The genetic basis of deafness in populations of African descent

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Abstract

Hearing loss is the most common sensorineural disorder worldwide and is associated with more than 1000 mutations in more than 90 genes. While mutations in genes such as *GJB2* (gap-junction protein β 2) and *GJB6* (gap-junction protein β 6) are highly prevalent in Caucasian, Asian, and Middle Eastern populations, they are rare in both native African populations and those of African descent. The objective of this paper is to review the current knowledge regarding the epidemiology and genetics of hearing loss in African populations with a focus on native sub-Saharan African populations. Environmental etiologies related to poor access to healthcare and perinatal care account for the majority of cases. Syndromic etiologies including Waardenburg, Pendred, and Usher syndromes are uncommon causes of hearing loss in these populations. Of the non-syndromic causes, common mutations in *GJB2* and *GJB6* are rarely implicated in populations of African descent. Recent use of next-generation sequencing (NGS) has identified several candidate deafness genes in African populations from Nigeria and South Africa that are unique when compared to common causative mutations worldwide. Researchers also recently described a dominant mutation in *MYO3a* in an African American family with nonsyndromic hearing loss. The use of NGS and specialized panels will aid in identifying rare and novel mutations in a more cost and time effective manner. The identification of common hearing loss mutations in indigenous African populations will pave the way for translation into genetic deafness research in populations of African descent worldwide.

Keywords: Genetic; Hearing loss; Deafness; African

1. **Introduction**

Hearing loss is the most common congenital sensorineural disorder worldwide, affecting about 1 in 1,000 children. In the US, genetic factors are responsible for approximately half of the cases of hearing loss (Mustapha et al., 2001), of which 30% are associated with syndromic causes and 70% are considered non-syndromic hearing loss (NSHL) – i.e., deafness alone with no associated features suggesting a clinical syndrome (Van Camp et al., 1997).

NSHL has been studied worldwide and more than 1000 mutations on over 90 genes have been identified, demonstrating that NSHL is highly heterogeneous (Van Camp et al., 1997; Van Camp and Smith, 2016). Mutations in the *GJB2* (gap-junction protein β 2) gene (13q11-q12) account for a high proportion of these cases in many populations and explain up to 50% of cases in the Mediterranean regions (Gasparini et al., 1997; Kelsell et al., 1997; Estivill et al., 1998). Specific *GJB2* mutations occur preferentially, but not exclusively, in distinct ethnic groups. The deletion variant g.3352GdelG (commonly designated c.30delG or c.35delG), for example, is by far the most frequent cause of autosomal recessive non-syndromic deafness among Caucasians (Zelante et al., 1997; Brobby et al., 1998; Kelley et al., 1998). Other frequent population-specific *GJB2* mutations include c.167delT in Ashkenazi Jews (Morell et al., 1998; Idan et al., 2013) and c.235delC in East Asians (Japanese, Koreans, and Chinese) (Abe et al., 2000; Park et al., 2000; Yan et al., 2003; Dai et al., 2009). On the other hand, the *GJB6* (gap-junction protein β 6) deletion mutation del(GJB6-D13S1830) is common in France, Spain, the United Kingdom and Israel, accounting for 6%–10% of all DFNB1 alleles (Del Castillo et al., 2003). Their identification has dramatically improved the clinical diagnosis and management of deaf and hard-of-hearing families. However, causative variants are found in less than 40% of cases, leaving a significant portion of deaf individuals (>60%) without a molecular diagnosis. This is significantly higher in US minorities that include African Americans (Shearer et al., 2013; Bademci et al., 2016; Yan et al., 2016). There is a very low frequency of deafness-causing variants in *GJB2/GJB6* in African American populations (Samanich et al., 2007). Likewise, Afro-Brazilians have a low incidence of specific *GJB2* mutations (c.35DelG) (Oliveira et al., 2004). In general, specific genetic causes of NSHL in populations of African descent have been more elusive.

There is therefore a need to identify genetic causes of hearing loss in populations of African descent. With recent developments in high-throughput sequence capture methods and next-generation sequencing (NGS), technologies now allow for the complete analysis of all known deafness-causing genes. Given the relative rarity of known deafness-causing genes in populations of African descent and the recent acceleration in identifying genetic causes of NSHL, the goal of the current paper is to review epidemiology, etiology, and genetic causes of deafness in African populations worldwide with a focus on indigenous African populations. We will then discuss current and future strategies to discover the genes responsible for deafness in African populations worldwide. A productive initial approach may be to describe the specific genetic causes of hearing loss in indigenous African populations with low foreign genetic admixture. Populations in Northern Africa have significant genetic admixture with European and Middle Eastern populations (Henn et al., 2012); we, therefore, will focus our attention on findings from populations in Sub-Saharan African, an area, which, despite European colonization, has remained relatively genetically isolated.

To identify the most relevant publications regarding the etiology of hearing loss in sub-Saharan Africa, we used the keywords 'hearing loss, deafness, genetics, sub-Saharan, and Africa,' in both PubMed and Google Scholar. We later expanded the search terms to include keywords for specific syndromes and genetic mutations. These search terms yielded numerous publications from 1966 to the present, and all applicable case series, reviews, and genetic studies were included. We included only publications with specific mention of indigenous African populations or populations of indigenous African descent in this manuscript as other populations have distinctly different genetic backgrounds from our population of interest. A summary of these papers is included in Table 1. We excluded those studies that are not relevant to our topic such as those dealing with specific environmental etiologies, newborn hearing screening studies, and audiometry studies.

