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Targeted genomic enrichment and massively parallel sequencing identifies novel nonsyndromic hearing impairment pathogenic variants in Cameroonian families

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To the Editor

In contrast to many world populations, in sub-Saharan Africa *GJB2*-related nonsyndromic hearing impairment (NSHI) is rare (1), and little is known about the contribution to hearing impairment (HI) by other known genes. Targeted genomic enrichment (TGE) and massively parallel sequencing (MPS), using a platform called OtoSCOPE®, have been shown as an efficient tool for comprehensive genetic testing for NSHI (2). In this study, we have investigated the clinical utility of this panel to resolve the genetic causes of autosomal recessive nonsyndromic hearing impairment (ARNSHI) in familial cases from Cameroon.

The study was approved by the Cameroon National Ethics Committee (REF 123/CNE/SE/2010), the Human Research Ethics Committee of the University of Cape Town (REF 455/2014), and the University of Iowa IRB (approval number 1035709). Patients were recruited from schools of the deaf in Cameroon (3). Clinical evaluation included a comprehensive questionnaire (exposure to noise, ototoxic agents, and familial history) and the diagnosis of sensorineural HI according to the current clinical standards (3). All probands and affected individuals were examined for syndromic features by a medical

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Supporting Information

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geneticist, and an ophthalmologist. Families with at least two individuals with ARNSHI who are negative for pathogenic variants in *GJB2* and *GJB6* were studied.

DNA samples of affected individuals were analyzed at the Molecular Otolaryngology and Renal Research Laboratories (MORL) at the University of Iowa's Carver College of Medicine; TGE and MPS were completed as previously described (2). The Exome Aggregation Consortium (ExAC) database was used to remove high frequency variants (i.e. minor allele frequency ≥ 0.1%) which were unlikely to be pathogenic and to obtain variant frequencies in Europeans, Asians and Africans. The conservation and deleteriousness of the variants were evaluated using the following bioinformatic tools: Likelihood ratio test (LRT), Mutation Assessor (MA), Mutation Taster (MT), PolyPhen2 (PP2), PROVEAN (PR), and SIFT (4). Population-specific allele frequencies were obtained by genotyping 250 ethnically matched Cameroonian control, using direct Sanger sequencing (Applied Biosystems, Foster City, CA). For the five novel variants identified in Cameroonian families, molecular modeling was performed using SWISS-MODEL and Phyre2; Specific templates were used for each protein or domain within which the novel variant lies (Table S1).

A total of 26 individuals from 10 families with congenital ARNSHI were studied. All the affected individuals had nonsyndromic, pre-lingual, bilaterally symmetric profound sensorineural HI, with hearing thresholds between 81 and 119 dB (Table S2, Supporting Information). In seven out of 10 families (70%), 12 putatively pathogenic variants were identified in six NSHI genes (*CHD23*, *LOXHD1*, *MYO7A*, *SLC26A4*, *OTOF*, and *STRC*) (Table 1). Five of the 12 variants (41.6%) have not been previously implicated in HI etiology whereas the remaining seven variants were shown to be involved in NSHI in populations outside of sub-Saharan Africa (Table 1). Most of the variants are not present or ultra-rare in the ExAC database, which has data on 60,706 individuals (Table 1). All identified variants segregate with the HI phenotype (Fig. S1). In three families (30%), no pathogenic variants were identified. For each of these five novel variants (Tables S1), molecular modeling revealed potential disruption of folding or inter-domain binding due to these variants, which are expected to result in changes to residue interactions within the same protein or with other proteins (Table S1, Fig. S2) and may explain the protein dysfunction that leads to hearing impairment.

This report is the first from sub-Saharan Africa to reinforce the value of TGE and MPS to determine the genetic cause of HI in this population (Table S3) (2, 5). The use of a comprehensive deafness-specific panel is state of the art and offers high diagnostic high sensitivity and specificity (5). Nevertheless, with small families, future studies in Cameroon should consider the use whole exome sequencing, in order to validate the co-segregation of an allele and a specific phenotype using the logarithm of the odds (LOD) score method. The absence of pathogenic variants in 30% of families suggests that novel hearing loss genes may be discovered among Africans, as supported by a recent report describing the lowest diagnostic rate for ARNSHI in African Americans (5). In aggregate, these results confirm the efficiency of comprehensive genetic testing in defining the causes of NSHI in Cameroon and highlight the value of African populations for the identification of novels genes associated with NSHI.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Variants identified in Cameroonian families using OtoSCOPE[a]

Family	#Aff	GT	Gene	Variant		ExAC all MAF	ExAC Afr MAF	Cameroonian controls MAF	Deleterious by bioinformatics	Known or novel
1	3	Het	*MYO7A*	c.5809_5811delCTC	p.Leu1937del	0	0	0	MT	Novel
				c.5886_5888delCTT	p.Phe1963del	0.00005	0	0	MT	Known (6)
2	3	Het	*CDH23*	c.6399C>A	p.Asp2133Glu	0	0	0	LRT, MA, MT, PP2, PR, SIFT	Novel
				c.8720T>C	p.Met2907Thr	0	0	0.002	LRT, MT, SIFT	Novel
3	2[b]	Het	*LOXHD1*	c.3371G>A	p.Arg1124His	0.00005	0	0.002	LRT, MA, MT, PP2, PR, SIFT	Novel
				c.3979T>A	p.Phe1327Ile	0.0001	0.0008	0	LRT, MA, MT, PP2, PR, SIFT	Novel
4	3	Hom	*OTOF*	c.766-2A>G	NA	0	0	0	MT	Known (7)
5	4	Het	*STRC*	20-kb del	NA	NA	NA	NA	NA	Known (8)
				CNV	NA	NA	NA	NA	NA	Known (8)
6	3	Het	*SLC26A4*	c.1678G>A	p.Asp560Asn	0.00002	0	0	LRT, MT, PP2, SIFT	Known (9)
				c.2007C>A	p.Asp669Glu	0	0	0	LRT, MA, MT, PP2, PR, SIFT	Known (10)
7	3	Hom	*MYO7A*	c.1996C>T	p.Arg666	0.00002	0	0.002	MT	Known (11)

Aff, affected; Afr, African; CNV, copy number variation; ExAC, exome aggregation consortium; GT, genotype; Het, heterozygous; Hom, homozygous; MAF, minor allele frequency; LRT, likelihood ratio test; MA, Mutation Assessor; MT, Mutation Taster; NA, not available; PP2, PolyPhen2 HVAR; PR, PROVEAN.

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

[b]Hearing-impaired individuals from family 3 are second-cousins, the rest are siblings.