Original Article

DNA Sequence Patterns in PAX3 and BMP4 for Differential Identification of Idoma and Igede Ethnic Groups in Benue State, Nigeria

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ABSTRACT

Background: Genes involved in craniofacial development may provide useful molecular markers for forensic identification and population studies. PAX3 and BMP4 regulate neural crest migration, facial morphogenesis, and skeletal patterning. This study evaluated sequence variation in these genes and their potential to differentiate the Idoma and Igede ethnic groups of Benue State, Nigeria.

Methods: Buccal-swab genomic DNA samples from 300 Idoma and Igede participants were analyzed for each gene. Target regions of PAX3 exon 8 and BMP4 exon 3 were amplified by polymerase chain reaction, producing expected fragments of 311 bp and 586 bp, respectively. The amplified products were sequenced using the Sanger method and aligned with National Center for Biotechnology Information reference sequences to identify synonymous and non-synonymous variations. Observed genetic variants were also assessed in relation to craniofacial measurements. Participants included males and females aged 15–29 years from the Idoma and Igede ethnic groups. All 300 samples were successfully amplified and sequenced. Successful amplification and sequencing were obtained for both target regions. In PAX3, 34 of the 300 samples analyzed exhibited synonymous sequence variations, representing a variant frequency of 11.3%. In BMP4, 22 of the 300 samples exhibited synonymous and non-synonymous variations, corresponding to a frequency of 7.3%. Selected participants carrying functionally relevant variants showed measurable deviations from population means in craniofacial dimensions, particularly nasal width, interendocanthal width, facial height, bizygomatic width, ear dimensions, and nasal index. However, sequence alignment and phylogenetic patterns showed substantial genetic homology between the Idoma and Igede groups, with no clear population-specific clustering at either locus. discriminatory ability for differentiating the Idoma and Igede populations as distinct ethnic groups.

Keywords: PAX3, BMP4, DNA sequence variation, Differential identification, Idoma and Igede

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Introduction

Human identification is one of the most important issues in the forensic science, biological anthropology and population genetics; it is the case when fingerprints, dental records, or personal documents are not accessible and/or reliable[1]. Molecular and craniofacial markers have gained growing significance in the process of determining the identity and affinity with the population in scenarios of mass disasters, violent conflict, decomposition, or skeletal recovery(2). More recent developments in the area of genetics have made this area even stronger as it has demonstrated that the shape of the face can not only be measured but that it is also highly dependent on inherited biological variation(3,4). DNA sequence patterns of genes related to facial development have thus far emerged as an enticing method in the investigation of human populations both in forensic and anthropological differentiation(5).

The interdisciplinary approach to craniomaxillofacial genetics has become a secondary discipline that interconnects molecular biology, developmental anatomy, forensic anthropology and morphometrics. Research has established that facial characteristics are very much heritable and are dependent on complicated interactions between genetic, developmental and environmental influences. A number of genes involved in the normal variation of faces have been discovered using genome-wide association studies; these genes are PAX3, DCHS2, RUNX2, EDAR and BMP4(6,7). The Implications of these findings on forensic identification are significant since it indicates that certain genetic variations may be involved in identifying population and individual differences towards craniofacial appearances that are identifiable. In this regard, the analysis of DNA sequence helps to provide another tier of evidence, as compared to conventional anthropometry.

Two particularly significant genes which are involved in cranio facial development are PAX3 and BMP4 since they are involved in regulative functions during early embryogenesis and morphogenesis of the face(8). PAX3 is an important developmental transcription factor, and plays a paramount role in the migration and variousiation of neural crest cells as well as in the shaping of facial mesenchyme(8). Crest cells of the nerves form various body parts of the faces such as the maxilla, mandible, nasal cartilages and face connective tissues. Normal facial diversity has been associated with variations in PAX3, and has been demonstrated in traits like nasal width, the LAC distance between the eyes and jaw formations, and disease-causing mutations have been identified to induce craniofacial defects similar to those seen in Waardenburg syndrome(9). Due to this dual applicability to normal variation as well as syndromic deformity, PAX3 is being considered a good candidate gene study of facial differentiation.

Likewise, BMP4 signaling molecule is one of the main molecules involved in the differentiation of embryonic tissues, skeletal development, and expression patterning of craniofacial structures(10). Cranial neural crest cells express BMP4 which is required in controlling development of the maxillary, mandibular and frontonasal processes in growth and shape development. Experimental and clinical research has demonstrated that a change in BMP4 expression can affect facial width, jaw size among other craniofacial dimensions whilst mutation of the gene has been linked with clefting, micrognathia and other facial defects(11). BMP4 is also able to interact with other key developmental regulators such as PAX3, SHH and MSX1 and thus it is at the centre of gene networks that regulate facial form(11). Because of this, the correlation of DNA sequences patterns in BMP4 would be helpful both when conducting population investigation and in a legal setting.

Single nucleotide polymorphisms have a forensic value of being abundant and stable and suitable to analyze DNA even in degraded form. SNPs have become one of the most frequently observed types of human genetic

variation and are finding applications in both ancestry inference and kinship analysis applications as well as in forensic DNA phenotyping(12,13). Thus, sequence variation in PAX3 and BMP4 presents a scientifically pertinent avenue, through which the differentiation of an ethnically group along closely related lines can be assessed based on their capacity to aid in differentiating the PAX3 and BMP4 gene sequences. *PAX3* and *BMP4* were selected because both genes play central roles in neural crest development and craniofacial morphogenesis and have previously been associated with variation in nasal, facial, orbital, and jaw morphology. Their established developmental functions make them suitable candidate genes for assessing genetic variation related to craniofacial differentiation.

This is especially critical in the Nigerian setting as the minority ethnic communities are continuing to be underrepresented in molecular and anthropometric data set. Despite increasing interest in craniofacial genetics, limited information is available on sequence variation in *PAX3* and *BMP4* among minority Nigerian populations. No previous study has specifically examined whether variations in these genes can differentiate the Idoma and Igede ethnic groups. This lack of population-specific molecular data limits the use of craniofacial genetic markers in forensic identification and anthropological classification within Benue State

Another valuable example in this context and case is the Idoma and Igede ethnicities of the Benue state. They are historically, linguistically and culturally different despite the similarities in culture, language and location, which are geographically close, and share similar ecological conditions. These populations present a continent opportunity to explore whether small variations in DNA sequences in craniofacial genes may aid in differentiation of the population.

Work on African communities has again and again highlighted the issue of underrepresentation by world craniofacial and genetic databanks, a lack that constrains effective forensic capabilities as well as anthropological knowledge(14). Since the Africa region is the richest place in human genetic diversity, a localized study is crucial in developing solid reference data. To this end, a DNA sequence pattern study of PAX3 and BMP4 in Idoma and Igede can not only assist in the field of forensic identifications, but also wider arguments of biological affinity, the history of populations, and diversity of the craniofacial features in Nigeria. Concentrating on genes that bear well-investigated functions in facial development and change assists the present study to build a scientific rationale of minority ethnic groups population-specific molecular identification and extends the currently accessible data on minority ethnic groups in West Africa. The molecular emphasis in the study, as is applied to PAX3 and BMP4, is highly pertinent as such locus was tested on variation (polymorphic one) where the sequence alteration observed contributed to individual-level variation as well as suggesting the close biological relation of the two groups.

Therefore, this study aimed to identify and compare DNA sequence variations in selected regions of the *PAX3* and *BMP4* genes among Idoma and Igede individuals and to determine whether the observed variations could support forensic and anthropological differentiation between the two populations.

The null hypothesis was that there would be no significant differences in *PAX3* and *BMP4* sequence patterns between the Idoma and Igede populations. The alternative hypothesis was that population-specific sequence variations would be present and could contribute to the genetic differentiation of the two ethnic groups.

