

Critical Review: Can carriers of a Cx26 mutation be detected through audiological assessment?

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The purpose of this critical review is to evaluate the current literature on audiological evaluation and identification of carriers of various mutations of the Cx26 gene which result in sensorineural hearing loss. Study designs include four randomized block designs and one between groups. Overall, the current literature provides very little evidence to demonstrate that carriers of a Cx26 mutation can be identified through audiological assessment using tools such as conventional audiometry, otoacoustic emissions, and auditory brainstem response. Future research needs to be completed to determine whether carriers of a mutation may be at greater risk for hearing loss due to environmental insult, and how that would impact clinical protocols.

Introduction

Deafness is the most common inherited disorder and congenital deafness affects approximately 1 in 1000 children (Morton, 1991). A number of genes contribute to different types of deafness; syndromic where a disease is present that is characterized by certain symptoms, and non-syndromic, where a hearing impairment is present with no other associated clinical features (Engel-Yeger et al., 2002). Eighty percent of the cases of hereditary deafness are recessive and non-syndromic.

Approximately 50% of cases of childhood non-syndromic recessive hearing loss is caused by mutations in the GJB2 gene which encodes the polypeptide components of gap junctions, which allow for the passive diffusion of water and small solutes between adjacent cells. Specifically, GJB2 encodes for the gap junction polypeptide known as connexin 26 (Cx26) (Resendes, 2001). More than 50 mutations have been reported for Cx26 with one of the most prevalent in the white population being a deletion of a single nucleotide in a string of six guanine residues that begins at nucleotide position 30 and ends at position 35 and is known as 35delG (Resendes, 2001). This creates a frame-shift mutation that results in premature translation termination.

Another mutation in Cx26 is W77R. This mutation consists of a thymine to cytosine transition, converting a tryptophan into arginine which leads to an inactive Cx26. The Cx26 produced by the W77R mutation causes an impaired intercellular coupling and it fails to assemble efficiently to form a gap junction channel (Engel-Yeger et al., 2002).

An additional nucleotide mutation in Cx26 is V37I, which comprises a guanine to adenine transition and results in an inactive Cx26 (Engel-Yeger, 2001). Cx26 is expressed within regions of the cochlea and plays a crucial role for its normal functioning as it occurs in gap junctions connecting all cell types in the cochlea. These gap junctions serve as the structural basis for recycling endolymphatic potassium ions that pass through the sensory cells during the transduction process.

The possible affected sites along the auditory pathway are not clear, and the features of each genetic group (non-carriers, carriers and homozygotes) or differences between the groups are not well defined. Outer hair cell impairment due to a Cx26 mutation may be indicated by otoacoustic emissions which are the signals from the cochlea occurring spontaneously and in response to sound stimulation. Auditory brainstem evoked potentials reflect auditory nerve and brainstem afferent activity initiated by the inner hair cells (Engel-Yeger et al., 2002).

The important role of the Cx26 gene in the pathogenesis of deafness emphasizes the need for a better understanding of the genotype-phenotype relationships in affected subjects. This would impact both clinical practice and genetic counseling for deafness. It is also important to investigate the group of carriers to determine any possible differences between carriers and non-carrier subjects, and determine whether carriers are a group in whom hearing thresholds may deteriorate more frequently than in non-carriers.

Objectives

The primary objective of this literature review is to critically evaluate the current literature on audiological evaluation and identification of carriers of

various mutations of the Cx26 gene which result in Sensorineural hearing loss. A secondary objective is to determine if carriers are a population more susceptible to environmental insults or progressive hearing loss, and how that would impact the clinical treatment of this group.

Methods

Search Strategy

Computerized databases including SCOPUS, MedLine, PubMed and Google Scholar were searched using the following search strategy: [(carriers) OR (heterozygotes) OR (obligate carriers)] AND [(connexin 26) OR (35delG) OR (hearing loss) OR (deafness)] AND [(identification) OR (DPOAE) OR (ABR) OR (threshold)]. The search was limited to the English language and human subjects. Reference lists in the obtained journals were also searched for any additional relevant articles.

Selection Criteria

Studies included in this critical review were required to investigate phenotypic differences between carriers of genetic hearing loss, non-carriers, and homozygotes. No limits were set on the methods of evaluation of phenotype or on the demographics of the research participants (age, gender, race, or socioeconomic status).

