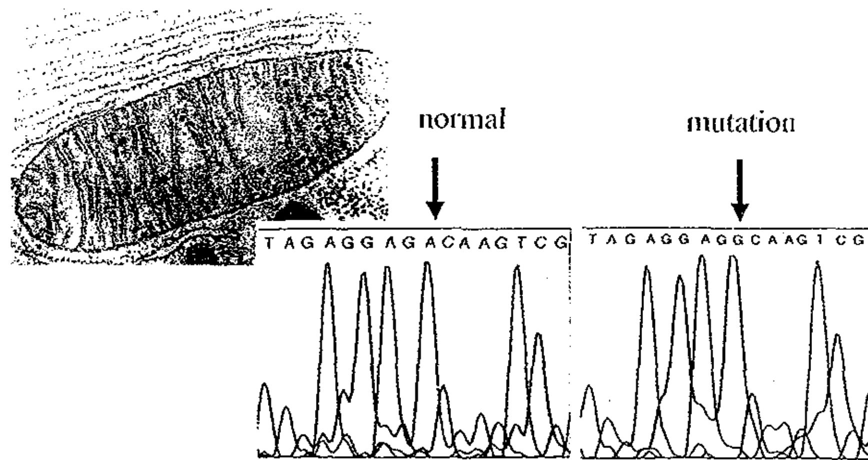

図 1 ミトコンドリア遺伝子 1555A→G 変異
直接シークエンス法で確認すると，正常では 1555 位は A（アデニン）であるが患者では G（グアニン）に変化している。

2. 局所の濃度

腎機能障害があるとアミノ配糖体抗生物質の排泄が遅れ副作用が出現しやすいといわれている。また，鼓室内投与では高濃度の薬物が種々の障害を起こしやすく注意が必要である。

3. 遺伝的要素

従来より，ストレプトマイシンによる難聴はある特定の家系に集積してみられることから，ストレプトマイシンによる感受性には遺伝的要因が関与することが示唆されてきた[2~6]。Tsuiki ら[3]はジヒドロストレプトマイシンによる難聴が家族内に発症した 16 家系を報告し，アミノ配糖体に対し受傷性が高い特定の家系が存在することを明らかにした。また，この易受傷性が母系遺伝することから，アミノ配糖体に対する受傷性にはミトコンドリア遺伝子が関与していることが示唆されていた[7]。Hutchin ら[8]は日本人および中国人のアミノ配糖体抗生物質による難聴患者のミトコンドリア遺伝子を解析し，1555A→G 変異の頻度が有意に高いことを報告した。また，時期を同じくして，中国およびアラブイスラエルの家系を用いた研究により，アミノ配糖体に対する高感受性がやはり 1555A→G 変異と関連があることが発表され[9,10]，この変異が難聴と関連していることが分子遺伝学的に明らかとなった（図 1)。当初，この変異はアジアを中心に報告があい次いだが，最近ではギリシャ，イギリス，イタリア，メキシコ，プエルトリコ，ベトナム人などからも同様の変異が報告されている[11]。

II. 難聴のメカニズム

従来より動物実験において，アミノ配糖体抗生物質投与により主として蝸牛コルチ器が障害を受けることが報告されているが[12]，1555A→G 変異をもつ難聴患者の自記オージオグラム，ABR，語音聴力検査などの聴覚検査の結果からも，難聴はおそらく内耳由来であることが推測されている[13]。近年，アミノ配糖体抗生物質投与により高度難聴をきたした 1555A→G 変異症例に人工内耳を施行し良好な成績が得られたことが報告されているが，これは 1555A→G 変異による難聴が蝸牛神経やその聴覚中枢によるものではなく蝸牛コルチ器に由来していることを示唆している[14]。蝸牛コルチ器では外有毛細胞および血管条中間細胞にミトコンドリアが豊富に存在することが知られている。ミトコンドリアは必要不可欠なエネルギーを産生する細胞内小器官であることから，難聴はこれらの細胞の機能障害と関連があることが推測されている。詳細なメカニズムはまだよく解明されていないのが現状であるが，ミトコンドリア遺伝子 1555 位の塩基が A から G に変異することによりバクテリアと類似した立体構造となり，アミノ配糖体抗生物質との結合性が高くなるという仮説が提唱されている[8,15]。ところが，実際難聴患者の臨床データを分析すると，患者の中にはアミノ配糖体抗生物質投与歴がなく，いわゆる特発性

難聴の形で難聴をきたす症例もかなり存在することから，この仮説ではアミノ配糖体投与歴のない難聴症例のメカニズムを説明することはできない。おそらく変異により細胞に必要十分なエネルギーが産生できず，他の因子，たとえば他の難聴遺伝子や調節遺伝子，あるいは環境因子（騒音やストレスなど）が加わることによって難聴になることが推測されている。最近われわれは，常染色体上にある難聴遺伝子 *GJB2* 遺伝子変異を合わせもつ家系を報告したが，興味あることに同じ家系内でも 2 つの遺伝子変異を両方もつ症例ではより高度な難聴を起こすことが明らかになった[16]。

III. 頻　度

われわれが行った頻度調査では，外来を訪れる感音難聴患者の約 3%に認められることが明らかになっており，この遺伝子変異による難聴患者あるいはハイリスク患者の数は全国的にかなり多いことが推測される[17]。また，患者のうちアミノ配糖体抗生物質による難聴患者に絞ると約 30%に見出されることが明らかとなった[17]。また，成人の人工内耳の埋め込み患者を対象に頻度を検討したところ人工内耳患者の約 10%に，またアミノ配糖体抗生物質により高度難聴をきたした人工内耳症例に限ると約 60%がこの変異をもっていた[17]。したがって，この変異は日本人の言語習得後失聴の重要な原因の 1 つであると考えられる。アミノ配糖体抗生物質を投与する場合には，その患者の遺伝的背景を考慮することが重要である。

IV. 診　断

この遺伝子変異による難聴の特徴は，母系遺伝することである（図 2）。これは受精の際に精子由来のミトコンドリアは特異的に排除されるため，母親由来のミトコンドリア DNA のみが子に伝えられるためである。難聴患者がミトコンドリア遺伝子変異をもつ場合，母親の家系に難聴者がいる場合が多く，問診の際に家族歴を聴取することが診断のポイントとなる。難聴の程度には個人差が大きく，同一家系内でも軽度から高度まで種々の程度の難聴がみられることが多いが，一般的には年齢が増すに従い高度になる場合が多い。難聴は

図 2　母系遺伝
子は母親のミトコンドリア遺伝子を受け継ぐ。したがって，母親が変異をもっているとその子供は全て変異を受け継ぐが，男の子から次世代には伝わらない。

一般的に両側性，対称性，高音障害型で，耳鳴を伴うことが多い[13,18]。また，アミノ配糖体抗生物質を中止した後も難聴が進行する症例があり注意が必要である。初期には 4,000～8,000 Hz のみが障害される場合が多く，自覚症状がなく聴力検査で初めて発見される場合も多い。アミノ配糖体抗生物質の投与により難聴をきたしたミトコンドリア遺伝子 1555A→G 変異患者の重ね合わせオージオグラムを図 3 に示すが，難聴は一般的に高度で中音域でも難聴を示すことが多い。アミノ配糖体抗生物質の投与により難聴をきたした 1555A→G 点変異をもつ患者に共通する点として，(1) 少量投与あるいは少ない投与回数でも難聴をきたしていること，(2) 投与後比較的早期に難聴をきたすことが挙げられる[13]。なお，難聴の程度とアミノ配糖体投与回数，投与時の年齢とは相関関係は認められていない。近年，副作用を軽減した新世代のアミノ配糖体抗生物質が発売されたが，筆者らはこの抗生物質の投与によって難聴をきたした 1555A→G 変異症例を経験しており，アミノ配糖体抗生物質を使用する際には常に患者の遺伝的背景を考えることが必要である[19]。

変異をもつ患者の中にはアミノ配糖体抗生物質の投与歴がなく，いわゆる特発性難聴の形で難聴をきたす症例もあるが，難聴の程度は一般的に軽度のことが多い（図 3）[13,20]。変異をもつ患者の中でめまいを訴える症例は少なく，温度眼振検査で

