



Autoinflammatory characteristics and short-term effects of delivering high-dose steroids to the surface of the intact endolymphatic sac and incus in refractory Ménière's disease[☆]

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ABSTRACT

Objective: To investigate immune-related genetic background in intractable Meniere's disease (MD) and the immediate results of a novel therapy by delivering steroids to the surface of the intact endolymphatic sac (ES) and incus in a sustainable manner.

Case report and methods: Candidate genes involved in immune regulation were sequenced using a next-generation sequencing method in a patient with intractable MD. Mutations were confirmed using the Sanger sequencing method. The ES was exposed, and gelatin sponge particles were immersed in high-dose methylprednisolone solution and placed onto the surface of ES. "L"-shaped gelatin sponge strips were immersed in dexamethasone solution and served as a guiding device for the steroids by touching the incus and gelatin sponge particles on the surface of the ES. Gelatin sponge particles immersed in dexamethasone solution were placed around the gelatin sponge strips and sealed using fibrin glue.

Results: Autoinflammation in the refractory MD case was indicated by genotype, including novel heterozygous mutations of PRF1, UNC13D, SLC29A3, ITCH, and JAK3, as well as phenotype. The vertigo was fully relieved immediately after operation. Tinnitus and aural fullness were resolved 3 weeks after operation, whereas hearing improved in 2 months postoperation. No recurrence was noted during the 5-month follow-up, and the final MRI supported the novel therapeutic hypothesis.

Conclusion: Autoinflammation was involved in a refractory MD. This novel therapy, which involves the delivery of steroids to the surface of the intact ES and incus, is effective in relieving vertigo and tinnitus and improves hearing function of refractory MD.

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1. Introduction

Ménière's disease (MD) is characterized by episodic vertigo, fluctuant hearing loss, tinnitus, and aural fullness. The illness is associated with the formation of endolymph in the inner ear, causing the so-called endolymphatic hydrops that are visible via

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MRI based on the work of Zou et al. in 2000 (Zou et al., 2000, 2003). The mechanism of MD remains largely unknown. However, an immune-inflammatory reaction has been suspected to be involved in the pathological progression; this hypothesis is supported by the works of Brookes in 1986 and Zou et al. in 1992 (Brookes, 1986; Zou, 1992). The endolymphatic sac (ES) plays critical roles in homeostatic and immune regulations in the inner ear. Several members of the solute carrier gene family were upregulated in human ES (Moller et al., 2015a). Takahashi & Harris identified T-cells, macrophages, and immunoglobulins throughout the ES (Takahashi and Harris, 1988), and molecules, such as Toll-like receptors (TLRs) (Moller et al., 2015b, 2017), which supports the hypothesis that the ES serves as an innate immune organ in the inner ear. Potent involvement of the ES in amplification of adaptive immune responses has been implicated based on the expression of tumor

necrosis factor- α and immune regulatory peptide transforming growth factor- β secondary to cochlear antigen challenge (Sato et al., 2003, 2006). The ES is also a target of immune injury that was demonstrated by histology in MD patients (Dornhoffer et al., 1993). Furthermore, immunoglobulin depositions (IgA, IgM and IgG) in the ES have been observed in a subpopulation of MD patients (Arnold et al., 1984; Soliman, 1996; Yazawa and Kitahara, 1989). Pro-inflammatory cytokines produced in the ES may diffuse to the cochlea via a mechanism similar to gadolinium movement through the endolymph in MD observed in an MRI study (Mandala et al., 2010). Gene mutations have been reported in MD patients (Martin-Sierra et al., 2016, 2017; Requena et al., 2015), but the genetic background of MD patients associated with immune regulation are still lacking in the literature with the exception of reports demonstrating effects of allelic variants in the MICA, TLR10, and NFKB1 genes on hearing loss progression in MD (Cabrera et al., 2014; Requena et al., 2013).

Favoring the suspected immune-inflammatory mechanism of MD, systemic administration and/or local perfusion of corticosteroids into the middle ear have been adopted as an alternative therapy for intractable MD (Sennaroglu et al., 1999). However, systemic administration of steroids had adverse effects, and intratympanic injection of dexamethasone in a group of patients with unilateral MD (Shea's stage IV) exhibited no benefit compared with placebo for the treatment of hearing loss and tinnitus (Silverstein et al., 1998). In 2001, Kitahara et al. reported the beneficial effect of exposing the opened ES to high-dose steroids to treat intractable MD (Kitahara et al., 2001). However, the hearing results were the same as those with nonsurgical treatments, and the long-term results in the vertigo control were similar to those of standard ES decompression and even sham decompression (Bretlau et al., 1989; Thomsen et al., 1981). Recently, Bojrab et al. injected dexamethasone into the intact ES of MD through ES decompression; however, no additional effects were noted compared with ES decompression without injection (Bojrab et al., 2018).

Regardless of steroid delivery, standard ES decompression, exposure of the opened ES to high concentrations of steroids, and endolymphatic duct blockage may activate endogenous damage-associated molecular patterns in the ES and further induce inflammation in the inner ear (Zou et al., 2015). Dexamethasone injection into the intact ES is actually harmful to the inner ear due to vascular leakage and inflammation upon exciting the transient receptor potential vanilloid-4 (TRPV-4) in the sac via an abrupt increase in the static pressure inside the ES (Dalsgaard et al., 2016; Matsumoto et al., 2018; Taguchi et al., 2007). Therefore, I designed a novel therapy by delivering high-dose steroids to the surface of the intact ES and incus in a sustainable manner to treat refractory MD cases. The whole immune genome of the patient who received the novel therapy was sequenced. The results are reported as follows.

2. Case presentation

2.1. Clinical data

A 57-year-old woman complained of episodic vertigo and fluctuant hearing loss, tinnitus, and aural fullness in the right ear with a 2-year duration, and the symptoms were exacerbated for greater than 2 months. Rotational vertigo occurred in early June 2016 and was accompanied by nausea, hearing loss and aural fullness in the right ear. The vertigo is automatically relieved in several hours, and the hearing recovered while the aural fullness resolved. However, high-tone tinnitus appeared in the right ear. The symptom cluster repeated every 3–4 months and had become more frequent in the most recent 2 months, and each episode lasted 5–8 h. Audiogram revealed low-frequency sensorineural hearing loss on 23-12-2016

and flattened mixed hearing loss on 21-5-2018 in the right ear (Fig. 1). A type As tympanogram appeared in both ears with double peaks in the right ear, and reflexes were induced on both sides (Fig. 1). Gadolinium-enhanced MRI demonstrated extreme endolymphatic hydrops in the right cochlea and significant endolymphatic hydrops in the right vestibule (Fig. 2), and the degrees were defined according to previous reports (Nakashima et al., 2009; Yang et al., 2018). Systemic administration of agents with the intent to improve blood circulation and neural function revealed insignificant effects. The patient visited me on 5-6-2018, and the diagnosis of MD was made according to the diagnostic criteria for MD jointly formulated by the classification committee of the Barany Society in 2015 (Lopez-Escamez et al., 2015) and the editorial board of Chinese Journal of Otorhinolaryngology-Head and Neck Surgery and the Society of Otorhinolaryngology-Head and Neck Surgery of the Chinese Medical Association in 2017 (Editorial Board of Chinese Journal of Otorhinolaryngology et al., 2017). The tympanic membranes appeared normal on both sides. Targeted delivery of dexamethasone onto the posterior tympanic medial wall (0.2 ml, 10 mg/ml, twice weekly for a total of 4 procedures) using a soft-tipped microirrigation catheter together with low-frequency vibration at 100 Hz that were delivered to the ipsilateral mastoid process for 30 min were performed (twice weekly for a total of 4 procedures) with a protocol that was modified from a previous report (Zou et al., 2011). In the modified protocol, a tiny penetration was made in the posterior upper quadrant of tympanic membrane adjacent to the pars flaccida, and the plastic catheter was inserted onto the posterior tympanic medial wall through the penetration. Blood was obtained for immune gene sequencing on 3-7-2018. The vertigo attack intensity was reduced but still recurred. More than 10 episodes occurred during July of 2018, and the patient insisted on surgical treatment. She was admitted on 30-7-2018 for high-dose steroid delivery to the surface of the intact ES and incus that was performed on 1-8-2018. The patient had also been diagnosed with hyperthyroidism in 1999 and hypothyroidism in 2016.

Autoinflammation was suggested by preoperative blood examinations. Routine blood tests revealed decreased white blood cell count ($3.25 \times 10^9/L$), neutrophils ($1.19 \times 10^9/L$, 36.7%) and hematocrit (35%) and increased lymphocytes (49.5%) and mononucleocytes (9.5%). Thrombin time was slightly high (14.5 s). Routine urine tests revealed that red blood cells (25/ μ l) and cystatin C were elevated (1.39 mg/L). Blood biochemistry demonstrated that albumin/globulin was slightly low (1.44), glucose was high (7.0 mmol/L), low-density lipoprotein was slightly high (3.63 mmol/L), apolipoprotein A1 was low (1.02 g/L), retinol-binding protein was slightly low (23 mg/L), and complement 3 was low (0.76 g/L). Autoantibody spectrum revealed negative results except that anti- β 2-glycoprotein 1 antibody was high (31.02 RU/ml) and circulating immunoglobulins were normal. The other blood immunological test revealed that thyroglobulin protein was low (<0.04 ng/ml), *Treponema pallidum* antibody was positive (chemiluminescence) (the patient denied any associated sexual history), and rheumatoid factor and C-reaction protein were in a normal range.

