULipP-N 11-1
N–Sn 1-10	ULipP–Sn 11–10	LLipP-ChinP 13–3

Table 1. List of points where biometric measurements are made (Numbers indicate the locations of 15 anatomical points on the face).

PCR

PCR Amplifications of ENNP1 (rs7754561), GHR (rs6180) SNPs were done. The PCR mixture was a total volume of 25µL. For each sample; 2ng/µL genomic DNA, 15.5µL dd H2O, 5µL Buffer, 0.5µL Taq DNA Polymerase and 1µL F/R primer were used. PCR was performed with the T100 Thermal Cycler instrument and 39 cycles were performed. Several experiments have been carried out for the optimization of the PCR protocol and the optimum protocol has been determined. For the PCR reaction, the binding temperatures of the Forward/Reverse primers used in the study were determined and the corresponding binding temperatures were applied for each primer [7].

Electrophoresis and sequencing

Electrophoresis and sequencing were carried out in the service procurement laboratory of "BMLabosis" company using POP7 polymer in ABI Prism 3130 XL Genetic Analyzer (Applied Biosystems) with a capillary length of 16 cm.

Analysis of data and evaluation of results

The sequence for each gene for each individual was screened and the sequence with the highest reliability was decided by the Multiple Sequence Alignment and the Pairwise Sequence Alignment [8,9]. SNPs were determined and statistical analysis was done for each SNP in

order to show their relationship with biometric measurements by using t-test, significance level is determined as $p<0.05$.

Results

We performed screens on specific polymorphisms of ENNP1 (rs7754561), GHR (rs6180) genes in order to evaluate their effects on facial morphology. 15 anatomic regions were identified on the face and 27 physical measurements were made between these 15 regions. We found that ENPP1 gene polymorphism (G>A) affects almost all biometric measures on face such as the height as shown in statistical analysis (Table 2). Individuals with genotype G had longer face lengths whereas individuals with genotype A had more oval, shorter and smaller face shapes shown in Figure 2. ENPP1 (rs7754561) polymorphism (G>A) and biometric measurements between the points; RAl (5) – RLipCorn (15), LAl (4)-LLipCorn (14), RLipCorn (15)–Sn (10), LLipCorn (14)–Sn (10), Sn (10)–ChiP (3), RLipCorn (15)–ChiP (3), LLipCorn (14)-ChiP (3), LAl (4)-ChiP (3), LLipP (13)–N (1), LLipP (13)-ChiP (3), RLipCorn (15)-N (1), LLipCorn (14)–N (1), ChiP (3)-N (1), ULipP (11)–N (1), ULipP (11)–Sn (10), ULipP (11)–LInCant (6), ULipP (11)–RInCant (8), ULipP (11) – RExtCan (9), ULipP (11) – LexCant (7) were found associated statistically significant (Table 2).

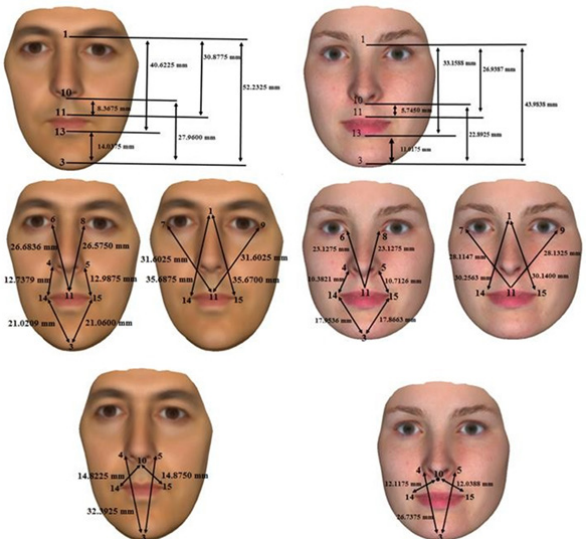

Figure 2. Measurements of male and female faces for ENPP1 gene. On left male face mask represents A genotype whereas on the right, female face mask represents to G genotype of ENPP1 gene.

