OMICS A Journal of Integrative Biology
Volume 18, Number 7, 2014

DOI: 10.1089/omi.2013.0166

In Search of Genetic Markers for Nonsyndromic Deafness in Africa: A Study in Cameroonians and Black South Africans with the *GJB6* and *GJA1* Candidate Genes

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Abstract

Deafness is the most common sensory disability in the world and has a variety of causes. Globally, mutations in *GJB2* have been shown to play a major role in nonsyndromic deafness, but this has not been seen in Africans. Two other connexin genes, *GJB6* and *GJA1*, have been implicated in hearing loss but have seldom been investigated in African populations. We set out to investigate the role of genetic variation in *GJB6* and *GJA1* in a group of Cameroonian and South African Blacks with nonsyndromic recessive hearing loss. A subset of 100 patients, affected with nonsyndromic hearing loss, from a cohort that was previously shown not to have *GJB2* mutation, was analyzed by Sanger sequencing of the entire coding regions of *GJB6* and *GJA1*. In addition, the large-scale *GJB6*-D3S1830 deletion was also investigated. No pathogenic mutation was detected in either *GJB6* or *GJA1*, nor was the *GJB6*-D3S1830 deletion detected. There were no statistically significant differences in sequence variants between patients and controls. Mutations in *GJB6* and *GJA1* are not a major cause of nonsyndromic deafness in this group of Africans from Cameroon and South Africa. Currently, there is no sufficient evidence to support their testing in a clinical setting for individuals of African ancestry.

Introduction

Deafness is one of the leading causes of disability globally and is most severe in the developing world (Stevens et al., 2013). While it is seen in fewer than 2 per 1000 births in Europe (Parving, 1999), it occurs in approximately 7 per 1000 births in Nigeria (Olusanya and Somefun, 2009) and 5.5 per 1000 births in South Africa (Swanepoel et al., 2009). Deafness is a highly variable and extremely heterogeneous condition that can range from mild to total hearing loss and present either as a single symptom or as one of many clinical features. Deafness can be caused by environmental conditions, genetics, aging, or a combination of these factors.

Currently, 65 different genes have been identified that contribute to nonsyndromic deafness and there are many more causative mutations (Van Camp and Smith, 2012). Genetic deafness can be inherited in a dominant, recessive, or mitochondrial manner, with some genes displaying more than one type of inheritance, depending on the change involved (Van Camp and Smith, 2012). Mutations in *GJB2* (connexin 26) have been shown to be a major contributor to deafness globally, but not in sub-Saharan Africa, with the exception of Ghana (Chan and Chang, 2014). Other potential candidate genes that could lead to nonsyndromic deafness in Africans are *GJB6* (connexin 30) and *GJA1* (connexin 43).

The second biggest genetic cause of nonsyndromic deafness in the European population is the *GJB6*-D13S1830 deletion identified by del Castillo et al. (2002, 2003) and present in up to 9.7% of patients in some European countries. Although originally considered to be a case of digenic inheritance, as connexins 26 and 30 are known to interact, other evidence suggests that the deletion includes an unidentified cis-regulatory region for *GJB2* (Rodriguez-Paris and Schrijver, 2009).

GJA1 emerged as a possible candidate gene for hearing loss in Black Africans when mutations in this gene were associated with nonsyndromic hearing loss in African Americans (Liu et al., 2001). However, subsequent analysis has shown that those results were due to failure to differentiate between *GJA1* and its pseudogene, and that the reported mutations occurred

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only in the pseudogene (Paznekas et al., 2003). Subsequent studies of *GJA1* and hearing loss have failed to provide convincing evidence of an association, either finding no causative variants or variants at very low frequencies (Uyguner et al., 2003; Yang et al., 2007, 2010; Kooshavar et al., 2012).

The mutation del(*GJB6*-D13S1830) in *GJB6* (Kabahuma et al., 2011) and variations *GJA1* are screened in some clinical settings in South Africa, especially in Black South African, but with insufficient evidence of their clinical utility. We aimed to validate the utility of testing for *GJB6* and *GJA1* in two carefully selected groups of Africans from Cameroon and South Africa, affected by nonsyndromic recessive hearing loss.

