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Craniofacial genetics as a differential identification tool: analysis of a subset of Yoruba-speaking population in Nigeria

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Abstract

Background Population-dependent genetics and normal-range variations in facial morphology have been reported across several populations, but there is paucity of literature on the African population. The study was designed to evaluate the potentials of craniofacial genetics as a differential identification tool amongst the Yoruba-Ethnic nationality in Nigeria. An anthropometric study of two thousand one hundred and nine (2109) randomly selected individuals (age 15–29 years) was conducted. The personal information of participants was obtained using semi-structured, self-administered questionnaires, while craniofacial parameters were measured from facial photographs with Digimizer software. DNA was isolated from buccal swabs samples obtained from three hundred participants (300) after careful stratifications to match the chosen ethnic group. Segments of two craniofacial-associated genetic markers (*PAX3* and *BMP4* genes) were amplified from participant's DNA samples using polymerase chain reaction technique. The amplified gene segments were purified, sequenced, and aligned with the reference sequences from the NCBI database.

Results A total of 45 samples were observed with synonymous and non-synonymous changes across the 2 genes. These genetic changes were not significantly associated with craniofacial differences in the study population. However, two participants, one each for *PAX3* and *BMP4*, displayed higher polymorphisms that were associated with values of different craniofacial linear parameters {Nasal width (al-al), Nasal height (n-sn), Morphological facial height (n-gn), Bizygomatic distance (zy-zy), Interendocanthal width (en-en), Ear width (t-pa), Ear height (sa-sba), Mandible height (sto-gn), Mouth width (ch-ch), Vermillion height (ls-sto), Eye fissure width@ R(en-ex)}, different from the general population not statistically significant.

Conclusion The slight differences in craniofacial parameter measurements in two individuals, with novel polymorphisms (SNPs) loci in *PAX3* and *BMP4* provides insights to baseline data for validation of cranio-genetic markers that may be useful in the population under study.

Keywords Craniofacial genetics, *PAX3*, *BMP4*, Novel polymorphisms, Facial identification, Yoruba population

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Introduction

Nigeria is a cosmopolitan country with multiple socially identifiable populations (largely Yoruba, Hausa, and Igbo), leading to more variations and complexities in the identification of forensic evidence. Thus, population-specific anthropometric data and genetic markers contributing to craniofacial differences are desperately needed. Facial morphological makeup is a vital

component of individualized identification, used globally in forensic examinations, ethnic and racial recognitions, or identity [1]. The differences in size, shape and spatial orientation of each of these facial features, such as eyes, nose, mouth, eyes, ears, and others, make an individual unique [2], with genetic compositions influencing the disparities [3].

Previous studies have found links between genetic variants and normal-range variation in facial morphology among humans [3], but not in a definitive African populations. Although the genetic basis that explains typical craniofacial morphology is yet to be clearly defined [3, 4], a recent study on fraternal twins [5] as well as the astounding similarity seen in monozygotic twins [6] and first-degree relatives [7] demonstrated the possible roles of genes in facial appearances. In the work of Sparks and Jantz's [8] on the relationship between craniofacial morphology and moderate genetic variation, craniometric characteristics exhibit average to moderate heritability, as with many other phenotypic traits, although the heritability of each craniometric feature varies greatly.

As violent crimes, road traffic accidents, and natural and human-mediated mass disasters including fires, floods, land border disputes, human trafficking, terrorist attacks, and other societal upheaval increases, there is a growing need for accurate identification of victims of these tragedies. Due to the dastardly and common nature of these unfortunate occurrences in some parts of Africa, the remains of many victims are often mutilated or charred leading to unregistered mass burials. In light of these difficulties and the devastating impact on families, governments, and society, there is a need to develop veritable tools that can efficiently identify the victims for easy closures, peace building and humanitarian purposes [9, 10]. Therefore, research in this area of forensic medicine may contribute to understanding the reasons why people differ from one another and assist with ethnic and racial identification, especially in multi-ethnic settings [1].

Methods

Ethical approval was obtained from the University of Ilorin Ethical Committee through the Faculty of Basic Medical Sciences Ethical Review Committee with reference number UERC/ASN/2019/1694. Written informed consent was obtained from each of the participants after providing detailed information on the scope and expected outcomes of the research. The study was carried out among University of Ilorin students of Yoruba sub-ethnic groups in Nigeria. The study population included males and females within the age range of 18 years to 29 years of the selected ethnic

groups (Lagos, Ogun, Ondo, Osun, Oyo, Kwara, Ekiti, and Kogi). A total of 2109 apparently healthy volunteers with no obvious facial abnormalities were selected using self-administered questionnaires to obtain their bio-data. Volunteers who met the inclusion criteria were randomly selected using stratified random sampling technique from Yoruba sub-ethnic groups of Nigeria.

Exclusion criteria

Participants with obvious facial abnormalities such as; cleft lips and palate; congenital abnormality of the head e.g. hydrocephalus; obvious asymmetry of the face; family history of craniofacial syndromes/ facial anomalies; and participants who have had severe facial injury in the past or of first-degree relatives were excluded from the study.

Pre-photographic capture

Participants were tagged with identifier numbers, and guided on how the facial pictures ought to be captured. All accessories were removed from the face and their hair groomed backwards.

Photographic capture

The photographs were captured using a digital camera (Amkov 3.0 Super AMOLED 24 mega pixel, ×4 zoom) following standard protocol [11]. An adjustable tripod stand was used to position the camera horizontally to the height of each participant [12], after which the photographic images were captured and downloaded onto a dedicated laptop computer. Photographs were captured from 2 angles: 1 frontal and 1 lateral view. The facial photographs were captured between 9 a.m. and 5 p.m., in order to avoid diurnal variations [13]. Participants were guided to stand upright behind a marked line (65 cm away from the camera) facing the lens of the camera with their forehead, neck, and ear clearly visible. The eyelids were opened (eye level at the Frankfurt position) [14], lips gently closed, neutral facial expression, heads held out straight in anatomical position [13, 15, 16]. All photographic images were captured against a white background.