2.2. Gene sequencing

All 232 candidate genes involved in immune regulation, which were selected according to Human Phenotype Ontology (<http://human-phenotype-ontology.github.io/>), were sequenced using next generation sequencing, a high-throughput method to foster novel discovery in biomedical research. Gene sequencing was performed using an Illumina HiSeq 2000 Sequencer (Illumina, California, USA) as previously reported (Zou et al., 2016). The data were analyzed using the following program: bwa-0.7.10, samtools-

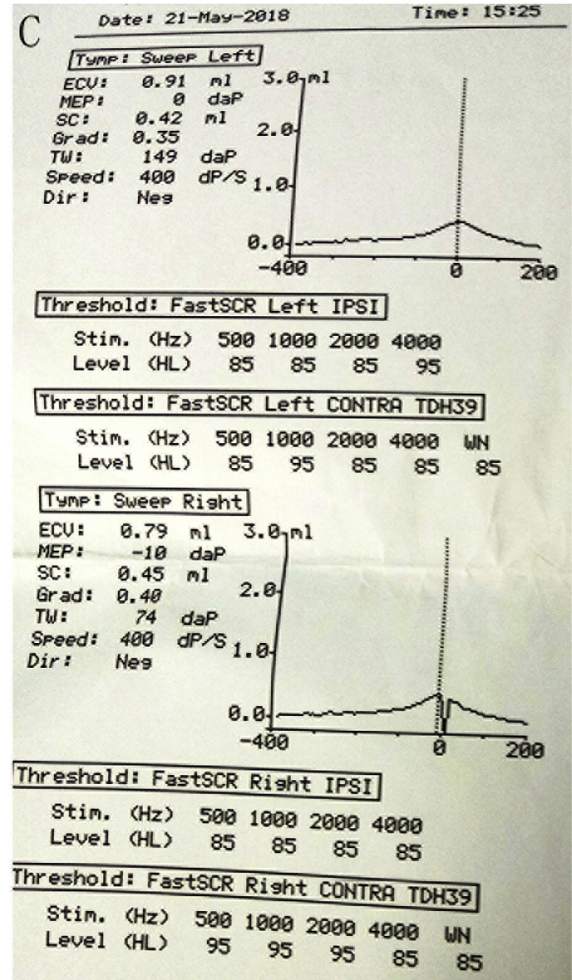
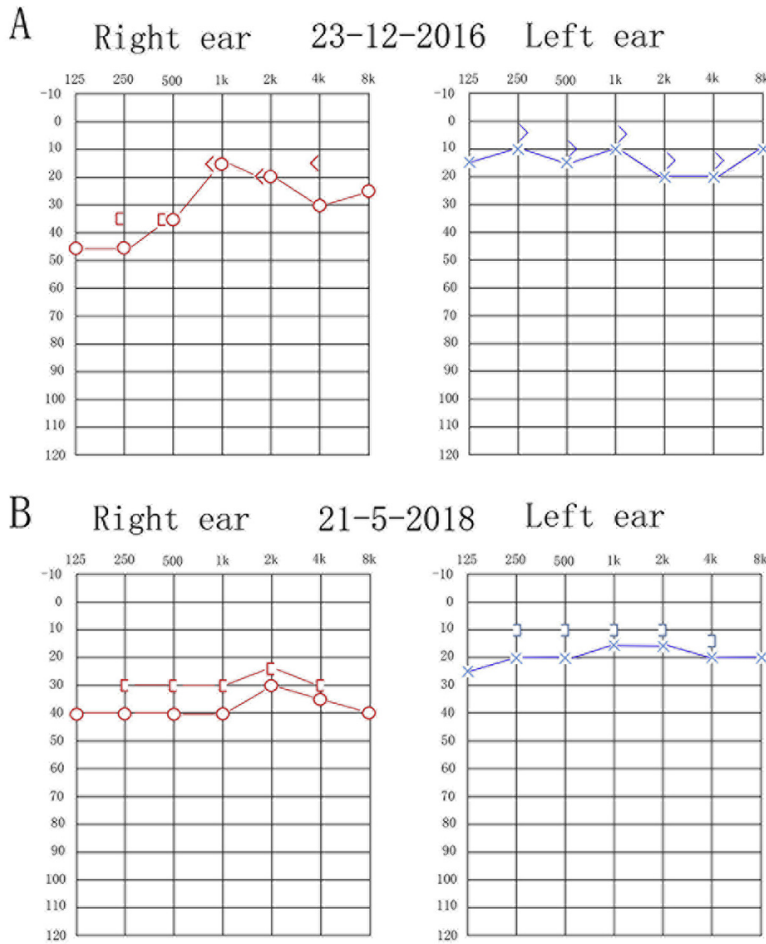


Fig. 1. Previous audiogram measured in other hospitals. Low-frequency hearing loss of the right ear (A) showed partial recovery on the second measurement (B). Type As tympanogram appeared on both ears with double peaks on the right ear (C).

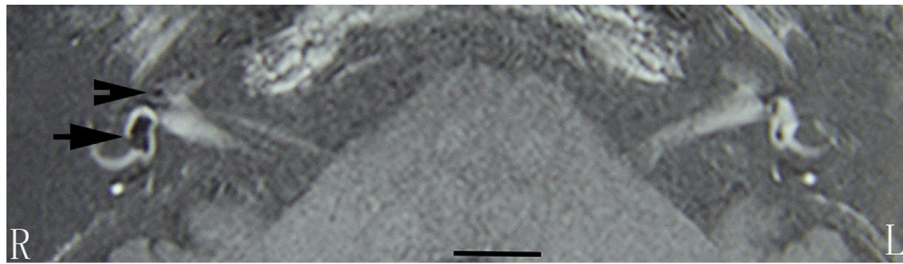


Fig. 2. Endolymphatic hydrops in both the cochlea and vestibule on the right side demonstrated by gadolinium-enhanced MRI. The images were obtained using 3D inversion-recovery turbo spin echo with real reconstruction sequence 4 h after intravenous injection of double doses of Gd-DTPA at Shanghai ENT hospital. In the right cochlea, marked hydrops was detected based on the bright semicircular appearance of the scala tympani and vestibuli (arrowhead) (Yang et al., 2018); in the vestibule, obvious endolymphatic hydrops was defined by an endolymph/total lymph ratio > 33% (=43%) (arrow) (Nakashima et al., 2009). Scale bar = 10.0 mm. L: left; R: right ear.

1.0, picard-tools-1.119, bamtools-2.3.0, Genome Analysis TK-3.3.0, Annovar-2014-11-12. REVEL (rare exome variant ensemble learner) software was used to predict the pathogenicity of missense variants on the basis of individual tools (Ioannidis et al., 2016). Mutations were confirmed using Sanger sequencing in both the patient and her daughter.

2.3. Surgery for steroid delivery to the surface of the intact endolymphatic sac and incus

The operation was performed under general anesthesia using

facial nerve monitoring, and a strict sterile work environment was guaranteed. The procedure is illustrated in Fig. 3. A simple mastoidectomy was performed through a retro-auricular incision, and the mastoid cavity was skeletonized by displaying the short process of the incus, superior part of the mastoid segment of the facial nerve canal, sinodural angle, and lateral and posterior semicircular canals. The ES was exposed by drilling the bones in the area between the sigmoid sinus and the inferior margin of the posterior semicircular canal (Fig. 4A). Gelatin sponge particles with dimensions of $5.0 \times 5.0 \times 5.0 \text{ mm}^3$ were immersed in a total of 80 mg methylprednisolone sodium succinate that was suspended in 1 ml

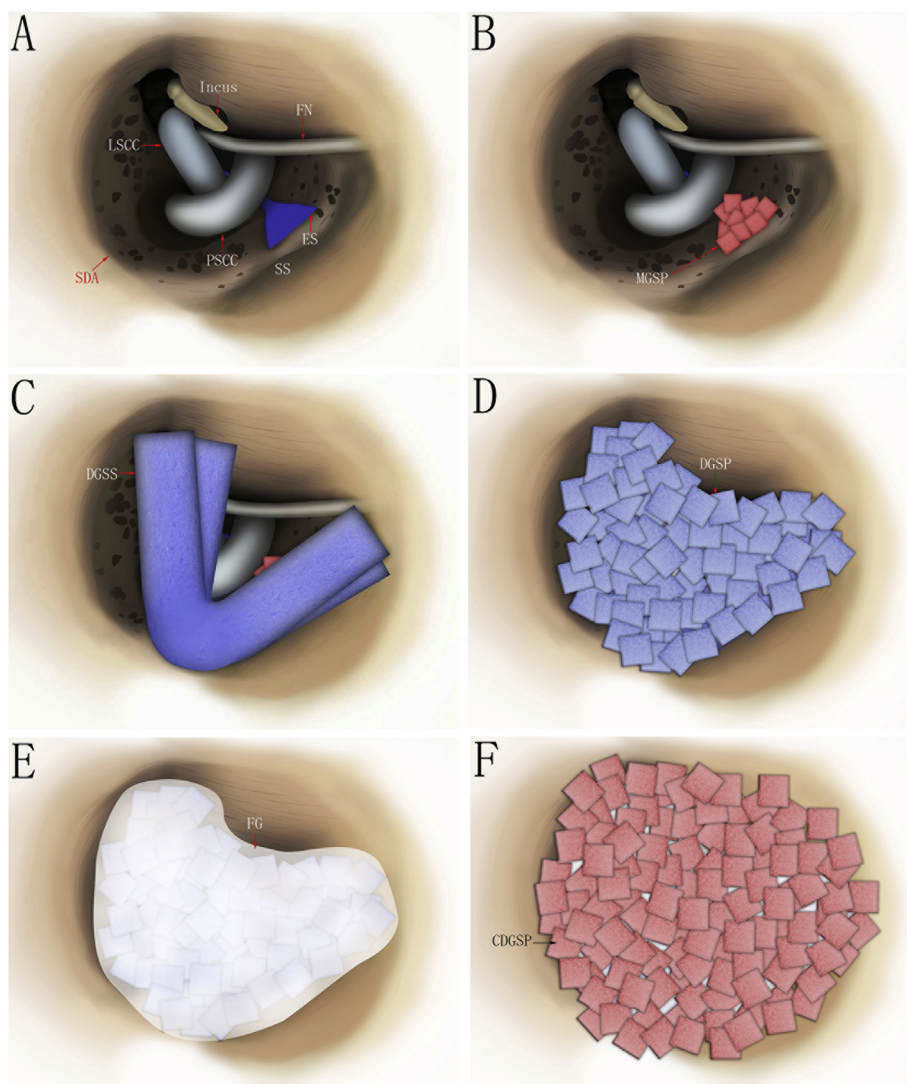


Fig. 3. Illustration of the delivery of high-dose steroids onto the surface of the endolymphatic sac (ES) and incus. The surgical landmarks are shown in A. Methylprednisolone sodium succinate-containing gelatin sponge particles (MGSP) were placed onto the surface of the intact ES (B). Dexamethasone-containing gelatin sponge strips (DGSSs) were installed to touch the short process of the incus and MGSP (C). Numerous dexamethasone-containing gelatin sponge particles (DGSP) were placed around DGSSs (D). DGSPs were sealed with fibrin glue (FG). The remainder of the mastoid cavity was filled by ciprofloxacin hydrochloride and dexamethasone-containing gelatin sponge particles (CDGSP).

saline and placed onto the surface of ES. Then, 2 strips of gelatin sponge with dimensions of $60.0 \times 5.0 \times 5.0 \text{ mm}^3$ were immersed in dexamethasone solution (8.3 mg/ml) and bent at 90° in the middle (“L” shape) to serve as guiding device for the steroids. The angular fragment was placed at the sinodural angle. One arm was positioned at the short process of incus, and the other arm was placed over the gelatin sponge particles on the surface of ES (Fig. 4B). Numerous gelatin sponge particles were immersed in the above-mentioned dexamethasone solution (a total of 3.6 ml solution was used), placed around the previous gelatin sponge (Fig. 4C), and sealed using fibrin glue (Fig. 4D). The remainder of the mastoid cavity was filled with gelatin sponge pieces containing a mixture of ciprofloxacin hydrochloride ear drops (3 mg/ml) and dexamethasone (5 mg/ml), and the wound was closed with skin sutures.

