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Original article

Otological aspects of *NLRP3*-related autoinflammatory disorder focusing on the responsiveness to anakinra

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Abstract

Objectives. Gradually progressive sensorineural hearing loss (SNHL) is a prevalent sensory defect. It is generally untreatable, making rehabilitation by hearing aid or cochlear implantation the only option. However, SNHL as one of the symptoms of the hereditary autoinflammatory systemic disease cryopyrin-associated periodic syndrome, or as the only symptom of the cochlea-specific form (DFNA34), was suggested to respond to IL-1 antagonist (anakinra) therapy, which ameliorates *NLRP3* variants-induced over-secretion of IL-1β. We analysed genotypic and phenotypic spectrum of cryopyrin-associated periodic syndrome or DFNA34, specifically focusing on the responsiveness of SNHL to anakinra.

Methods. Seventeen families diagnosed with either cryopyrin-associated periodic syndrome or DFNA34 were recruited. Genotyping and phenotyping including audiogram, MRI findings, and *in vitro* IL-1β assay were performed.

Results. Our cohort had an etiologic homogeneity of 94.1% to *NLRP3* variants and a high *de novo* occurrence (84.6%). We identified the second DNFA34 pedigree worldwide with a novel *NLRP3* variant supported by *in vitro* analysis. Significant improvement of hearing status against the natural course, showing response to anakinra, was identified in three probands, one of whom used to have severe SNHL. Hearing threshold worse than 60 dB at the start of anakinra and cochlear enhancement on brain MRI seemed to be related with poor audiologic prognosis and responsiveness to anakinra therapy despite stabilized systemic symptoms and inflammatory markers.

Conclusion. We propose a constellation of biomarkers comprising *NLRP3* genotypes, hearing status at diagnosis, and cochlear radiological findings as prognostic factors of hearing status after anakinra treatment and possibly as sensitive parameters for treatment dosage adjustment.

Key words: sensorineural hearing loss, *NLRP3*, cryopyrin-associated periodic syndrome, autoinflammatory, anakinra

Rheumatology key messages

- Even severe hearing loss in CAPS could be reversed by anakinra in selected cases.
- Hearing might remain as a sensitive biomarker for CAPS after stabilization of inflammatory markers.
- Hearing loss in CAPS can be a potential target of precision medicine.

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BASIC SCIENCE

Introduction

In a previous study, a subset of progressive sensorineural hearing loss (SNHL) cohort, with unknown aetiology and unresponsiveness to corticosteroids, has shown improvement to therapy with the IL-1 receptor antagonist, a anakinra (Kineret; Amgen, Cambridge, UK), in relation to IL-1β plasma levels [1]. Recently, another research group identified a non-syndromic SNHL [DFNA34, (MIM: 617772)] caused by *NLRP3* [NLR family, pyrin domain containing three (MIM: 606416)]-related localized cochlear autoinflammation which is responsive to anakinra therapy [2]. They further characterized the audiometric phenotype of DFNA34 due to cochlear autoinflammation as gradual symmetric progressive bilateral SNHL, beginning in the late 2nd to 4th decade of life [3].

Cryopyrin-associated periodic syndrome (CAPS) is an autosomal-dominantly inherited autoinflammatory disease with clinical spectrum of varying degree: chronic infantile, neurological, cutaneous and articular [CINCA, (MIM: 607115)] syndrome; Muckle–Wells syndrome [MWS, (MIM: 191900)]; and familial cold autoinflammatory syndrome [FCAS, (MIM: 120100)], in an order of decreasing severity [4–6]. CAPS is known to be caused by *NLRP3* variant via a gain-of-function mechanism and most patients diagnosed with CINCA syndrome or MWS present with progressive SNHL [7, 8]. Regarding pathogenesis of this disease entity, alterations in *NLRP3* are known to cause constitutive activation of the NLRP3 inflammasome, leading to an increase of IL-1β (a potent proinflammatory cytokine) production, eventually resulting in autoinflammation [4, 6–9].

Although germline hypermorphic, gain-of-function variants of *NLRP3* were well known to cause CAPS, *NLRP3*-related somatic mosaicism has recently been repeatedly reported to contribute to the pathogenesis of CAPS and presents with milder neurologic symptoms compared with the germline variant [10–13]. Given the possibility of mosaicism or somatic variants that might be restricted to inner ear resident macrophages, conventional sequencing technology using the DNA derived from circulating blood cells might not reach the adequate genetic evaluation, deviating the accurate genetic diagnosis and delaying the appropriate therapy.