Image processing

The various landmarks were chosen following the method adopted by Farkas [17] and annotated by the Digimizer software (version 5.3.5, MedCalc, Belgium). A total of 12 linear craniofacial distances were digitally located and measured on each facial photograph image. Out of the 12 craniofacial distances that were measured, 4 craniofacial indices were calculated following a modification of the methods adopted by Barash et al. [18]. Additional phenotypic traits (weight, height, body

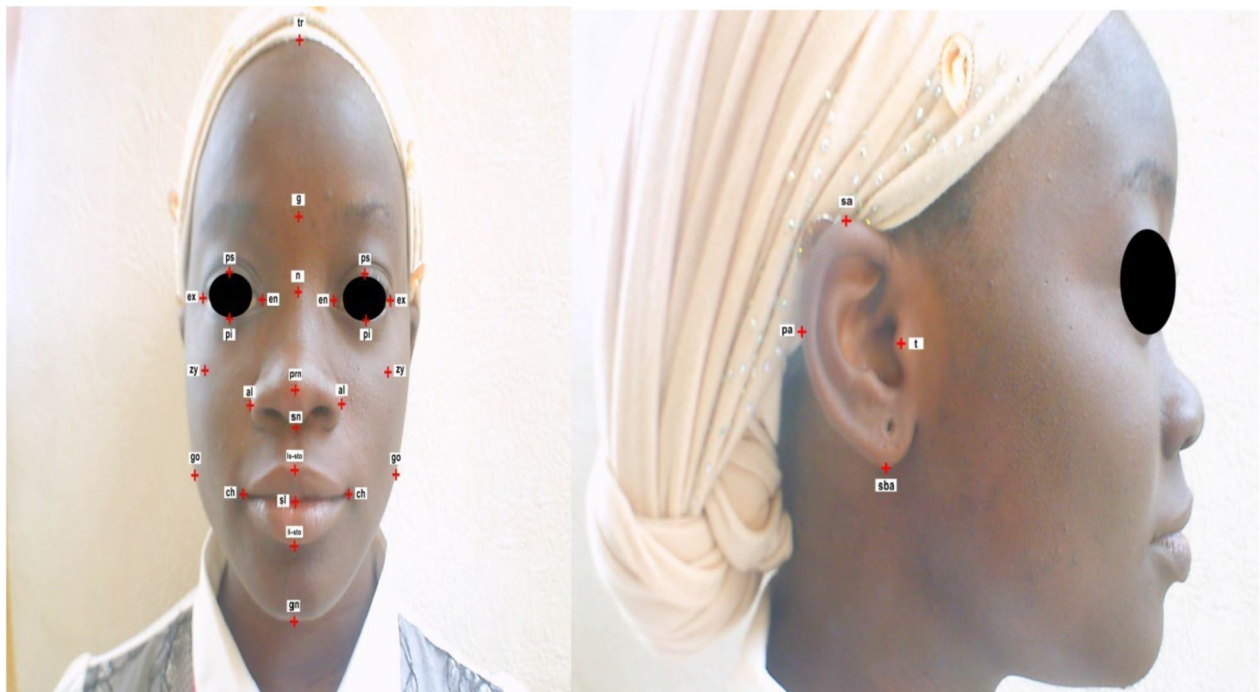


Fig. 1 Glabella (g); Nasion (n); Endocanthion (en) L/R; Exocanthion (ex) L/R; Palpebraic superius (ps) L/R; Palpebraicinferius (pi) L/R; Pronasale (prn); Subnasale (sn); Alare (al); Labrale superius (ls); Labraleinferius (li); Cheillion (ch) L/R; Pogonium (pg) L/R; Crista phitrum (cph) L/R; Trichion (tr); Gonium (go); Superaurale (sa-); Subaurale (sba); Postaurale (pa); Palpebral superioris (ps); Palpebral inferioris (pi); Tragus (t), Gnathion (gn)

mass index (BMI), sex, age, and ancestry) were collected and analyzed. The phenotypic data collection was done by a trained examiner for each of the parameters.

Samples collection and processing

The genetic association analysis employed a total of 300 samples. These 300 samples were drawn from the total number of participants (2019) who met the study's criteria. Buccal swabs samples were obtained from the participants for genomic DNA extraction and analysis. Briefly, each participant was provided clean water to rinse mouth prior to sample collection. This was done to ensure that there was no food material in the mouth that could contaminate the sample. The samples were obtained using sterile stick-swabs, by scraping the inner corners of the cheeks. The cotton tips of the sticks were broken off and suspended in 5 mL Eppendorf tubes containing the digestion buffer. The tubes were labeled with coded tags, and stored at -20°C until processed. The sample collection took place between July 2019 and December 2019. DNA was extracted using a DNA extraction kits (NORGEN, BIOTEK CORP, Canada) following the manufacturers protocol. Isolated genomic DNA samples were subjected to amplification of segments of PAX3 (exon 8) and BMP4 (exon 3) genes using referenced primers [19, 20]. These genes were selected based on

previous reports of association of genetic changes and craniofacial differences in distinct population [2, 3]. PAX3 gene forward sequence primers 5'-CTGTTTITA GGTAATGGGAC-3', and reverse primer 5'-GGACCT TCCCTATTTGAG-3' while the forward primer for the BMP4 gene was 5'- TGCCCTCCATTCTAGC-3' and 5'- ACTGGGGCTTTGATGTAACC-3' for the reverse primer. PCR conditions for PAX3 gene was as follows: 95°C for 5 min; 95°C for 25 s; 49.5°C for 1 min; 72°C for 1 min; 72°C for 5 min; 15°C ∞ , while the reaction conditions for BMP4 gene was 95°C for 5 min; 95°C for 30 s; 51°C for 45 s; 72°C for 1 min; 72°C for 5 min; 15°C ∞ . Both reactions were subjected to 40 repeated cycles. The amplicons were resolved on 2% agarose gel electrophoresis and documented appropriately. The amplicons were purified using a genomic purification kit (Qiagen, Germany) following the manufacturer's recommended protocol.

Sanger sequencing

Purified amplicons were forwarded for sequencing using Sanger's sequencing platform (Integrated DNA Technologies, Singapore) using the forward and the reverse primers shown above. The sequence data obtained were aligned with reference sequences of human PAX3 and BMP4 full length sequences from the NCBI database

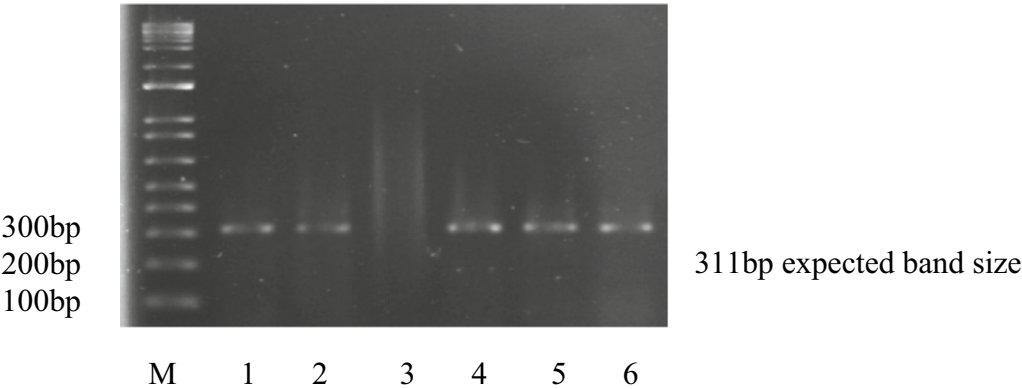


Fig. 2 Representative agarose gel electrophoresis resolution image of amplified amplicons for segments of exon 8 of PAX 3 gene resolved at 311 bp

