Objective: To evaluate difficulties encountered in genetic counseling in deaf children carrying connexin 26 gene (CX26 or GJB2) mutations.

Design: Prospective study.

Setting: Outpatients, tertiary referral center.

Patients: Ninety-six unrelated deaf children in whom CX26 mutations had been detected consecutively. Children were recruited to a center for genetic counseling for deaf children, and all had congenital deafness, sporadic or familial.

Results: In 63 children, deafness was clearly a DFNB1 form with autosomal recessive inheritance: 47 of the 63 were homozygous for the most frequent mutation, the deletion of G at position 35 (35delG); 16 of 63 carried on both alleles of CX26 frameshift or stop mutations, or missense mutations affecting a critical region of the gene. In 33 of 96 children, genetic counseling was difficult: 21 of 33 had a single mutation detected, 11 of 33 had new missense mutations or mutations whose pathogenicity remains debated in the literature, and 1 of 33 had a genotype with both a recessive mutation (35delG) and a mutation acting as a dominant mutation.

Conclusions: Interpretation of results for the molecular diagnosis of mutations in the connexin 26 gene is difficult in almost one third of cases. Close collaboration between geneticists familiar with deafness and otolaryngologists is essential to provide a high standard of genetic advice.


SENSONEURAL DEAFNESS present at birth affects 1 child in 1000 in developed countries.1 Until a few years ago, genetic forms were thought to account for 30% to 45% of cases of congenital deafness, about two thirds of these cases being isolated, ie, nonsyndromic, genetic forms.2-4 The remaining cases were attributed to environmental causes (27%-35%) or considered sporadic cases of deafness for which no cause had been identified (25%-35%).2-4

See also pages 937 and 941

In 1997, an unexpected discovery concerning nonsyndromic forms modified the knowledge of the causes of congenital deafness and provided a new tool for investigating these causes. The nonsyndromic genetic cases are mainly autosomal recessive forms (DFNB forms). Twenty-eight DFNB genes (called DFNB1 to DFNB29) have so far been mapped to the human chromosomes; 7 of them are identified,3 one of which is the connexin 26 gene (CX26 or GJB2) underlying the DFNB1 form.6 We and others showed in 1997 that this gene, CX26, was responsible for half of the DFNB forms in a multicentric analysis performed on families from France, the United Kingdom, and New Zealand, and in a study of families from Spain and Italy.7-9 One specific mutation, 30delG, actually referred to as 35delG (the deletion of G at position 35), creating a frameshift at the beginning of the protein, was found to account for about 70% of all CX26 mutations.7 Studies of the prevalence of 35delG in Mediterranean countries and the United States showed that this mutation has a high prevalence in the control population (normal-hearing heterozygous carriers, 2%-4%), similar to that of the most frequent disease mutation reported to date, the ΔF508 mutation of the cystic fibrosis transmem-
PATIENTS AND METHODS

PATIENTS

We recruited patients from genetic counseling consultation for deaf people at the Pasteur Hospital and at the Armand-Trousseau Children’s Hospital, Paris, France, from September 1, 1997, through December 31, 1999. The CX26 mutations were identified in 96 unrelated families (126 deaf subjects tested for CX26 mutations) affected by a nonsyndromic mild to profound prelingual deafness (ie, with a supposed onset before 2 years of age). Forty-six families had a single deaf child (sporadic case of deafness). Affected members had mild deafness in 4 families, moderate in 8, severe in 13, and profound in 35, and 16 families had members with different degrees of deafness. In 50 families, 2 or more individuals were affected: 47 (74 deaf individuals) had an autosomal recessive, 1 an autosomal dominant (2 deaf individuals), and 2 an uncertain mode of inheritance (4 deaf individuals).

METHODS

The protocol of this prospective study was accepted by the Consultative Committee for People Protection in Biomedical Research according to the French legislation, and informed consent was obtained from all subjects and from parents of underaged patients.

In each patient, a complete medical history was obtained to determine the age at onset of deafness and to exclude the possibility of environmental causes, such as maternal fetal infection, perinatal complications, meningitis, mumps, prenatal or postnatal drug ototoxic effects, and acoustic trauma. The deaf subjects underwent an otorhinolaryngologic and a general examination with a systematic search for signs suggestive of a syndromic form of deafness (especially dysmorphosis, integumentary disorders, and branchial, cardiac, and thyroid anomalies). They also underwent an ophthalmologic evaluation (including funduscopy) and a search for hematuria and proteinuria.

