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SLC26A4 p.T410M homozygous mutation in a patient with a cystic cochlea and an enlarged vestibular aqueduct showing characteristic features of incomplete partition type I and II

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Abstract

Mutations of *SLC26A4* are associated with incomplete partition type II (IP-II) and isolated enlargement of the vestibular aqueduct (EVA). We experienced a congenitally deaf patient with a rare p.T410M homozygous mutation of *SLC26A4*. The patient had unusual inner ear malformations on both sides, in which the vestibules and vestibular aqueducts were identical to those in IP-II, but the cochleae lacked a bony modiolus and resembled those in incomplete partition type I. These results suggest that homozygous mutations in *SLC26A4* are always associated with EVA, but that the severity of cochlear malformation may vary depending on the type of *SLC26A4* mutation.

Keywords: *SLC26A4*, homozygous mutation, hearing loss, inner ear malformation, incomplete partition, enlarged vestibular aqueduct

1. Introduction

Severe or profound hearing loss occurs in approximately 1-2 of 1000 newborn children and genetic causes account for more than half of cases of congenital deafness [1, 2]. Mutations of the *SLC26A4* gene, which encodes Pendrin, a member of the solute carrier family, are the second leading cause of autosomal recessive hearing loss and are strongly associated with inner ear malformation, including isolated enlargement of the vestibular aqueduct (EVA) and incomplete partition type II (IP-II), which is defined by a cochlea with confluence of the middle and apical turns, a minimally dilated vestibule, and EVA [3, 4]. Mutations of *SLC26A4* result in non-syndromic or syndromic hereditary hearing loss [5, 6]. *SLC26A4* was originally identified as the causative gene of Pendred syndrome, an autosomal recessive hereditary disease characterized by congenital hearing loss and early adult-onset thyroid goiter [7]. Subsequently, mutations of *SLC26A4* have also been found in patients with familial non-syndromic sensorineural hearing loss with EVA [5].

Pendrin is expressed in the inner ear, thyroid, and kidney [8] and functions as a transmembrane $Cl^-/I^-/OH^-/HCO_3^-$ exchanger across the plasma membrane in vitro [9-11]. Pendrin is thought to mediate Cl^-/I^- exchange in the thyroid [9] and $Cl^-/HCO3^-$ exchange in the inner ear [12]. To date, more than 170 mutations of *SLC26A4*, including missense, nonsense, frameshift, and splice site mutations, have been identified and these mutations are located throughout the coding region of Pendrin [13]. Interestingly, the most common mutation differs among countries [13]. A recent

genetic study in the Japanese population showed that the p.H723R mutation accounted for more than 50% of identified *SLC26A4* mutations, while the frequency of p.T410M is less than 10% and relatively rare, but is found in Asian, European and American populations [14-18]. Compound heterozygotes of p.T410M and another mutation, as well as the mono-allelic p.T410M mutation in *SLC26A4* have been associated with isolated EVA or IP-II [19, 20]. However, only a few p.T410M homozygous patients have been described and the clinical features and radiographic findings in these cases have not been widely investigated [17, 21]. Here, we present the case of a congenitally deaf patient who was homozygous for p.T410M *SLC26A4* and had an unusual inner ear malformation that was partly similar to IP-II, the typical malformation associated with *SLC26A4* mutations, but clearly differed from IP-II because of cystic cochleae lacking a bony modiolus.

2. Case report

The patient was an otherwise healthy 1-year-old boy who was referred to our hospital because of failure to pass newborn hearing screening. He had no family history of hearing loss and no history of prenatal or perinatal problems. He started to use bilateral hearing aids at 5 months old, but did not respond to surrounding sounds after 6 months of use of the hearing aids. An otoscopic examination revealed no abnormality in his tympanic membranes and ABR testing exhibited no response at 105 dB on both sides, indicating bilateral profound sensorineural hearing loss. This

patient showed neither developmental delay nor associated psychophysiological abnormalities, except for delayed speech development due to the profound sensorineural hearing loss. The Invader assay for screening of 47 mutations of 13 known deafness genes that are common in the Japanese population [22] identified a p.T410M homozygous mutation in the *SLC26A4* gene.

