THYROID RESEARCH

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Influence of dietary iodine deficiency on the thyroid gland in *Slc26a4*-null mutant mice

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Abstract

Background: Pendred syndrome (PDS) is an autosomal recessive disorder characterized by sensorineural hearing impairment and variable degree of goitrous enlargement of the thyroid gland with a partial defect in iodine organification. The thyroid function phenotype can range from normal function to overt hypothyroidism. It is caused by loss-of-function mutations in the *SLC26A4* (*PDS*) gene. The severity of the goiter has been postulated to depend on the amount of dietary iodine intake. However, direct evidence has not been shown to support this hypothesis. Because *Slc26a4*-null mice have deafness but do not develop goiter, we fed the mutant mice a control diet or an iodine-deficient diet to evaluate whether iodine deficiency is a causative environmental factor for goiter development in PDS.

Methods: We evaluated the thyroid volume in histological sections with the use of three-dimensional reconstitution software, we measured serum levels of total tri-iodothyronine (TT3) and total thyroxine (TT4) levels, and we studied the thyroid gland morphology by transmission electron microscopy.

Results: TT4 levels became low but TT3 levels did not change significantly after eight weeks of an iodine-deficient diet compared to levels in the control diet animals. Even in *Slc26a4*-null mice fed an iodine-deficient diet, the volume of the thyroid gland did not increase although the size of each epithelial cell increased with a concomitant decrease of thyroid colloidal area.

Conclusions: An iodine-deficient diet did not induce goiter in *Slc26a4*-null mice, suggesting that other environmental, epigenetic or genetic factors are involved in goiter development in PDS.

Background

Pendred syndrome (PDS) is an autosomal recessive disorder characterized by sensorineural hearing impairment, presence of goiter, and a partial defect in iodine organification [1]. The goiter in PDS is variable in its presentation; it can develop at any age (although generally after puberty), but may be totally absent in some affected individuals [2]. Also, there is substantial intrafamilial and regional variation, and nutritional iodine intake may be a significant modifier of the thyroid phenotype [1]. Kopp *et al.* suggested that under conditions of sufficient iodine intake, thyroid enlargement may be very mild or absent, and hence these patients are often simply categorized as having enlarged vestibular aqueduct [1]. Sato *et al.* also suggested

that even in patients with impaired iodide transport, high iodine intake may prevent the development of goiter [3].

Slc26a4-null (*Slc26a4^{-/-}*) mutant mice were generated by Everett *et al.* [2]. *Slc26a4^{-/-}* mice are profoundly deaf with vestibular dysfunction, but they lack goiter and thyroid histological abnormalities. We hypothesized that the absence of goiter and hypothyroidism in *Slc26a4^{-/-}* mice was due to a sufficient iodine intake, and that goiter and hypothyroidism might be induced by iodine deficiency. We, therefore, performed this study to investigate the influence of iodine intake on serum thyroid hormone levels and the histology and volume of the thyroid gland in *Slc26a4^{-/-}* mice.

Materials and methods

Slc26a4-null mice

An *Slc26a4*-null (*Slc26a4^{-/-}*) mouse colony was established and bred with homozygotes and heterozygotes imported from the National Institutes of Health

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(Rockville, Maryland) [2]. The line was maintained on a 129/SvEv background.

Breeding and Diet

Matings were performed between $Slc26a4^{-/-}$ and $Slc26a4^{-/-}$, and between $Slc26a4^{+/-}$ and $Slc26a4^{+/-}$ mice. These mice were fed a control diet (CLEA Japan Inc.). F1 offspring at two months of age were paired for mating. The mice were fed iodine-deficient chow (CLEA Japan Inc. T-08514) or control chow (CLEA Japan Inc. T-08513) from the beginning of the mating. Each chow was comprised solely of artificial materials. According to an analysis by the Laboratories for Food & Environmental Science, Tokyo, Japan, the iodine level was less than the sensing threshold (< 0.02 mg%) in iodine-deficient chow (ICD) whereas it was 0.51 mg% in control chow (CCD). F2 offspring were fed with the same diet as their parents for 12 to 16 weeks after weaning. $Slc26a4$ genotyping was performed on DNA prepared from tail specimens obtained at the time of sacrifice of the mice. There were six groups comprising one of three different genotypes ($Slc26a4^{-/-}$, $Slc26a4^{+/-}$, and $Slc26a4^{+/+}$) and either of ICD or CCD. Thirty-one 12 to 16 week-old males were used for this study (Table 1). Females were not analyzed in order to avoid the effect of menstrual cycles on hormone levels. The experimental protocol was approved by the Experimental Animal Management Committee, Nagoya University, Graduate School of Medicine.

Serum thyroid hormones

After deep anesthesia by intraperitoneal injection of pentobarbital sodium, blood was collected from the inferior vena cava. Serum total tri-iodothyronine (TT3) and total thyroxine (TT4) levels were measured by an electrochemiluminescence immunoassay (TT3: DRG® T-3 ELISA, TT4: DRG® T-4 ELISA, DRG International, East Mountainside, New Jersey USA).