The role of *NLRP3* gene is well known in the pathogenesis of CAPS by regulating IL-1β, and thus IL-1 receptor antagonist has been effective in controlling the representative systemic symptoms of the disease, including periodic fever and a rash [14]. SNHL observed in CAPS has also been reported in several papers to be modestly responsive to anti-IL-1 therapy [2, 15]. Cochlear implantation (CI), which replaces the normal acoustic hearing process via the cochlea with an electrical signal to stimulate the auditory nerve directly, has been a huge success in auditory rehabilitation of patients with severe-to-profound hearing loss. However, considering the prominent expression of *NLRP3* in the spiral ganglion neurons of mouse cochlea and the maximally increased IL-1β level in response to the stimulation [lipopolysaccharides (LPS)+ATP] observed in auditory nerve tissue, both of which are located distal or on the site of spiral ganglion stimulation by CI [2, 16], outcomes of CI would be undesirable or at least unpredictable, and well-evidenced strategy to manage the SNHL associated with autoinflammatory disease is still not established.

With the advent of deep sequencing technology, genetic diagnosis became more affordable and doable in a real clinic setting and this change has the potential to shift the paradigm of management of autoinflammatory disease-related hearing loss, making it the promising target of precision medicine. In addition, previous studies revealed the crucial role of IL-1β in the development of inflammation and hearing loss by NLRP3 inflammasome, and the individualized approach using IL-1β from patient-derived peripheral blood mononuclear cells (PBMC) culture would also be a good candidate for precision medicine.

This study aims to investigate the potential biomarkers for disease progression and predictors for efficient anti-IL-1 therapy by reviewing the clinical characteristics, and to suggest a comprehensive management strategy for autoinflammatory hearing loss by analysing the genotypic and phenotypic spectrum of the largest Korean cohort of *NLRP3*-related autoinflammatory disorder.

Methods

Subjects and clinical data

Our cohort [19 subjects (17 families)] comprised: 17 clinically diagnosed CAPS subjects (16 families), including two monozygotic twin boys; and two subjects (mother and daughter) from a DFNA34 family, segregating autoinflammatory type hearing loss (AIHL) in an autosomal dominant fashion. Patients were thoroughly examined and their symptoms were extensively characterized to evaluate if they were CINCA syndrome, MWS, FCAS or DFNA34. In addition, two 'seemingly AIHL' patients without any family history were enrolled for comparison to CAPS patients in a subset of factors with the working definition as follows: Bilateral, fluctuating SNHL, which is steroid-resistant [1], with/without cochlear enhancement on fluid-attenuated inversion recovery (FLAIR) sequence of MRI [17], and without definite autoimmune markers.

Clinical data including gender, age, medical history, physical examination, audiological test results and imaging data were obtained. The hearing threshold was calculated by averaging the thresholds of 0.5, 1, 2 and 4 kHz, and hearing level was classified into four categories: mild (26–40 dB), moderate (41–55 dB), moderately severe (56–70 dB), severe (71–90 dB), and profound (>90 dB).

All steps in this study were approved by the Institutional Review Boards of Seoul National University Hospital (IRBY-H-0905-041-281) and Seoul National

University Bundang Hospital (IRB-B-1007-105-402). Written informed consent was obtained from all subjects. In the case of minors, written informed consent was obtained from parents or guardians.

Molecular genetic diagnosis

Genomic DNA was extracted from the peripheral blood or buccal swab of all probands and, if possible, their siblings and parents for the segregation study per manufacturer's protocol. Then, whole *NLRP3* gene was screened to identify the causative variant. If the potential candidate variant was identified through *NLRP3* screening, segregation study was performed to confirm the genetic diagnosis. In cases with no detection of any potentially pathogenic *NLRP3* variant, exome sequencing was done to investigate other possible candidate genes, which was followed by the filtering process using bioinformatics analysis [18–22]. Detailed information is described in Supplementary Material, section Methods, available at *Rheumatology* online [21, 22].

Clinical evaluation: audiologic and radiologic data review

Physical examination was done to document the clinical characteristics and to diagnose CAPS by two experienced paediatric rheumatologists and two otologists. Audiologic assessment was performed depending on test eligibility (age-dependent): Pure-tone audiometry and/or Auditory brainstem response, and/or Auditory steady-state response. Internal auditory canal protocol MRI including FLAIR sequence was performed in a subset of the cohort to assess whether tumourous condition or inflammation existed in cerebellopontine angle, internal auditory canal or cochlea.

ELISA assay of IL-1β in cultured PBMCs

PBMCs were collected from peripheral venous blood samples from four individuals [normal control 1 (NC01), Cases 17–1 and 17–2, AIHL 2] and isolated by Ficoll density gradient separation. Plastic-adherent PBMCs were stored at −80 degrees by freezing container and cultured with serum-free RPMI media in 12-well culture plates (2×10^6 cells per well) for 20 min. The media were replaced with 1 ml of RPMI with 10% FBS with or without LPS (1X, from Escherichia coli 026: B6, 00–4976-03; Thermo Fisher, Waltham, MA, USA) for 3 h. Then, media were replaced with 500 μl serum-free RPMI with or without 1 mM $CaCl_2$ for 60 min. The sample supernatants were collected and 35 μl of samples were analysed using IL-1β ELISA kit (BMS224-2, Invitrogen, Carlsbad, CA, USA) by measuring absorbance at 450 nm. Test result for each individual was calculated as fold change standardized by one normal control (NC01) for comparison. Bar graph depiction of IL-1β secretion determined by ELISA assay was created for each individual, and IL-1β secretion in response to LPS or LPS+$CaCl_2$ was statistically analysed.

