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Clinical comparison of hearing impaired patients with DFNB1 against heterozygote carriers of connexin 26 mutations

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Abstract

Objective—The aim of the study is to assess clinical characteristics of individuals with non-syndromic sensorineural hearing loss (NSSNHL) with genetic mutations in *GJB2* and/or *GJB6*. We describe and compare one group with biallelic mutations against a group of heterozygote mutation carriers.

Subjects and Methods—350 patients between the ages of 3 months and 80 years referred to a tertiary care outpatient otology practice for NSSNHL were screened for genetic mutations. Direct sequencing of *GJB2* and PCR analysis of *GJB6* was performed and clinical data from history and physical, audiologic testing and radiographic studies were reviewed.

Results—Thirty two patients were found to have bi-allelic mutations (incidence of 9.1%). Twenty five patients were found to have only one *GJB2* mutation (incidence of 7.1%). Severe to profound hearing loss occurred in 85% of the homozygote group and 38% of the heterozygote group. Both groups similarly had a propensity towards bilateral, symmetric, non-progressive hearing loss with rare inner ear malformations on radiologic imaging.

Conclusions—These two patient populations have similar incidences in a cohort of patients evaluated for NSSNHL, which is higher than general population heterozygote carrier rates. Heterozygote mutation carriers had less hearing impairment but most other factors demonstrated no differences. These results support the theory of an unidentified genetic factor contributing to hearing loss in some heterozygote carriers. Therefore, genetic counseling should consider the complexity of their genetic factors and the limitations of current screening.

Level of Evidence 1b

Keywords

Connexin 26; connexin 30; GJB2; GJB6; DFNB1; non-syndromic sensorineural hearing loss; computed tomography; cochlear implant; molecular genetic testing

Introduction

The diagnosis of DFNB1 depends in the identification of biallelic deafness-causing mutations in *GJB2* and/or *GJB6* in subjects with non-syndromic inherited pattern hearing

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loss. The vast majority of patients with DFNB1 are either homozygotes or compound heterozygotes with two mutations in *GBJ2*, the gene that encodes connexin 26. Rarely, patients can have homozygous mutations in *GJB6*, the gene that encodes for connexin 30 or can carry one mutated allele from each gene in which case they are considered digenic heterozygotes.

Determining the etiology of congenital hearing loss is generally challenging. Risk factors elicited by clinical history and inner ear malformations are some of the ways to determine the cause of a patients hearing loss. More recently, molecular genetic testing has given clinicians an additional tool in the evaluation of these patients. Screening for mutations in connexin 26 and connexin 30 was previously available at large academic centers. Today, an increasing number of laboratories are offering these tests and it is likely to be offered to many more patients.

Molecular genetic screening for single-gene disorders such as DFNB1 provides a wealth of information. Besides providing a cause for their hearing loss, it can help with prognostication of the course of their disease as well as response to various treatment options. Additionally, a patient's parents will better understand the chances of having children with DFNB1, a carrier state or no mutations at all. The patient themselves will also understand the risks to their children.

As more patients with hearing loss are diagnosed with DFNB1, we are also identifying a group of individuals with hearing loss and only one *GJB2* mutation. These DFNB1 carriers pose a diagnostic dilemma. While these patients can have hearing loss and coincidentally be a carrier of a *GJB2* mutation, they can also have hearing loss with DFNB1 secondary to a novel non-*GJB2*, non-complementary mutation at the DFNB1 locus[1]. The role of unrecognized mutations is supported by the fact that mutation carrier rates in the general population are lower than the rate of heterozyous *GJB2* mutation carriers in the deaf population[2].

The purpose of this study is to determine the clinical characteristics of DFNB1 carriers presenting with hearing loss and compare them to patients found to have DFNB1. If the groups are overall very different, it would support the notion that these are true DFNB1 carriers with coincidental hearing loss and if the converse were true, it would support the hypothesis of an unrecognized genetic factor contributing to their hearing loss. This information would be helpful to the clinician and patient when molecular genetic testing fails to identify a clear etiology of hearing loss.

