Presymptomatic Diagnosis of Nonsyndromic Hearing Loss by Genotyping

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**Background:** Nonsyndromic hearing loss (NSHL) is the most common type of hereditary hearing impairment (HHI). It is genetically heterogeneous, and although the exact number of genes is not known, 38 loci have been identified. By cloning the relevant genes and studying the function of the encoded proteins at the molecular level, it may be possible to impact the habitation of persons at risk for HHI. Currently, for select families, presymptomatic diagnosis of NSHL by genotyping is possible.

**Objective:** To provide presymptomatic diagnosis of HHI to individuals in select families who have participated in linkage studies.

**Design:** In 2 large families with autosomal dominant HHI, genes for NSHL were mapped to chromosomes 6 (DFNA10) and 19 (DFNA4). In each family, the phenotype is one of progressive sensorineural hearing loss that begins in the individual’s mid-30s and progresses to a severe-to-profound loss requiring amplification. Presymptomatic diagnosis was requested by, and provided to, 19 at-risk persons in these kindreds.

**Results:** By reconstructing haplotypes through the use of short tandem repeat polymorphisms tightly linked to the disease gene, risk calculations and genetic counseling were provided to these persons.

**Conclusions:** By simple Mendelian genetics, the risk of inheriting a fully penetrant autosomal dominant NSHL gene from a single affected parent is 50% for each offspring. However, by reconstructing haplotypes in families in which an HHI gene has been localized, this risk can be changed substantially.


PROFOUND HEARING loss affects 1 in 1000 newborns in the United States, and by puberty, an equal number of children are similarly affected. With increasing age, prevalence figures continue to increase to such a degree that approximately 50% of octogenarians have hearing impairment. Causality can be difficult to determine. In approximately 50% of newborns with deafness, hereditary hearing impairment (HHI) is implicated. Similar epidemiologic studies for adults are lacking, and although environmental factors are clearly important, autosomal dominant forms of nonsyndromic hearing loss (NSHL) are common.

Within a family, the diagnosis of HHI typically evokes a flood of emotions, ranging from guilt and anger to acceptance or denial. Genetic counseling can help families by ensuring that the mechanisms of inheritance are properly explained and by offering impartial guidance and advice. Often, young adults at risk for hearing impairment or hearing-impaired parents with at-risk children wish to know whether hearing loss will develop. Risk calculations for at-risk persons may be determined by applying Bayes theorem to data reconstructed from family linkage studies (see Rosner and Ott for discussion of Bayes theorem; see Jorde et al, Terwilliger and Ott, and Conneally and Rivas for a discussion of linkage analysis).

Two large families in which postlingual, autosomal dominant NSHL is segregating participated in linkage studies. The results of these studies led to the localization of 2 different loci for autosomal dominant NSHL, DFNA4 and DFNA10 (the 4th and 10th autosomal dominant NSHL loci mapped, respectively). Markers tightly linked to these loci were used to reconstruct haplotypes, permitting presymptomatic diagnosis of HHI in family members not yet hearing impaired. The experience of the Molecular Otolaryngology Research Laboratories, University of Iowa, Iowa City, with presymptomatic diagnosis and its limitations are described.

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MATERIALS AND METHODS

DFNA4 AND DFNA10 FAMILIES

Families were ascertained through the Department of Otolaryngology at the University of Iowa Hospitals and Clinics. Localization of DFNA4 and DFNA10 is as described previously.11,12 Presymptomatic diagnosis was provided at no charge to family members who requested this service. All procedures were approved by the institutional review board.

GENOTYPING

DNA was prepared from peripheral blood lymphocytes. Amplification of short tandem repeat polymorphisms (STRPs) was performed by polymerase chain reaction using 30-ng DNA template and 1 µL of each STRP-specific primer (50 µmol/L). (Sequences for all primers are listed in the Genome Database.) DNA was denatured for 30 seconds at 95°C, annealed with primers at 55°C for 30 seconds, and extended with 1 µL of unlabeled deoxyadenosine triphosphate labeled with sulfur 35 (1 µL) was added with 1 µL each of 10-mmol/L deoxycytidine triphosphate and deoxyguanosine triphosphate. Deoxyadenosine triphosphate labeled with sulfur 35 (1 µL) was added with 1 µL of unlabeled deoxyadenosine triphosphate (0.1 mmol/L). Reaction products were resolved on a 6% polyacrylamide gel, followed by drying and autoradiography.

HAPLOTYPE RECONSTRUCTION

Once linkage between a polymorphic marker and the disease locus was established, flanking markers were determined, and within the candidate region, additional STRPs were analyzed. Haplotypes were reconstructed for all persons, and the chromosomal region that contained the disease gene was traced through the pedigree. For persons too young to have developed hearing impairment but who carry the affected chromosomal region, relative risk was calculated, assuming a recombination event on either side of the disease gene, thereby leaving flanking markers unchanged.

