

A Genomic and Protein–Protein Interaction Analyses of Nonsyndromic Hearing Impairment in Cameroon Using Targeted Genomic Enrichment and Massively Parallel Sequencing

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Abstract

Hearing impairment (HI) is one of the leading causes of disability in the world, impacting the social, economic, and psychological well-being of the affected individual. This is particularly true in sub-Saharan Africa, which carries one of the highest burdens of this condition. Despite this, there are limited data on the most prevalent genes or mutations that cause HI among sub-Saharan Africans. Next-generation technologies, such as targeted genomic enrichment and massively parallel sequencing, offer new promise in this context. This study reports, for the first time to the best of our knowledge, on the prevalence of novel mutations identified through a platform of 116 HI genes (OtoSCOPE[®]), among 82 African probands with HI. Only variants *OTOF* NM_194248.2:c.766-2A>G and *MYO7A* NM_000260.3:c.1996C>T, p.Arg666Stop were found in 3 (3.7%) and 5 (6.1%) patients, respectively. In addition and uniquely, the analysis of protein–protein interactions (PPI), through interrogation of gene subnetworks, using a custom script and two databases (Enrichr and PANTHER), and an algorithm in the *igraph* package of R, identified the enrichment of sensory perception and mechanical stimulus biological processes, and the most significant molecular functions of these variants pertained to binding or structural activity. Furthermore, 10 genes (*MYO7A*, *MYO6*, *KCTD3*, *NUMA1*, *MYH9*, *KCNQ1*, *UBC*, *DIAPH1*, *PSMC2*, and *RDX*) were identified as significant hubs within the subnetworks. Results reveal that the novel variants identified among familial cases of HI in Cameroon are not common, and PPI analysis has highlighted the role of 10 genes, potentially important in understanding HI genomics among Africans.

Keywords: nonsyndromic hearing impairment, genomics, protein–protein interaction, Africans, Cameroon

Background

HEARING IMPAIRMENT (HI) is one of the leading causes of disability in the world with the highest rate for age-standardized disability-adjusted life-years (DALYs). For example, the HI-related DALYs is approximately one and a half time to twice greater than congenital heart disease (Murray et al., 2015). It has a global prevalence of about 1 per 1000 live births in developed countries, with a much higher rate in the developing world, as high as 6 per 1000 in sub-Saharan Africa (Olusanya et al., 2014).

Genetic factors are estimated to account for 50% of variability in the congenital HI phenotype with a mostly

nonsyndromic and autosomal recessive (AR) mode of inheritance (Hutchin et al., 2005). To date, 90 genes have been implicated in nonsyndromic hearing impairment (NSHI) (<http://hereditaryhearingloss.org/main.aspx?c=HHH&n=86163>, accessed December 1, 2016). In many populations, pathogenic variants in *GJB2* (connexin 26 gene) are a major contributor to autosomal recessive NSHI (ARNSHI) (Chan and Chang, 2014). However, the prevalence of *GJB2*- or *GJB6*-related NSHI is approximating to zero in most sub-Saharan African populations and little is known about the contribution to HI by other known NSHI genes (Bosch et al., 2014a, 2014b; Gasmelseed et al., 2004; Javidnia et al., 2014; Kabahuma et al., 2011; Lasisi et al., 2014).

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Targeted genomic enrichment (TGE) and massively parallel sequencing (MPS), for example, with OtoSCOPE[®], include 116 HI genes, and has been shown to be an efficient tool for genetic testing for NSHI (Shearer et al., 2010). Using OtoSCOPE, genetic testing of ten multiplex Cameroonian families segregating NSHI identified pathogenic variants in 7 out of 10 families (70%) (Lebeko et al., 2016); within the 7 families, 12 pathogenic variants in 6 genes previously implicated in NSHI were identified, of which 5 were novel (41.6%). The report of five novel pathogenic variants highlights the importance of ethnic-specific filtering (Sloan-Heggen et al., 2016) and underscores the need to further investigate these variants among nonfamilial Cameroonian cases of NSHI.

This study investigated the prevalence of these newly identified mutations in a cohort of HI patients from Cameroon and South Africa. In addition, to explore the interaction of various HI genes that could potentially play a role in clinical penetrance and variable expression of the phenotype, as well the epidemiology of HI in the general population, we investigated the relationships between variants identified by OtoSCOPE using bioinformatics analysis of a human protein–protein interaction (PPI) network.

Materials and Methods

Ethics approval and consent to participate

This study was approved by the Human Research Ethics Committee of the University of Cape Town (ethics approval HREC REF: 455/2014) and the Cameroon National Ethics Committee (ethics approval N°123/CNE/SE/2010). Written informed consent was obtained from participants 18 years or older and from parents/guardians of minors with verbal assent from the children.

Patients and inclusion criteria

Patients were recruited from (1) schools of the deaf and outpatient clinics in Cameroon, as previously reported (Wonkam et al., 2013), and (2) from South Africa at the Effata School of the Deaf in Umtata Eastern Cape and were self-identified as Black Xhosa. As previously described, we included, in this study, patients of Black African descent with NSHI of either (1) putative genetic origin, as revealed by one or more affected family members or consanguinity, or (2) unknown origin. We excluded patients with syndromic HI and those with obvious environmental causes such as meningitis, rubella, mumps, measles, severe prematurity and/or birth weight less than 1500 g, neonatal hyperbilirubinemia, neonatal asphyxia, ototoxicity, or severe head trauma (Bosch et al., 2014a).