CT images revealed a bilaterally symmetrical inner ear malformation with (1) a cystic cochlea without a bony modiolus, (2) a dilated vestibule, and (3) EVA (Fig. 1A-D), indicating characteristic features of IP-I (Fig. 1E, F) and IP-II (Fig. 1G, H). The cystic cochleae without a bony modiolus resembled those in IP-I, while the dilated vestibule and EVA were similar to those in typical IP-II found in patients with homozygous mutations of p.H723R, the most common mutation of *SLC26A4* in Japan [14]. To confirm this finding, we measured the size of each part of the malformed inner ears (Table 1). The length of the basal portion and the height of the cochleae were 8.81 mm and 4.93 mm, respectively, on the right side, and 9.10 mm and 5.51 mm, respectively, on the left side, which are similar to the width of 8.59 ± 0.41 mm and height of 5.31 ± 0.41 mm for normal cochleae [23]. The normal external dimensions of the cochleae and complete absence of the bony modiolus indicated cochleae similar to those observed in IP -I [4]. The widths of the vestibule were 4.03 mm and 4.18 mm on the right and left sides, respectively, which were shorter than the typical length of 5.74 ± 0.68 mm in IP-I, but similar to the length of 4.26 ± 0.38 in IP-II [23]. The widths of the EVA at the midpoint of the vestibular aqueduct were 3.73 mm and 4.33 mm on the

right and left sides, respectively, which were larger than the criteria of 1.5 mm for EVA [4]. These findings showed that, unlike the cochleae, the vestibule and vestibular aqueduct on each side were typical of those found in IP-II. CT images also revealed a bony defect between the fundus of the IAC on both sides (Fig. 1 A, C).

T2-weighted MRI revealed that soft tissue separated the fundus of the IAC from the malformed cochlea on the right side, but that the IAC communicated with the cystic cochlea on the left side (Fig. 2A, B). A modiolus-like structure with low intensity on T2-weighted images was observed at the center of the right malformed cochlea, suggesting that neural tissue was present in the center of the cochlea, despite the lack of a bony modiolus, while the left side showed a complete empty cochlea. Interestingly, MRI showed that the endolymphatic sac was not enlarged, even though the vestibular aqueduct was significantly enlarged (Fig. 2A, B). In IP-II and isolated EVA, an EVA is always associated with an abnormally large endolymphatic sac, which is a clear contrast to the observations in the present case (Fig. 2C).

The patient underwent sequential bilateral cochlear implantation: the first implantation on the right side at 14 months old and the second on the left side at 6 years old. A perimodiolar hugging electrode array with half-banded electrodes and a straight electrode array with full banded electrodes (Nucleus CI24RECS and Nucleus CI24REST, respectively; Cochlear Ltd., Sydney, Australia) were inserted via a cochleostomy on the right and left sides, respectively. A cerebrospinal fluid (CSF)

gusher occurred during the left side implantation, but not on the right side, as predicted by the MRI findings showing a lack of the lateral end of the IAC on the left side, as described above. The CSF gusher on the left side was easily controlled by plugging of soft tissue at the cochleostomy. The patient understood conversation without lip-reading with a familiar talker at 14 months after the first implantation and scored 90% on a Japanese infant word discrimination test. No delay in language development was observed before the second implantation on the left side performed at 6 years old.

3. Discussion

The patient in this case report had p.T410M homozygous mutation in the *SLC26A4* gene and showed bilateral symmetric inner ear malformation on CT imaging, with a cystic cochlea, a dilated vestibule, and EVA. With regard to each part of the bony labyrinth, the cystic cochlea without a bony modiolus was similar to that observed in IP-I, while the dilated vestibule and EVA were identical to those in IP-II. MRI showed that a basement membrane-like structure divided the membrane labyrinth into the scala tympani and scala vestibuli in the left malformed cochlea, suggesting that this cochlea was more differentiated than the completely empty cochlea observed in typical IP-I [4, 23]. MRI also showed that each side of the endolymphatic sac was not dilated, even though the vestibular aqueduct was as wide as those in isolated EVA and IP-II.