Materials and Methods

Patient evaluation

Subjects were recruited from an outpatient otology practice at the University of Miami. A total of 350 patients with non-syndromic sensorineural hearing loss (NSSNHL) agreed to molecular genetic testing for mutations of *GJB2* and *GJB6* by giving informed consent. This study was approved by the University of Miami IRB committee and the recruitment period was between 2002 and 2006.

Each patient underwent a full medical history and physical focusing on their otologic complaints. A questionnaire was completed by the patient or their family at the time of their enrollment with specific questions pertaining to their hearing loss, family history, previous radiologic evaluation and hearing habilitation.

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Age specific pure tone audiometry was obtained when possible, using equipment in accordance with International Standards Organization (8253-1-3) standards. Pure tone average (PTA) in decibels (dB) was calculated for each ear by averaging thresholds at three frequencies (0.5, 1, and 2 kHz) in accordance with recommendations of the GENDEAF study group and previous reports from our group[3].4.

Severity of hearing loss was then categorized as mild (21–40 dB), moderate (41–70 dB), severe (71–95 dB) and profound (95 dB) based on binaural PTA. Asymmetric hearing loss was defined as interaural PTA difference greater than 10 dB in at least two frequencies. Progression of hearing loss was defined as a greater than 15 dB loss in binaural PTA within a 10-year period.

Computed tomography (CT) was done with high resolution 1 mm contiguous axial and coronal images of the temporal bones. When available, images were reviewed by an otologist for inner ear abnormalities. Otherwise, radiologist reports were reviewed for pertinent findings.

Mutation Screening

All patients were screened for allelic variants in the coding exon (exon 2) of *GJB2* using polymerase chain reaction (PCR) amplification. For patients with heterozygous or no mutations in *GJB2*, further analysis of the non-coding exon (exon 1) was performed using direct sequencing. All patients were also screened for the 342-kb deletion of GJB6 using PCR amplification. DFNB1 was diagnosed if patients had either biallelic pathologic mutations of *GJB2*, biallelic deletions of *GJB6* as described above or one GJB2 pathologic mutation with the 342-kb deletion of *GJB6*. Patients with one identified DFNB1-associated mutation were DFNB1 carriers. To rule out a mitochondrial mutation cause of NSSNHL, all patients were also screened for the mitDNA A1555G and A7445G mutations.

Statistical Analysis

Descriptive analysis, one-way ANOVA and Fischer's exact test were used to compare DFNB1 patients and DFNB1 carriers. The level of significance used was $p = 0.05$. Statistical calculations were performed using Analyse-It computer software (Analyse-it Software, Ltd. Leeds, United Kingdom)

Results

A total of 350 patients were screened for genetic mutations of connexin 26 and connexin 30 after being evaluated for NSSNHL. Thirty two patients were identified with biallelic pathologic mutations in connexin genes (9.1%). From that cohort, only one patient had homozygous mutations for connexin 30 and two were digenic heterozygotes with mutations in connexin 26 and connexin 30. Twenty five patients were found to have only one allele pathologic mutation and classified as DFNB1 carriers (7.1%). All of these patient's mutations were in the *GJB2* gene. The DFNB1 carrier group had a slightly more males (64%) compared to the DFNB1 group (53%) but the difference was not significant ($p=0.58$). The spectrum of mutations for the DFNB1 is shown in Table I and for the DFNB1 carrier group in Table II.

Baseline hearing level

Overall the two groups demonstrated a wide range of hearing loss. Binaural pure tone average levels were calculated from the first complete audiogram. The DFNB1 group had significantly worse hearing (mean = 90dB HL; SD = 26) compared to the DFNB1 carrier group (mean = 62 dB HL; SD = 33) ($p < 0.01$). This difference is again demonstrated when

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categorizing the two groups by severity (mild/moderate versus mild/moderate). The vast majority of DFNB1 patients had severe/profound hearing loss (85%) compared to DFNB1 carrier patients (38%) ($p < 0.01$).