Clinical evaluation included a comprehensive questionnaire (exposure to noise, ototoxic agents, and familial history) and the diagnosis of sensorineural HI according to current clinical standards, as previously reported (Wonkam et al., 2013). All probands and affected individuals were examined for syndromic features by a medical geneticist and an ophthalmologist. All probands were negative for pathogenic variants in *GJB2*, *GJB6*, and *GJA1*. The total number of patients included in this study was 25 Black Xhosa South Africans and 57 Cameroonians.

Control participants

A total of 250 ethnically matched Cameroonian controls without HI and negative for a family history were recruited, from apparently healthy blood donors in Yaoundé.

Molecular analysis

DNA preparation and polymerase chain reaction amplification. DNA was extracted from whole blood as previously described (Bosch et al., 2014a, 2014b; Wonkam et al., 2013). Polymerase chain reaction (PCR) primers were designed to amplify between 150 and 400 bps encompassing each mutation. Patients' samples were genotyped through singleplex snapshot PCR using a SNaPshot Multiplex Ready Reaction Mix (Applied Biosystems, Foster City, CA, USA). Fragments were resolved on an ABI 3130xl Genetic Analyzer (Applied Biosystems) with the data being analyzed on GeneMapper, Bioedit Sequence Alignment Editor v 7.2.5 23, and Finch TV v 1.4.0 (Washington, DC, USA). Results of genotypes were confirmed by direct Sanger sequencing using a BigDye Terminator v 3.1 Cycle Sequencing Kit (Applied Biosystems) (Fig. 1), at the Division of Human Genetics, Faculty of Health Sciences, University of Cape Town, South Africa.

Sequencing analysis and identification of causative mutations. From the previously reported study using OtoSCOPE, the average coverage for the 350,160 targeted base pairs was 99.60% at a greater than 10X depth of coverage. Sequencing reads were aligned to the reference Human genome (Hg19) using the Burrows-Wheeler Aligner (BWA) algorithm as reported (Shearer et al., 2010). The Exome Aggregation Consortium database was used to remove high-frequency variants (i.e., minor allele frequency $\geq 0.1\%$), which were unlikely to be pathogenic and obtain variant frequencies in Europeans, Asians, and Africans (Lebeko et al., 2016). The conservation and deleteriousness of the variants were evaluated using the following tools: likelihood ratio test (LRT), mutation assessor, mutation taster, PolyPhen2, PROVEAN, and sorting intolerant from tolerant (SIFT) (Wang et al., 2010).

Molecular modeling

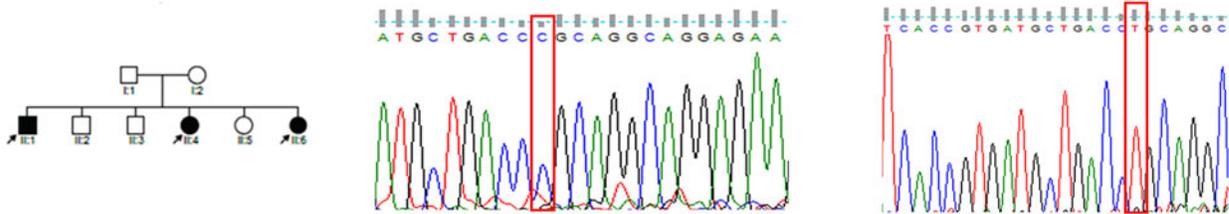
For the five novel variants identified in Cameroonian families, molecular modeling was performed using SWISS-MODEL (Biasini et al., 2014) and Phyre2 (Kelley et al., 2015) (Fig. 2; Supplementary Fig. S1). Specific templates that were used for each protein or domain within which the novel variant lies were previously reported (Lebeko et al., 2016).

Network analysis

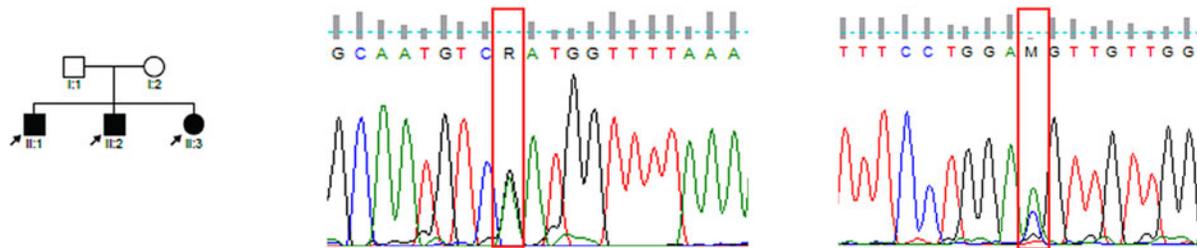
Network and enrichment analysis. We used a comprehensive human PPI network (Chimusa et al., 2016; Wu et al., 2009) to understand how the set of casual variants are layered in a biological network. We performed enrichment analysis to examine how these variants and genes within the identified subnetwork are associated with human phenotypes and determine their potential biological processes, pathways, and molecular functions. For this, we used a custom script and two databases: Enrichr (www.amppharm.mssm.edu/Enrichr) and PANTHER (<http://pantherdb.org>).