2. Material and Methods

2.1 Ethical Consideration and Consent

The University of Ilorin Ethical Committee, through the Faculty of Basic Medical Sciences Ethical Review Committee, gave their Ethical approval. The reference number for this approval is UERC/ASN/2025/3143. Each participant also gave their informed consent

after being told what the research was about and how it will help the community and society as a whole. When performing this study, such ethical principles as beneficence, non-maleficence, autonomy, and justice were considered.

Beneficence: The study made sure that the participants' interests were taken into account by making sure that the results would be good for both the people being studied and the country as a whole. To preserve and defend the rights of the participants, moral principles were also taken into account (15).

Non-maleficence: the study made sure that the participants did not get hurt by not causing them pain or suffering during the research process (15).

Autonomy: The study afforded participants the opportunity to make an educated decision regarding their participation, as well as the option to withdraw at any point if they were unable to proceed (15) The study was fair since all of the subjects were treated the same way. No one was given too much work or treated differently than anyone else (15).

2.2 Research Materials and Equipment

Compared to other advanced imaging methods like computed tomography (CT) scan and magnetic resonance imaging (MRI), 2D photo-image analysis allows for faster image processing and the use of less expensive equipment (16,17). Consequently, photography was factored into this research. The Digimizer software program (version 5.3.5, MedCalc, Belgium) made it easy to look at all the measurements and indices used in this study, though measuring the 14 craniofacial distances for each participant took a lot of time and effort. However, the outcomes from these instruments are reproducible and can be preserved for an extended duration, in contrast to direct measurements (e.g., the use of vernier calipers, rulers, etc.), where the investigator lacks access to the volunteers post-initial measurement (18).

The study used the following tools and materials:

I. **For the measurements of the head and face:** We used the Amkov 3.0 Super Amoled 24 Mega Pixel Digital Camera (x4 Zoom), Digimizer software (version 5.3.5, MedCalc, Belgium), a tripod stand (DSLR Camera holder), a stadiometre rule (2 metre Stature Meter), and a weighing scale (Toughened Glass Electronic Digital Scale-LWG-2018A).

2.3 Study Design

Participants for the study were recruited using stratified random sampling techniques.

2.4. Study area

The study was conducted in Benue State, which lies in Nigeria's North-Central geopolitical region. Benue State is found between 6°30′ and 8°10′ north of the Equator and from 7°30′ and 10°00′ east of the Greenwich Meridian (**S1**). The state shares boundaries with Nasarawa State to the north, Taraba State to the east, Cross River State to the south, Enugu State to the southwest, and Kogi State to the west. The administrative and commercial hub of the region is Makurdi, where the state capital is located. Benue State occupies a strategic geographical location within Nigeria. Because of the state's large number of farmers working to achieve high productivity in agriculture, it has been dubbed "Food Basket of the Nation.

2.5 Study Population

The research was conducted with students from the Idomas and Igedes ethnic groups at the Federal University of Health Sciences Otukpo and college of Education Oju in Nigeria. The participants in the study comprised both males and females aged 15 to 29 years. To gather biodata, 3000 healthy volunteers without apparent facial abnormalities were selected through semi-structured, self-administered questionnaires(19). The WHO classification put the participants into three age groups.

Group 1: Adolescent 15 to 19

Group 2 is made up of people who are 20 to 24

years old (young adults).
Group 3: Adults ages 25 to 29

2.6. Research Population and Sample Size Determination

The Academic Record office at the Federal University of Health Sciences Otukpo and college of Education Oju stated that there were about 7000 and 5000 respectively for the 2024–2025 school year, which was when recruitment took place.

Sample Size (SS) Determination

Using the formula:

$SS = (Z^2 \times p \times q) / d^2$

with Z = 1.96, p = 0.5, q = 0.5, and applying the finite population correction for a population of N = 3000:

$SS = (1.96^2 \times 0.5 \times 0.5) / (0.063^2)$

$SS = 242.1$

$n = SS / (1 + (SS - 1) / 3000)$

$n = 242.1 / (1 + 241.1 / 3000)$

$n = 242.1 / 1.0804$

$n = 224.1$

Rounding and allowing for approximation gives a sample size of about **227 respondents.**

2.7 . Sampling Technique and Subject Selection.

2.7.1 Sampling Technique

Volunteers who fulfilled the inclusion criteria were randomly chosen by a stratified random sample method from the Idomas and Igedes ethnic groups of Nigeria.

2.7.2 Inclusion Criteria

The volunteers, aged 18 to 30, belonged to the pure Idomas and Igedes ethnic groups, traceable to their grandparents (up to the third generation). They were selected from the Federal University of Health Sciences Otukpo and college of Education Oju and had no apparent facial abnormalities, having accepted to participate in the research.

2.7.3 Exclusion Criteria

Participants exhibiting pronounced facial deformities, including cleft lips and palates; congenital disorders impacting the head or face (e.g., hydrocephalus, holoprosencephaly); evident facial asymmetry; a familial history of craniofacial syndromes or facial anomalies; and those with significant facial injuries in the past or relatives within the same nuclear family were excluded from the study.

2.7.4 Procedure for Data Collection and Ethical Considerations

Informed permission was obtained from each participant after a thorough explanation of the research and its societal benefits, in accordance with the World Medical Declaration of Helsinki Ethical Principles for Medical Research Involving Human Subjects (World Medical Association, 2024)(20).

2.8. Samples Collection and Processing

The total number of samples used in genetic association study was 300. These 300 samples were sampled out of the total amount of 3,000 people who met the requirements of this study. The research DNA samples were collected using buccal swabs. All the participants received clean water (purified sachet water) to wipe their mouths prior to sample collection. This was done to ensure that no food particles are in the mouth that can affect the integrity of the sample. Sterile swabs were used to collect the samples scraping the inner corners of the cheeks. The ends of the sticks of cotton were cut and put in 5ml Eppendorf tubes filled with the digesting buffer. The tubes were coded with an unidentified number and stored at -20 o C awaiting further handling. DNA analysis was performed in the virology lab at the central research laboratory, Federal University of Health Sciences Otukpo and college of Education Oju, using the methodology outlined on the packet of the DNA extractor (NORGEN Biotek Corp.). The period of sample collection was between July 2019 and December 2019 where semi-structured self-administered

questionnaires were used to obtain the required information about the sample.

2.8.1. DNA Extraction (Spin Column Extraction)

Buccal swabs in digestion buffer were frozen and genomic DNA was isolated using DNA extraction kit as recommended by the manufacturer (NORGEN Biotek Corp.). It was extracted in the Virology Laboratory of the Central Research Laboratory, Federal University of Health Sciences, Otukpo and college of Education Oju under aseptic conditions. The following stages were used in the extraction process:

1) Cell lysis to liberate the DNA: The cells in the sample were gently vortexed to create a homogenized mixture and incubated for 5 minutes at ambient temperature.
2) Isolating DNA from proteins and other cellular debris: This was achieved by adding proteinase K into the solution, therefore degrading the proteins associated with the DNA molecules. The samples were then incubated for one hour at 55°C.
3) Precipitating DNA with alcohol: Ice-cold 96-100% ethanol was meticulously introduced to the sample to precipitate the DNA.
4) Purification of DNA: The precipitate underwent many washing with wash solutions to eliminate the adhered contaminants.
5) Column binding: 600 µl of the mixture was dispensed into the kit's spin column assembly, centrifuged for 3 minutes at 52,000 x g to ensure complete passage of the lysate through the column, and the flow-through was discarded.
6) Washing bound DNA: 500 µl of wash solution A was introduced to the spin column, centrifuged for 1 minute at 14,000 x g, followed by a further wash with 500 µl of wash solution A, centrifuged for 2 minutes at 14,000 x g. The flow-through was discarded, and the column containing the genomic DNA was allowed to dry.
7) Elution of purified DNA: 200 µl of elution buffer B was added in the column resin bed and centrifuged at 1 minute 3000 x g to allow any solubilized DNA flow in the column. The genomic DNA was harvested into fresh 1.5 ml Eppendorf tubes and this was put at -20 o C till further processing.
8) Confirmation of the presence and integrity of the DNA: gel electrophoresis was performed to show the presence of DNA in the sample and also give an idea about the quality of the DNA.