Thyroid histology and volume

After intracardiac infusion of 4% paraformaldehyde, thyroid glands were excised together with adjacent tracheae and immersed in the same fixative for 24 hours at 4°C. The specimens were placed in 10% EDTA for seven days, washed with phosphate buffered saline (PBS), embedded in paraffin, and sectioned at 4-μm thickness for collection of every fifth section. The sections were stained with hematoxylin-eosin. The serial sections were observed with a light microscope system (BZ-8000, Keyence, Tokyo, Japan) and saved as digital images. A digital image of the whole thyroid was reconstructed and the volume was measured using three-dimensional reconstruction software, ZedView (LEXI, Tokyo, Japan).

Ultrastructural evaluation

One mouse was selected randomly for electron microscopic observation of the thyroid gland from each group of $Slc26a4^{-/-}$ CCD, $Slc26a4^{-/-}$ ICD, $Slc26a4^{+/-}$CCD and $Slc26a4^{+/-}$ ICD. The electron microscopic observation was done according to the method described previously [4]. Two-mm^3 thyroid specimens were excised, fixed in 2.5% glutaraldehyde for 24 hours at 4°C, washed in 0.1M phosphate buffer (pH = 7.0), and fixed again in 1% osmium tetroxide for 3 hours at 4°C. The samples were dehydrated in a graded series of ethanol and embedded in epoxy resin. Ultrathin sections were cut, double stained with uranyl acetate and lead citrate, and examined using a JEOL JEM100S electron microscope (JEOL, Tokyo, Japan).

Statistics

Statistical analysis was performed using SPSS Statistics ver.19.0 (SPSS Inc., Chicago, IL). One-way ANOVA and Mann-Whitney U-testing were used for statistical analysis. A P value less than 0.05 defined a significant difference.

Results

Volume of thyroid gland

The thyroid volume in each animal is shown in Table 1. Mean thyroid volumes of $Slc26a4^{-/-}$, $Slc26a4^{+/-}$ and $Slc26a4^{+/+}$ mice fed with CCD were 1.8 ± 1.0 mm^3, 1.9 ± 0.9 mm^3, 1.4 ± 0.2 mm^3 respectively. The mean thyroid volumes of $Slc26a4^{-/-}$, $Slc26a4^{+/-}$ and $Slc26a4^{+/+}$ mice fed with ICD were 1.0 ± 0.3 mm^3, 1.5 ± 0.6 mm^3, 1.1 mm^3 respectively. There were no significant differences in mean thyroid volumes between ICD and CCD groups for any genotype. The thyroid images reconstructed by three-dimensional reconstruction software are shown in Figure 1.

Histological findings

Figure 2 demonstrates light microscopic observation of the thyroid gland of ICD and CCD groups for the three different $Slc26a4$ genotypes. The size and height of epithelial cells increased with a concomitant decrease of colloidal area in ICD thyroid glands as compared to those of CCD animals among all genotypes. Electron microscopic observations in $Slc26a4^{-/-}$ and $Slc26a4^{+/-}$ thyroid glands were consistent with these findings (Figure 3).

Serum thyroid hormone levels

Serum concentrations of TT3 and TT4 in each animal are shown in Table 1. In the CCD group, the average TT3 levels were 1.26 μg/dl, 1.39 μg/dl and 1.53 μg/dl in $Slc26a4^{-/-}$, $Slc26a4^{+/-}$ and $Slc26a4^{+/+}$ mice, respectively. In the ICD group, the average TT3 levels were 0.92 μg/dl, 0.93 μg/dl and 1.07 μg/dl in $Slc26a4^{-/-}$, $Slc26a4^{+/-}$ and

Table 1 Total tri-iodothyronine (TT3) and total thyroxine (TT4) levels and thyroid volumes in *Slc26a4*-null mice eating control chow (CCD) or iodine-deficient chow (ICD)

Genotype	Diet	Body weight (g)	TT3 (ng/ml)	TT4 (µg/dl)	Volume (mm³)
Slc26a4⁻ᐟ⁻	CCD	25.6	2.80	8.70	0.95
		22.5	1.24	5.10	1.03
		19.8	0.74	4.68	EM
		28.9	0.76	3.99	2.27
		29.7	0.78	3.78	2.93
	ICD	25.4	0.87	3.60	0.67
		22.5	0.79	3.58	1.28
		21.3	0.99	2.77	0.88
		23.0	1.01	3.52	1.28
		20.9	0.86	3.42	0.75
		25.7	1.04	3.12	1.07
		24.6	0.88	1.79	EM
Slc26a4⁺ᐟ⁻	CCD	25.6	1.30	6.30	1.48
		23.8	1.35	5.90	1.47
		23.5	1.45	4.03	1.28
		27.4	2.40	8.10	1.03
		28.2	1.60	5.05	2.82
		25.1	0.82	3.83	EM
		38.0	0.81	4.13	3.22
	ICD	24.8	1.02	4.29	1.31
		23.4	0.84	3.11	0.66
		29.2	0.87	2.64	EM
		33.3	0.82	2.41	2.04
		31.7	1.12	2.92	1.92
Slc26a4⁺ᐟ⁺	CCD	26.8	1.55	3.97	1.63
		24.5	1.60	3.88	1.44
		23.7	2.10	8.40	1.38
		25.0	1.60	5.95	1.31
		26.7	0.79	3.45	1.24
	ICD	23.4	0.93	4.26	1.11
		23.8	1.20	4.35	UM