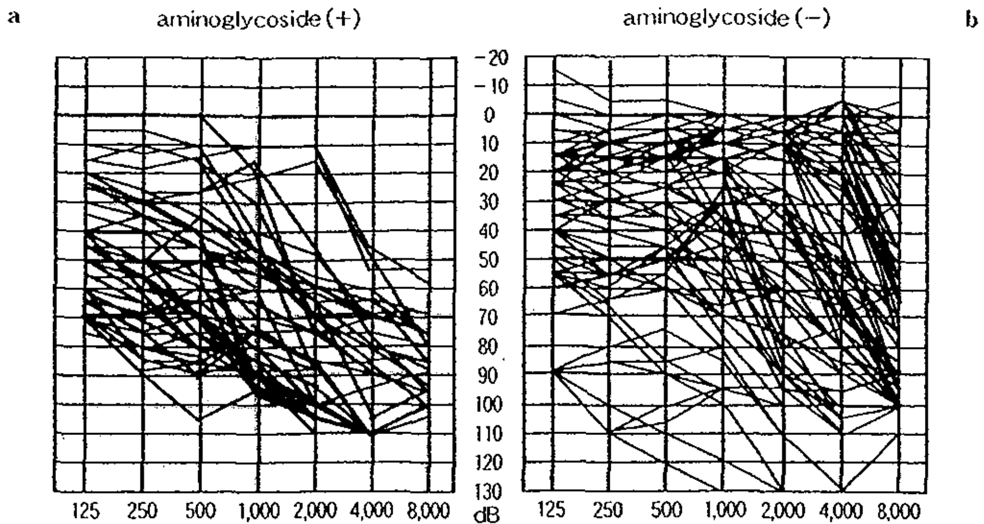

図 3 ミトコンドリア 1555A→G 変異をもつ患者の聴力像（文献 20 より引用）
アミノ配糖体投与歴のある患者群 (b) とない患者群 (a) の聴力を比較すると，投与歴のある患者群のほうが一般に難聴の程度が高度なことがわかる。

も異常を示さないことが多い[13,21]。アミノ配糖体抗生剤は聴覚障害とともに前庭障害をきたすことが知られている。特に硫酸ストレプトマイシンは前庭障害をきたすことが多いことが知られているが，この遺伝子変異によりなぜ聴力のみが顕著に障害をきたすのかは明らかになっていない。

最終的な診断の決め手は遺伝子診断である。遺伝子検索には制限酵素を用いる方法，直接シークエンスを用いる方法，MASA 法を用いる方法などがあるが[13,22]，最近臨床検査の 1 つとしても検査が可能になった（株ビー・エム・エル：受託検査項目)。

V. 治療と予防

難聴は非可逆的で，いったん難聴をきたすと残念ながら難聴の回復は困難である。中等度難聴に関しては補聴器が，また補聴効果の認められない高度難聴に関しては人工内耳が適応となる[14]。このミトコンドリア 1555A→G 変異に伴う難聴に関してはアミノ配糖体抗生物質の投与を避けることにより高度難聴はある程度予防が可能であることから，現在筆者の施設ではミトコンドリア遺伝子変異のスクリーニングシステムを確立するとともに薬物カードを配付し予防に努めている[22]。

VI. おわりに

アミノ配糖体抗生物質による聴覚障害，特に最近の分子遺伝学により明らかになったミトコンドリア 1555A→G 変異に関し紹介した。

表 2 どうやってハイリスク患者をみつけ出すか

(1) 家族歴：母系に難聴者がいる
(2) 両側高音障害型難聴に注意
(3) 特発性難聴に注意
(4) 遺伝子検査

最近，十分な家族歴の聴取なしにミトコンドリア 1555A→G 変異をもった患者にアミノ配糖体抗生物質の投与を行い，さらに副作用が出た後も漫然と使用した結果，難聴を生じ進行した患者・家族が病院側を訴え病院側が非を認めた事例があった。今後，このような事例が増えていくことが予想されるが，医師サイドでも患者の遺伝的背景には十分留意することが必要である。表 2 にハイリスク患者をみつけ出すポイントについてまとめたが，前述のようにアミノ配糖体抗生物質による難聴患者の約 33％がこの変異をもっていることを考えると，患者にアミノ配糖体抗生物質を投与する場合には，患者の遺伝的背景に留意し副作用を避ける必要がある。

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著者は，日本で初めてがん患者の「痛み」を緩和させるために，モルヒネの大量投与の有効性を「WHOがん疼痛救済プログラム」から示した。それはまさに「がんの痛みからの解放」であった。20年にわたって，日本ばかりではなく，世界を駆け巡ってきた著者の，医療用モルヒネ使用によるがんの痛みからの解放の軌跡をたどる本書は，がん医療に携わる医療職，教育者，学生，「がん」を考える市民にとっても「バイブル」となりうる書である。

CASE REPORT

Dennis H. Kraus, MD, *Section Editor*

PENDRED'S SYNDROME WITH GOITER AND ENLARGED VESTIBULAR AQUEDUCTS DIAGNOSED BY *PDS* GENE MUTATION

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Abstract: *Background.* Pendred's syndrome (PS) is an autosomal recessive disorder characterized by goiter and congenital sensorineural hearing loss. Recent advances in molecular biology revealed the gene responsible for PS *(PDS)* and provided an important aid for the diagnosis of this condition.

Methods. A case of PS with huge goiter and congenital hearing impairment was diagnosed by mutational analysis of the *PDS* gene.

Results. Physical examination and computer tomography CT revealed a diffuse swelling of the thyroid gland. Thyroid function tests were normal, and the perchlorate discharge test was negative. Audiologic examination confirmed sensorineural hearing loss, and temporal bone CT revealed bilateral enlarged vestibular aqueducts. The mutational analysis revealed that the patient was homozygous for His 723 Arg (2168A→G) in exon 19, a missense mutation.

Conclusions. The results of thyroid function tests in PS patients are usually normal, and the positive perchlorate discharge test has been used for the diagnosis. However, this is a nonspecific test and is not sensitive enough for PS. In our case, despite a negative perchlorate test, the patient was diagnosed by mutational analysis and received total thyroidectomy to relieve respiratory distress caused by thyroid enlargement. This is the first report of a mutation detected in the thyroid tissue and clearly shows that the mutation caused histopathologic change in that gland. © 2002 Wiley Periodicals, Inc. *Head Neck* 24: 710–713, 2002

Keywords: Pendred's syndrome; enlarged vestibular aqueducts; *PDS* gene mutation; goiter; perchlorate discharge test

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Pendred's syndrome (PS) is an autosomal recessive disorder characterized by goiter and congenital sensorineural hearing loss.[1] Thyroid enlargement is caused by an impairment of thyroxine synthesis caused by defective organic binding of iodone.[2] The positive perchlorate discharge test has been used for the diagnosis of PS, but this is a nonspecific test, the sensitivity is unclear. Recent advances in molecular biology revealed the responsible gene for PS *(PDS)* and provided a new diagnostic aid for the identification of this disorder.[3] We report a rare case of PS with huge goiter and congenital hearing impairment. The perchlorate discharge test was negative, and the patient was diagnosed by mutational analysis of the *PDS* gene. The medical treatment with

thyroxine was not effective, and the patient received total thyroidectomy to relieve respiratory distress caused by thyroid enlargement. The treatment of goiter and the relation between the genetic defect and clinical manifestations are discussed.

CASE REPORT

This patient was born in 1926 with congenital hearing loss and had goiter a develop at puberty. Her family history revealed two younger sisters who were affected by congenital hearing loss and goiter since childhood. The family pedigree indicated that the patient's parents, who both had the heterozygous H723R mutation, were consanguineous (Figure 1). The patient was seen in our department in November 1997. Tracheostomy for respiratory distress, caused by the enlarged thyroid gland, had already been performed in another hospital. Physical examination revealed 13 × 12 cm in the right lobe and 12 × 10 cm in the left lobe of the thyroid; it was soft, and no induration was palpable (Figure 2). CT showed a diffuse swelling of the thyroid gland (Figure 3). Thyroid function tests showed euthyroidism, and the serum thyroid antibodies were negative. The perchlorate discharge test was negative; it did not show a decrease in the radioactive iodine content of thyroid from the baseline. Audiologic examination confirmed sensorineural hearing loss (Figure 4), and temporal bone CT revealed bilateral enlarged vestibular aqueducts (Figure 5).

Because she had a high risk for surgical treatment such as atrial fibrillation, drug treatment with thyroxine was selected. However, the goiter enlarged gradualy, and she had massive bleeding from the stoma when the tracheal tube was changed. In November 1999, a total thyroidectomy was performed. The size of the thyroid specimen was 13 × 10 cm in the right lobe and 11 × 8 cm in the left lobe, and the total weight was 620 g. Histologic examination revealed hyperplastic adenomatous goiter (Figure 6). After the operation, the patient's heart condition deteriorated, but she recovered with medical treatment.

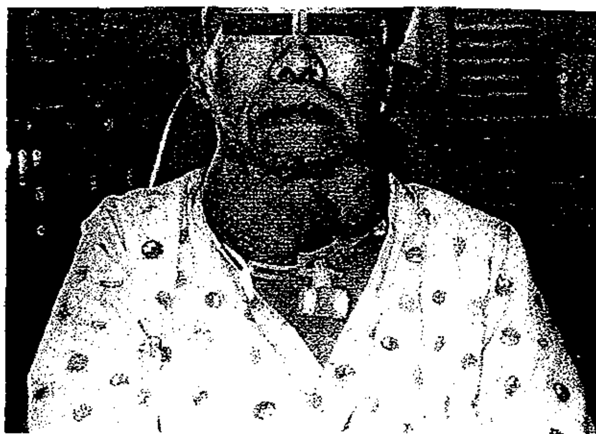

FIGURE 2. Diffuse swelling of the thyroid gland. Tracheostomy had been performed.