Methods

Ethical considerations

Recruitment of patients from Cameroon was approved by Cameroon's National Ethics Committee, authorization number N°123/CNE/SE/2010. Ethics approval for the *GJB6* and *GJA1* research was granted by the University of Cape Town's Human Research Ethics Committee, reference numbers 042/2013 and 080/2011, respectively. Written informed consent was obtained from all participants, if they were 18 years or older, or from the parents/guardians with verbal assent from the children.

Patient selection

Cameroonian patients were recruited from seven of the ten regions of Cameroon, mainly from schools for the deaf, and those procedures including participants' medical and family history general systemic and otological examination have been reported previously (Wonkam et al., 2013). South African patients, all from the Xhosa ethnic group, were recruited from Efata School for the Blind and Deaf in the Eastern Cape Province, South Africa.

For the present study, a subset of 100 patients was chosen in order to maximize the probability of finding a genetic cause of nonsyndromic deafness, as revealed by one or more affected family members or consanguinity, or deafness of unknown origin, that were shown not to have mutations in *GJB2* (unpublished data). This group of patients selected for the present study included six Cameroonian patients from consanguineous marriages, 52 familial Cameroonian cases (patients coming from families that had more than one patient affected with nonsyndromic hearing loss), five familial South African cases, two patients with heterozygous *GJB2* mutations, 15 prelingual Cameroonian sporadic cases, and 20 South African sporadic cases. All individuals, with the exception of four Cameroonian patients, had been previously genotyped for *GJB2*. The inclusion of Cameroonian patients was carefully recruited throughout the country, with the intended attempt to serve as a proxy for the multiple ethnolinguistic background found in Africa. Indeed, Cameroon is called ''Africa in miniature'' as the Cameroonian population has been shown, not only at the cultural level but also at the population genetic and linguistic level, to mimic the genetic diversity that is observed in Africa (Tishkoff et al., 2009). Ethnically matched controls from Cameroon and South Africa were recruited from data-based DNA, in the Division of Human Genetics, Faculty of Health Sciences, University of Cape Town, South Africa.

Molecular methods

At the Molecular Diagnosis Laboratory of the Gyneco-Obstetric and Paediatric Hospital of Yaoundé, Cameroon, genomic DNA samples were extracted from peripheral blood of the patients, following instructions on the available commercial kit [Puregene Blood Kit® (Qiagen, USA)]. At the Division of Human Genetics, Faculty of Health Sciences, University of Cape Town, DNA was purified from saliva (Oragene® kit; DNA Genotek®, USA) according to the manufacturer's instructions.

Detection of del(*GJB6*-D13S1830) was performed using the method and primers described by del Castillo et al. (2002, 2003). The entire coding region of *GJB6* was amplified using the method described by Chen et al. (2012). A 1348 bp fragment consisting of the entire *GJA1* coding region was amplified using the F1 (5′ – GAA ATA CGT GAA ACC GTT GG – 3′) and R3 (5′ – CCT GGT GCA CTT TCT ACA GC – 3′) primers described by Huang et al. and which were designed to distinguish between *GJA1* and its pseudogene (Huang et al., 2011). Amplified products were sequenced, using both the forward and reverse primers, on an ABI 3130XL Genetic Analyser (Applied Biosystems, Foster City, CA). The same primers were used for amplification and sequencing.

Bioinformatic and statistical analyses

Chromatogram files were manually checked using FinchTV 1.3.1 (GeoSpiza) and aligned in BioEdit 7.0.5.3 to the *GJB6 and GJA1* reference sequence (Ensembl transcripts, retrieved 31 August 2012). Detected variations were checked against dbSNP (Sherry et al., 2001), and the effects of nonsynonymous mutations were predicted using Polyphen-2 (Adzhubei et al., 2010). Differences in allele, genotype, and haplotype frequencies between cases and controls were assessed using SHEsis (http://analysis2.bio-x.cn/myAnalysis.php) (Shi and He, 2005; Li et al., 2009). The Chi-square test and the Fisher's exact test were used to compared SHEsis results, and a p value of less than 0.05 was considered statistically significant.