These radiographic findings imply that the inner ear malformation in this case had

characteristic features of both IP-I and IP-II and could be classified between IP-I and IP-II using the classification of inner ear malformations published in 2010 by Sennaroglu [4]. In this classification, EVA is observed in cochlear hypoplasia type II other than IP-II and isolated EVA. Cochlear hypoplasia type II is defined by (1) smaller dimensions of the cochlea with no modiolus and interscalar septa, (2) normal external architecture of the cochlea, (3) a minimally dilated vestibule, and (4) EVA [4]. The malformed inner ears in our case resembled cochlear hypoplasia type II, but did not meet the first criterion. The outer diameters of the cochleae were as large as those of normal cochleae and based on this finding we concluded that the malformed inner ears were not cochlear hypoplasia type II, but rather were located between IP-I and IP-II.

To our knowledge, this is the first reported case with bi-allelic *SLC26A4* mutations and a cystic cochlea without a bony modiolus. Many studies have shown that homozygous or compound heterozygous mutations in *SLC26A4* are associated with LVAS and IP-II [24], in which the cochlea is normal and mildly malformed, respectively, and the bony modiolus is always present. The results in our patient with a p.T410M homozygous *SLC26A4* mutation suggest that EVA is a universal phenotype among *SLC26A4* mutations [25], while the severity of cochlear malformations may vary widely between patients with bi-allelic *SLC26A4* mutations. The p.T410M mutation in *SLC26A4* has been found in Asian, European, and American populations, but the frequency is not high among *SLC26A4* mutations, suggesting that patients with a p.T410M homozygous mutation are rare [15-17,

26]. To date, 8 patients with *SLC26A4* p.T410M homozygous mutations from 5 families have been reported in the English literature [15-18, 26]. Among these, only a report from Spain described no inner ear malformation except for EVA in 4 patients of the same family, but CT images were not shown [17]. The other reports did not mention abnormalities other than EVA.

For genetic testing in the present case, we used the Invader assay, which is optimized for screening for 47 common mutations of 13 causative genes in the Japanese deafness population [14]. For *SLC26A4*, the Invader assay evaluates 19 mutations including p.H723R, p.Y530H, p.T416P, p.Q514K, c.919-2A>G, and c.1001+1G>A, which are the most common mutations in the Asian, European, and American populations [14, 15, 17, 27-29]. In our case, the Invader assay detected only a p.T410M homozygous mutation of *SLC26A4* among the 47 mutations in the assay, but additional mutations may have been present because we did not perform other tests, including direct sequencing of *SLC26A4*. Other mutations or polymorphisms in *SLC26A4* and mutations in genes such as *FOXI1*, which regulates *SLC26A4* expression, may exacerbate the phenotype of p.T410M homozygotes [30]. Therefore, the causal relationship between p.T410M homozygous *SLC26A4* and the type of inner ear malformation in our case remains uncertain. An *in vitro* study showed that the p.T410M mutation eliminated the transport function of Pendrin [11]. Given that the $Cl^-/HCO3^-$ exchange function of Pendrin in the endolymphatic sac is essential for normal inner ear development [31], the complete loss of function of Pendrin caused by the p.T410M mutation may result in the

severe malformation in the cochlea. Recent studies have failed to identify a relationship between sites of mutations in *SLC26A4* and phenotypes in patients with hearing loss, but most of these studies focused on clinical findings such as progression and fluctuation of hearing thresholds, goiter, and unilateral or bilateral EVA, rather than on cochlear malformations [18, 25, 32]. Thus, further studies are needed to evaluate the relationship between the severity of cochlear malformations and types of *SLC26A4* mutations.