Our review includes 26 case reports, case series, reviews, and cross-sectional studies detailing the etiology of hearing loss for a total of roughly 10,500 patients with hearing loss of any cause, from Sub-Saharan Africa or of African descent. Collectively, 1.5% of these subjects had syndromic deafness, while 29.8% had suspected non-syndromic genetic deafness, and 68.7%

Table 1. Studies discussing hearing loss in populations of African descent, 1966 - Present

Ethnic group	Sample description	Gene or mutation tested	Result	Reference
African American	Two parents and three children (aged 5–9) with post-lingual progressive autosomal dominant NSHL	*MYO3A*	p.Gly488Glu in *MYO3A*; novel interaction between *MYO3A* and *PCDH15*.	Grati et al., 2016
African American	23 African American simplex cases from New York	*GJB2*, *GJB6*, *mtDNA*	*GJB2* benign polymorphisms in two African Americans; no *GJB6* or *mtDNA* mutations.	Samanich et al., 2007
Angolan	Two dizygotic twins with KID syndrome	*GJB2*, *GJB6*	Two patients with a rare lethal form of KID are found to be heterozygous for c.134G>A (p.Gly45Glu) mutation in *GJB2*	Jonard et al., 2008
Bantu	Two brothers	N/A	Case report of two brothers with Pendred syndrome	Levin, 1966
Brazilian	100 African Brazilians	*GJB2*, g.3352delG	1/100 found to be heterozygous for g.3352delG	Oliveira et al., 2004
Cameroonian	70 children with severe to profound pre-lingual deafness	*GJB2*, *MT-RNR1*	*GJB2* mutation p.Asn62Asn in two patients (3.28%); *MT-RNR1* variants detected (m.1503G>A, m.1018G>T, m.959C>T, and m.1048C>T) and one novel variant (m.1462G>T)	Trotta et al., 2010
Cameroonian	582 subjects aged 1–32 with onset at <15 years old.	N/A	Hearing loss is due to genetic (14.8%), environmental (52.6%), unknown (32.6%) causes. Genetic causes include non-syndromic (86.1%) and syndromic hearing loss (13.9%) with Waardenburg syndrome representing 7% of all genetic cases.	Wonkam, 2013; Noubiap et al., 2014
Cameroonian and South African	205 black patients with NSHL	*GJB2*, *GJB6*, *GJA1*	No pathogenic mutations were detected in *GJB2*, *GJB6*, *GJA1*, or *GJB6*-D3S1830.	Wonkam et al., 2015
Cameroonian and South African	205 black patients with NSHL	*GJB2*	No pathogenic mutations were found.	Bosch et al., 2014
Djiboutian	One six-year-old boy	N/A	Case report of Leopard syndrome	Massoure et al., 2012
Gambian	259 severe to profoundly deaf children aged 2–10 years.	N/A	Postnatally acquired hearing loss (i.e. infection, *n* = 167), congenital (*n* = 5), unknown (*n* = 81), other (*n* = 6)	McPherson, 1985
Ghanaian	21 deaf subjects from 11 families	*GJB2*	All affected subjects homozygous for *GJB2* mutation p.R143W.	Brobby et al., 1998
Ghanaian	365 unrelated individuals aged 6–10 years with profound NSHL.	*GJB2*, p.R143W	51 individuals homozygous, 4 individuals heterozygous, 4 compound heterozygous.	Hamelmann et al., 2001
Kenyan and Sudanese	589 Sudanese (*n* = 162) and Kenyan (*n* = 406) children with pre-lingual NSHL.	*GJB2*	10 novel variants identified: g.3318–6T>A, g.3318–15C>T, g.3318–34C>T, g.3318–35T>G, g.3455_3460del, g.3512C>A (p.Tyr65X), g.3395C>T (p.Thr26Thr), g.3503C>T (p.Asn62Asn), g.3627A>C (p.Arg104Arg) and g.3816C>A (p.Val167Met).	Gamelseed et al., 2004
Multiple	Normal hearing individuals from multiple regions including North Africa/African Americans	*GJB2*, g.3352delG	Identified in Jews of North African origin, but not in African Americans.	Gasparini et al., 2000

Nigerian, Yoruba tribe	44 individuals aged 8 months to 45 years with NSHL	*GJB2*, *GJB6*, *MT-RNR1*	No pathogenic mutations were found.	Lasisi et al., 2014
Nigerian	One three-day-old male child	N/A	Case report of Goldenhar syndrome	Adeoye, 2002
North African	N/A	730,000 DNA sites	History of migrations, involving at least five ancestral populations, into North Africa.	Henn et al., 2012
North American, Mixed	Survey including 50 African Americans from hearing-impaired families	*GJB2*, *GJB6*	*GJB2* mutations accounting for 22.2% of deafness across all ethnicities.	Pandya et al., 2003
South African and Nigerian	342 *GJB2*-negative deaf probands from multiple countries	180 genes	Etiologic diagnostic rate for families from Nigeria and South Africa is 4%. No *GJB2*, *GJB6*, or *mtDNA* mutations were found.	Yan et al., 2016
South African (Limpopo)	182 children aged 5–21.	*GJB2*, *GJB6*, *MT-RNR1*	No mutations in *GJB2, GJB6*-D13S1380, or *mtDNA* were found.	Kabahuma et al., 2011
South African	1 family with 18 members, mixed ancestry	*MT-RNR1,* m.1555A>G	The *MT-RNR1* m.1555A>G was homoplasmic in nine affected family members following streptomycin exposure and wild-type in nine normal hearing with no streptomycin exposure.	Gardner et al., 1997
South African	52 black MDR-TB patients on aminoglycosides with 112 black controls	*MT-RNR1*	One MDR-TB patient with m.827A>G mutation. The m.1555A>G was found in 0.9% of black controls.	Human et al., 2010
South African	One fifteen-month-old female	N/A	Case report of Goldenhar syndrome	Naidoo and Stephen, 1998
South African – Sotho, Zulu, Xhosa tribes	12 indigenous and 3 mixed ancestry with Usher; 6 with retinal degenerative disease/hearing loss but no Usher diagnosis.	*MYO7A*, c.6377delC	Homozygous *MYO7A* mutation c.6377delC underlies 43% of USH cases; 60% of these are Usher Type II.	Roberts et al., 2015
Southern African	2410 profoundly deaf black children	N/A	Genetic syndromes (8%), undifferentiated familial deafness (12%), acquired (25%) and unknown (55%).	Beighton et al., 1991
Southern African	264 pediatric cochlear implant recipients with hearing loss before 5 years old.	N/A	10% of children (*n* = 23) had a genetic syndrome (Waardenburg, Ushers, Pierre Robin, Leopard). Of 90 children with Waardenburg syndrome, 33 (2.6%) were black.	Le Roux et al., 2015
Southern African	1226 deaf black children	N/A	33 (2.6%) black children with Waardenburg syndrome.	Sellers and Beighton, 1983
Sub-Saharan African	One child	N/A	Case report of KID syndrome	Barruet et al., 2011
Sub-Saharan African	N/A	N/A	Review of causes of hearing loss including environmental, syndromic and non-syndromic causes.	Lebeko et al., 2015
Togolese (West Africa)	1 child of consanguineous marriage	N/A	Case report of KID syndrome	Kombate et al., 2015
Ugandan	126 simplex cases aged 2–25 years with NSHL	*GJB2*, *GJB6*	One pathogenic variant in *GJB2* (c.208C>G, p.P70A); three variants (c.–22–12C>T, c.–15C>T, c.–6T>A). two novel variants (c.–23+12G>A, c.–23+37C>T).	Javidnia et al., 2014

