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A Recessive Mendelian Model to Predict Carrier Probabilities of DFNB1 for Nonsyndromic Deafness

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Abstract

Mutations in the DFNB1 locus, where two connexin genes are located (*GJB2* and *GJB6*), account for half of congenital cases of nonsyndromic autosomal recessive deafness. Because of the high frequency of DFNB1 gene mutations and the availability of genetic diagnostic tests involving these genes, they are the best candidates to develop a risk prediction model of being hearing impaired. People undergoing genetic counseling are normally interested in knowing the probability of having a hearing impaired child given his/her family history. To address this, a Mendelian model that predicts the probability of being a carrier of DFNB1 mutations, using family history of deafness, has been developed. This probability will be useful as additional information to decide whether or not a genetic test should be performed. This model incorporates Mendelian mode of inheritance, the age of onset of the disease, and the current age of hearing family members. The carrier probabilities are obtained using Bayes' theorem, in which mutation prevalence is used as the prior distribution. We have validated our model by using information from 305 families affected with congenital or progressive nonsyndromic deafness, in which genetic analysis of *GJB2* and *GJB6* had already been performed. This model works well, especially in homozygous carriers, showing a high discriminative power. This indicates that our proposed model can be useful in the context of clinical counseling of autosomal recessive disorders.

Keywords

hearing loss; recessive Mendelian model; predicting carrier probabilities; DFNB1; Bayes' theorem; *GJB2*; *GJB6*

INTRODUCTION

Hearing loss is the most common sensory defect in developed countries [Davis, 1997] and it is estimated that about 60% of the cases without an obvious environmental origin have a genetic basis [Marazita et al., 1993]. One infant in 650 is born with severe or profound deafness [Mehl and Thomson, 2002], and in the general population, the prevalence of hearing loss increases with age. Single-gene mutations can lead to hearing loss. In these cases, hearing loss is a monogenic disorder with an autosomal dominant, autosomal recessive, X-linked, or mitochondrial mode of inheritance [Willems, 2000]. About 80% of cases of congenital deafness are inherited in an autosomal recessive way [Van Camp et al., 1997].

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It is believed that more than one hundred genes could be involved in hearing impairment. Despite the fact that nearly 70 loci have been described for nonsyndromic autosomal recessive deafness (DFNB), a single locus, DFNB1 (MIM# 220290), accounts for a half of congenital hearing loss cases, with variable incidence among populations [Zelante et al., 1997]. The genes responsible for DFNB1 locus-linked deafness cases are *GJB2* (MIM# 121011) and *GJB6* (MIM# 604418), which encode the gap junction protein connexin 26 (Cx26) and connexin 30 (Cx30), respectively. Over 100 distinct mutations have been described in gene *GJB2* that are associated with deafness of different severity; most of these are early-onset (Connexins and Deafness Home-page; <http://davinci.crg.es/deafness>). The DFNB1 locus has also been linked to hearing loss with deletions involving the *GJB6* gene associated with hearing loss [del Castillo et al., 2002, 2005]. These deletions have been reported to cause deafness both in the homozygous and heterozygous state with a *GJB2* point mutation [del Castillo et al., 2002, 2005]. One particular Cx26 mutation, called 35delG, represents about 70% of all Cx26 mutations in the Caucasian population with relative frequencies ranging from 28 to 94% depending on the population [Denoyelle et al., 1997; Estivill et al., 1998; Kelley et al., 1998; Scott et al., 1998]. Due to the high prevalence of mutations in the DFNB1 locus, genetic counseling for deafness may be a beneficial outcome of deafness research (35delG mutation is present in over 40% of familial and sporadic cases of congenital deafness). This is particularly remarkable when the family history is compatible with an autosomal recessive mode of inheritance. Although two different genes are involved in the DFNB1 locus, they are located close enough to each other to consider them as a single locus. In addition, in several cases, one mutation in each of the two genes lead to a phenotype indistinguishable from the phenotype observed when two mutations in one of the genes are found [Snoeckx et al., 2005].

