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CASE

The H723R Mutation in the *PDS/SLC26A4* Gene Is Associated with Typical Pendred Syndrome in Korean Patients

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Inherited as an autosomal recessive trait, Pendred syndrome is a disease that shows congenital sensorineural hearing loss and goiter, with a positive finding in the perchlorate discharge test. Pendred syndrome results from various mutations in the *PDS/SLC26A4* gene that cause production of an abnormal pendrin protein. More than 90 mutations in the *PDS/SLC26A4* gene have been reported throughout the world. A recent study of 26 Korean patients with a relatively high frequency (65%) of a mutated *PDS/SLC26A4* gene exhibited nonsyndromic deafness and an enlarged vestibular aqueduct. We report two patients with characteristics of typical Pendred syndrome, a 26-yr-old female and a 61-yr-old male, who were both homozygous for a previously reported missense mutation, H723R (Histidine 723Arginine) in the *PDS/SLC26A4* gene.

Key Words: Pendred syndrome; pendrin; *PDS/SLC26A4* gene.

Introduction

Pendred syndrome (PS) is a rare genetic disease reported for the first time in 1896 by Vaughan Pendred who described sisters with goiter and congenital deafness *(1)*. This syndrome is inherited as an autosomal recessive trait, and its characteristics include congenital sensorineural hearing loss, goiter, and a positive perchlorate discharge test indicating an iodide organification defect *(2)*.

In most cases, thyroid function is normal or shows only mild hypothyroidism *(3)*. Goiter is the most variable component of this disorder, with some individuals developing large goiters, while others present with minimal to no enlargement. This variability in the phenotype is thought to be influenced by nutritional iodide intake *(4)*. In most cases, PS presents as bilateral sensorineural hearing loss.

Recently, it has been revealed that the cause of this syndrome is due to the mutation in the *PDS/SLC26A4* gene. The *PDS/SLC26A4* gene, which is located on the chromosome 7q31, is composed of 21 coding exons *(5)*. The protein coded by the *PDS/SLC26A4* gene, pendrin, acts as a chloride/iodide transport protein *(6)*. The approx 5-kb *PDS/SLC26A4* transcript showed striking tissue-specific expression, being highly expressed in the thyroid, kidney, and inner ear *(7)*. The critical role of pendrin in the inner ear has been corroborated by targeted disruption of the *Pds* gene in mice *(8)*. Analysis of the inner ear in these mice revealed dilated endolymphatic ducts and sacs beyond embryonic d 15, presumably as a consequence of defects in anion and fluid transport. These *Pds*–/– mice show profound deafness with vestibular dysfunction, although they lack goiters *(8)*. These observations are in line with the enlargement of the endolymphatic system observed in human patients with PS *(9)*. Another study using *Pds*–/– mice demonstrated that pendrin is expressed in a restricted subset of cells in the inner ear, all of which are in contact with endolymph and are believed to play a role in regulating the unusual ionic composition of this fluid *(10)*. The presence of pendrin in these cells is essential for auditory and vestibular function *(10)*. At the inner ear level, a defect in chloride transport could lead to an altered endolymph composition, resulting in damage of the neuroepithelium and enlargement of the membranous labyrinth structures with an osmotic and toxic mechanism *(11)*. The structural malformation of the inner ear including the endolymphatic duct occurs, and endolymphatic sac is enlarged, consequently developing hearing impairment *(12)*.

In patients who present with sensorineural hearing loss and possibly goiter, the perchlorate discharge test and images showing an enlarged vestibular aqueduct (EVA) are helpful in diagnosing PS. However, typical goiter may not be present, depending on the age of patients, and the perchlorate discharge tests are frequently nonspecific. Therefore, some cases may not be detected as true PS. It is not always

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feasible to completely diagnose this syndrome. Thus, molecular analysis of the *PDS/SLC26A4* gene would be useful for making a definite diagnosis *(13)*.

In PS, the spectrum of mutation appears to show geographic differences *(14)*. Park et al. reported that most deaf patients with EVA in Korea and Japan carried mutations in the *PDS/SLC26A4* gene *(15)*. In two patients who clinically presented with typical PS, we confirmed the mutation of the *PDS/SLC26A4* gene (H723R) by direct DNA sequencing.