Deaf children and their parents underwent pure-tone audiometry with a diagnostic audiometer in a sound-proof room, with recording of pure-tone air- and bone-conduction thresholds. Air-conduction pure-tone average (ACPTA) threshold in the conversational frequencies (0.5, 1, and 2 kHz) was calculated for each deaf ear and was used to define the severity of deafness: mild (20 dB<ACPTA≤39 dB), moderate (40 dB<ACPTA≤69 dB), severe (70 dB<ACPTA≤89 dB), or profound (ACPTA ≥90 dB). The severity of deafness in each child was defined by the degree of hearing loss for the best ear.

Blood samples were obtained from deaf children and their parents, and the DNA was extracted. The entire coding phase of CX26 (exon 2) and flanking acceptor splicing site were amplified by polymerase chain reaction, with the use of primers GAP1F (5’-CCTAT-GACAACTAAGTTGGTTC-3’) and P50, antisense (5’-GACAGCTGACCGGGTTGCCT-3’). The CX26 exon 1 and flanking donor splicing site were amplified with primers PPI18 (5’-TCCGTAACCTTTCCCGTCTCCGAGGAGG-3’) and PP21, antisense (5’-CCAAAAGAGGCTGTGTTGGTCAGGCCCC-3’). The polymerase chain reaction products were sequenced. Experimental conditions for polymerase chain reaction and sequencing were as previously described.7 After January 31, 1999, the molecular diagnosis of CX26 mutations was performed by the Laboratory Pasteur-Cerba, Cergy-Pontoise, France, by the same methods.

A set of control DNA samples from 116 unrelated individuals living in France (232 chromosomes) was screened for mutations in the coding part of CX26.

brane conductance regulator gene responsible for cystic fibrosis.9,12 Two other CX26 mutations are highly prevalent in other populations: the deletion of T at position 167 (167delT) in Ashkenazi Jews (4%) and the deletion of C at position 235 (235delC) in Japan (1%-2%).11,13

In a prospective analysis performed on families from France, we observed that 51% of the families with prelingual DFNB forms of deafness were DFNB1 forms caused by biallelic CX26 mutations.16 Moreover, we showed that DFNB1 forms accounted for 31% of the sporadic cases of congenital deafness classified as being of unknown origin. We can estimate that the majority of congenital cases of deafness are genetic autosomal recessive forms. These data demonstrate the value of looking for CX26 mutations in sporadic cases to document the genetic nature of the disorder and thereby inform the families of the risk of other affected children.

New epidemiologic studies of factors causing congenital deafness will have to consider this high proportion of DFNB1 forms among sporadic cases and to include, in the etiologic evaluation, the search for CX26 mutations.

Molecular diagnosis of CX26 mutations is now available in the majority of developed countries, and this new ability to investigate the etiology of congenital deafness has profoundly modified daily medical practice. This test forms part of the investigations of the etiology of congenital deafness, as long as the families agree to it. After clinical examination and laboratory tests to identify an extrinsic cause or a syndrome associated with the deafness (see the “Patients and Methods” section), the molecular diagnosis of CX26 mutations can be proposed if the phenotype of the deafness is compatible with DFNB1 forms: nonsyndromic congenital deafness with no associated radiologic anomaly of the inner ear and no vestibular symptoms (walking before 18 months, no episode of vertigo, and, if available, normal results of vestibular calorick tests).16

However, detection of CX26 mutations does not always indicate the involvement of the gene in the cause of the deafness: some deaf patients only have a mutation on one allele, while others have mutations that are not known to be definitely pathologic. Clinicians treating deaf children must be aware of these diagnostic pitfalls and be very careful in the information they provide to the families. We detail in this prospective study the various mutations found in 96 unrelated families. The interpretation of the results are discussed to help genetic counseling in deaf individuals carrying CX26 mutations.
RESULTS

RESULTS IN DEAF INDIVIDUALS FROM THE 96 AFFECTED FAMILIES

Considering 1 deaf individual in each family and allowing 1 independent allele in the 2 consanguineous families, we found 169 of 190 mutated alleles, and the 35delG mutation accounted for 75.1% (127/169) of the mutated alleles.

Deafness was clearly of a DFNB1 form in 63 (65.6%) of the 96 families. In 47 of these 63 families, deaf individuals were homozygous for the 35delG mutation. The homozygous genotype 35delG was detected in 27 (58.7%) of the 46 families with autosomal recessive mode of inheritance, 18 (38.3%) of the 47 families with a sporadic case of deafness, and 2 of 2 families with uncertain mode of inheritance. In 16 of 63 families, deaf individuals carried, on both alleles, mutations considered to be pathologic because they either produced a premature stop codon or affected the splicing site or initiator codon (Table 1).