Medical geneticists may need to be prepared to respond to possible requests for prenatal diagnosis. Thus, a model specially designed for predicting the probability of being the carrier of a mutation could help them to determine whether to perform genetic tests. On the other hand, they may be interested not only in determining the probability of being deaf but also knowing as soon as possible if a child will develop the disorder. Thus, the quality of life and cognitive and psychosocial development of deaf children may improve if adequate educational plans and hearing aids are implemented at an early age. In general, people who are concerned with a family history of disease may seek genetic counseling to assess the risk of carrying a genetic mutation [Murphy and Mutalik, 1969]. In the case of families with a high proportion of hearing impaired individuals, genetic counseling's implications are not very dramatic. However, its knowledge may help decide correct management of the disease. So far, genetic risk calculations in families with autosomal recessive hereditary hearing loss are normally performed using empirical risk assessment with information only from parents of the proband. As an example, the subsequent offspring of a hearing couple with one deaf child and an otherwise negative family history of deafness has been estimated to be 18% of deafness in future children (www.geneclinics.org/profiles/deafness-overview/details.html).

Genetic counselors normally use statistical models that predict the probability that a counselee carries a mutation by using the counselee's reported family history of disease. Two different classes of modeling approaches for risk prediction are empirical or regression-based models and Mendelian models. The main difference between these approaches is that empirical approaches model the conditional distribution of testing positive (since the genotype is not observed) given the phenotype, while Mendelian approaches model the conditional distribution of the genotype given the phenotype and are built upon the conditional distributions of phenotypes linked to a given genotype (penetrance), and the marginal distribution of genotypes (prevalence) [Chen et al., 2004]. The probabilities for counseling derived from these approaches use both Bayes' rule and Mendel's law [Murphy and Mutalik, 1969; Elston and Stewart, 1971; Parmigiani et al., 1998; Antoniou et al., 2000].

Mendelian models have been applied in some syndromes caused by deleterious germline mutations of individual genes, mainly in cancer settings. As an example, a comparative study between eight empirical and Mendelian predictive models for predicting mutations in the *BRCA1* (MIM# 113705) and *BRCA2* (MIM# 600185) genes involved in breast cancer showed that Mendelian models perform better than empirical models [Marroni et al., 2004]. So far, neither empirical nor Mendelian models have been developed to predict mutations in families with a high prevalence of deafness.

The aim of the work presented in this report is to develop a Mendelian model to determine the probability that a proband carries a mutation at the *DFNB1* locus taking into account his/her family history of deafness. The model is a modification of the methodology described by Lange [1997] and implemented and discussed in Parmigiani et al. [1998] and in Chen et al. [2004] to accommodate the recessive Mendelian mode of inheritance. We validate our proposed Mendelian model by testing 305 families in which genetic information in the *DFNB1* locus is known.

MATERIALS AND METHODS

Notation and Assumptions

In this section we establish the notation and assumptions found in the work by Parmigiani et al. [1998] and Chen et al. [2004]. The family history includes the proband and his/her first- and second-degree relatives. For each member, we ascertain: 1) whether he or she has been diagnosed with deafness; and 2) for deaf individuals, the age at diagnosis, and, for hearing individuals, the current age or age at death. We assume that individuals inherit two *DFNB1* alleles, one from each parent, and that alleles are either normal or mutated. As *GJB2* and *GJB6* are only 50 kb apart, we considered that they are inherited together and that recombination between them is very unlikely. We also assume a recessive mode of inheritance of mutations, supported by the results obtained by Estivill et al. [1998].

At each locus, an individual can have zero, one, or two mutations. We denote by f the frequency of mutations in the allele population, for *DFNB1*. If we do not consider information about family history, the probabilities that an individual inherits a given number of mutated copies of *DFNB1* are $p(\text{DFNB1} = 2) = f^2$, $p(\text{DFNB1} = 1) = 2f(1 - f)$, and $p(\text{DFNB1} = 0) = (1 - f)^2$.

Let g_0 be the genotype of the counselee, denoting $g_0 = 2$ that the individual is a homozygous carrier, $g_0 = 1$ is a heterozygous carrier, and $g_0 = 0$ for wild-type status at the locus *DFNB1*. Let R be the number of relatives of the counselee and g_r ($r = 1, \dots, R$) their corresponding genotype vectors for the locus. Similarly, we define $h_0, h_1, \dots, h_R$ as the deafness status and ages of onset of the counselee and relatives. Thus, given a pedigree and a counselee, our goal is to compute the probability of the counselee genotype given the family history and pedigree structure, that is

$$p(g_0 | h_0, h_1, \dots, h_R). \quad (1)$$

We notice that previous probability may also depend on some covariates $X_1, X_2, \dots, X_R$ such as being of different ethnic origin (see Parmigiani et al. [1998] or Chen et al. [2004] for further details), but in our case we do not include this information although the model may deal with this information without problems.