The presence or absence of an entire modiolus, which consists of a bony modiolus and soft tissue including neural elements, is clinically important when considering cochlear implantation. As pointed out previously, a CSF gusher is associated with inner ear malformations lacking an entire cochlear modiolus due to communication between the IAC and the malformed cochlea [4, 23, 33]. In our patient, we encountered a CSF gusher during implantation on the left side, but not on the right side, as predicted by MRI findings indicating a lack of the entire modiolus and the fundus of the IAC only on the left side. Evaluation of the presence of a modiolus is also useful to select an appropriate type of electrode array for the implant. CT failed to detect a bony modiolus on both sides in the present case, but MRI identified soft tissue (probably neuronal elements) at the center of the right cochlea. Therefore, we used a modiolar hugging electrode array on the right side to stimulate the putative neuronal tissue at the core of the cochlea using the closely positioned electrodes. It should be noted that identification of soft tissue at the core of the cystic cochlea does not necessarily

indicate a need for use of a modiolar hugging electrode array because the precise distribution of neuronal elements is uncertain in this type of malformed cochleae. However, when the entire modiolus is absent, as for the left cochlea in our patient, the standard straight electrode array is recommended because neuronal tissue may be distributed near the inner wall of the cystic cochlea, as we previously showed in patients with a common cavity deformity [34].

The patient underwent sequential cochlear implantation on the right side at 1 year old and on the left side at 6 years old. His implant-aided word discrimination score was excellent and reached 90% at 14 months after the first implantation, and his language development had caught up with that of children with normal hearing before the second implantation. Differences in severity of cochlear malformations may be associated with differences in cochlear implant outcomes. However, the short follow-up period after the second implantation and the 5-year gap between implantations made it difficult to evaluate the negative impacts of the more severe left cochlear malformation on implant-aided auditory performance.

4. Conclusions

We have described the case of a congenitally deaf patient with a rare p.T410M homozygous mutation of the *SLC26A4* gene. This patient had an unusual inner ear malformation that was classified between IP-I and IP-II. The dilated vestibule and EVA were similar to those typically

seen in IP-II, which is associated with *SLC26A4* mutations, but the cystic cochleae without a bony modiolus were similar to features seen in IP-I. The causal relationship between a p.T410M homozygous mutation of *SLC26A4* and this type of inner ear malformation remains unclear. However, these results suggest that homozygous mutations in *SLC26A4* are always associated with EVA, but that the severity of cochlear malformations may vary widely depending on the type of *SLC26A4* mutation.

5. Financial disclosures

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7. Conflict of interest

None.

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9. Figure legends

Fig.1. Axial CT images showing the right and left malformed inner ears in the present case who had a p.T410M homozygous mutation in the *SLC26A4* gene (A-D), IP-I (E and F), and IP-II found in a patient who had a p.H723R homozygous mutation in the *SLC26A4* gene (G and H). The upper column shows malformed cochleae in each inner malformation (arrow heads). An interscalar septum is observed in IP-II (G, black arrow), while the others show a cystic cochlea without a bony modiolus (A, C, and E). The present case has a minimally dilated vestibules (B and D, *), which are similar to that seen in IP-II (H, *), but differ from the large cystic vestibule in IP-I (F, black arrow). Both *SLC26A4* mutations are associated with EVA (B, D, and H, white arrows). Scare bars: 5 mm.

Fig.2. Axial T2-weighted MRI shows that soft tissue with low intensity is present at the center of the cochlea on the right side in the present case who had a p.T410M homozygous mutation in *SLC26A4* (A, arrow). On the other hand, the left side showed a complete empty cochlea without an entire modiolus and the lateral wall of the IAC is absent on left side (B, arrow). The endolymphatic sac is

considerably dilated in IP-II, but not in the present case (A, B, and C, arrow heads). Scare bars: 5 mm.

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Table(s)

Table 1: Measurements of each part of malformed inner ears

	Length of the basal portion of a cochlea (mm)	Height of a cochlea (mm)	Width of a vestibule (mm)	Width of EVA at the midpoint of a vestibular aqueduct (mm)
p.T410M Rt	8.81	4.93	4.03	3.73
p.T410M Lt	9.10	5.51	4.18	4.33
IP-I*	8.45 ± 0.68	5.04 ± 0.46	5.74 ± 0.68	N.E.
IP-II*	8.10 ± 0.46	4.87 ± 0.19	4.26 ± 0.38	3.68 ± 0.36
Normal*	8.59 ± 0.41	5.31 ± 0.41	3.40 ± 0.28	N.E.

p.T410M Rt and Lt indicate the right and left inner ears of the present case, respectively.

