

Original Research

The Identification and Classification of Novel Genetic Variants in the *MCPH1* Gene Suggest Association with Non-Syndromic Hearing Impairment

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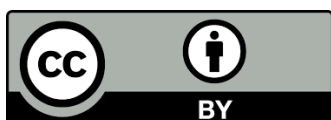
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Abstract

Human mouse orthologous hearing impairment genes were investigated in African patients for causal variants. A homozygous mutation in exon 13 of the microcephalin1 (*MCPH1*) gene, which encodes the BRCA1-carboxyl terminal 2 domain (BRCT2), was reported in non-syndromic hearing impairment (NSHI). The present study aims to investigate further the emerging roles of *MCPH1* in the genetics of NSHI in African patients in the new and larger cohorts. This study screened multiplex families and isolated cases, including 90 patients and 212 controls from Cameroon (n = 106) and South Africa (n = 106) using the Sanger sequencing technique with PCR. Subsequently, computational analyses were conducted to assess the level of relevance of the gene and the effects of genetic variations within it. The estimated mode of inheritance for the familial cases was 34.8% autosomal recessive, 34.8% autosomal dominant, 21.74% mitochondrial, and 8.66% X-linked. Four rare missense variants and seven novel variants were identified in the *MCPH1* gene. The homozygous variants *MCPH1* c.2222G>A p.(Arg741Gln) and *MCPH1* c.2234A>C p.(His745Pro) were identified in two probands; one of the probands had an affected sibling who is a heterozygous carrier of *MCPH1*



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c.2234A>C p.(His745Pro). Computational analysis suggests that these variants are potentially pathogenic, as they occur in mutational hotspots within *MCPH1* and a domain susceptible to missense loss-of-function mutations. The evolutionary analyses revealed that the *MCPH1* protein evolved in 150 taxa, while about 28 condensed into a phylogeny cluster that indicated similar substitution rates, divergent lengths, and positive selection, particularly in the two closest taxa to humans (chimpanzee and gorilla), suggesting that *MCPH1* is a stable gene. The protein modelling and surface hydrophobicity analyses indicate a change in atomic charges at the helix-loop that mediates dimerization and DNA binding, such that the wildtype equilibrates at 0.072 nm. In contrast, the mutant equilibrates at 0.042 nm *in-silico*. The study further reveals an association between the *MCPH1* gene and NSHI. The aberrations in the *MCPH1* gene are emerging with multiple conditions, understanding its genetic variations in different populations will be very important in genomic medicine.

Keywords

Hearing impairment; microcephalin1; Africa; heredity; phylogeny

1. Introduction

Hearing impairment (HI), hearing loss, or deafness can be defined as the total or partial inability to hear sounds. HI is a leading cause of disability worldwide, affecting an estimated 466 million people [1, 2]. Disabling HI is classified as HI above 40 decibels (dB) in adults (15 years or older) or above 30 dB in children (0 to 14 years) [1, 2]. Disabling HI is defined as hearing loss above 40 dB in adults (≥ 15 years) and above 30 dB in children (< 15 years). The World Health Organization reports a high prevalence of HI in sub-Saharan African countries, including South Africa, Cameroon, Kenya, Ghana, Mali, and Senegal [3, 4]. Genetic factors contribute 50% to the causes of HI, with 70% of genetic cases being diagnosed as non-syndromic hearing impairment (NSHI) [5]. The inheritance patterns of NSHI can be autosomal dominant or recessive, X-linked or mitochondrial-linked [6]. Genetic variants are associated with both syndromic and non-syndromic cases [7]. The most common mode of inheritance of NSHI is autosomal recessive, with over 50% of cases currently being mapped to mutations in the *DFNB1* gene locus, which is associated with deafness, autosomal recessive B1 (*DFNB1*). The prominent HI genes in this locus are *GJB2* and *GJB6* [6]. Identified NSHI mutations only account for most cases in the European and Asian populations [6]. There is a low pickup rate of these mutations in African populations [8-11]. Also, *GJB2* mutations in NSHI that showed founder effects in Senegal and Ghana were recently reported [12, 13], but not in Mali [14]. There is an ongoing effort to implement precision medicine globally. However, the current reality reveals that only a few actionable genetic biomarkers or targets are available for implementing precision medicine [15, 16], particularly in genetically diverse and underrepresented populations represented in public genomic databases. There is a need to identify ethnic-specific disease-associated genes and understand their mechanisms and recurrence to determine if they are actionable mutations. Over the years, we have developed research capacity in Africa to identify novel pathogenic variants in NSHI through biospecimen storage, genomics, and phenotype ontologies [17, 18]. In our previous study, we screened 34 novel human-mouse orthologous HI

genes in African cohorts [19], these genes were initially reported in mouse HI studies [20]. Our analysis identified a homozygous mutation in the Microcephaly 1, primary, autosomal recessive (*MCPH1*) gene in human studies. Microcephaly 1, primary, autosomal recessive (*MCPH1*) is a DNA damage response protein involved in the regulation of checkpoint kinase 1 (CHK1) and breast cancer 1 gene (BRCA1). It has been implicated in chromosome condensation and cellular responses induced by DNA damage [21]. It may play a role in neurogenesis by coordinating the cell cycle and the centrosome cycle [21]. Exposure to biological and physical stress is an essential mechanism in the aetiologies of HI and brain-associated disorders [22, 23]. The human *MCPH1* gene contains 14 coding exons. The BRCT1 domain comprising exons 1-4 of the *MCPH1* has been the hotspot for causative mutations associated with primary microcephaly [24]. The present study aims to investigate further the emerging roles of *MCPH1* in the genetics of NSHI in African patients in the new and larger cohorts.

