

Spectrum of *GJB2* Mutations in a Cohort of Nonsyndromic Hearing Loss Cases from the Kingdom of Saudi Arabia

Mohammed H. Al-Qahtani,¹ Ibtessam Baghlab,¹ Adeel G. Chaudhary,¹ Adel M. Abuzenadah,¹ Afaf Bamanie,² Kamal J. Daghistani,³ Malek Safieh,⁴ Loai Fida,⁵ and Ashraf Dallo¹

Nonsyndromic hearing loss (NSHL) affects a substantial proportion of newborns in the world every year. This proportion increases proportionally with the degree of consanguineous marriages in any society. In the Kingdom of Saudi Arabia, consanguineous marriages are common practice and this is associated with a noticeably high frequency of inherited conditions affecting the resulting progeny, including NSHL. Until now there is no published data on the genetic causes of NSHL in Saudi Arabia, which greatly hindered the ability of local genetic counseling and family planning centers to distinguish between hereditary and nonhereditary forms of NSHL and subsequently could not give information on the possible inheritance of deafness. In addition, the lack of validated genetic tests for NSHL delayed the detection of deafness in affected individuals and may have lowered the efficiency of later medical interventions. Further, the population covered in this study is likely to have a multi-ethnic background caused by decades of religious and economic migration to this region. To address such problems, we undertook the task of unraveling the genetic causes of hearing loss in Saudi Arabia, starting with identifying the *GJB2/DFNB1* mutation spectrum in a cohort of unrelated individuals suffering from mild to profound NSHL. A total of 12 reported *GJB2* mutations were identified in 17 out of 109 (15.59%) NSHL cases. Biallelic *GJB2* mutations were identified in 11 out of the 109 NSHL cases (10.09%), with c.35delG being the most common (7/11, 63.63%). The remaining six patients were found to have monoallelic *GJB2* mutations. Interestingly, biallelic *GJB2* mutations were not detected in patients of Arab tribal origins, reflecting the genetic heterogeneity of the western area of the Kingdom of Saudi Arabia. Therefore, ethnically targeted genetic screening for *GJB2* mutations could be a useful tool toward the management of NSHL in this area.

Introduction

HEARING LOSS is one of the most common afflictions in the world, affecting about one in every 1000 newborns (Petersen and Willems, 2006). Provision of specialized care and education to those children require a significant amount of resources and dedication. In addition, the social stigma usually associated with a deaf child may affect his or her response to treatment or care. Genetic factors are estimated to be the underlining cause of more than half of the hearing loss cases, which means that deafness is hereditary in a significant proportion in any given population. Hereditary hearing loss can be associated with syndromes affecting other functions of the body. However, the majority of hearing loss cases is not associated with syndromes (nonsyndromic) that can be transmitted in an autosomal recessive, autosomal dominant, or

X-linked modes of inheritance (Petersen and Willems, 2006). Autosomal recessive hereditary nonsyndromic hearing loss (the most common type of hereditary hearing loss) can be caused by mutations affecting any one of the 62 deafness loci identified so far, thus making NSHL a very heterogeneous trait and complicating diagnosis and genetic counseling (Hilgert *et al.*, 2009). However, on average, at least half of the NSHL cases are caused by mutations in the *GJB2/CONNEXIN26* gene (Hereditary Hearing Loss Home Page, <http://webh01.ua.ac.be/hhh/>). This places *GJB2* as the prime suspect and the first candidate NSHL-causing gene to be analyzed when studying the molecular etiology of deafness in any new ethnic population (Hilgert *et al.*, 2009).

GJB2/CONNEXIN26 is a member of a large family of gap junction proteins that function in mediating cell-to-cell contact and communication. Located at chromosome 13q12.11, *GJB2*

¹Center of Excellence in Genomic Medicine Research, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia.

²Audiology Section, Department of Otolaryngology, KAUH, Jeddah, Kingdom of Saudi Arabia.

³Ear, Nose, Throat (ENT)—Head and Neck Surgery Department, Erfan-Bagedo Hospital, Jeddah, Kingdom of Saudi Arabia.

⁴Jeddah Institute for Speech and Hearing, Jeddah, Kingdom of Saudi Arabia.