To identify potential gene hubs in the network of HI, we queried our reported genes (Lebeko et al., 2016) alongside all

D Family 4



E Family 6



F Family 7

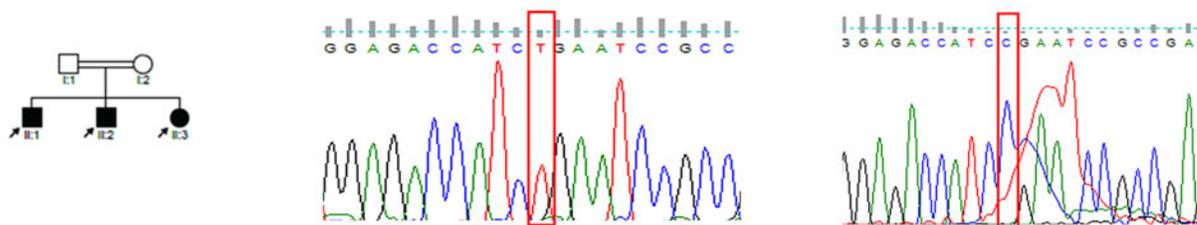


FIG. 1. Pedigrees of selected families (4, 6 and 7) and sequence data showing variants that are causal for hearing impairment. Detected variants include the following: homozygous *OTOF* NM_194248.2:c.766-2A>G (**panel D**); compound heterozygous *SLC26A4* NM_000441.1:c.1678G>A, p.Asp560Asn and NM_000441.1:c.2007C>A, p.Asp669Glu (**panel E**); and homozygous *MYO7A* NM_000260.3:c.1996C>T, p.Arg666Stop (**panel F**).

other known genes associated with HI in the PPI network; these are the genes which were screened through the OtoSCOPE panel (116 in total). We applied a clustering algorithm in the *igraph* package of R (www.r-project.org) to determine community structure from the obtained subnetwork of hearing loss interactive genes.

Prioritizing variants on the merged data from all family members. We additionally prioritized variants using the aggregated data of all interrogated family members. We merged all single variants calling files (vcf) from each family member and annotated the resulting merged vcf using ANNOVAR (Wang et al., 2010). Based on prediction approaches implemented in ANNOVAR, we filtered and retained only variants (Supplementary Table S1) that have a functional prediction using 10 approaches (SIFT, PolyPhen 2, MutationTaster, MutationAssessor, LRT, FATHMM, MetaSVM, MetaLR, GERP++, and PhyloP). Functional prediction status included “Deleterious/probably or damaging/disease_causing” (D) or “disease_causing_automatic” (A). Enrichment analysis was performed using two databases, including Enrichr

(www.amppharm.mssm.edu/Enrichr) and PANTHER (<http://pantherdb.org>). We estimated and aggregated the minor allele frequencies (MAF) of single nucleotide polymorphisms (SNPs) downstream and upstream (0 kb, respectively) within each of these retained genes using exome data from 105 Cameroonian control population, all African, European, and East Asian populations from the 1000 Genome Phase 3 Project.

Results

Patients' description

The patients' descriptions are summarized in Table 1. The total number of samples analyzed were from 82 patients with a skew toward male participants (58.5%, $n=48$). The majority of affected individuals had nonsyndromic, prelingual, bilaterally profound sensorineural HI, with hearing thresholds between 81 and 119 dB.

Prevalence of previously identified pathogenic variants

Mutations identified through OtoSCOPE in our previous study were not prevalent in this group of patients with NSHI