EM, examined with electron microscopy.
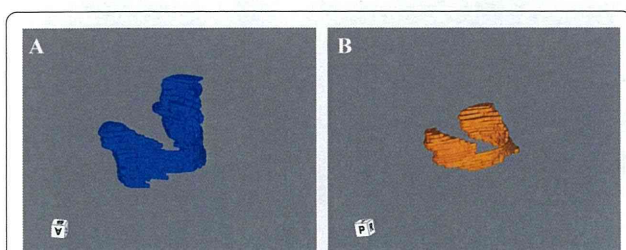UM, unmeasurable due to failure to prepare adequate thin sections.

Figure 1 The thyroid images reconstructed by three-dimensional reconstruction software. A, *Slc26a4⁻ᐟ⁻* mouse thyroid with iodine-deficient chow (ICD) (reconstructed with three-dimensional software, Zed View). B, *Slc26a4⁻ᐟ⁻* mouse thyroid with control chow (CCD) (reconstructed with three-dimensional software, Zed View).

Slc26a4⁺ᐟ⁺ mice, respectively. The average TT4 levels in the CCD group were 5.25 µg/dl, 5.33 µg/dl and 5.13 µg/dl in *Slc26a4⁻ᐟ⁻*, *Slc26a4⁺ᐟ⁻* and *Slc26a4⁺ᐟ⁺* mice, respectively. The average TT4 levels in the ICD group were 3.11 µg/dl, 3.07 µg/dl and 4.31 µg/dl in *Slc26a4⁻ᐟ⁻*, *Slc26a4⁺ᐟ⁻* and *Slc26a4⁺ᐟ⁺* mice, respectively. One-way ANOVA did not reveal a significant difference in TT3 and TT4 levels among the three genotypes.