Statistical analysis

Statistical analysis was performed using Prism v.8.0 software (GraphPad Software, Inc. San Diego, CA, USA) and SPSS Statistics v.24 (IBM, Armonk, NY, USA) for Windows. Fisher's exact test was used to determine the association between cochlear enhancement on Brain MRI and hearing outcome. Kruskal–Wallis test was used to compare the IL-1β secretion in response to LPS and LPS+$CaCl_2$ treatment depending on each individual's medical situation (normal control, DFNA34, AIHL) and Bonferroni adjustment was conducted for *post hoc* test. $P<0.05$ was considered statistically significant.

Results

Genotypic characteristics of patients with autoinflammatory hearing loss

Genotypic and phenotypic spectrum of all 19 subjects (17 families) with either clinically diagnosed CAPS or suspected DFNA34 was summarized (Table 1). Among the 19 subjects, genetic diagnosis was made in 18 subjects (94.7%) (16 families), in whom three novel variants of *NLRP3* were found with one variant occurring twice in two genetically unrelated individuals (c.1217T>C in Cases 5 and 9). Another variant, c.1709A>G was also detected in two unrelated individuals (Cases 2 and 6). Among 18 subjects (16 families) carrying a pathogenic *NLRP3* variant, 13 families [including one monozygotic twin pedigree (Cases 13–1 and 13–2) and a mother-daughter pedigree (Cases 17–1 and 17–2)] had complete trio samples for segregation study, revealing strikingly high *de novo* occurrence of the variant [84.6% (11/13)] in our cohort (Fig. 1). Notably, autosomal dominant inheritance of the *NLRP3* variant from mother to child was shown from the other two families (Cases15 and 17): one with CINCA syndrome (Case15) and the other with non-syndromic feature (DFNA34) (Case17). Two seemingly AIHL subjects turned out not to carry *NLRP3* variants.

There was a preponderance of the pathogenic *NLRP3* variant in specific domains. Specifically, 10 out of 16 families with detectable *NLRP3* variants had pathogenic variants in the NACHT domain of *NLRP3*, four subjects had theirs in the NAD, and one proband (two monozygotic twins) had a pathogenic variant between NAD and LRR, while one non-syndromic DFNA34 pedigree had a pathogenic variant in the LRR domain (Supplementary Fig. S1, available at *Rheumatology* online). Mosaicism was also identified in one proband (Case 11) with a variant in DNA from blood cells but not from buccal samples. Detailed information of each variant is summarized in Supplementary Table S1, available at *Rheumatology* online.

Audiologic phenotype as a potential biomarker predicting the disease severity and treatment response

Among 19 subjects, two [Cases 1 (FCAS) and 4 (CINCA syndrome)] have never been tested for hearing and

Table 1 Genotypic and phenotypic spectrum of subjects with autoinflammatory hearing loss