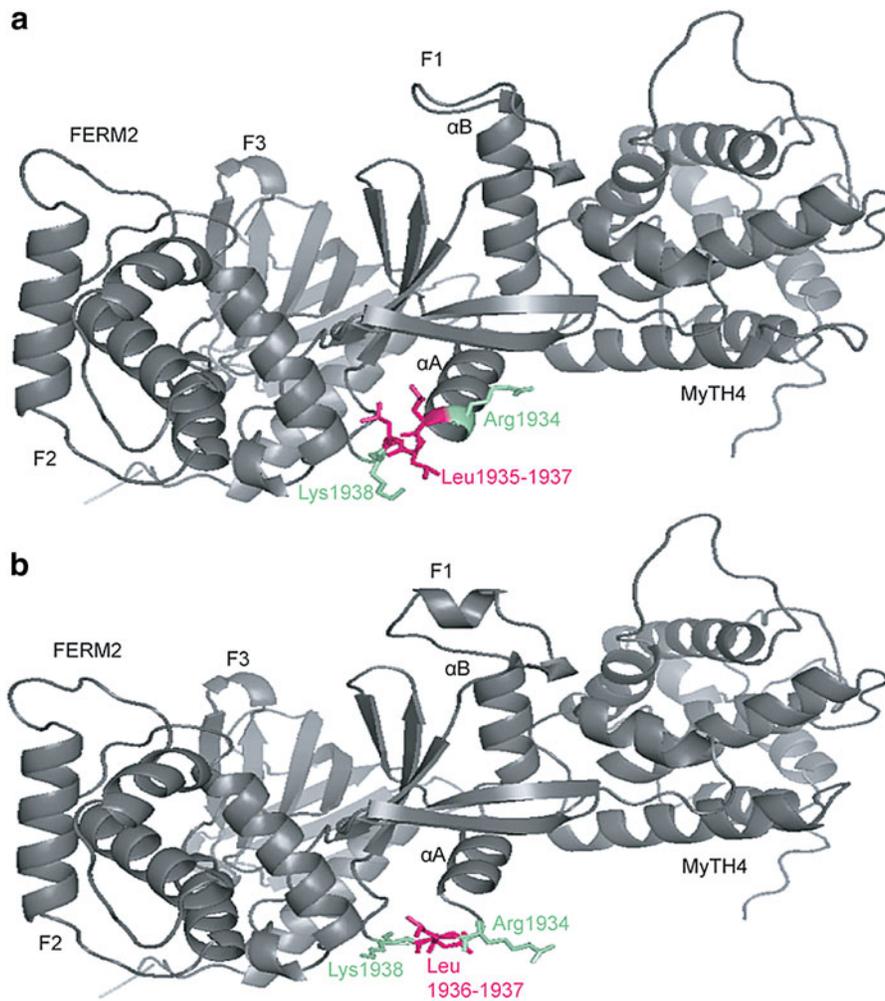


FIG. 2. Molecular modeling of MYO7A p.Leu1935del comparing wild-type (a) and mutant (b) proteins. Loss of one out of three leucine residues (pink) at the edge of the αA helix of the F1 lobe within the FERM2 domain of the MYO7A protein is predicted to result in unraveling not just of the αA_{F1} helix but also of the αB_{F1} helix. The variant is expected to cause misfolding of the F1 lobe and poor contact between F1 and the central/CEN1 domain of SANS protein.

(Table 2). Among Cameroonian individuals with HI, only *OTOF* NM_194248.2:c.766-2A>G was found in 3 (3.7%) patients with an MAF=0.02, and *MYO7A* NM_000260.3:c.1996C>T, p.Arg666Stop was found in 5 (6.1%) patients with an MAF=0.04 (Table 2). All these variants were in a heterozygous state in all the cases and are thus unlikely to explain, alone, the cause of HI in these patients. None of these variants were found among South African patients. None of the variants previously reported in *MYO7A*, *CDH23*, *LOXHD1*, and *SLC26A4* were identified in this group of patients with HI (Table 2).

Molecular modeling

Previous molecular modeling data reported changes to the *LOXHD1* protein that affected binding (Lebeko et al., 2016). The effect of the novel *MYO7A* variant results in a disruption of the folding of the FERM2 domain needed for binding to the SANS protein central domain disrupting their dimerization (Fig. 2) (Wu et al., 2011). The p.Asp2133Glu change in *CDH23* results in a loss of calcium binding site which is

integral to the functioning of the protein (Supplementary Fig. S1b) (Sotomayor et al., 2010). Coupled with the change in linker regions caused by the p.Met2907Thr substitution (Supplementary Fig. S1d) (Patel et al., 2006), the protein is rendered nonfunctional.

Gene network analysis and enrichment of reported genes

Querying the genes reported in Table 2 in the human PPI network, we identified 16 pairs of interactive genes (Fig. 3A). Interestingly, the novel mutation identified in *MYO7A* appears to be the central hub of the subnetwork, indicating a major functional role that the variant may play in HI. The subnetwork (Fig. 3A) was not found to be significantly associated with any biological pathway; however, from the OMIM (Online Mendelian Inheritance of Man database) disease database (www.omim.org), the whole subnetwork is enriched with Usher Syndrome and deafness variants ($p=4.134e-7$ and 0.0001138, respectively). Two biological processes were found to be significantly associated with the

TABLE 1. SUMMARY OF PARTICIPANTS' DEMOGRAPHICS

Demographic	Category	n (%)
Sex	Male	48 (58.5)
	Female	33 (40.2)
	N/A	1 (1.22)
Age of onset	Congenital/prelingual	56 (68.3)
	Perilingual	6 (7.32)
	Postlingual	7 (8.54)
	N/A	13 (15.85)
Cause/transmission	Familial	37 (45.1)
	Sporadic (unknown)	37 (45.1)
	N/A	8 (9.75)
Type of hearing loss (dB)	Severe I (71–80)	1 (1.22)
	Severe II (81–90)	1 (1.22)
	Profound I (91–100)	12 (14.63)
	Profound II (101–110)	9 (10.98)
	Profound III (111–120)	5 (6.1)
	Total HL (>120)	1 (1.22)
	Asymmetric	23 (28.05)
	N/A	31 (37.08)
Previous testing	<i>GJB2</i> , <i>GJB6</i> , <i>GJA1</i> , and variants identified by OtoSCOPE®	82 (100)

N/A, no data available.

subnetwork, including sensory perception of mechanical stimulus (GO: 0050954, $p = 1.430e-8$) and sensory perception of sound (GO: 0007605, $p = 1.246e-8$). Furthermore, 60% of the molecular functions (Fig. 3B) of these variants are in binding (GO: 0005488) and/or structural molecule activity (GO: 0005198) pathways.

From the compiled gene network of 116 hearing loss genes, we identified 10 hubs (the most import gene node, harboring the major connectivity within the subnetwork), in-

cluding *MYO7A*, *MYO6*, *KCTD3*, *NUMA1*, *MYH9*, *KCNQ1*, *UBC*, *DIAPH1*, *PSMC2*, and *RDX* (Fig. 4A). The subnetwork (Fig. 4B) is significantly associated with known biological pathways such as proteasome degradation, Tumor necrosis factor (TNF)-alpha signaling pathway, and transforming growth factor (TGF)-beta signaling pathway (Fig. 4B). Sixty-three percent of the genes within the subnetwork (Table 3) have a molecular function in binding (GO: 0005488) and/or catalytic activity (GO: 0003824) pathways, and are significantly enriched with deafness ($p = 4.22e-15$).