* Sennaroglu and Sacci 2004 [23]

N.E.: not evaluated due to no EVA

Figure1

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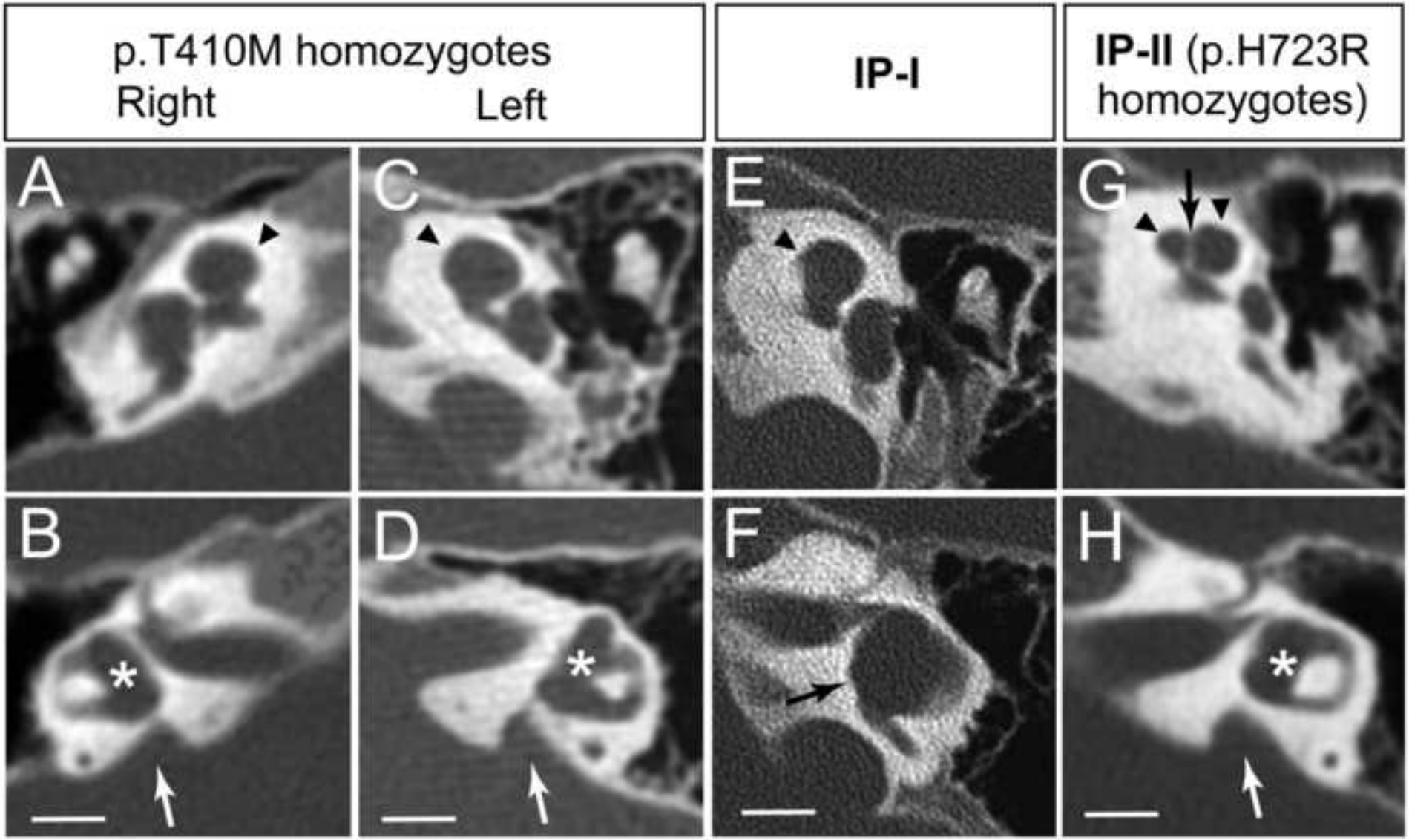

Figure2

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