As shown in Figure 4, Mann-Whitney U-testing revealed that serum TT4 level was lower in the ICD group than in the CCD group both in *Slc26a4⁻ᐟ⁻* and *Slc26a4⁺ᐟ⁻* mice ($p = 0.004$ and $p = 0.019$, respectively). In *Slc26a4⁺ᐟ⁺* mice, Mann-Whitney U-testing was not
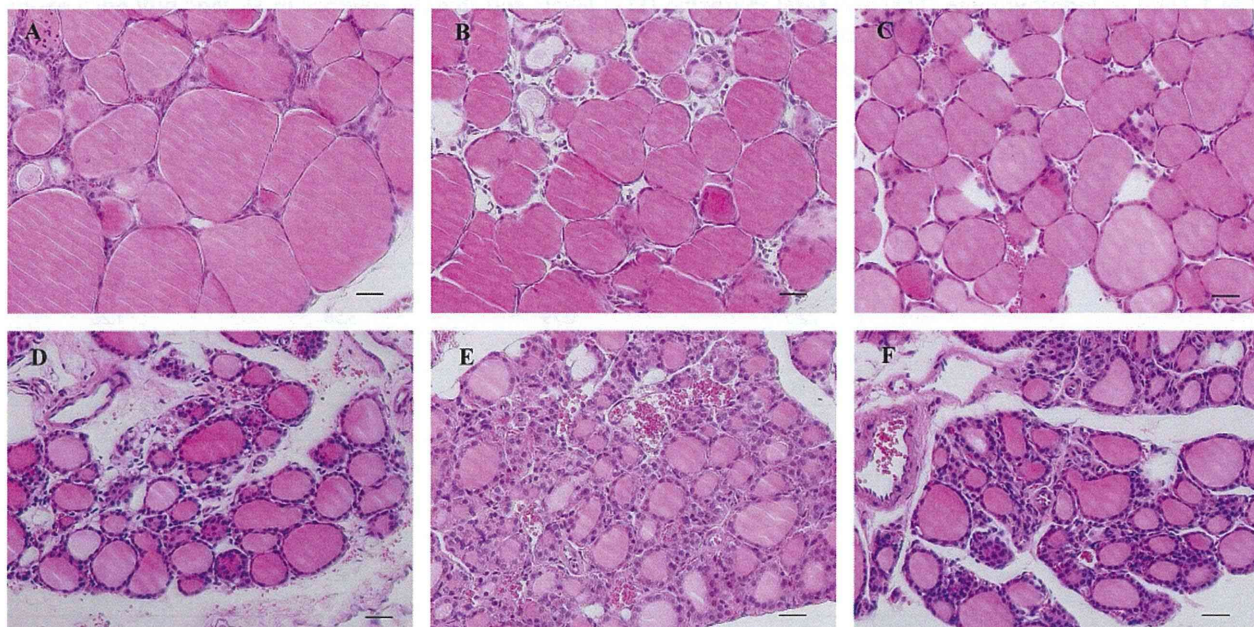
Figure 2 Light microscopic findings of the thyroid gland. A, *Slc26a4*^{-/-} control chow (CCD); B, *Slc26a4*^{+/-} CCD; C, *Slc26a4*^{+/+} CCD; D, *Slc26a4*^{-/-} iodine-deficient chow (ICD); E, *Slc26a4*^{+/-} ICD; F, *Slc26a4*^{+/+} ICD. Scale bars: 30 μm.
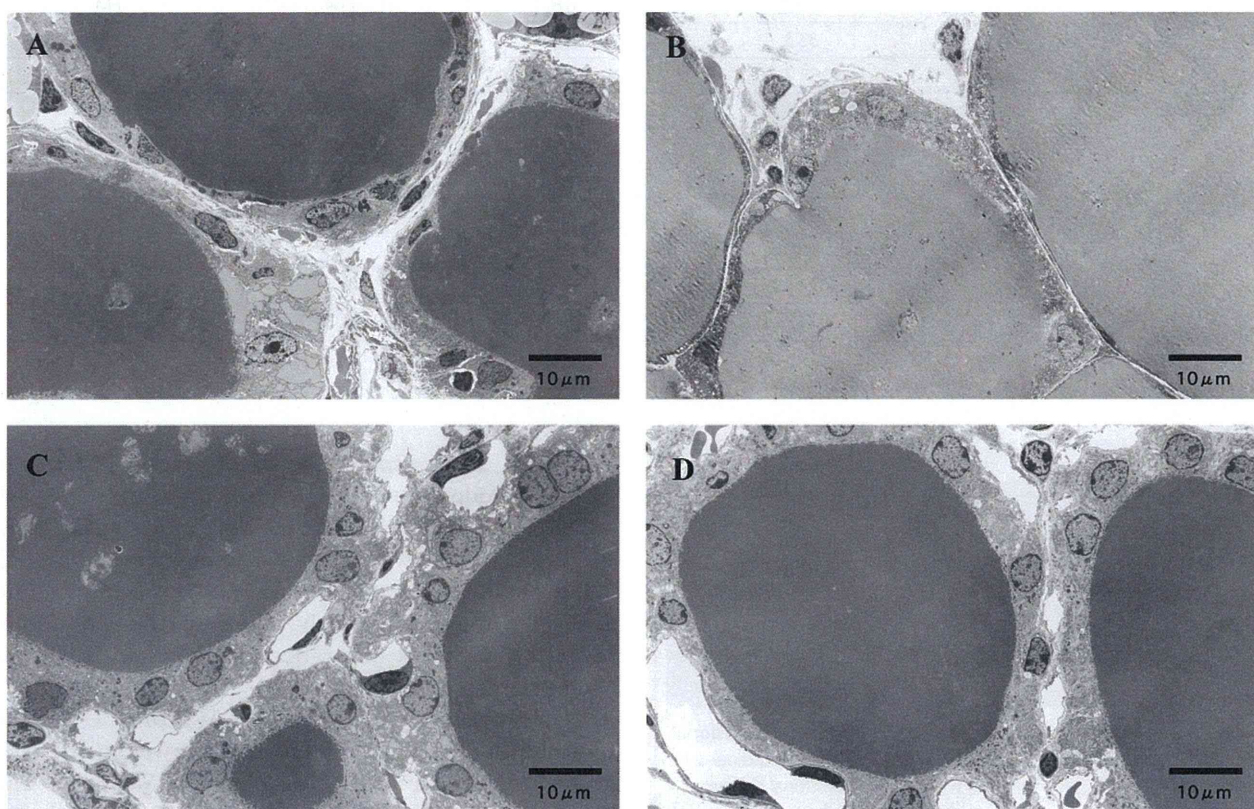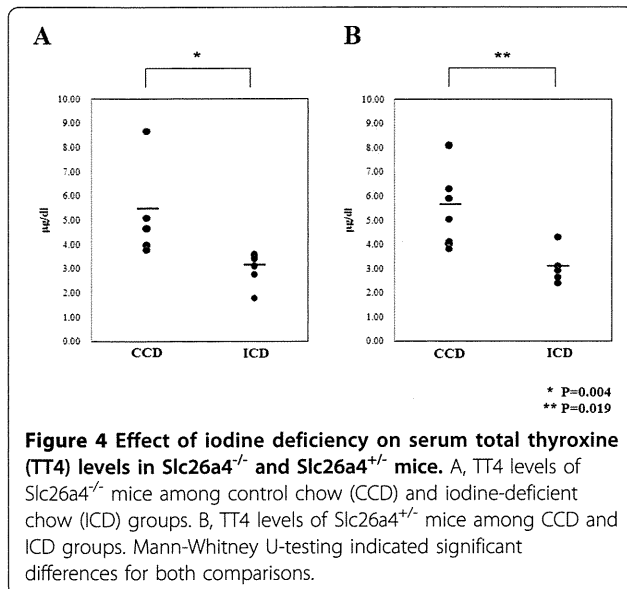