Leveraging the combined data in prioritizing variants and gene enrichment

We merged the data of nine Cameroonian family members, and filtered and prioritized variants using the approach and criteria described above. We replicated OtoSCOPE findings in two genes, *OTOF* and *LOXHD1* (Supplementary Fig. S2 and Supplementary Table S1), and identified other genes in linkage disequilibrium (LD) with those reported in Table 2. Furthermore, we used a comprehensive human PPI network (Chimusa et al., 2016; Wu et al., 2009) to derive their biological network positions. We identified 29 pairs of interacting genes (Supplementary Fig. S2A). From the OMIM disease database (www.omim.org), the whole subnetwork is enriched with HI variants ($p = 0.000001870$ and adjusted $p = 0.000013$).

The highest proportion of variants in the subnetwork (Supplementary Fig. S2A) is associated with endoplasmic reticulum pathways (Supplementary Fig. S2B) and 60% of the molecular functions (Supplementary Fig. S2C) of these variants are related to binding (GO: 0005488) and/or catalytic activity (GO: 0003824). Using exome data of 105 Cameroonian controls, as well as data extracted from the 1000 Genomes project, results displayed in Supplementary

TABLE 2. PREVALENCE OF MUTATIONS IDENTIFIED BY OTOSCOPE AMONG CAMEROONIAN PATIENTS WITH NONSYNDROMIC HEARING IMPAIRMENT

Gene	Biological function/pathway	Chromosomes	Exon/ Intron	cDNA change	Protein change	Allele frequency in patients (no. of chromosomes)	
						Cameroon	South Africa
<i>MYO7A</i>	Intracellular protein transport	11q13.5	Exon 40	c.5806_5808 delCTC	p.Leu1935del	0 (0/114)	0 (0/25)
			Exon 40	c.5880_5882 delCTT	p.Phe1963del	0 (0/114)	0 (0/25)
<i>CDH23</i>	Calcium-dependent cell adhesion	10q22.1	Exon 46	c.6399C>A	p.Asp2133Glu	0 (0/114)	0 (0/25)
			Exon 59	c.8720T>C	p.Met2907Thr	0 (0/114)	0 (0/25)
<i>LOXHD1</i>	Targeting of proteins to plasma membrane	18q21.1	Exon 22	c.3371G>A	p.Arg1124His	0 (0/114)	0 (0/25)
			Exon 26	c.3979T>A	p.Phe1327Ile	0 (0/114)	0 (0/25)
<i>OTOF</i>	Calcium ion binding	2p23.3	Intron 6–7	c.766-2A>G	N/A	0.02 (3/114)	0 (0/25)
<i>SLC26A4</i>	Regulation of membrane potential	7q22.3	Exon 15	c.1678G>A	p.Asp560Asn	0 (0/114)	0 (0/25)
			Exon 17	c.2007C>A	p.Asp669Glu	0 (0/114)	0 (0/25)
<i>MYO7A</i>	Intracellular protein transport	11q13.5	Exon 17	c.1996C>T	p.Arg666	0.04(5/114)	0 (0/25)

RefSeq#: *MYO7A*, NM_000260.3; *SLC26A4*, NM_000441.1; *OTOF*, NM_194248.2; *LOXHD1*, NM_144612.6; *CDH23*, NM_022124.5.

cDNA, complementary DNA.