ID Gender/ Age at diagnosis	Clinical diagnosis	*NLRP3* variant [NM_001243133.1] DNA source: blood	Domain	Inheritance pattern	Hearing status at last visit	MRI finding	Anakinra therapy	CI
Case 1 (M/17 years)	FCAS	c.910G>A:p.Glu304Lys	NACHT	Assumed *de novo*	Never tested	—	+	—
Case 2 (F/1 month)	CINCA syndrome	c.1709A>G:p.Tyr570Cys[a]	NAD	*De novo*	Profound (Aggravation)	Cochlear enhancement	+	+
Case 3 (F/2 years)	CINCA syndrome	Not detected	N/A	N/A	Moderate	No cochlear enhancement	+	—
Case 4 (M/2 months)	CINCA syndrome	c.907G>C:p.Asp303His	NACHT	Assumed *de novo*	Never tested	—	—	—
Case 5 (M/7 months)	CINCA syndrome	c.1217T>C:p.Met406Thr[a] novel	NACHT	*De novo*	Normal but with HFHL	—	+	—
Case 6 (F/8 months)	CINCA syndrome	c.1709A>G:p.Tyr570Cys[a]	NAD	*De novo*	Mild (Improvement)	No cochlear enhancement	+	—
Case 7 (M/2 years)	MWS	c.1985T>C:p.Met662Thr	NAD	*De novo*	Profound (Aggravation)	Cochlear enhancement	+	+
Case 8 (M/27 months)	FCAS	c.908A>G:p.Asp303Gly	NACHT	*De novo*	Normal but with HFHL	No cochlear enhancement	+	—
Case 9 (F/6 months)	CINCA syndrome	c.1217T>C:p.Met406Thr[a] novel	NACHT	*De novo*	Mild	No cochlear enhancement	+	—
Case 10 (F/23 months)	CINCA syndrome	c.1718T>C:p.Phe573Ser	NAD	*De novo*	Moderately severe	—	+	—
Case 11 (M/14 months)	CINCA syndrome	c.926T>C:p.Phe309Ser	NACHT	*De novo* Blood sample (+) Buccal sample (-) Mosaicism likely	Normal (Improvement)	—	+	—
Case 12 (F/11 years)	FCAS	c.778C>T:p.Arg260Trp	NACHT	*De novo*	Normal	—	+	—
Case 13-1 (M/22 months)	CINCA syndrome	c.2062G>A:p.Glu688Lys	NAD-LRR	*De novo*	Normal but with HFHL	No cochlear enhancement	+	—
Case 13-2 (M/22 months)	CINCA syndrome			(monozygotic twins)	Moderate (Aggravation)	No cochlear enhancement	+	—
Case 14 (M/18 years)	MWS	c.1000A>G:p.Ile334Val	NACHT	Assumed *de novo*	Moderately severe (Improvement)	No cochlear enhancement	+	—
Case 15 (M/5 years)	CINCA syndrome	c.1076T>C:p.Leu359Ser novel	NACHT	Vertical transmission (AD inheritance)	Normal but with HFHL	No cochlear enhancement	+	—
Case 16 (M/4 months)	CINCA syndrome	c.1213A>C:p.Thr405Pro	NACHT	*De novo*	Normal	No cochlear enhancement	+	—
Case 17-1 (F/34 years)	Nonsyndromic	c.2752C>T:p.R918X	LRR	Vertical transmission	Severe	No cochlear enhancement	—	+
Case 17-2 (F/18 months)	Nonsyndromic[b]	novel		(AD inheritance)	Mild	—	—	—
AIHL 1 (M/44 years)	Seemingly auto-inflammatory HL	Not detected	N/A	N/A	Severe	Cochlear enhancement	—	+
AIHL 2 (F/40 years)	Seemingly auto-inflammatory HL	Not detected	N/A	N/A	Severe	Cochlear enhancement	—	+

[a]Variant occurring twice. [b]Too young to be clearly determined to be nonsyndromic. AD: autosomal dominant; CI: cochlear implantation; FCAS: familial cold autoinflammatory syndrome; HFHL: high frequency hearing loss; N/A: not applicable; —: not done.

Fig. 1 Pedigrees with *de novo* inheritance and Sanger sequencing chromatograms

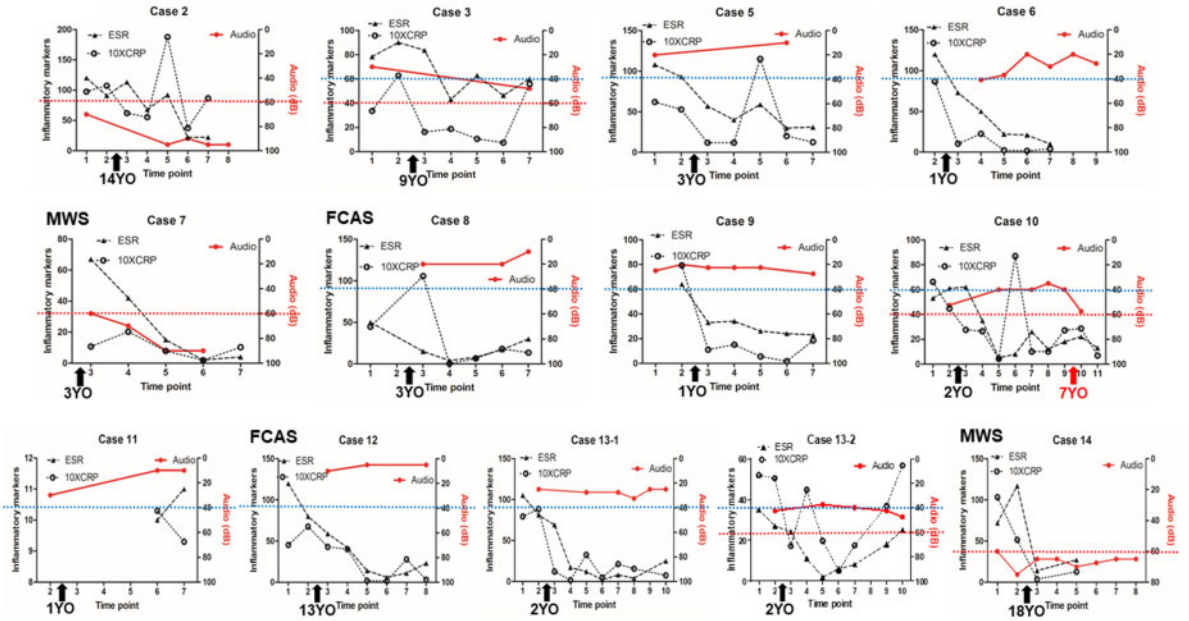
A variant in each family as confirmed by Sanger sequencing chromatograms co-segregates with phenotypes including hearing loss. Sanger sequencing traces of parents of Cases 11 and 13 are not available, because sequencing of parental DNA was performed in other institutes, which confirmed *de novo* inheritance.