In the remaining families (33/96), genetic counseling was difficult: deaf individuals had either mutations on a single allele (21 families) or biallelic mutations of which one or both were missense mutations of uncertain pathogenicity (12 families) (Table 2). One of these families had a deletion of a guanosine in a sequence of 6 G extending from position 30 to 35.

Table 1. Biallelic Mutations in 63 of 96 Families Indicating DFNB1-Type Deafness

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Description of Mutations (Except 35delG)</th>
<th>No. of Families</th>
<th>Mode of Inheritance</th>
<th>Degree of Deafness (Best Ear)</th>
</tr>
</thead>
<tbody>
<tr>
<td>35delG/35delG</td>
<td>...</td>
<td>47</td>
<td>AR, 27; SP, 183;</td>
<td>Pr, 29; S, 10; mod, 7; mild, 1</td>
</tr>
<tr>
<td>35delG/-3170 G to A</td>
<td>G to A position +1 of intron 1 (splicing site)</td>
<td>2</td>
<td>AR, 1; SP, 1</td>
<td>Pr, 1; S, 1</td>
</tr>
<tr>
<td>35delG/M1V</td>
<td>G to A at nucleotide 1</td>
<td>2</td>
<td>AR</td>
<td>Pr, 1; S, 1</td>
</tr>
<tr>
<td>35delG/31del38</td>
<td>Deletion of 38 nucleotides at 31</td>
<td>1</td>
<td>AR</td>
<td>Mod</td>
</tr>
<tr>
<td>35delG/E47X</td>
<td>G to A at nucleotide 139</td>
<td>2</td>
<td>SP</td>
<td>Pr</td>
</tr>
<tr>
<td>35delG/O57X</td>
<td>C to T at nucleotide 169</td>
<td>1</td>
<td>AR</td>
<td>Pr</td>
</tr>
<tr>
<td>35delG/167delT</td>
<td>Deletion of T at nucleotide 167</td>
<td>1</td>
<td>AR</td>
<td>Mod</td>
</tr>
<tr>
<td>35delG/269insT</td>
<td>Insertion of T at nucleotide 269</td>
<td>1</td>
<td>AR</td>
<td>Pr</td>
</tr>
<tr>
<td>35delG/312del14</td>
<td>Deletion of 14 nucleotides from 312</td>
<td>2</td>
<td>AR, 1; SP, 1</td>
<td>Pr</td>
</tr>
<tr>
<td>35delG/333delAA</td>
<td>Deletion of A at nucleotides 333 and 334</td>
<td>1</td>
<td>AR, 1; SP, 1</td>
<td>Pr, 1; S, 1</td>
</tr>
<tr>
<td>35delG/31del38</td>
<td>Insertion of A at nucleotide 509</td>
<td>1</td>
<td>SP</td>
<td>S</td>
</tr>
<tr>
<td>167delT312del14</td>
<td>Deletion of T at nucleotide 167/deletion of 14 nucleotides from nucleotide 312</td>
<td>1</td>
<td>SP</td>
<td>Pr</td>
</tr>
</tbody>
</table>

*AR indicates autosomal recessive; SP, sporadic case; Pr, profound; S, severe; and mod, moderate.
†The frequent mutation 35delG is the deletion of a guanosine in a sequence of 6 G extending from position 30 to 35.
‡Consanguinity in 1 family.

Table 2. Mutations Detected on a Single Allele and Missense Mutations on One or Both Alleles in 33 of 96 Families