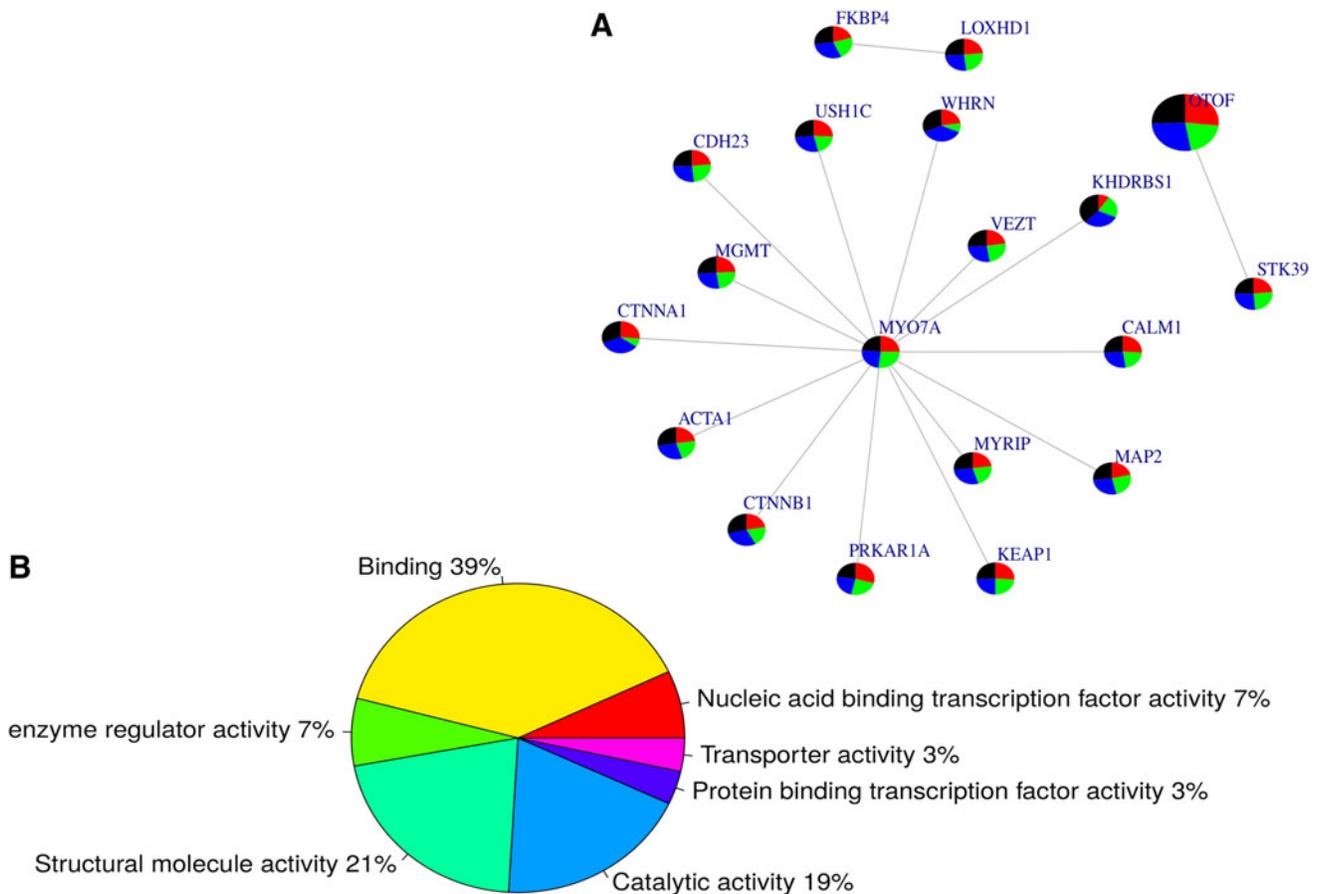


FIG. 3. Subnetwork of interactive genes with potential mutations associated with hearing loss. From the identified causal mutations occurring in genes displayed in Table 2 and Supplementary Table S1, we used a human protein–protein interaction network to interrogate how these genes are positioned in a biological network: we identified 16 pairs of interacting genes (**panel A**). The whole subnetwork is enriched with Usher syndrome and deafness gene variants ($p=4.134e-7$ and 0.0001138 , respectively). Interestingly, the *MYO7A* gene that had a novel identified mutation appears to be the central hub of that subnetwork, suggesting a major role that variant may play in hearing impairment. Two biological processes were found to be significantly associated with the subnetwork, including sensory perception of mechanical stimulus (GO: 0050954, $p=1.430e-8$) and sound (GO: 0007605, $p=1.246e-8$); furthermore, 60% of the molecular functions (**panel B**) of these variants are annotated to binding (GO: 0005488) and/or structural molecule activity (GO: 0005198).

Table S1 indicate that the variants in the majority of these hub genes displayed almost similar MAFs across various populations, with the exception of variants in *MYO1A*, which were significantly more prevalent among East Asians, and variants in *GIPC3*, which were relatively rare among the East Asian populations.

Discussion

HI is one of the highest contributors to disability worldwide, impacting on the social, economic, and psychological well-being of the affected individual (Olusanya, 2011). This is particularly true in low-income regions such as sub-Saharan Africa, which also carries one of the highest burdens of this disease (WHO, 2012). Despite this, there are limited data to support the most prevalent genes or mutations that cause HI among patients from this region. Using TGE and MPS in 10 Cameroonian families recently demonstrated the importance of this approach in the resolution of genetic

aetiology of HI in Africa and its role in uncovering novel variants (Lebeko et al., 2016). Follow-up research among nonfamilial cases of HI, potentially of genetic origin as presented in this study, revealed that the aforementioned variants are not common in other nonfamilial cases of HI in Cameroon, and absent from a small sample of Xhosa patients from South Africa.

The findings of the heterozygous state of *OTOF* NM_194248.2:c.766-2A>G found in three patients, and *MYO7A* NM_000260.3:c.1996C>T, p.Arg666Stop found in 5 (6.1%) patients (Table 2) suggest that these eight individuals are most likely compound heterozygotes, with second mutations to be investigated in these genes. The relatively high number of NSHI genes and novel variants among a relatively small sample of Cameroonians (Lebeko et al., 2016) demonstrates the high level of genetic heterogeneity, and could reflect genetic diversity found in Cameroons, which mimics many populations in sub-Saharan Africa (Lambert and Tishkoff, 2009).