Case 1 (YS-1)

This 26-yr-old female first presented in 2005 in our department for evaluation of a diffuse goiter that had been present since she was 1 yr old. Her medical history revealed that she became deaf at the age of 6 yr. At the age of 10 yr, she underwent cochlear prosthesis surgery for sensorineural deafness, and, during the follow-up observation at another hospital, was transferred to our hospital for a detailed test of the goiter. Her mother was taking levothyroxine for a multinodular goiter. Her only younger brother had a pigeon chest from birth and underwent an operation in 1985. Also the brother's right eyelid was absent, which was operated on in 1987, but hearing loss and goiter were absent.

On physical examination, her blood pressure was 120/72 mmHg, pulse 92 beat per minute, respiration rate 15 times per minute, and temperature 36.6°C. She was not acutely ill and had a goiter, although there were no palpable neck lymph nodes. Chest and abdominal diagnosis findings were normal, edema in the lower limbs was absent.

Routine biochemical blood tests were all within normal range. In the thyroid function test, T3 was 123.65 ng/dL (80–220), free T4 was 1.20 ng/dL (0.73–1.95), TSH was 0.68 μIU/mL (0.34–3.5), and anti-thyroglobulin antibody and anti-microsomal antibody tests were both negative.

In a perchlorate discharge test, performed by measuring radiolabeled iodide thyroid uptake before and after oral administration of 1 g of $KClO_4$, her radioiodide content decreased to 76.33% of the maximal value after 15 min, to 70.24% after 90 min, and then to 65.11% after 120 min. These findings suggest impairment of iodide organification. The temporal computed tomography (CT) revealed a bilateral EVA (Fig. 1). Neck ultrasonography showed a diffuse enlargement of the thyroid gland, heterogeneous echotexture, and the presence of several benign cysts in the left thyroid.

Case 2 (YS-2)

This 61-yr-old male was first admitted to our department in 2005 for the detailed examination and management of a huge goiter whose size had increased over the previous 15 yr. His medical history revealed that he had hearing loss since the age of 2 yr; however, no special treatments for it were administered. He was recently diagnosed as glucose intolerant 1 mo prior to admission, and he was undergoing lifestyle modification without any medication. The patient