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Description of Mutations (Except 35delG)</th>
<th>No. of Families</th>
<th>Mode of Inheritance</th>
<th>Degree of Deafness (Best Ear)</th>
</tr>
</thead>
<tbody>
<tr>
<td>35delG/+</td>
<td></td>
<td>11</td>
<td>SP, 7; AR, 4 (2 AR discord)</td>
<td>Pr, 9; S, 2</td>
</tr>
<tr>
<td>V27I-E114G/+</td>
<td>G to A at nucleotide 79–A to G at nucleotide 341</td>
<td>1</td>
<td>SP</td>
<td>Pr</td>
</tr>
<tr>
<td>L90P/+</td>
<td>T to C at nucleotide 269</td>
<td>1</td>
<td>SP</td>
<td>Pr</td>
</tr>
<tr>
<td>M34T/+</td>
<td>T to C at nucleotide 101</td>
<td>3</td>
<td>SP, 2; AR, 1</td>
<td>Mild, 2; mod, 1</td>
</tr>
<tr>
<td>N206S/+</td>
<td>A to G at nucleotide 617</td>
<td>1</td>
<td>SP</td>
<td>Pr</td>
</tr>
<tr>
<td>V37I/+</td>
<td>G to A at nucleotide 109</td>
<td>1</td>
<td>AR</td>
<td>S</td>
</tr>
<tr>
<td>M163V/+</td>
<td>A to G at nucleotide 487</td>
<td>1</td>
<td>SP</td>
<td>Pr</td>
</tr>
<tr>
<td>R127H/+</td>
<td>G to A at nucleotide 380</td>
<td>2</td>
<td>SP, 1; AR, 1 discord</td>
<td>Mild</td>
</tr>
</tbody>
</table>

Missense Mutations on One or Both Alleles

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Description of Mutations (Except 35delG)</th>
<th>No. of Families</th>
<th>Mode of Inheritance</th>
<th>Degree of Deafness (Best Ear)</th>
</tr>
</thead>
<tbody>
<tr>
<td>35delG/L90P</td>
<td>T to C at nucleotide 269</td>
<td>2</td>
<td>SP, 1; AR, 1</td>
<td>S, 1; mod, 1</td>
</tr>
<tr>
<td>35delG/P175T</td>
<td>C to T at nucleotide 523</td>
<td>1</td>
<td>AD</td>
<td>Mod</td>
</tr>
<tr>
<td>35delG/S139N</td>
<td>G to A at nucleotide 416</td>
<td>1</td>
<td>AR</td>
<td>Mild</td>
</tr>
<tr>
<td>35delG/V37I</td>
<td>G to A at nucleotide 109</td>
<td>1</td>
<td>SP</td>
<td>S</td>
</tr>
<tr>
<td>35delG/R32H</td>
<td>G to A at nucleotide 95</td>
<td>1</td>
<td>SP</td>
<td>Pr</td>
</tr>
<tr>
<td>35delG/R32C</td>
<td>C to T at nucleotide 94</td>
<td>1</td>
<td>AR</td>
<td>S</td>
</tr>
<tr>
<td>35delG/V153I</td>
<td>G to A at nucleotide 457</td>
<td>1</td>
<td>SP</td>
<td>Pr</td>
</tr>
<tr>
<td>V37I/V37I</td>
<td>T to C at nucleotide 101</td>
<td>2</td>
<td>SP</td>
<td>Mild, 1; mod, 1</td>
</tr>
<tr>
<td>M34T/M34T</td>
<td>G to A at nucleotide 109/A to G at nucleotide 617</td>
<td>1</td>
<td>SP</td>
<td>Pr</td>
</tr>
</tbody>
</table>

*Plus sign indicates wild-type allele; SP, sporadic case; AR, autosomal recessive; AR discord, mutations were not detected in all the deaf children of the family; AD, autosomal dominant; Pr, profound; S, severe; and mod, moderate.
†The frequent mutation 35delG is the deletion of a guanosine in a sequence of 6 G extending from position 30 to 35.
‡Consanguinity in 1 family.
In each case, the mutations found in deaf individuals were also present in the parents who were heterozygous carriers. However, 5 normal-hearing parents of children homozygous for 35delG had mutations on both alleles of the connexin 26 gene: 35delG/V153I (2 cases), 35delG/M34T (2 cases), and 35delG/R127H (1 case), suggesting that a polymorphism is associated with the 35delG mutation. The normal-hearing father with the 35delG/R127H genotype had a congenitally deaf mother on the other allele in normal-hearing parents, and one can regard these changes as polymorphisms. As found in 2 families in this study (Table 1), R127H has been previously described in deaf patients as the sole mutation found in CX26, and this change affects a residue that is not highly conserved among β-connexins.7,23 This is in keeping with the nonpathogenic nature of this mutation.

The data presented herein point out the difficulties encountered in interpreting the result of molecular diagnosis in about one third of the families carrying CX26 mutations. Deaf individuals in these families carry either mutations whose pathogenicity is uncertain on one or both alleles of CX26, or a single mutated CX26 allele.

The 35delG mutation is detected in about three fourths of the mutated alleles, which is similar to the proportion reported in other studies in white populations. Because of the high prevalence of 35delG heterozygous carriers in the general population, pedigrees with false dominance (Figure 1) should suggest a DFNB1 form of deafness.