seven (Cases 5, 8, 11, 12, 13–1, 15 and 16) had overall normal hearing thresholds, while four (Cases 5, 8, 13–1 and 15) of the seven subjects with overall normal hearing thresholds had mild hearing loss limited to only high frequency, which might suggest that high frequency hearing is most vulnerable in CAPS patients. Interestingly, four subjects (Cases 3, 5, 6 and 13–2) showed asymmetric hearing loss (a difference of >15 dB between the right and left ears). Audiologic phenotype in relation to inflammatory markers was analysed in the 13 patients with available audiograms and lab data (Cases 2–3, Cases 5–14).

Changes in hearing threshold and inflammatory markers, including ESR and CRP, were plotted on time domain (clinic visits) focusing on anakinra use to examine the role of hearing threshold as a potential biomarker for disease progression and responsiveness to anti-IL1 therapy (Fig. 2). Contrary to prompt and consistent response of inflammatory markers to the therapy, hearing thresholds showed differential responses to anakinra. In detail, seven genetically confirmed *NLRP3*-related syndromic patients (Cases 5, 6, 8, 9, 11, 12 and 13–1) starting initially with normal or mild hearing loss, demonstrated stable or slightly improved hearing status in response to anakinra therapy. Additionally, one MWS patient (Case 14) with initially severe SNHL, showed gradual hearing improvement despite delayed anakinra therapy. Although the improved status did not reach serviceable hearing (40 dB), at least the patient avoided CI and was well rehabilitated with hearing aid use. It turned out that this subject (Case 14) manifested the most severe hearing loss responding to anakinra therapy in literature. In summary, hearing status has apparently improved by one level in response to anakinra in three subjects with *NLRP3*-related CAPS, from mild hearing loss to normal (Cases 11), from moderate to mild hearing loss (Case 6) and from severe to moderately severe hearing loss (Case 14).

On the contrary, hearing thresholds from one CINCA syndrome patient (Case 2) and MWS patient (Case 7) which initially were within the range of moderately severe hearing loss eventually progressed to profound hearing loss despite anakinra therapy, and ended up requiring CI. Another patient (Case 3) with obvious CINCA syndrome manifestations but without any definite *NLRP3* pathogenic variant, initially demonstrated mild hearing loss that later progressed to moderate hearing loss despite continued anakinra therapy. More intriguingly, two monozygotic twins (Cases 13–1 and 13–2) with CINCA syndrome caused by the same *NLRP3* variant, manifested different audiologic phenotypes and divergent responses to anakinra therapy. In detail, Case 13–2 showed gradual aggravation of hearing on one side even with anakinra therapy contrary to Case 13–1 whose hearing status was stable throughout the follow-up period. We (otolaryngologists and paediatric rheumatologists) increased the dose of anakinra in an attempt to stop further aggravation of hearing loss, which was not successful. One patient (Case 10) with initially moderate SNHL (52.5 dB) had improved to mild

Fig. 2 Hearing thresholds relative to blood levels of inflammatory markers (ESR/CRP) over time (clinic visits)



ESR (mm/hr)/ CRP (mg/dl), Start of Anakinra is designated as an upward pointing arrow (↑) with the age at start. Red upward arrow (↑) in case 10 shows the point where dose of anakinra increased. YO, year-old

The X-axis shows visits over time: visits 1 & 2, most recent before anakinra use; visits 3 & 4, within 6 months after ankinra use; visits 3 or more, every visit where inflammatory markers or audiograms were obtained. The right Y-axis shows hearing threshold assessed by either PTA or ABR, plotted inversely to an audiogram (higher numbers correspond to worse hearing). The left Y-axis shows blood levels of ESR and 10×CRP (number of CRP blood level is relatively smaller than that of ESR, thus it was multiplied 10 times for scale adjustment in a graph). Blue dotted line and red dotted line denote hearing thresholds of 40 dB and 60 dB, respectively. PTA: pure-tone audiometry; ABR: auditory brainstem response.

hearing loss (35 dB) with anakinra therapy, but it aggravated again even worse (57.5 dB) despite increase of anakinra dose. Case 17–1 with nonsyndromic DFNA34 manifested continued progression of hearing loss, finally leading to severe to profound hearing loss requiring CI. This subject never had a chance to take anakinra treatment before CI due to late molecular genetic diagnosis and thus showed the worst hearing status among nonsyndromic DFNA34 subjects in the literature.

CI was performed in two CINCA syndrome and one DFNA34 patients, which led to a successful outcome from all three subjects.

Radiologic phenotype as a potential biomarker predicting the disease severity and treatment response

Fourteen subjects, including two AIHL subjects, underwent MRI with FLAIR sequence of the brain, including the cochlea and internal auditory canal (Table 1). Cochlear enhancement on FLAIR sequence had been observed in four subjects (Cases 2 and 7/AIHL 1 and 2) (Fig. 3), who eventually underwent CI due to profoundly aggravated hearing status. Statistically significant association was noted between poor hearing status (>60 dB) and cochlear enhancement on FLAIR sequence of brain MRI (P=0.015 by Fisher's exact test).