The genotype probability of Eq. [1] may be computed using an instance of Bayes' theorem as follows:

$$p(g_0|h_0, h_1, \dots, h_R) = \frac{p(g_0)p(h_0, h_1, \dots, h_R|g_0)}{p(h_0, h_1, \dots, h_R)}. \quad (2)$$

In Eq. [2], the unconditional carrier probability $p(g_0)$ obtained from prevalence studies as is updated to incorporate information from the pedigree. The term $p(h_0, h_1, \dots, h_R|g_0)$ is the probability of observing the phenotypes for the whole pedigree (deaf or hearing) given the genotype of the proband. This probability may be complex to evaluate, but we can obtain a simplified form using the law of total probability as follows:

$$p(h_0, h_1, \dots, h_R|g_0) = \sum_{g_1 \dots g_R} p(h_0, \dots, h_R|g_0, g_1, \dots, g_R)p(g_1, \dots, g_R|g_0).$$

Then, we can assume that individual histories are conditionally independent given the genotypes and obtain:

$$p(h_0, h_1, \dots, h_R|g_0) = \sum_{g_1 \dots g_R} \left\{ \prod_{r=0}^R p(h_r|g_r) \right\} p(g_1, \dots, g_R|g_0). \quad (3)$$

This Eq. [3] provides the basic idea of how we decompose genotype information based on Mendel's law and deafness information based on known penetrances. The version of the integrating algorithm currently implemented in BayesMendel [Chen et al., 2004] is recursive, which is in theory the same as the peeling algorithm. (C code is available at <http://astor.som.jhmi.edu/BayesMendel/Rpackage.html> upon request). The term $p(g_1, \dots, g_R|g_0)$ may be obtained from Mendel's laws, in our case assuming that the mode of inheritance is recessive. On the other hand, when $p(h_r|g_r)$ is calculated for an autosomal dominant syndrome Chen et al. [2004] assume that both censoring process and death of causes unrelated to the syndrome are independent of latent time of phenotype. They also assume that censoring and deaths of other causes or time of censoring are the same for both wild types and mutation carriers. Both assumptions are very important when dealing with aggressive diseases such as cancer. However, in deaf patients, these assumptions are not as important since the probability of dying is independent of hearing impairment.

Chen et al. [2004] also stated that, $p(h_r|g_r)$ the phenotype-specific net penetrance. In the case of a recessive syndrome, and in particular if we consider profound hearing loss, the mutation in DFNB1 locus may be considered fully penetrant. Therefore, the likelihood contribution of an individual homozygous carrier ($g = 2$) is

$$f(t; g = 2) = \begin{cases} 1 & \text{if individual diagnoses at } [0, 1) \\ 0 & \text{if not} \end{cases} \quad (4)$$

where $f(t; g) = F(t + 1; g) - F(t; g)$, and $F(t; g)$ denotes the net penetrance of genotype g by age t . The net penetrance may be defined as the latent time to phenotype or, in other words, as the time to the development of a deafness (or other phenotypes) if there were no death or censoring [Chen et al., 2004]. The phenotype associated to biallelic DFNB1 mutations is usually nonprogressive but variable in the degree of hearing loss, mainly due to the nature of the mutations [Snoeckx et al., 2005]. Whereas in the most severe cases, hearing loss is usually detected during the first months of life, in mild cases hearing loss may be diagnosed at a later age. Thus, to model the different phenotypes (mild or moderate hearing loss), Eq. [4] could be modified considering the penetrance as age-dependent. As an example, in our cohort of patients we have observed that most of the cases with two DFNB1 mutations are diagnosed at birth. However, there is a small fraction that is diagnosed later (up to the age of 3 years) mainly due

to difficulties in realizing hearing impairment before the onset of speech. In order to take into account this fact we modified Eq. [4] as follows:

$$f(t; g = 2) = \begin{cases} 0.95 & \text{if individual diagnoses at } [0, 1) \\ 0.03 & \text{if individual diagnoses at } [1, 2) \\ 0.02 & \text{if individual diagnoses at } [2, 3) \\ 0 & \text{in other cases,} \end{cases} \quad (5)$$

which corresponds to the probabilities of being deaf at each age interval for patients with biallelic DFNB1 mutations in our data.