⁵Medical Supervision, Department of Education, Jeddah, Kingdom of Saudi Arabia.

is a relatively small gene with two exons and one intron and the entire coding region is comprised only of exon 2 (Martinez *et al.*, 2009). Mutations in *GJB2* account for up to 50% of NSHL cases in Europe and the United States, with a single mutation (c.35delG) accounting for at least one-third of those cases (Gasparini *et al.*, 2000; Lucotte and Mercier, 2001; Minarik *et al.*, 2003; Roux *et al.*, 2004; Lucotte and Dieterlen, 2005; Putcha *et al.*, 2007). Relatively high frequencies of *GJB2* mutations, other than c.35delG, have been reported for other ethnic populations, including c.235delC in Japanese (Abe *et al.*, 2000), p.Arg143Trp in Africans (Broby *et al.*, 1998), and c.167delT in Ashkenazi Jews, indicating founder events (Morell *et al.*, 1998). Interestingly, in Lebanon, *GJB2* mutation levels and type (c.35delG) are equivalent to that in western Europe (Mustapha *et al.*, 2001). This observation supports the founder effect of the c.35delG mutation, considering a recent paper on the genetic backgrounds of the Lebanese population showing that a significant percentage has southern/western European origins (Zalloua *et al.*, 2008).

Consanguineous marriages are frequent in Saudi Arabia; over half of the marriages are between related partners, often first-cousin relatives (El Mouzan *et al.*, 2008). Consanguinity can exacerbate the frequency of genetic diseases and it is also expected to do so in hereditary deafness. The prevalence of hearing impairment was significantly higher in children whose parents were either first cousins or relatives when compared with the children whose parents were not related, thus demonstrating a definite role of consanguinity in the etiology of childhood hearing impairment in Saudi Arabia (Jamal *et al.*, 2002). In the neighboring Sultanate of Oman, 70% of the deaf children were born to parents of consanguineous marriages (Khabori and Patton, 2008). *GJB2* mutations were ruled out as a cause of hereditary NSHL in Oman (Simsek *et al.*, 2001), making genetic counseling for hearing loss a difficult task to accomplish.

The objective of this study was to determine the frequency and spectrum of *GJB2* mutations in the Saudi population, particularly from the ethnically mixed western region of the Kingdom of Saudi Arabia. The overall aim was to understand the molecular etiology of NSHL in this region, to be able to offer genetic counseling to the affected families and aid in the care of the hearing-impaired individuals.

Materials and Methods

Samples from patients with NSHL were collected following the appropriate local ethical protocols and guidelines from the Audiology Clinic of the King Abdulaziz University Hospital in Jeddah or the Audiology Department of the Jeddah Institute for Speech and Hearing as well as from the Al-Amal Institute for the Deaf in Jeddah. Genomic DNA was prepared from peripheral blood isolated from the subjects using the Qiagen (Valencia, CA) Blood DNA extraction kit according to the manufacturers' protocol. In addition, DNA obtained from 37 apparently normal hearing subjects was also used to determine the carrier frequency for *GJB2* mutations. *GJB2* exon 1 was amplified using a forward primer (5'-CGTAACTTTCCCAGTCTCCG-3') and reverse primer (5'-CCAAGGACGTGTGTTGGTC-3'), generating a 358-bp polymerase chain reaction (PCR) product. *GJB2* exon 2 was amplified using forward primer (5'-AGATTTAAGCATGCTTGCTTAC-3') and reverse primer (5'-CTCATGCTGTCTATTCTTAATCTAA-3'), gen-

erating an 838-bp PCR product. The PCR conditions used for the amplification of either exon 1 or exon 2 were one denaturation cycle at 95°C for 15 min followed by eight touchdown cycles of denaturation at 95°C for 30 s, and annealing for 30 s at 60°C for the first two cycles and a 2°C reduction every two cycles. This is followed by 35 cycles of denaturation at 95°C for 30 s, and annealing at 53°C for 30 s and extension at 72°C for 1 min. The final extension step was done at 72°C for 10 min. The MgCl₂ concentrations used for the amplification of exon 1 and exon 2 were 3 and 5 mM, respectively. In addition, the exon 1 amplification reaction was aided by the addition of 15% glycerol. All PCR reactions were performed using the Qiagen HotStarTaq DNA Polymerase on the Bio-Rad MyCycler thermal cycler (Bio-Rad, Hercules, CA). The PCR products were purified using the GFX columns (GE Healthcare, Little Chalfont, United Kingdom) prior to performing the cycle sequencing reaction using the ABI BigDye v3.1 and the ABI 3130 capillary DNA analyzer (ABI, Foster City, CA). For the detection of the Δ (GJB6-D13S1830), we used the primers *GJB6-1R* (5'-TTTAGGGCATGATTGGGGTGATTT-3') and reverse primer *BKR-1* (5'-CACCATGCGTAGCCTTAACCATTTT-3') using the conditions reported by del Castillo *et al.* (2002). A positive sample for the deletion, kindly provided by Dr. del Castillo and Dr. Guy Van Camp, was used as a positive control.