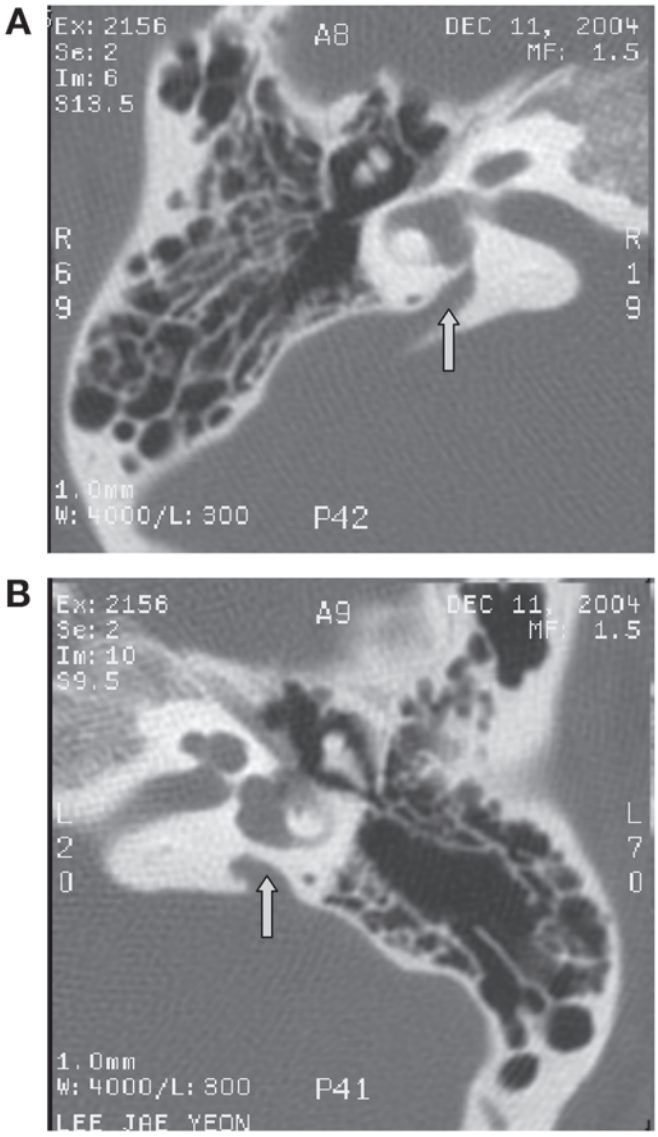

Fig. 1. Axial computed tomographic images of the temporal bones showing enlarged vestibular aqueduct in the right (**A**) and left (**B**) inner ear of YS-1. The enlarged vestibular aqueduct is indicated by the arrow.

has one daughter and one son, and his wife is also a deaf. His daughter was diagnosed as a deaf at the age of 3 yr.

Upon physical examination his blood pressure was 104/52 mmHg, pulse rate 92 times per minute, respiration rate 16 times per minute, temperature 36.5°C, and he appeared healthy. However, a large goiter was detected in the anterior neck (Fig. 2). The result of chest and abdomen examination was normal, and edema in the lower limbs was not detected.

Routine biochemical blood tests were all within normal range. A thyroid function test showed a T3 of 177.86 ng/dL (80–220), a free T4 of 0.82 ng/dL (0.73–1.95), and a TSH of 2.01 μIU/mL (0.34–3.5). An anti-thyroglobulin antibody was negative and anti-microsomal antibody was 863.87 IU/mL (<60). His fasting blood sugar was 87 mg/dL (75–115), and his HbA1C was 5.2% (3.8–6.4).

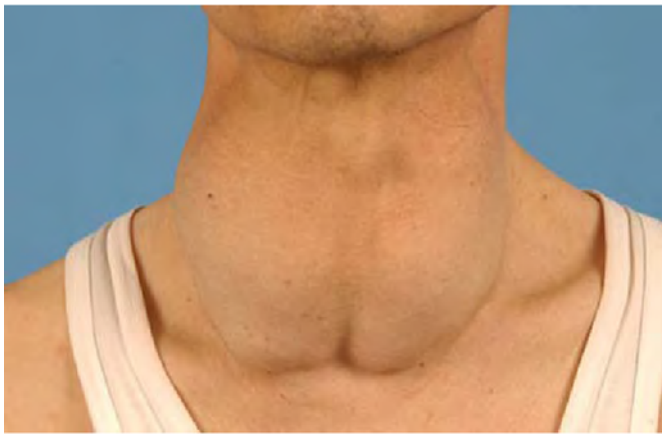

Fig. 2. General appearance of YS-2 showing a protruding anterior huge neck mass (anterior view).