On the other hand, the likelihood contribution of an asymptomatic individual (noncarrier or heterozygous) of age t years is proportional to $1 - F(t; g \neq 2)$. This function may be seen as the deafness rates or penetrance in the heterozygous and noncarrier population, as a function of age. This information may be obtained from the literature or from epidemiological studies as we will illustrate in the next section.

Genetic Parameters and Deafness Rates

In general, we may be interested in computing the probability that the counselee is a carrier of two mutations in the DFNB1 locus mutation, given his/her family history. This is really useful in clinical practice because once the presence of two mutations is ascertained, then it is known that the child will be deaf (complete penetrance). That is,

$$\pi \equiv p(g_0 = 2 | h_0, h_1, \dots, h_r).$$

To compute this probability we have to know the mutation frequencies and deafness rates for wild-type and heterozygous carriers, who have been considered to have the same penetrance. Regarding the mutation frequency, carrier frequencies of *GJB2* have been extensively studied showing a wide heterogeneity depending on population group [Denoyelle et al., 1997; Gasparini et al., 1997, 2000; Zelante et al., 1997]. As an example, it has been observed that the mutations in this gene are more frequent in the south of Europe than in the north, showing an increasing gradient of 35delG frequencies from north to south [Lucotte and Mercier, 2001; Lucotte and Dieterlen, 2005]. Gasparini et al. [2000] determined that the frequency of genetic mutation in the *GJB2* gene ranged from 0.026 to 0.031 in Southern Europeans and from 0.018 to 0.007 in Northern and Central Europeans. On the basis of this, we have estimated a normal probability distribution of f , with a mean of 0.0284 for Southern Europeans and a mean of 0.0126 for Northern and Central Europeans, with 95% confidence intervals of 0.026–0.031 and 0.018–0.007, respectively. The assumed distributions of *GJB2* for two different European populations are shown in Figure 1. In the Results section, we carry out a sensitivity analysis in order to observe how this value can affect to the probability of being a carrier. On the other hand, no studies have yet been reported regarding the carrier frequency of *GJB6* deletions. In any case, its allelic frequency would be negligible with respect to *GJB2* mutations, as the allelic frequency in a hearing-impaired sample has been reported to be 1 out of 600 [del Castillo et al., 2005]. Thus, we assume that the allelic frequency in the DFNB1 locus to be similar to that of *GJB2* gene.

Deafness rates or penetrance for normal and heterozygous individuals might be estimated to compute π . To do so, information about epidemiological studies may be used. Estimates of the prevalence of hearing impairment vary widely across studies and age of the individuals. In addition, depending on the categorization criteria used, prevalence estimates may vary substantially [Niskar et al., 2001; Flamme et al., 2005]. Thus, in order to get accurate estimates of probabilities in Eq. [2], incidence deafness rates should be obtained from the country where

genetic counseling is planned to be implemented. In our case, we describe how to obtain this information from the Spanish National Disability, Impairment, and Handicap Survey. This study was conducted by the National Statistics Institute (Instituto Nacional de Estadística) in 1999 [Instituto Nacional de Estadística, 2001a]. The sample comprised residents in 80,000 dwellings. Information on the existence, type, and severity of disability for each member of the dwelling was recorded. Other additional sociodemographic variables were also obtained. Sampling was conducted in two stages, with census sections (stratified by province, size, and type of town or city) and socioeconomic level being selected in the first stage, and family dwellings in the second stage. For the calculation of hearing disability rates, intercensus Spanish population projections drawn up by the National Statistics Institute were used as the denominator [Instituto Nacional de Estadística, 2001b]. The numerator comprises those persons with total deafness in both ears (code 21 in the survey) that can be considered as suffering a form of severe deafness. In addition, we have also estimated the rates for moderate hearing loss (code 22 with severity 3 in the survey) in order to be able to incorporate uncertainty of disease rates. At this point, we should highlight that, in the model above described, only age-specific deafness rates (penetrances) are relevant up to age 3 years when calculating the probability of carrying the mutation. However, it is useful to have estimations for the whole range of ages to model other phenotypes as we have previously outlined.