2. Materials and Methods

2.1 Ethical Approval

The study received approval in South Africa by the Human Research Ethics Committee (Ethics approval number HREC Ref: 104/2018) of the University of Cape Town, South Africa and by the Institutional Research Ethics Committee for Human Health of the Gynaeco-Obstetric and Paediatric Hospital of Yaoundé, Cameroon (Ethics Approval No723/CIERSH/DM/2018).

2.2 Study Participants

Patients from South Africa and Cameroon were diagnosed with pure tone audiometry. The hearing level was classified according to the World Health Organization (WHO) recommendations. The hearing level is profound, indicating a significant loss of hearing. The audiometric readings ranged from 90-120 dB using the KUDUwave Audiometer eMoyo, manufactured by Radioear (Northcliff, Johannesburg, South Africa). Both verbal and signed consent were obtained from all participants or their parents/guardians before recruitment into the study, with guaranteed anonymity for the strict scientific use of the samples and data generated.

2.3 DNA Extraction, Quantification, and Integrity Analysis

Blood samples obtained from each patient and an unaffected relative were collected. DNA was extracted using the Chemagic 360 robot (Perkin Elmer®, Waltham, Massachusetts, United States of America (USA)) and diluted to a working concentration of 150 ng/μl using SABAX H₂O (Adcock Ingram, South Africa (SA)).

2.4 Genotyping

The screening of the Exon 13 BRCT2 domain was done using Sanger sequencing. The primers were designed with Primer3Plus (<https://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi>), IDT Oligo Analyzer 3.1 (<https://eu.idtdna.com/pages/tools/oligoanalyzer>), and NCBI Primer Blast (<https://www.ncbi.nlm.nih.gov/tools/primer-blast/>) (Table S1). Polymerase chain reaction (PCR)

was performed using the optimized conditions for each primer pair to amplify the regions of interest. The sequencing reaction was performed with a BigDye® Terminator kit (Applied Biosystems, USA), and capillary electrophoresis was performed using the ABI PRISM® 3130xl Genetic Analyzer (Applied Biosystems, USA).

2.5 Variant Annotation

Sequencing alignment and analyses were performed using the UGENE v.40 software. The *MCPH1* transcript ID used was ENST00000344683.10. Once a variant had been found in a particular sample, the sequence went through Ensembl Blast (<https://www.ensembl.org/index.html>) and the Varsome database (<https://varsome.com/>) to investigate if the single nucleotide polymorphisms (SNP) had been previously reported or if it was a novel variant. The variants previously reported or novel were analysed to predict their pathogenicity using the Varsome database (<https://varsome.com/>) as well as the variant annotation tools SIFT (<https://sift.bii.a-star.edu.sg/>), CADD (<https://cadd.gs.washington.edu/score>), and PolyPhen2 (<http://genetics.bwh.harvard.edu/pph2/>). The novel deleterious variants in the *MCPH1* gene with a consensus prediction were selected for further analyses with high-resolution melt.

2.6 High-Resolution Melt (HRM)

The HRM was performed with PCR preparation on the samples with SYTO9 fluorescence dye (ThermoFisher UK). The PCR was performed to amplify the region of interest as per the previously stated protocol. The samples were transferred to the BioRad CFX96™ real-time PCR detection system (BioRad Laboratories) for HRM. The samples were subjected to temperatures between 75 and 95°C at 0.1°C increments, with measurements taken every 2 seconds. The results were then analysed by the BioRad Precision Melt Analysis™ software (BioRad Laboratories, USA).

2.7 Protein Modelling of Novel Homozygous *MCPH1* c.2234A>C p.(His745Pro)

The process of protein homology modelling involves target identification, sequence alignment, model building, and model refinement. We retrieved the protein sequence of the *MCPH1* canonical isoform 1 from Ensembl. The target template used for the modelling was queried on the Protein Data Bank database (<https://www.rcsb.org/>) and the AlphaFold database (www.alphafold.ebi.ac.uk). The modelled residue number 3TIN template was downloaded in PDB format, i.e., the 'A' chain sequences. The template was modeled using the target with the Swiss model tool. The top model was selected based on their Discrete Optimised Protein Energy scores. The model was analysed on PyMol. The sequence was truncated to introduce the novel *MCPH1* c.2234A>C p.(His745Pro) variant in the appropriate position. The same approach was used for the modelling of the 3D structure of the wild type to determine the protein structure and characteristic changes as previously described. We converted the amino acid sequences (wild type and mutant) into a programme, ProtScale, that generated the hydrophobicity plot. We utilised the 20-length amino acid residues spanning the mutated amino acid for the computational analysis of the helical wheel projection to determine the physicochemical properties and relevance to therapeutic targets.

2.8 Evolutionary Analyses

We retrieved the protein sequence of *MCPH1* isoform 1 NM_024596 for the evolutionary analyses for amino acid rate distribution, time tree (tree topology) and conserved regions. Also, we selected the two closest taxa to humans for the *MCPH1* gene-positive selection analysis. The *MCPH1* DNA sequences of patients with rare variants were also utilised in the evolutionary mapping with controls, only for the domain of interest i.e. BRCT2. The analyses were conducted in MEGA11 (Molecular Evolutionary Genetic Analysis).