Data Collection

A review of the literature yielded five articles consistent with the selection criteria: two case control studies, two quasi-experimental studies and one between groups all of which provide a grade III level of evidence. Although the five studies provide good levels of evidence, they are not from very diverse research groups. Two of the five are from M. Wagenaar's research group in the Netherlands, while another two are from B. Engel-Yeger's group in Israel. The intent of this critical review was to evaluate all current literature available regarding genetic hearing loss, and phenotypic differences between carriers and non-carriers.

Results and Discussion

Case control study #1. Wagenaar, Rahe, van Aarem, Huygen, Admiraal, Bleeker-Wagemakers, Pinckers, Kimberling and Cremers (1995) evaluated seventeen carriers of autosomal recessive Usher syndrome type I with conventional pure tone audiometry measured at 0.5, 1, 2, 4 and 8 kHz through air and bone conduction. Genetic evaluation confirmed linkage to one of three mutations of Usher syndrome type I, and carriers were matched for age and sex with normal controls. Results revealed sensorineural hearing loss in all seventeen

carriers with an average threshold loss of 5 dB, however the methods were found to not be specific enough to be used for individual carrier identification of Usher syndrome type I.

This study failed to perform any audiological assessment beyond conventional audiometry. Additionally, the author's did not include the methods used to confirm linkage to a Cx26 mutation. To improve the design of this study, a larger group size, more detailed methods, and explanation of statistical analysis would be required.

Case control study #2. Wagenaar, Snik, Kimberling and Cremers (1996) further investigated audiometric evaluation of carriers of Usher syndrome type IB. Nine carriers comprising five women and four men were the parents of one or more children diagnosed with Usher syndrome type I. These participants were chosen based on gene linkage data from the Boys Town National Research Hospital, and all pedigrees showed linkage to chromosome 11q13.5, and therefore designated USHIB. The control group was comprised of 25 individuals (15 women and 10 men) with no history of hearing impairment.

Conventional Audiometry

Pure-tone audiometry was conducted using the automated Hughson-Westlake procedure with an Audioscan audiometer and headphones. The 50th and 90th percentiles for threshold values for presbycusis for each carrier and control participant were calculated at 1, 2, 4 and 8 kHz. A pure-tone average (PTA) of 20dB HL or less was considered normal. Results revealed that eight of the nine carriers had a PTA of 20dB HL or less, indicating normal thresholds, however four of the carriers were found to have thresholds exceeding 20dB HL at 4 and/or 8kHz, indicating the presence of a high frequency hearing loss. The individual thresholds were compared with the age and sex matched P50 values at 1, 2, and 8 kHz, and the average differences were calculated. The average measured thresholds of the carriers were 2.2dB above the P50 values with a standard deviation of 9.0 dB. A t-test was performed and revealed that the difference was not statistically significant. Measured thresholds of the control group were also compared to P50 values, and a difference of 2dB was found. These results suggest that carriers of the Usher syndrome type IB do not show characteristic audiometric abnormalities that would enable their identification by standard audiometric evaluation.

This study provided no statistically significant evidence that carriers of Usher syndrome type IB could be identified through characteristic audiometric abnormalities. Although it was concluded that carrier identification was not possible through audiometric

measures, it is notable that the participant count is low in this study, and various audiometric measures were not performed, such as otoacoustic emissions or auditory brainstem responses.

Quasi-experimental study #1. Engel-Yeger, Zaaroura, Zlotogora, Shalev, Hujeirat, Carrasquillo, Barges and Pratt (2002) examined differences in conventional audiometry between carriers of the 35delG mutation and non-carriers. Fifty six participants aged 10-80 years were divided into groups based on molecular findings: non-carriers (n=20), 35delG carriers (n=20), and 35delG homozygotes (n=16). All participants underwent pure tone audiometry, DPOAE evaluation and ABR testing.

Conventional Audiometry

Participants underwent pure tone audiometry at 250, 500, 1000, 2000, 4000 and 8000 Hz, with thresholds lower than 25dBHL considered normal. Thresholds of the two groups were comparable and within normal limits at all frequencies.