2.4. Inner ear MRI to detect EH

The patient was seen on 30-11-2018 (4 mon post operation) for MRI to detect potent EH. Briefly, 0.1 ml of 20-fold diluted gadolinium-diethylenetriamine pentaacetic acid (Gd-DTPA) was

delivered onto the posterior upper portion of the tympanic medial wall of each ear as previously report (Zou et al., 2018). After each injection, the patient was instructed to lie on the bed in a lateral position with the injected ear upward and turned approximately 30° backward for 15 min. Inner ear MRI was performed 24 h after Gd-DTPA administration using a 3T MR machine (Skyra, Siemens, Munich, Germany) and a 20-channel Tim 4G head/neck coil (Siemens, Munich, Germany). Potent EH was detected using a modified 3D-real inversion recovery (m3D-real IR) based on T2-sampling perfection with application-optimized contrasts using a flip angle evolution (SPACE) sequence. The parameters for SPACE were as followings: TR 4400 ms, TE 544 ms, echo spacing 5.43 ms, allowed delay 30 s, bandwidth 434 Hz/Px, flip angle (FA) 120° , strong fat suppression, FOV $196 \times 84.4 \text{ mm}$, slice thickness 1.0 mm, averages 1.8, slices per slab of either 104, and scanning time 3.4 min. The parameters for m3D-real IR were as followings: TR 16000 ms, TE 663 ms, echo spacing 5.43 ms, inversion time (TI) 2700 ms, FA 180° , strong fat suppression, FOV read $196 \times 165.4 \text{ mm}$, matrix 384×324 , slice thickness 1.0 mm, and averages 2. Slices per slab of either 104 or 60 were selected, which corresponds to

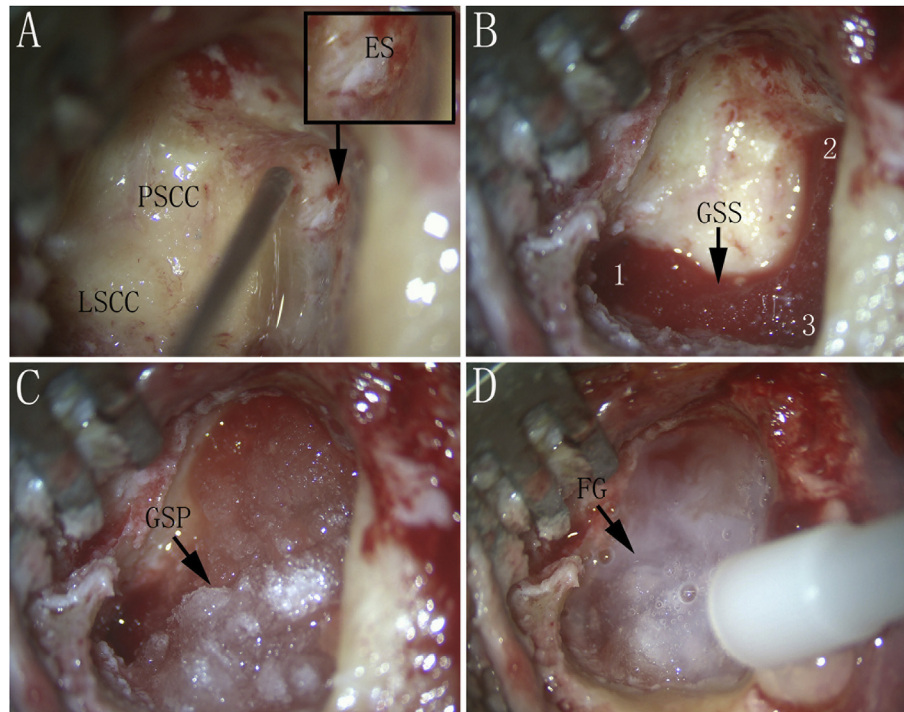


Fig. 4. Main steps of the surgery for high-dose steroid delivery to the surface of the intact endolymphatic sac (ES) and incus. A. The ES was exposed. B. Dexamethasone-containing “L”-shaped gelatin sponge strips (GSSs) were placed onto the surface of the short process of incus (1), outer surface of the ES that was covered by methylprednisolone sodium succinate-containing gelatin sponge particles (GSP) (2) and the sinodural angle (3). C. Numerous dexamethasone-containing GSPs were piled around the previous gelatin sponge materials. D. Dexamethasone-containing GSPs were sealed using fibrin glue (FG). LSCC: lateral semicircular canal; PSCC: posterior semicircular canal.

scanning time of either 15 min 11 s or 8 min 50 s. Potent EH was judged according to previous reported suggestions by measuring the area using ImageJ (version 1.50i, National Institute of Health, USA) (Nakashima et al., 2009; Yang et al., 2018).

3. Results

3.1. Candidate gene variants

Rare coding heterozygous variants of unknown significance were detected in *PRF1*, *UNC13D*, *SLC29A3*, *ITCH*, and *JAK3* using next generation sequencing and were confirmed in both the patient and her daughter using Sanger sequencing (Table 1, Fig. 5). The sequences of variants in *PRF1*, *SLC29A3*, and *ITCH* have not been reported in the literature to date. The REVEL order is as follows: *PRF1* > *UNC13D* > *SLC29A3* > *ITCH* > *JAK3*.

3.2. Symptom changes

The patient had insomnia the first night after operation that was subsequently resolved by taking compound diazepam tablets. The

vertigo was fully relieved immediately after operation, and tinnitus and aural fullness resolved 3 w after operation. She had hearing loss on the operated ear that started to improve 2 w after operation. A tuning fork test indicated conductive hearing loss on the operated ear. In 1 mon postoperation, a type B tympanogram was displayed, and an audiogram demonstrated hearing improvement at higher frequencies (2, 4, and 8 kHz) but loss at low frequencies (250 and 500 Hz) for both air and bone conduction. No hearing change was noted on the contralateral side (Fig. 6). The patient informed me via the internet 2 mon postoperation that her hearing recovered. The audiogram in 2 mon revealed that hearing improved at all frequencies compared with that before operation with an average improvement of 15 dB at 0.5, 1, and 2 kHz. A type As tympanogram with slightly double peaks was detected and was similar to that before operation (Fig. 7). MRI in 4 mon demonstrated mild vestibular hydrops on both sides and extreme cochlear hydrops on the right side. The endolymphatic sacs were symmetric bilaterally (Fig. 8). The patient experienced tinnitus in the right ear and transient headache on the left forehead approximately 5 h after Gd-DTPA injection. The latest follow-up was performed in 5 mon, and no vertigo attack was noted, but the tinnitus still existed.

Table 1

Candidate gene variants detected the patient and her daughter.

Chr position	Gene	Exon	Variants	Status	1000G	gnomAD-exome EAS	Polyphen2	SIFT	Note
chr10-72358767	<i>PRF1</i>	3	c.710C > A(p.T237N)	het	none	UA	D(0.997)	D (0.0)	novel
chr10-73122036	<i>SLC29A3</i>	6	c.1099G > A(p.A367T)	het	none	0.000	D(0.821)	D (0.048)	novel
chr20-33068519	<i>ITCH</i>	19	c.1934A > G(p.N645S)	het	none	0.0000599	D(0.911)	D (0.028)	novel
chr19-17943330	<i>JAK3</i>	19	c.2678C > T(p.P893L)	het	none	0.0004893	D(0.45)	D (0.299)	Not MD*
chr17-73832723	<i>UNC13D</i>	14	c.1228A > C(p.I410L)	het	0.0028	0.01588	B(0.021)	T (1.0)	Not MD*

1000G: 1000 genome; gnomAD-exomeEAS: gnomAD-exome EAS: the population frequencies of exome variant data in the eastern Asian descent provided by Genome Aggregation Database (gnomAD); het: heterogenous; Polyphen2: Polymorphism Phenotyping v2; SIFT: sorting intolerant from tolerant; UA: data unavailable.

*These variants were not reported in Meniere's disease.