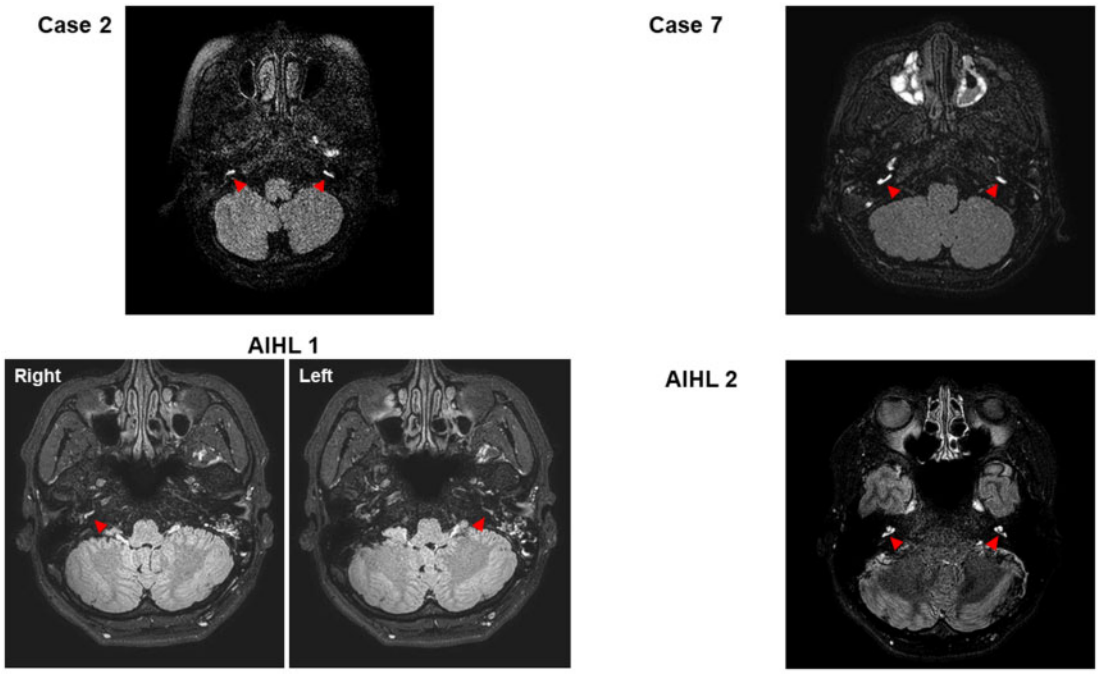
Serum cytokine measurement (ELISA assay)

Levels of IL-1β secretion from the cultured PBMCs were compared among control (NC-01), non-syndromic autoinflammatory hearing loss (DFNA34) (Cases 17–1 and 17–2) and one AIHL subject (AIHL 2) under three conditions: no stimulation, stimulation with LPS, or stimulation with LPS+$CaCl_2$. IL-1β level upon stimulation with LPS in Case 17–2 was significantly higher than those in NC01 and Case 17–1 (P=0.008 and P=0.016, respectively). Likewise, IL-1β secretion of Case 17–2 in response to LPS+$CaCl_2$ was higher than that of NC01 (P=0.031 by Kruskal–Wallis test and Bonferroni correction) (Fig. 4).

Discussion

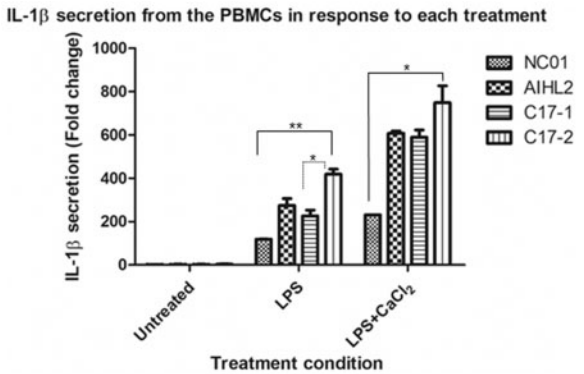
In this study, we tried to characterize the otological aspects of *NLRP3*-related autoinflammatory disorder, specifically focusing on the responsiveness to anakinra and also on the applicability of precision medicine in this disease entity. Here we report the second DFNA34 variant in the LRR domain of *NLRP3* as well as the second DFNA34 family without any syndromic feature at all in the literature [2] and showed for the first time that aggravation of hearing loss related to nonsyndromic DFNA34 could reach the point where CI was mandated

Fig. 3 Cochlear enhancement on brain MRI



Enhancement is observed after contrast administration on the mostly basal turn of cochlea (red arrowhead), suggesting localized inflammation (FLAIR image of Cases 2, 7, AIHL1 and 2). FLAIR: fluid-attenuated inversion recovery.