Evaluation of Otoacoustic Emissions

Each group underwent DPOAE testing, and each ear was tested twice with the better test used for statistical analysis. DPOAEs were classified according to response level as follows: less than -15dB SPL = no response, level 0; -15 to -11 dB SPL = hyporesponse, level 1; -10 to -5 dB SPL = hyporesponse, level 2; -4 to 4 dB SPL = normal response, level 3; 5 to 11 dB SPL = hyperresponse, level 4; 12 to 20 dB SPL = hyperresponse, level 5. For each group, the mean and standard deviation of the DPOAE response level for each frequency was calculated.

Effects of group on response levels were assessed using analysis of variance and the percentage of individuals responding in each frequency were determined. A significant difference in response levels between carriers and non-carriers was found at all frequencies with carriers having a lower response level than non-carriers. Among the carriers, 33-52% of responding subjects were within normal limits (levels 3-4) between 1000 and 4000Hz. Between 5000 and 10 000 Hz, carriers had the highest percentage of no response (47-90%).

Auditory Brain Stem Response

ABR waveforms of their fifty six participants were also investigated in an effort to determine if any differences exist in the latency of peaks I, III, and V, or the interpeak latencies between III and I and V and I between carriers of the 35delG mutation and non-carriers. Alternating polarity clicks were presented at 90dBnHL through insert earphones at rates of 10/sec and 50/sec. Potentials were recorded by 9mm silver

disk electrodes with the following placement: forehead (non-inverting), mastoid ipsilateral to the stimulated ear and contralateral mastoid (ground). Potentials during the 10ms following each click were filtered (100-3000 Hz), amplified (x100 000) and averaged (1024 sweeps). Each recording was made twice to assess reproducibility.

Group effects for each stimulus rate, and interaural effects were assessed by ANOVA with probabilities below 0.05 considered significant. Results indicated peak latencies and interpeak latencies for carriers and non-carriers within normal limits. No significant effects of subject group on latencies of peaks I, III and V, or on interpeak latency differences between III and I, V and I, or V and III at each stimulus rate were found, nor were they found for the effects of increasing stimulus rate.

This study provides level III evidence (Dollaghan, 2007) indicating that significant differences in DPOAE levels exist between carriers and non-carriers. A fairly large number of participants were included, and all underwent genetic testing to identify carriers and non-carriers, and stringent exclusion criteria were applied to prevent confounds such as pre-existing hearing loss. Appropriate statistical analyses also provide strong support for results suggesting that DPOAE level differences exist but concluding that conventional audiometry and ABR results do not differ among groups.

Quasi-experimental study #2. Engel-Yeger, Zaaroura, Zlotogora, Shalev, Hujeirat, Carrasquillo, Saleh and Pratt (2003) further investigated differences between carriers and non-carriers of various mutations that result in hearing loss. In the present study, carriers of three different Cx26 mutations were evaluated, 35delG, W77R and V37I. The methods used were the same as in their 2002 study with the exception that the participants were divided into the following six groups; non-carriers (n=24), carriers of the 35delG mutation (n=34), carriers of the W77R mutation (n=12), carriers of the V37I mutation (n=19), homozygotes to one of the mutations (n=24), and compound heterozygotes who carried two different mutations (n=15).

Conventional Audiometry

Results revealed that non-carriers and carriers had similar pure tone audiograms with thresholds that fell within normal limits across frequencies and indicated no significant differences between carriers and non-carriers.

Otoacoustic Emissions

Each group underwent DPOAE recordings, using the same methods and measurements as in their 2002 study. For each group, the mean and standard deviation of the DPOAE response level for each frequency were calculated.

Effects of group on response levels were assessed using analysis of variance and the percentage of individuals responding in each frequency were determined. A significant difference in response level of DPOAEs between non-carriers and all groups was found at most frequencies with carriers showing lower response levels than non-carriers. Differences across mutations were also found with carriers of the V77I mutation having significantly lower DPOAEs compared to non-carriers between 1000 and 7000Hz and carriers of both 35delG and W77R mutations having significantly lower DPOAEs compared to non-carriers between 1000 and 9000Hz. No significant level differences were found between the carrier groups.

Auditory Brainstem Response

ABR waveforms between carriers and non-carriers of three different Cx26 mutations: 35delG, W77R, and V37I were also analyzed. Following the same methods and testing protocol as outlined in their 2002 study, 128 carriers of one Cx26 mutation were examined. As previously found in their last study, no significant effects of group or stimulus rate were found.

This study provides further support that differences in DPOAE levels may provide insight into carrier status. By extending their 2002 study to include multiple Cx26 mutations, they provide level III evidence that carriers have lower DPOAE levels than non-carriers while pure tone thresholds and ABR's remain within normal limits.