Figure 2 shows smoothed cumulative rates of hearing impairment by age group, sex, and grade of severity for the general Spanish population. We have subtracted from the total number of individuals suffering deafness, the number that is probably due to homozygous carriers in *DFNB1* in order to estimate the penetrance for normal and heterozygous individuals. This number was 1 out of 650 newborns as estimated by Mehl and Thomson [2002]. In Figure 2, we can observe that males tend to have higher incidence rates than females and rates dramatically increase with age. This pattern is similar to those observed in other populations [Wilson et al., 1999; Flamme et al., 2005; Hannaford et al., 2005]. As an example, annual age-adjusted rate of severe hearing loss is 1.67 per 1,000 males aged from 6 to 65 years old, while the same rate for moderate hearing impairment is 2.35 per 1,000 males.

RESULTS

In this section we first illustrate our approach by using two families arising from a cohort of Spanish deaf families that will be later described in detail. Then, we discuss the validation study we carried out, including genetic information about this cohort.

We have developed an R function called “*dfnbpro*” that computes the probability of being a carrier of mutations in the *DFNB1* locus given a family history of deafness, as described in Eq. [1]. This function uses tools developed in the R library called “*BayesMendel*” and it will be available in version 2.0. A similar function for dealing with two genes is also available upon request. This package was originally designed specifically to address autosomal dominant syndromes. In particular, Mendelian models for two major cancer syndromes such as the breast-ovarian cancer (BRCA1/2) and the hereditary nonpolyposis colorectal cancer (HNPCC) syndrome have been developed. The package was designed to evaluate Eq. [2] using the “*aveG*” function for any arbitrary disease. Here, we have updated this function to accommodate the case of an autosomal recessive syndrome. Further details of the *BayesMendel* package and how it works can be found at <http://astor.som.jhmi.edu/BayesMendel> or in Chen et al. [2004]. The calculations, with a simple example, are described in the Supplementary Appendix (also includes Supplementary Table S1; available online at <http://www.interscience.wiley.com/jpages/1059-7794/suppmat>).

Application to Two Families With History of Hearing Impairment

We use two families to illustrate how our Mendelian model predicts the probability of being homozygous for the DFNB1 locus. Figure 3 corresponds to a family in which a recessive mode of inheritance is observed. This family will also be seen later as an example for sensitivity analysis. On the other hand, Figure 4, which corresponds to a family history with a dominant mode of inheritance, will be useful to validate how our model works when the genetic cause involves other genes different from those described in the DFNB1 locus. We also performed a probabilistic sensitivity analysis, varying allelic frequencies and incidence rates.

For Family 1, we computed the probability of being a homozygous carrier for the proband (indicated by “dfn-”) under five different scenarios with varying allele frequency. The baseline scenario makes reference to the prevalences estimated in the Spanish population. The other scenarios correspond to confidence intervals of prevalences estimated in northern and southern Europe. Table 1 shows that the probability of being a carrier of two mutations does not change over the different scenarios and we observe that, with a very high probability, the counselee would be considered to be carrying two mutations in the DFNB1 locus. These results agree with genetic testing that reveals he is homozygous in the *GJB2* gene. Regarding Family 2, similar results are obtained, taking into account that in this case the proband is a noncarrier in the DFNB1 locus (see Table 2).

We now consider a modified pedigree for Family 1, in which there is no information about the disease status of the proband's sister. We observe that the absence of this information highly decreases the probability of being a carrier (see Table 3). To analyze further how our model makes predictions, let us now consider that we are interested in predicting the probability of the proband's mother, who is a heterozygous carrier for the DFNB1 locus, being a carrier. Our model predicts that the probability that the proband's mother is heterozygous at the DFNB1 locus is 99.4% or 95.8% depending on using severe or moderate deafness rates (data not shown).

Finally, Figure 5 shows a probabilistic sensitivity analysis based on the distribution of allelic frequency given in Figure 1. Box plots represent the distribution of the probability that the proband of Family 1 and 2 carries two mutations in the DFNB1 gene, with their age at onset being changed. Each box plot is based on the same Monte Carlo sample size of 500. The observed variability is due to uncertainty in deafness rates and in allelic frequency. We deal with this uncertainty as follows: for each Monte Carlo sample, we consider that deafness rates may be either those corresponding to severe hearing impairment or those corresponding to moderate hearing impairment that are shown in Figure 2. In addition, in order to take into account the high heterogeneity observed in the allelic frequency of the *GJB2* gene, a random value from a normal distribution with parameters observed in Europe (including both Southern and Northern Europe) is used in each simulated sample. We observe that in Family 1, the probability of being homozygous strongly depends on the age of onset. This probability is negligible if the age of onset is 5 years of age, and more than 80% for those cases diagnosed at 0, 1, or 2 years old. Furthermore, we also observe big differences between these years regarding their confidence limits. Considering Family 2, we realize that unless the proband is assumed to be deaf at age 0 (that is, congenital deafness) the probability of being a carrier is negligible.