Table 1 shows the values of craniofacial measurements of the affected male participant with corresponding changes in the DNA sequence of the PAX3 gene in comparison with other populations under study

Participants craniofacial parameters	Values for affected male participant (mm)	Mean ± std. deviation of the non affected male participants (mm)	Changes in PAX3 gene seen in the affected participant
Nasal width (al–al)	15.52	21.61 ± 4.99	22 Non-synonymous changes were seen at exon 8 of target region of interest
Nasal height (n–sn)	21.36	26.26 ± 5.63	
Morphological facial height (n–gn)	133.63	142.42 ± 34.77	
Bizygomatic distance (zy–zy)	113.84	119.64 ± 21.49	
Interendocanthal width (en–en)	10.44	13.44 ± 2.93	
Ear width (t–pa)	8.06	12.97 ± 3.62	
Ear height (sa–sba)	30.50	42.71 ± 10.76	
Mandible height	4.84	12.21 ± 2.02	
Mouth width (ch–ch)	29.53	38.75 ± 8.26	
Vermillion height (ls–sto)	6.16	8.31 ± 2.20	
Eye fissure width@R (en–ex)	12.52	14.26 ± 2.73	

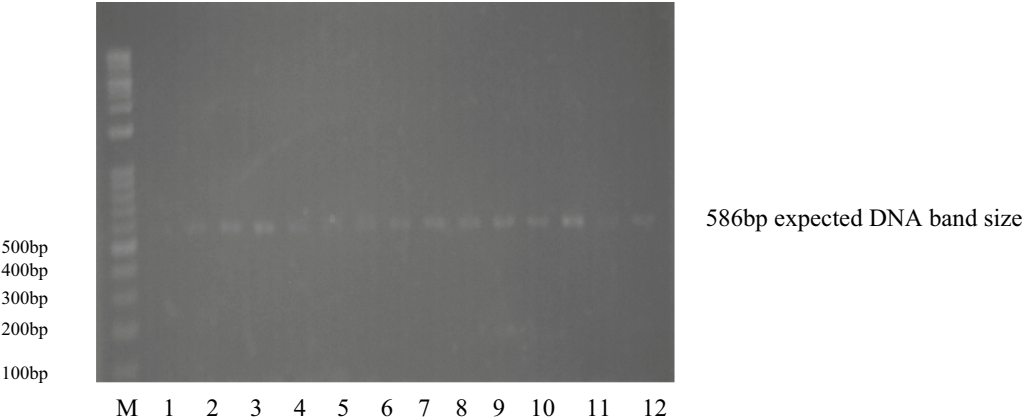


Fig. 3 Representative agarose gel electrophoresis resolution image of amplified segment of exon 3 of BMP4 gene from participants genomic DNA resolved at 586 bp

Table 2 shows the values of craniofacial measurements of the affected female participant with corresponding changes in the DNA sequence of the BMP4 gene in comparison with other populations under study

Participants craniofacial parameters	Values for affected female participant (mm)	Mean ± standard. deviation of the non affected female participants (mm)	Changes in BMP4 gene seen in the affected participant
Nasal width (al-al)	17.88	18.88 ± 4.45	8Non-synonymous and 2 synonymous Changes were seen at Exon 3 In addition; a stop codon was found upstream of the target sequence
Morphological facial height (n-gn)	90.53	73.35 ± 26.09	
Bizygomatic distance (zy-zy)	104.33	109.07 ± 20.76	
Interendocanthal width (en-en)	10.62	12.39 ± 3.00	
Ear width (t-pa)	10.20	12.49 ± 3.37	
Ear height (sa-sba)	39.34	35.44 ± 8.93	
Mandible height (sto-gn)	7.97	8.60 ± 1.08	
Vermillion height (ls-sto)	7.42	7.57 ± 2.08	
Eye fissure width® (en-ex)	13.28	12.86 ± 2.85	

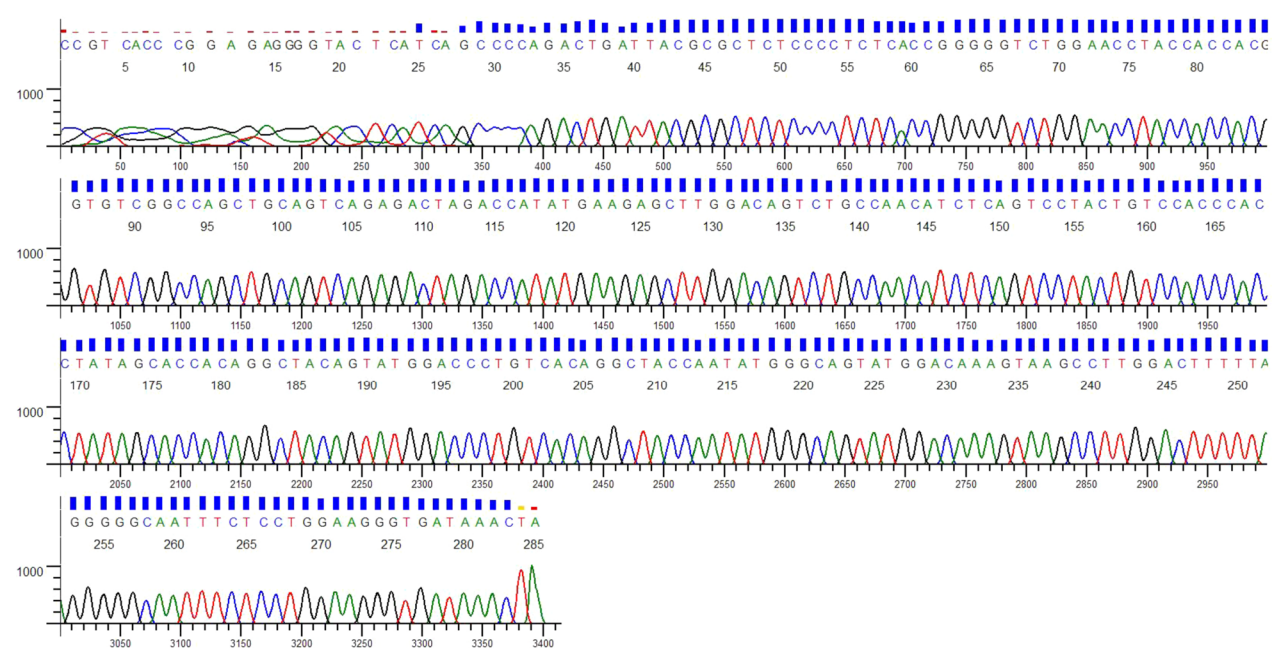


Fig. 4 Electropherogram of exon 8 segment of the *PAX3* gene of a participant. The sequence reads shows a positive and clean Sanger sequencing run of the samples

using web-based software, clustal Omega. To assess the phenotypic-genetic association, the craniometrics of the affected participants with genetic differences were compared with the average values of the population under study.