either had acquired hearing loss or were unable to be determined based on thorough review of the article. In this review, we included 159 patients of African descent for whom an etiologic NSHL diagnosis is known. The geographic distribution included is primarily sub-Saharan Africa, defined as all African countries located south of the Sahara Desert, with most patients from Cameroon, Ghana, Kenya, Sudan, and South Africa. Several of the papers included in this review describe populations of African descent in North and South America, including cohorts from North America and Brazil, as well as North African subjects with Arab influences.

2. Environmental causes of congenital deafness

Congenital hearing loss may be environmental or genetic. In developed countries with modern prenatal care and vaccination, deafness occurs in 1 in 1000 births with genetic forms of hearing loss being the leading cause of congenital deafness. In developing countries, however, the rate of deafness is 3 in 1000 births, with environmental causes continuing to be the main contributor to congenital hearing loss. In developing countries of Sub-Saharan Africa with predominantly indigenous African populations, this rate of congenital deafness is higher, 6 per 1000 births in South Africa and 7 per 1000 births in Nigeria for example, suggesting a higher burden of environmental deafness in these populations (Lebeko et al., 2015). This can be attributed to multiple factors in the perinatal and early childhood periods including limited healthcare access, lack of vaccination, maternal infection and malnutrition during pregnancy, birth trauma, and perinatal infections such as bacterial meningitis. With the current low rate of neonatal hearing screening in Africa, the resultant hearing loss may only be diagnosed months or years later, confounding the classification of the hearing loss into congenital or acquired.

In a study among 582 deaf patients in Cameroon to determine etiology, a review of family history including pedigree drawing and identification of consanguinity, and a comprehensive retrospective chart review were performed. The results attributed the majority of cases (53%) to environmental causes, 15% to genetic components, and the remaining 32% to unknown causes. Of those cases due to genetics, 13% were attributed to a syndromic form of hearing loss (Wonkam et al., 2013).

Similar findings were seen in a study of 259 Gambian hearing impaired children aged from 2 to 10 years. Based on a detailed birth, medical, and family history, the study demonstrated that environmental exposure to illness accounted for the majority of cases, with nearly 32% attributed to meningitis or meningitis-like symptoms and an additional 25% to other common childhood illness (e.g., rubella, fever). Genetic factors contributed to a low 8% of the cases and for the remainder the etiologies were unknown (McPherson and Holborow, 1985).

However, both of these studies relied heavily on clinical history rather than medical testing, perhaps underestimating the number of cases due to genetic factors. This high rate of environmental deafness compounded with a lack of complete medical records has complicated the task of deciphering the genetic contribution to congenital hearing loss in African populations.

3. The impact of cultural and social practices

The reliance on traditional forms of healing and on traditional healers has at times delayed patients from seeking formal medical attention in a timely fashion. For example, seeking tribal medicine for a child with a fever due to meningitis will delay the administration of suitable antibiotics, leading to sequelae such as deafness.

Single parent homes and disruption of family structures due to migratory labor practices are both commonplace in many parts of sub-Saharan Africa. Children are then brought up by single mothers, grandparents or other relatives. While taking a medical history, valuable information may be unknown to the primary caregiver and therefore may be missed. On the other hand, the discouragement of close family mating and consanguineous mating among sub-Saharan populations protects against the clustering of defective genes in the population.

4. Syndromic deafness

Among cases of genetic or familial hearing loss, several syndromic conditions have been encountered in Sub-Saharan Africa. As early as 1977, Sellars and Beighton studied 499 deaf black children from three special schools for the deaf in Transkei, Ciskei, and the eastern Orange Free State of Southern Africa and identified 21 children aged 6 to 18 years with genetic syndromes. Based on clinical and otologic examinations, researchers identified Waardenburg (*n*

= 13), vitiligo deafness (*n* = 2), treacher collins (*n* = 2), Hunters (*n* = 1), Crouzon's (*n* = 1), Pendred's (*n* = 1), and trichorhinophalangeal (*n* = 1) syndromes (Sellars, 1977). Additional reports of syndromic deafness in Sub-Saharan Africa include cases of Usher's, Pierre Robinson, and Leopard syndromes (le Roux et al., 2015). While these syndromes account for the majority of syndromic hearing loss cases in the developed world, they are infrequent causes of hearing loss in African populations and are not considered to be strong risk factors for hearing loss in these populations (Friderichs et al., 2012).