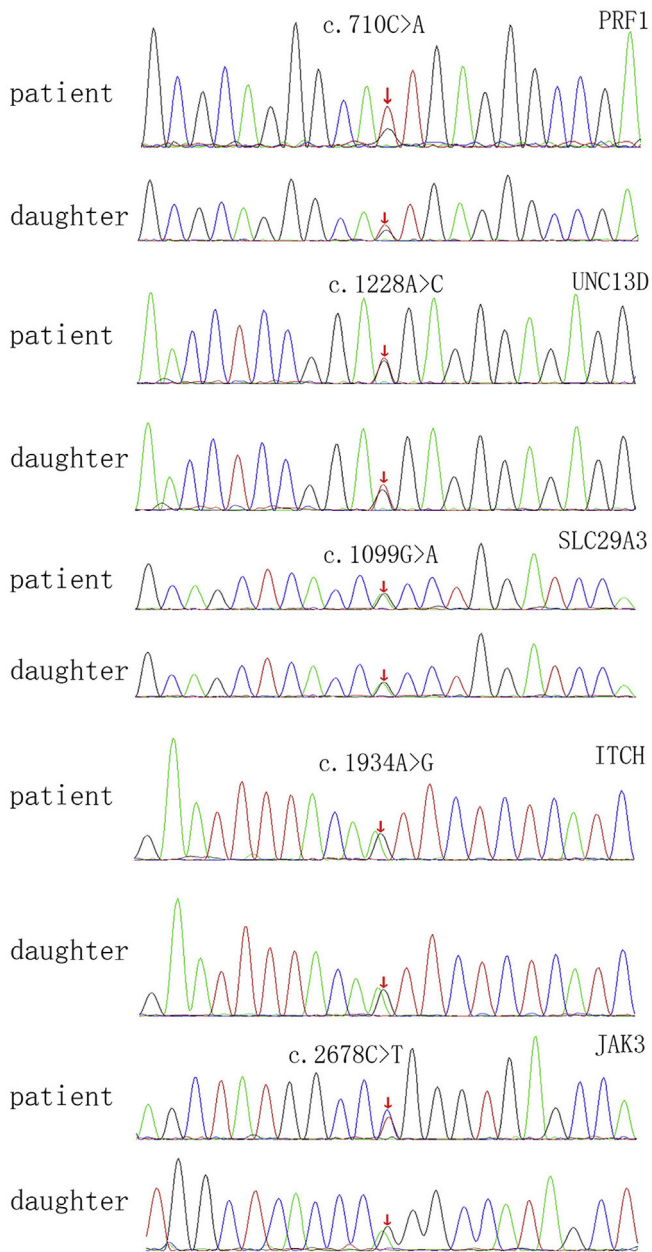


Fig. 5. Confirmation of gene mutations using Sanger sequencing. The same gene mutations were noted in the patient and her daughter.

4. Discussion

The involvement of the immune response in this patient with MD was supported by the finding of novel rare coding variants in *PRF1*, *UNC13D*, *SLC29A3*, *ITCH*, and *JAK3* and the comorbidity of hyperthyroidism suggesting autoimmune MD type 5. The regulation of the expression of the receptor Fn14 and the transcription factor NF- κ B is mediating inflammation in the carrier of the non-coding risk allelic variant rs4947296 (Frejo et al., 2017). *PRF1* heterozygous mutations, such as c.1091T>G, c.282C>A/p.N94K, and c.1349C>T/p.T450M, are associated with familial hemophagocytic lymphohistiocytosis type 2 (Kim et al., 2017; Liu et al., 2018). A compound heterozygous mutation in *UNC13D* gene could be detrimental in familial hemophagocytic lymphohistiocytosis (Yoon et al., 2010; Zhizhuo et al., 2012). Hemophagocytic lymphohistiocytosis is an immune disorder caused by the uncontrolled

activation of T lymphocytes and macrophages, which demonstrates a group of syndromes characterized by infiltration of multiple organs and reduction of hemocytes due to excessive generation of inflammatory cytokines. Autoinflammatory disease, such as adult-onset hemophagocytic lymphohistiocytosis, may also occur in adults and may be triggered by infection (Algahtani et al., 2018; Brito-Zeron et al., 2016). The current patient exhibited elevated anti- β 2GPI antibodies and reduced hemocytes in blood. Anti- β 2GPI antibodies may trigger the TLR4-MyD88 signaling pathway or stimulate DNA sensors in the cytoplasm through binding to cell surface β 2GPI and lead to activation of inflammatory signaling pathways and expression of tissue factor (TF) (Virachith et al., 2018). The patient was also diagnosed with hyperthyroidism and secondary hypothyroidism, which is suggestive of Graves' disease, a subtype of autoimmune thyroid disease (Li et al., 2019). However, antibodies against thyroglobulin protein, thyroid peroxidase, and thyroxine receptor were in normal range, indicating that an autoinflammatory rather than autoimmune reaction was involved in the thyroid disease of the current patient (Martinez-Quiles and Goldbach-Mansky, 2018). Heterozygous mutations in these 2 genes might have caused autoinflammation in the inner ear of the current patient. McDermot et al. proposed an autoinflammatory phenotype which results from impaired downregulation of membrane tumor necrosis factor receptor 1 (TNFR1) and diminished shedding of potentially antagonistic soluble receptor (McDermot et al., 1999). Six categories of autoinflammatory disease have been defined according to the molecular pathophysiology (Masters et al., 2009). The present results indicate a novel autoinflammatory MD which might be distinguished from autoimmune MD type 5. In a previous report, the best predictors for clustering were autoimmune history, familial MD, migraine, and the onset of hearing loss, and type 5 MD is heterogeneous. Ninety-five patients remained unclassified because of incomplete clinical data (Frejo et al., 2016). A subset of MD patients with high basal levels of cytokines and negative autoantibodies was suggested as an autoinflammatory condition involving the inner ear (Frejo et al., 2018).

The *SLC29A3* gene encodes human equilibrative nucleoside transporter 3 (hENT3), a member of a largely conserved group of solute carrier transporters that is located in the endosome/lysosome and mitochondrial membrane. *SLC29A3* mRNA expression has also been detected in the inner ear of mouse embryo (Morgan et al., 2010). hENT3 regulates T cell homeostasis by coordinating lysosomal function with nucleoside availability (Wei et al., 2018), and hENT3 spectrum disorder impairs nucleoside transport, protein localization, and stability (Kang et al., 2010). The patients display symptoms such as cutaneous hyperpigmentation, hypertrichosis, cardiac abnormalities, hearing loss, hepatomegaly hypogonadism, and pigmented hypertrichotic dermatosis with insulin-dependent diabetes (Kang et al., 2010). The E3 protein ubiquitin ligase *ITCH* gene encodes 854 amino acids with a relative molecular weight of 113 kDa. *ITCH* plays key roles in different cellular contexts given its functionally distinct substrates. *ITCH* targets are categorized in 2 main classes: transcription factors and growth factor receptors. Aberrant accumulation of several signaling proteins, such as the Jun family members and Notch, due to the loss of *ITCH* protein critically contributes to autoimmunity (Melino et al., 2008; Parravicini et al., 2008). *JAK3* mutation is associated with cytotoxic T lymphocyte antigen-4-dependent immune dysregulation syndrome (Sic et al., 2017). The overall effects of above gene mutations might cause autoinflammation, autoimmunity, and even direct impairment in the inner ear; however, the biological effects of heterogenic mutations of the above genes require further investigation. The patient experienced her first autoinflammatory disease at 38 years old (hyperthyroidism in 1999). Her daughter does not have any associated diseases to date at the age of 31; however, she carries the

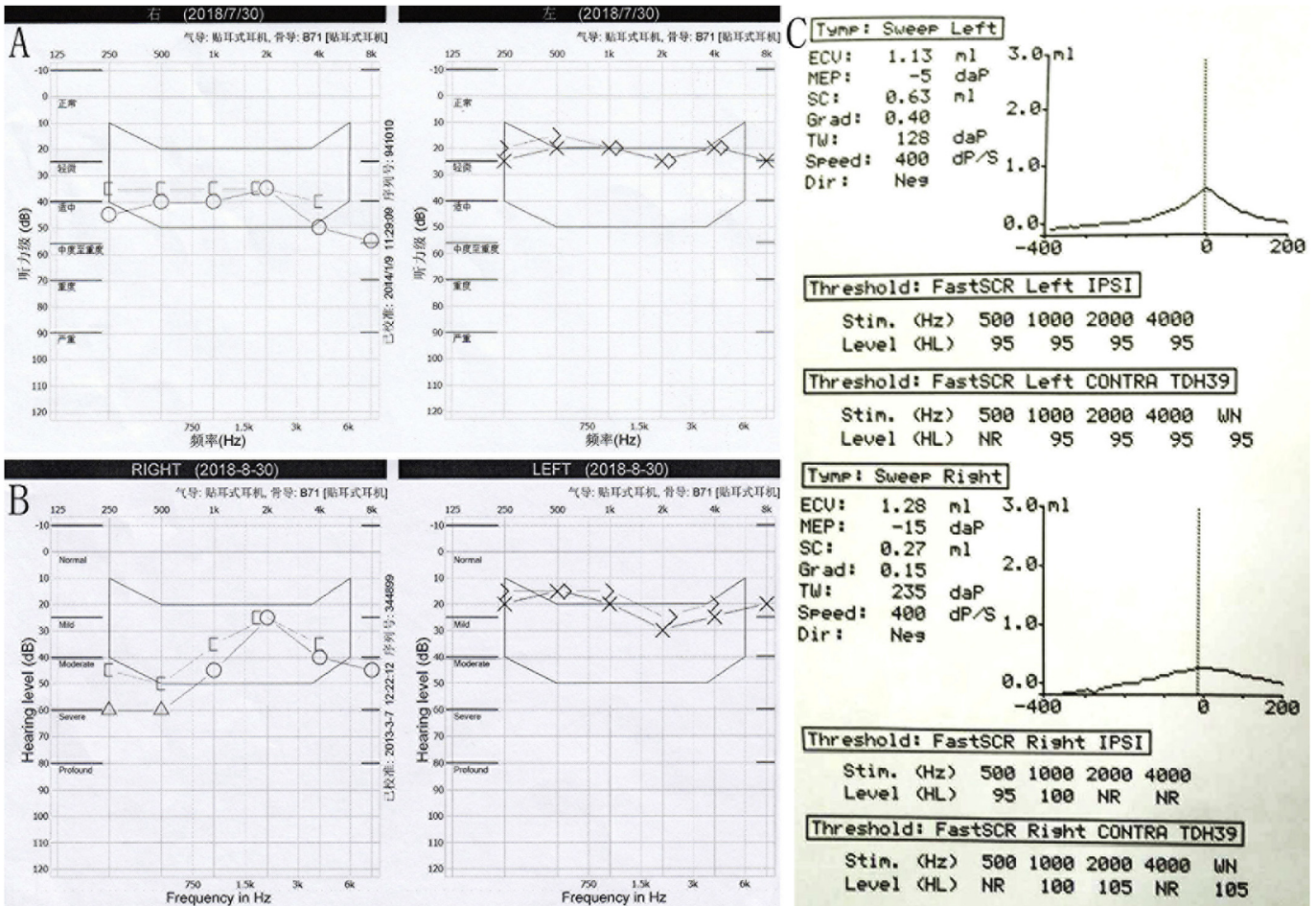


Fig. 6. Perioperative audiograms demonstrating hearing changes. Improvements at higher frequencies were noted, but loss at the frequencies of 250 and 500 Hz in the right ear 1 month after operation (B) was noted compared with that before the operation (A). B type tympanogram indicating middle ear fluids on the right side (C).