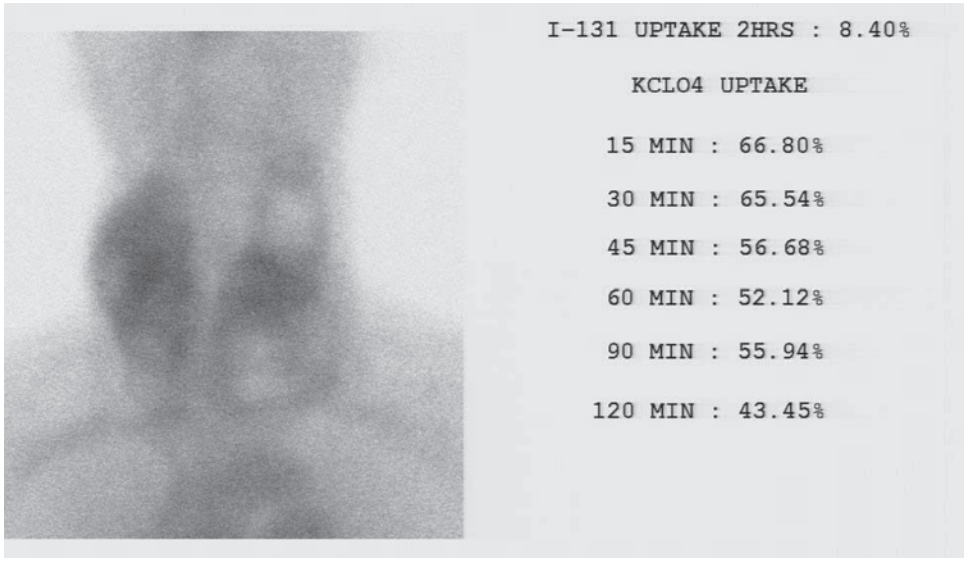

Fig. 3. Perchlorate discharge test of YS-2 shows extensive discharge of radioactive iodine from thyroid after the challenge with 1 g of $KClO_4$ consistent with an organification defect in the thyroid.

In a perchlorate discharge test, the radioiodide content in the thyroid decreased to 66.80% of the maximal level 15 min after administering of 1 g of $KClO_4$ to 55.94% after 90 min, and to 43.45% after 120 min. These findings are consistent with an iodide organification defect (Fig. 3). Neck ultrasonography revealed several large thyroid nodules in the anterior portion; therefore, fine needle aspiration was performed on the right upper portion of the tumor.

Fine needle aspiration was performed on the right thyroid nodule under the guidance of ultrasonography, and thyroid follicular neoplasm was suspected. A bilateral thyroidectomy was performed; the right lobe of the thyroid was 160 g in weight and 11 × 7 × 5 cm in volume, multiple cystic masses were present, and the largest one was 5 × 4 cm. The left lobe was 247 g in weight, 13.5 × 7.5 × 5 cm in volume, and several masses with accompanying hemorrhage and calcification were present (the largest being 5 × 4 cm). The final pathology of several nodules was consistent with nodular adenomatous hyperplasia.

Genetic Testing of the Patient

DNA was extracted from the immediate family members of both patients. Exons 2 to 21 of the *PDS/SLC26A4* gene were amplified by polymerase chain reaction (PCR) using previously reported primers *(5)*. The PCR products were purified with Centricon 100 columns (Amicon, Beverly, MT) and both strands were sequenced with an ABI prism rhodamine dye primer cycle sequencing kit following the protocol of the supplier using a 3773A sequencer (Applied Biosystems, Foster City, CA).

The genetic analysis was performed on the first degree family members of both patients. After obtaining informed consent, in both patients, the H723R mutation (2169A>G)