RESULTS

The exact locations of DFNA10 and DFNA4 are not known; however, the STRPs that flank the intervals that contain these genes are known. The STRPs within these intervals were used to reconstruct haplotypes. These STRPs divide the respective deafness intervals into smaller intervals of known distances. Although the position of the disease gene cannot be placed between specific pairs of STRPs (other than between the 2 flanking STRPs), a false linkage result can only occur in the case of a double recombination between adjacent STRPs, 1 on each side of the gene. The highest probability that this type of recombination will occur is within the largest interval of the haplotype, and can be at most equal to the distance of this interval. If each recombination is treated as an independent event, then the probability of a double recombination equals the product of 2 single recombinations, or the square of the largest interval. This assumption was used to calculate recurrence risks. Nineteen individuals were provided presymptomatic diagnosis (8 individuals from the DFNA4 kindred; 11 individuals from the DFNA10 kindred).

In nuclear family 1, affected by DFNA10, affected individual II1 has identical alleles for the STRPs D6S457, D6S270, and D6S292. Individual II1 in family 2 has identical alleles for the flanking STRP D6S292. Risk calculations must take this into account. Closed symbols indicate affected; open symbols, unaffected; question mark, phenotype unknown; squares, male; circles, female; and cM, centimorgans.

Figure 1. Genetic mapping for calculation of risk for hereditary hearing impairment in families 1 and 2. In the DFNA10-affected nuclear families, individual II1 in family 1 has identical alleles for short tandem repeat polymorphisms (STRPs), D6S457, D6S270, and D6S292. Individual II1 in family 2 has identical alleles for the flanking STRP D6S292. Risk calculations must take this into account. Closed symbols indicate affected; open symbols, unaffected; question mark, phenotype unknown; squares, male; circles, female; and cM, centimorgans.

| FIGURE 1 | Genetic mapping for calculation of risk for hereditary hearing impairment in families 1 and 2. In the DFNA10-affected nuclear families, individual II1 in family 1 has identical alleles for short tandem repeat polymorphisms (STRPs), D6S457, D6S270, and D6S292. Individual II1 in family 2 has identical alleles for the flanking STRP D6S292. Risk calculations must take this into account. Closed symbols indicate affected; open symbols, unaffected; question mark, phenotype unknown; squares, male; circles, female; and cM, centimorgans. |
not be affected is 10%. Alternatively, individual II2 has inherited the unaffected haplotype. The posterior probability that he will be affected will be 10%, while his children have a 5% probability of being affected. His grandson IV1 has a 2.5% chance of being affected. In nuclear family 2, the situation is similar, affected individual I1 has identical alleles for the flanking STRP, D6S292 (Figure 1). The posterior probability that his offspring II1 will be affected, given inheritance of the affected haplotype, is 90%. Individuals I2, I3, and I4 have not inherited the affected haplotype. The posterior probability that they will be affected is 10%.

In nuclear family 3, affected by DFNA4, the greatest distance between adjacent STRPs is 11.5 cM (Figure 2). As the probability of recombination is directly proportional to the genetic map distance, the DFNA4 gene may be arbitrarily placed within this interval to obtain the most liberal recurrence risks. The probability of a recombination between D19S208 and APOC2 can be at most 11.5/100, if the gene is very close to APOC2. Alternatively, the probability of a recombination between APOC2 and the gene can be at most 11.5/100. Treating the recombinations as independent events, the probability of a double recombination would be (11.5/100)\(2\). Individual I1 has inherited the unaffected haplotype. The posterior probability that he will be affected is 132.25/7964.5 or 1.66%. The probability that his son, II1, will be affected is (1/2)(132.25/7964.5) or 0.83%. In nuclear families 4 and 5, affected individuals II1 both have identical alleles for the flanking STRP, D19S412, similar to nuclear families 1 and 2 (Figure 2). Posterior probabilities are as shown in the adjacent table. In family 6, the affected individual, II1, has identical alleles for the spanning STRP, D19S208, eliminating it as an informative STRP. The largest effective interval within the haplotype would then be 17.5 cM, making the probability of a double recombination (17.5/100)\(2\). The posterior probability that individual II1 would be affected is 306.25/7112.5 or 4.31%, while for individual II2, it is 6806.25/7112.5 or 95.69%.

### COMMENT

In 1975, the American Society for Human Genetics defined genetic counseling as a process to help individuals (1) comprehend medical facts, (2) appreciate how inheritance contributes to a given disorder, (3) understand the concept of risk recurrence, (4) choose a course of action, and (5) make the best possible adjustment to the disorder and the recurrence risk. The most important elements of the counseling process include obtaining the best possible clinical and genetic diagnosis and establishing good rapport between the genetic counselor and the counselee.