Figure 3 Electron microscopic findings of the throid gland. A, *Slc26a4*^{-/-} CCD; B, *Slc26a4*^{+/-} CCD; C, *Slc26a4*^{-/-} ICD; D, *Slc26a4*^{+/-} ICD.

Figure 4 Effect of iodine deficiency on serum total thyroxine (TT4) levels in Slc26a4$^{-/-}$ and Slc26a4$^{+/-}$ mice. A, TT4 levels of Slc26a4$^{-/-}$ mice among control chow (CCD) and iodine-deficient chow (ICD) groups. B, TT4 levels of Slc26a4$^{+/-}$ mice among CCD and ICD groups. Mann-Whitney U-testing indicated significant differences for both comparisons.

adequate to compare between ICD group and CCD group because the number of ICD animals was two. On the other hand, the TT3 level was not different significantly between the ICD and CCD groups.

Discussion

Mutations of the *SLC26A4* (*PDS*) gene can cause sensorineural hearing loss with goiter (PDS) or non-syndromic recessive deafness with enlarged vestibular aqueduct [5,6]. To date, more than 150 mutations in the *SLC26A4* gene have been reported in patients with PDS or nonsyndromic deafness with enlarged vestibular aqueducts (http://www.healthcare.uiowa.edu/labs/pendredandbor/slcMutations.htm). According to previous reports, the H723R missense substitution accounts for up to 75% of *SLC26A4* mutations in Japanese families with EVA [6,7]. There are many cases without goiter associated with the H723R mutation [3]. Madeo *et al.* found that thyroid gland volume is primarily *SLC26A4* genotype-dependent in children but is age-dependent in adults [8]. These reports suggest that the variable degree of thyroid dysfunction and goiter associated with *SLC26A4* mutations may be caused by factors unrelated to *SLC26A4* genotype. It is noteworthy that reported homozygotes for the H723R mutation were mainly from Japan and Korea where daily iodine intake should be comparatively high [3,7,9]. We therefore hypothesized that the amount of iodine intake influences the thyroid phenotype associated with PDS, leading us to study the effect of dietary iodine deficiency on thyroid gland structure and function in *Slc26a4*-null mutant mice.

TT4 levels were lower in the ICD group than in the CCD group. This difference was observed regardless of genotype, and these results suggest the thyroid function

of *Slc26a4*$^{-/-}$ mice is approximately the same as of *Slc26a4*$^{+/-}$ and *Slc26a4*$^{+/+}$ mice. While we were preparing the manuscript, we found a similar report by Calebiro *et al* [10]. In their report they also confirmed that dietary iodine restriction did not induce goiter in *Slc26a4*$^{-/-}$ mice. However, Calebiro *et al.* reported that total TT4 levels did not differ significantly between mice fed a low-iodine diet in comparison to those fed a standard diet [10].

The reason why TT3 levels did not decrease might be because incompletely iodinated thyroglobulin (Tg) in the thyroid colloid is accompanied by an increase in monoiodotyrosine (MIT) on Tg molecules, resulting in preferential T3 synthesis [11]. Therefore, TT3 levels may have been maintained despite the decline in TT4 levels in iodine-deficient mice. Another explanation why TT3 was unchanged in mice fed an iodine-deficient diet is an increase of type 1 iodothyronine 5'-deiodinase (D1) activity in the thyroid gland. Pedraza *et al.* reported that thyroidal D1 activity was increased with an iodine-deficient diet [12].

Other factors may compensate for defective iodine transport in both patients with PDS and *Slc26a4*$^{-/-}$ mice. Van den Hove *et al.* have reported that the ClCn5 (chloride channel 5) protein localizes at the apical membrane of thyrocytes. The thyroidal phenotype in ClCn5-deficient mice is similar to that in Pendred syndrome, suggesting that ClCn5 could participate in mediating apical iodine efflux or iodine/chloride exchange [13,14]. Suzuki *et al.* reported that thyroglobulin, by mediating differential expression of several thyroid-specific genes including TSHR, NIS, and TPO, TG, PAX8, TTF1, and TTF2 regulates the rate of iodide efflux into the follicular lumen and may thus play an important role in regulating thyroid function under constant levels of TSH [13,15].