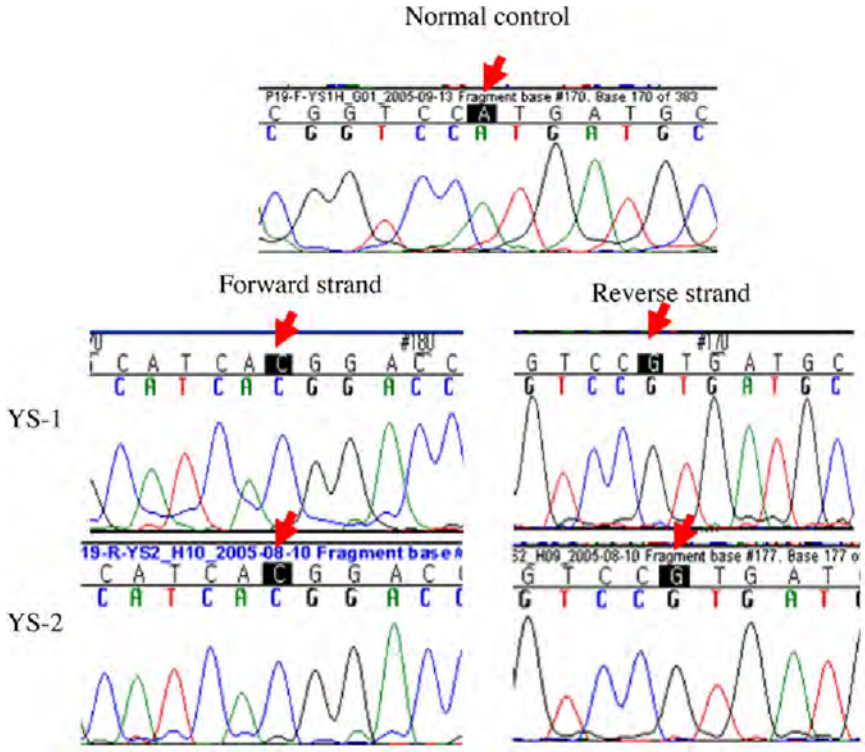

Fig. 4. DNA analysis of the PDS gene mutation described in YS-1 and YS-2 patients. The homozygous 2169 A>G mutation are indicated by arrows in the forward and reverse strand. For comparison, there is a sequence of same site in a normal control on the top.

was detected in both alleles (Fig. 4). The nucleic acid sequence analysis of the genes of mother, father, and brother of the YS-1 revealed that the patient's other family members were a heterozygous carriers. The result for family members of YS-2 showed that both children of the patient were heterozygous carriers of the H723 mutation. Among them, the daughter had hearing loss; therefore, it is possible that she has an undetected mutation in the *PDS/SLC26A4* gene and possibility of a compound heterozygous mutation. The husband of the daughter was also deaf, and the husband was found to have the bialleic *GJB2* mutation (OMIM 121011). All phenotypes of the grandchildren of the patient were normal, and only one of four granddaughters was a heterozygous carrier (Fig. 5).

Discussion

PS is an autosomal recessive disease with a incidence of 7.5 to 10 per 100,000 individuals, and it comprises 10% of all cases of hereditary deafness *(2)*. It is the most frequent type among syndromic deafness, and together with congenital or progressive sensorineural hearing loss, patients have a goiter with a positive perchlorate discharge test *(2)*. Based on the typical enlargement of the endolymphatic system in patients with PS and the *Pds* knockout mouse *(8)*, pendrin is probably also involved in anion and fluid transport in the inner ear. However, the exact role of pendrin in the inner ear remains to be defined *(16)*. A previous study by Gillam et al. *(16)* suggests that the impairment of iodide transport through the apical membrane of thyroid cells into the colloid lumen is the cause of the thyroid functional impairment in the thyroid. Using polarized mammalian cells, it was demonstrated that pendrin protein mediates the apical iodide efflux *(16)*. The possible role of pendrin in the inner ear is less clear; it has been hypothesized that the chloride transport function of pendrin is important in the homeostasis of the endolymph with ionic imbalance potentially responsible for the auditory developmental malformation *(8)*. EVA is associated with fluctuating and sometimes progressive sensorineural hearing loss *(17)* and is known as the most common form of inner ear abnormality among the hearing impaired population *(18)*. Therefore, when the *PDS/SLC26A4* gene is mutated, the abnormal pendrin protein develops, which has been reported to induce goiter and EVA, i.e., PS *(12)*.

Clinical symptoms and signs with additional imaging tests have been used for the diagnosis of PS. Subtle changes in iodide organification defect of the thyroid gland without typical goiter or nonspecificity of the perchlorate discharge test hinder the definite diagnosis of PS. In Hashimoto thyroiditis, ^{131}I-treated thyrotoxicosis, total iodine organification deficiency, and other diseases, false positive results from the perchlorate discharge test can also be detected, and actual PS patients with a negative perchlorate test have been reported *(19)*. Because of these reasons, radiologic and molecular studies should be performed for confirmation of

