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Common genes for non-syndromic deafness are uncommon in sub-Saharan Africa: A report from Nigeria

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ABSTRACT

Introduction: Little is known about the molecular epidemiology of deafness in sub-Saharan Africa (SSA). Even in Nigeria, the most populous African nation, no genetic studies of deafness have been conducted. This pioneering work aims at investigating the frequencies of gene mutations relatively common in other parts of the world (i.e. those in *GJB2*, *GJB6*, and mitochondrial DNA) among subjects from Nigeria with hearing loss (HL) with no evidence of acquired pathology or syndromic findings. In addition, we review the literature on the genetics of deafness in SSA.
Method: We evaluated 81 unrelated deaf probands from the Yoruba tribe residing in Ibadan, a suburban city in Nigeria, for the aetiology of their deafness. Subjects underwent genetic testing if their history was negative for an environmental cause and physical examination did not find evidence of a syndrome. Both exons of *GJB2* and mitochondrial DNA flanking the 1555A>G mutations were PCR-amplified followed by Sanger sequencing. *GJB6* deletions were screened via quantitative PCR.
Result: We identified 44 probands who had nonsyndromic deafness with no environmental cause. The age at study time ranged between 8 months and 45 years (mean = 24 years) and age at onset was congenital or prelingual (<age 2 years) in 37 (84%) probands and postlingual in 7 (16%) probands. Among these, 35 probands were the only affected members of their families (simplex cases), while there were at least two affected family members in nine cases (multiplex). Molecular analyses did not show a pathogenic variant in any one of the 44 probands studied.
Conclusion: *GJB2*, *GJB6* and mitochondrial DNA 1555A>G mutations were not found among this initial cohort of the deaf in Nigeria. This makes imperative the search for other genes in the aetiology of HL in this population.

1. Introduction

Hearing loss (HL) is the most common sensory disorder. One out of every 500 newborns has bilateral permanent sensorineural HL, with the prevalence increasing to 2.7 per 1000 by the age of 5 and 3.5 per 1000 during adolescence [1]. Epidemiological surveys of the deaf have consistently shown that currently about 50% of childhood deafness in developed countries can be attributed to genetic causes [1]. In fact, the causative genomic variants have been documented for most types of deafness in developed countries and efforts are now focused on determining the phenotype–genotype correlation for many of the known genes/variants [1]. In contrast, the literature from sub-Saharan Africa (SSA) on the genetic aetiology of deafness is sparse even though sub-Saharan Africa has a high (1.8%) prevalence for hearing loss affecting communication in children, second only to the south Asia region (2.3%) [2]. Earlier surveys of deafness in Gambia [3] and Nigeria [4] revealed meningitis and chronic middle ear infections as the major diseases causing deafness, while familial factors accounted for less than 10% of the childhood deafness [3]. Consequently, it was recommended that a primarily preventive approach was the most rational way of helping the deaf in these countries [2–5]. However, it is to be noted that genetic services, in particular hearing genetics research, are still at elementary stages in most parts of SSA [6–8]. Hence, the lack of genetic facilities in the investigation of HL in these earlier studies would have resulted in failure to identify genetic factors as a major contributor to the aetiology. In addition, these earlier works reported a high proportion (32% and 54.4%) of deafness whose 'cause is unknown'

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leading to the conclusion that these cases of deafness in the unknown category may well be of genetic origin [2–5]. Thus, there is a need to explore the possibility of a genetic aetiology for the unknown cause category. In developed countries, with the availability of molecular diagnosis, the cause of childhood deafness is unknown in less than 50% [9,10]. Furthermore, improvement in health care services, especially the vaccination programme in SSA, has resulted in the control of many infectious diseases, including measles and mumps [11,12]. By inference, this reduction in the prevalence of these infectious diseases will reduce their contribution as a cause of deafness, thus increasing the relative contribution of genetics. A few molecular analyses in SSA have identified a few genetic mutations as possible etiological factors for deafness in the sub-region [13–23]. It is noted that most of the studies of the molecular genetics of deafness in SSA have been driven by research in the specialist/tertiary hospitals. Notably, there has not been any study on the genetic epidemiology of deafness in Nigeria, the most populous African nation. Hence this pioneering work aims at investigating the frequencies of gene mutations relatively common in other parts of the world (i.e. those in *GJB2*, *GJB6*, and mitochondrial DNA) in this population. In addition, this work will review the literature on the genetics of deafness in SSA.