Results

Patients

The Cameroonian cohort was evenly distributed in terms of gender and well phenotyped. Full sociodemographic data of the participants is presented in Table 1. Ten patients in the Cameroonian cohort were from consanguineous marriages and all presented with severe to total (≥71 db) bilateral hearing loss (Table 2). The majority of the Cameroonian patients (85%) had sensorineural deafness, one had mixed hearing loss, and the rest were undetermined.

GJB6

GJB6 amplification was unsuccessful in two Cameroonian patients, and none of the remaining 98 patients presented with the *GJB6*-D13S1830 deletion. Only one variant (rs145762940) was detected, in the heterozygous state, in the coding region of *GJB6,* leading to the synonymous c.480G > A change. No variations in *GJB6* were detected in 31 controls (12 South African and 19 Cameroonian).

Table 1. Patient Sociodemographic Information

	South Africa Case (frequency)	*Cameroon Case (frequency)*
Gender		
Male	20 (0.80)	39 (0.52)
Female	4 (0.16)	35 (0.47)
Unknown	1 (0.04)	1 (0.01)
Age		
Average	13.95	12.11
Unknown	4 (0.16)	2 (0.03)
Age of Onset		
Prelingual (<2 Years)	3 (0.12)	70 (0.93)
Perilingual (2–4 Years)	6 (0.24)	0
Postlingual (>4 years)	3 (0.12)	4 (0.05)
Unknown	13 (0.52)	1 (0.01)
Transmission		
Familial	5 (0.20)	52 (0.69)
Unknown/unknown	20 (0.80)	23 (0.31)
	N=25	*N*=75

GJA1

It was not possible to amplify the *GJA1* gene in 10 participants (two South African and eight Cameroonian). Five variants were detected in *GJA1* (Table 3), one of which occurred in the intron, but none of which are known to be pathogenic. Forty-one controls (17 South African, 24 Cameroonian) were also sequenced, but only the synonymous c.717G>A change was detected. In addition, there were no statistically significant differences between cases and controls.

Discussion

Identification of genetic markers of deafness may lead to early detection and advise the choice of intervention. However, it has become apparent that the genetic variants associated with deafness in different populations are not the same. Thus, this study aimed to fully sequence and characterize, for the first time to our knowledge, the role of variation in *GJB6* and *GJA1* in a group of sub-Saharan Africans with nonsyndromic hearing loss.

As in previous studies amongst Chinese (Chen et al., 2012), Indians (Padma et al., 2009), Turkish (Tekin et al., 2003), and both African American and Caribbean Hispanics

Table 2. Audiological Data from the Cameroonian Cohort

Severity of deafness	*Left ear (frequency)*	*Right ear (frequency)*
Severe 1 (71–80)	1 (0.01)	2 (0.03)
Severe 2 (81–90)	3 (0.04)	4 (0.05)
Profound 1 (91–100)	24 (0.32)	22 (0.29)
Profound 2 (101–110)	22 (0.29)	23 (0.31)
Profound 3 (111–119)	10 (0.13)	11 (0.15)
Total (120)	3 (0.04)	1 (0.01)
Unknown	12 (0.16)	12 (0.16)
	N=75	*N*=75

Table 3. Genotypes of Patients and Controls with Respect to Variations in *GJA1*

				South Africa					*Cameroon*					
				Case		*Control*			*Case*			*Control*		
Transcript	*Protein*	*Domain*	*RS number*	*Het. (freq.)*	*WT (freq.)*	*Het. (freq.)*	*WT (freq.)*	P *Value*	*Hom. (freq.)*	*Het. (freq.)*	*WT (freq.)*	*Het. (freq.)*	*WT (freq.)*	P *Value*
c.-67A>G	NA	Intron	rs189167598	0	23 (1.000)	0	17 (1.000)	NA	1 (0.015)	0	66 (0.985)	0	24 (1.000)	0.547
c.189T>C	p.=	EC1	rs139688042	1 (0.043)	22 (0.957)	0	17 (1.000)	0.384	0	0	67 (1.000)	0	24 (1.000)	NA
c.366T>C	p.=	IC2	Novel	1 (0.043)	22 (0.957)	0	17 (1.000)	0.384	0	0	67 (1.000)	0	24 (1.000)	NA
c.717G>A	p.=	IC3	rs57946868	2 (0.087)	21 (0.913)	3 (0.176)	14 (0.824)	0.397	0	11 (0.164)	56 (0.836)	2 (0.083)	22 (0.917)	0.332
c.758C>T	p.(A253V)	IC3	rs17653265	1 (0.043)	22 (0.957)	0	17 (1.000)	0.384	0	0	67 (1.000)	0	24 (1.000)	NA

EC, extracellular domain; freq, frequency; Het., heterozygous; Hom., homozygous; IC, intracellular domain; WT, Wildtype.