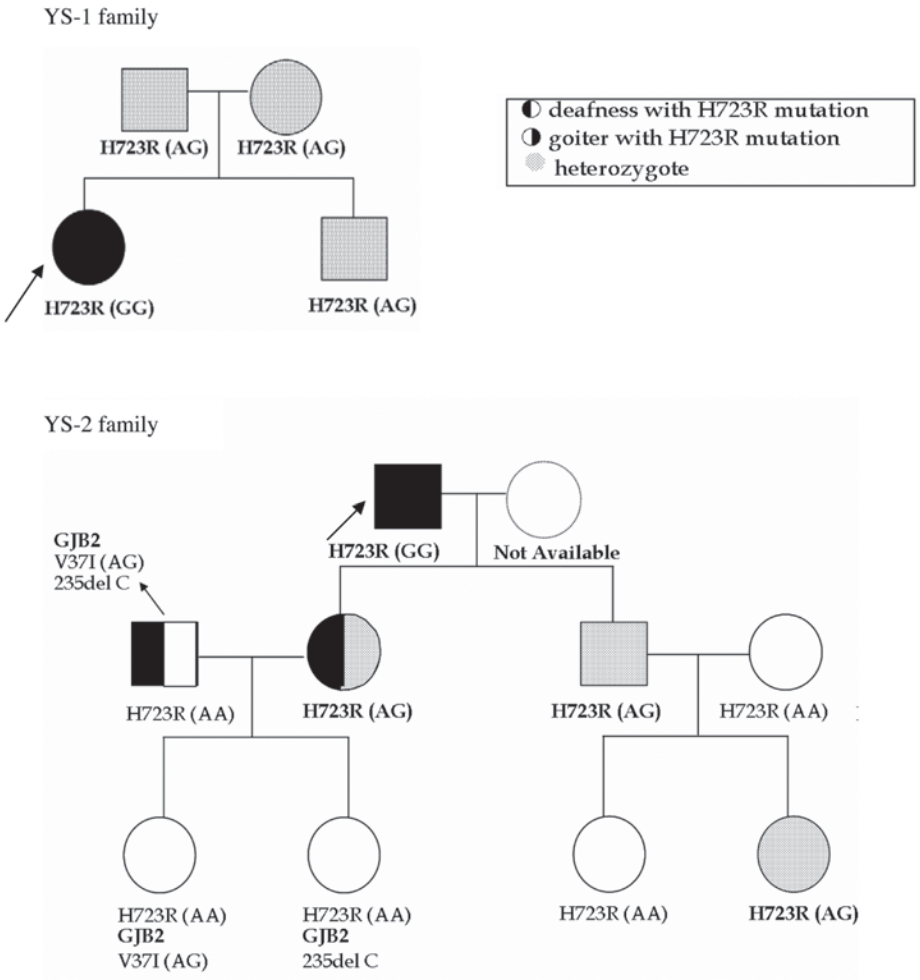

Fig. 5. Family pedigree, phenotype, and detected mutations in the PDS gene. The nucleic acid sequence analysis of the genes of mother, father, and brother of the YS-1 revealed that the rest of the family members were heterozygous carriers. The results of YS-2 family member showed that both children of the patient were heterozygous carriers. All phenotypes of the grandchildren of the patient were normal, and only one granddaughter was a heterozygous carrier among four granddaughters.

PS *(20)*. In the patients presented in this paper, homozygous mutations of the *PDS/SLC26A4* gene (H723R) were detected and the findings were consistent with the clinical signs such as goiter and sensorineural hearing loss with EVA and the positive perchlorate discharge test. Consequently, we could definitely diagnose PS. The clinical pattern of PS appears to be diverse among families, and it is different even in the same family *(13)*. This is thought to be due to the fact that phenotypes induced by the mutations in the *PDS/SLC26A4* gene vary considerably within families *(12)*. The variability in phenotype may be dependent on the nutritional iodide intake and modifier genes. According to the long-term follow-up result of 14 PS patients carried out by Sugiura et al. *(21)*, patients with the H723R mutation were less likely to have goiter and profound hearing loss compared with previously described patients with a PDS mutation other than the H723R mutation *(21)*. Also, patients with the H723R mutation have a mild impairment level of iodide organification in the perchlorate discharge test *(21)*. Iodine deficiency causes goiter, and the daily intake of iodine varies widely according to eating habits *(22)*. However, the daily iodine intake of an adult Korean is about 61–4086 μg because of their high intake of fish and seaweed *(23)*.