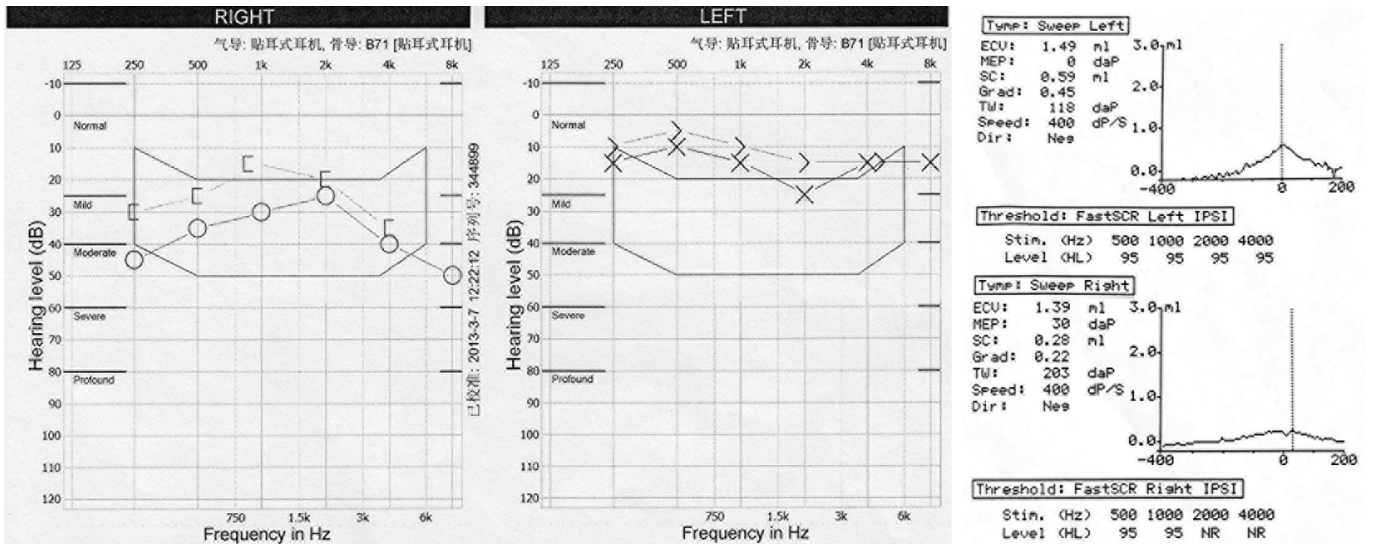


Fig. 7. Audiogram and tympanogram 2 months post-operation.

reported gene mutations. It is possible that multiple pathways are involved in disease development, including genetic mutations and environmental impacts.

The vertigo was immediately relieved after operation. This excellent result might be attributed to the powerful anti-inflammatory effect of the novel high-dose steroids delivered to

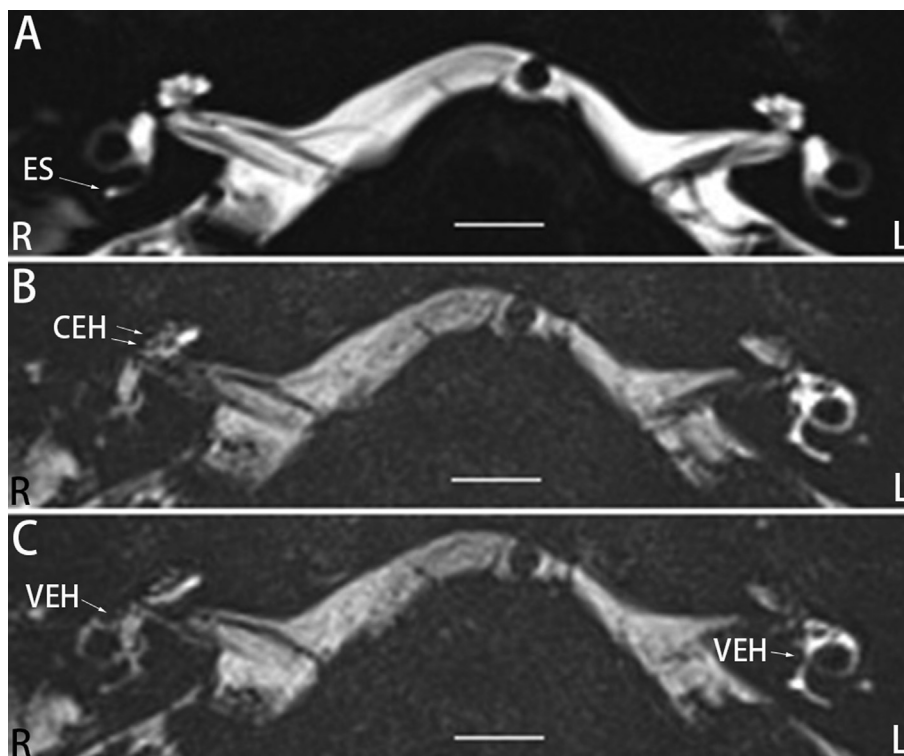


Fig. 8. Inner ear MRI of potent endolymphatic hydrops 4 mon after operation. Images were acquired using modified 3D-real inversion recovery sequence 24 h after targeted transtympanic injection of Gd-DTPA. The endolymphatic sacs (ES) were symmetric bilaterally (A). There were extreme cochlear endolymphatic hydrops (CEH) (B) in the right ear (R) and mild vestibular endolymphatic hydrops (VEH) on both sides (C). L: left ear.

the surface of both the intact ES and incus. The surgery itself likely did not contribute to symptom relief given that the ES was symmetric bilaterally and EH existed 4 mon after operation without symptoms. It is possible that the endolymphatic pressure was reduced, attributed to inflammation inhibition. In addition, the Reissner's member remained in the extended position, which potentially resulted from a plasticity that was observed in an animal model (Zou et al., 2003). At 1 mon after operation, hearing improvements greater than 2 kHz might be associated with anti-inflammation treatment. Worse hearing at 250 and 500 Hz may result from vibration during drilling of the temporal bone, in which the low frequencies are transmitted into the skull more efficiently compared with higher frequencies (Sutinen et al., 2007; Zou et al., 2001). Although the third window effect was suspected to contribute to low-tone air-bone gaps after endolymphatic sac surgery (Kitahara et al., 2011), the conductive hearing loss in the present case seems to result from the fluids in the middle ear cavity after steroid delivery, which was indicated by the type B tympanogram (Fig. 6E). This hypothesis was supported by hearing recovery on 53 d postoperation. The improvements in hearing over all frequencies at 2-mon postoperation compared with before surgery suggested that the mechanism of hearing loss might be associated with impairment of the cell populations that are capable of regeneration, such as lateral wall structures, rather than hair cells. The elimination of tinnitus is possibly associated with the anti-inflammatory effect. The recurred tinnitus after Gd-DTPA injection might be associated to psychiatric reaction to the MRI study, which was suggested by the transient headache on the left forehead after Gd-DTPA injection. The patient told me that she worried about the last MRI study when she had no symptom appeared.

The fibrin glue-sealed gelatin sponge worked as a natural sustained-release vehicle. A previous study reported that a neurotrophin-3/fibroin-coated gelatin sponge scaffold generated

sustainable cargo release up to 28 d (Li et al., 2016). One of the arms delivered steroids to the ES surface to modify the immune status of the ES. The more porous subepithelial capillary of ES due to inflammation facilitates the penetration of steroids throughout the ES layers after delivery onto the surface (Friis et al., 2013). The excellent therapeutic effect of the present method may work through modifying macrophage polarizations in the inner ear, especially in the ES. Macrophages play critical roles in the immune reaction by differentiating into two subsets in response to environmental stimuli: classically (M1) or alternatively (M2) activated macrophages. M1 polarization facilitates proinflammatory function and efficient antigen presentation in macrophages. In contrast, M2 macrophages play anti-inflammatory and scavenger roles (Castro et al., 2011; Hirano et al., 2003; Meagher et al., 1996; Wyllie, 1980). Adoptive transfer of M2 macrophages successfully prevented type 1 diabetes and experimental colitis progression in mice, indicating the beneficial effects of M2 macrophages in controlling autoimmune diseases (Hunter et al., 2010; Parsa et al., 2012). High-dose dexamethasone or all-trans-retinoic acid restores the balance of macrophages towards M2 in immune thrombocytopenia (Feng et al., 2017). High-dose dexamethasone also induced lipopolysaccharide-stimulated rat alveolar macrophage apoptosis with downregulation of tumor necrosis factor- α and interleukin (IL)-12 and upregulation of IL-10 and transforming growth factor- β (Zeng et al., 2017). The human sac is endowed with a variable number of leucocytes, and there is a continuous recirculation of immunocompetent cells in this area of the inner ear (Rask-Andersen et al., 1991).