Genomic DNA samples from the patient's thyroid and peripheral blood leukocytes from her sisters were obtained with informed consent and analyzed to determine the *PDS* mutations. The mutational analysis, performed as previously reported,[4] revealed that the patient was homozygous for His723Arg (2168 A→G) in exon 19, a missense mutation (Figure 7).

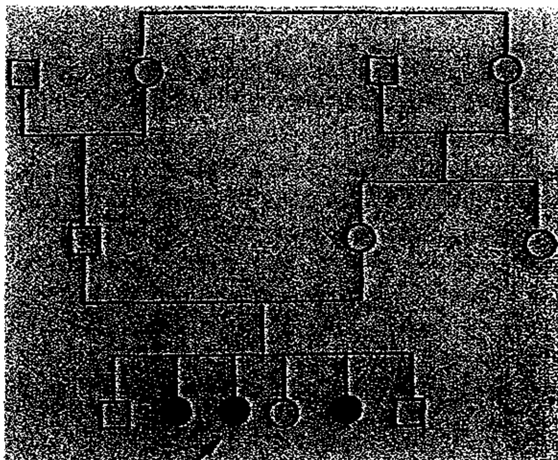

FIGURE 1. Pedigrees of the family with PS. □, male; ○, female. A filled mark indicates the affected patient.

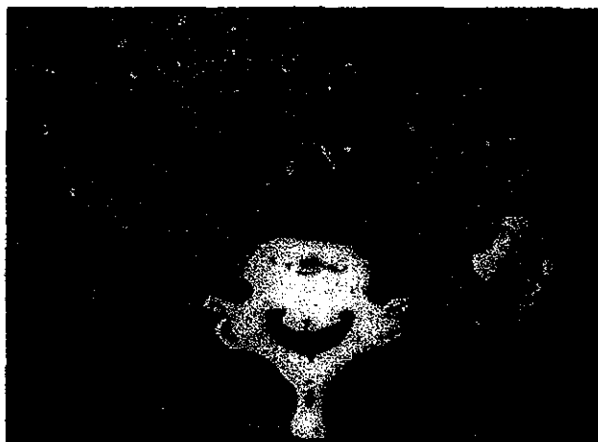

FIGURE 3. Axial CT showing diffuse swelling of the thyroid gland.

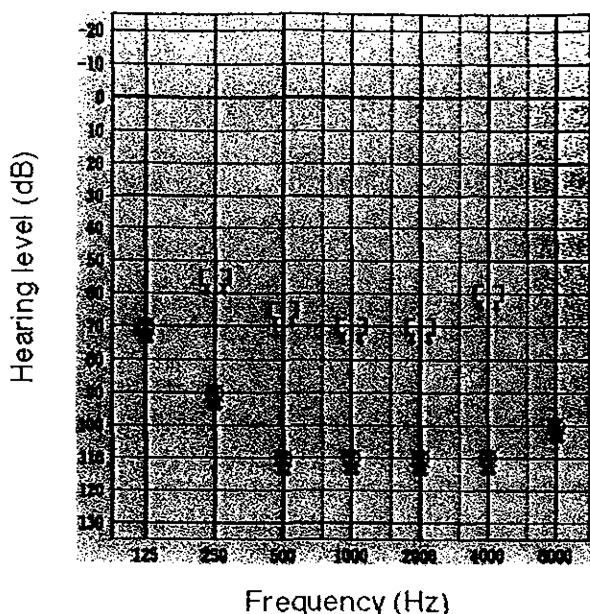

FIGURE 4. Audiologic examination showing bilateral deafness.O, right air conduction hearing; x, left air conduction hearing; [, right bone condution hearing;], left bone conduction hearing.

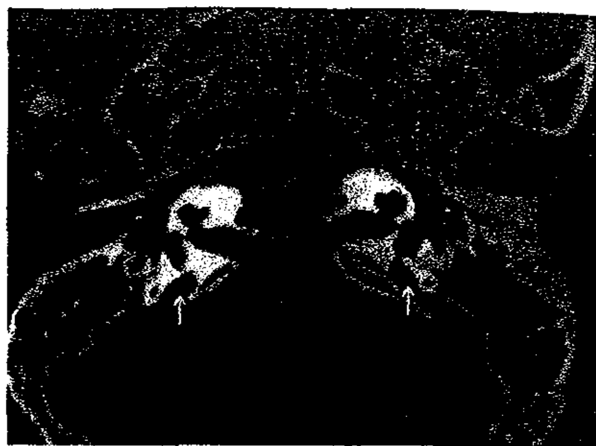

FIGURE 5. Axial CT showing bilateral enlarged vestibilar aqueducts (white arrows).

DISCUSSION

PS has been characterized by goiter and sensorineural hearing loss in association with inner ear malformation. Enlargement of the thyroid usually develops at puberty or late.[5] The goiter is soft and diffuse, and it may increase considerably in size. Drug therapy is sometimes effective for the treatment of the goiter. Reardon et al[6] have reported 27 PS patients with goiter who received thyroxine treatment. Fifteen patients were controlled by conservative treatment only, and 12 patients progressed to surgical intervention. It has been reported that the regression of the goiter might occur if the drug treatment is started early in life[7]. Failure to respond to thyroxine requires surgical treatment. The goiter often recurs after partial thyroidectomy, and many patients undergo repeat surgery.[8] Thyroid carcinoma has also been seen at reoperation in two patients with PS.[9,10] Therefore, initial total thyroidectomy has been recommended.[7] In our case, the tracheostomy had been performed in another hospital because of the airway distress caused by thyroid enlargement. Conservative treatment was not effective, and therefore total thyroidectomy was performed.

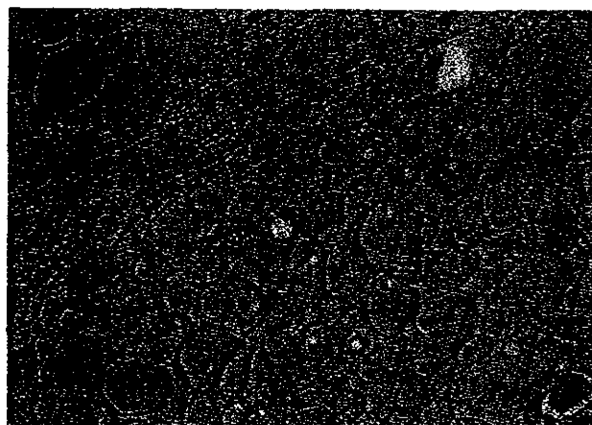

FIGURE 6. Histologic examination shows normal follicular formation, including colloid pooling and the pathologic diagnosis is adenomatous goiter. CH & E stain, original magnification (×200).

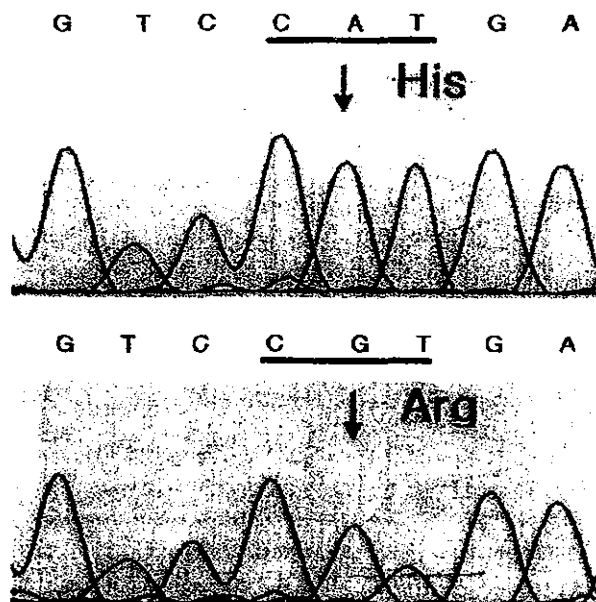

FIGURE 7. The electrophoretogram showing H723R mutation. The A to G mutation (arrow) at position 2168 leads to the replacement of a histidine (His, H) residue at position 723 by arginine (Arg, R).