4.1 Waardenburg syndrome (WS)

Waardenburg syndrome is a group of genetic conditions that cause pigmentation changes in the hair, skin, and eyes, and has been noted as the most common cause of syndromic hearing loss in Sub-Saharan populations (Lebeko et al., 2015). However, it still only accounts for approximately 3%–5% of genetic cases (Beighton et al., 1991). In 1983, Sellers and Beighton evaluated 3006 school children and identified 90 (3.0%) as having Waardenburg Syndrome. This study included 1226 indigenous African children, of whom 33 were affected (2.7%) (Sellars and Beighton, 1983). Waardenburg Syndrome typically follows an autosomal dominant inheritance pattern; however, a number of *de novo* cases have been described in a Cameroonian cohort (Noubiap et al., 2014).

4.2 Usher syndrome (USH)

Usher syndrome is clinically characterized by hearing loss and retinitis pigmentosa. It has three clinical subtypes and is a common cause of combined hearing loss and blindness worldwide. To date, 11 genes have been associated with USH, with numerous autosomal recessive mutations reported in *USH1C*, *MYO7A*, and *CDH23* (Lebeko et al., 2015). USH is less common in Sub-Saharan Africa, representing only 1%–2% of genetic cases; however, researchers recently described a rare c.6377delC (p.Pro2126Leufs*5) mutation in the *MYO7A* gene in two unrelated African individuals. Further analysis revealed that this is the causative mutation in 12 additional African individuals with hearing loss. This particular mutation has only been reported once before in a heterozygous Caucasian individual (Roberts et al., 2015).

4.3 Pendred syndrome

Pendred syndrome is a form of sensorineural hearing loss (SNHL) associated with thyroid goiter that accounts for <1% of genetic causes of hearing loss in Sub-Saharan Africa (Beighton et al., 1991; Wonkam et al., 2013). There are rare case reports in the literature, with the first cases in Africa described by Levin and Glugman in 1966 in two South African Bantu brothers (Levin and Klugman, 1966).

Additionally, there have been case studies describing less frequently encountered syndromes (Lebeko et al., 2015). Two cases of Goldenhar, or oculo-auriculo-vertebral, syndrome have been described in a 3-day-old child in Nigeria (Adeoye, 2002) and a 15-month-old child in South Africa (Naidoo and Stephen, 1998). The genetics of this syndrome is not well understood, and its inheritance is believed to be multifactorial. Though only about 100 cases have been reported worldwide, several cases of keratitis-ichthyosis-deafness (KID) syndrome, defined by cornea, skin, and hearing abnormalities, have been described in Sub-Saharan Africa including Togo (Barruet et al., 2011; Kombate et al., 2015) and Cameroon. In Cameroon, two unrelated patients, ages 5 and 2, were found to have a sporadic p.Asp50Asn mutation in the *GJB2* gene, previously described only in a single African patient from the Emirates (Wonkam et al., 2013). Additionally, a set of African monozygotic twins, one referred at birth and one at 3 months, have been described with a p.Gly45Glu (G45E) mutation in the *GJB2* gene, coding for a lethal form of the disease within the first year of life (Jonard et al., 2008). The extremely rare Leopard syndrome, due to a defect in the protein tyrosine phosphatase non-receptor 11 gene (*PTPN 11*) on chromosome 12, is characterized by abnormalities of the skin, heart, and ear and has been described in one 6-year-old Djiboutian boy (Massoure et al., 2012).

5. Non-syndromic deafness

Non-syndromic hearing impairment accounts for the majority of inherited hearing loss, approximately 70%. In the US, autosomal-recessive inheritance is responsible for about 80% of cases of non-syndromic hearing impairment, while autosomal-dominant genes cause 20%, and less than 2% of cases are caused by X-linked and mitochondrial genetic malfunctions. Comprehensive review of the relatively sparse literature of NSHL in populations of African descent shows 12 different pathogenic genes, including 34 different pathogenic or likely pathogenic mutations in 159 individuals with a confirmed etiology of genetic NSHL (Table 2).

Table 2. Mutations associated with non-syndromic hearing loss in populations of African descent

Mutation	Ethnic group	Affected/sample size	Number of pathogenic or likely pathogenic alleles	Frequency of mutated allele*	Variant classification	Description of hearing loss	Reference
***GJB2* (n^{**} = 189) – gap junction in cochlea involved in ion recycling**							
g.3352delG (c.35delG)	Kenyan/Sudanese	5/589 (hom[#])	10	0.041	Pathogenic, frameshift mutation	Not discussed	Gasmelseed et al., 2004
	African American	7/50	7	0.029		Not discussed	Pandya et al., 2003
	Afro-Brazilian	1/100	1	0.004		Carrier only (frequency = 1%, *q* = 0.005); HL not assessed.	Oliveira et al., 2004
g.3455_3460del (c.138_143del, p.Asp46_Gln48delinsGlu)	Kenyan/Sudanese	1/589 (het[##])	1	0.004	Pathogenic, novel frameshift mutation	Not discussed	Gasmelseed et al., 2004
g.3512C>A (c.195C>A, p.Tyr65X)	Kenyan/Sudanese	1/589 (het)	1	0.004	Pathogenic, novel stop codon	Not discussed	Gasmelseed et al., 2004
c.208C>G (p.Pro70Ala)	Ugandan	1/115	1	0.004	Pathogenic	Bilateral, severe to profound, pre-lingual HL	Javidnia et al., 2014
g.3741_2743delTTC (p.F142del)	Cameroon	1/205	1	0.004	Pathogenic	Not discussed	Bosch et al., 2014
g.3744C>T (c.427C>T, p.R143W)	Ghana	21/21 (hom)	42	0.174	Pathogenic, compound het (p.R143W/35insG; p/R143W/I203K; p.R143W/L79P; p.R143W/L214P)	Bilateral, severe to profound, congenital, nonsyndromic, sensorineural HL	Brobby et al., 1998
		4/365 (het), 4/365 (comp het[###]), 51/365 (hom)	110	0.456			Hamelmann et al., 2001
	African American	1/50	1	0.004			Pandya et al., 2003
g.3795G>A (c.478G>A, p.Gly160Ser)	Kenyan/Sudanese	1/589 (het)	1	0.004	Pathogenic, nonsynonymous mutation	Not discussed	Gasmelseed et al., 2004
g.3816C>A or g.3816G>A (p.Val167Met)	Kenyan/Sudanese	4/589 (het)	4	0.017	Pathogenic, novel nonsynonymous mutation	Not discussed	Gasmelseed et al., 2004
	Cameroon	1/205 (het)	1	0.004	Pathogenic	Not discussed	Bosch et al., 2014
g.3850T>C (c.533T>C, p.Val178Ala)	Ghana	2/365 (hom)	4	0.017	Pathogenic, novel missense mutation, recessive	Bilateral, profound, nonsyndromic, sensorineural HL in childhood. Unspecified onset or progression.	Hamelmann et al., 2001
g.3868G>A (c.551G>A, p.Arg184Gln)	Ghana	1/365 (het)	1	0.004	Pathogenic, novel missense mutation, dominant		
g.3906G>T (c.589G>T, p.Ala197Ser)	Ghana	1/365 (het)	1	0.004	Pathogenic, novel missense mutation		