In order to concentrate the specimen, it was centrifuged in a microtubes. Supernatant is cleared off and re-suspended in the lysis buffer. This is warmed up so as to lysise the cell and release the DNA. Short centrifugation is done to remove cell debris and then the transfer of the supernatant into a fresh microtube is done followed by immediate use of the supernatant in the downstream applications **(21)**.

2.8.2 DNA Quantification

A nanodrop spectrophotometer was used to measure the amount of DNA in each tube, and the concentrations was recorded.

2.8.3 Primer Design

During the execution of polymerase chain reaction (PCR), primers short nucleotide sequences or single-stranded DNA fragments that serve as initiation points for DNA synthesis were initially designed with modifications based on published studies concerning the PAX3 gene and regarding the BMP4 gene(22). The primer pairs were verified against the published sequences of the targeted genes using Snapgene software (version: 5.0.7.0). The unpaired primers were re-engineered and synthesized. The synthesized primer sequences for the analyzed genes are:

***PAX3* Gene:** Forward Primer (F): 5´-CTGTTTTTTAGGTAATGGGAC-3´
Reverse Primer (R): 5´-GGACCTTCCCTCTATTTGAG-3´

***BMP4* Gene:** Forward primer (F): 5´-TGCCCCTCCATTTCTAGC-3´

Reverse Primer (R): 5′-ACTGGGGCTTTGATGTAACC-3′

2.8.4 DNA Amplification (Polymerase Chain Reaction {PCR})

PCR is a technique used to amplify or generate several copies of a given DNA segment (23). The procedure has three primary stages, necessitating Taq polymerase (a thermostable DNA polymerase) and primers. Distinct conditions were used for the two genes of interest.

The amplification of the target gene segments (Paired Box 3 {PAX3} and Bone Morphogenetic Protein 4 {BMP4}) was performed via polymerase chain reaction (PCR) utilizing a thermo-gradient cycler (PTC 200, MJ Research, USA), which precisely regulates the temperature fluctuations necessary for denaturation, annealing, and extension, as well as the cycle count (24).

Denaturation: it is the process in which the template DNA or genetic material is denatured; the strands of its helix are unraveled and separated by heating to 94-96 °C (Arya, 2018). During annealing, primers, which are small, single-stranded DNA molecules, bind to the new single-stranded DNA to create complementary strands. The reaction mixture is promptly cooled to a temperature below the melting point of the particular primers (68°C)(23).

In this third stage, the reaction temperature is raised to the optimum range for the polymerase (68–72°C). The polymerase catalyzes the synthesis of new DNA, starting with the primer. The polymerase interprets a template strand and rapidly synthesizes complimentary nucleotides. The outcome is two new helices replacing the original, each consisting of one of the initial strands together with its newly synthesized complimentary strand (23).

2.8.5 Primer Preparation and Polymerase Chain Reaction (PCR) Set-up

The primers are prepared by re-suspending the lyophilized stock primer in DNA-free water to create a 100µM stock solution. This was executed in accordance with the manufacturer's prescribed methodology. A 10µM working solution of each primer was then made from the stock primer solution by a 10-fold dilution in DNase-free water.

A master mix of the PCR components was prepared in a 1.5 ml Eppendorf tube, designed for a 25 µl reaction volume for each amplification. Utilizing the NEB one-Taq fast load 2X master mix, the estimated quantities of the forward and reverse primers were included into the quick load solution and adjusted with water to the requisite amount in a 1.5ml Eppendorf tube on ice. Twenty-four microliters (24 µl) of the PCR master mix was allocated into pre-labeled PCR tubes, and 1 µl of the extracted genomic DNA sample was introduced into each tube, matching to the respective labels of the samples.

PCR reaction condition for *PAX3*

Genomic DNA	1 µl
PAX3 F	1 µl
PAX3 R	1 µl
NEB one-Taq quick load 2X master mix	12.5 µl
Deionized water	9.5 µl
Total volume	25 µl

PCR reaction condition for *BMP4*

Genomic DNA	1 µl
BMP4 F	1 µl
BMP4 R	1 µl
NEB one-Taq quick load 2X master mix	12.5 µl
Deionized water	9.5 µl
Total volume	25 µl

2.8.6 Materials and Conditions for PCR

PCR conditions for *PAX3* Gene

Step 1--- 95°C for 5minutes

2--- 95°C for 25seconds
3--- 49.5°C for1 minute
4--- 72°C for 1 minute
5--- go to step 2 repeat 35cycles
6--- 72°C for 5 minutes
7--- 15°C ∞
8--- End

PCR conditions for *BMP4* Gene

Step 1 – 95°C for 5minutes
2 – 98°C for 30seconds
3 – 51°C for 45seconds
4 - 68°C for 1 minute
5 - Go to step 2 repeat 35 cycles
6 - 68°C for 5 minutes
7 - 15°C for 4minutes
8 - End

2.8.7 Resolution of Amplified DNA by Agarose Gel Electrophoresis

The amplified products (amplicons) were examined using gel electrophoresis after resolution on an agarose gel stained with ethidium bromide solution. The distinct DNA bands were then seen under ultraviolet (UV) light.

2.8.8 Preparation and Casting of Gel

A 0.5X electrolyte buffer (Ethylene-Diamine-Tri-acetic acid, EDTA[TAE]) was produced from a 50X stock solution. One gram of agarose powder (Fisher's Scientific Company) was measured on weighing paper and dissolved in 100 milliliters of TAE in a conical flask. The suspension was then microwaved for 3 minutes at moderate heat until all the powder was fully solubilized. Upon cooling to about 60°C on a surface, the agarose solution was transferred into a casting tray containing a sample comb and let to solidify at ambient temperature. The comb was then extracted to form a cavity inside the solidified gel. The gel with the casting tray was positioned horizontally in the electrophoresis tank, which was filled almost to capacity with 0.5X TAE buffer. This approach adhered to the procedure outlined (25).

2.8.9 Gel Electrophoresis Configuration

This study involved pipetting 2 µl of each amplified DNA sample mixed with a loading dye buffer into the sample wells of the electrophoretic chamber, while 1 µl of DNA marker ladder (NEB 1Kb plus ladder) was loaded into the first well of each gel to serve as a comparative control for the migrating DNA. The tank was sealed, and the positive and negative electrode terminals were linked to the power supply (Biorad power pack (Singapore)). The electrophoretic separation was performed at a power rating of 100V for 40 minutes using a specific power supply system (Biorad power pack, Singapore). To serve as a control, 0.5g of a 1Kb+ ladder comprising a series of DNA pieces of established sizes was introduced into well 1. The velocity of DNA molecule migration is directly proportional to the size of the DNA. Smaller DNA molecules have increased mobility and gravitate towards the gel's bottom. Subsequent to the removal of the gel, it was immersed in a 5% ethidium bromide solution for staining and DNA-ethidium bromide intercalation.