Validation Study

This section addresses the study of performance of the model proposed in a cohort of Spanish deaf families. Totally we collected 446 unrelated cases, 316 families (with at least six family members), and 130 sporadic cases affected with different degrees of sensorineural hearing loss from different clinical centers. Family history was recorded using information provided by the

proband. The collection and analysis of the samples was performed in accordance with the approved ethics rules for genetic studies. Informed consent for analysis was obtained from all members who participated in the study. Among the 316 families from our cohort, 215 (68.0%) of the pedigrees showed an autosomal recessive segregation pattern, 97 (30.7%) were autosomal dominant, one (0.3%) was X-linked, and in three (1.0%) families we cannot establish the mode of inheritance by looking at the pedigree. A total of 11 of the families were syndromic, so we finally analyzed a total of 305 families affected with congenital or progressive nonsyndromic deafness.

Total DNA was extracted from peripheral blood using standard procedures. The samples were tested for the presence of mutations in the coding region of the *GJB2* gene and deletions in the *GJB6* gene. Mutation detection for *GJB2* was performed by direct sequencing of the entire coding region. To detect *GJB6* deletions, a specific PCR assay was used [del Castillo et al., 2005]. The prevalence of DFNB1 mutations in our sample was the following: 27 probands (8.8%) carried two mutations at DFNB1. Of these, 25 had two mutations in the *GJB2* gene (18 [72%] 35delG homozygote) and the other two are a compound heterozygote *GJB2/GJB6*.

The probability of carrying a DFNB1 mutation, π , was calculated in each pedigree using the Mendelian model previously described. We then compared these probabilities with the results obtained from genetic testing. The receiver operating characteristics (ROC) curve area, sensitivity, specificity, negative predictive value (PV-), and positive predictive value (PV+) were calculated in order to study the accuracy of the predicted probabilities. Figure 6 shows the ROC curve for our model. The area under the ROC curve is 0.78 and the sensitivity and specificity are 76.0% and 83.1%, respectively. The PV- using the same threshold is 97.3% whereas the PV+ is 30.2%. To further investigate how the model performs, it is interesting to study the characteristics of families where the predicted probability of being homozygous does not agree with the observed genotype for DFNB1 locus. First, there are 6 out of 27 probands with two identified DFNB1 mutations with a low predicted probability of being homozygous. Four of the cases are carriers of either 35delG and another nontruncating mutation in *GJB2* gene or homozygous for the M34 T mutation, which has been associated with a milder phenotype compared to homozygous 35delG cases [Snoeckx et al., 2005]. In two other cases the predictions are not correct because the pedigree is not informative. Second, most of the probands who were negative in the genetic test but with a high predicted probability of being homozygous for the DFNB1 locus were congenital cases with a clear recessive pattern of inheritance; these are probably due to mutations in other genes.

DISCUSSION

In this report we have proposed a model to compute the probability that a proband is a carrier of a genetic mutation in the DFNB1 locus that has been associated with nonsyndromic deafness. This model incorporates information about a proband's family history of deafness and ages of onset in first- and second-degree relatives. We modified a Bayesian approach developed in the context of autosomal dominant diseases such as breast or colorectal cancer to accommodate an autosomal recessive inheritance. This model also considers uncertainties about family members' genetic status and uncertainties about the prevalence of mutations.

The penetrance and prevalences used by the model are stored in an external file, which is the input to the program. Thus, the user can modify these values to customize computation for any population. As an example, one can modify the prevalence mutation parameters to compute carrier probabilities in a Northern European country where very different prevalences for DFNB1 mutations are observed. In that way, our proposed model may also be useful to model other diseases in which a recessive mode of inheritance can be assumed, just by changing the allelic frequency and penetrance for the normal and heterozygous carriers.

