

FORUM

GENETICS IN MEDICINE

No evidence for clinical utility in investigating the connexin genes *GJB2*, *GJB6* and *GJA1* in non-syndromic hearing loss in black Africans

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Background. Deafness is the most common sensory disability in the world. Globally, mutations in *GJB2* (connexin 26) have been shown to play a major role in non-syndromic deafness. Two other connexin genes, *GJB6* (connexin 30) and *GJA1* (connexin 43), have been implicated in hearing loss, but these genes have seldom been investigated in black Africans. We aimed to validate the utility of testing for *GJB2*, *GJB6* and *GJA1* in an African context.

Methods. Two hundred and five patients with non-syndromic deafness from Cameroon and South Africa had the full coding regions of *GJB2* sequenced. Subsequently, a carefully selected subset of 100 patients was further sequenced for *GJB6* and *GJA1* using Sanger cycle sequencing. In addition, the large-scale *GJB6*-D3S1830 deletion was investigated.

Results. No pathogenic mutations that could explain the hearing loss were detected in *GJB2*, *GJB6* or *GJA1*, and the *GJB6*-D3S1830 deletion was not detected. There were no statistically significant differences in genomic variations in these genes between patients and controls. A comprehensive literature review supported these findings.

Conclusion. Mutations in *GJB2*, *GJB6* and *GJA1* are not a major cause of non-syndromic deafness in black Africans and should not be investigated routinely in clinical practice.

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Deafness is one of the leading causes of disability in the world. The prevalence of hearing loss is highest in South Asia and sub-Saharan Africa, attributable to poor healthcare systems where complications at birth as well as infections result in loss of hearing in the newborn.^[1]

Screening for hearing loss in newborns is standard practice in many countries and is the most effective way of detecting hearing problems and reducing the negative effects of hearing loss. South Africa (SA) does not have a national screening programme. At best, only 7.5% of public hospitals provide screening for hearing loss, with only 1% providing universal screening.^[2] The situation is even worse in the rest of sub-Saharan Africa.^[3] Clinical presentation of hearing loss is extremely heterogeneous, ranging from mild to total hearing loss and presenting either as a single symptom or as one of many clinical features. The causes of hearing loss can be genetic or environmental. A recent review of the aetiology of childhood hearing loss showed that 48.3% of cases of hearing loss were of unknown cause, 30.4% were genetic and 19.2% were acquired.^[4] In developing communities, the environment contributes significantly more to congenital hearing loss than in the developed world. In Africa, bacterial meningitis contributes to cases of hearing loss in infants and young children.^[5] With improved healthcare there will be a

reduction in cases of hearing loss caused by disease and an increase in the proportion attributable to genetics, the majority of which are non-syndromic.^[1]

To date, 65 different genes, with many different causative mutations, have been identified that contribute to non-syndromic deafness.^[5] Mutations in gap junction (GJ) genes, specifically *GJB2* (connexin 26), have been shown to be the major contributors to deafness globally.^[6]

GJ proteins (connexins) regulate functions of the cochlea

GJs are intercellular channels that allow ions, second messengers and small metabolites to be exchanged by adjacent cells. Connexins form intercellular channels by combining in groups of six to form a structure called a connexon.^[7] Connexons from adjacent cells join together to form GJs (Fig. 1, A). The connexin proteins are named according to their weight; connexin 26 is a protein with a molecular weight of 26 kDa. The two major groups of connexins are the alpha and beta connexins, based on sequence similarity of the cytoplasmic loop. *GJB2* was the second beta connexin gene to be identified. *GJB2* is located on chromosome 13 and codes for the GJ protein connexin 26. Connexin 26 is involved in the transport of potassium ions and other small molecules and is expressed in the cochlea (Fig. 1, B).^[8] It