In conclusion, the ICD did not induce goiter in *Slc26a4*-null mice whereas, in humans, *SLC26A4* mutations sometimes lead to goiter and even hypothyroidism. Mice may be different from humans in their ability to transport iodide into the follicular lumen or mice may respond differently to altered iodine availability. It is also possible that our results result from the use of male experimental animals since goiter and hypothyroidism are more prevalent among human females than males. The genetic strain background may also influence the penetrance and expressivity of the thyroid phenotype associated with *Slc26a4* mutations. These may be some of the factors involved in the development of goiter in PDS.

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Authors' contributions
TI designed and coordinated the study, performed the experiments and drafted the manuscript; TY and MT supervised all experimental procedures, participated in performing experiments, and helped to draft the manuscript; YM participated in coordination of the study and helped to draft the manuscript; YH participated in performing experiments. YK participated in coordination of the study. TN and AJG, the senior author, drafted the manuscript. All authors have read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Endolymphatic hydrops and blood–labyrinth barrier in Ménière's disease

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Abstract

Conclusions: The blood–labyrinth barrier is impaired in association with the hydrops grade in Ménière's disease. *Objectives:* To investigate the relationship between endolymphatic hydrops and the clinical characteristics of patients with Ménière's disease revealed by 3 T magnetic resonance imaging (MRI). *Methods:* A double dose of gadoteridol (Gd; 0.2 mmol/kg) was injected intravenously in 12 patients with Ménière's disease. We performed three-dimensional fluid attenuated inversion recovery MRI and three-dimensional real inversion recovery MRI 4 h later using a 3 T MRI unit. Ten patients had unilateral and two had bilateral Ménière's disease. *Results:* Fourteen ears with Ménière's disease showed intense Gd contrast on MRI compared with that in the 10 asymptomatic contralateral ears of patients with unilateral Ménière's disease (1.12 ± 0.36 vs 0.82 ± 0.15). The hydrops grade was correlated significantly with the contrast effect. The 14 ears with Ménière's disease had endolymphatic hydrops. Of the 10 contralateral ears of patients with unilateral Ménière's disease, 2 had endolymphatic hydrops in the cochlea and 6 had endolymphatic hydrops in the vestibule.

Keywords: *3 T magnetic resonance imaging, three-dimensional fluid attenuated inversion recovery, 3D FLAIR, contrast effect, signal intensity ratio, contralateral ear*

Introduction

Ménière's disease is an inner ear disorder with various recurrent symptoms, such as fluctuating hearing loss, tinnitus, and vertigo. It has been reported that Ménière's disease is related to pathological endolymphatic hydrops of the inner ear, but endolymphatic hydrops is also occasionally observed in the asymptomatic contralateral ear [1,2].

Because an ordinary amount of gadoteridol (Gd) intravenous injection is often insufficient to acquire enough contrast enhancement of inner ear, we injected a double dose of Gd to obtain clear inner ear magnetic resonance imaging (MRI) [3,4]. The advantage of intravenous Gd injection is very useful for the evaluation of bilateral Ménière's disease.

In this study, we investigated the relationship between endolymphatic hydrops revealed with 3 T MRI and the clinical characteristics of Ménière's disease. We also evaluated the blood–labyrinth barrier in ears with and without Ménière's disease.

Material and methods

Patients

Twelve patients with Ménière's disease were enrolled in this study (Table I), including three previously reported patients [3]. Patient no. 10 had undergone surgery for acoustic neurinoma of her right ear when aged 58 years and had lost the hearing in her right ear completely. Our group previously reported that

Table I. Patients' clinical characteristics.

Case no.	Gender	Age (years)	Side	Hearing fluctuation	Vertigo attacks	Low frequency hearing level at worse and better (dB)	Hearing level at 1000 Hz at worse and better (dB)	High frequency hearing level at worse and better (dB)	Time from onset of symptoms (months)	AAO–HNS classification
1	M	41	Right	Yes	Rotatory	45, 25	15, 15	27, 17	61	Definite
			Left	No		15, 10	5, 5	10, 10		
2	F	67	Right	Yes	Rotatory	75, 68	60, 60	70, 60	6	Definite
			Left	No		22, 25	10, 10	10, 10		
3	M	68	Right	Yes	Rotatory	50, 25	25, 15	70, 57	2	Definite
			Left	No		27, 23	10, 10	43, 30		
4	M	36	Right	No	Rotatory	20, 17	10, 10	23, 17	28	Definite
			Left	Yes		67, 38	60, 30	45, 33		
5	F	42	Right	No	Rotatory	15, 15	10, 5	12, 5	78	Definite
			Left	Yes		20, 15	10, 10	12, 12		
6	F	46	Right	Yes	Rotatory	57, 42	85, 75	90, 90	48	Definite
			Left	Yes		33, 35	55, 50	82, 80		
7	M	62	Right	Yes	Rotatory	60, 50	45, 50	58, 57	1	Probable
			Left	No		27, 25	15, 20	55, 65		
8	M	38	Right	Yes	Rotatory	32, 20	10, 20	5, 8	1	Probable
			Left	Yes		25, 25	25, 25	7, 7		
9	F	74	Right	No	Rotatory	40, 47	55, 50	80, 78	20	Probable
			Left	Yes		50, 45	60, 50	72, 68		
10	F	60	Right	No	Nonrotatory	90, 90	110, 110	107, 107	8	Possible
			Left	Yes		87, 77	85, 60	90, 80		
11	F	36	Right	Yes	Nonrotatory	28, 22	30, 10	45, 17	1	Possible
			Left	No		15, 20	10, 10	5, 7		
12	M	55	Right	Yes	Nonrotatory	25, 20	20, 20	57, 50	30	Possible
			Left	No		23, 22	15, 15	43, 40		