Statistical analysis

Different craniofacial parameters were measured using digimizer soft-wares and the data was collated and the mean values were calculated analyzed using the students't-test, with the confidence level set at 95%, with

$P \leq 0.05$ considered statistically significant. Linear regression analysis were conducted to test the genetic association between the suspected facial shape-associated SNPs and facial phenotypes.

Results
Craniofacial phenotypic measurement

Using the facial photographs of 2109 individuals, we accessed the 12 craniofacial phenotypic measurements of adolescents and young adults of Yoruba Nigeria, shown in Fig. 1.

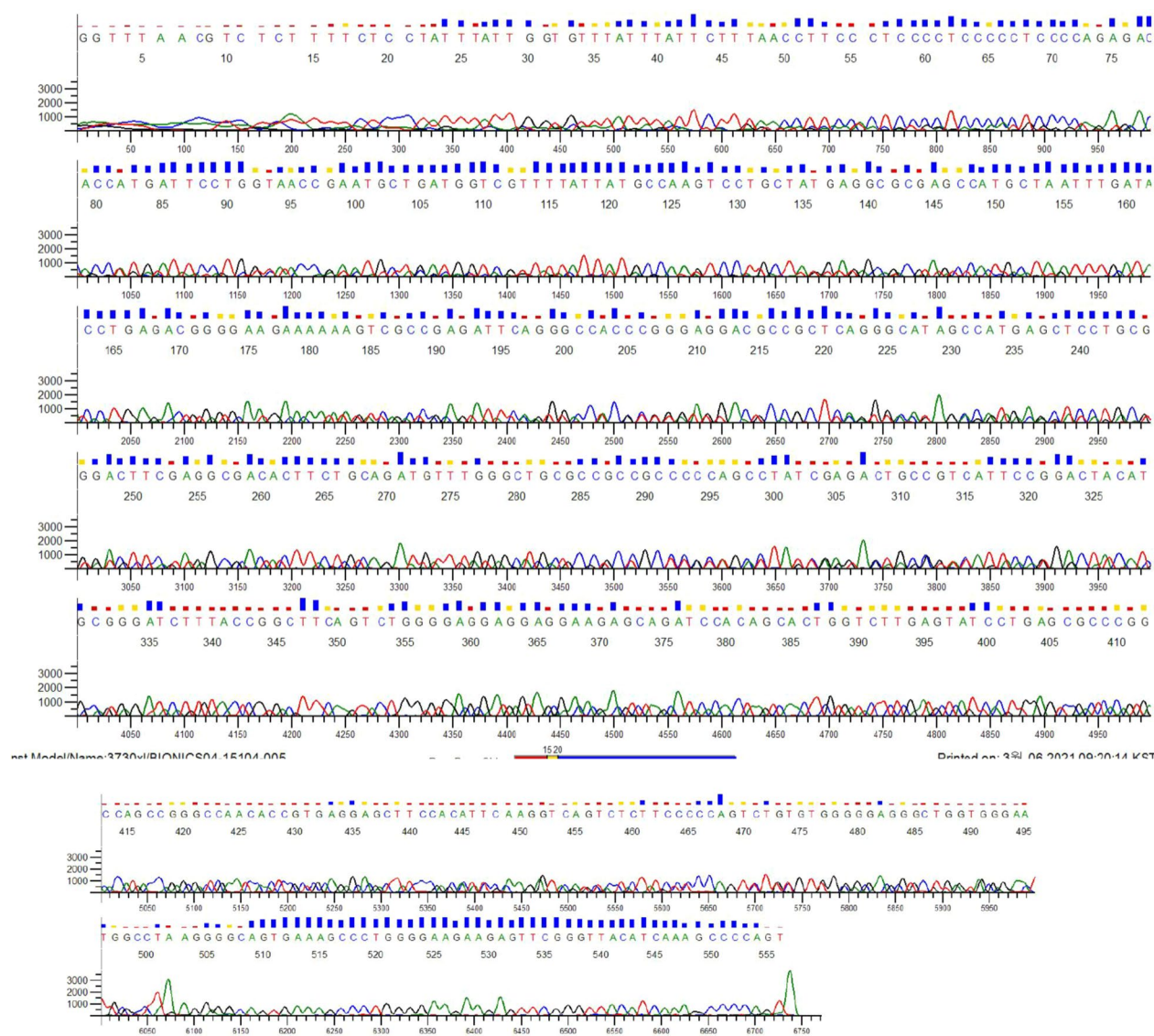


Fig. 5 Electrophoregram of exon 3 segment of the *BMP4* gene of participant. The sequence reads shows a positive and clean sanger sequencing run of the samples

1. Nasal width – is the maximum horizontal distance between the alare (al)
2. Nasal height – is the straight distance between Nasion (n) and subnasale (sn).
3. Vermillion height – is the vertical distance between labralesuperiorisistomium (ls-sto) – Labraleinferiorisistomium (li – sto)
4. Mouth width – is the horizontal distance between cheillion (ch) – cheillion (ch)
5. Mandible height – stomium(li-sto) – gnathion (gn)
6. Mandible width – gonium (go) – gonium (go)
7. Morphological facial height- nasion (n) – gnathion (gn)
8. Bizygomatic distance – zygium (zy) –zygium (zy)
9. Interendocanthal width – (en) – (en)
10. Interexocanthal width – (ex) – (ex)
11. Ear height- supaurale (sa) – subaurale (sba)
12. Ear width- tragus (t) – postaurale(pa)

Description of the landmarks

- al-The most protruded point of each alar contour
- n- The deepest point on the nasal bridge



Fig. 6 Chromatograph representation of sequenced-PAX3-gene. Sequence alignment of amplified PAX3 gene against the NCBI PAX3 gene database (Gene ID: 5077, MIM: 606,597). PAX3 represents the database sequence (Size of PAX3 gene = 99112 bp located on 2q36.1). While P1-P6 represents

amplified samples from study participants

Key: bright green-represents the positions of non-synonymous changes in the PAX3 gene of the affected participant
 red - represents the codons on the human PAX3 gene NCBI database sequence

- sn- The midpoint of the angle at the columella base where the lower border of the nasal septum and the surface of the upper lip meet
- ls- The midpoint of the upper vermillion line
- li- The midpoint of the lower vermillion line

- go-The most lateral point on the mandibular angle close to the bony gonion
- sto-The imaginary point at the crossing of the vertical facial midline and the horizontal labial fissure between gently closed lips, with teeth shut in the natural position
- ch- The point located at each labial commissure
- gn-The lowest anterior midpoint of the chin, located on the skin surface in front of the identical landmark of the mandible
- sn-The midpoint of the angle at the columnella base where the lower border of the nasal septum and the surface of the upper lip meet.
- en-The point at the inner commissure of the eye fissure
- ex-The point at the outer commissure of the eye fissure
- zy-The most lateral point of each zygomatic arch
- sa-The highest point on the free margin of the ear lobe
- sba-The lowest point on the free margin of the ear lobe
- t-The notch on the upper margin of the tragus
- pa- The most posterior point on the free margin of the ear [3]

Genetic analysis result

The amplification of exon 8 segment of the PAX 3 gene was achieved with an expected DNA band size of 311 bp as shown in Fig. 2. From the 300 samples sequenced and analyzed, a total of 22 samples were observed with synonymous and non-synonymous changes across the exon 8 of PAX3 gene. The sequence profile from one male participant displayed higher polymorphisms in the exon 8 segment, in manners distinctively different from others in the study population. The polymorphic changes in the particular sample coincidentally displayed differences in mean values of craniofacial parameters measured (Table 1), as considered for the population under study.