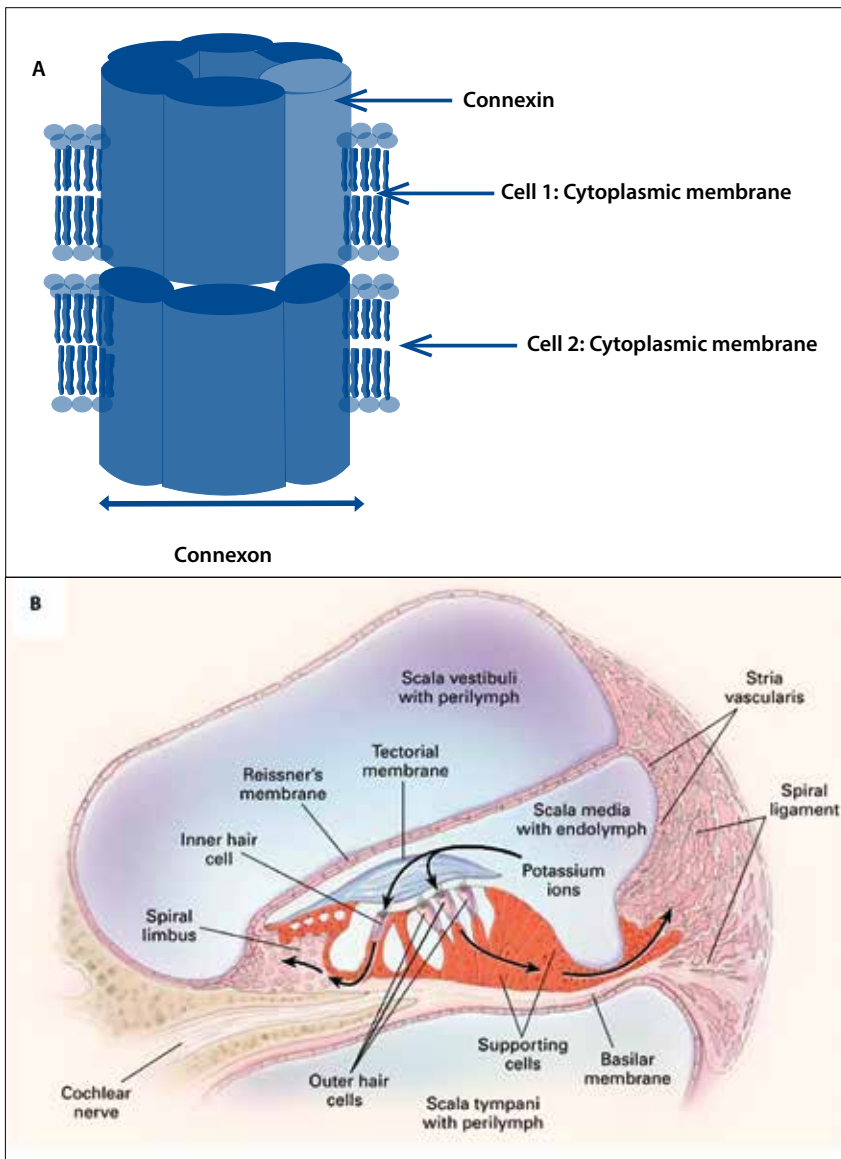


Fig. 1. GJ proteins (connexins) and the inner ear. A: Connexins form intercellular channels by combining in groups of six to form a structure called a connexon; two connexons from adjacent cells join together to form GJs. B: Cross-section through the cochlea. Red cells express connexin 26 (adapted from Willems et al.^[8]). (GJ = gap junction.)

is believed that mutations in *GJB2* (connexin 26 gene) affect its ability to transport potassium ions and therefore regulate the endocochlear potential required for nerve impulses involved in hearing, but the mode of action is not definitively elucidated.^[9]

No mutations in connexin genes *GJB2*, *GJB6* or *GJA1* among Cameroonians and Xhosa South Africans

With the exception of Ghana,^[10] mutations in *GJB2* (connexin 26) have not been shown to be a major contributor to deafness in sub-Saharan Africa.^[6] Could other potential candidate genes, *GJB6* (connexin 30) and

GJA1 (connexin 43), lead to non-syndromic deafness in Africans? The *GJB6*-D13S1830 deletion is present in up to 9.7% of people of European descent,^[11] and represents the second leading genetic cause of non-syndromic deafness. When *GJA1* mutations were detected in African Americans,^[12] *GJA1* emerged as a possible candidate for hearing loss in indigenous Africans. However, failure by investigators to differentiate between *GJA1* and its pseudogene led to this hypothesis being discarded.^[13]