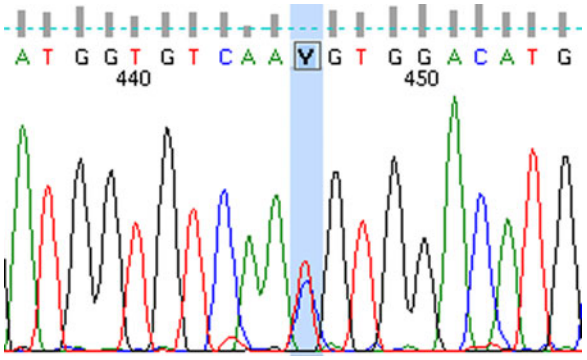

FIG. 1. Novel *GJA1* variant, c.366T>C (p.=) detected in one South African patient.

without *GJB2* mutations (Samanich et al., 2007), this study did not find either the *GJB6*-D13S1830 deletion or coding region variations. This supports the hypothesis that the *GJB6*-D13S1830 deletion is the result of a founder effect (del Castillo et al., 2003).

Although variants were detected in *GJA1*, there were no significant differences between patients and controls. We report a novel c.366T>C (p.=) *GJA1* variant (Fig. 1) which has not, to our knowledge, been described before. Only one variant, the c.758C>T (p.(A253V)) change, was nonsynonymous. However, c.758C>T is a known change that is not considered to be pathogenic. It has been reported before in both cases and controls in various studies on *GJA1* (Alexandrino et al., 2009; Paznekas et al., 2009; Kooshavar et al., 2012; Van Norstrand et al., 2012), but has been suggested to modify disease severity in certain cases (Cella et al., 2006).

We are aware of several limitations in this study, including that our cohort is too small to provide reliable information on the contribution of rare variants. In addition, although the diversity of Cameroonian patients provides strength to the study, by allowing us to examine variations that may occur throughout Africa, it also introduces a weakness by possibly introducing too much variation. This is not the case with the Xhosa population from South Africa, and results from other African studies should always be kept in mind. Despite these limitations, the data presented here support the conclusion that neither *GJA1* nor *GJB6* is a major cause of deafness in Africans.

As the two major genetic causes of global nonsyndromic deafness, *GJB2* and *GJB6,* as well as *GJA1,* have not been shown to be associated with nonsyndromic deafness in Africans studies here, the focus should turn to the other 65 candidate genes (Van Camp and Smith, 2012). The most effective approach would be to use methods such as massively parallel sequencing that can screen multiple genes at once (Shearer et al., 2010) or Whole Exome Sequencing (WES), which has proven successful at elucidating the causes of deafness in a variety of genes and populations, even in small families (Diaz-Horta et al., 2012). The use of targeted massively parallel sequencing approach or the WES approach offers the best chance of uncovering the genetic causes of deafness in a setting with the genetically diverse populations found in Africa.

Conclusion

Our results do not support a link between mutations in either *GJB6* or *GJA1* and nonsyndromic deafness in sub-Saharan Africans from Cameroon and South Africa. At present, there is no sufficient evidence to support their testing in a clinical setting for individuals of African ancestry.

Acknowledgments

We would like to thank Efata School for the Deaf and Blind for their assistance and the patients and their families for their participation.

Funding; The research was funded by the University of Yaoundé for clinical phenotyping and DNA extraction and the National Health Laboratory Services (NHLS), South Africa, for the recruitment of the South African samples, DNA extraction, and molecular analysis. Jason Bosch was supported by funding provided by the University of Cape Town through the Ada and Bertie Levenstein Scholarship.

Author Disclosure Statement

The authors declare that no competing financial interests exist.

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