The amplification of exon 3 segment of the BMP4 gene was achieved with an expected DNA band size of 586 bp as shown in Fig. 3. Out of the 300 samples that were analyzed for BMP4, 23 samples were observed with synonymous and non-synonymous changes across the exon 3 segment of the BMP4 gene. The sequence profile from one female participant displayed higher polymorphisms in the exon 3 segment, in manners also distinctively different from others in the study population. The polymorphic changes in the sample of the participant were associated with differences in craniofacial parameter values from the average (Table 2) considered for the population under study (Fig. 4).

The sequence alignment analysis of all other amplified samples displayed homology across the study population for both genes (Fig. 5).

Phenotypic-genetic association analysis

A total of 32 polymorphic sites were detected by sequence analysis. It was noted that the two participants who displayed significant polymorphic changes in the two genes of interest (one for PAX3 and the other for BMP4) also displayed marked differences (though not statistically significant) in the values of the linear craniofacial measurements when compared differentially with the generality of the participants under study. The mutations were either due to deletions or single-base substitution of the nucleic acid sequences (Fig. 6).

Discussions

Due to the diverse ethnic background of the Nigerian population and the high prevalence of unidentified and unclaimed remains of victims in crisis prone areas in Nigeria[21], there is a need for an efficient tool or technology that can help with identity recognition. Anthropometric measurements are widely used in most of Sub-Saharan Africa, including Nigeria, with little effort made to explore the roles of genetic markers affecting normal facial morphology. This study presents a proof of concept in combining genetic markers that determine normal phenotypic appearance with craniofacial measurements to identify a subset of the Yoruba ethnic group in Nigeria (Fig. 7).

The choice of these age groups is also in accordance with Barash [22], where younger age has little or no impact on face morphology, and Bishara et al. [23], wherein it was observed that significant changes in facial morphology occur more later in life, after the mid-twenties. Furthermore, earlier research revealed that criminal behaviors are predominant in adolescence or early adulthood. Also, persons within the age grouping used in this study have been reported to have lessened facial laxity and the complete growth at the basocranium [22].

We report differences in the values of affected participants when compared with the normal average unaffected population in the following craniofacial linear measurements (Nasal width (al-al), nasal height (n-sn), morphological facial height (n-gn), bizygomatic distance (zy-zy), inter-endocanthal width (en-en), ear width (t-pa), ear height (sa-sba), mandible height, mouth width (ch-ch), Vermillion height (ls-sto), and eye fissure width (en-ex). However, these associations are often considered speculative [3]. In this study, we identified 22 non-synonymous changes at exon 8 of

B3	----GGTTTAACGTCTCTTTTCTCCTATTTATTTGGTGTGTTATTTATCTTTAACCTT-CC	55
B4	GGAAATAAAGATCATCATTTCTCCTATTTATTTGGTGTGTTATTTATCTTTAACCTTCCA	60
B2	GGAGTTAAATAATATCATTTCTCCTATTTA-TTGGTGTGTTATTTATCTTTAACCTTCCA	59
<i>BMP4</i>	-----	0
B1	GGGACTAAGCTATTACTTACTCCCTATTTATTTGGAGTTTCAGTTTATCTTTAACCTTCCA	60
B3	CTCCCCCTCCCCCTCCCCAGAGACACCATGATTCTCTGGTAACCGAATGCTGATGGTCGTTT	115
B4	CTCCCCCTCCCCCTCCCCAGAGACACCATGATTCTCTGGTAACCGAATGCTGATGGTCGTTT	120
B2	CTCCCCCTCCCCCTCCCCAGAGACACCATGATTCTCTGGTAACCGAATGCTGATGGTCGTTT	119
<i>BMP4</i>	-----ATGATTCTCTGGTAACCGAATGCTGATGGTCGTTT	34
B1	CTCCCCCTCCCCCTCCCCAGAGACACCATGATTCTCTGGTAACCGAATGCTGATGGTCGTTT	120

B3	TATTATGCCAAGTCCTGCTA TGA GGCGCGAGCCATGCT AAT TTGATACCTGAGACGGGGA	175
B4	TATTATGCCAAGTCCTGCTAGGAGGCGCGAGCCATGCTAGTTTGATACCTGAGACGGGGA	180
B2	TATTATGCCAAGTCCTGCTAGGAGGCGCGAGCCATGCTAGTTTGATACCTGAGACGGGGA	179
<i>BMP4</i>	TATATGCTGCAATCCTGCTAGGAGGCGCGAGCCATGCTAGTTTGATACCTGAGACGGGGA	94
B1	TATTATGCCAAGTCCTGCTAGGAGGCGCGAGCCATGCTAGTTTGATACCTGAGACGGGGA	180

B3	AGAAAAAAGTCGCCGAGATTCAAGGCG GAG CGG GAGCGCCGCTCAGGC AAT AGCCATG	235
B4	AGAAAAAAGTCGCCGAGATTCAAGGCGCACGCGGAGGAGCGCCGCTCAGGCGAGGCCATG	240
B2	AGAAAAAAGTCGCCGAGATTCAAGGCGCACGCGGAGGAGCGCCGCTCAGGCGAGGCCATG	239
<i>BMP4</i>	AGAAAAAAGTCGCCGAGATTCAAGGCGCACGCGGAGGAGCGCCGCTCAGGCGAGGCCATG	154
B1	AGAAAAAAGTCGCCGAGATTCAAGGCGCACGCGGAGGAGCGCCGCTCAGGCGAGGCCATG	240

B3	AGCTCTGCGGGACTTCGAGGCGACACTTCTGCAGATGTTTGGGCTGCGCCGCCG CCCC	295
B4	AGCTCTGCGGGACTTCGAGGCGACACTTCTGCAGATGTTTGGGCTGCGCCGCCGCCCGC	300
B2	AGCTCTGCGGGACTTCGAGGCGACACTTCTGCAGATGTTTGGGCTGCGCCGCCGCCCGC	299
<i>BMP4</i>	AGCTCTGCGGGACTTCGAGGCGACACTTCTGCAGATGTTTGGGCTGCGCCGCCGCCCGC	214
B1	AGCTCTGCGGGACTTCGAGGCGACACTTCTGCAGATGTTTGGGCTGCGCCGCCGCCCGC	300