The results of thyroid function tests in PS patients are usually normal,[5] and the positive perchlorate discharge test is usually used for the diagnosis.[6] In a normally functioning thyroid gland, inorganic iodine is immediately organized by binding to thyrogloblin, and perchlorate unmasks defects of organification by provoking the discharge of inorganic iodine. In PS patients with defective organic binding of iodine, between 15% and 80% of accumulated iodine is discharged on administration of perchlorate compared with less than 10% in normal PS.[11] An abnormal thyroid response is recognized in other thyroid disorders, such as Hashimoto's thyroiditis, total iodine organification deficiency, and ^{131}I-treated thyrotoxicosis.[12] False-negative cases for perchlorate discharge tests have also been reported.[13]

Recently, the gene responsible for PS *(PDS)* has been identified,[3] and 38 mutations have been reported (see The Human Gene Mutation Database[14]). The molecular analysis of the *PDS* gene has become an important aid for the diagnosis of PS. In our case, despite a negative perchlorate discharge test, the patient was diagnosed with PS by mutational analysis of the *PDS* gene. A missense mutation, His723Arg (2168A-G), was found in exon 19. This type of mutation has already been reported in one PS family[15] and in three families with nonsyndromic hearing loss associated with enlarged vestibular aqueducts.[4] A recent study with imaging MRI revealed that all PS patients had a large vestibular aqueduct with an enlarged endolymphatic sac and duct.[16] The recessive inherited nonsyndromic hearing loss with enlarged vestibular aqueducts was mapped to chromosome 7q31 containing the *PDS* gene region[17] and later reported to be caused by *PDS* gene mutations.[4] Therefore, it is currently thought that both categories of diseases are a continuous condition caused by mutations in the *PDS* gene. It is possible that different combinations of mutations may lead to different phenotypes.[18] However, the question has been raised why the same mutation (His723Arg) resulted in different conditions. There may be modifying genes, or secondary *PDS* mutations may also be related to the phenotypic expression. In fact, phenotypic variability in two families carrying the same *PDS* missense mutation (L445W) has been reported.[19]

This is the first report of a mutation detected in the thyroid tissue and clearly shows that the mutation caused the histopathologic change in the thyroid. The *PDS* gene encodes a putative sulfate transporter called pendrin.[4] Although the precise function of pendrin remains unclear, it may serve as an ionic transporter both in the inner ear and in the thyroid gland.[18] The enlarged endolymphatic sac is a characteristic finding of the inner ear, and it may be caused by a defect in transmembrane iodine/chloride transport.

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RAPID COMMUNICATION

A Mutational Hot Spot in the *KCNQ4* Gene Responsible for Autosomal Dominant Hearing Impairment

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Several different mutations in the KCNQ4 K^+ channel gene are responsible for autosomal dominant nonsyndromic hearing impairment (DFNA2). Here we describe two additional families originating from Europe and Japan with a KCNQ4 missense mutation (W276S) that was previously found in one European family. We compared the disease-associated haplotype of the three W276S-bearing families using closely linked microsatellite markers and intragenic single nucleotide polymorphisms. Differences between the haplotypes were found, excluding a single founder mutation for the families. Therefore, the W276S mutation has occurred three times independently, and most likely represents a hot spot for mutation in the KCNQ4 gene. Hum Mutat 20:15–19, 2002. © 2002 Wiley-Liss, Inc.

KEY WORDS: hereditary hearing impairment; *KCNQ4*; mutational hotspot; DFNA2

DATABASES:
KCNQ4 – OMIM: 603537; 600101 (DFNA2); GenBank: XM_002010;
http://www.uia.ac.be/dnalab/hhh/ (Hereditary Hearing Loss Homepage)

INTRODUCTION

Hearing impairment is the most frequent sensory handicap, and genetic factors play a leading role in its etiology. In a limited number of cases, hearing loss is part of a recognizable syndrome. Several genes for syndromic hearing loss have been identified over the last 10 years. In the majority of cases, hearing impairment occurs without additional clinical abnormalities (nonsyndromic). Before 1994, very little was known about the genes responsible for hearing impairment, but since then enormous progress has been made. At this moment more than 70 chromosomal loci for nonsyndromic hearing impairment have been localized, and for more than 20 of these the responsible genes have been identified (Hereditary Hearing Loss Homepage, www.uia.ac.be/dnalab/hhh/).

Despite this progress, molecular diagnostics of patients with hearing loss remain complicated due to the high genetic heterogeneity. With the exception of a single gene (connexin 26, also called *GJB2*; MIM# 121011), each of these genes is responsible in only a limited number of families. In some populations connexin 26 accounts for 20% of all childhood hereditary hearing loss, and the fact that a single mutation (35delG) is responsible for a majority of disease alleles has led to the first useful genetic test for autosomal recessive hearing impairment in Caucasoids [Estivill et al., 1998; Green et al., 2000].

For nonsyndromic autosomal dominantly inherited hearing impairment (DFNA), most genes have been identified in only a single family. However, mutations in the voltage gated potassium channel gene *KCNQ4* (MIM# 603537) have been identified in seven independent families. These include a French family

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[Kubisch et al., 1999], a Belgian, two Dutch, and an American family [Coucke et al., 1999], another Dutch family [Van Hauwe et al., 2000], and another American family [Talebizadeh et al., 1999]. Each of these families showed a different mutation; in six families a missense mutation was found, and in one family there was an inactivating small deletion early in the gene.

KCNQ4 is a member of a large family of K^+ channel genes that have six transmembrane domains and a single pore loop region that is believed to be responsible for K^+ selectivity. *KCNQ4* is expressed in the sensory hair cells of the cochlea [Beisel et al., 2000], where it is thought to be involved in K^+ recycling, as well as in the central auditory pathway [Kharkovets et al., 2000].

We report here two new families with autosomal dominant progressive hearing impairment originating from the Netherlands and Japan. Both families harbor the same *KCNQ4* mutation (827G→C), which already had been found previously in a Dutch family affected by DFNA2 (MIM# 600101). We compared the chromosomal haplotype carrying the 827G→C mutation in these three families with flanking microsatellite markers as well as intragenic single nucleotide polymorphisms and found several differences among the families. These results indicate that the 827G→C mutation must have arisen independently in these three families, making this site a hot spot for mutation in the *KCNQ4* gene.

MATERIALS AND METHODS

Clinical Diagnosis

Family members underwent otoscopic and audiological examination following common clinical standards, and were interviewed regarding their medical history and environmental factors that may be implicated in hearing impairment. The clinical diagnosis was based upon the audiometric thresholds in comparison to standard values in function of age and sex (International Organization for Standardization, 1984), as well as clinical and environmental data, as described previously [Van Camp et al., 1997]. Family members with an atypical audiometric pattern were excluded from the analysis.

Families

The pedigree of this extended Dutch family with autosomal dominant progressive hearing loss is presented in Figure 1a. Patients showed a postlingual progressive hearing impairment, most pronounced in the high frequencies. Linear regression analysis of longitudinal as well as cross-sectional data revealed threshold progression of approximately 1 dB per year for all frequencies. However, offset thresholds were higher for the high frequencies.

The pedigree of the Japanese family, with six affected members across four generations, is shown in Figure 1b. The patients presented with progressive hearing impairment very similar to the Dutch family.

Genetic Analysis

Blood samples were obtained from all family members of both families participating in this study after informed consent. Genomic DNA was isolated from peripheral blood lymphocytes using standard protocols. Genotyping of microsatellite markers was performed using a fluorescent technique. One of the primers was synthesized with a 19-bp sequence complementary to M13 at the 5′ end. A 5′-IRD labeled (800 nm) M13 primer was included in the PCR reaction, thus labeling the PCR product. Pattern visualization of the different markers was performed by electrophoresis using a LICOR 4200-DNA analyzer (LI-COR Biosciences, Lincoln, NE). Detailed information for the microsatellite markers D1S432, D1S2743, GATA P32043, D1S2645, D1S2861, and A289YD1, closely linked to the DFNA2 region, can be found in the Genome Database (www.gdb.org). For the haplotype comparison, seven single nucleotide polymorphisms located inside *KCNQ4* have also been analyzed [Talebizadeh et al., 1999]. Disease haplotypes were determined by direct sequencing of PCR-amplified fragments for several members of each family. PCR products were purified by filtration (Concert Nucleic Acid Purification System, Invitrogen, Carlsbad, CA) and sequenced using the ABI Prism Big Dye Termination Kit (Applied Biosystems, Foster City, CA). Linkage analysis was performed with the Linkage software package (version 5.1) [Lathrop and Lalouel, 1984] as described [Van Camp et al., 1997]. Mutation analysis of *KCNQ4* was performed as described previously [Coucke et al., 1999].

RESULTS

Dutch Family

We analyzed a new family with autosomal dominant nonsyndromic progressive hearing loss, originating

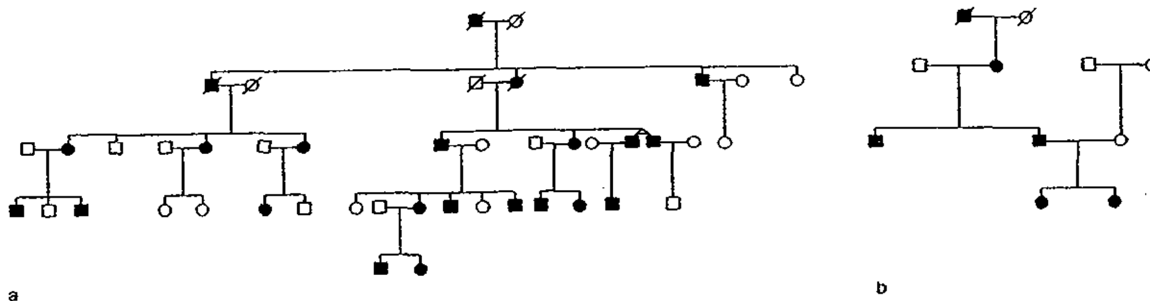

FIGURE 1. Pedigrees of two new families with autosomal dominant hearing impairment caused by the W276S mutation in the *KCNQ4* gene, originating from (a) The Netherlands (Dutch 4) and (b) Japan. Only individuals included in the genetic analysis are represented.

from the Netherlands (Fig. 1a). Because of phenotypic similarities with other DFNA2 families, we carried out linkage analysis with markers from the DFNA2 region. Several markers gave LOD scores of over +3.0 (data not shown), giving conclusive evidence of linkage to the DFNA2 locus. We subsequently screened two affected family members for all previously identified KCNQ4 mutations [Coucke et al., 1999; Kubisch et al., 1999; Talebizadeh et al., 1999], and found that both were heterozygous for the known G to C mutation at position 827 (827G→C). Analysis of the complete family indicated that the 827G→C mutation was present in all affected individuals. Because this is the fourth Dutch family with a *KCNQ4* mutation, we named this family Dutch 4.