g.3426G>A (c.109G>A, p.Val37Ile)	Kenyan/Sudanese	1/589 (het)	1	0.004	Likely pathogenic	Not discussed	Gasmelseed et al., 2004
g.3697G>A (c.380G>A, p.Arg127His)	Kenyan/Sudanese	1/589 (het)	1	0.004	Likely pathogenic, nonsynonymous mutation	Not discussed	
***MARVELD2* (*n* = 2) – establishing epithelial barrier at tight junctions in organ of Corti**							
c.1555-1G>A (splice)	Limpopo Province, South Africa	1/91	2	0.008	Novel, likely pathogenic	Not discussed	Yan et al., 2016
***MYO3a* (*n* = 4) – autosomal dominant mutation affecting ATPase of motor domain in inner ear stereocilia**							
rs145970949:G>A (p.Gly488Glu)	African American	4/4	4	0.017	Pathogenic	Bilateral, progressive, post-lingual, sensorineural HL	Grati et al., 2016
***MYO6* (*n* = 2) – unconventional myosin aids in intracellular transport**							
c.1477_1487delCAAGAACTCTA (p.Q493Sfs*8)	Yoruba tribe (Ibadan, Nigeria)	1/90	2	0.008	Novel, pathogenic	Mild to profound, congenital or pre-lingual, nonsyndromic HL	Yan et al., 2016
***MYO7A* (*n* = 7) – unconventional myosin aids in intracellular transport**							
c.6375delC (p.P2126Lfs*5)	Limpopo Province, South Africa	1/91	1	0.004	Pathogenic	Mild to profound, congenital or pre-lingual nonsyndromic HL	Yan et al., 2016
c.1118G>A (p.R373H)	Limpopo Province, South Africa	1/91	1	0.004	likely pathogenic		
c.1142C>T (p.T381M)	Limpopo Province, South Africa	1/91	1	0.004	likely pathogenic		
c.1554+7C>T (splice)	Limpopo Province, South Africa	1/91	1	0.004	Likely pathogenic		
c.5326+7G>A (splice)	Limpopo Province, South Africa	1/91	1	0.004	Likely pathogenic		
c.287C>T (p.T96M)	Yoruba tribe (Ibadan, Nigeria)	1/90	1	0.004	Novel, likely pathogenic		
c.1708C>T (p.R570*)	Yoruba tribe (Ibadan, Nigeria)	1/90	1	0.004	Novel, pathogenic		
***POU3F4* (*n* = 1) – transcription factor that regulates proliferation of neural stem cells**							
c.986G>C (p.R329P)	Limpopo Province, South Africa	1/91	1	0.004	Likely pathogenic, hemizygous	Mild to profound, congenital or pre-lingual, nonsyndromic, HL	Yan et al., 2016
***SIX1* (*n* = 1) – transcription factor**							
c.373G>A (p.G125L)	Limpopo Province, South Africa	1/91	1	0.004	Likely pathogenic	Mild to profound, congenital or pre-lingual, nonsyndromic HL	Yan et al., 2016

***SLC26A4* (n=4) – tansporting chloride and iodine to maintain pH of endolymph**							
c.737delA (p.N246Tfs*43)	Yoruba tribe (Ibadan, Nigeria)	1/90	2	0.008	Novel, pathogenic	Mild to profound, congenital or pre-lingual, nonsyndromic HL	Yan et al., 2016
c.164+1G>C (splice)	Yoruba tribe (Ibadan, Nigeria)	1/90	1	0.004	Novel, pathogenic, splice		
c.2171A>T (p.D724V)	Yoruba tribe (Ibadan, Nigeria)	1/90	1	0.004	Novel, likely pathogenic		
***TRIOBP* (*n* = 2) – actin binding protein involved in stabilization**							
c.572delC (p.P191Rfs*50)	Limpopo Province, South Africa	1/91	1	0.004	Novel, likely pathogenic	Mild to profound, congenital or pre-lingual, nonsyndromic HL	Yan et al., 2016
c.3510_3513dupTGCA (p.P1172Cfs*13)	Limpopo Province, South Africa	1/91	1	0.004	Novel, likely pathogenic		
***MT-RNR1 (Mitochondrial)* (*n* = 27) – predisposing to aminoglycoside ototoxicity**							
m.1555A>G	Black South African	1/115	1	0.004	pathogenic		Human et al., 2010
		18/18	18	0.075	pathogenic, homoplasmic	Aminoglycoside-induced, bilateral	Gardner et al., 1997
961delT+insC(n)	Black South African	8/115	8	0.033	pathogenic		Human et al., 2010
***MT-ND3 (Mitochondrial)* (*n* = 1) – encoding an NADH dehydrogenase involved in mitochondrial respiratory chain**							
m.10114T>C (p.Ile19Thr)	Black South African	1/115	1	0.004	Likely pathogenic	Aminoglycoside-induced	Human et al., 2010
***MT-CYB (Mitochondrial)* (*n* = 1) – encoding the cytochrome B protein involved in mitochondrial respiratory chain**							
m.15312T>C (p.Ile18ThrT)	Black South African	1/115	1	0.004	Likely pathogenic	Aminoglycoside-induced	Human et al., 2010

*The mutation frequency is calculated based on this review of the literature. It is not a reported value from the original publications. ** *n* = number of subjects with a mutation in the gene of interest identified in review of the literature. # hom, homogeneous mutation; ## het, heterogeneous mutation; ### comp het, composite heterozygous mutation; HL, hearing loss.