Low frequency hearing level: average at 125, 250, and 500 Hz. High frequency hearing level: average at 2000, 4000, and 8000 Hz. Patient no. 10 had had an acoustic neurinoma of her right ear and the hearing data were excluded from the analysis. F, female; M, male.

increased permeability of the blood–labyrinth barrier in acoustic neurinoma [5]. Therefore, we excluded the hearing data and signal intensity ratio (SIR) for her right ear from our analysis in this study. Ménière's disease was diagnosed according to the criteria of the 1995 American Academy of Otolaryngology–Head and Neck Surgery (AAO–HNS) [6].

MRI

A double dose (0.4 ml/kg, 0.2 mmol/kg) of gadoteridol (ProHance®; Eisai, Tokyo, Japan) was injected intravenously. Four hours later, an MRI was performed using a 3 T MRI unit with a 32-channel array coil to obtain a high signal-to-noise ratio [4,7]. Heavily T2-weighted three-dimensional (3D) constructive interference in steady-state imaging was obtained for anatomical reference and 3D fluid attenuated inversion recovery (3D FLAIR) MRI was then performed to detect perilymph enhancement while suppressing the signal from the endolymph. Finally, we carried out 3D real inversion recovery MRI to visualize the endolymph, perilymph, and bone separately on a single image. The details of the MRI protocol have been described previously [3,4,8].

Image evaluation of the endolymphatic space

Gd administered intravenously enters the perilymph but does not enter the endolymph. The difference makes it possible to visualize the endolymphatic space. The degrees of endolymphatic hydrops in the vestibule and cochlea were classified into three groups: none, mild, and significant, according to the criteria described previously [9]. A radiologist who

was blinded to the patients' clinical data evaluated the hydrops grade. Example images are shown in Figure 1.

Evaluation of Gd contrast effects

The contrast effects on the cochlear fluid were evaluated semiquantitatively. This method has been reported previously in patients with sudden deafness [4]. The SIR was measured three times and the average SIR value was calculated for each ear. The results of simple SIR measurements have been reported to correlate well with those based on a quantitative method [10].

Ethics review

The protocol for the study was approved by the Ethics Review Committee of the Nagoya University School of Medicine (approval nos 369, 369-2, 369-3, and 369-4). All patients gave their informed consent to their participation in this study. Their written informed consent was attached to their electronic medical records after permission was obtained from the patients.

Statistical analysis

The data were analyzed with SPSS 17.0 for Windows (SPSS Inc., Chicago, IL, USA). Differences in SIR were assessed with the Mann–Whitney U test and those in categorical variables were assessed with the χ^2 test. Spearman's correlation coefficient was used to investigate the relationship between hearing level or SIR and hydrops grade (none 0, mild 1, significant 2). A p value of < 0.05 was considered statistically significant.