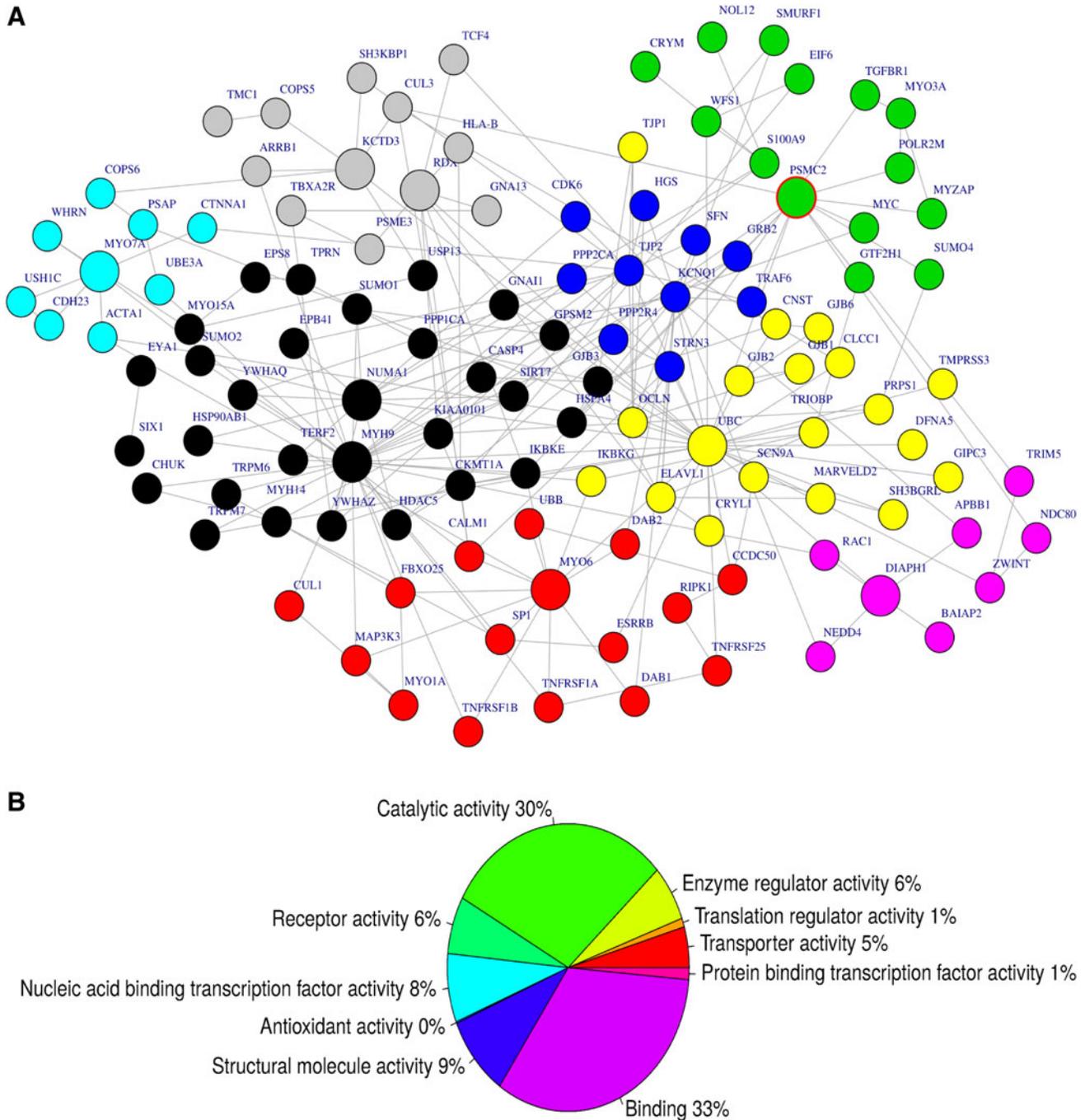


FIG. 4. Subnetwork of interacting genes with mutations potentially associated with hearing loss. We identified 10 hubs from the subnetworks extracted from protein-protein interaction database (**panel A**), including *MYO7A*, *MYO6*, *KCTD3*, *NUMA1*, *MYH9*, *KCNQ1*, *UBC*, *DIAPH1*, *PSMC2*, and *RDX*. The molecular functions of the subnetwork are significantly associated with known biological pathways such as Proteasome Degradation, TNF-alpha signaling pathway, and TGF-beta signaling pathway (**panel B**). TNF, tumor necrosis factor; TGF, transforming growth factor.

TABLE 3. TOP ASSOCIATED BIOLOGICAL PATHWAYS FOR THE IDENTIFIED SUBNETWORK

Term	Overlap	p	Adjusted p	Z-score
Proteasome degradation	25/62	5e-13	7e-11	-1.90
TNF-alpha signaling pathway	28/92	5e-12	5e-10	-1.8
TGF-beta signaling pathway	30/132	4e-10	2e-08	-1.8
Calcium regulation in the cardiac cell	29/149	1e-08	7e-05	-1.8

Pathway enrichment using Enrichr (www.amppharm.mssm.edu/Enrichr), based on genes in the subnetwork in Figure 4A; 63% of the genes within the subnetwork are annotated to the molecular functions binding (GO: 0005488) and/or catalytic activity (GO: 0003824) and are significantly enriched with deafness ($p=4.22e-15$).

TNF, tumor necrosis factor; TGF, transforming growth factor.