2.8.10 Staining of DNA Molecules

The electrophoresed DNA molecules on the gel were then transferred to a plastic dish containing 0.5X TAE buffer and 250 µl of ethidium bromide (intercalating dye). As DNA fragments traverse the gel, they absorb the dye, enhancing their visibility. Ethinium bromide has mutagenic properties, necessitating the exercise of care throughout its use.

2.8.11. Visualization of DNA Molecules

A transilluminator, or ultraviolet light box, is used to see the stained electrophoresed DNA samples inside the gel. Ultraviolet light induces fluorescence in the intercalated dye inside the DNA fragments, allowing for comparative assessment of the separated DNA molecules against a control "ladder.

2.8.12 Purification of Amplified DNA

The amplified DNA samples were purified with a genomic purification kit (Qiagen) in accordance with the manufacturer's guidelines. Fifty microliters of binding buffer was added to five microliters of amplified DNA material. The mixture was centrifuged at 5000 rpm for one minute. The flow-through was discarded,

followed by centrifugation three times for one minute at 8000 rpm, and then washed three times with 500 µl of wash buffer. The flow-through was discarded again and centrifuged at 8000 rpm for one minute. Subsequently, 30 µl of elution buffer was added, and the column was positioned in a 1.5 ml elution tube, centrifuged at 10,000 rpm for 12 minutes, and the flow-through was preserved as pure amplicons, stored at -20°C until sent for sequencing analysis

2.8.31 DNA Sequencing (Sanger Sequencing Method)

Purified DNA samples were sent for sequencing using the Sanger sequencing technology from Integrated DNA Technologies, Singapore. Primer synthesis was conducted by Inqaba Biotek (West Africa Limited, Africa's Genomics Company). The sequences of the primers are as follows:

***PAX3* Gene:** Forward Primer (F): 5´-CTGTTTTTTTAGGTAATGGGAC-3´

Reverse Primer (R): 5´-GGACCTTCCCTCTATTTGAG-3´

***BMP4* Gene:** Forward primer (F): 5´-TGCCCCTCCATTTCTAGC-3´

Reverse Primer (R): 5´-ACTGGGGCTTTGATGTAACC-3´

The PCR products were purified with a Qiagen genomic purification kit in accordance with the manufacturer's specified technique. The composition of the reaction mixture was as follows: 50 µl of the primer stock solution, 45 µl of DNA-free water, and 2 µl of the purified PCR result. The mixture underwent centrifugation at 5000 rpm for one minute, and one nanogram of the mixture was sent for sequencing. The sequencing data was obtained from the server connection.

2.8.14. Sequencing Quality Control and Variant Calling

Sanger sequencing chromatograms were visually inspected to assess peak clarity and sequence quality. Low-quality terminal regions and ambiguous base calls were excluded before alignment. Sequences were compared with the corresponding NCBI reference sequences using Clustal Omega. Variants were accepted only when supported by clear, distinct chromatogram peaks and consistent alignment at the same nucleotide position. Samples with overlapping peaks, excessive background noise, or insufficient readable sequence length were excluded from variant analysis. Where necessary, uncertain variants were confirmed by repeat amplification and sequencing. Forward and reverse reads were compared when available to improve confidence in variant detection. Sequencing success was calculated as the proportion of amplified samples that produced interpretable reads suitable for alignment and variant classification. Detected variants were classified as synonymous or non-synonymous based on their predicted effects on the encoded amino acid sequence.

2.8.15. Genetic Association Studies

Gene marker mutations were evaluated by matching the sequence with homologous sequences from the National Center for Biotechnology Information (NCBI) human genome nucleotide sequence database with the Clustal Omega sequence alignment software(26). All detected mutational differences were verified for synonymous or non-synonymous amino acid alterations at the corresponding locations and recorded for profiling purposes.

2.9. Data analysis

The raw data from this research was imported into Microsoft Excel 2016 for efficient processing. The analysis was conducted using IBM Statistical Package for Social Sciences version 23.0. The mean, standard deviation (SD), and standard error (SE) were recorded when necessary, mostly for the craniofacial indices. Frequencies and percentages were used to characterize independent variables (socio-demographic factors) and other categorical variables (such as body mass indices). ANOVA, chi-square, correlation, and

regression analyses were used to ascertain differences and connections among the variables and covariates.

3. Results:

3.1. Genetic Analysis of PAX3 Gene in Idoma and Igede Population and Correlation with Craniofacial Indices

The genetic work on the PAX3 gene demonstrates that the molecular work was effective and results are biologically significant. The PCR amplification of target PAX3 region has yielded the required 311 bp fragment, which established that the PCR amplification had amplified the locus of interest with the right size. This can be seen in Figure 1S where positive bands can be seen at the correct size with respect to the DNA ladder, and the negative control validates the specificity of the amplification. Figure 2S (a representative electropherogram of the exon 8 segment of PAX3) and Figure 3S (the chromatography makes representative of the sequenced PAX3 gene Figure 4S) are also provided that show that the sequencing output was sufficient. Combined, Figures 1S, 2S, and 3S suggest that optimally, the downstream interpretation is founded on technically sound amplification and sequencing data.

This analysis showed that sequential alignment of amplified samples had only slight variations, majority of which were synonymous and that 34 out of 300 samples investigated further had synonymous mutations in the exon 8 region. This trend suggests that the examined PAX3 locus is highly preserved in the population and, thus, lacks evidence of significant ethnic separations at the locus. That is, the finding on PAX3 does not decisively distinguish between Idoma and Igede, but rather only contributes to the more general observation of a high level of molecular homology, and biological similarity, between the two populations..

The deviation of this overall trend is however the most crucial in participant 15 (Px15). The analysis reported that further matching with NCBI reference sequence revealed the participant with the disorder (identified as P15)-had 22 non-synonymous changes in the targeted region of PAX3 and it is demonstrated in Figure 3. These non-synonymous SNPs are biologically important as opposed to synonymous substitutions since they are likely to change protein sequence and have the potential to modify protein activity. The craniofacial parameters were also found to have significant deviations among the same participant as compared to the population mean in Table 1. Notably, this extends to differing in spatial terms of the width of the nose and in the part of the head of the eye known as cante, as the study observes that participants with PAX3 mal adaptations exhibited deviations of the nose and other craniofacial sizes, and more broadly that individual differences as a result of the PAX3 relationship can be quantified in terms of the nose and face, the cante, Participant 15 thus demonstrates a clear example where SNP variation had been found to correlate with craniofacial variation which is measurable in terms of width of the nose and canthal dimensions associated with dimensions.

We have also observed this effect, at the individual level, using the affected-participant tables, such as Table 2, Table 3, and Table 4 tables which compares the participants with affected versions of PAX3 with their subgroup averages. As an illustration, Table 5 reveals that afflicted Igede females recorded more than the general female mean on most dimensions including nasal width, interendocanthal width and nasal index implying that PAX3-related variation can take place in both nasal and canthal measurements. Similarly, the research observes that the affected Idoma male-participant in Table 3 loses bigger measurements in comparison to the overall mean of males in the various variables, such as; nasal width, nasal height, morphological facial height, bizygomatic distance, and nasal index.

3.2. Population structure of Pax3 gene sequence alignment by Phylogeny

The PAX3 findings interpretation is enhanced by the phylogenetic finding in Figure 1. The

analysis posits the PAX3 tree to be tightly clustered and shallowly branched, and that the sequences are closely related, and do not represent deep divergence between the two populations. Some of the substitutions and missing-base tracts were clustered into a few samples and the study reported that some of those individuals had enlarged nasal width than the other members. This helps a model of close common ancestry and a low amount of within-population variation. Consequently, the PAX3 phylogenetic outcome can be most convincingly interpreted as the gene being mostly shared between the two populations, yet there are some rare non-synonymous variants, particularly in participant 15, that are moderating craniofacial deviations, such as changes in nasal width and the canthal area.