Japanese Family

Initially, 16 Japanese families with autosomal dominant nonsyndromic hearing loss were tested for linkage to the known autosomal dominant loci (data not shown). In a single family the analysis was compatible with linkage to DFNA2. Mutation analysis of the complete *KCNQ4* gene by genomic sequencing of exons revealed the presence of the 827G→C mutation in all living affected individuals. The mutation was not present in unaffected individuals.

Haplotype Comparison

To determine whether the two new families from this study (Dutch 4 and Japanese) were related to each other or to a previously identified family with the same *KCNQ4* mutation (Dutch 1), we looked for haplotype sharing of the disease chromosome in these families. First, six closely linked microsatellite markers were analyzed, three on each side of KCNQ4. Four of these markers (D1S2743, D1S2130, D1S2645, and D1S2861) are located on BAC contig of approximately 1 Mb surrounding *KCNQ4* [Coucke et al., 1999]. The two terminal markers (D1S432 and AFMA289YD1) are located on a YAC contig of approximately 2 Mb surrounding *KCNQ4* [Coucke et al., 1999], and are separated by 40 cR on the NCBI radiation hybrid map [Agarwala et al., 2000]. Marker D1S2645 is located on the same BAC as *KCNQ4* [Coucke et al., 1999]. The microsatellite haplotyping revealed a different haplotype for all three families (Fig. 2a).

In addition to the microsatellite markers, seven intragenic *KCNQ4* SNPs [Talebizadeh et al., 1999] were analyzed. For all these SNPs, one of the alleles has a high frequency in the Caucasian population, while the allele frequencies in the Japanese population have not been determined. Therefore, it was not surprising that for most of the SNPs an identical allele (the most frequent allele) was found by chance. Nevertheless, differences were found for two SNPs. The Dutch 4 family differs from the two others for the polymorphism 1657C→T, whereas the Japanese family differs from both Dutch families for the *KCNQ4* polymorphism 777C→T (Fig. 2b).

DISCUSSION

We identified two additional families with autosomal dominant progressive hearing loss caused by a *KCNQ4* mutation, one from Dutch and one from Japanese origin. Statistical analysis of the audiograms showed progression of 1 dB per year for all frequencies, but higher offset thresholds in the high frequencies. Because this type of hearing impairment had been found previously in several other Dutch families with *KCNQ4* mutations [Marres et al., 1997; Kunst et al., 1998; Ensink et al., 2000], we performed linkage analysis with markers from the DFNA2 region in the Dutch family. When linkage to DFNA2 was found, *KCNQ4* mutation analysis was carried out. *KCNQ4* mutation analysis was also carried out in one out of 16

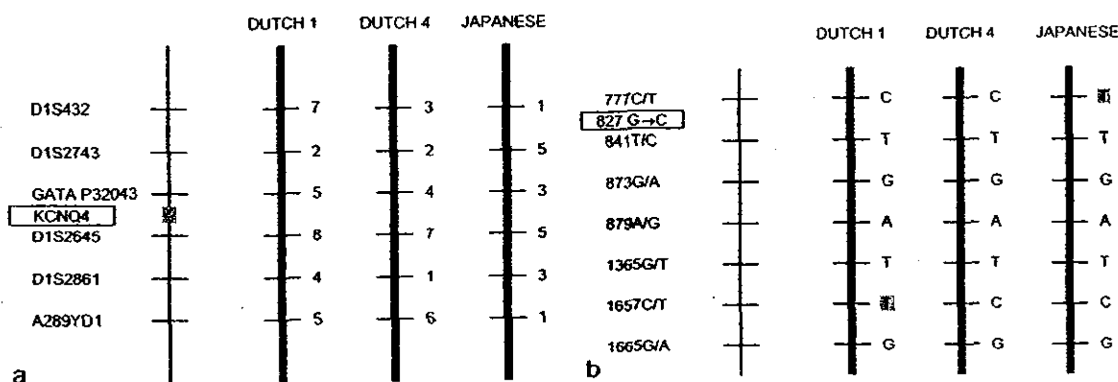

FIGURE 2. Haplotype analysis in the three families with the W276S *KCNQ4* mutation. Family Dutch 1 has been described before [Coucke et al., 1999; Marres et al., 1997]. a: The haplotypes for the mutation-carrying chromosome for six microsatellite markers closely linked to the *KCNQ4* gene are given. Alleles of different lengths were assigned different numbers. b: The disease haplotypes for seven intragenic *KCNQ4* single nucleotide polymorphisms are presented.

Japanese families with autosomal dominant sensorineural high frequency hearing impairment for which the analysis of genetic markers from the DFNA2 region was compatible with linkage. Remarkably, an identical *KCNQ4* mutation (827G→C) was found in both families. The same mutation had previously been found in a Dutch DFNA2 family (Dutch 1) [Coucke et al., 1999]. In the *KCNQ4* protein, the 827G→C mutation leads to the replacement of a tryptophan (Trp, W) residue at position 276 by serine (Ser, S). This W276S mutation is located in the functionally important pore region of *KCNQ4*. It has been shown that this Trp residue is involved in maintaining the channel pore at a correct diameter [Doyle et al., 1998]. In addition, Trp at this position is conserved in a wide variety of K^+ channels of different species. As the mutation changes a conserved and functionally important residue of a critical domain of the *KCNQ4* protein, it is expected to have important consequences on the channel function.

At this moment, the W276S mutation has been found independently in three families, and it is the only *KCNQ4* mutation that has been found more then once. From the seven different *KCNQ4* mutations that have been found up to now in families with sensorineural hearing impairment, one is located in the N-terminal intracellular region, five are located in the channel pore region, and one is located in the sixth transmembrane domain. As the *KCNQ4* gene is a highly conserved gene, there must be numerous possibilities for *KCNQ4* mutations leading to hearing impairment. Therefore, it is very unlikely that the same mutation has been found three times merely by chance, and there must be some explanation for this. The first and most obvious explanation is that the mutation could have arisen in a common ancestor of the three families. For the two Dutch families, this was a priori a very plausible explanation, but it is harder to imagine a common ancestor for the Dutch and the Japanese families. A founder mutation can be tested by the analysis of genetic markers close to the disease gene, as these markers will be co-inherited with the mutation. In each meiosis, the probability of having a recombination between the mutation and a marker located on the same chromosome is equal to the recombination fraction between them. For markers in close proximity to the mutation, the recombination fraction will be very small, and the allele for this marker will not change by recombination for many generations. We compared the mutation-carrying haplotypes of the three families with the W276S mutation by analyzing microsatellites closely flanking the *KCNQ4* gene. Because these markers are highly polymorphic, they have a high chance of being informative. A different haplotype was found in all three families, strongly arguing against a common founder. Because the microsatellite markers that were used were located very close to the *KCNQ4* gene (up to a location on the same BAC for marker D1S2645), a common ancestor can be excluded for a large number of generations. However, if a mutation is very old, the region that is identical by descent surrounding the mutation can be very small. For example, after 100 generations, the average region identical by descent flanking a disease gene will be about 0.7 cM, while 1000 generations will reduce it to about 0.07 cM, which comes close to the size of an average gene. In this case, analysis of markers flanking the gene would not pick up the region identical by descent. To exclude a very old mutation, markers located inside the gene have to be analyzed. Intragenic SNPs can be used, but as these are less polymorphic then microsatellites, they may often be uninformative. Nevertheless, we found differences among the families. The Japanese family differs in a SNP that is only 50 bp away from the mutation, while the two Dutch families differ in a SNP located 830 bp downstream from the mutation.

The second possible explanation for the triple occurrence of the 827G→C mutation is a mutational hot spot. Under this hypothesis, the mutation has occurred several times independently due to an exceptionally high mutation rate of the G at position 827 of the *KCNQ4* gene. A mutational hot spot has been put forward in many publications, but strong evidence to support it is lacking in many cases. A strong example of a mutational hot spot is the G380R mutation in the Fibroblast Growth Factor Receptor 3 gene (*FGFR3*; MIM# 134934), leading to achondroplasia [Wilkin et al., 1998]. Nearly all cases of achondroplasia are novel mutations, and more then 97% are caused by a mutation at position 1138 of *FGFR3*. However, it currently remains unknown what mechanism is responsible for the highly increased mutation rate at position 1138. Position 827 in the sequence of *KCNQ4* is the first G of a stretch of 5 Gs (CTC TGG TGG GGG ACG ATT). It is noteworthy that a sequence of 18 or 17 bp surrounding this position is also present in *Paramecium bursaria* Chlorella virus, Herpes simplex virus type 2, and *C. elegans*, but no arguments can be found that may point to a possible mechanism. As a hot spot for mutation is the most likely explanation for our data, it is likely that this mutation will be detected in additional families with hearing impairment in the future.