We performed a series of molecular investigations and reviewed the literature with the aim of validating the clinical utility of testing for *GJB2*, *GJB6* and *GJA1* in the African context. As part of this validation,

we recruited a total of 205 patients affected with non-syndromic hearing loss from a well-described Cameroonian cohort^[3] and newly recruited black South Africans of Xhosa ancestry, the majority (85%) of whom had sensorineural deafness. All 205 patients were investigated for *GJB2* gene, as previously reported.^[14] A subset of 100 selected patients, with deafness likely to be of genetic cause (mostly familial cases) and who did not have any mutation in *GJB2* gene, were investigated for mutations in the *GJB6* and *GJA1* genes.^[15]

All the coding regions of *GJB2*, *GJB6* and *GJA1* were amplified and detection of *del(GJB6-D13S1830)* was also investigated.^[14,15] In the *GJB2* gene, two likely pathogenic mutations were detected in two unrelated Cameroonian participants, g.3741_3743delTTC (p.F142del) and g.3816G>A (p.V167M) in a single individual each and in the heterozygous state (Table 1). No pathogenic mutation was detected among the SA patients.^[14] Phylogeny analysis of the sequence data from the Cameroonian and SA controls, together with that of various populations extracted from the 1000 Genomes Project, shows as expected that the SA patients and Cameroonian controls grouped with the other African populations. There was a low variance when comparing sequences in *GJB2* in Africans with that of other population groups: the principal component analysis explains only 40% of the variations.^[14] Specific sequence variants in the *GJB2* gene in Africans could therefore not explain the low occurrence of mutations associated with non-syndromic deafness in this population.

In the *GJB6* gene, none of the patients had the *GJB6*-D13S1830 deletion. Only one variant (rs145762940) was detected, in the heterozygous state, in the coding region of *GJB6*, leading to the c.480G>A (p.G160=) change.^[15] Equally, in the *GJA1*, five variants were detected; one of these occurred in the intron, but none were known to be pathogenic.^[15]

Few mutations in *GJB2*, *GJB6* or *GJA1* genes among other populations of African ancestry

Our report and review of the literature confirmed that *GJB2* gene is of little significance in non-syndromic hearing loss in populations of African descent (Table 1).^[10,14,16-20] In addition, by combining data from previously unstudied deaf Xhosa patients in SA, Cameroonian patients, previous studies in Africans and the 1000 Genomes Project,

Table 1. Comparison of pathogenic mutations found in *GJB2* in a few populations of African ancestry

Variations		Country (observed/total alleles)				
Genomic	Coding	Cameroon	Ghana [†]	Kenya/Sudan [§]	South Africa [‡]	USA (African Americans)
g.3352_3353insG	c.35dupG	-	1/730	-	-	-
g.3352delG	c.35delG	-	-	10/1 178	-	7/100**
g.3396C>T	c.79G>A	-	-	-	-	2/46 [†] , NA
g.3419T>C	c.101T>C	-	-	-	-	NA
g.3426G>A	c.109G>A	-	-	1/1 178	-	-
g.3455_3460del	c.138_143del	-	-	1/1 178	-	-
g.3512C>A	c.195C>A	-	-	1/1 178	-	-
g.3553T>C	c.236T>C	-	1/730	-	-	-
g.3566C>G	c.249C>G	-	-	-	-	1/100**
g.3586_3587insT	c.269_270insT	NA [*]	-	-	-	-
g.3658A>G	c.341A>G	-	-	-	-	NA
g.3697G>A	c.380G>A	-	-	1/1 178	-	-
g.3741_3743delTTC	c.424_426delTTC	1/360 [†]	-	-	-	-
g.3744C>T	c.427C>T	-	110/730	-	-	1/100**
g.3795G>A	c.478G>A	-	-	1/1 178	-	NA
g.3816G>A	c.499G>A	1/360 [†]	-	4/1 178	-	NA
g.3850T>C	c.533T>C	-	4/730	-	-	-
g.3868G>A	c.551G>A	-	1/730	-	-	-
g.3906G>T	c.589G>T	-	1/730	-	-	-
g.3925-3926delinsAA	c.608_610delinsAA	-	2/730	-	-	-
g.3958C>T	c.641T>C	-	1/730	-	-	-

NA = variations found during the study, but only in the control group. Variant information was obtained through the relevant paper's own results and a combination of the Deafness Variation Database (<http://deafnessvariationdatabase.org/>) and the Connexin-Deafness Homepage (<http://davinci.crg.es/deafness/index.php>).