Fig. 4 Comparison of IL-1β response to each treatment condition among cohort



IL-1β secretion from the cultured PBMC was compared among normal control (NC01), DFNA34 (C17-1 and C17-2), AIHL 2 under three conditions: untreated, LPS, LPS+$CaCl_2$. C17-2 shows greater response than normal control under LPS and LPS + $CaCl_2$ condition. *$P<0.05$: **$P<0.01$. LPS: lipopolysaccharide; PBMC: peripheral blood mononuclear cell.

and actually performed (Case 17–1). Further, we also presented a case of the worst hearing condition (severe degree) that ever responded to anakinra so far in literature. Collectively, these suggest a sufficiently wide time window in terms of degree of hearing loss, during which we can potentially intervene in the progression of hearing loss. Unlike several inflammatory markers and systemic symptoms/signs, hearing loss was inconsistent and varied in response to anakinra. Based on this, we propose *NLRP3* genotypes, auditory thresholds at diagnosis and radiological findings of the cochlea as potential predictive and prognostic factors of future hearing status and progression of hearing loss for the hearing-impaired cases.

Specifically, audiological response to anakinra therapy and the potential role of hearing threshold as a biological marker were thoroughly investigated in relation to the change of inflammatory markers and anakinra use. Initial hearing threshold at the start of anakinra was elucidated to be an important parameter. As seen in Fig. 2, initial hearing thresholds worse than 60 dB (below red dotted line) did not recover better than 60 dB. In one subject (Case 14), hearing improved from severe SNHL to moderately severe SNHL in response to anakinra use and with apparent subjective improvement, but it did not get better than 60 dB. Nonetheless, this subject has been recorded as having the worst hearing threshold ever that indeed responded to anakinra to date in literature. On the contrary, initial hearing threshold better than 40 dB (above blue dotted line) did not get worse except in one subject (Case 3), who did not carry a confirmed pathogenic *NLRP3* variant. This might reflect the possible role of anakinra in preventing aggravation of cochlear autoinflammation. Given this, there might be a time window during which hearing loss can be reversible with anakinra therapy. In addition, older age at the start of anakinra therapy seems to be related to worse hearing

in selected cases (Cases 2, 3, 7 and 14), in accordance with a previous report [15]. For Case 3 without a definite detectable genetic cause, hearing has worsened and did not respond to anakinra therapy, potentially suggesting presence of a pathophysiologic pathway other than IL-1β pathway, although we could not exclude a possibility of mosaicism. As is seen in Cases 5, 8, 13–1 and 15, the high frequency region of hearing seems to be more vulnerable to the pathogenic effect of gain-of-function variant of *NLRP3* in CAPS [23, 24]. Meanwhile, patients with fewer and milder systemic symptoms (FCAS: Cases 8 and 12) maintained good hearing. Inflammatory markers and other systemic symptoms became well controlled soon after the start of anakinra therapy, and were kept relatively controlled with maintenance dose of anakinra. However, hearing sometimes worsened even under well-controlled inflammatory markers and systemic symptoms (Case 7), raising the possibility that the anakinra dosage required for controlling activated cochlear resident macrophage is highest amongst the various tissues in the human body, and therefore, hearing status may be the most sensitive biomarker reflecting the progression of CAPS. Given this, regular assessment of hearing is necessary for predicting disease progression, and hearing status can be regarded as a sensitive biomarker of disease severity, treatment response and dose (anti IL-1 therapy) adjustment.

Analysis of brain MRI and hearing status revealed significant association between cochlear enhancement and poor hearing outcome related to anakinra use. One subject (Case 17–1) without cochlear enhancement on MRI might be a good responder to IL-1 antagonist therapy based on the result of low IL-1β secretion after treatment (Fig. 4). However, she underwent CI after rapid aggravation of hearing thresholds before consideration of anakinra treatment due to late molecular genetic diagnosis. Thus, response to anakinra therapy could not be evaluated in this case.

A new genotype-phenotype correlation was also proposed, suggesting the LRR domain might be related to a milder phenotype (nonsyndromic DFNA34 in our cohort) in accordance with findings from the previous paper [2]. Based on this, sequencing of this LRR domain should be prioritized when DFNA34 is suspected. However, the fact that even monozygotic twins (Cases 13–1 and 13–2) demonstrate divergent auditory phenotypes and frequent asymmetry even between both ears also indicates phenotypic heterogeneity or variable expressivity in this disease entity, precluding a confirmative genotype-phenotype correlation at this point. Further, initial mild manifestation of hearing loss from the DFNA34 subject (Case 17–1) in our cohort eventually progressed to severe or profound hearing loss requiring CI in her thirties. This suggests that the strategy of attenuation of hearing loss progression by anakinra use, based on molecular genetic diagnostics, should be considered for all rapidly progressive hearing loss irrespective of the degree of hearing loss. As in Case 14, early detection and anakinra use could be powerful enough to change the method of hearing rehabilitation.