3.3. Translation of the genetic changes in Pax3 exon 8

The genetic changes in the PAX3 exon 8 were translated to suggest that the majority of the recognized nucleotide changes did not translate into amino acid replacements. The reason behind this is that most of the mutations detected in the sequence alignment process were synonymous in nature, that is, although there was change of the base, the amino acid coded was the same because of the degenerate nature of the genetic code. This may indicate that the functional integrity of PAX3 protein was mostly maintained in the study population. Nevertheless, in few persons, specifically the participants 15 (Px15) multiple non-synonymous substitutions are observed meaning there may be change in the protein structure. These alterations could affect the biological functions of PAX3 which is a gene that is known to be important in the development of the face. The observed deviations in craniofacial measurements, especially in the nasal and canthal areas, are thus explained on a molecular basis in association with the variation in the genotype and the variation in a particular phenotype in relation to genotype, at the individual level.

3.4 Genetic Analysis of BMP4 gene in Idoma anad Igede Population and Correlation with Craniofacial Indices

The BMP4 outcome is similar to that of PAX3, albeit with its own attendant molecular and craniofacial ramifications. PCR amplification of the locus was evidenced by amplification of targeted BMP4 exon 3 region with the expected 586 bp fragment. This is demonstrated in Figure 2 which displays the agarose gel electrophoresis representative resolution of the amplified BMP4 gene. The quality of the sequences is further depicted in Figure 5S, the electropherogram of the BMP4 exon 3 fragment and Figure 6S indicates the alignment of the amplified BMP4 sequence to the NCBI reference sequence, and Figure 3 illustrates the phylogeny of the few BMP4 exon 3 sequences of choice. Collectively, Figures 3, and 4S5S, and Figure 3, suggest that the BMP4 analysis was technically sound and could be interpreted. It also demonstrates that 22 among the 300 samples studied had synonymous and non-synonymous differentiations in the exon 3 of BMP4. This implies that BMP4, similar to PAX3, does not provide a firm set of differentiation between Idoma and Igede at the general ethnic level. Instead, the finding contributes to the more general finding that there is significant biological relatedness between the two populations and that most of the variation seen is within a generally maintained background of development.

The greatest variation in BMP4 was observed when one of the selected affected individuals was considered, whereas the study is not completely consistent in the identification of such an individual C (B15).. This study indicates that this participant with the affected mutation has 8 non-synonymous and 2 synonymous mutations in the BMP4 exon 3 region and an upstream stop codon and that these mutations were marked in the sequence alignment in Figure 5S. There were also craniofacial deviations in this participant versus the group mean of measurements as summarised in Table 5. Due to the inconsistency between B3 and B15 in the thesis,

the safest is to say that the study has found a single selected affected BMP4 with high polymorphism and respective craniofacial deviation, as the label of the participants is inconsistent.

The phenotypic significance of the BMP4 difference is summative in Table 5 that compares the female and male BMP4 participants who are affected with the means of overall participants. The paper reveals that the BMP4 participants who were affected exhibited larger-than-mean values in some facially craniofacial variables, such as morphological facial height, bizygomatic distance, interendocanthal width, ear dimensions, mandible height and nasal index. The trend motivates that variants of BMP4 provided craniofacial phenotype when such variation is functionally consequential such as variants of PAX3. The mentioning of interendocanthal width implies that a change in BMP4, as in a change in PAX3 might also be being measured in the canthal area and in larger parts of the face and nose. Therefore, it seems that BMP4 is a factor that contributes to small but significant idiosyncrasy of the craniofacial appearance among the otherwise very similar population.

3.5. Amplification, Sequence Alignment and Phylogenetic Analysis of the BMP4 Gene

Amplification of the exon 3 region of the BMP4 gene yielded the expected DNA fragment size of 586 bp, as shown in Figure 3. Sequence alignment of the amplified samples showed a high level of homology across the study population, with only minor base variations, most of which were synonymous and considered insignificant. Of the 300 samples analysed, 22 samples exhibited synonymous and nonsynonymous variations within the exon 3 region of the BMP4 gene. The electropherogram of the amplified BMP4 gene segment is presented in Figure **5S**.

The sequence profile of participant B3 revealed a higher level of polymorphism within the exon 3 region when compared with other individuals in the study population. The alignment showed that participant B3 had the most distinct sequence variation among the selected samples. The other individual with the mutation had 8 synonymous exonic 3 mutations in the BMP4 gene. Also, there was a stop codon found in the upstream of the target sequence.

Clustering of the amplified BMP4 gene sequences into a phylogenetic tree also showed that B1, B2, and B4 were closely clustering with a NCBI BMP4 reference sequence with B3 branching away, showing that it exhibited the greatest polymorphism of all chosen participants. This trend substantiates the finding of the sequence alignment and also, it confirms the unique molecular variation of participant B3. This pattern of polymorphic variations was found to correlate with deviations of craniofacial measurements when compared with the total population means as shown in Table 5. The affected participants had greater mean-values than mean in various parameters of craniofacial such as morphological facial height, bizygomatic distance, interendocanthal width, ear width, ear height, mandible height, ear fissure width, and nasal index. This implies that differences in the BMP4 gene can be linked with changes that are visible

4. Discussion

Based on the primary evidence and evidence presented in supplementary data, this discussion explores the findings in forensic, anthropological, and developmental basis. The additional gels, sequence alignments, electropherograms, and mapping of the area under study build robust trust in the technical validity and applicability of the craniofacial and molecular patterns observed to populations. The molecular findings relating to PAX3 are among the most critical aspects of the study. Amplification of the PAX3 gene produced the expected 311 bp fragment, confirming the reliability of the laboratory procedure. Of the 300 samples analyzed, 34 showed synonymous mutations, while selected affected individuals also exhibited non-synonymous changes associated with deviations in craniofacial

measurements. This result is important for several reasons. First, the predominance of synonymous mutations suggests that the PAX3 locus studied is highly conserved in both populations. This agrees with the known biological importance of PAX3 in craniofacial development, neural crest migration, and facial patterning (9,26). Genes with critical developmental roles are often relatively conserved, and variation within them may have limited functional effect unless it alters protein structure or gene regulation.

Second, the presence of selected non-synonymous changes associated with measurable craniofacial deviations is consistent with earlier and current studies linking PAX3 to facial morphology, especially in the nasal and periorbital regions. Previous work has shown that PAX3 is associated with both normal facial variation and syndromic craniofacial anomalies such as those seen in Waardenburg syndrome (27). The present study is similar in showing that variation in PAX3 may influence craniofacial form. It differs, however, in showing that these effects are more evident at the individual level than at the population level. In other words, PAX3 variation did not strongly separate Idoma from Igede as entire groups, but it did help explain craniofacial departures among specific individuals. This is a very important finding. It means that in this context, PAX3 is more useful as a contributor to individual craniofacial variation than as a broad ethnic marker. This has forensic relevance because it suggests that genotype–phenotype analysis may strengthen individual identification or explain unusual facial traits, even when two populations are generally similar.

The BMP4 findings follow a similar pattern. Amplification produced the expected 586 bp fragment, and 22 of the 300 samples analyzed showed synonymous and non-synonymous variations. Variant-bearing individuals displayed craniofacial deviations from the population mean, again indicating a relationship between molecular variation and phenotype. This finding aligns with earlier and current developmental biology studies showing that BMP4 plays a major role in craniofacial growth, facial width, mandibular patterning, and bone morphogenesis (28). Variations in BMP4 expression or sequence have been linked in earlier studies to both normal facial diversity and craniofacial anomalies. The present study therefore agrees with the broader literature in showing that BMP4 is relevant to facial form.