5.1 GJB2

Mutations of *GJB2* are the most common cause of non-syndromic genetic deafness worldwide in non-African populations. Specific mutations are associated with specific populations: c.35delG in Europeans, c.167delT in Ashkenazi Jews, c.235delC in East Asians. In contrast, the few studies available suggest that *GJB2* is not a major cause of deafness in most African populations studied to date.

With the notable exception of Ghana (Chan and Chang, 2014), *GJB2* has not been found to be a prominent genetic cause of hearing loss in sub-Saharan Africa. We have previously shown that none of the reported deafness-causing mutations in *GJB2* nor any novel pathogenic mutations in the coding region were detected in an indigenous African population of the Limpopo Province of South Africa. The *GJB6*-D13S1830 deletion and the mitochondrial mutations were also not observed in this group (Kabahuma et al., 2011). *GJB2* mutations were also absent among deaf probands from the Yoruba tribe residing in Ibadan, a suburban city in Nigeria (Lasisi et al., 2014). Similarly, an analysis of a large cohort of Ugandan deaf patients identified none of the common *GJB2* deletions (Javidnia et al., 2014). Despite low rates of common *GJB2* mutations in the Ugandan population, complete sequencing of 115 of the 126 individuals revealed one pathogenic variant in *GJB2* (c.208C>G; p.Pro70Ala) (Javidnia et al., 2014). Analysis of the coding region of the *GJB2* gene in 205 Cameroonian and Xhosa South Africans with congenital, non-syndromic deafness, also revealed low frequencies of common mutations but identified two monoallelic pathogenic mutations (p.F142del and p.V167M) in two unrelated Cameroonian participants (Bosch et al., 2014).

Most African populations do not show a high prevalence of *GJB2* mutations. However, there is one specific *GJB2* mutation, p.R143W (c.427C>T), with a relatively high prevalence in the Ghanaian population of the Adamorobe village, an inbred community (Hamelmann et al., 2001). These individuals presented with severe to profound bilateral congenital SNHL. Secondly, other *GJB2* variants have been identified in 12.7% and 6.5% of Kenyan and Sudanese deaf persons, respectively. Collectively, the population's hearing loss is described as bilateral, sensorineural, non-syndromic, pre-lingual, and of severe to profound severity; hearing characteristics of

specific variants are not described. Variants found include p.Tyr65X, p.Val167Met, c.35delG, p.Val37Ile, p.Val153Ile, p.Gly160Ser (Gasmelseed et al., 2004). It is noted that, in this study, there is no description of the population characteristics of the study group. The northern parts of Kenya bordering onto Sudan and Somalia are inhabited by individuals of Arab and Somali descent who migrated from the north. As such, this population group is not of clear African genetic makeup and may reflect a genetic admixture from Arabic populations further north.

We have previously reported that none of the 182 deaf African individuals from the Limpopo Province of South Africa carried any of the known disease causing mutations of *GJB2*, including c.35delG, or any other potentially pathogenic *GJB2* mutations. There was, however, a high frequency of two *GJB2* variants, C>T at position g.3318–3315 and C>T at p osition g.3318–3334, which occurred in 21.4% and 46.2% of the deaf cohort respectively, and in 35% and 42.6% of a normal hearing control group, respectively, suggesting that these are common non-pathogenic polymorphisms (Kabahuma et al., 2011). These variants were also found with high frequency in populations from Cameroon, Uganda, Kenya, and Sudan, supporting their role as benign polymorphisms (Gasmelseed et al., 2004; Bosch et al., 2014; Javidnia et al., 2014).

Interestingly, the c.35delG mutation, common in Europeans, has been identified in 5/139 Sudanese deaf children and 1/100 Afro-Brazilians. In both cases, it has been suggested that the presence of this mutation is from genetic admixture with European populations rather than inherent in each population (Gasparini et al., 2000; Gasmelseed et al., 2004).

The c.35delG mutation is also rare among African American populations. Gaparini et al. (2000) assessed the carrier frequency of the c.35delG mutation in various populations, including 190 African Americans, and did not identify any carriers in this cohort. Another study examined 173 non-deaf African Americans for the c.35delG variant and 171 non-deaf African Americans for the 167delT variant and did not identify any carriers for either, suggesting that these alleles are of very low frequency (Morell et al., 1998).

One study identified *GJB2* pathologic variants in 4% of 50 African American deaf patients, as compared to 26% of Caucasians and 11% of Hispanics in the same study, with c.35delG as the

most common mutation in all ethnicities (Pandya et al., 2003). However, most deafness-causing genes in African individuals within the United States have yet to be identified.

Overall, more than 30 variants in the *GJB2* gene have been described in deaf patients of African descent. Approximately 13 are thought to be pathogenic or likely pathogenic. These mutations along with their frequencies, if available, are listed in Table 2. Among them, 78% of all pathogenic or likely pathogenic NSHL mutations in populations of African descent included in this review are found on *GJB2*. However, much of this contribution is from the Ghanaian population alone. If Ghana is excluded, *GJB2* mutations comprise only 37.3% of NSHL mutations in this review, with most studied populations of African descent having a complete absence of pathogenic *GJB2* mutations.