To date, descriptions of 49 CX26 mutations found in deaf individuals have been published in the literature, including the 3 new missense mutations (R32H [G to A at position 95], R32C [C to T at position 94], and N206S [A to G at position 617]) detected in this study in association with the 35delG mutation.7-11,13-24 The pathogenicity of the stop or frameshift mutations (including the mutations reported in Table 1), usually leading to a protein truncated before the third transmembrane domain, is highly probable.

The pathogenicity of missense mutations is more difficult to determine. Segregation analysis with the polymorphic markers of DFNB1 has not been performed systematically in the families carrying this type of mutation.7-11,13-24 These mutations are usually presumed to be pathologic when they affect amino acids conserved among connexins and when they are not detected in the general population. Three CX26 missense mutations (M34T, W44C [G to C at position 132], and W77R [T to C at position 229]) have been tested in vitro expression systems (Xenopus oocytes or noncommunicating HeLa cell models), showing impaired intercellular coupling and abnormalities of trafficking and targeting of the connexin 26.25-26 However, the problems encountered in the interpretation of the M34T mutation (see below) point out the difficulty in extrapolating in vitro functional results to the consequences of the mutation in vivo.

The results of this study allow better interpretation of the significance of certain missense mutations. The mutations R127H (a C-to-T transition at position 523 that creates a proline-to-threonine substitution at codon 175), V153I (G to A at position 457), and M34T were detected in association with the 35delG mutation on the other allele in normal-hearing parents, and one can regard these changes as polymorphisms. As found in 2 families in this study (Table 1), R127H has been previously described in deaf patients as the sole mutation found in CX26, and this change affects a residue that is not highly conserved among β-connexins.7,23 This is in keeping with the nonpathogenic nature of this mutation.

Figure 1. Pedigree of the family with a dominant mode of inheritance and audiometric curves correlated to the genotype. Solid symbols represent congenitally deaf individuals; open symbols, unaffected individuals; and plus signs, the wild-type allele. In audiometric curves, the dashed line represents the right-ear air conduction threshold and the solid line, the left-ear air conduction thresholds.
The V153I mutation was not detected in our study in the control population but was associated with a 35delG mutation in one deaf child with a sporadic case of deafness. We decided that this change was not a causative factor in the deafness.

The effect of the M34T mutation continues to be debated. It was first described as a dominant mutation, and functional studies in in vitro expression systems support the hypothesis of dominant negative effect. However, several authors described normal hearing in M34T heterozygous carriers, and M34T presented as a recessive mutation in one family. The current evidence is in favor of M34T being a polymorphism. Heterozygous carriers are found in the control population, with a carrier frequency of 1% to 2.9% (0.86% in this study). Despite this high prevalence in the general population, deaf subjects homozygous for M34T have not been described in the series published to date, except the case reported herein (one deaf individual with a sporadic deafness). Moreover, we report herein for the first time, to our knowledge, that 2 subjects (parents of deaf children) who were compound heterozygotes (M34T/35delG) had normal hearing.

Another pitfall in genetic counseling is highlighted by the pedigree shown in Figure 1. The P175T mutation acts here as a dominant mutation. Involvement of this mutation in deafness is probable: first, P175T has never been described in control populations, and second, the proline at codon 175 is highly conserved among human, rat, and Xenopus connexins and is located in the second extracellular loop, which is the major determinant for compatibility between connexins. However, in the family described herein, we cannot exclude the possibility that P175T is a recessive mutation and that the father’s and grandmother’s deafness are due to another cause. We consider that there is no indication to search for CX26 mutations in families affected by autosomal dominant deafness when the affected members have a similar deafness phenotype. However, we sometimes encounter pedigrees, as shown in Figure 1, in which 2 or more very different deafness phenotypes coexist: we can suspect different causes of deafness in the same family and consider that deafness can be a DFNB1 form in congenitally affected members. The search for CX26 mutations is warranted in those cases.

The role of the V37I mutation is also contentious. This mutation, described as a polymorphism, has a high prevalence in the general population in Japan (1%-3% of the control alleles) and has a similar frequency in alleles of congenitally deaf individuals (1/70 [1.4%]). In our series, V37I was homozygous in 2 sporadic cases, one of which was associated with parental consanguinity and the other associated with 35delG. Rabionet et al described another homozygous V37I sporadic case of deafness, and V37I was not detected in the control subjects from Italy and Spain. The V37I mutation has been iden-
identified here in 6 (3.6%) of 169 mutated alleles from deaf subjects (taking into account a single mutated allele in a case of consanguinity) and 1 (0.4%) of 232 alleles in our control population. Screening of very large samples of control population is needed to determine the frequency of V37I heterozygous carriers in the general white population and to determine the status of this mutation.