However, as with PAX3, the key insight here is that the detected BMP4 variations did not produce a clear ethnic boundary between Idoma and Igede. Rather, they appeared to account for measurable individual deviations within a shared population background. This is significant because it shows that relevant craniofacial genes may influence phenotype without necessarily functioning as strong population-level separators in closely related groups. The study therefore supports a more **sophisticated** model of craniofacial genetics. Important genes such as BMP4 do not simply divide populations into distinct biological blocks. Instead, they help shape the range of facial variation observed within and across populations, with some variants becoming especially important in individuals who differ from the average pattern (28).

A major strength of the present study is the convergence between the anthropometric and molecular findings. Both lines of evidence point toward the same overall conclusion: the Idoma and Igede populations share close biological affinity. The craniofacial indices show strong overlap and similar dominant morphological categories, while the PAX3 and BMP4 analyses show high molecular similarity with only limited individual-level variation. This convergence strengthens the validity of the findings. When two different approaches yield similar conclusions, the resulting interpretation becomes more convincing. In the present case, both anthropometry and genetics support the argument that the two populations are better

understood as biologically overlapping neighbours rather than sharply distinct craniofacial groups. The combined evidence also clarifies the usefulness of craniofacial genetics as a differential identification tool. The findings suggest that such tools are valuable, but not necessarily in the narrow sense of rigid ethnic classification. Instead, their greatest value may lie in building local databases, describing regional biological profiles, identifying sex-related patterns, and explaining individual deviations. This is a more realistic and scientifically defensible interpretation of the data.

4.1. Molecular and Forensic implications of study.

The forensic implications of the study are largely based on the molecular characterization of the locus of PAX3 and BMP4 as far as the Idoma and the Igede populations are concerned. The two genes were successfully sequenced and amplified, which proves the technical soundness of the laboratory process. The major portion of sequence alterations observed was synonymous meaning that respectively the studied regions are highly conserved in both groups. This high level of conservation demonstrates that these loci are of limited usefulness in discrimination of ethnicity, by themselves.

However, non-synonymous variants that are found in the selected individuals are still vital at the individual level. Their applicability is enhanced by the fact that they are accompanied by craniofacial calculations using the S3 dataset which contains the raw measures used to arrive at the aforementioned nasal width (al-al), nasal height (n-sn), morphological facial height (n-gn), bizygomatic width (zy-zy), the interendocanthal width (en-en), the eye fissure width (en-en) By doing so the molecular variants do not receive individual treatment but towards a deviation that can be quantified in the form of craniofacial deviation with regard to the wider study sample.

To this end, the significant forensic implication would be that PAX3 and BMP4 has proven to be more effective in understanding individual molecular specificity and other phenotypic variation rather than provide general ethnic identity by two closely related populations. This is one way of adopting a forensic skepticism whereby developmental gene variants are applied as supplementary markers, as opposed to separating ethnicities.

4.2. Implications of the Study at the Molecular and Anthropological level.

There is an implication from a molecular anthropological viewpoint that sociocultural difference does not necessarily reflect high divergence at the loci of development with which the current findings may be characterized. The patterns of sequences in PAX3 and BMP4 show that there is a high homology between Idoma and Igede participants implying that both have the same biological inclination. This conclusion is also in line with the craniofacial calculations using S3 wherein the underlying facial variables exhibit smooth overlap in the two populations as opposed to the separate distributions.

The S3 additional spreadsheet gives the craniofacial measurements of the major phenotype using the raw craniofacial perimeter measurements. These will be those dimensions directly most discussed as pertaining to the molecular variants, most notably the nasal width, nasal height, morphological facial height, bizygomatic distance, interendocanthal width, eye fissure width and the measures of the ears. Overall Idoma and Igede have similar deriv indices of these variables as well. As an illustration, an average nasal index value in S3 data generate a close range of values between Idoma and Igede males and females and the same trend of overlapping occurs in the relation of facial height and width to ears. This close phenotypic overlap makes the molecular conclusion that these two groups are more homozygous than heterozygous at the locus in these studies.

Non-synonymous changes observed in some of the participants are thus only best explained as part of within-population craniofacial diversity as opposed to showing an evident separation

between populations. This anthropologically is significant since it advocates a more sophisticated paradigm where developmental genes can be used to support the subtle individual variation to a common biological foundation.

4.3. Summary

This research looked at how variations in sequence of PAX3 and BMP4 were compared between Idoma and Igede of the Benue State, Nigeria. The two genes were successfully amplified and sequenced to give quality molecular data to be used in interpretations. Majority of the observed changes in sequences were synonymous by nature which implies that the loci studied under analysis are highly conserved amongst the sampled.

The proportion of the number of participants with non-synonymous variants are smaller and the interpretation of the variants is reinforced in the S3 supplementary dataset which includes the craniofacial measurements on the calculation of phenotypes. These computations involve nasal, facial, canthal and auricle variables which aid in putting the molecular results into senses. The general trend of the molecular and additional craniofacial data is that of high similarity in between Idoma and Igede and with only slight yet significant variation on an individual level.

Therefore, identifying acute ethnic molecular demarcations is not the key contribution of the study but showing that PAX3 and BMP4 variants could be useful in the context of least part of the phenotypic deviation of individuals that would likely share a common developmental program.

This study has several limitations. Participants were recruited from selected institutions and may not fully represent all Idoma and Igede communities. The genetic analysis was limited to selected regions of *PAX3* and *BMP4*, so other relevant craniofacial genes and variants were not assessed. Potential confounding factors, including population admixture, environmental influences, age, sex, nutrition, and measurement variability, may also have affected the observed associations. Furthermore, the cross-sectional design limits causal interpretation. Future studies should include larger, geographically diverse samples, broader genomic panels, ancestry-informative markers, and multivariable analyses to validate these findings and improve population discrimination.

Conclusion

The study principal value is the results obtained from the molecular evidence of the PAX3 and BMP4. The fact that the majority of mutations were synonymous in nature suggests that the regions of interest in the two Idoma and Igede individuals are highly maintained and therefore lends to the explanation that there is close biological similarity and small ability to differentiate between extended ethnic groups.

This study is reinforced by the additional S3 dataset which gives the craniofacial measurements on which the phenotype calculated are based on and which are included in the molecular interpretation. Signs, such as nasal width, nasal height, facial height, bizygomatic width, interendocanthal width, eye fissure width, ear height, ear width, and indices (derived) demonstrate that there is an extensive overlap of both populations in variables like nares width, optimal nares height, facial height, bizygomatic width, interendocanthal width, eye fissure, ear height, ear Simultaneously, the small number of non-synonymous deviations found in the few samples, indicate that these genes still have a potential to help in the explanation of individual deviations of the craniofacial phenotype of a population.

On the whole, PAX3 and BMP4 can be considered in the context of this study as indicators of personal variation in development of a common population rather than a powerful ethnic indicator of a specific population. The observed PAX3 and BMP4 variations contribute to individual craniofacial diversity but provide limited ability to distinguish the Idoma and Igede populations. Therefore, these genes should not be used independently for ethnic identification. Their practical value is

primarily as supplementary markers in broader forensic, anthropological, and population-genetic analyses that incorporate additional genetic loci, ancestry-informative markers, and craniofacial data. Further validation in larger and geographically diverse populations is required before routine forensic application.

Declarations

Guidelines followed while conducting the study: This study was conducted and reported in accordance with the **Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement**

Ethics approval: The University of Ilorin Ethical Committee, through the Faculty of Basic Medical Sciences Ethical Review Committee, gave their Ethical approval. The reference number for this approval is UERC/ASN/2025/3143.