Study references ^{*}Trotta *et al.*,¹⁶ [†]Bosch *et al.*,¹⁴ [‡]Kabahuma *et al.*,¹⁷ [§]Gasmelseed *et al.*,¹⁸ ^{||}Hamelmann *et al.*,¹⁰ ^{||}Samanich *et al.*,¹⁹ and ^{**}Pandya *et al.*²⁰ indicate that the mutation was found neither in patients nor in controls.

the analysis further supported the limited contribution of *GJB2* genes in non-syndromic hearing loss in Africans. Interestingly, we reported two cases of keratitis-ichthyosis-deafness (KID) syndrome in two Cameroonian patients,^[21] caused by mutations in *GJB2*. In both cases the mutation found (p.Asp50Asn) was the most common in many populations globally.^[5,22] Adding to the established founder effect of the *GJB2* mutations reported in European and Asian populations,^[5] the data indicate that the high frequency of *GJB2* mutations in non-syndromic hearing loss have evolved in Eurasian populations after their migration out of Africa, and spread with population migrations. Finally, at the genetic level, the Cameroonian population diversity mimics that of various ethnolinguistic groups in African populations;^[23] it is anticipated that results from a carefully selected sample in this population could capture those of many other populations on the African continent.

As in previous studies in Africans,^[17] African Americans and Caribbean Hispanics with *GJB6* mutations,^[19] we did not find either the *GJB6*-D13S1830 deletion or coding region changes. Similarly, no pathogenic variants were detected in *GJA1*, suggesting their non-implication in hearing loss among the Cameroonians and black South Africans studied,^[15] as has been reported in African Americans.^[13]

Clinical implications and research perspectives

From our analysis, there is no evidence that mutations in *GJB2*, *GJB6* or *GJA1* are associated with non-syndromic deafness in sub-

Saharan African patients. We therefore recommend against routine use of either gene for clinical testing in patients of African ancestry. We suggest that future research should take advantage of the power of massively parallel sequencing to screen multiple genes at once. This approach has previously been shown to offer the best chance of uncovering the genetic causes of deafness in settings with a genetically diverse populations.^[24]

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1. Stevens G, Flaxman S, Brunskill E, Mascarenhas M, Mathers CD, Finucane M; Global Burden of Disease Hearing Loss Expert Group. Global and regional hearing impairment prevalence: An analysis of 42 studies in 29 countries. *Eur J Public Health* 2013;23(1):146-152. [<http://dx.doi.org/10.1093/eurpub/ckr176>]
2. Swanepoel D1, Störbeck C, Friedland P. Early hearing detection and intervention in South Africa. *Int J Pediatr Otorhinolaryngol* 2009;73(6):783-786. [<http://dx.doi.org/10.1016/j.ijporl.2009.01.007>]
3. Wonkam A, Noubiap JN, Djomou F, Fieggen K, Njock R, Toure GB. Aetiology of childhood hearing loss in Cameroon (sub-Saharan Africa). *Eur J Med Genet* 2013;56(1):1-6. [<http://dx.doi.org/10.1016/j.ejmg.2012.09.010>]