After reviewing the family members of PS patients, Hiyoshi & Yamane *(24)* reported that some PS patients have sensorineural hearing loss, goiter, and a positive perchlorate discharge test simultaneously, while others have only sensorineural deafness or a positive perchlorate discharge test *(24)*, thus showing the very diverse clinical patterns of this disease *(24,25)*. Besides congenital hearing loss, some PS patients are negative for the perchlorate discharge test or goiter. The first manifestation of PS is typically hearing loss at early ages, whereas the cases in which a goiter was detected at puberty are most frequent *(13,26,27)*.

The first analysis of the *PDS/SLC26A4* gene mutation in Korea focused on patients presenting with hearing loss, i.e., nonsyndromic EVA (15). According to the analysis of the *PDS/SLC26A4* gene in Asian hearing loss patients by Park et al. *(15)*, 6 out of 92 hearing loss patients in Korea had *PDS/SLC26A4* mutation, and four patients among them

had the H723R mutation (one patient was homozygous and three pateints were heterozygous). Park et al. reported that the result of *PDS/SLC26A4* gene analysis in 26 hearing loss patients who showed EVA phenotype was that 14 types of mutations, including nine novel mutations, were discovered among them *(28)*. The two patients presented here were both homozygous for the H723R mutation. Even though the mother and father of YS-1 were heterozygous carriers, showing the H723R mutation only in one allele of *PDS/SLC26A4* gene, YS-1 had a homozygous genotype. An interesting point to be mentioned here is the result that two individuals among the 120 individuals who served as a control group were heterozygous carriers for the H723R mutation *(15)*. This further implies that the H723R mutation is not rare and that the genetic test for *PDS/SLC26A4* is not simply a diagnostic tool for definite diagnosis, but also an essential test that predicts the possibility of disease development in offsprings of the patient.

It has been reported that the hearing loss in PS patients can sometimes only manifest several years after birth and then progress gradually *(13,26,29)*. Therefore, it is thought that the genetic analysis of PS patients and their family is an important tool to assess the possibility of their offspring developing hearing impairment and its progression level, thus providing an opportunity to minimize the harm of deafness by early detection and treatment of hearing impairment.

The second patient in our study was a case in which thyroid follicular neoplasm was suspected, therefore, surgery was performed by ATA guidelines *(30)*, although the postsurgical final pathological result was found to be benign. In fact, the development of thyroid cancer in PS is rare, but according to several reports, most thyroid cancer cases are follicular cancers, with a more aggressive histological pattern *(31)*. In thyroid tumors, an aberrant hypermethylation of the *PDS/SLC26A4* gene was observed *(32)*. Thyroid cancers have different iodide metabolism defects, which may occur at each step of the iodide pathway, thus including defects of the iodide transporters *(33)*. In the cases with concomitant PS and thyroid cancer, it is speculated that most patients did not receive appropriate thyroid hormone treatment for the hypothyroidism. Therefore, they were chronically exposed to thyroid stimulatory hormone. As a consequence, a large goiter develops, where thyroid cells continuously proliferate, and the opportunity of damage in the genes which control proliferation is increased, thus resulting in thyroid cancer *(34)*.

Through the analysis of typical PS patients, a specific gene lesion was found to cause the syndrome. Over 90 different *PDS/SLC26A4* mutations have been reported, and each ethnic population has its own distinctive mutant allele series with a few prevalent founder mutations *(35,36)*. H723R mutations accounted for 40% of the deaf Korean patients with EVA *(28)*. Therefore, it is predicted that a molecular approach on the specific site of the *PDS/SLC26A4* gene in Korean patients may improve the detection of PS.

In reviewing these two cases, definite diagnosis by genetic test seemed to be important. Furthermore, because differences of the mutation of the *PDS/SLC26A4* gene are dependent on race and because the phenotype is influenced by this mutation, analysis of the of *PDS/SLC26A4* gene mutation pattern may heighten the understanding of phenotype and genotype patterns in Koreans. Genetic tests on family members of patients are important to facilitate early diagnosis and treatment of individuals who may be at high risk of developing the disease.

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