Consent for publication: Informed permission was obtained from each participant after a thorough explanation of the research and its societal benefits, in accordance with the World Medical Declaration of Helsinki Ethical Principles for Medical Research Involving Human Subjects (World Medical Association, 2024).

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Authors contribution: Co**nceptualization, and research formulation** BAO, KSB, AAS, DEU, AMS: **Data analysis and interpretation** IA, SAA, DEU**: writing original draft**: BAO, **Supervision** AMS: **Reviewing and editing: All authors**

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process: During the preparation of this work the author(s) used Grammarly & AI to frame the grammar and English editing. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the published article.

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Table 1: Idoma Male Participants (Affected PAX3 vs Overall Mean)

Parameter	Affected PAX3 Male Mean	Overall Male Mean ± SD	Difference
Morphological facial height (n–gn)	119.8	114.2 ± 5.9	+5.6
Bizygomatic distance (zy–zy)	133.6	128.3 ± 5.2	+5.3
Interendocanthal width (en–en)	33.0	30.8 ± 2.3	+2.2
Ear width (t–pa)	35.3	33.1 ± 2.2	+2.2
Ear height (sa–sba)	63.5	60.1 ± 3.4	+3.4
Mandible height (sto–gn)	71.2	67.9 ± 4.0	+3.3
Vermillion height (ls–sto)	10.0	8.9 ± 1.2	+1.1
Eye fissure width (en–ex)	31.0	29.0 ± 2.0	+2.0
Nasal index	92.8	87.4 ± 4.6	+5.4

***Table 2**: Idoma Female Participants (Affected PAX3 vs Overall Mean)*

Parameter	Affected PAX3 Male Mean	Overall Male Mean ± SD	Difference
Morphological facial height (n–gn)	119.8	114.2 ± 5.9	+5.6
Bizygomatic distance (zy–zy)	133.6	128.3 ± 5.2	+5.3
Interendocanthal width (en–en)	33.0	30.8 ± 2.3	+2.2
Ear width (t–pa)	35.3	33.1 ± 2.2	+2.2
Ear height (sa–sba)	63.5	60.1 ± 3.4	+3.4
Mandible height (sto–gn)	71.2	67.9 ± 4.0	+3.3

Vermillion height (ls–sto)	10.0	8.9 ± 1.2	+1.1
Eye fissure width (en–ex)	31.0	29.0 ± 2.0	+2.0
Nasal index	92.8	87.4 ± 4.6	+5.4

Table 3: Igede Male Participants (Affected PAX3 vs Overall Mean)

Parameter	Affected PAX3 Male Mean	Overall Male Mean ± SD	Difference
Morphological facial height (n–gn)	119.8	114.2 ± 5.9	+5.6
Bizygomatic distance (zy–zy)	133.6	128.3 ± 5.2	+5.3
Interendocanthal width (en–en)	33.0	30.8 ± 2.3	+2.2
Ear width (t–pa)	35.3	33.1 ± 2.2	+2.2
Ear height (sa–sba)	63.5	60.1 ± 3.4	+3.4
Mandible height (sto–gn)	71.2	67.9 ± 4.0	+3.3
Vermillion height (ls–sto)	10.0	8.9 ± 1.2	+1.1
Eye fissure width (en–ex)	31.0	29.0 ± 2.0	+2.0
Nasal index	92.8	87.4 ± 4.6	+5.4

Table 4: BMP4. SNPS IN BMP4 GENE

S/N	Original	SNP	Nucleotide position	Percentage	Codon Change
1	A**G**T	A**A**T	481	45/150= 30%	S25N
2	**C**GG	**A**GG	571	59/150= 39.3%	R55

on the faces of the involved participants.

Table 5: Female and Male Participants of Idomas and Igedes with Affected BMP4 Gene Values in Comparison to Overall Participant Mean Values

Parameter	Number of Participants	Affected Female (BMP4)	Mean ± Standard Deviation	Affected Male (BMP4)	Mean ± Standard Deviation
Morphological facial height (n–gn)	300	116.7	111.1 ± 5.4	119.8	114.2 ± 5.9
Bizygomatic distance (zy–zy)	300	129.8	124.0 ± 4.8	133.6	128.3 ± 5.2
Interendocanthal width (en–en)	300	32.1	30.0 ± 2.1	33.0	30.8 ± 2.3
Ear width (t–pa)	300	34.5	32.0 ± 2.0	35.3	33.1 ± 2.2
Ear height (sa–sba)	300	62.0	58.3 ± 3.1	63.5	60.1 ± 3.4
Mandible height (sto–gn)	300	69.5	65.4 ± 3.7	71.2	67.9 ± 4.0
Vermillion height (ls–sto)	300	9.5	8.6 ± 1.1	10.0	8.9 ± 1.2
Eye fissure width® (en–ex)	300	30.1	28.2 ± 1.9	31.0	29.0 ± 2.0
Nasal index	300	91.2	86.2 ± 4.3	92.8	87.4 ± 4.6

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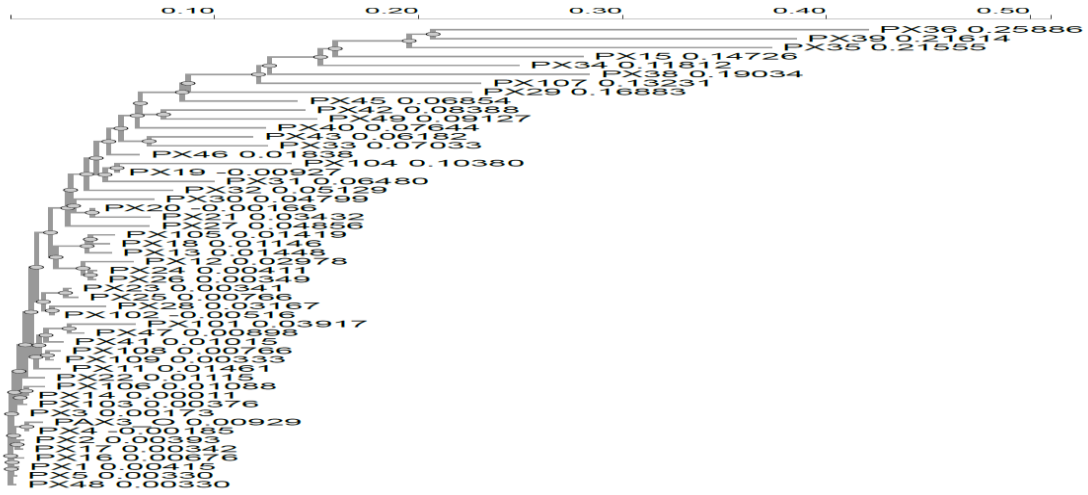


Figure 1: Phylogenetic tree of the Pax3 ancestral reference and the population sample sequences highlighting the population structure and lineage clustering