Results

We have obtained informative DNA sequencing data from 109 individuals. *GJB2* was amplified by PCR and subjected to automated DNA sequencing for affected individuals, followed by confirmation of the presence of the mutation in the parents and siblings where available. Twelve genetic variations in the *GJB2* sequence were found in 17 members of our cohort. Eleven patients with NSHL exhibited biallelic *GJB2* mutations, whereas six were found to have monoallelic *GJB2* variations. The mutations found were all previously reported (Fig. 1 and Table 1). The common, biallelic c.35delG mutation routinely found in patients of southern European or Mediterranean origins was found in seven patients in our cohort who were diagnosed with profound NSHL. The p.Trp24X truncation mutation, which is common in India and in the Gypsy population, was found in one patient with profound NSHL from our cohort. In addition, another patient exhibited compound heterozygote missense changes (c.11G>A, p.Gly4Asp and c.109G>A, p.Val37Ile). However, this patient suffered mild NSHL. A biallelic c.-34C>T change in the 5'-UTR was found in two unrelated patients. However, one of these patients (and his brother) had a homozygous p.Trp77Arg mutation. A p.Glu47X nonsense mutation was found in one patient but in a heterozygous state. A heterozygous c.-6T>A change in the 5'-UTR of *GJB2* was detected in one family, with the father, three boys, and one girl all exhibiting the same change. Despite this mutation, the father was found to have apparently normal hearing. A heterozygous c.-3179G>A splice site mutation in the first intron was detected in one patient with profound hearing loss. A monoallelic change at c.487A>G, p.Met163Val was detected in one patient, whereas another exhibited a c.35G>T, p.Gly12Val substitution as opposed to deletion. We could not detect the Δ (GJB6-D13S1830) mutation in our cohort. When consanguinity was examined, consanguineous marriages were the case for 8 out of 10 affected families. In addition,