5.2 GJB6

The most common mutation in *GJB6* is the 342-kb *GJB6*-D13S1830 deletion, which causes NSHL when homozygous, or when present on the opposite allele of a *GJB2* mutation. The *GJB6*-D13S1830 mutation, which is most frequent in Spain, France, the United Kingdom, Israel and Brazil (5.9%-9.7% of all DFNB1 alleles), is less frequent in the USA, Belgium and Australia (1.3%-4.5% of all DFNB1 alleles), and very rare in Southern Italy (Del Castillo et al., 2003). In Northern Italy, frequencies are similar to those of other European countries.

Studies among African populations reported that pathologic *GJB6* mutations have been widely screened but not identified. Specifically, they were found to be absent in African populations within South Africa (Kabahuma et al., 2011), Nigeria (Lasisi et al., 2014), Cameroon (Bosch et al., 2014), Brazil (Batissoco et al., 2009) and the United States (Pandya et al., 2003; Samanich et al., 2007).

5.3 MTRNR1 (mitochondrial 12S ribosomal RNA)

Mitochondrial DNA (mtDNA) mutations have been associated with non-syndromic and aminoglycoside-induced hearing loss. Mutations in *MTRNR1* gene, m.1555A>G, m.961delT/insC, m.961T>G, m.1095T>C, and m.1494C>T, have been found in some patients with aminoglycoside-induced hearing loss or hereditary non-syndromic NSHL. Among these, the

commonest predisposing mutation is m.1555A>G. This mutation has been detected in low frequency among African populations, including 18 family members of mixed ancestry in South Africa where nine of these family members had profound bilateral post-lingual hearing loss following aminoglycoside use, while the remaining nine were found to be carriers of the mutation but had not received aminoglycosides (Gardner et al., 1997). Human et al. (2010) later conducted a study that included 115 MDR-TB (multi-drug resistant tuberculosis) patients all on aminoglycosides and 439 controls representative of the main ethnic groups in South Africa. Data analysis revealed the presence of homoplasmic mutations in controls (m.1555A>G in 0.9% of Black controls and m.827A>G in 1.1% of Afrikaner controls), suggesting that a significant proportion of the South African population is genetically predisposed to developing aminoglycoside-induced hearing loss. The 961 delT+insC(*n*) (7.1% of Black controls) and m.961T>G (2.9% of Afrikaner controls) variants were found at frequencies ranging from 1.1% to 7.1%. However, the incidence of ototoxicity in the MDR-TB patients harboring these mutations is not discussed in the review.

In those black patients for whom aminoglycoside induced ototoxicity was confirmed, researchers identified several novel variants in the *MT-ND3* and *MT-CYB* genes including two likely pathogenic mutations m.10114T>C (I19T) and m.15312T>C (I189T) and three benign variants m.10128C>A (L24M), m.11318T>C (S187P), and m.15735C>T (A330V) (Human et al., 2010). Although m.10114T>C and m.10128C>A have been described in a South African pediatric population with neuromuscular mitochondrial respiratory chain disease, neither of these variants have otherwise been implicated in hearing loss (van der Walt et al., 2012).

Among 78 children with severe to profound prelingual deafness from Cameroon, researchers identified six mitochondrial variants including four known polymorphisms of the *MTRNR1* gene in the African population (m.1503G>A, m.1018G>T, m.959C>T, and m.1048C>T) and one novel variant (m.1462G>T). This novel variant was also identified in deaf patients from two additional studies, suggesting a pathologic role in hearing loss (Trotta et al., 2011).

Overall, mutations on *MTRNR1* account for 11.2% of NSHL in populations of African descent included in this review, with the m.1555A>G mutation comprising 70% of these mutations (7.8% of mutations in this study).

5.4 MYO3A

Several recessive, loss of function mutations in *MYO3A* causing hearing loss have been described in populations worldwide. Although mutations in this gene have not been described in indigenous African populations, researchers recently reported a dominant mutation responsible for a new form of NSHL in an African American family. Grati et al. (2016) performed exome sequencing on five African American family members with post-lingual, progressive, NSHL. Mutational analysis revealed a single novel variant, p.Gly488Glu, in the *MYO3A* gene (Grati et al., 2016). *MYO3A* is responsible for 1.7% of mutations in populations of African descent included in this review.

6. Application of next-generation sequencing (NGS) in non-syndromic hearing loss

NGS is rapidly expanding the knowledge of non-syndromic genetic deafness, particularly in previously under-studied populations. In use since the late 1990s, NGS encompasses several techniques of genome sequencing that produce thousands or millions of parallel and overlapping DNA sequences that allows for rapid and relatively inexpensive analysis of large segments of DNA.

As part of a recent multi-ethnic genetic deafness study, two deaf indigenous *GJB2*-negative sub-Saharan African populations were tested for genetic deafness using an NGS custom capture panel (MiamiOtoGenes, USA) (Yan et al., 2016). The study included 91 indigenous deaf persons from the Limpopo province of South Africa and 90 from the Yoruba tribe in Ibadan, Nigeria. Tunisia, a north African country, was also included in their study but will not be included in this discussion as the ethnic admixture in Tunisia is significantly higher given its European and Arabic genetic contribution.

The authors detected an etiologic genetic deafness diagnosis in 4% of families from both South Africa and Nigeria. This is a relatively low rate compared to non-Sub-Saharan African

populations (28%). This may be due in part to the larger number of simplex cases (one family member affected) *versus* the multiplex cases (in which multiple members of the family are affected) among the South African and Nigerian cohorts in comparison to the other ethnic groups. This study further reported solved rates of 7% for simplex families compared to 25% for multiplex families overall, suggesting that a positive family history of deafness may be an important indicator for a genetic etiology.