The second arm delivers dexamethasone to the vestibule and cochlea to further modify the immune status in the inner ear. In an MRI study, attic delivery of gadolinium-chelate diffused along the ossicular chain towards the oval window and then efficiently entered the vestibule and cochlea in rats (Zou, 2010; Zou et al.,

2012). Although the histology of the human oval window may differ from that of rats, the surface chemistry of the ossicular chain should be similar between human and rats. The efficient passage of gadolinium-chelate through the human oval window has been demonstrated in our previous study using MRI (Shi et al., 2014). Therefore, sufficient dexamethasone might be supplied to the vestibule and cochlea via the second arm of the “L”-shaped gelatin sponge. The cochlea is active in inducing the immune reaction and inflammation. The lateral wall structures, including stria vascularis and spiral ligament, have been reported as the primary site harboring macrophages in the inner ear of human and mouse (O'Malley et al., 2016; Zhang et al., 2012). Nonsensory supporting cells of Corti's organ play an irreplaceable role in immune surveillance of the inner ear (Ladrech et al., 2007; Rio et al., 2002; Sun et al., 2015). In response to lipopolysaccharide, spiral ligament fibrocytes exhibited TLR2-dependent nuclear factor- κ B signaling activation and monocyte chemoattractant proteins-1 upregulation, resulting in monocyte migration and consequential infiltration (Moon et al., 2007; Woo et al., 2010). Our previous study demonstrated that ubiquitin-editing protein A20 and RING finger protein11 played roles in maintaining cochlear homeostasis via negative regulation of the expression of inflammatory cytokines (Feng et al., 2016). Dexamethasone inhibits tumor necrosis factor- α -induced cytokine secretion from spiral ligament fibrocytes (Maeda et al., 2005). Glucocorticoid receptors were broadly expressed in the cochlear cell populations and respond to dexamethasone in a manner suggestive of dose-induced translocation (Kil and Kalinec, 2013).

Some recognized limitations of the present report should be noted. The clustering and molecular pathophysiology of MD associated with autoinflammation is unknown. There was only one case in the observation, and the follow up period was short (5 mon). MD is often a chronic condition, and recurrence within months is possible. In general, a randomized controlled trial (RCT) including a placebo treatment provides the strongest evidence. However, it is impractical to performed an RCT study in China, especially for surgery. Given that the symptom attacks did not recur despite the lack of additional steroid supply indicates an unknown mechanism of macrophage plasticity within the inner ear. Therefore, a long-term follow-up and large sample investigation is necessary to draw final conclusions regarding this novel therapy in the future.

5. Conclusions

Autoinflammation in the refractory MD case was indicated by the genotypes of novel heterozygous mutations of PRF1, UNC13D, SLC29A3, ITCH, and JAK3; blood test characteristics; and previous diagnoses of hyperthyroidism and secondary hypothyroidism. It is necessary to complete the clustering and molecular pathophysiology of MD in the future. The preliminary results suggested that the novel therapy that delivers steroids to the surface of the intact endolymphatic sac and incus effectively relieves vertigo and tinnitus and improves hearing function of refractory MD, opening a new avenue of treatment in the clinic.

Declaration of conflicting interests

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Appendix A. Supplementary data

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References

- Algahtani, H., Absi, A., Bassuni, W., Shirah, B., 2018. Adult-onset hemophagocytic lymphohistiocytosis type 2 presenting as a demyelinating disease. *Mult Scler Relat Disord* 25, 77–82.
- Arnold, W., Altermatt, H.J., Gebbers, J.O., Laissue, J., 1984. Secretory immunoglobulin A in the human endolymphatic sac. An immunohistochemical study. *ORL J Otorhinolaryngol Relat Spec* 46, 286–288.
- Bojrab 2nd, D.I., LaRouere, M.J., Bojrab, D.I., Babu, S.C., Sargent, E.W., Chan, E.Y., Hong, R.S., 2018. Endolymphatic sac decompression with intra-sac dexamethasone injection in Meniere's disease. *Otol. Neurotol.* 39, 616–621.
- Bretlau, P., Thomsen, J., Tos, M., Johnsen, N.J., 1989. Placebo effect in surgery for Meniere's disease: nine-year follow-up. *Am. J. Otol.* 10, 259–261.
- Brito-Zeron, P., Bosch, X., Perez-de-Lis, M., Perez-Alvarez, R., Fraile, G., Gheitasi, H., Retamozo, S., Bove, A., Monclus, E., Escoda, O., Moreno, A., Lopez-Guillermo, A., Khamashta, M.A., Ramos-Casals, M., Group, B.S., 2016. Infection is the major trigger of hemophagocytic syndrome in adult patients treated with biological therapies. *Semin. Arthritis Rheum.* 45, 391–399.
- Brookes, G.B., 1986. Circulating immune complexes in Meniere's disease. *Arch. Otolaryngol. Head Neck Surg.* 112, 536–540.
- Cabrera, S., Sanchez, E., Requena, T., Martinez-Bueno, M., Benitez, J., Perez, N., Trinidad, G., Soto-Varela, A., Santos-Perez, S., Martin-Sanz, E., Fraile, J., Perez, P., Alarcón-Riquelme, M.E., Batuecas, A., Espinosa-Sanchez, J.M., Aran, I., Lopez-Escamez, J.A., 2014. Intronic variants in the NFKB1 gene may influence hearing forecast in patients with unilateral sensorineural hearing loss in Meniere's disease. *PLoS One* 9, e112171.
- Castro, R., Zou, J., Secombes, C.J., Martin, S.A., 2011. Cortisol modulates the induction of inflammatory gene expression in a rainbow trout macrophage cell line. *Fish Shellfish Immunol.* 30, 215–223.
- Dalsgaard, T., Sonkusare, S.K., Teuscher, C., Poynter, M.E., Nelson, M.T., 2016. Pharmacological inhibitors of TRPV4 channels reduce cytokine production, restore endothelial function and increase survival in septic mice. *Sci. Rep.* 6, 33841.
- Dornhoffer, J.L., Waner, M., Arenberg, I.K., Montague, D., 1993. Immunoperoxidase study of the endolymphatic sac in Meniere's disease. *Laryngoscope* 103, 1027–1034.
- Editorial Board of Chinese Journal of Otorhinolaryngology, H., Neck, S., Society of Otorhinolaryngology, H., Neck Surgery Chinese Medical, A., 2017. Guideline of diagnosis and treatment of Meniere disease (2017). *Zhonghua er bi yan hou tou jing wai ke za zhi* 52, 167–172.
- Feng, H., Pyykko, I., Zou, J., 2016. Involvement of ubiquitin-editing protein A20 in modulating inflammation in rat cochlea associated with silver nanoparticle-induced CD68 upregulation and TLR4 activation. *Nanoscale Res Lett* 11, 240.
- Feng, Q., Xu, M., Yu, Y.Y., Hou, Y., Mi, X., Sun, Y.X., Ma, S., Zuo, X.Y., Shao, L.L., Hou, M., Zhang, X.H., Peng, J., 2017. High-dose dexamethasone or all-trans-retinoic acid restores the balance of macrophages towards M2 in immune thrombocytopenia. *J. Thromb. Haemostasis* 15, 1845–1858.
- Frejo, L., Requena, T., Okawa, S., Gallego-Martinez, A., Martinez-Bueno, M., Aran, I., Batuecas-Caletrio, A., Benitez-Rosario, J., Espinosa-Sanchez, J.M., Fraile-Rodrigo, J.J., Garcia-Arumi, A.M., González-Aguado, R., Marques, P., Martin-Sanz, E., Perez-Fernandez, N., Pérez-Vázquez, P., Perez-Garrigues, H., Santos-Perez, S., Soto-Varela, A., Tapia, M.C., Trinidad-Ruiz, G., Del Sol, A., Alarcón Riquelme, M.E., Lopez-Escamez, J.A., 2017. Regulation of Fn14 receptor and NF- κ B underlies inflammation in Meniere's disease. *Front. Immunol.* 8, 1739.
- Frejo, L., Soto-Varela, A., Santos-Perez, S., Aran, I., Batuecas-Caletrio, A., Perez-Guillen, V., Perez-Garrigues, H.S., Fraile, J., Martin-Sanz, E., Tapia, M.C., Trinidad, G., Garcia-Arumi, A.M., González-Aguado, R., Espinosa-Sanchez, J.M., Marques, P., Perez, P., Benitez, J., Lopez-Escamez, J.A., 2016. Clinical subgroups in bilateral Meniere disease. *Front. Neurol.* 7, 182.
- Frejo, L., Gallego-Martinez, A., Requena, T., Martin-Sanz, E., Amor-Dorado, J.C., Soto-Varela, A., Santos-Perez, S., Espinosa-Sanchez, J.M., Batuecas-Caletrio, A., Aran, I., Fraile, J., Rossi-Izquierdo, M., Lopez-Escamez, J.A., 2018. Proinflammatory cytokines and response to molds in mononuclear cells of patients with Meniere disease. *Sci. Rep.* 8, 5974.
- Friis, M., Thomsen, A.R., Poulsen, S.S., Qvortrup, K., 2013. Experimental hyperactivity of the endolymphatic sac. *Audiol. Neuro. Otol.* 18, 125–133.
- Hirano, S., Asano, K., Namba, M., Kanai, K., Hisamitsu, T., Suzuki, H., 2003. Induction of apoptosis in nasal polyp fibroblasts by glucocorticoids in vitro. *Acta Otolaryngol.* 123, 1075–1079.
- Hunter, M.M., Wang, A., Parhar, K.S., Johnston, M.J., Van Rooijen, N., Beck, P.L., McKay, D.M., 2010. In vitro-derived alternatively activated macrophages reduce colonic inflammation in mice. *Gastroenterology* 138, 1395–1405.
- Ioannidis, N.M., Rothstein, J.H., Pejaver, V., Middha, S., McDonnell, S.K., Baheti, S., Musolf, A., Li, Q., Holzinger, E., Karyadi, D., Cannon-Albright, L.A., Teerlink, C.C., Stanford, J.L., Isaacs, W.B., Xu, J., Cooney, K.A., Lange, E.M., Schleutker, J., Carpten, J.D., Powell, I.J., Cussenot, O., Cancel-Tassin, G., Giles, G.G., MacInnis, R.J., Maier, C., Hsieh, C.L., Wiklund, F., Catalona, W.J., Foulkes, W.D., Mandal, D., Eeles, R.A., Kote-Jarai, Z., Bustamante, C.D., Schaid, D.J., Hastie, T., Ostrander, E.A., Bailey-Wilson, J.E., Radvoyac, P., Thibodeau, S.N.,