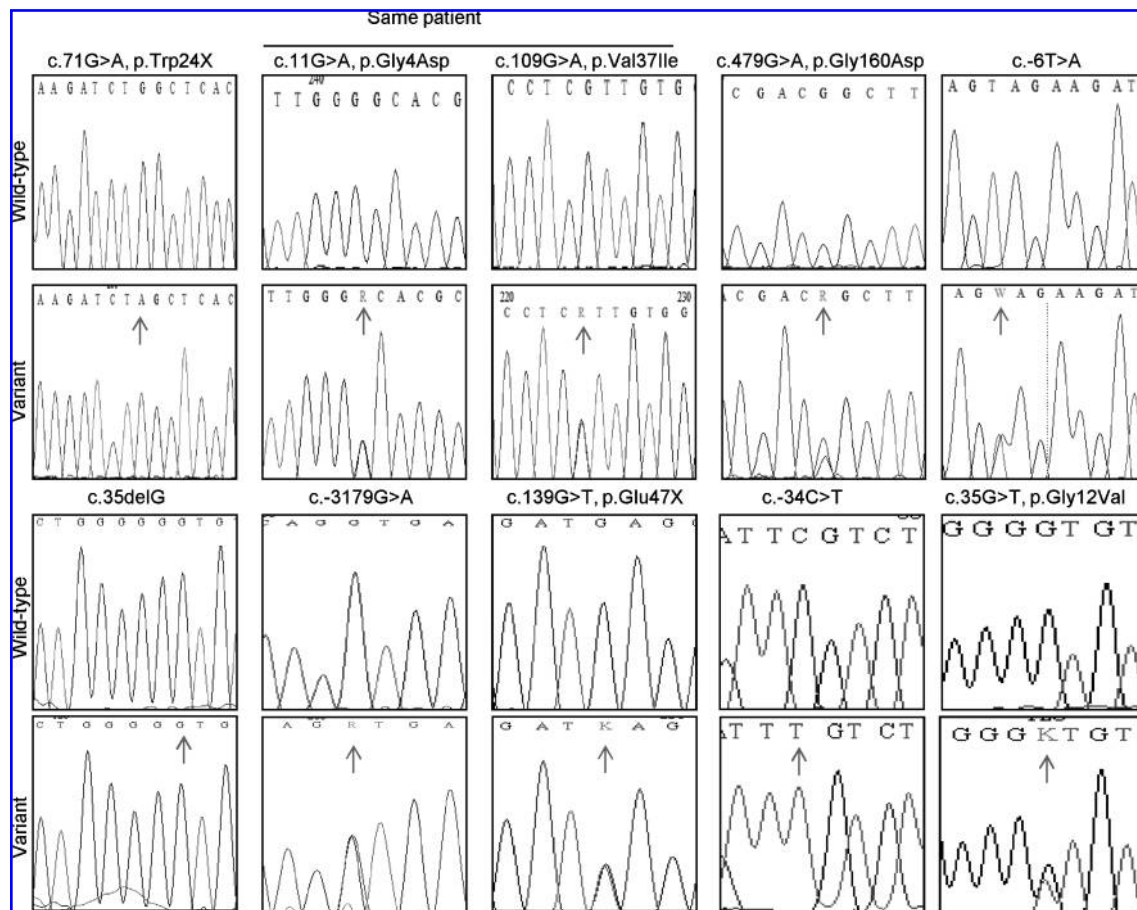


FIG. 1. Sequence chromatographs of the GJB2 sequence variants identified in our cohort. Nucleotide numbering is based on the ATG translation start site, with A considered as +1. Arrows indicate the site of nucleotide change.

another family had a same-tribe marriage. Taken together, *GJB2* mutations were detected in 17 unrelated deafness cases out of 109 (15.59%). Biallelic mutations, which are more likely to be the underlying cause of deafness, were detected in 11/109 (10.09%). The homozygous c.35delG mutation was found in 7/11 affected individuals (63.63%). For genotype–phenotype correlation, all the biallelic mutations identified were associ-

ated with profound hearing loss, with the exception of compound heterozygous p.Gly4Asp and p.Val37Ile which were associated with mild hearing loss only. The c.35delG mutation was found in the monoallelic state in 2 out of 37 individuals with apparently normal hearing (5.4%). The p.Val27Ile SNP was also identified in the heterozygous state in 2 out of 37 individuals with apparently normal hearing (5.4%).

TABLE 1. THE GJB2 MUTATION FREQUENCY IDENTIFIED IN OUR COHORT

Codon	Nucleotide change ^a	Affected individuals (n = 109)	Monoallelic/biallelic	Degree of NSHL	References
—	c.35delG	7	Biallelic	Profound	Kelsell <i>et al.</i> (1997)
p.Trp24X	c.71G>A	1	Biallelic	Profound	Kelsell <i>et al.</i> (1997)
p.Trp77Arg ^b	c.229T>C	1	Biallelic	Profound	Carrasquillo <i>et al.</i> (1997)
—	c.-34C>T ^b	2	Biallelic	Profound	Gasmelseed <i>et al.</i> (2004)
p.Gly4Asp ^c	c.11G>A	1	Monoallelic	Mild	Hwa <i>et al.</i> (2003)
p.Val37Ile ^c	c.109G>A	1	Monoallelic	Mild	Abe <i>et al.</i> (2000)
p.Met163Val	c.487A>G	1	Monoallelic	Profound	Marlin <i>et al.</i> (2001)
p.Gly12Val	c.35G>T	1	Monoallelic	Mild	Rabionet <i>et al.</i> (2000)
p.Gly160Asp	c.479G>A	1	Monoallelic	Profound	Janecke <i>et al.</i> (2002)
—	c.-3179G>A	1	Monoallelic	Profound	Denoyelle <i>et al.</i> (1999)
—	c.-6T>A	1	Monoallelic	Profound	Gasmelseed <i>et al.</i> (2004)
p.Glu47X	c.139G>T	1	Monoallelic	Profound	Denoyelle <i>et al.</i> (1997)

^aNucleotide numbering is based on the ATG translation start site with the A considered as +1.