B3	AGCCT ATCGAGACT GCCGTCAATCCGGACTACATGCGGGATCTTTACCGGCTTCAGTCTG	355
B4	AGCCTAGCAAGAGTGCCGTCAATCCGGACTACATGCGGGATCTTTACCGGCTTCAGTCTG	360
B2	AGCCTAGCAAGAGTGCCGTCAATCCGGACTACATGCGGGATCTTTACCGGCTTCAGTCTG	359
<i>BMP4</i>	AGCCTAGCAAGAGTGCCGTCAATCCGGACTACATGCGGGATCTTTACCGGCTTCAGTCTG	274
B1	AGCCTAGCAAGAGTGCCGTCAATCCGGACTACATGCGGGATCTTTACCGGCTTCAGTCTG	360

B3	GGGAGGAGGAGGAAGAGCAGATCCACAGCACTGGTCTTGAGTATCCTGAGCGCCCGGCCA	415
B4	GGGAGGAGGAGGAAGAGCAGATCCACAGCACTGGTCTTGAGTATCCTGAGCGCCCGGCCA	420
B2	GGGAGGAGGAGGAAGAGCAGATCCACAGCACTGGTCTTGAGTATCCTGAGCGCCCGGCCA	419
<i>BMP4</i>	GGGAGGAGGAGGAAGAGCAGATCCACAGCACTGGTCTTGAGTATCCTGAGCGCCCGGCCA	334
B1	GGGAGGAGGAGGAAGAGCAGATCCACAGCACTGGTCTTGAGTATCCTGAGCGCCCGGCCA	420

B3	GCCGGGCCAACACCGTGAGGAGCTTCCAC ATTCAA GGTCAGTCTCTTCCCCAGTCTGTG	475
B4	GCCGGGCCAACACCGTGAGGAGCTTCCACCACGAAGGTCAGTCTCTTCCCCAGTCTGCG	480
B2	GCCGGGCCAACACCGTGAGGAGCTTCCACCACGAAGGTCAGTCTCTTCCCCAGTCTGCG	479
<i>BMP4</i>	GCCGGGCCAACACCGTGAGGAGCTTCCACCACGAAGGTCAGTCTCTTCCCCAGTCTGCG	394
B1	GCCGGGCCAACACCGTGAGGAGCTTCCACCACGAAGGTCAGTCTCTTCCCCAGTCTGCG	480

B3	TGGGGGAGGGCTGGTGGGAATGGCCTAAGGGGCAGTGAAAGCCCTGGGGAAGAAGAGTTC	535
B4	TGGGGGAGGGCTGGTGGGACTGGCTAGAGGGGCAGTGAAAGCCCTGGGGAAGAAGAGTTC	540
B2	TGGGGGAGGGCTGGTGGGACTGGCTAGAGGGGCAGTGAAAGCCCTGGGGAAGAAGAGTTC	539
<i>BMP4</i>	TGGGGGAGGGCTGGTGGGACTGGCTAGAGGGGCAGTGAAAGCCCTGGGGAAGAAGAGTTC	432
B1	TGGGGGAGGGCTGGTGGGACTGGCTAGAGGGGCAGTGAAAGCCCTGGGGAAGAAGAGTTC	540

B3	GGGTTACATCAAAGCCCCAGT-----	556
B4	GGGTTACATCAAAGCCCCAGTAAA	565
B2	GGGTTACATCAAAGCCCCAGTATT-	563
<i>BMP4</i>	-----	432
B1	GGGTTACATCAAAGCCCCAGTA---	562

Fig. 7 Sequence alignment of amplified *BMP4* gene against the NCBI database (Gene ID: 652, MIM: 112,262 located on 14q22.2 with size of 7156 bp). *BMP4* represents the database sequence of human. While B1-B4 represents amplified samples from study participants

Key:

- red**: represents the codons on the human *BMP4* gene NCBI database sequence
- Bright green**: represents the positions of non-synonymous changes in the *BMP4* gene of the affected participant.
- Aqua**: represents the positions of synonymous changes in the *BMP4* gene of the affected participant.
- Deep mauve**: represents the position of stop codon in the *BMP4* gene of the affected participant.
- Yellow**: represents the start codon on the human *BMP4* gene NCBI database sequence.

the PAX3 gene relatable with variation in the values of affected participants when compared with the general population under consideration. These mutations were either due to deletion or single-base substitution of the sequence when compared with the human PAX3 NCBI sequence. Deletion of cytosine occurred at positions 178 and 179 for one participant with single-base substitution occurring at other positions for the same participants in recognizable manner. The single base substitutions found in this participant is consistent with previous research [24]. These point mutations (non-synonymous changes / synonymous changes) seen within the region of interest for PAX3 gene may result in changes in the polypeptide array, possibly influencing protein folding and function and subsequently affecting the phenotypic expression of such gene [3]. This ultimately can affect the phenotypic appearance of the participant. The synonymous SNPs typically do not affect protein structure or function but recent studies [25] suggested that they may cause major impact in potential phenotypes. Point mutations on the segment of interest of the PAX3 gene may represent a wide spectrum of mutations that can cause Waardenburg syndrome type 1, characterized by dystopia canthorum, broad nasal root, square-tipped nose, square jaw, among others [18]. The results of our study of affected participants also displayed measurements suggestive of variation compared to the general population under study though not significant. It is however worthy of note that, the singular individual identified with values that differ from the average values of the population under consideration had subtle facial features of Waardenburg syndrome with sparsely defined classic facial phenotype [26]. It is thus possible that the polymorphisms in PAX3 gene of the participant may not be fully manifest in conditions wherein the recessive form of the gene is inherited [26]. This may be possible for the insignificant changes in craniofacial measurement compared with the general population despite pronounced genetic changes. Also, the differences in the values of the affected participants (lower but not statistically significant) when compared with the average value of unaffected participants, for mandible height, may suggest square jaw as a feature of Waardenburg syndrome associated with mutation in the exon 8 of PAX3 gene [19]. This is corroborated by the finding of a previous study [27] wherein connections between disordered and normal variation are likely a result of genetic variation and associated typical facial appearance. The results of our study is also in line with previous findings on the association of the PAX3 gene with the shape of the nasal root area [4, 28–32] and facial width [33]. Our finding

also aligns with the report of Mark et al. [3], wherein a noticeable but not significant association was described between nasal area, maximum facial width (zy-zy), upper lip width, and ear height and; Kohn [34]. These studies reported a marked difference in nose width and bizygomatic distance with inheritance markers, implying that facial morphology is largely shaped by genetics even though the phenotypic values may vary across ethnic and racial lines. Previous studies [2, 4, 29] have also documented significant effect on a decrease nasion to mid endocanthal point distance in European and Latin American populations. In addition, nose width and bizygomatic width (facial width) are the most prominent deviant variables in humans. Thus, our findings of the genetic differences may corroborate the anthropometric findings that the PAX3 gene could be a useful genetic marker for identification of Yoruba population in Nigeria.