3. Results

3.1 NSHI Mode of Inheritance Analysis

Figure 1 describes the demographics of the patients diagnosed with sensorineural NSHI. There were thirty-nine (n = 39) (43%) male patients and fifty-one (n = 51) (57%) female patients in this study cohort. The number of isolated cases is thirty-seven (n = 37) (41%), contrasting with familial cases being fifty-three (n = 53) (58%). The figure also shows the distribution of the mode of inheritance amongst the patient cohort. The cohort included thirty-one (n = 31) (34.8%) autosomal recessive cases, thirty-one (n = 31) (34.8%) autosomal dominant cases, twenty (n = 20) (21.74%) mitochondrial cases and eight (n = 8) (8.66%) X-linked cases of NSHI (Figure 1).

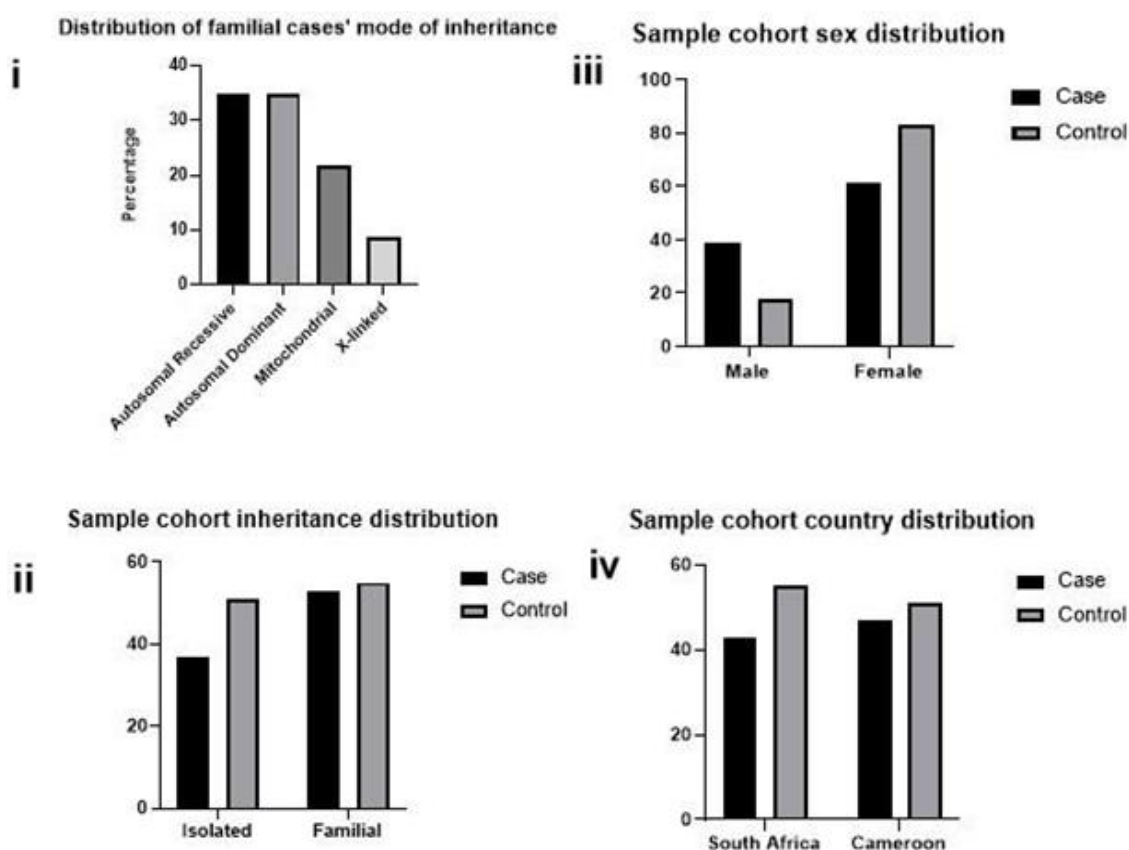


Figure 1 The results of the pedigree analyses and the demographics of the study participants. The graphs showed the mode of inheritance, disease status, and gender. The analyses are essential to our study to ascertain the genetic profile. Statistical test for differences was not performed.

3.2 Identification of Novel Variants in MCPH1

The previously reported mutation under investigation *MCPH1* c.2311C>G p.(Pro771Ala) was not identified in this study. After screening across the entire amplified region, we identified four rare missense variants and seven novel variants (Table 1). A homozygous pathogenic missense variant, *MCPH1* c.2222G>A p.(Arg741Gln), was identified in a single Cameroonian patient. The patient had congenital NSHI with two affected brothers. The pedigree analysis revealed an autosomal recessive mode of inheritance (Figure S1). The homozygous variant was not found in the affected relatives. Other heterozygous variants were identified in six Cameroonian patients, all of whom were isolated cases. The homozygous variant *MCPH1* c.2234A>C p.(His745Pro) identified in another proband was also not present in any affected relatives, but one affected sibling is a heterozygous carrier of *MCPH1* c.2234A>C p.(His745Pro). In terms of allele frequencies, the heterozygous *MCPH1* c.2222G>A; p.(Arg741Gln) variant has one entry in the gnomAD v3.1.2 database (rs779231385). The global allele frequency is 0.00006549 and was reported in an admixed American. The SIFT, Polyphen, and CADD predictions for this variant are deleterious with high impact. We are unable to ascertain the hearing condition of the gnomAD control. The *MCPH1* c.2234A>C p.(His745Pro) is novel. The multiple sequence alignments and phylogenetic analysis of the variants revealed that the two positions are substantially conserved at the protein level across various mammalian species (Figure S2). Furthermore, the demographic information and the descriptions of hearing loss level and the variant(s) identified in the prioritized study participants are presented in Table 2.