^bThese two mutations were found in the same patient.

^cThese two mutations were found in the same patient.

NSHL, nonsyndromic hearing loss.

Discussion and Conclusion

There is an increasing demand for genetic testing of hereditary hearing loss among the population of Saudi Arabia. Lack of information about the genetic factors that predispose to NSHL in this population have so far hindered offering this service as part of the tasks of molecular diagnostic and genetic testing centers. Therefore, we have undertaken this study to examine the spectrum and frequency of mutations in the *GJB2/DFNB1* locus in the Saudi population to aid genetic counseling for families with NSHL as well as early intervention when inactivating mutations are discovered in newborns. According to our analysis, *GJB2* biallelic mutation frequency in the western region of the Kingdom of Saudi Arabia is estimated to be 10.09%. This frequency is more in line with the *GJB2* mutation frequency in non-European populations. However, the mutations found give a snapshot of the genetic heterogeneity of the urban population of the western province of Saudi Arabia caused by centuries of Hajj pilgrimage migration as well as economic migration and settlement in the port city of Jeddah and the surrounding areas from around the world. The c.35delG mutation is thought to be of southern European origin, whereas the p.Trp24X is mostly found in the Indian and Gypsy populations (Bouwer *et al.*, 2007). The c.-6T>A and c.-34C>T variations have been previously detected in Sudan and Kenya (Gasmelseed *et al.*, 2004). The c.229C>T, p.Trp77Arg, was previously reported in Palestine (Carrasquillo *et al.*, 1997). Identifying a case with compound homozygous *GJB2* mutations (-34C>T and p.Trp77Arg) is unusual and reflects either a case of chromosome 13 uniparental disomy (Yan *et al.*, 2007) or an extreme case of consanguinity and multiple generations of in-breeding. Unfortunately, for such a case, DNA from parents was not available for this study. Our cohort consisted mainly of Saudi nationals (92/109) and the others were from Yemen (12/109), Pakistan (2/109), Oman (1/109), Palestine (1/109), and Egypt (1/109). The selection of the Saudi patients in our cohort was not biased toward any particular ethnic origin and we attempted to have a representative sample of the population in the area. Interestingly, only the heterozygous p.Glu47X and the p.Met163Val carriers belonged to a family from Arab tribal areas, which may indicate that another genetic factor(s) other than *GJB2* is involved in causing the NSHL in the Arab tribal population. In addition, 5 out of 11 biallelic mutations identified were found in patients of Yemeni descent. This suggests that when deciding whether to screen a patient with hearing loss for *GJB2* mutation, the ethnic origin should be taken into consideration, that is, patients from the Arab tribes are not likely to carry *GJB2* mutations, whereas other Saudi patients of other ethnic origins, especially from Yemen or the Mediterranean, should be screened for *GJB2* mutations. Unfortunately, determining the ethnic origin of Saudi patients remains a sensitive subject that should be approached with care and consideration on a case-by-case basis. *GJB2* mutations were identified mostly in those patients with NSHL who had a family history of hearing loss and consanguineous marriages. Therefore, we recommend that NSHL sufferers be screened, along with their parents and siblings, for *GJB2* mutations only when hereditary NSHL is indicated by family history and consanguinity. Carriers should be provided with genetic counseling to help them make informed decisions regarding family planning. This is the first published report

on the genetic causes of NSHL in the Kingdom of Saudi Arabia. As *GJB2* mutations are not the major underlining genetic cause of NSHL in Saudi Arabia, the search is currently on for identifying such factors in the local population which should aid the genetic testing and diagnosis of NSHL in this community.

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Disclosure Statement

The authors have no conflicting commercial interests.

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Address correspondence to:
Ashraf Dallol, Ph.D.

Center of Excellence in Genomic Medicine Research
King Abdulaziz University
P.O. Box 80216
Jeddah 21589
Kingdom of Saudi Arabia
E-mail: adallol@kau.edu.sa

Mohammed H. Al-Qahtani, Ph.D.
Center of Excellence in Genomic Medicine Research
King Abdulaziz University
P.O. Box 80216
Jeddah 21589
Kingdom of Saudi Arabia
E-mail: mhalqahtani@kau.edu.sa