2. Materials and methodology

2.1. Samples

This study has been approved by the Ethics Committee of University of Ibadan (Nigeria) and IRB at the University of Miami (USA). Signed informed consents were collected from all participants or parents. We evaluated 81 unrelated deaf probands from the Yoruba tribe residing in Ibadan, a sub-urban city in Nigeria, for the aetiology of their deafness. In order to have deaf subjects from diverse groups, the study participants were selected from various vocational and professional groups, schools and religious groups. The criteria for recruitment was (i) lack of evidence for an environmental cause of HL such as meningitis, measles, mumps or cerebral malaria, as reported by the pupil's parents or guardians, (ii) lack of evidence for a syndrome obtained by physical examination, and (iii) audiometric findings compatible with a severe to profound sensorineural HL. The study included 44 deaf subjects whose histories were negative for an environmental cause and physical examination did not show syndromic findings. The remaining 37 subjects were excluded because their deafness was either syndromic or secondary to meningitis, viral infection, or associated with other neurological abnormalities such as cerebral palsy. The probands or their representatives were asked for permission to communicate the results of the genetic testing. DNA was extracted using standard procedures with a Qiagen extraction kit at the Institute of Medical Research and Training in University of Ibadan and the DNA samples were subsequently transferred to the Hussman Institute for Human Genomics at the University of Miami for laboratory studies.

2.2. Sanger sequencing and CNV detection

Both exons of *GJB2* and mitochondrial DNA flanking the 1555A>G mutation were PCR-amplified followed by Sanger sequencing [24]. Previously reported four large genomic deletions involving *GJB6* [25–27] were screened via quantitative PCR. CNV analysis for the genomic region of the *GJB6* gene was performed with a TaqMan predesigned probe (Hs03843749, **Chr13:20961484 on NCBI build 37)** by using a previously described protocol [28]. For the Sanger sequencing, PCR reactions included 25 μg of genomic DNA with Taq DNA polymerase (Roche). Corresponding DNA fragments were amplified using a touchdown protocol. PCR products were visualized on agarose gels cleaned over Sephadex columns or with NucleoFast 96 PCR plates (Clontech) in accordance with the manufacturer's protocols. Sequence analysis was performed with the ABI PRISM Big Dye Terminator Cycle Sequencing V3.1 Ready Reaction Kit and the ABI PRISM 3730 DNA Analyzer (Applied Biosystems). Sequence traces were analyzed using the Sequencher 4.7 program (Gene Codes Corporation).

3. Results

Among the 44 probands, there were 32 males and 12 females with age ranging between 8 months and 45 years (mean = 24 years). Age at onset was congenital or prelingual (<age 2 years) in 37 (84%) probands and postlingual in 7 (16%) probands. Among these, 35 probands were the only affected members of their families (simplex cases), while there were at least two affected family members in nine cases (multiplex) with likely autosomal recessive and X-linked patterns of inheritance in 8 and 1 families, respectively. Molecular analyses did not identify a pathogenic or polymorphic variant in *GJB2* gene studied in the 44 probands.

4. Discussion

Our report of the genetics of deafness in Nigeria shows that the DFNB1 locus (containing *GJB2* and *GJB6* genes) and the mitochondrial 1555A>G mutation are not major genetic causes of deafness among Nigerians. This is in a sharp contrast to the results of many other populations of the world but, not surprisingly, similar to those studies conducted in other sub-Saharan African populations [29,30].

Previous studies on the genetics of deafness in SSA have been mainly from South Africa and Cameroun, with fewer reports from Sudan, Zaire, and Ghana [13–23]. Table 1 shows the detected *GJB2* variants reported from SSA and Fig. 1 shows the contribution of pathogenic mutations of *GJB2* to non-syndromic deafness among

Table 1
Pathogenic variants of *GJB2* reported among deaf individuals in SSA.

Genotype (cDNA)	Protein change	Number affected	Sample size	Ethnic/country	References
c.427T>C/c.427T>C;	p.R143W/p.R143W	11	11	Adamarobe/Ghana	Brobby et al. [15]*
c.427T>C/c.427T>C	p.R143W/p.R143W	51	365	Ashanti, Central, Eastern, Greater Accra, Upper East, Upper West, Volta,	Hamelmann et al. [18]
c.427T>C/c.35insG	p.R143W/p.V13fs	1			
c.533T>C/c.533T>C	p.V178A/p.V178A	2			
c.236T>C/wt	p.R184Q/wt (Dominant)	1			
c.427T>C/p.641T>C	p.R143W/p.L214P	1			
c.35delG/c.35delG	p.G12fs	5	162	Sudanese/Sudan	Gasmelseed et al. [22]
–	–	0	406	Kenya	
–	–	0	182	Pedi, Venda and Tsonga groups in Limpopo, S/A	Kabahuma et al. [23]

* Ref. [15] total number of families/subjects studied is unknown.