Between Groups #1. Franze, Caravelli, Di Leva, Marciano, Auletta, D'Aulos, Saulino, Esposito, Carella and Gasparini (2005) examined carriers of the 35delG mutation for audiometric abnormalities. Thirty-one carriers for the 35delG and 28 normal hearing controls, matched for age, sex, geographical origin, and social class were included in the study. Molecular analysis of blood and saliva samples was performed to determine the presence and/or absence of the 35delG mutation through direct sequencing of amplified PCR fragments of the coding region of the GJB2 gene. Tonal audiometry was also performed through air conduction at 0.125, 0.25, 0.5, 1, 2, 3, 4, 6 and 8 kHz and through bone conduction at 0.125, 0.25, 0.5, 1, 2, 3, and 4 kHz. Measures were repeated three times at each frequency and were conducted in a sound booth with the same operator and equipment across participants. The mean and standard deviations of hearing loss were calculated at each frequency, and differences between means were

assessed using analysis of variance (ANOVA) with test significance set at 0.05. Main significant effects were further evaluated using the Newman-Keuls test at the level of $P=0.05$. Results revealed thresholds within normal limits across frequencies for the control group while the carrier group had thresholds within normal limits only up to 4 kHz with audiometric abnormalities characterized by hearing loss higher than 25dBHL at 6 and 8 kHz. The threshold differences at 6 and 8 kHz between the carriers and normal controls were statistically significant with p values of 0.00039 and 0.00035 for the left ear results and p values of 0.00018 and 0.00039 for the right ear results.

This study provides level III evidence that threshold differences exist between carriers and non-carriers, contrary to past research. Subjects underwent molecular analysis to confirm carrier or non-carrier status, and the two groups were matched for age, sex, geographical origin and socioeconomic status, allowing for accurate comparison of results between the two groups. Additionally, after the null hypothesis was rejected by the ANOVA, they accurately investigated the main effects with the Newman-Keuls post-hoc method. To further their results, DPOAE measures could have been made to determine whether significant differences in pure tone thresholds were consistent with differences in DPOAE levels.

Conclusion

Each of the five studies examined measured pure tone thresholds of carriers and non-carriers in an effort to determine if statistically significant differences exist. Of the five, only one study found there to be statistically significant differences in pure tone thresholds between 4kHz and 8kHz. The inconsistency in these findings suggests that carriers of a Cx26 mutation cannot be reliably identified by any type of threshold shift as measured by conventional pure tone audiometry. Auditory brainstem responses were another measure evaluated in two of the five studies. In both studies, no significant differences in morphology or latencies were found, ruling out ABR as a method of identifying carriers.

Distortion product otoacoustic emissions were measured and compared in three of the five studies, and significant differences in DPOAE levels between groups were found in two of the three. Both studies that found significant differences in DPOAE levels provided strong support and statistical analysis of the results, however, it is important to note that both studies were conducted by the same research group.

Another interesting finding across the studies is that significant differences in DPOAE levels were found at extended high frequencies, above those tested with pure tone audiometry. Future research could also examine extended high frequency audiograms in carriers and non-carriers to determine whether a threshold shift exists similar to the differences found in DPOAE levels. Since none of the studies performed conventional audiometry above 8000 Hz, it is unknown whether carriers have threshold shifts above this frequency. Although this may not provide sufficient evidence to suggest that high frequency audiometry would be able to detect carriers of a Cx26 mutation, it would provide some support to the differences in the DPOAE findings.

Clinical Implications

The identification and detection of carriers of genetic hearing loss are important in the diagnosis of congenital hearing loss of unknown aetiology, in counseling family members with regard to their likelihood of acquiring a hearing loss, or their likelihood of having children with congenital hearing loss (Stephens et al., 1995). The current literature tends to question whether carriers of genetic hearing loss are more susceptible to environmental insults such as noise or ototoxic chemicals, however, none have furthered their studies to investigate these possibilities. Should carriers have a higher susceptibility to noise induced hearing loss, or presbycusis, clinical management of these individuals would evolve to include more specific counseling and possibly more stringent steps taken to prevent exposure to such agents. Future research needs to examine these topics and results should be incorporated into clinical protocols regarding counseling and management of families that carry a Cx26 mutation.

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