Interestingly, among families with confirmed pathogenic variant and parental samples available, *NLRP3* variants arose *de novo* from nearly all families, except two with one nonsyndromic and one CINCA syndrome case. Although autosomal dominantly inherited or *de novo* gain-of-function mutation has been reported before [7, 25], this strikingly high percentage of *de novo* inheritance pattern is surprising. We speculated why *de novo* variants of *NLRP3* are predominant in this Korean population, as prejudice towards those with disabilities might hinder the patients from getting married and prevent passing on the genetic traits, which needs to be further investigated.

Somatic *NLRP3* mosaicism has been an important issue in the diagnosis of CAPS, and several papers mentioned the substantial contribution of somatic mosaicism to this disease entity, specifically in cases of mutation-negative CAPS [10, 11]. In our study, one case of mosaicism was identified; a pathogenic *NLRP3* variant was detected in DNA sample from blood but not from buccal mucosa (Case 11). However, whether it is somatic or germline mosaicism remains elusive, awaiting further clarification. In one subject (Case 3), genetic diagnosis was not made although the patient showed the apparent features of CINCA syndrome, suggesting the possibility of mosaicism. The cochlea-specific mosaic *NLRP3* variants might explain the hearing loss phenotype of Case 3 (moderate HL), AIHLs 1 and 2 (severe to profound HL) and cochlear enhancement in brain MRI from AIHLs 1 and 2, mandating further investigation of hidden mosaicism in such cases. Alternatively, phenotypes of these subjects can be accounted for by other molecular aetiology than *NLRP3* variants.

Due to the small sample size, interpretation of the results of IL-1β assay in PBMC is very limited. However, at least one DFNA34 subject (Case 17–2) showed higher secretion of IL-1β than normal control (NC01) in response to LPS and LPS+$CaCl_2$ with statistical significance (Fig. 4), confirming pathogenic potential of p.R918X of *NLRP3*. Interestingly, in response to LPS, Case 17–2 showed greater response than Case 17–1. Case 17–2 (daughter of Case 17–1) was found to have mild hearing loss (35 dB) when she had been retrospectively investigated because of her mother's diagnosis (DFNA34). She is still too young to fully develop other systemic symptoms and thus her diagnosis might change from nonsyndromic (DFNA34) to CINCA syndrome later on. From this point of view, higher responsiveness observed in Case 17–2 than Case 17–1 might be understood and re-evaluated in the future.

There have been a couple of reports regarding CI in CAPS, showing variable hearing outcomes [26–28]. Due to the rarity of this disease entity, its management has not been spotlighted so far. However, as the genetic pathophysiologic mechanism regarding *NLRP3* has been elucidated, management of CAPS has become a good candidate of precision medicine and genetic analysis could provide a clue to determine whether to

choose medical treatment using IL-1β antagonist or surgical treatment such as CI. Patient-derived lymphoblastoid cell line might be used to predict the responsiveness to anakinra therapy, further aiding in the choice of treatment strategy.

Findings in this study might implicate idiopathic SNHL. Corticosteroids have been the only option for medical treatment of SNHL, which was not always successful [29]. SNHL is a heterogeneous disorder in terms of aetiology, where IL-1β over-secretion might be one important pathophysiologic mechanism for hearing loss, being a good target of anakinra therapy [1]. More elaborate and delineated classification of SNHL would expand the application of anakinra in this disease entity. Canakinumab (ILARIS; Novartis, Basel, Switzerland), which is a fully human monoclonal antibody targeted selectively at IL-1β, is different from anakinra, which is a recombinant inhibitor of the IL-1 receptor. Canakinumab has advantages over anakinra in that it has a longer half-life than anakinra, and thus can be administered every 8 weeks to control the symptoms of the disease, although it is significantly more expensive than anakinra. Partial efficacy of early canakinumab therapy for SNHL in MWS was already reported [25], and further extension of indication of canakinumab as well as anakinra to selected cases of SNHL is anticipated [30].

In summary, when managing CAPS and probably DFNA34 subjects, current hearing status and radiological findings, together with inflammatory markers and systemic symptoms like fever, arthritis and rash etc., should also be considered as important factors in choosing the treatment strategy and dose adjustment of IL-1 antagonist therapy *vs* proceeding to CI. After additional data collection, redefinition of disease entities based on genetic aetiologies and/or IL-1β response assay could be made: IL-1β hyper-secreting CINCA syndrome *vs* IL-1β non-secreting CINCA syndrome, or anakinra-responsive CINCA syndrome *vs* anakinra-unresponsive CINCA syndrome.

Further well-controlled prospective studies will enable us to refine the treatment strategy by systematically analysing factors including genotype, hearing status, radiologic markers and IL-1β assay in broad spectrum disease from autoinflammatory hearing loss to CAPS including CINCA syndrome.

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Supplementary data

Supplementary data are available at *Rheumatology* online.

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