Table 1 Variants and pathogenicity information identified in the *MCPH1* exon 13 after sequence alignment - NM_024596.5.

rsID	Position	Coding	AA.change	Variant type	Zygosity	CADD score	SIFT score	Polyphen2 score	Pathogenicity	Supporting evidence	Country	No of patients	HI diagnosed	MOI
rs779231385	8:6478982	c.2222G>A	p.Arg741Gln	Missense	Homozygous	25.3	0.03	0.523	Pathogenic	Strong	CAM	6	Profound	AR
rs768268033	8:6478987	c.2227G>A	p.Glu743Lys	Missense	Heterozygous	16.51	0.1	0.09	Likely benign	Strong	CAM	1	Profound	AR
rs373658774	8:6479014	c.2254C>A	p.Gly753Glu	Missense	Heterozygous	22.4	0.01	0.291	Uncertain significance	Strong	CAM	1	Profound	AR
rs376082728	8:6479032	c.2272G>A	p.Asp758Asn	Missense	Heterozygous	10.50	0.4	0.007	Likely benign	Strong	CAM	1	Profound	AR
Novel	8:6478994	c.2234A>C	p.His745Pro	Missense	Homozygous	10.25	0.11	0.474	Likely pathogenic	Strong	CAM	1	Profound	AR
Novel	8:6478994	c.2234A>G	p.His745Arg	Missense	Heterozygous	0.870	N/A	N/A	Uncertain significance	Moderate	CAM	1	Profound	AR
Novel	8:6479008	c.2248C>T	p.Pro750Ser	Missense	Heterozygous	14.34	0.04	0.006	Uncertain significance	Strong	CAM	1	Profound	AR
Novel	8:6479018	c.2258G>A	p.Gly753Glu	Missense	Heterozygous	24.1	0.01	0.998	Uncertain significance	Strong	CAM	1	Profound	AR
Novel	8:6479018	c.2258G>C	p.Gly753Ala	Missense	Heterozygous	23.4	0.02	0.807	Uncertain significance	Strong	CAM	1	Profound	AR
Novel	8:6479044	c.2284A>C	p.Met762Leu	Missense	Heterozygous	15.11	0.04	0.54	Uncertain significance	Strong	CAM	1	Profound	AR
Novel	8:6479106	c.2346C>G	p.Cys782Try	Missense	Heterozygous	18.15	0	1	Uncertain significance	Strong	CAM	1	Profound	AR

The variants listed in the Table are predicted to be pathogenic, likely pathogenic, or having uncertain significance. The supporting evidence is moderate to strong according to the ACMG criteria. The majority are absent in the population databases, the prevalence of the variant in the affected cases (i.e., six, for a particular variant), and 1 each for others. In contrast, none of the variants are identified in the unaffected. Also, the domain is a mutational hotspot (for missense truncating mutations) and has the most important functional roles in *MCPH1* biological activities.

Table 2 Demographic information and the descriptions of hearing loss level and variant(s) identified in the selected study participants.

Participants	Sex	Age	City	Description	Mechanism	Degree of hearing loss – Left	Degree of hearing loss – Right	Consanguineous	Cause	Variant
1	Male	27	Yaoundé	Congenital/Prelingual	Perception	P3 (111-119)	P3 (111-119)	No	Familial	p.Arg741Gln
2	Female	13	Garoua	Congenital/Prelingual	Perception	P1 (91-100)	P1 (91-100)	No	Familial	p.Arg741Gln
3	Male	16	Bafoussam	Congenital/Prelingual	Not determined	P2 (101-110)	P2 (101-110)	No	Unknown	p.Arg741Gln
4	Female	12	Garoua	Prelingual	Perception	P2 (101-110)	P2 (101-110)	No	Familial	p.Arg741Gln
5	Male	6	Garoua	Prelingual	Perception			No	Familial	p.Arg741Gln
6	Male	12	Garoua	Congenital/Prelingual	Perception	P1 (91-100)	P1 (91-100)	No	Familial	p.Arg741Gln
7	Female	12	Yaoundé	Congenital/Prelingual	Perception	P1 (91-100)	P1 (91-100)	No	Familial	p.Arg741Gln
8	Female	8	Yaoundé	Congenital/Prelingual	Perception	P1 (91-100)	P1 (91-100)	No	Familial	p.Met762Leu
9	Female	16	Yaoundé	Congenital/Prelingual	Perception	P1 (91-100)	P2 (101-110)	No	Familial	p.Cys782Try
10	Male	10	Douala	Congenital/Prelingual	Perception	P2 (101-110)	P3 (111-119)	No	Unknown	p.Glu743Lys
11	Female	16	Bafoussam	Congenital/Prelingual	Perception	P2 (101-110)	P3 (111-119)	No	Unknown	p.Pro750Ser
12	Male	10	Bafoussam	Congenital/Prelingual	Perception	P1 (91-100)	S1 (71-80)	No	Unknown	p.Pro750Ser
13	Male (proband)	13	Bafoussam	Congenital/Prelingual	Perception	T (120)	S2 (81-90)	No	Unknown	p.His745Pro
14	Male	11	Douala	Congenital/Prelingual	Perception	P1 (91-100)	P1 (91-100)	No	Familial	p.Cys782Try
15	Male (brother)	10	Bafoussam	Congenital/Prelingual	Not determined	P2 (101-110)	P2 (101-110)	No	Unknown	p.His745Arg
16	Male	10	Yaoundé	Congenital/Prelingual	Perception	P2 (101-110)	P2 (101-110)	No	Familial	p.Gly753Glu
17	Female	11	Yaoundé	Congenital/Prelingual	Not determined	S2 (81-90)	P1 (91-100)	No	Familial	p.Gly753Ala
18	Female	13	Bertoua	Congenital/Prelingual	Perception	P1 (91-100)	P1 (91-100)	No	Familial	p.Cys782Try