We also discovered polymorphisms in the BMP4 gene that have been linked to craniofacial abnormalities. We report for the first time, the description of a participant with changes in the segment of BMP4 gene displaying values suggestive of craniofacial abnormality different from members of the population (Yoruba) under consideration. The changes (8 non-synonymous and 2 synonymous) identified within exon 3 of the BMP4 gene in addition to a stop codon were distinct in the population under study. The non-synonymous alterations/ SNPs found within the region of interest for the BMP4 gene may bring about a change in polypeptide sequence, thereby altering protein scaffolding and, as a result, disrupting a participant's inter-endocanthal width (en-en) as observed in the particular participant within the study population. This may suggest a correlation that participants with mutated BMP4 gene may have parameters suggestive of eye structural defect, as exemplified by Dunn et al., [35] where participants with mutated BMP4 genes may have craniofacial malformation with measurements insignificant from the general population. Thus, the genetic changes highlighted in this singular participant in our study (though not marked by significant craniofacial measurements) may corroborate the anthropometric findings that the BMP4 gene is also a useful genetic marker for identification of Yoruba population in Nigeria [36].

With samples from 300 participants out of 2109 enrolled, analyzed for genetic changes, this study may be limited in sample size to identify latent association of the genetic markers with the craniofacial changes. However, the approach showed potential in mapping segments of PAX3 and BMP4 genes that may affect the population. Future studies may consider genome wide association

study (GWAS) to unravel linkages in sequence arrays that this study was unable to espouse. These studies could target larger geo-ethnic zones in Nigeria to further validate the efficiency of the selected marker genes. In addition to the craniofacial parameters measured in this study, other major and minor criteria[37] may be assessed and correlated with genetic differences in such studies. The affected participants with either BMP4/PAX3 genetic differences or anthropometric correlates identified in this study can be tracked, and assisted in improving their health and preventing future health problems. They can also be screened for genetic abnormalities that may be hereditary. Such participants may be probands with facial phenotype that are crucial for craniofacial syndrome diagnosis.

Conclusion

The study found that PAX3 and BMP4 genes are potential genetic markers that may be considered for prediction of the facial appearances of the Yoruba population. In addition, the study has provided baseline data for craniofacial measurements that is relevant to forensic investigators, clinicians, plastic surgeons, and genetic counselors in the diagnosis and treatment of craniofacial abnormalities in Yoruba population. This is buttressed by the homogeneity or similarities in the genetic sequences observed within the population, though, comparison with other ethnic nationalities was not carried out.

Frankfurt position- a plane connecting the highest point of the opening of the external auditory canal with the lowest point on the lower margin of the orbit, used to orient a human head so that the plane is horizontal.

Abbreviations

Anthropometric terms

N	Nasion
En	L/R- Endocanthion (left/right eye)
Ex	L/R- Exocanthion (left/right eye)
Sn	Subnasale
Al	Alare
Ls	Labrale superius
Li	Labraleinferius
Ch	L/R- Cheilion (left/right)
Go	Gonium
Sa	Superaurale
Sba	Subaurale
Pa	Postaurale
Ps	Palpebral superioris
Pi	Palpebral inferioris
T	Tragus
Gn	Gnathion
al-al	Nasal width
n-sn	Nasal height
sto-gn	Mandible height
go-go	Mandible width
n-gn	Facial height
zy-zy	Bizygomatic distance/face width
ch-ch	Mouth width

Is-sto	Vermillion height
en-en	Interendocanthal width
ex-ex	Inter-exocanthal width
en-ex [®]	Eye fissure width (right eye)
sa-sba [®]	Ear height
t-pa	Ear width

Population genetics terms

GWAS	Genomic wide association studies
SNPs	Single nucleotide polymorphisms
nsSNPs	Non-synonymous single nucleotide polymorphisms
NCBI	National centre for biotechnology information
PAX3	Paired box gene 3
BMP4	Bone morphogenic protein 4
DNA	Deoxy-ribo nucleic acid

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Author contributions

SBK, OAI, ASA, OA and MSA designed the study, SBK carried out the craniofacial-genetic studies and the data collection. AI and MYA carried out the statistical analysis and interpretation. SBK, MYA and OA drafted the manuscript, and all authors read and approved the final draft. MSA supervised the research work.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The participants provided their written informed consent to participate in the study, which was approved by the University of Ilorin Ethical Committee through the Faculty of Basic Medical Sciences Ethical Review Committee and this was approved with reference number UERC/ASN/2019/1694.

Consent for publication

“Written informed consent for the publication of the findings of this study was obtained from all the participants of the study. The finding of this study has also been communicated and discussed with the study participants without identification of personal data”.

Competing interests

The authors declare that there is no conflict of interest.

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References

1. Maalman RS, Stansie C, Darkoa N (2019) Facial types and morphology : a study among Sisaala and Dagaaba adult population in the upper west region, Ghana. Facial types and morphology : a study among Sisaala and