Mutations were categorized as either protein truncating (nonsense mutations and deletions or insertions leading to frameshifts) or non-truncating (missense mutations). The DFNB1 group was therefore divided in to three sub-groups: homozygous truncating (81%), homozygous non-truncating (13%) and heterozygous truncating and non-truncating mutations (6%). The DFNB1-carrier group was divided into two sub-groups: truncating (56%) or non-truncating (44%). One way ANOVA was performed to compare the PTA for each group but the difference only approached significance ($p=0.054$) indicating that no two groups had significantly different means. The largest difference in mean PTA level was between DFNB1 homozygote for truncating mutations and DFNB1 carriers with truncating mutations (34dB HL). The smallest difference was between the two sub-groups of the DFNB1 carrier group (5 dB HL). DFNB1 carriers with truncating mutations had better hearing than those with non-truncating mutations.

Progression of hearing loss

Progression of hearing loss could be determined for patients with serial audiograms or if a patient with hearing loss had previously passed their newborn hearing screening test. In the DFNB1 group, four patients had progression of hearing loss documented with serial audiograms and one patient had hearing loss despite passing their newborn hearing screening test. In the DFNB1 carrier group, one patient had progression of hearing loss documented with serial audiograms and one patient had hearing loss despite passing their newborn hearing screening test. This difference in rate of progression of hearing loss between the DFNB1 and DFNB1 carrier groups was not statistically significant ($p = 0.69$). Patients found to have progression of hearing loss in the DFNB1 group lost an average of 20 dBHL over an average of 57 months compared to those in the DFNB1 carrier group who lost an average of 15 dB HL over an average of 42 months.

Incidence of asymmetry

Asymmetric hearing loss was an uncommon finding in both groups. Five patients with DFNB1 presented with asymmetric hearing loss compared to seven patients who are DFNB1 carriers. There was no statistically significant difference between groups ($p=0.42$).

Radiographic evaluation

The vast majority of patient underwent radiographic studies to evaluate for inner ear malformations. All patients for which results were available, temporal bone computed tomography (CT) was performed except for one patient with DFNB1 who was evaluated with magnetic resonance imaging (MRI) for asymmetric hearing loss. Many patients were initially evaluated with CT imaging and then by MRI as part of the work-up preceding cochlear implantation.

Out of 27 patients in the DFNB1 group with available radiographic records, three patients had inner ear malformations (11%) including one Mondini malformation, one internal auditory canal with bulbous shape and one dilated vestibule and lateral semi-circular canal. In a similar and non-significant ($p = 1.0$) rate, one out of 18 patients (6%) in the DFNB1 carrier group had a Mondini inner ear malformation. All abnormalities from both groups were bilateral.

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Hearing habilitation

The majority of patients in this study were referred for a hearing aid evaluation upon diagnosis of hearing loss. One patient from the heterozygous group with mild hearing loss was rehabilitated using an FM system in school. Eventually, a significant number of patients received cochlear implants. In the DFNB1 group, fifteen of twenty six patients with recorded habilitation method received a cochlear implant (58%) in contrast to six of twenty four patients (25%) in the DFNB1 carrier group. This difference was statistically significant ($p < 0.05$).

Discussion

An evaluation of hearing loss entails a complex work up involving careful histories and physicals, audiologic testing and radiographic imaging. Molecular genetic testing offer a new tool to determine inherited causes for a patient's hearing loss and its availability is rapidly growing. There are many reports of the hearing characteristics of patients diagnosed with DFNB1[4,5], but none that focus of hearing impaired patients who are carriers for DFNB1 related genes. This group presents a complex set of issues because they could be coincidental carriers for the mutation or have an unrecognized second mutated allele at the DFNB1 locus.