In the group of mutations associated with 35delG in deaf children, we identified, in addition to the V37I and P175T mutations, a previously described mutation, L90P (T to C at position 269), which has also been reported in Spain and Italy in 0.7% of the CX26 alleles from deaf individuals and never in control populations. In this group we also detected 3 novel changes, and their involvement in disease can be assumed: R32C, R32H, and S139N (G to A at position 416) affect amino acids that are highly conserved among connexins and located in the first (R at position 32) and third (S at position 139) transmembrane domains. These mutations have never been detected in control populations in the literature or in the present study.

Finally, in the group of 21 families in which deaf individuals had a single CX26 mutation, we detected, in addition to the 35delG mutation (11 families) and the 3 mutations M347T, V37I, and R127H (7 families) whose importance was discussed already, the V27I and E114G (A to G at position 341) polymorphisms highly prevalent in Japan (detected in 36%-39% and 24%-28% of the Japanese control alleles and 2 new mutations, N206S and M163V (A to G at position 487). The N206S mutation affects an asparagine conserved among connexins and located in the fourth transmembrane domain, and M163V, a methionine of the second extracellular loop, conserved among β-connexins. Their pathogenicity is yet to be established given the absence of any identified mutation on the other CX26 allele and of families in whom the segregation of the mutation and the deafness can be studied.

Among the families with only one detected mutation, the majority (14/21) were cases of sporadic deafness. Moreover, 7 of 21 families had an autosomal recessive deafness, but in 3 of these 7 families, only 1 of the 2 deaf children had the mutation (two 35delG heterozygotes and 1 R127H heterozygote). If one compares the group of 21 families with only 1 detected mutation with the group of 47 families homozygous for 35delG, the proportion of sporadic deafness or familial discordance was significantly different (17/21 [81%] vs 18/47 [38%]; P=.01). The involvement of the CX26 gene is not likely in the majority of these families.

The molecular diagnosis of CX26 has substantially improved genetic counseling for hearing impairment, because it allows the identification of a genetic cause to be established in many sporadic cases for which an etiology diagnosis had been impossible before.

The discovery in a deaf patient of a biallelic stop or frameshift mutation allows the clinical geneticist to assert the genetic nature of the hearing loss, to establish recurrence risks, to reassure the patient that the defect is isolated, and to detect possible heterozygous relatives. The identification of the role of CX26 in the etiology allows the clinician to predict a low risk of progression of the hearing defect. However, at this time, this molecular diagnosis cannot guide the approach to treatment and rehabilitation. In the future, it will be imperative to compare the results of different treatments with the genotype.

On the contrary, the identification of biallelic mutations considered to be polymorphisms allows one to rule out a role for CX26 in the cause of the audiologic defect and to search for another cause.

Genetic counseling becomes much more difficult when the molecular diagnosis shows only a monoallelic mutation or biallelic mutations for which the pathogenicity has not been proved in the absence of familial segregation analysis. If biallelic missense mutations are found in a deaf individual, the clinical geneticist could be helped by looking at CX26 mutations in both parents to determine if these mutations were inherited from 2 different chromosomes. The pathogenicity of a mutation could be established by the CX26 genotyping of hearing or deaf relatives of the proband. In these cases, the definition of the phenotype becomes very important. To prevent situations where interpretation is difficult, a molecular diagnosis should be proposed only after checking that the phenotype is compatible with a role for CX26. However, if the phenotype is compatible and if the pathogenicity of the mutations could not be determined, the clinician has to be very careful with respect to the cause of the hearing impairment, and other genetic tests should be performed when possible.

With the advent of the molecular diagnosis of CX26 mutations, new mutations are being described every month, and the status of many of the previously reported missense mutations remains uncertain. Clinical geneticists, otolaryngologists, and audiologists are all likely to be confronted with results, the interpretation of which will be difficult. The various medical specialists who treat deaf children must be aware of these difficulties and be very careful about what information they provide the families.

Accepted for publication February 6, 2001.

Presented at the 15th annual meeting of the American Society of Pediatric Otolaryngology, Orlando, Fla, May 17, 2000.

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