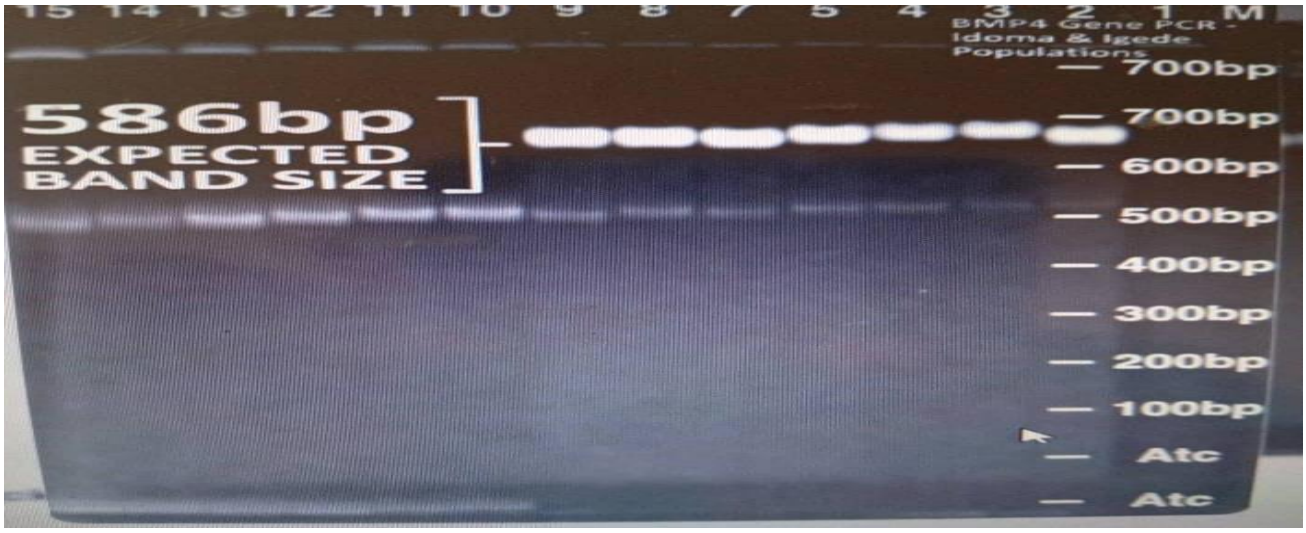


Figure 2: *Representative agarose gel electrophoresis resolution of amplified BMP4 gene from participants genomic DNA. This shows that Amplified samples for BMP4 gene tested positive at 586bp*

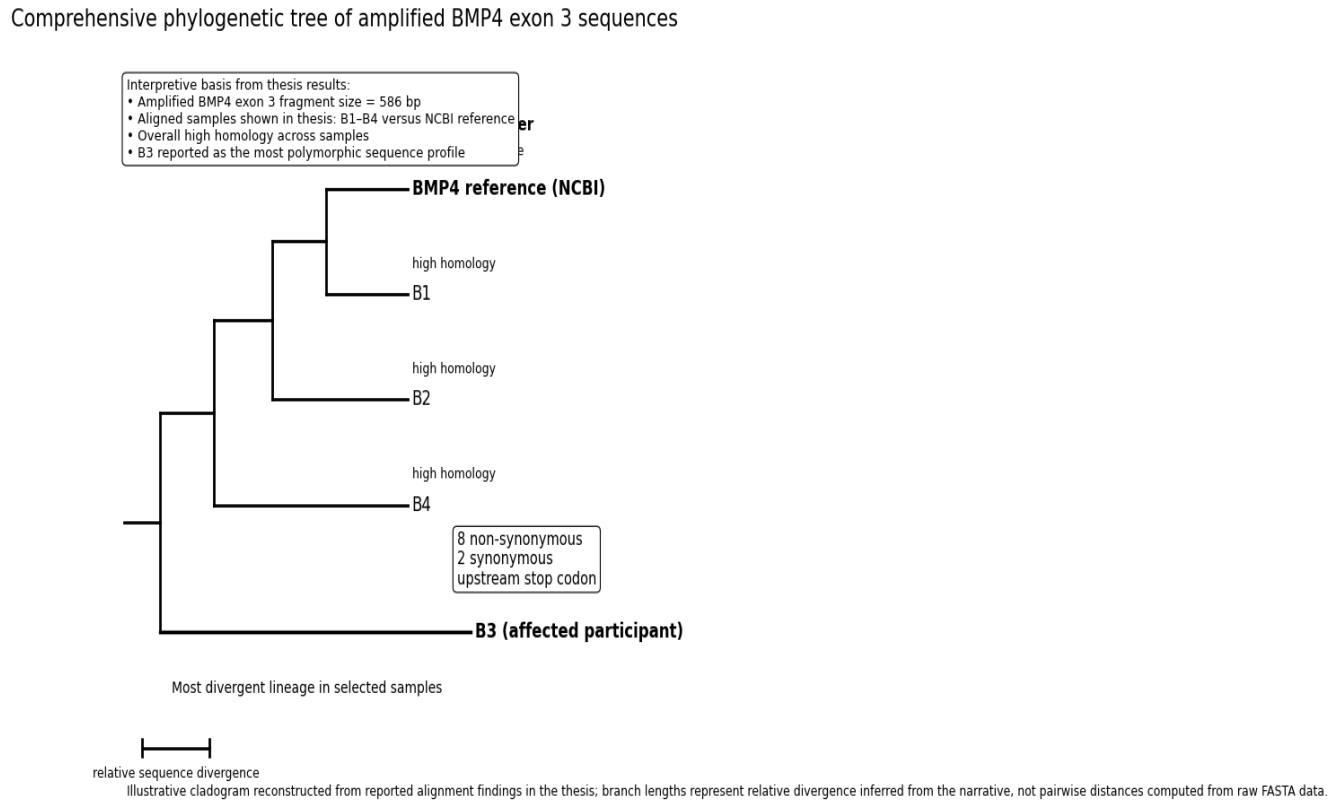
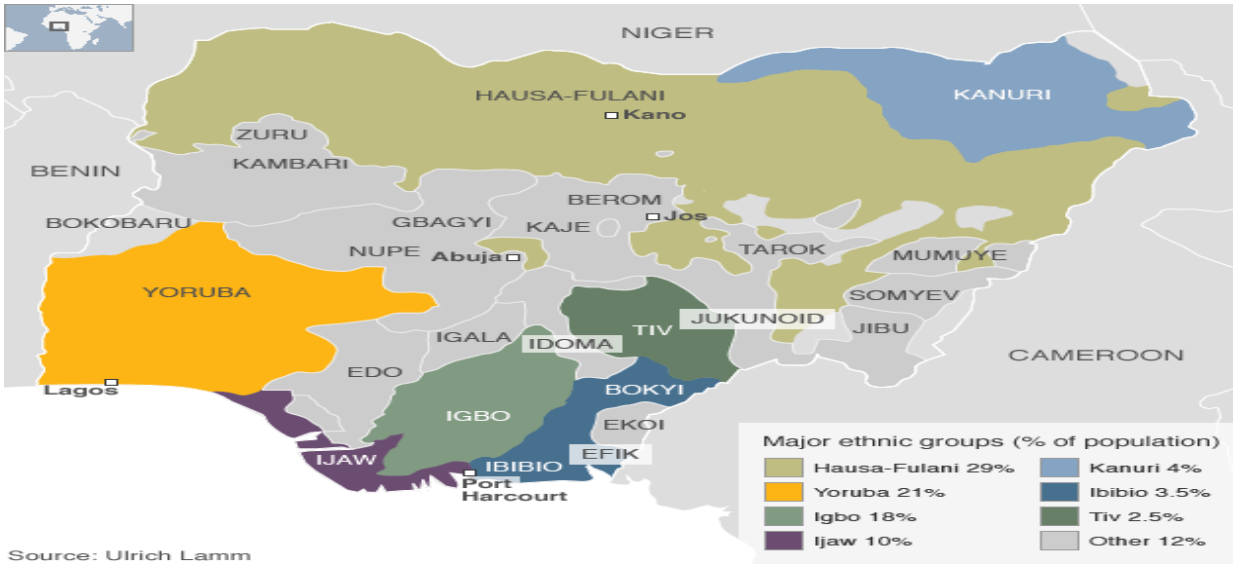


Figure 3: Phylogenetic tree of amplified BMP4 exon 3 gene sequences from selected participants (B1–B4) and the NCBI reference sequence.



S1: Map of Nigeria showing major ethnic groups (% population) with study area of idoma and Igede population (21%) located in the south-west Nigeria.