In our study, the DFNB1 group had worse hearing loss and underwent treatment with cochlear implantation more frequently. These two variables are surely interrelated since profound hearing is an indication for cochlear implantation. This could be attributable to the higher frequency of truncating mutations in the DFNB1 group which is associated with worse hearing loss. The DFNB1 carrier group was a more heterogeneous cohortin terms of the broader spectrum of degree of hearing loss and proportion of truncating to non-truncating mutations. It is possible that patients from this group with better hearing may indeed be coincidental carriers while patients with worse hearing may possess an unrecognized mutation. Unfortunately, defining a clear boundary between the two may be difficult because of the variation of hearing loss both groups results in significant overlap.

The remaining variables studies demonstrated remarkable similarity between groups. No differences were noted in the incidence of asymmetry, inner ear radiographic abnormalities and patients who had progression of their hearing loss. This supports the theory that their hearing loss is of similar etiology.

The conclusions that could be drawn from these results are clinically valuable. Counseling patients with biallelic connexin mutations has been described. No literature outlines how to proceed with counseling of connexin mutation carriers who have hearing loss. Overall, they will have better hearing, end up with cochlear implants less frequently and rarely have inner ear malformations on imaging. Patients with non-DFNB1 related hearing loss have significantly more inner ear abnormalities than DFNB1 patients[6]. If patients present with better than profound hearing loss, a large proportion can suffer from progression of their hearing loss making frequent follow up necessary.

Advising DFNB1 carrier patients regarding the risks to future generations is also important. Patients and their parents must be advised that these screening tests lack 100% sensitivity. If there is indeed an unrecognized mutation responsible, then parents can have a 25% chance of having another hearing impaired child and the patient's offspring would be obligate carriers. Regardless, the siblings of a DFNB1 carrier should be evaluated for hearing loss to avoid a delay in diagnosis.

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Finally, when patients with hearing loss are found to be DFNB1 carriers, they should be considered for DNA banking. DNA extracted from white cell could be stored for future analysis. In situations where the sensitivity of genetic screening is not perfect, these patients may find consolation in the fact that future advances in testing may explain their hearing loss.

Conclusion

The rapidly expanding use and acceptance of genetic testing for NSSNHL adds a valuable tool to our diagnostic algorithm. Due to the variability of hearing loss, there is no clear and effective way to predict patients who will have DFNB1 and those who will be carriers. However, the similarity of other clinical data suggests that these two groups may share genetic etiologies for their hearing loss. Therefore, it is important to offer molecular genetic screening to all patients with NSSNHL and council patients who are DFNB1 carriers about the implications of these tests.

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Table I

Genotypes in 32 DFNB1 patients

allele pairs	number	type of mutation
35delG/35delG	21	Truncating/Truncating
35delG/Cx30del	2	Truncating/Truncating
167delT/167delT	1	Truncating/Truncating
35delG/IVS1+1G>A	1	Truncating/Truncating
Cx30del/Cx30del	1	Truncating/Truncating
35delG/W77R	1	Truncating/Non-Truncating
35delG644delT	1	Truncating/Non-Truncating
W44X/V37I	1	Truncating/Non-Truncating
Q124X/W77R	1	Truncating/Non-Truncating
G4D/V37I	1	Non-Truncating/Non-Truncating
P58R/H100Y	1	Non-Truncating/Non-Truncating

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Table II

Genotypes in 25 DFNB1 carriers

allele pairs	number	type of mutation
35delG/Wild Type	8	Truncating
167delT/Wild Type	5	Truncating
M34T/Wild Type	3	Non-Truncating
N206S/Wild Type	2	Non-Truncating
235delC/Wild Type	1	Truncating
A171T/Wild Type	1	Non-Truncating
L79Va/Wild Type	1	Non-Truncating
L90R/Wild Type	1	Non-Truncating
R143W/Wild Type	1	Non-Truncating
V37A/Wild Type	1	Non-Truncating
V37I/Wild Type	1	Non-Truncating