- Dagaaba adult population in the upper west region ,Ghana. *Sci African* 3:00071. <https://doi.org/10.1016/j.sciaf.2019.e00071>
2. Richmond S, Howe LJ, Lewis S, Stergiakouli E, Zhurov A (2018) Facial genetics: a brief overview. *Front Genet*. <https://doi.org/10.3389/fgene.2018.00462>
 3. Mark B, Philipp EB, van Angela D (2018) Identification of the single nucleotide polymorphisms affecting normal phenotypic variability in human craniofacial morphology using candidate gene approach. *J Genet Genome Res*. <https://doi.org/10.23937/2378-3648/1410041>
 4. Adhikari K, Fuentes-Guajardo M, Quinto-Sánchez M, Mendoza-Revilla J, Camilo Chacón-Duque J, Acuña-Alonzo V et al (2016) A genome-wide association scan implicates DCHS2, RUNX2, GLI3, PAX1 and EDAR in human facial variation. *Nat Commun* 7(May):1–11
 5. Martínez-abadías N, Esparza M, Sjøvold T, González- R 2018 Heritability of human cranial dimensions : comparing the evolvability of different cranial regions 1–24.
 6. Weinberg SM, Parsons TE, Marazita ML, Maher BS (2013) Heritability of face shape in twins: a preliminary study using 3D stereophotogrammetry and geometric morphometrics. *Dent* 3000 1(1):7–11
 7. Roosenboom J, Hens G, Mattern BC, Shriver MD, Claes P (2016) Exploring the underlying genetics of craniofacial morphology through various sources of knowledge. *BioMed Research International*. Hindawi Publishing Corporation
 8. Sparks CS, Jantz RL (2002) A reassessment of human cranial plasticity: Boas revisited. *Proc Natl Acad Sci U S A* 99(23):14636–14639
 9. Alvarez-Cubero MJ, Saiz M, Martinez-Gonzalez LJ, Alvarez JC, Eisenberg AJ, Budowle B et al (2012) Genetic identification of missing persons: DNA analysis of human remains and compromised samples. *Pathobiology* 79(5):228–238
 10. Sommer J, Hinsberger M, Elbert T, Holtzhausen L, Kaminer D, Seedat S et al (2017) The interplay between trauma, substance abuse and appetitive aggression and its relation to criminal activity among high-risk males in South Africa. *Addict Behav* 64:29–34. <https://doi.org/10.1016/j.addbeh.2016.08.008>
 11. Ozioko OM, Egwu OA, Ozioko US (2020) Photoanthropometric Auricular Morphology for Identification in Southeast Nigerians. *Int J Inf Secur Res* 10(1):924–931
 12. Moshkelgosha V, Fathinejad S, Pakizeh Z, Shamsa M, Golkari A (2015) Photographic facial soft tissue analysis by means of linear and angular measurements in an adolescent Persian population. *Open Dent J* 9:346–356
 13. Anjankar VP, Nair SK, Anjankar VP, Singh S, Bindra M (2014) The study of cephalic index of medical students of central India. *Asian J Biomed Pharmaceut Sci* 04(28):48–50
 14. Sforza C, Elamin F, Tommasi DG, Dolci C, Ferrario VF (2013) Morphometry of the soft tissues of the orbital region in Northern Sudanese persons. *Forensic Sci Int* 228(13):180.e1–180.e11. <https://doi.org/10.1016/j.forsciint.2013.02.003>
 15. Sha and Jadhav, 2004-Cephalic Index In Gujarat Students.
 16. Simmons LW, Rhodes G, Peters M, Koehler N (2004) Are human preferences for facial symmetry focused on signals of developmental instability? *Behav Ecol* 15(5):864–871
 17. Farkas LG (1994) Anthropometry of the head and neck. *Head Neck* 17(2):427. <https://doi.org/10.1002/hed.2880170222>
 18. Barash AM, Bayer PE, Van DA (2016) Candidate gene scan for single nucleotide polymorphisms involved in the determination of normal variability in human craniofacial morphology. *BioRxiv* 6:289
 19. Baldwin CT, Hoth CF, Macina RA, Milunsky A (1995) Mutations in PAX3 that cause Waardenburg syndrome type I: ten new mutations and review of the literature. *Am J Med Genet* 58(2):115–122
 20. Bakrania P, Efthymiou M, Klein JC, Salt A, Bunyan DJ, Wyatt A et al (2008) Mutations in BMP4 cause eye, brain, and digit developmental anomalies: overlap between the BMP4 and hedgehog signaling pathways. *Am J Hum Genet* 82(2):304–319
 21. Silali MB, Odero W, Rogena E (2021) Studies on DNA molecules in identification of unclaimed or missing dead bodies presumed death in Western Kenya utilizing physical comparison standards. *Highlights Med Med Sci* 2(1):24–45
 22. Barash M. 2014 Identification of single nucleotide polymorphisms (SNPs) involved in the determination of craniofacial morphology
 23. Bishara SE, Jakobsen JR, Hession TJ (1959) Soft tissue profile changes from 5 to 45 years of age. *Am J Orthodont Dentofac Orthoped* 114:698–706
 24. Hoth CF, Milunsky A, Lipsky N, Sheffer R, Clarren SK, Baldwin CT (1993) Mutations in the Paired Domain of the Human PAX3 Gene Cause Klein-Waardenburg Syndrome (WS-III) as well as Waardenburg Syndrome Type I (WS-I). *Am J Human Gene* 52:455–462
 25. Sauna ZE, Kimchi-sarfaty C (2011) Understanding the contribution of synonymous mutations to human disease. *Nat Publ Gr* 12(10):683–691. <https://doi.org/10.1038/nrg3051>
 26. Gurovich Y, Hanani Y, Bar O, Fleischer N, Gelbman D, Basel-Salmon L, et al. 2018 DeepGestalt - Identifying Rare Genetic Syndromes Using Deep Learning. Available from: <http://arxiv.org/abs/1801.07637>
 27. Winter RM (1996) What's in a face? *Nat Genet* 12(2):124–129. <https://doi.org/10.1038/ng0296-124>
 28. Liu F, van der Lijn F, Schurmann C, Zhu G, Chakravarty MM, Hysi PG, et al. 2012 A Genome-Wide Association Study Identifies Five Loci Influencing Facial Morphology in Europeans. *PLoS Genet* 8(9).
 29. Paternoster L, Zhurov AI, Toma AM, Kemp JP, St Pourcain B, Timpson NJ 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(3):478–485. <https://doi.org/10.1016/j.ajhg.2011.12.021>
 30. Claes P, Hill H, Shriver MD (2014) Forensic science international : genetics toward DNA-based facial composites : preliminary results and validation. *Forensic Sci Int Genet* 13:208–216. <https://doi.org/10.1016/j.fsigen.2014.08.008>
 31. Shaffer JR, Orlova E, Lee MK, Leslie EJ, Raffensperger ZD, Heike CL et al (2016) Genome-wide association study reveals multiple loci influencing normal human facial morphology. *PLoS Genet* 12:1006149
 32. Wilson-nagrani C, Richmond S, Paternoster L (2018) Non-syndromic cleft lip and palate polymorphisms affect normal lip morphology. *Front Gene* 9:1–12
 33. Sinigerova S, Nowak S, Boehringer S, Van Der LF, Liu F, Gu M et al (2011) Genetic determination of human facial morphology : links between cleft-lips and normal variation. *Eur J Human Gene* 19:1192–1197
 34. Kohn L (1991) The role of genetics in craniofacial morphology and growth. *Ann Rev Anthropol* 20:261–278
 35. Dunn NR, Winnier GE, Hargett LK, Schrick JJ, Fogo AB, Hogan BLM (1997) Haploinsufficient phenotypes in Bmp4 heterozygous null mice and modification by mutations in Gli3 and Alx4. *Develop Biol* 247:235–247
 36. Kayser M, Schneider PM (2009) Forensic science international : genetics DNA-based prediction of human externally visible characteristics in forensics : motivations, scientific challenges, and ethical considerations. *Foren Sci Int: Gene* 3:154–161
 37. Odogu V, Chukwuka I, Chinawa N (2017) Waardenburg syndrome in an 8 year old African child: case report. *Br J Med Med Res* 21(9):1–5

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