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Identification of 605ins46, a novel *GJB2* mutation in a Japanese family

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Abstract

Connexin 26 gene (*GJB2*) mutations are known to be responsible for a significant portion (30–80%) of autosomal recessive congenital severe to profound deafness. More than 60 recessive mutations in *GJB2* have been reported and most consist of point mutations of a nucleotide. We report here a novel insertional *GJB2* mutation consisting of a long repetitive nucleotide sequence. As compound heterozygotes of this mutation with 235delC express sensorineural hearing loss of variable severity, further analysis of the phenotype–genotype relationship is required.
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Keywords: Connexin 26; *GJB2*; 605ins46; Insertional mutation

1. Introduction

Congenital severe to profound hearing loss affects one in a thousand neonates [1] and genetic factors are involved in half of those cases [2]. Approximately 80% of this total, shows autosomal recessive inheritance and more than 60 recessive loci have been reported (Van Camp G., Smith R.J.H. Hereditary Hearing Loss Homepage; http://dnalab-www.uia.ac.be/dnalab/hhh). A large proportion of recessive severe to profound hearing loss has been linked to the DFNB1 locus and it has been demonstrated that mutations in *GJB2* lead to hearing impairment at this locus [3]. *GJB2* gene mutations are involved in roughly 50% of recessive inherited hearing loss in many populations [4–6]. Mutation analysis in Japanese non-syndromic hearing loss patients also indicated that *GJB2* is the most prevalent gene causing recessive inherited non-syndromic hearing loss [7]. With regard to the frequent mutations, however, the Japanese population has different combinations of *GJB2* mutations in comparison with other populations [7]. Therefore, it is likely that the spectrum of *GJB2* mutations in Japanese is quite different from that found in populations with European ancestry. The current report presents a novel *GJB2* mutation in a Japanese family with autosomal recessively inherited deafness.

2. Subjects and methods

The proband was a 7-year-old Japanese boy who had a history of congenital hearing loss. He visited one of our affiliated clinics to consult about his severe hearing loss with his 48-year-old mother who also had congenital hearing loss. The proband had a 9-year-old sister who was also affected by congenital hearing loss. Neither child had any abnormal history during pregnancy or delivery.

Informed consent was obtained from all participants in the study. DNA samples from the study participants and controls were extracted from the peripheral blood collected at our institution or at affiliated institutions.

2.1. Mutation analysis

The entire coding region for *GJB2* was amplified from genomic DNA samples using the primer pair

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Cx48U/Cx1040L (5′-GGTGAGGTTGTGTAAGAGTTGG-3′/3′-AGCAGAGCTCATTGTGGCATC-5′) and used as a template for sequencing. Cx433U (5′-CTGCAGCTGATCTTCGTGTCC-3′) was also used for sequencing as an additional primer.

PCR conditions were as follows: 5 min denaturation at 95 °C followed by 37 three step cycles of 95 °C for 30 s, 60 °C for 30 s and 72 °C for 2.5 min, followed by 72 °C for 10 min, ending with a holding period at 4 °C in a Perkin–Elmer thermal cycler. PCR products were directly sequenced after unincorporated dNTPs and primers were removed by incubation at 37 °C for 30 min with 50–100 ng PCR products with 0.1 μl exonuclease I (Amersham Life Science Inc, Cleveland, USA) and 1 μl shrimp alkaline phosphatase (Amersham Life Science Inc). The enzymes were heat inactivated for 15 min at 80 °C. Four pmol of either primer Cx48U or Cx433U were used in standard cycle sequencing reactions with ABI BigDye terminators and run on an ABI 377 sequencer. DNA samples from 96 normal hearing unrelated Japanese were used as controls.

3. Results

A novel mutation, 605ins46, was identified (Fig. 1). The length of the mutation was confirmed by sequencing backward as shown in Fig. 2. This insertional mutation has a tandem repeat of 46 nucleotides (corresponding to the positions 559–604 of the Cx26 DNA sequence) at the position 605. A stop codon (TGA) is produced at the 202nd amino acid, leading to the premature truncation in the series of polypeptide synthesis.

In the present family, the sister, parents, and the sibs of both parents expressed sensorineural hearing loss. DNA sequence analysis revealed that the proband and the mother had the compound heterozygous mutation of 235delC and 605ins46 whereas his sister was homozygous for 235delC (Fig. 3).

No 605ins46 mutations were detected in the control subjects.

4. Discussion

Many mutations have been identified in the *GJB2* gene. The deletion of one guanosine residue from a stretch of six between nucleotide positions 30 and 35 (30delG or 35delG) in the DNA sequence is the most common mutation found in over two-thirds of patients affected with DFNB1 in many ethnic groups (in sporadic patients and autosomal recessive families from Italy, Spain, Portugal, France, Great Britain, Israel, Lebanon, Morocco, Tunisia, Algeria, and New Zealand, and in Caucasian American families of northern and southern European origin) [4–6,8–10]. On the other hand, 235delC is the most prevalent mutation in the Japanese and Koreans [7,11], and the 167delT mutation is the most common in Ashkenazi Jews [12]. The highly frequent deafness-causing allele variant, 35delG, with premature protein truncation at codon 13, was recently revealed to be due to a founder effect rather than a mutational hot spot [13].

To date, 75 recessive and five dominant mutations in non-syndromic deafness and four dominant mutations in syndromic deafness have been identified in *GJB2* (Rabionet R., Gassparini P., Estivill X. Connexins and deafness Homepage: http://www.iro.es/cx26deaf.html, 2001). In contrast to the number of point mutations, the most common type reported, mutation by the insertion of the nucleotide(s) is rare with only six found: 35insG, 51del12insA, 269insT, 486insT, 504insAAGG, and 509insA. The current report is, therefore, the first of a connexin 26 insertional mutation with a long nucleotide sequence. This mutation's peculiarity is also seen in it's repetition of the DNA sequence.

The possible cause of this tandem duplication may be a slipped mispairing or homologous unequal recombination [14]. In COL2A1 variations of partial duplication of 45-bp have been reported [15] and the same mechanism could be applied to this 46-bp tandem repeat.

The schema of connexin 26 (*GJB2*) (Fig. 4) indicates the location of the mutations involved in this family in which 235delC is sited in the second transmembrane domain and 605ins46 is located at the fourth transmembrane domain. The 235delC and 605ins46 heterozygote

605ins46

ccacg/gagaagactgtcttcacagtgttcatgattgcagtgtctggaatt/gagaagactgtcttcacagtgttcatgattgcagtgtctggaattt/gcatcctgctgaatgtcactgaattgtgttatttgctaattagata/ttgttct

Normal sequence

559 ... 605

ccacg/gagaagactgtcttcacagtgttcatgattgcagtgtctggaattt/gcatcctgctgaatgtcactgaattgtgttatttgctaattagata/ttgttct

Fig. 1. Top, DNA sequence of 46-bp insertion as a tandem repeat. Boxed region represents a stop codon. Shaded region is duplicated nucleotides as an insertion. Bottom, normal sequence and its position.