The absence of a mutation in 30% of Cameroonian families previously studied indicates the possibility of discovering new HI-associated genes in Africans (Lebeko et al., 2016). Supporting this, recent studies using MPS that looked at the exons of 180 genes in various populations across the globe, including two sub-Saharan African populations, showed the lowest number of resolved cases, reported at 4% and 17%, among Nigerian and South African populations, respectively (Yan et al., 2016). A similar trend with only 26% resolution rate was reported among African-Americans, the lowest when compared to that of other ethnic groups (Sloan-Heggen et al., 2016). Thus, in an effort to address the genetic causes of HI among patients of African descent, the use of whole-exome sequencing should be the next approach. Estimates indicate that at least 1000 NSHI genes remain to be identified (OMIM) (Hertzano and Elkon, 2012), and African populations could be important in this endeavor.

The *MYO7A* variant c.5880_5882 delCTT (p.Phe1963del) was previously identified in a French patient with Usher syndrome (Roux et al., 2006), and bioinformatics analysis performed in this study also revealed that the subnetwork associated with this *MYO7A* variant is enriched with Usher syndrome annotation ($p = 4.134e-7$). While surveillance will determine whether retinitis pigmentosa will develop in the Cameroonian hearing-impaired probands and affected siblings, it is possible that the compound heterozygous variants in *MYO7A* are causal of NSHI. Two previously reported *MYO7A* tail variants caused NSHI rather than Usher syndrome (Riazuddin et al., 2008), like the novel *MYO7A* p.Leu1935del variant reported in this study.

Likewise, for *CDH23* compound heterozygous variants (Table 2), the occurrence of an NSHI variant *in trans* with an Usher allele is predicted to cause NSHI only (Schultz et al., 2011). Similarly, the compound heterozygous *SLC26A4* variants identified in one of the Cameroonian families (family 6) (Fig. 1; Table 2) were previously identified in Chinese NSHI probands (Jiang et al., 2015; Yasunaga et al., 2000; Yuan et al., 2012) and are unlikely to cause Pendred syndrome. A homozygous splice mutation, *OTOF* [c.766-2A>G], identified in this study (family 4; Fig. 1) was previously reported in a southwest Indian family segregating NSHI (Yasunaga et al., 2000).

The bioinformatics interrogation of genes in the human PPI network performed in this study could give us new insight into biological process involved in HI, in understanding variable clinical phenotypes, and hopefully, investigating new routes for therapeutic interventions. As expected, there was a significant association between the reported subnetwork and the biological processes' sensory perception of sound and mechanical stimulus. Specifically, the network analysis identified 10 hubs that involved genes that could happen in future studies to be of importance in Cameroon. In addition, the analysis has reaffirmed the importance of *MYO7A* in HI. *MYO7A* is a large gene that spans 87 kb of the genome and plays an important role in nonsyndromic as well as syndromic HI (i.e., Usher syndrome) (Gibbs et al., 2010). OtoSCOPE interrogates the exons of genes known to be associated with HI, thus any variants identified within the genes could potentially have clinical implications (Shearer et al., 2013).

The above PPI analysis results on the variants reported by OtoSCOPE, in addition to analysis of data extracted from the 1000 Genomes with a community network analysis across the

different populations, have also shown that for some targeted HI genes, the overall MAFs at the gene level were population-specific common (Supplementary Table S1). Last, prioritization of genes and their variants from merged data replicated *OTOF* and *LOXHD1* as important genes in HI, which also appeared to be occurring in LD with other reported variants. Much like *MYO7A*, *OTOF* carries variants that are reported in multiple populations (Table 2) and could prove to be important genes in HI across different populations, and their roles and interactions should be further investigated.

Conclusions and Outlook

The result reveals that novel variants recently identified among selected familial case of HI in Cameroon variants are not common in isolated cases of NSHI. PPI network analysis has highlighted the potential role of 10 genes in HI genomics and phenotypes among Cameroonians that need further investigation. It is anticipated that future ramifications of the study will include study of exomes data of families segregating HI, isolated probands with HI, as well as control populations without HI from Africa, with the anticipation that the genes highlighted by the present studies could play prominent roles in the etiology and epidemiology of HI in sub-Saharan Africa. Such knowledge will assist in establishing appropriate molecular diagnosis from this condition, as well as clinical and molecular clues to be used for anticipatory guidance in clinical settings, based on genotypes to phenotypes correlation studies.

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A.W. conceived the study, participated in its design, interacted with the patients, coordinated the blood sample collection, and drafted the article. K.L., M.N., E.C., N.M., C.D., and A.W. carried out the genetic studies, performed the data analysis, and contributed to drafting the article. K.L. and C.D. participated in its design, interacted with the patients, coordinated the blood sample collection, and helped to draft the article. E.C., N.M., and A.W. performed the bioinformatics analysis, coordinated the data interpretation and statistical analysis, and helped to draft the article. All authors read and approved the final article.

Author Disclosure Statement

The authors declare that no competing financial interests exist.

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Abbreviations Used

AR = autosomal recessive
 ARNSHI = autosomal recessive nonsyndromic hearing impairment
 cDNA = complementary DNA
 DALYs = disability-adjusted life-years
 ExAC = Exome Aggregation Consortium
 HI = hearing impairment
 LD = linkage disequilibrium
 LRT = likelihood ratio test
 MAF = minor allele frequencies
 MPS = massively parallel sequencing
 N/A = no data available
 NSHI = nonsyndromic hearing impairment
 OMIM = Online Mendelian Inheritance of Man database
 PCR = polymerase chain reaction
 PPI = protein–protein interaction
 TGE = targeted genomic enrichment
 vcf = variants calling files