Parameter	Mean	Mean	P	t value
RAl (5)–RLipCorn (15)	12.9875	10.7126	0.0002	5.8896
LAl (4)-LLipCorn (14)	12.7379	10.3821	0.0002	5.8396
RLipCorn (15)–Sn (10)	14.875	12.0388	0.0001	7.2972
LLipCorn (14)–Sn (10)	14.8225	12.1175	0.0001	7.5916
Sn (10)–ChiP (3)	27.96	22.8925	0.0003	5.3236
RLipCorn (15)–ChiP (3)	21.06	17.8663	0.0043	3.6746
LLipCorn (14)-ChiP (3)	21.0209	17.9536	0.0043	3.6746

LAI (4)-ChiP (3)	32.3925	26.7375	0.0001	7.3223
LLipP (13)–N (1)	40.6225	33.1588	0.003	3.8821
LLipP (13)-ChiP (3)	14.0375	11.0175	0.0017	4.24
RLipCorn (15)-N (1)	35.67	30.14	0.0083	3.2818
LLipCorn (14)–N (1)	35.6875	30.2563	0.0094	3.2088
ChiP (3)-N (1)	52.2325	43.9838	0.001	6.7399
ULipP (11)–N (1)	30.8775	26.9387	0.0147	2.943
ULipP (11)–Sn (10)	8.3675	5.745	0. 0036	3.7851
ULipP (11)–LInCant (6)	26.6836	23. 1272	0.0139	3.356
ULipP (11)–RInCant (8)	26.575	23.1275	0.0073	3.3863
ULipP (11)–RextCan (9)	31.6025	28.1325	0.0235	2.6264
ULipP (11)-LExCant (7)	31.6025	28.1147	0.0235	2.6264

Table 2. Mean measures (mm), p-values and t-values for ENPP1 (rs7754561) gene polymorphism.

GHR gene polymorphism was associated with the width of the lips. Individuals with genotype A, the distance between the right and left lip corners is longer, whereas in individuals with genotype C, this distance is shorter as shown in Figure 3. LLipCorn (14) – RLipCorn (15) were also found associated statistically significant as shown in Table 3.

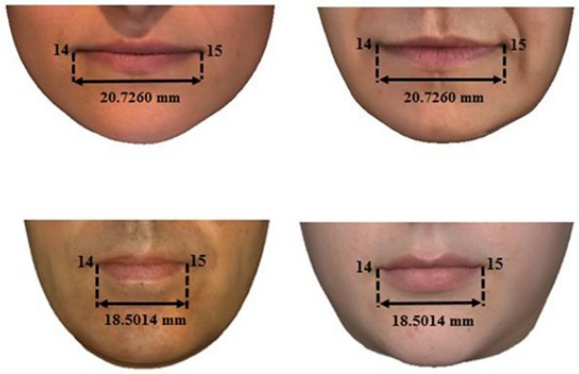

Figure 3. Measurements of male and female faces for GHR gene. Upper lip models represent A genotype whereas bottom lip models represent to C genotype of GHR gene.

	ALLELE A ALLELE C		
Parameter	**Mean**	**Mean**	**P**
LLipCorn (14)–RLipCorn (15)	20.726	18.5014	0.0246

Table 3. Mean measures (mm), p-values and t-values for GHR (rs6180) gene polymorphism.

Discussion

Human facial morphology is a highly complex composite structure controlled by genetic, environmental, mechanical and epigenetic factors. The face is a visible feature that plays an important role in social interaction, medical diagnosis, and potential judicial problems in order to identify individuals.

In our study, we used biometric measurements and morphology related SNPs to determine the relationship between genes and facial morphology. We used digital photography to display the face shapes and we performed biometric measurements using "Image J" program. We performed a correlation study between specific SNPs (ENNP1-rs7754561, GHR-rs6180) and biometric measurements. Our findings showed that rs7754561 in the ENPP1 gene and rs6180 in the GHR gene are associated with morphometric facial features.