3.3 Confirmation of Allele Frequency Through Genotyping of Variants in Controls

The normalized melt curves from HRM suggest that the fragments between the NSHI patients and their ethnically matched controls are different due to the fragments melting to varying temperatures during melting (Figure 2a, b, c, d). HRM provided evidence that showed a difference in the DNA between the NSHI patients and unaffected controls, suggesting the variant was not present in the controls. Further analyses using Sanger sequencing confirmed the presence of the variants in the ethnically matched controls for all the variants. Still, the *MCPH1* c.2222G>A p.(Arg741Gln) and *MCPH1* c.2234A>C p.(His745Pro) were not present in controls.

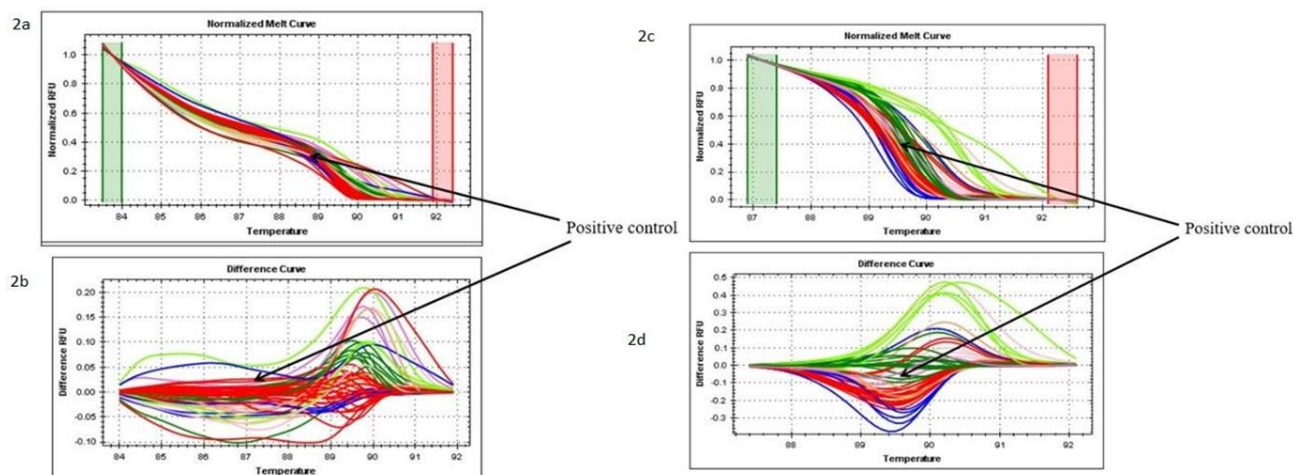


Figure 2 The (a) graph shows the results of the HRM for the rs779231385, *MCPH1* c.2222G>A p.(Arg741Gln) previously identified. The arrows point toward the melting curve for the positive controls, respectively, in a normalised melt curve graph (a), and difference curve (b) for the same reaction. It indicates the absence of the variant in the controls. Each colour represents a different cluster. The BioRad Precision Melt Analysis™ software allocates samples to a particular cluster based on similarity, but not necessarily identical melting curves and melt temperatures. The (c) graph is the HRM result of the novel *MCPH1* c.2234A>C p.(His745Pro). The arrows point toward the melting curve of the positive controls, respectively, in a normalised melt curve graph (c), and difference curve (d) for the same reaction. It indicates the absence of the variant.

3.4 The Protein Modelling

The protein modelling revealed that the position of this residue is at the deep end of the protein backbone in one of the alpha-helix fragments. The wild type (His) contributed a charge of -0.06 to the protein structure stability, whereas the mutant charges were -0.03. The mutant was defined by 7 atoms, while 10 atoms determined the wild type. However, final calculations of the atom-state-level properties were unaffected, suggesting that the mutant could be tolerated. Indeed, the Packpred (<http://cospi.iiserpune.ac.in/packpred/>) prediction of the functional consequences of the variant in the protein was used in this study, and it ranked the variant as neutral or likely to be pathogenic. Also, the ProtScale algorithm detects an increase in hydrophobicity for this variant (Figure 3a and 3b).