- Whittemore, A.S., Sieh, W., 2016. REVEL: an ensemble method for predicting the pathogenicity of rare missense variants. *Am. J. Hum. Genet.* 99, 877–885.
- Kang, N., Jun, A.H., Bhutia, Y.D., Kannan, N., Unadkat, J.D., Govindarajan, R., 2010. Human equilibrative nucleoside transporter-3 (hENT3) spectrum disorder mutations impair nucleoside transport, protein localization, and stability. *J. Biol. Chem.* 285, 28343–28352.
- Kil, S.H., Kalinec, F., 2013. Expression and dexamethasone-induced nuclear translocation of glucocorticoid and mineralocorticoid receptors in Guinea pig cochlear cells. *Hear. Res.* 299, 63–78.
- Kim, M.S., Cho, Y.U., Jang, S., Seo, E.J., Im, H.J., Park, C.J., 2017. Familial hemophagocytic lymphohistiocytosis type 2 in a Korean infant with compound heterozygous PRF1 defects involving a PRF1 mutation, c.1091T>G. *Ann Lab Med* 37, 162–165.
- Kitahara, T., Takeda, N., Mishiro, Y., Saika, T., Fukushima, M., Okumura, S., Kubo, T., 2001. Effects of exposing the opened endolymphatic sac to large doses of steroids to treat intractable Meniere's disease. *Ann. Otol. Rhinol. Laryngol.* 110, 109–112.
- Kitahara, T., Horii, A., Mishiro, Y., Kawashima, T., Imai, T., Nishiike, S., Inohara, H., 2011. Low-tone air-bone gaps after endolymphatic sac surgery. *Auris Nasus Larynx* 38, 178–184.
- Ladrech, S., Wang, J., Simonneau, L., Puel, J.L., Lenoir, M., 2007. Macrophage contribution to the response of the rat organ of Corti to amikacin. *J. Neurosci. Res.* 85, 1970–1979.
- Li, G., Che, M.T., Zhang, K., Qin, L.N., Zhang, Y.T., Chen, R.Q., Rong, L.M., Liu, S., Ding, Y., Shen, H.Y., Long, S.M., Wu, J.L., Ling, E.A., Zeng, Y.S., 2016. Graft of the NT-3 persistent delivery gelatin sponge scaffold promotes axon regeneration, attenuates inflammation, and induces cell migration in rat and canine with spinal cord injury. *Biomaterials* 83, 233–248.
- Li, Q., Wang, B., Mu, K., Zhang, J.A., 2019. The pathogenesis of thyroid autoimmune diseases: new T lymphocytes - cytokines circuits beyond the Th1-Th2 paradigm. *J. Cell. Physiol.* 234, 2204–2216.
- Liu, C., Li, M., Wu, X., Yao, X., Zhao, L., 2018. Type 2 familial hemophagocytic lymphohistiocytosis in half brothers: a case report. *Medicine (Baltim.)* 97, e11577.
- Lopez-Escamez, J.A., Carey, J., Chung, W.H., Goebel, J.A., Magnusson, M., Mandala, M., Newman-Toker, D.E., Strupp, M., Suzuki, M., Trabalzini, F., Bisdorff, A., Classification Committee of the Barany, S., Japan Society for Equilibrium, R., European Academy of O., Neurology, Equilibrium Committee of the American Academy of O.-H., Neck, S., Korean Balance, S., 2015. Diagnostic criteria for Meniere's disease. *J. Vestib. Res.* 25, 1–7.
- Maeda, K., Yoshida, K., Ichimiya, I., Suzuki, M., 2005. Dexamethasone inhibits tumor necrosis factor- α -induced cytokine secretion from spiral ligament fibrocytes. *Hear. Res.* 202, 154–160.
- Mandala, M., Colletti, L., Carner, M., Cerini, R., Barillari, M., Mucelli, R.P., Colletti, V., 2010. Induced endolymphatic flow from the endolymphatic sac to the cochlea in Meniere's disease. *Otolaryngol. Head Neck Surg.* 143, 673–679.
- Martin-Sierra, C., Gallego-Martinez, A., Requena, T., Frejo, L., Batuecas-Caletrio, A., Lopez-Escamez, J.A., 2017. Variable expressivity and genetic heterogeneity involving DPT and SEMA3D genes in autosomal dominant familial Meniere's disease. *Eur. J. Hum. Genet.* 25, 200–207.
- Martin-Sierra, C., Requena, T., Frejo, L., Price, S.D., Gallego-Martinez, A., Batuecas-Caletrio, A., Santos-Perez, S., Soto-Varela, A., Lysakowski, A., Lopez-Escamez, J.A., 2016. A novel missense variant in PRKCB segregates low-frequency hearing loss in an autosomal dominant family with Meniere's disease. *Hum. Mol. Genet.* 25, 3407–3415.
- Martinez-Quiles, N., Goldbach-Mansky, R., 2018. Updates on autoinflammatory diseases. *Curr. Opin. Immunol.* 55, 97–105.
- Masters, S.L., Simon, A., Aksentijevich, I., Kastner, D.L., 2009. Horror autoinflammaticus: the molecular pathophysiology of autoinflammatory disease. *Annu. Rev. Immunol.* 27, 621–668.
- Matsumoto, K., Yamaba, R., Inoue, K., Utsumi, D., Tsukahara, T., Amagase, K., Tominaga, M., Kato, S., 2018. Transient receptor potential vanilloid 4 channel regulates vascular endothelial permeability during colonic inflammation in dextran sulphate sodium-induced murine colitis. *Br. J. Pharmacol.* 175, 84–99.
- McDermott, M.F., Aksentijevich, I., Galon, J., McDermott, E.M., Ogunkolade, B.W., Centola, M., Mansfield, E., Gadina, M., Karenko, L., Pettersson, T., McCarthy, J., Frucht, D.M., Aringer, M., Torosyan, Y., Teppo, A.M., Wilson, M., Karaarslan, H.M., Wan, Y., Todd, I., Wood, G., Schlimgen, R., Kumarajeewa, T.R., Cooper, S.M., Vella, J.P., Amos, C.I., Mulley, J., Quane, K.A., Molloy, M.G., Ranki, A., Powell, R.J., Hitman, G.A., O'Shea, J.J., Kastner, D.L., 1999. Germline mutations in the extracellular domains of the 55 kDa TNF receptor, TNFR1, define a family of dominantly inherited autoinflammatory syndromes. *Cell* 97, 133–144.
- Meagher, L.C., Cousin, J.M., Seckl, J.R., Haslett, C., 1996. Opposing effects of glucocorticoids on the rate of apoptosis in neutrophilic and eosinophilic granulocytes. *J. Immunol.* 156, 4422–4428.
- Melino, G., Gallagher, E., Aqeilan, R.I., Knight, R., Peschiaroli, A., Rossi, M., Scialpi, F., Malatesta, M., Zocchi, L., Browne, G., Ciechanover, A., Bernassola, F., 2008. Itch: a HECT-type E3 ligase regulating immunity, skin and cancer. *Cell Death Differ.* 15, 1103–1112.
- Moller, M.N., Kirkeby, S., Caye-Thomasen, P., 2017. Innate immune defense in the inner ear - mucines are expressed by the human endolymphatic sac. *J. Anat.* 230, 297–302.
- Moller, M.N., Kirkeby, S., Vikesa, J., Nielsen, F.C., Caye-Thomasen, P., 2015a. Gene expression in the human endolymphatic sac: the solute carrier molecules in endolymphatic fluid homeostasis. *Otol. Neurotol.* 36, 915–922.
- Moller, M.N., Kirkeby, S., Vikesa, J., Nielsen, F.C., Caye-Thomasen, P., 2015b. Gene expression demonstrates an immunological capacity of the human endolymphatic sac. *Laryngoscope* 125, E269–E275.
- Moon, S.K., Woo, J.I., Lee, H.Y., Park, R., Shimada, J., Pan, H., Gellibolian, R., Lim, D.J., 2007. Toll-like receptor 2-dependent NF- κ B activation is involved in non-typeable Haemophilus influenzae-induced monocyte chemotactic protein 1 up-regulation in the spiral ligament fibrocytes of the inner ear. *Infect. Immun.* 75, 3361–3372.
- Morgan, N.V., Morris, M.R., Cangul, H., Gleeson, D., Straatman-Iwanowska, A., Davies, N., Keenan, S., Pasha, S., Rahman, F., Gentle, D., Vreeswijk, M.P., Devile, P., Knowles, M.A., Ceylaner, S., Trembath, R.C., Dalence, C., Kismet, E., Koseoglu, V., Rossbach, H.C., Gissen, P., Tannahill, D., Maher, E.R., 2010. Mutations in SLC29A3, encoding an equilibrative nucleoside transporter ENT3, cause a familial histiocytosis syndrome (Faisalabad histiocytosis) and familial Rosai-Dorfman disease. *PLoS Genet.* 6, e1000833.
- Nakashima, T., Naganawa, S., Pyykko, I., Gibson, W.P., Sone, M., Nakata, S., Teranishi, M., 2009. Grading of endolymphatic hydrops using magnetic resonance imaging. *Acta Otolaryngol. (Suppl.)* 5–8.
- O'Malley, J.T., Nadol Jr., J.B., McKenna, M.J., 2016. Anti CD163+, Iba1+, and CD68+ cells in the adult human inner ear: normal distribution of an unappreciated class of macrophages/microglia and implications for inflammatory otopathology in humans. *Otol. Neurotol.* 37, 99–108.
- Parravicini, V., Field, A.C., Tomlinson, P.D., Basson, M.A., Zamoyska, R., 2008. Itch- α and gammadelta T cells independently contribute to autoimmunity in Itchy mice. *Blood* 111, 4273–4282.
- Parsa, R., Andresen, P., Gillett, A., Mia, S., Zhang, X.M., Mayans, S., Holmberg, D., Harris, R.A., 2012. Adoptive transfer of immunomodulatory M2 macrophages prevents type 1 diabetes in NOD mice. *Diabetes* 61, 2881–2892.
- Rask-Andersen, H., Danckwardt-Lilliestrom, N., Friberg, U., House, W., 1991. Lymphocyte-macrophage activity in the human endolymphatic sac. *Acta Otolaryngol* 485 (Suppl.), 15–17.
- Requena, T., Cabrera, S., Martin-Sierra, C., Price, S.D., Lysakowski, A., Lopez-Escamez, J.A., 2015. Identification of two novel mutations in FAM136A and DTNA genes in autosomal-dominant familial Meniere's disease. *Hum. Mol. Genet.* 24, 1119–1126.
- Requena, T., Gazquez, I., Moreno, A., Batuecas, A., Aran, I., Soto-Varela, A., Santos-Perez, S., Perez, N., Perez-Garrigues, H., Lopez-Nevo, A., Martin, E., Sanz, R., Perez, P., Trinidad, G., Alarcon-Riquelme, M.E., Teggi, R., Zagato, L., Lopez-Nevo, M.A., Lopez-Escamez, J.A., 2013. Allelic variants in TLR10 gene may influence bilateral affection and clinical course of Meniere's disease. *Immunogenetics* 65, 345–355.
- Rio, C., Dikkes, P., Liberman, M.C., Corfas, G., 2002. Glial fibrillary acidic protein expression and promoter activity in the inner ear of developing and adult mice. *J. Comp. Neurol.* 442, 156–162.
- Satoh, H., Firestein, G.S., Billings, P.B., Harris, J.P., Keithley, E.M., 2003. Proinflammatory cytokine expression in the endolymphatic sac during inner ear inflammation. *J. Assoc. Res. Otolaryngol.* 4, 139–147.
- Satoh, H., Billings, P., Firestein, G.S., Harris, J.P., Keithley, E.M., 2006. Transforming growth factor beta expression during an inner ear immune response. *Ann. Otol. Rhinol. Laryngol.* 115, 81–88.
- Sennaroglu, L., Dini, F.M., Sennaroglu, G., Gursel, B., Ozkan, S., 1999. Transtympanic dexamethasone application in Meniere's disease: an alternative treatment for intractable vertigo. *J. Laryngol. Otol.* 113, 217–221.
- Shi, H., Li, Y., Yin, S., Zou, J., 2014. The predominant vestibular uptake of gadolinium through the oval window pathway is compromised by endolymphatic hydrops in Meniere's disease. *Otol. Neurotol.* 35, 315–322.
- Sic, H., Speletas, M., Cornacchione, V., Seidl, M., Beibel, M., Linghu, B., Yang, F., Sevdali, E., Germentis, A.E., Oakeley, E.J., Vangrevelinghe, E., Sailer, A.W., Traggiai, E., Gram, H., Eibel, H., 2017. An activating janus kinase-3 mutation is associated with cytotoxic T lymphocyte antigen-4-dependent immune dysregulation syndrome. *Front. Immunol.* 8, 1824.
- Silverstein, H., Isaacson, J.E., Olds, M.J., Rowan, P.T., Rosenberg, S., 1998. Dexamethasone inner ear perfusion for the treatment of Meniere's disease: a prospective, randomized, double-blind, crossover trial. *Am. J. Otol.* 19, 196–201.
- Soliman, A.M., 1996. A subpopulation of Meniere's patients produce antibodies that bind to endolymphatic sac antigens. *Am. J. Otol.* 17, 76–80.
- Sun, S., Yu, H., Yu, H., Honglin, M., Ni, W., Zhang, Y., Guo, L., He, Y., Xue, Z., Ni, Y., Li, J., Feng, Y., Chen, Y., Shao, R., Chai, R., Li, H., 2015. Inhibition of the activation and recruitment of microglia-like cells protects against neomycin-induced ototoxicity. *Mol. Neurobiol.* 51, 252–267.
- Sutinen, P., Zou, J., Hunter, L.L., Toppila, E., Pyykko, I., 2007. Vibration-induced hearing loss: mechanical and physiological aspects. *Otol. Neurotol.* 28, 171–177.
- Taguchi, D., Takeda, T., Kakigi, A., Takumida, M., Nishioka, R., Kitano, H., 2007. Expressions of aquaporin-2, vasopressin type 2 receptor, transient receptor potential channel vanilloid (TRPV)1, and TRPV4 in the human endolymphatic sac. *Laryngoscope* 117, 695–698.
- Takahashi, M., Harris, J.P., 1988. Anatomic distribution and localization of immunocompetent cells in normal mouse endolymphatic sac. *Acta Otolaryngol.* 106, 409–416.
- Thomsen, J., Bretlau, P., Tos, M., Johnsen, N.J., 1981. Meniere's disease: endolymphatic sac decompression compared with sham (placebo) decompression. *Ann. N. Y. Acad. Sci.* 374, 820–830.
- Virachith, S., Saito, M., Watanabe, Y., Inoue, K., Hoshi, O., Kubota, T., 2018. Anti-beta2-glycoprotein I antibody with DNA binding activity enters living monocytes via cell surface DNA & induces tissue factor expression. *Clin. Exp. Immunol.* <https://doi.org/10.1111/cei.13229> [Epub ahead of print].