This study also detected a high number of variants of unknown significance (VUS), especially among the sub-Saharan African cohorts. South Africa has the highest number of VUS (23), followed closely by Nigeria (18). The main difference is seen among the simplex families, with 15 and 12 for South Africa and Nigeria, respectively. As a comparison, ethnic cohorts in Tunisia, Turkey, Iran, and India show no VUS, with only two VUS identified in USA and Guatemalan cohorts.

It is, however, the unsolved numbers that reflect the true picture of the state of genetic deafness research among different ethnic groups. The data coming out of sub-Saharan African populations show a high number of unsolved variants compared to the non-sub-Saharan cohorts. The South African cohort reported a total of 64 unsolved variants (53 simplex, 11 multiplex), while Nigeria reported 68 unsolved variants (51 simplex and 17 multiplex). Compared to the USA which had almost equal numbers of simplex and multiplex unsolved variants (18 and 17 respectively), to Tunisia with 20 unsolved variants (0 simplex and 20 multiplex), or to Guatemala with four unsolved variants (two simplex, two multiplex), this is a significant finding.

Two issues must be clarified if one is to make logical conclusions from this data. One, all the deaf probands were recruited by experienced and highly trained otolaryngologists who are based in those countries (South Africa and Nigeria), and who understand both the cultures and the languages of the local people. Second, based on the foregoing, it can be accepted that all these deaf individuals were correctly assessed and that environmental causes for the hearing loss were excluded, leaving only genetic causes for the hearing loss as reported. From these observations, it therefore becomes apparent that the high number of unsolved cases are all due to a genetic

cause whose genes and mutations have either not yet been identified or fall outside of the gene pool included in the gene panel used in this study.

To support this conclusion, it is notable that the unsolved cases from the countries that practice consanguineous mating or where inbreeding is prevalent, such as Tunisia, India, Iran, and Turkey, were found mainly in the multiplex families. The sub-Saharan communities in South Africa and Nigeria, where the deaf probands were recruited, discourage consanguineous mating. When one considers that large endogamous populations were instrumental in mapping genes for NSHL (Keats and Berlin, 1999), and the extreme heterogeneity of genetic hearing loss, these findings are to be expected. In the sub-Saharan populations, natural spread of mutations and variations is expected, leading to a larger number of simplex cases compared to multiplex cases. A high number of *de novo* mutations are therefore to be expected. The reverse holds for inbred communities where clustering of mutations occurs due to inbreeding and consanguineous mating. Recessive genetic defects will cluster in the latter communities, as exemplified by the founder effect.

Despite these limitations, this was the first study to successfully identify several likely pathogenic deafness-causing variants in sub-Saharan populations (Yan et al., 2016). Additionally, several other genes found to have VUS in these South African and Nigerian populations will require further investigation.

7. Future work

Deafness research in Africa has long lagged behind the developed world due to lack of diligent medical records, poor access to health care, limited infrastructure, low number of skilled workers along with the outflow of skilled researchers to developed countries, funding, and limited access to high-impact journals for relatively lesser-known African researchers. Additionally, the environmental impact on deafness and the widespread avoidance of consanguinity in African societies, further minimize the genetic contributions to disease (McPherson and Swart, 1997) and increase the burden of genetic data needed for pathologic mutation identification. However, genetic deafness research in populations of African descent has advanced tremendously over the

last two decades with the advent of NGS and the increasing level of collaboration between African universities with genetic research centers abroad.

South Africa is addressing the issue of poor continuity of patient care in Africa as well as the suboptimal medical records by the development of a unique healthcare identifier system, currently in its pilot phase. In this system, the patient will retain a unique identifier throughout life and his or her medical records are accessible in all public hospitals nationally. The anticipated effects in medical management, patient tracing, and clinical research are expected to be transforming.

Common variants responsible for genetic deafness in populations are not common in individuals of African descent; in fact, some authors argue that there is no clinical utility in screening for *GJB2, GJB6,* or *GJA1* in black Africans (Wonkam et al., 2015). Recent work by Yan et al (2016) showed promising use of a large screening deafness gene panel (MiamiOtoGenes) for detecting rare or novel mutations in a rapid and cost-effective manner. In patients shown to be negative for *GJB2, GJB6,* and mitochondrial mutations, NGS with the MiamiOtoGenes panel revealed several candidate deafness genes for the first time in sub-Saharan African populations (Nigeria and Limpopo, South Africa).

In those patients for whom a diagnosis is still not obtained, whole-exome sequencing or even whole-genome sequencing is then the next step. This type of sequential genetic screening approach will become increasingly important for both research and clinical applications. Much work remains to elucidate the genetic causes of hearing loss in populations of African descent worldwide, but with the development of NGS and efficient deafness gene-screening protocols, the present and future of genetic deafness research has never looked brighter.

8. Conclusion

Globally, acquired causes of hearing loss remain the predominant etiology of hearing loss in patients of African descent. With improved preventative care, including vaccination and perinatal care, genetic causes of hearing loss will take on a larger proportion of the hearing loss burden in this population. Syndromic hearing loss in Africa is primarily caused by Pendred,

Waardenburg, and Usher Syndromes. Specific genetic causes of NSHL have been elusive in populations of African descent. Review of the global literature shows 12 genes and 34 mutations known to be responsible for NSHL in these populations, with most individuals having bilateral, severe to profound hearing loss of prelingual onset. *GJB2* remains a relatively uncommon contributor to NSHL in most of these populations, with the exception of Ghana. In at-risk populations of African descent, screening for mitochondrial mutations predisposing to aminoglycoside toxicity may prevent hearing loss. The remainder of identified genes causing NSHL in these populations are all relatively rare but may warrant incorporation into population-specific gene panels (*MYO7A*, *MYO3A*, *SLC26A4*, *MARVELD2*, *MYO6*, *POU3F4*, *SIX1*, and *TRIOBP*). With recent advancement in sequencing technologies like NGS and population-specific gene panels, we are poised for rapid advance in diagnosis of genetic hearing loss in populations of African descent.

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