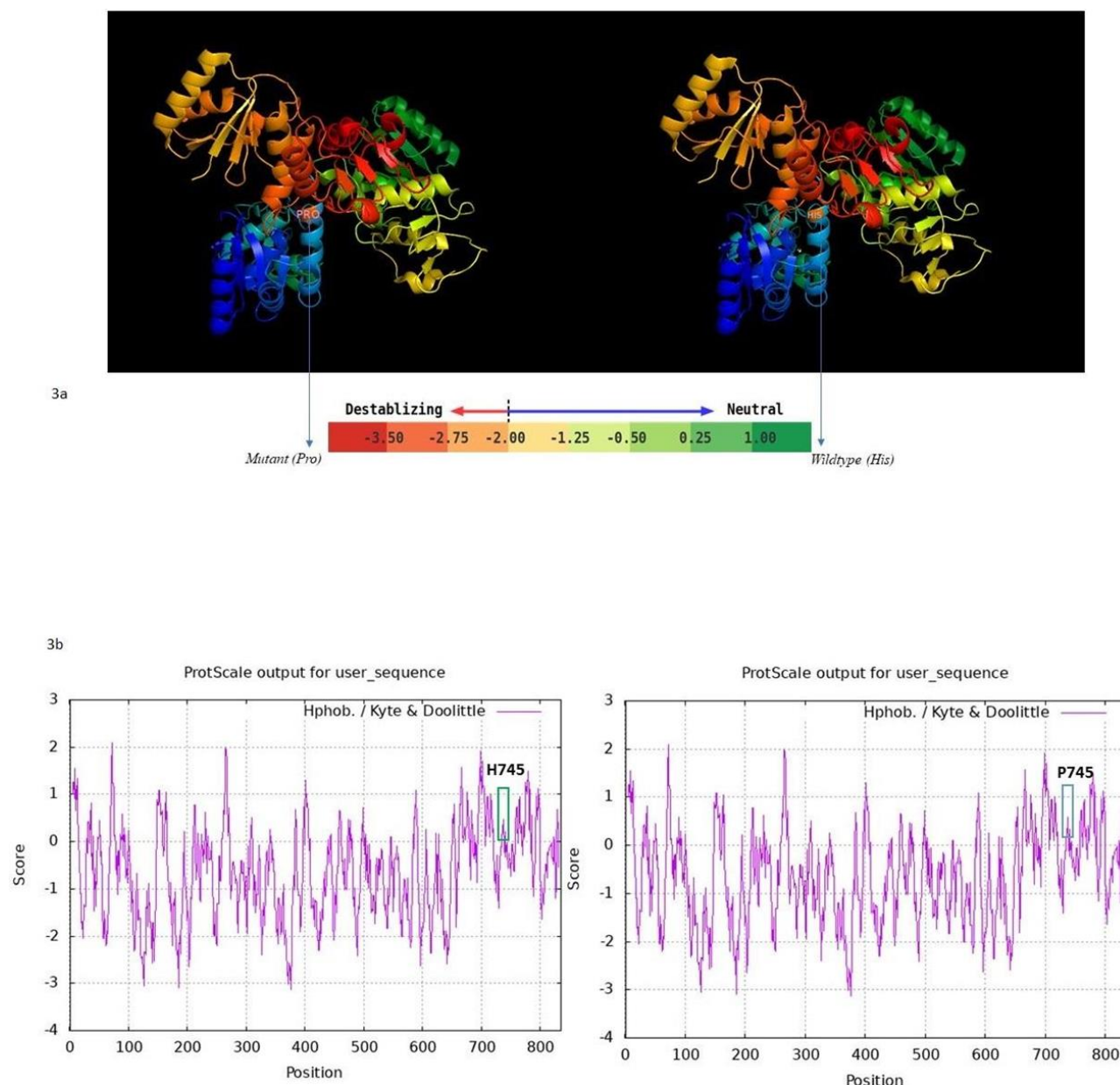


Figure 3 (a) The figure displays the protein modelling of novel homozygous *MCPH1* c.2234A>C p.(His745Pro). The root mean square deviation (RMSD) values show that wildtype equilibrates at 0.072 nm while the mutant equilibrates at 0.042 nm. (b) Depicts the hydrophobicity profile of wildtype and the predicted pathogenic heterozygous single-nucleotide polymorphisms (SNP) rs779231385, i.e., *MCPH1* c.2222G>A p.(Arg741Gln). The interactions between hydrophobic and hydrophilic molecules have a significant influence on the three-dimensional structure. Protein surface contact with water, followed by protein folding, may be influenced by hydrophilic modifications.