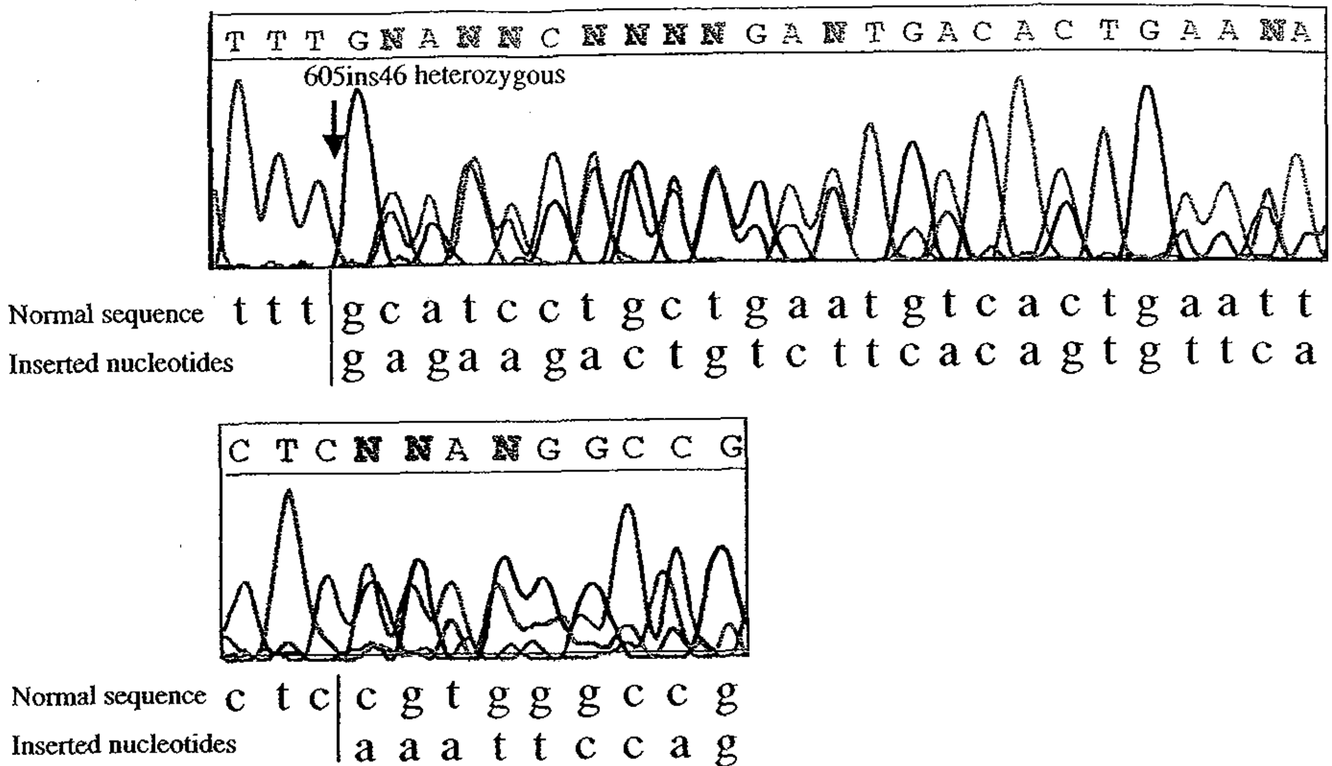

Fig. 2. Direct sequence analysis of patients for 605ins46. Top, partial sequence of the proband. Bottom, the end-point of the insertion identified by the backward direct sequence.

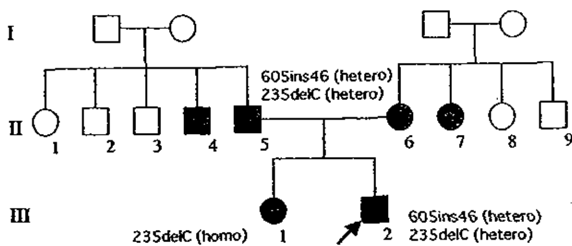

Fig. 3. The pedigree and sequence results for the family.

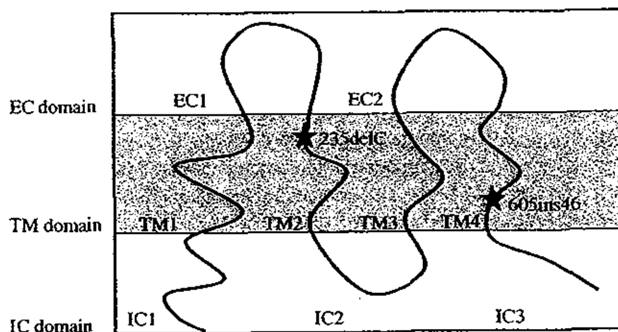

Fig. 4. Schematic representation of Cx26 and its mutations: Stars, two recessive mutations found in the family studied; TM, transmembrane domains; EC, extracellular domains; IC, intracellular domains.

(frameshift leads to 81X for 235delC, C202X for 605ins46) produces a longer polypeptide than in 235delC. The heterozygote seems to have less severe hearing impairment. However, audiograms of the family members (Fig. 5) revealed severe sensorineural hearing loss in the mother (235delC and 605ins46 heterozygote) and the sister (235delC homozygote), and moderate sensorineural hearing loss in the proband (235delC and 605ins46 heterozygote). This is consistent with the reported intrafamilial variability [9,16].

The high variability and phenotype independence in *GJB2* mutations indicate the presence of either environmental factors or modifying genes that can affect the particular *GJB2* phenotype. The second connexin gene might share partial functional redundancy with *GJB2* or other unknown gene mutations may produce a synergistic effect on the severity, as indicated in the case of mitochondrial mutations [17].

The reason for genotype–phenotype discrepancy in this family might be attributed not only to the influence of the senescence or the environmental factors but also to the transcriptional and/or post-transcriptional modification. This leads to difference in the molecular masses or isoelectric point changes of the protein. In the same way, as already reported in the protein product of the *COCH* gene, altered tertiary structure could lead to a functional disturbance [18].

Thus, given the currently accumulated knowledge of the *GJB2* gene, future research should be aimed at

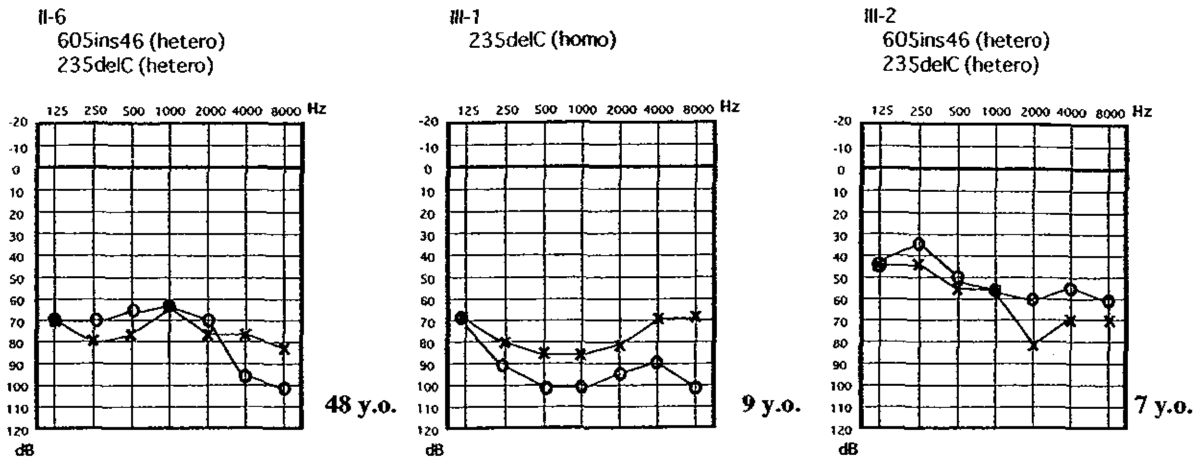

Fig. 5. Audiograms of the family members and the mutation analysis results.

analyzing the internal and external subsidiary factors by focusing on the protein interactions.

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Gene Therapy and Therapeutic Interventions

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Molecular Diagnosis of Deafness: Impact of Gene Identification

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Key Words
Gene · Deafness · *GJB2* (connexin 26) · *PDS* · Mitochondria

Abstract
Recent progress in identifying genes responsible for hearing loss enables the ENT clinician to apply molecular diagnosis by genetic testing. This article focuses on three genes, which are prevalent and therefore commonly encountered in the clinic. *GJB2* (connexin 26) is currently recognized as the most prevalent gene responsible for congenital hearing loss in many countries. A series of reports revealed that different combinations of *GJB2* mutations exist in different ethnic populations, indicating that ethnic background should be considered when performing genetic testing. *GJB2* mutations will be of particular interest in combination with universal infant hearing screening programs, because it has been shown that early identification of hearing loss and early intervention are crucial for language development. Progress in genetic analysis has changed the concept of diseases. The present review introduces the example of two historically distinct categories of disease, Pendred syndrome and nonsyndromic hearing loss associated with enlarged vestibular aqueduct, which are currently considered to be a continuum of diseases caused by the same gene, *PDS*. This review also emphasizes that some hearing impairment can be prevented. The 1555A→G mitochondrial mutation, the most prevalent mitochondrial mutation found in the hearing-impaired population, was found in approximately 3% of the outpatients. The 1555A→G mutation is known to be associated with a susceptibility to aminoglycoside antibiotics. There may be a considerably large high-risk population and to avoid possible side effects in this group, a rapid mass screening system and careful counseling are recommended.

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Introduction

Approximately 1 in 500–1000 children is affected by prelingual deafness [Morton, 1991] and it has been estimated that a significant portion (at least 50%) of hearing impairment is genetically determined [Morton, 1991; Marazita et al., 1993; Kimberling, 1999].

Hearing impairment is a genetically highly heterogeneous disorder, and it is estimated that many genes are responsible for a similar phenotype. Much effort has been made to identify the loci including the responsible genes for deafness, mainly by means of linkage analysis. More than 70 loci have already been demonstrated for nonsyndromic hearing impairment and 21 genes have been identified [Van Camp G, Smith RJH: Hereditary Hearing Loss Homepage: http://www.uia.ac.be/dnalab/hhh].