Results

The clinical characteristics of the 12 patients are shown in Table I. Ten patients had unilateral Ménière's disease and two had bilateral disease. All patients had hearing fluctuations in at least one ear. The worst mean low frequency hearing levels in the diseased ears and contralateral ears were 46.7 ± 20.7 dB and 22.7 ± 8.1 dB, respectively. The mean worst hearing levels at 1000 Hz in the diseased ears and contralateral ears were 41.8 ± 26.1 dB and 15.6 ± 15.1 dB, respectively. The mean worst high frequency hearing levels in the diseased ears and contralateral ears were 52.1 ± 29.7 dB and 31.2 ± 25.6 dB, respectively. The differences in the hearing levels of the diseased and contralateral ears at low frequencies and 1000 Hz were significant but these differences were not significant at high frequencies. According to the AAO–HNS classification, six patients were diagnosed with definite Ménière's disease. Three patients had fluctuating hearing loss with rotatory vertigo, but without definitive episodes of vertigo (probable Ménière's disease). Three patients had fluctuating hearing loss with nonrotatory vertigo (possible
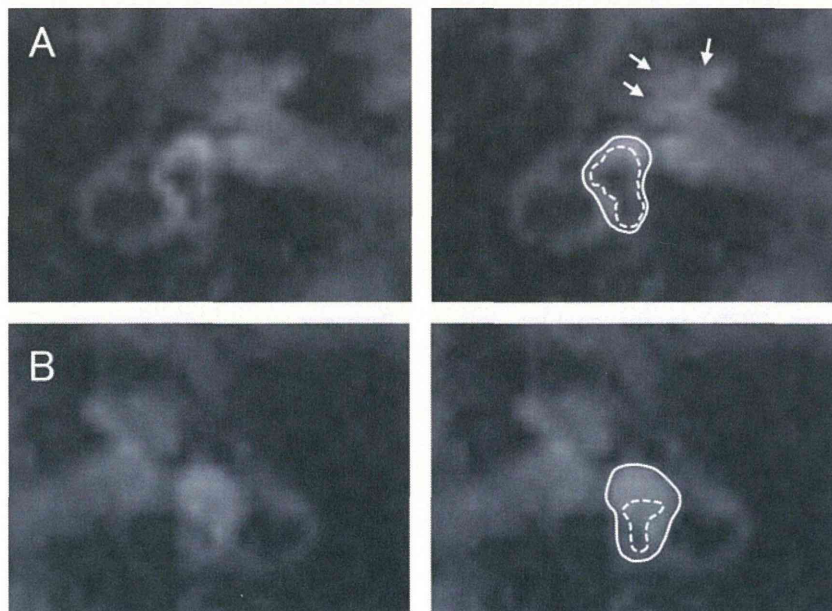

Figure 1. 3D FLAIR MRI of patient no. 3. Significant hydrops was observed in the cochlea (arrows) and vestibule of the diseased ear (A). The dotted line indicates the endolymphatic space and the solid line indicates the total fluid space in the vestibule. No hydrops was visible in the cochlea or vestibule of the contralateral ear (B).

Table II. Imaging data for each patient.

Case no.	Side	Diseased ear	Cochlear hydrops	Vestibular hydrops	SIR
1	Right	○	Significant	Significant	2.18
	Left		None	None	0.55
2	Right	○	Significant	Significant	1.33
	Left		None	Mild	1.04
3	Right	○	Significant	Significant	1.00
	Left		None	None	0.88
4	Right		None	Mild	0.65
	Left	○	Significant	Significant	0.71
5	Right		Mild	Mild	0.83
	Left	○	Significant	Significant	1.00
6	Right	○	None	Significant	0.99
	Left	○	None	Significant	0.93
7	Right	○	None	Significant	1.05
	Left		None	Mild	0.74
8	Right	○	None	Significant	0.94
	Left	○	Mild	Mild	0.81
9	Right		Mild	Mild	0.97
	Left	○	Significant	Significant	1.27
10	Right		None	None	2.79★
	Left	○	Significant	Significant	1.09
11	Right	○	Significant	Significant	1.44
	Left		None	None	0.88
12	Right	○	None	Mild	0.93
	Left		None	Significant	0.84

SIR, signal intensity ratio between the basal turn and cerebellar hemisphere.
★Patient no. 10 had had an acoustic neurinoma of her right ear and the SIR data were excluded from the analysis.

Ménière's disease). Table II shows the imaging data for each patient.

Of the 14 diseased ears, 8 showed significant hydrops (57%) and 1 mild hydrops (7%) in the cochlea, and 12 showed significant hydrops (86%) and 2 mild hydrops (14%) in the vestibule (Table III). Thus, the diseased ears showed hydrops in either the cochlea or vestibule. In the 10 contralateral ears, 2 showed mild hydrops (20%) in the cochlea and 1 showed significant hydrops (10%) and 5 mild hydrops (50%) in the vestibule. The diseased ears had more significant hydrops than the contralateral ears, in both the cochlea and vestibule.

The 14 ears with Ménière's disease had intense Gd contrast on MRI compared with that in the 10 contralateral ears in the patients with unilateral Ménière's disease (Figure 2; 1.12 ± 0.36 vs 0.82 ± 0.15).

The low frequency hearing level correlated significantly with the hydrops grade in the cochlea ($r = 0.448$) and vestibule ($r = 0.682$; Figure 3). The hearing level at 1000 Hz also correlated significantly

with the hydrops grade in the cochlea ($r = 0.434$) and vestibule ($r = 0.605$). The high frequency hearing level was not related to the hydrops grade in the cochlea, but it was related to the hydrops grade in the vestibule ($r = 0.470$). SIR correlated with the hydrops grade in

Table III. Grading of hydrops in diseased ear and contralateral ear.

Parameter	Diseased ear	Contralateral ear	p value★
Number	14	10	
Cochlear hydrops			
Significant	8 (57%)	0 (0%)	0.019
Mild	1 (7%)	2 (20%)	
None	5 (36%)	8 (80%)	
Vestibular hydrops			
Significant	12 (86%)	1 (10%)	0.001
Mild	2 (14%)	5 (50%)	
None	0 (0%)	4 (40%)	

★χ^2 test.