3.5 MCPH1 Evolutionary Analyses

The *MCPH1* phylogeny tree for the ancestral reconstruction with 150 taxa showed a condensed tree that identified 28 close taxa to the query sequences (Figure 4). Also, the maximum likelihood estimates of the gamma parameter for the site rate distribution estimated from the protein sequences are 1.0168. Substitution pattern and evolutionary rate differences among sites showed

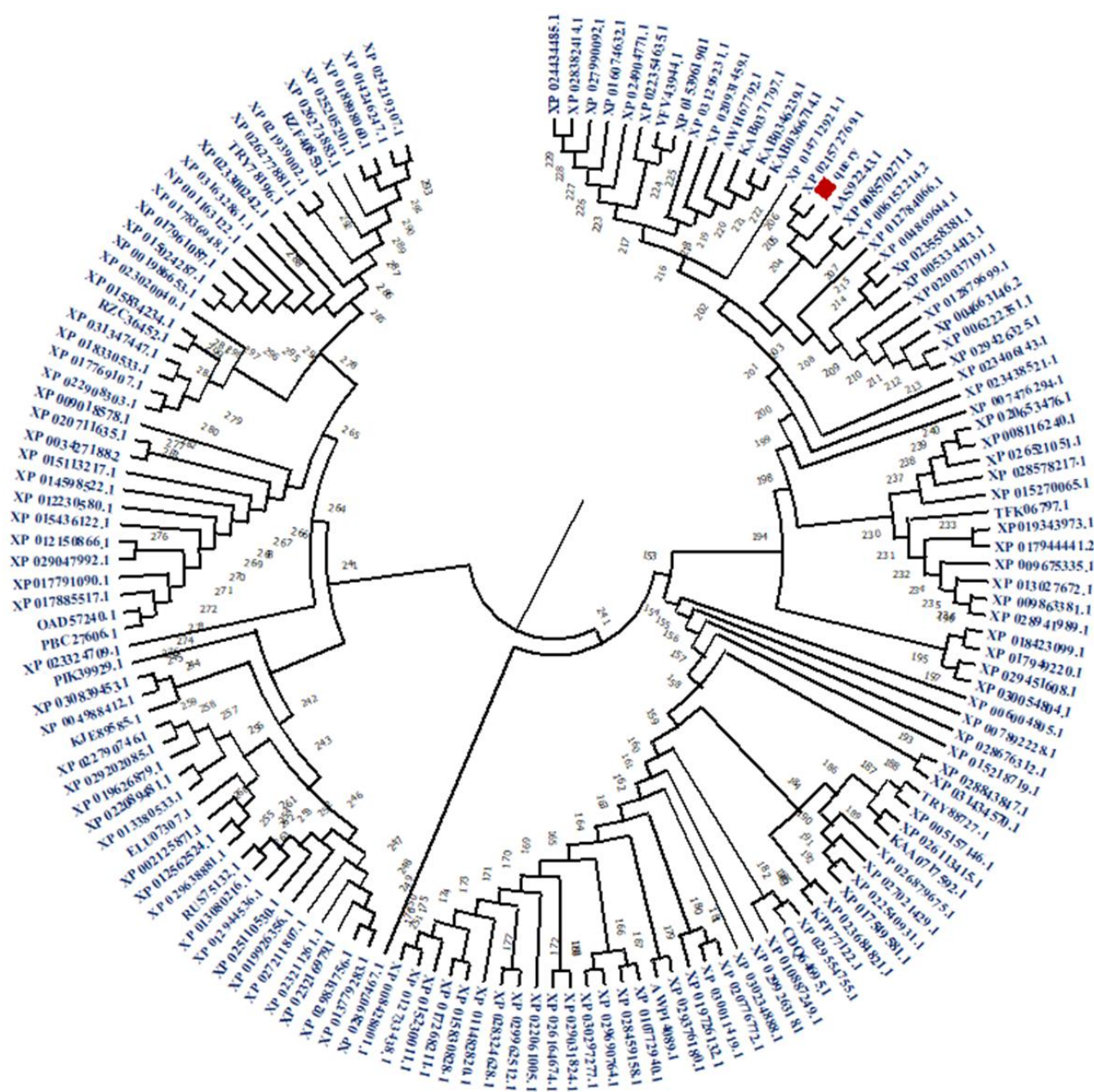


Figure 4 The phylogeny-based analysis showing ancestral reconstruction of the protein amino acid sequences using the FireProt and MEGA algorithms. The image shows the human *MCPH1* phylogeny tree with 150 taxa. By computing the condensation tree based on sequence differences 28 close taxa to the query sequence, based on length/distance were clustered/condensed. File S1 shows the conservation and missing amino acids in the cluster.

Table 3 Results of the relative rate test for the *MCPH1*.

The configuration	Count
Identical sites in all three sequences	732
Divergent sites in all three sequences	0
Unique differences in Sequence A	10
Unique differences in Sequence B	10
Unique differences in Sequence C	4

The equality of evolutionary rate between sequences **A** (*Human*) and **B** (*Gorilla*), with sequence **C** (*Chimp*) used as an outgroup in Tajima's relative rate test. The χ^2 test statistic was 0.00 ($P = 1.00000$ with 1 degree[s] of freedom) P -value less than 0.05 is used to reject the null hypothesis of equal rates between lineages. This analysis involved 3 amino acid sequences. All positions containing gaps and missing data were eliminated. There was a total of 756 positions in the final dataset, of which 732 are unique and identical to the three species.