ENPP1 (ecto-nucleotide pyrophosphatase/phosphodiesterase 1), encoding a transmembrane ecto-enzyme, is a promoter that controls the hydrolysis of inorganic pyrophosphate and thus inhibits the formation of hydroxyapatite. ENPP1 expression is associated with osteoblast differentiation, and osteoblast cultures overexpressing ENPP1 contain high amounts of PPi and exhibit reduced mineral formation. The ENPP1 gene is crucial for maintenance and repair of appropriate bone development in bone mineralization [10,11]. For this reason, the regulatory network that controls craniofacial growth may form an important node. Several polymorphisms of ENPP1 have been also associated with changes in long bone geometry as well as their role in facial bone geometry [5].

In this study, we performed 27 linear measurements on 15 anatomical points on the facial shape for the ENPP1 rs7754561 polymorphism.

As a result of these measurements we found a significant difference between almost all points such as RAI (5)-RLipCorn (15). We have seen that the linear distance between the nose and mouth tips is shorter in the individuals carrying the genotype A than in the G genotype. When the distance between the nose edges and the mouth tips is short, it is shortened at the distance between the nose and the lip and affects the face height. In addition, measurements were also made at the points of LLipCorn (14)-Sn (10) and RLipCorn (15)-Sn (10) points and shortening between the sub-nasal and lip corners was observed. Individuals with genotype G had a longer distance than individuals with genotype A. Measurements were made between Sn-ChiP (10-3) and RLipCorn-ChiP (15-3). It was found that individuals with genotypes at these points were shorter while those with genotype G were longer. Thus, the ENPP1 rs7754561 polymorphism was associated with the lower face height in the measurements between the nasal floor, nose wings, lip corners and chin. Individuals carrying the genotype A were found to have shorter distances than those carrying the genotype G, and this difference was related to the phenotype of the genotype. In addition, in order to determine the effects on the upper face height of ENPP1 gene, the measures between ULipP (11)-RInCant (8), ULipP (11)-RExtCan (9), LLipCorn (4)-N (1) and ChiP (3)-N (1) were analyzed. As a result of the measurements, we found that the distance between these points was shorter in individuals with genotype A than in genotype G, and that the ENPP1 gene was associated with upper face height. Based on these observations, we assume that the functional variant in the ENPP1 gene affects the stability of the mRNA. This effect influences Pi/PPi levels and bone mineralization. Thus, ENPP1 rs7754561 polymorphism causes generalized effects on bone development, affecting facial morphological characteristics.

GHR polymorphism has a strong genetic correlation with mandibular morphology and craniofacial morphology. It regulates the size and angular relations of the craniofacial structures directly and indirectly in the growth and development of the craniofacial complex. The disproportionate growth of skull base structures and jaws can lead to facial retrognathia, resulting in a proportionally smaller posterior for the anterior face. Bayram and his colleagues performed studies on the GHR genome and associated it with mandibular prognathism (6). Studies in GHR knockout mice have reported that the GH → GHR → insulin-like growth factor 1 system is important in postnatal growth and that GHR plays a role in the protection of proportional skeletal growth. In these rats, the height of the mandibular ramus decreased significantly and disproportionate skeletal growth was observed.

In our study, we performed measurements between 27 points considering this data. As a result of the measurements, we found a significant relationship between LLipCorn (14) – RlipCorn (15) points. It has been observed that the width of the mouth crack and the distance between the mouth corners are shortened in the individuals carrying the C genotype in place of the A genotype.

Conclusion

Collecting the biological evidence remaining in the crime scene is crucial to the success of the investigation. In particular, DNA evidence alone would be sufficient to find the person of interest. Face morphology might be determined by the genetic inheritance of the individual. Thus, it might be possible to use genetic tools to identify a person. Finally, due to genetic differences between different populations, screening studies of SNP biomarkers among local populations are of importance in forensic

genetic studies. Therefore, Genetic tools would be more powerful in future investigations.

We believe that the data obtained from this study will be a basis for the development of more powerful genetic biomarkers for identification of persons from DNA in forensic sciences. The database to be created in this way would help to get information for face morphology of the person from the biological evidence at the crime or crash scene. Moreover, the information obtained from DNA analysis might be used for drawing the 2D or 3D robot images. If the genes affecting the facial morphology and the variants of these genes were well understood, it would be possible to reach the person whose DNA could not be identified via databases.

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