- Wei, C.W., Lee, C.Y., Lee, D.J., Chu, C.F., Wang, J.C., Wang, T.C., Jane, W.N., Chang, Z.F., Leu, C.M., Dzhalgalov, I.L., Hsu, C.L., 2018. Equilibrative nucleoside transporter 3 regulates T cell homeostasis by coordinating lysosomal function with nucleoside availability. *Cell Rep.* 23, 2330–2341.
- Woo, J.I., Pan, H., Oh, S., Lim, D.J., Moon, S.K., 2010. Spiral ligament fibrocyte-derived MCP-1/CCL2 contributes to inner ear inflammation secondary to nontypeable *H. influenzae*-induced otitis media. *BMC Infect. Dis.* 10, 314.
- Wyllie, A.H., 1980. Glucocorticoid-induced thymocyte apoptosis is associated with endogenous endonuclease activation. *Nature* 284, 555–556.
- Yang, S., Zhu, H., Zhu, B., Wang, H., Chen, Z., Wu, Y., Chen, B., Shi, H., Li, Y., Zou, J., Yin, S., 2018. Correlations between the degree of endolymphatic hydrops and symptoms and audiological test results in patients with Meniere's disease: a reevaluation. *Otol. Neurotol.* 39, 351–356.
- Yazawa, Y., Kitahara, M., 1989. Immunofluorescent study of the endolymphatic sac in Meniere's disease. *Acta Otolaryngol* 468 (Suppl), 71–76.
- Yoon, H.S., Kim, H.J., Yoo, K.H., Sung, K.W., Koo, H.H., Kang, H.J., Shin, H.Y., Ahn, H.S., Kim, J.Y., Lim, Y.T., Bae, K.W., Lee, K.O., Shin, J.S., Lee, S.T., Chung, H.S., Kim, S.H., Park, C.J., Chi, H.S., Im, H.J., Seo, J.J., 2010. UNC13D is the predominant causative gene with recurrent splicing mutations in Korean patients with familial hemophagocytic lymphohistiocytosis. *Haematologica* 95, 622–626.
- Zeng, S., Qiao, H., Lv, X.W., Fan, D., Liu, T., Xie, D., 2017. High-dose dexamethasone induced LPS-stimulated rat alveolar macrophages apoptosis. *Drug Des. Dev. Ther.* 11, 3097–3104.
- Zhang, W., Dai, M., Fridberger, A., Hassan, A., Degagne, J., Neng, L., Zhang, F., He, W., Ren, T., Trune, D., Auer, M., Shi, X., 2012. Perivascular-resident macrophage-like melanocytes in the inner ear are essential for the integrity of the intrastrial fluid-blood barrier. *Proc. Natl. Acad. Sci. U. S. A.* 109, 10388–10393.
- Zhizhuo, H., Junmei, X., Yuelin, S., Qiang, Q., Chunyan, L., Zhengde, X., Kunling, S., 2012. Screening the PRF1, UNC13D, STX11, SH2D1A, XIAP, and ITK gene mutations in Chinese children with Epstein-Barr virus-associated hemophagocytic lymphohistiocytosis. *Pediatr. Blood Canc.* 58, 410–414.
- Zou, J., 1992. A preliminary study on the mechanism of immune injury of Meniere's disease—type V collagen play the role of antigen. *Zhonghua Er Bi Yan Hou Ke Za Zhi* 27, 14–16, 60.
- Zou, J., 2010. The efficient oval window passage of gadolinium chelate. In: 4th Annual Meeting of NanoEar, Chania, Greece.
- Zou, J., Yoshida, T., Ramadan, U.A., Pykko, I., 2011. Dynamic enhancement of the rat inner ear after ultra-small-volume administration of Gd-DOTA to the medial wall of the middle ear cavity. *ORL J Otorhinolaryngol Relat Spec* 73, 275–281.
- Zou, J., Poe, D., Ramadan, U.A., Pykko, I., 2012. Oval window transport of Gd-DOTA from rat middle ear to vestibulum and scala vestibuli visualized by in vivo magnetic resonance imaging. *Ann. Otol. Rhinol. Laryngol.* 121, 119–128.
- Zou, J., Pykko, I., Bjelke, B., Bretlau, P., Tayamaga, T., 2000. Endolymphatic Hydrops Is Caused by Increased Porosity of Stria Vascularis? Barany Society Meeting, Uppsala, Sweden.
- Zou, J., Bretlau, P., Pykko, I., Starck, J., Toppila, E., 2001. Sensorineural hearing loss after vibration: an animal model for evaluating prevention and treatment of inner ear hearing loss. *Acta Otolaryngol.* 121, 143–148.
- Zou, J., Pykko, I., Bretlau, P., Klason, T., Bjelke, B., 2003. In vivo visualization of endolymphatic hydrops in Guinea pigs: magnetic resonance imaging evaluation at 4.7 tesla. *Ann. Otol. Rhinol. Laryngol.* 112, 1059–1065.
- Zou, J., Duan, X., Zheng, G., Zhao, Z., Chen, S., Dai, P., Zheng, H., 2016. A novel PIK3CD C896T mutation detected in bilateral sudden sensorineural hearing loss using next generation sequencing: an indication of primary immunodeficiency. *J. Otolaryngol.* 11, 78–83.
- Zou, J., Feng, H., Pykko, I., 2015. TLR signaling pathway regulation in association with autophagy in the diseased inner ear. *J. Mol. Transl. Med.* 1, 008.
- Zou, J., Wang, Z., Chen, Y., Zhang, G., Lu, J., Zheng, H., 2018. Optimization of delivering minimum Gd-DTPA at the posterior upper point on tympanic medial wall and hT2W-3D-FLAIR sequence for detecting endolymphatic hydrops. *Zhonghua er bi yan hou tou jing wai ke za zhi* 53, 931–938.