4. Discussion

MCPH1 is a DNA repair gene that is ubiquitously expressed in adult human tissues, including the brain, testes, pancreas, and liver. The *MCPH1* regulation of the DNA repair mechanisms maintains genome integrity. An extensive DNA damage leads to apoptosis through the selective destabilization of the cell membrane [23]. Studies have shown that genetic mutations, exposure to toxic environments may result to developing various neurological diseases like Parkinson's disease and Alzheimer's disease [23, 25, 26]. The impairment of DNA repair machinery in a cell often manifests biologically as pathological stress and inflammation. These factors are the well-established hallmarks of many diseases that can be biologically ameliorated by various bioactive compounds previously studied [22, 27-32]. *MCPH1* is mainly associated with primary microcephaly and other neurological deficits like epilepsy, learning disabilities, speech delays, and infertility [27, 33]. The gene has been linked to HI due to the variant's predisposition to otitis media [34]. The study showed that knockout mice exhibited mild to moderate (HI) with a penetrance level of 70%, in which the mice suffered from otitis media [34]. The role of *MCPH1* in the regulation of mitochondrial activity and metabolism was reported [27], suggesting an association with various biological processes and a pleiotropic role for *MCPH1*. Hence, given the *MCPH1*'s role in multiple conditions, understanding its genetic variations in different populations is crucial.

This study used genetic analyses to identify likely pathogenic *MCPH1* variants in Cameroonian patients. These variants were absent in both related and unrelated unaffected controls. The homozygote *MCPH1* variant identified in the proband was not present in the affected full sibling. Another causal variant might co-segregate with the disease in the family. The proband in the study has a pathogenic homozygous variant in the *MCPH1* biallelic gene, confirming why the variant is prioritised in our research. Mutations have been primarily linked to the BRCT2 domain. The domain is described as very active in the LacZ vector experiment of exons 12 and 13 of the gene [35]. With the advent of gene editing, gene therapy, and anti-sense oligonucleotide therapies, it might be possible to ameliorate the *MCPH1* disease-linked genes.

Furthermore, the evolutionary analyses indicated that significant evolutionary conservation and favorable selection rates occurred over this gene in the evolutionary clock. The evolutionary studies

of *MCPH1* have shown that it is highly conserved in many species. Tajima's relative rate test demonstrated this by analyzing the translated sequences of humans and two closely related taxa [36]. Usually, at the molecular level, positive selection occurs when a particular DNA variant becomes more common because of its effect on the organisms that carry it. Amino acids and nucleic acids are frequently preserved as coding sequences to maintain a protein's structure and function [37, 38]. Site-directed mutagenesis of an existing gene is a well-established practice for studying the relationships between protein structure and function [39]. The protein modeling shows that the variant can alter protein functions by changing the physicochemical properties of the amino acid.

The different patterns of NSHI inheritance observed in patients signify that the genetics of HI studies in Africa require different approaches due to population diversity. A previous study had described Africa as the most diverse population [40]. Our findings from this study contribute to ethnic-specific genetic studies of African origin. While resources for genetic studies are still limited in Africa, several studies have employed the same approach to identify disease genes in the population. For example, we recently used a similar approach in South Africa to confirm the case of POIKTMP syndrome [41]. Based on literature findings, it is increasingly evident that aberrations in the *MCPH1* gene are emerging with multiple conditions; therefore, understanding the genetic variations associated with *MCPH1* in different populations will become crucial [42]. Hence, by focusing on *MCPH1* and other novel genes, we can understand disease mechanisms and target treatment, or at least improve genetic counseling services to prevent the genetic transmission of NSHI. One possible mechanism could be the DNA-damage immune response of the inner ear, which has been linked to macrophage infiltration [43]. In light of this discussion, studies investigating the potential role of *MCPH1* in macrophage-related immune responses in NSHI would be essential to support previous findings relating to the plausible association between DNA repair and immune response mechanisms.

In conclusion, putative deleterious variants were identified in the *MCPH1* gene in NSHI patients recruited in Cameroon. All prioritized variants identified through in-silico analyses were also found in Cameroonian patients. The study indicates that the *MCPH1* gene harbours variants for NSHI cases. The *MCPH1* should be studied in NSHI in a population-specific manner, as the level of admixture and the sample size may contribute to the allele frequencies of the identified variants.

Author Contributions

OGO conceptualised the study, provided the methodology and supervised the work, K.J carried out the experiments for data collection. OGO and K.J. analysed and interpreted the results data, and KJ drafted the first version of the manuscript. OGO wrote, reviewed, and edited the manuscript. A.W. was the principal investigator and provided clinical expertise and relevant information for the research.

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Competing Interests

There is no conflict of interest to declare.

Data Availability Statement

The datasets generated and/or analyzed during the current study are available in the European Nucleotide Archive repository with the accession number PRJEB53404 (ERA15321558).

Additional Materials

The following additional materials are uploaded at the page of this paper.

1. Figure S1: This figure shows the pedigree of the proband found to have a novel homozygous *MCPH1* c.2234A>C p.(Arg741Gln), and the chromatogram. The pedigree reflects an autosomal recessive mode of inheritance in the family line. The variant is not present in the affected brother or the unaffected relatives.
2. Figure S2: This figure shows the sequenced alignments for the selected patients. The alignments and the chromatograms show the novel homozygous variant *MCPH1* c.2234A>C p.(His745Pro) found in proband and the *MCPH1* c.2222G>A p.(Arg741Gln) found in six Cameroonian patients, as well as the evidence for the conservation of the wildtype amino acids in different species.
3. Table S1: The primer information for *MCPH1* exon 13 sequencing. This table shows the primer information for *MCPH1* exon 13 sequencing.
4. File S1: This table shows the conservation map of Human *MCPH1* matched with 28 condensed taxa. A few missing amino acids and many conserved amino acids in other mammals indicate the stability of this gene over the evolutionary processes.

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