Meanwhile, consequent progress has also been made in the study of the key molecules encoded by deafness genes

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[Steel and Bussoli, 1999; Willems, 2000; Steel and Kros, 2001, for review]. These molecules are being extensively studied from the morphological as well as physiological viewpoints. The development of knockout mice for some genes has also made progress and has provided valuable information regarding the functional role of the coded proteins. The identification of genes that are responsible for hearing loss is indeed a breakthrough approach and has advanced the knowledge of the biology of hearing.

One of the remarkable aspects associated with the identification of the genes involved in hearing is that the clinician can possibly make a molecular diagnosis by genetic testing. This is a highly accurate diagnosis and results in considerable improvement in genetic counseling. Our series of studies indicated that mutations in these genes are frequently found among the hearing-impaired population, and thus much attention should be paid to the genetic background of these patients. The ENT clinician will be able to use the molecular genetic information to help decide the strategy of assistance with hearing, i.e., whether a child should receive cochlear implantation or hearing aids. Three genes, which are prevalent and therefore commonly encountered in the clinic, are reviewed in this article.

GJB2: The Most Prevalent Gene Responsible for Congenital Hearing Loss

GJB2, which codes the gap junction protein connexin (Cx) 26, was the first gene reported to be responsible for nonsyndromic hearing impairment [Kelsell et al., 1997]. As shown in figure 1, Cx 26 is known to be distributed in the spiral ligament, basal cells of the stria vascularis, various supporting cells, and limbal fibrocytes, and is thought to constitute a major system of intracellular communication. It is currently accepted that Cx 26 has an important role in recycling potassium ions. Recent remarkable progress in genetic approaches revealed several genes that may be involved in maintaining the ionic environment of the cochlea. The focus has been on the molecules coded by genes including *KCNQ4, GJB2, GJB3, GJB6, Pou3f4, Slc12a2, KCNQ1,* and *KCNE1,* that recycle potassium ions [Steel and Bussoli, 1999; Steel and Kros, 2001]. Mutations in *GJB2,* the gene responsible for DNFB1 and DFNA3, have recently been of particular interest because these mutations account for up to 50% of congenital deafness in European countries as well as in the United States [Kelsell et al., 2001, for review]. To date, more than 50 disease-causing mutations in *GJB2* have been reported for both recessive as well as dominant forms of nonsyndromic hearing loss [Rabionet R, Gasparini P, Estivill X: Connexins and Deafness Homepage: http//www.iro.es/cx26deaf.html]. One mutation, namely 35delG, has been the most commonly detected in many countries [Kelsell et al., 2001, for review]. Mutation analysis for *GJB2* in Japanese nonsyndromic hearing loss patients is also in line with the previous reports and indicated that *GJB2* is the most prevalent responsible gene as a cause of recessive inherited nonsyndromic hearing loss. With regard to the frequent mutations, however, the Japanese population has different combinations of *GJB2* mutations in comparison with other populations, 235delC, Y136X, and R143W being the most important in Japanese patients with hearing loss [Abe et al., 2000]. Of these, the most frequent mutation, 235delC, accounted for 73% of mutated alleles. The 35delG mutation, although known as the most common *GJB2* mutation in people of European ancestry, was not found in our mutation screening of Japanese subjects. Phylogenetic analysis indicated the evidence of a founder effect for the 235delC mutation [manuscript in preparation]. This indicates that ethnic background may be important and should be considered when performing genetic testing.

Universal infant hearing screening programs are the current trend, because it has been shown that early identification of hearing loss and early intervention are crucial. Significantly better language development was achieved in children whose hearing losses were identified by 6 months of age [Yoshinaga-Itano et al., 1998]. Children with congenital hearing impairment associated with *GJB2* mutations who received cochlear implantation showed remarkable improvement in auditory skills and development of speech production compared to those with deafness due to other etiology [Fukushima et al., personal communication]. This indicates that determination of the cause of deafness by genetic testing may be useful in anticipating the prognosis of language development. *GJB2,* which accounts for significant proportions of congenital genetic hearing impairment in children, is a relatively small gene with the coding region contained within one exon, facilitating the development of a screening system. An inexpensive, rapid, high-throughput, large-scale screening method is the goal. Recent technological advances have made it possible to apply microarray for detection. We have succeeded in detecting the above three frequent *GJB2* mutations using oligonucleotide microarray [Asamura, in preparation]. Future improvements in diagnostic accuracy using microarray technology will contribute to early diagnosis and better counseling.

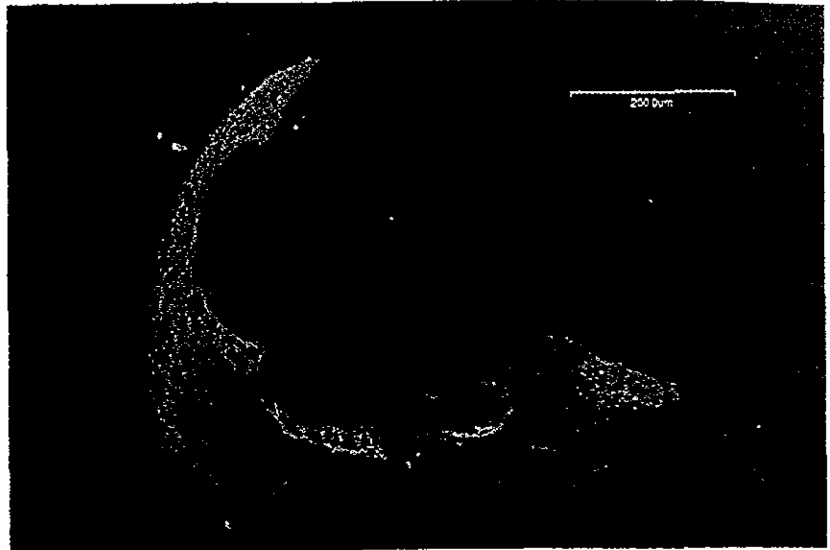

Fig. 1. Immunoreactivity for Cx 26 in the rat organ of Corti. Bar = 200 μm.

PDS: A New Concept of Disease

Based on genetic evaluations, the historical concept of two distinct categories of disease, Pendred syndrome and nonsyndromic hearing loss associated with an enlarged vestibular aqueduct (EVA), has changed and they are currently considered to be a continuum of diseases caused by the same gene.

Various types of inner ear anomaly have been reported to be associated with hearing loss. The most common is EVA [Urman and Talbot, 1990; Mafee et al., 1992] (fig. 2). EVA can be easily evaluated by CT scan, and the enlarged endolymphatic duct and sac can be confirmed by MRI. As this anomaly is associated with characteristic clinical findings, it is of particular interest. These findings include high-frequency involvement, the conductive component at the lower frequencies, fluctuating and sometimes progressive hearing loss, and disequilibrium symptoms in about one third of patients [Abe et al., 1997] (fig. 3). Other clinical manifestations, including palpable goiter and/or positive perchlorate discharge test, which are diagnostic criteria of Pendred syndrome, are not associated with EVA. There have been reports on families with unaffected parents but with 2 affected sibs, indicating that sensorineural hearing loss associated with EVA is inherited in a recessive manner [Griffith, 1996; Abe, 1997]. Subsequent linkage analysis was initiated to determine the location of the gene that causes this specific form of hearing loss. Multipoint linkage indicated a responsible gene localized in a region overlapping the region that contains the gene responsible for Pendred syndrome, *PDS*

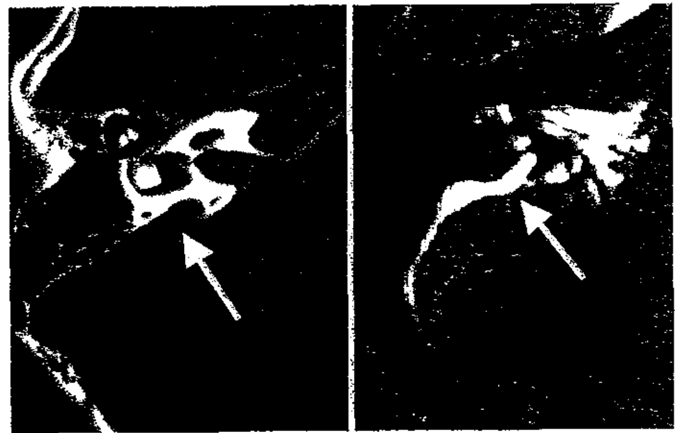

Fig. 2. CT findings and MR images of EVA (arrows). T_2-weighted MRI showing enlarged bilateral endolymphatic sac and duct. Note that the shape of the endolymphatic sac complements the shape of the bony labyrinth shown in CT.

[Abe et al., 1999], which was reported in 1997 [Everett et al., 1997]. Pendred syndrome is an autosomal recessive disorder originally defined as associated with deafness and goiter, and is also known to be associated with the Mondini anomaly. Mutation screening revealed mutations in the *PDS* gene in families of nonsyndromic hearing loss associated with EVA, confirming our hypothesis that mutations in *PDS* cause a broader phenotypic spectrum, from typical Pendred syndrome to nonsyndromic hearing loss associated with EVA [Usami et al., 1999b].

Pendrin, the *PDS*-encoded protein, is thought to be involved in transportation of iodide, chloride, formate,