4. Korver AM, Admiraal RJ, Kant SG, et al. Causes of permanent childhood hearing impairment. *Laryngoscope* 2011;121(2):409-416. [http://dx.doi.org/10.1002/lary.21377]
5. Van Camp G, Smith RJH. Hereditary Hearing Loss Homepage. <http://hereditaryhearingloss.org/> (accessed 3 May 2012).
6. Chan DK, Chang KW. GJB2-associated hearing loss: Systematic review of worldwide prevalence, genotype, and auditory phenotype. *Laryngoscope* 2014;124(2):E34-E53. [http://dx.doi.org/10.1002/lary.24332]
7. Meşe G, Richard G, White TW. Gap junctions: Basic structure and function. *J Invest Dermatol* 2007;127(11):2516-2524. [http://dx.doi.org/doi:10.1038/sj.jid.5700770]
8. Willems PJ. Genetic causes of hearing loss. *N Engl J Med* 2000;342(15):1101-1109. [http://dx.doi.org/10.1056/NEJM200004133421506]
9. Iossa S, Marciano E, Franzé A. GJB2 gene mutations in syndromic skin diseases with sensorineural hearing loss. *Curr Genomics* 2011;12(7):475-785. [http://dx.doi.org/10.2174/138920211797904098]
10. Hamelmann C, Amedofu GK, Albrecht K, et al. Pattern of connexin 26 (GJB2) mutations causing sensorineural hearing impairment in Ghana. *Hum Mutat* 2001;18(1):84-85. [http://dx.doi.org/10.1002/humu.1155]
11. Del Castillo I, Villamar M, Moreno-Pelayo MA, et al. A deletion involving the connexin 30 gene in nonsyndromic hearing impairment. *N Engl J Med* 2002;346(4):243-249. [http://dx.doi.org/10.1056/NEJMoa012052]
12. Liu XZ1, Xia XJ, Adams J, et al. Mutations in GJA1 (connexin 43) are associated with non-syndromic autosomal recessive deafness. *Hum Mol Genet* 2001;10(25):2945-2951. [http://dx.doi.org/10.1093/hmg/10.25.2945]
13. Paznekas WA, Boyadjev SA, Shapiro RE, et al. Connexin 43 (GJA1) mutations cause the pleiotropic phenotype of oculodentodigital dysplasia. *Am J Hum Genet* 2003;72(2):408-418. [http://dx.doi.org/10.1086/346090]
14. Bosch J, Noubiap JN, Dandara C, et al. Sequencing of GJB2 in Cameroonians and black South Africans and comparison to 100 Genomes Project data support the need to revise strategy for discovery of nonsyndromic deafness genes in Africans. *OMICS* 2014;18(11):705-710. [http://dx.doi.org/10.1089/omi.2014.0063]
15. Bosch J, Lebeko K, Nziale JJ, Dandara C, Makubalo N, Wonkam A. 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. *OMICS* 2014;18(7):481-485. [http://dx.doi.org/10.1089/omi.2013.0166]
16. Trotta L, Iacona E, Primignani P, et al. GJB2 and MTRNR1 contributions in children with hearing impairment from Northern Cameroon. *Int J Audiol* 2011;50(2):133-138. [http://dx.doi.org/10.3109/14992027.2010.537377]
17. Kabahuma RI, Ouyang X, Du LL, et al. Absence of GJB2 gene mutations, the GJB6 deletion (GJB6-D13S1830) and four common mitochondrial mutations in nonsyndromic genetic hearing loss in a South African population. *Int J Pediatr Otorhinolaryngol* 2011;75(5):611-617. [http://dx.doi.org/10.1016/j.ijporl.2011.01.029]
18. Gasmelseed NMA, Schmidt M, Magzoub MMA, et al. Low frequency of deafness-associated GJB2 variants in Kenya and Sudan and novel GJB2 variants. *Hum Mutat* 2004;23(2):206-207. [http://dx.doi.org/10.1002/humu.9216]
19. Samanich J, Lowes C, Burk R, et al. Mutations in GJB2, GJB6, and mitochondrial DNA are rare in African American and Caribbean Hispanic individuals with hearing impairment. *Am J Med Genet A* 2007;143A(8):830-838. [http://dx.doi.org/10.1002/ajmg.a.31668]
20. Pandya A, Arnos KS, Xia XJ, et al. Frequency and distribution of GJB2 (connexin 26) and GJB6 (connexin 30) mutations in a large North American repository of deaf probands. *Genet Med* 2003;5(4):295-303. [http://dx.doi.org/10.1097/01.GIM.0000078026.01140.68]
21. Wonkam A, Noubiap JN, Bosch J, Dandara C, Toure GB. Heterozygous p.Asp50Asn mutation in the GJB2 gene in two Cameroonian patients with keratitis-ichthyosis-deafness (KID) syndrome. *BMC Med Genet* 2013;14(1):81-87. [http://dx.doi.org/10.1186/1471-2350-14-81]
22. Gasparini P, Rabionet R, Barbujani G, et al. High carrier frequency of the 35delG deafness mutation in European populations. *Eur J Hum Genet* 2000;8(1):19-23. [http://dx.doi.org/10.1038/sj.ejhg.5200406]
23. Tishkoff SA, Reed FA, Friedlaender FR, et al. The genetic structure and history of Africans and African Americans. *Science* 2009;324(5930):1035-1044. [http://dx.doi.org/10.1126/science.1172257]
24. Shearer AE, Black-Ziegelbein EA, Hildebrand MS, et al. Advancing genetic testing for deafness with genomic technology. *J Med Genet* 2013;50(9):627-634. [http://dx.doi.org/10.1136/jmedgenet-2013-101749]

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