The assessment of hearing impairment must include a thorough family history as distant as second-degree relatives. The possibility of syndromes should be explored by inquiries directed at psychomotor development, general health, and the presence of physical abnormalities, such as pigmentary alterations (hair, skin, eyes); structural malformations of the external ears, eye

<table>
<thead>
<tr>
<th>STRP</th>
<th>Distance to Next STRP cM</th>
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<tr>
<td>D19S414</td>
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<td>3</td>
</tr>
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</tr>
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<td>2</td>
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<td>7</td>
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<td>4</td>
</tr>
<tr>
<td>D19S412</td>
<td>...</td>
<td>6</td>
</tr>
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**Figure 2.** Genetic mapping for calculation of risk for hereditary hearing impairment in families 3 through 6. In the DFNA4-affected nuclear families, families 3 and 6 have haplotypes that require a double recombination event to yield a false result, while only a single recombination event is needed in families 4 and 5. STRP indicates short tandem repeat polymorphism; closed symbols, affected; open symbols, unaffected; question mark, phenotype unknown; squares, male; circles, female; and cM, centimorgans.
diseases, the presence of a cleft lip or palate, congenital heart disease, kidney malformations, or skeletal abnormalities. The obstetric history should clarify the health of the expectant mother, noting medications taken during pregnancy and any untoward perinatal events. Audiometry should be performed on the conselees, both normal-hearing and hearing impaired, including pure tones and otoacoustic emissions, and vestibular function should be evaluated. This information, complemented with a thorough physical examination, should establish whether a syndromal cause of hearing loss is likely, as the implications for recurrence risk and counseling issues differ compared with NSHL.

If NSHL is diagnosed, genetic counseling is possible using risk calculations based on empiric recurrence risks data derived from family studies. These calculations may be refined much further in families that have participated in linkage studies by using STRPs tightly linked to the disease locus to reconstruct the “affected” haplotype. However, the use of linkage studies in genetic counseling or presymptomatic diagnosis has limitations. A recombination event during meiosis may occur between a linked marker and the disease locus, yielding a false-positive result. This error may be minimized by using either 2 tightly linked markers to flank the disease locus or numerous STRPs within the region of interest, although a double recombination event may occur, again resulting in a falsely “affected” haplotype. Ideally, mutations should be sought in the relevant genes, but to date, this type of screening is possible for only a few types of NSHL.

Prenatal diagnosis also is possible, although it raises much more complicated and controversial questions than does presymptomatic diagnosis. Ramifications affect both medical ethics and personal moral values, with the focal point of this controversy lying in the possibility of pregnancy termination. Fritz Fuchs, MD, the codeveloper of amniocentesis, has stated that there is little point to prenatal diagnosis if prospective parents are unwilling to act on the results. This sentiment is echoed in the 1990 Ethical Issues Policy Statement on Huntington's Disease. However, the “targeted” genetic conditions were much more physically debilitating than HHI. In fact, most members of the deaf community do not believe they have a physical disability. This viewpoint is obviously at odds with the perception that genetic counseling and prenatal diagnosis have the “hidden agenda” of eliminating hearing impairment. The Council on Ethical and Judicial Affairs of the American Medical Association has stated that it is permissible for physicians to participate in genetic selection to prevent, cure, or treat genetic disease; however, the main goal of genetic counseling is not to reduce the incidence of genetic diseases, but to help individuals understand their genetic condition and make informed decisions. Therefore, regardless of the decision that may be chosen, the genetic counselor is ethically obligated to inform the prospective parents if a genetic problem could exist, even if the chosen course of action is in conflict with the counselor’s own moral values.

Genetic counseling for NSHL is unique, and persons who provide this service must be especially sensitive to the cultural, linguistic, and ethnic differences of the deaf community. Proficiency with American Sign Language is desirable, as more than 90% of deaf or hard-of-hearing individuals who seek genetic counseling prefer American Sign Language as a means of communication. Counseling must be nondirective—the difficulties faced by hearing parents of a deaf child should not be minimized, but the potential for a deaf person to lead a full and rewarding life must be made equally clear.

When deaf persons are provided accurate information to correct misconceptions about genetic counseling, many seek this service to learn more about their condition. In a survey of 659 individuals at Gallaudet University, Washington, DC, who sought counseling, most did so to learn the cause of their deafness and to know their chances of having deaf or hearing children. Although reproductive decisions were not altered, counseling did help prospective parents choose an appropriate environment in which to raise a family, as educational, social, and employment opportunities for the deaf vary greatly across the country.

Postlingually deafened persons requested genetic counseling for somewhat different reasons. Because hearing is often lost after several decades, most affected persons are less likely to integrate into the deaf community. They typically marry hearing spouses and seek counseling for the purpose of presymptomatic diagnosis for their children and close relatives to better prepare for the future.

In the large kindreds that participated in linkage studies that led to the localization of DFNA4 and DFNA10, the HHI did not present in affected individuals until middle age. Presymptomatic diagnosis was provided for several young adults who were at risk for hearing impairment; however, the majority of the diagnoses were provided for hearing-impaired parents with at-risk children who wished to know whether hearing loss would develop in their progeny. One parent expressed it well when she wrote that presymptomatic diagnosis would allow her children “to care for their hearing now and learn as much as they can both verbally and socially.” This knowledge also gave her the opportunity to teach her children “what they will have to learn while they still have their hearing (ie, lip reading and sign language).” But more important, it gives children “time to accept what is going to happen, hopefully, [so] give them a feeling of control [so] they can be successful in spite of [their hearing impairment].”

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REFERENCES