We have focused our work on the DFNB1 locus, where two connexins genes are located, *GJB2* and *GJB6*, which are the genes most frequently associated with hearing impairment [Kenneson et al., 2002]. The phenotype associated with the DFNB1 locus is normally characterized by a moderate to severe congenital deafness. However 10% of persons with *GJB2*-related deafness have mild to moderate hearing loss [Snoeckx et al., 2005]. The detection of DFNB1 carriers with mild hearing impairment could not be as accurate as for a more extreme phenotype. In addition, another limitation of our model is the existence of additional genes with a similar mode of inheritance that can modify the probability of being homozygous for these genes, as we have observed in the validation study.

Due to the increasing knowledge regarding the molecular genetics of deafness in recent years, it is likely that diagnostic, carrier identification and possibly, prenatal genetic testing for deafness-causing genes will become part of routine clinical practice. Therefore, the development of an accurate pretest determination of carrier probability will enable us to detect families with a high probability of having deaf children due to DFNB1 mutations. It is specially relevant in predicting a priori the probability of having a hearing impaired child, as it is extremely important to detect the defect as early as possible to take the appropriate actions in terms of educational resources or hearing aids. This model could help genetic counselors not accustomed to dealing with risk assessment calculations based on family history to estimate gene mutation probability in a more consistent way. Moreover, this estimation would be an important initial task for risk counselors, allowing for more focused management of patients. The model developed here is robust and gives good results, especially when homozygous DFNB1 cases are considered. A negative result, however, does not imply a total absence of risk, as genetic susceptibility cannot be ruled out because other deafness genes may be involved.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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FIGURE 1.
Assumed probability distribution of *GJB2* mutation frequencies by European region.

FIGURE 2.

Cumulative net penetrance for both non- and heterozygous carriers (estimated from prevalence study) per 1,000 inhabitants of deafness in Spain by grade of severity and sex.

ID: 51 Name: S26

FIGURE 3.

Hearing impairment pedigree number 1, in which a recessive mode of inheritance can be assumed. Solid symbols indicate clinically deaf individuals and open symbols unaffected individuals. The number corresponds either to current age or age of onset, depending on whether deafness has been diagnosed.

FIGURE 4.

Hearing impairment pedigree number 2, in which a dominant mode of inheritance can be assumed. Solid symbols indicate clinically deaf individuals and open symbols unaffected individuals. The number corresponds either to current age or age of onset, depending on whether deafness has been diagnosed.

FIGURE 5.

Probabilistic Monte Carlo sensitivity analysis for Family 1 and 2. Box plots represent the distribution of probability that the proband carries two mutations at DFNB1 locus, with his age of onset changing. The variability is due to uncertainty in the deafness rates and in the allele frequency.

FIGURE 6.
ROC curve of 305 Spanish families affected with hearing impairment.

TABLE 1

Predicted Probabilities for Proband in Family 1 Varying Allelic Frequency*

Allele frequency	DFNB1		
	Homozygous	Heterozygous	Noncarrier
Baseline (Spain) 0.0250	0.997	0.001	0.002
South Europe 0.0312	0.997	0.001	0.002
South Europe 0.0256	0.997	0.001	0.002
North Europe 0.0182	0.995	0.001	0.004
North Europe 0.0070	0.976	0.001	0.023

* The proband was homozygous for the 35delG mutation.

TABLE 2
 Predicted Probabilities for Proband in Family 2 Varying Allelic Frequency*

Allele frequency	DFNB1		
	Homozygous	Heterozygous	Noncarrier
Baseline (Spain) 0.0250	0	0.044	0.956
South Europe 0.0312	0	0.053	0.947
South Europe 0.0256	0	0.045	0.955
North Europe 0.0182	0	0.033	0.967
North Europe 0.0070	0	0.014	0.986

* The proband was a noncarrier in the DFNB1 locus.

TABLE 3

Predicted Probabilities for Proband in Family 1 in Which There is no Information About the Disease Status of the Proband's Sister*

Allele frequency	DFNB1		
	Homozygous	Heterozygous	Noncarrier
Baseline (Spain) 0.0250	0.94	0.02	0.04
South Europe 0.0312	0.96	0.01	0.03
South Europe 0.0256	0.94	0.02	0.04
North Europe 0.0182	0.90	0.02	0.08
North Europe 0.0070	0.46	0.03	0.51

* Results varying allelic frequency and degree of deafness. The proband was homozygous for the 35delG mutation.