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Clinical Characteristics of Hearing Loss Caused by *P2RX2* Gene Variants

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Academic Editor: Marc Via

Received: 15 October 2026
Revised: 30 October 2026
Accepted: 3 November 2026
Published: 5 November 2026

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Abstract

Background/Objectives: The *P2RX2* gene encoding an ATP-gated ion channel purinoreceptor is a genetic cause of autosomal dominant non-syndromic hearing loss (DFNA41). *P2RX2* is a rare causative gene and its detailed clinical characteristics remain unclear. **Methods**: In this study, we performed next-generation DNA sequencing analysis covering 158 genes previously reported to be associated with non-syndromic and syndromic hearing loss in 18,475 patients. **Results**: We identified 29 patients from 22

independent families carrying candidate *P2RX2* variants. All of the patients showed late-onset progressive hearing loss. In addition, several cases showed asymmetric hearing deterioration. Among the 29 patients, 23 from 19 unrelated families carried the same c.1057G>A variant, which was identified as a founder mutation by SNP-based haplotype analysis. **Conclusions**: Our findings indicate that late-onset asymmetric progressive hearing deterioration is a characteristic/unique feature of *P2RX2*-associated hearing loss, which may be related to noise exposure. Our results may be useful in further clarifying the natural history of and optimizing the management strategies for *P2RX2*-related hearing loss.

Keywords: *P2RX2*; DFNA41; autosomal dominant; hearing loss; next-generation sequencing

1. Introduction

Hearing loss (HL) is among the most frequent sensory impairments in humans. Genetic factors are a major cause of sensorineural HL, and it has been reported that more than half of congenital or prelingual-onset HL cases are attributable to genetic causes [1–3]. In addition, approximately 20–30% of late-onset HL cases are also attributable to genetic causes [4,5]. Among individuals with hereditary HL, approximately 75% of patients suffer non-syndromic HL (NSHL) without any additional abnormalities, whereas approximately 25% of patients suffer syndromic HL, which is characterized by HL accompanied by other symptoms or malformations [6]. According to the Hereditary Hearing Loss homepage, over 150 genes have been identified as causes of NSHL, and about 90 genes have been identified as causes of autosomal dominant non-syndromic HL (ADNSHL) [7].

The *P2RX2* gene, located on chromosome 12q24, has recently been reported as a genetic cause of ADNSHL (DFNA41) [8]. The P2X2 receptor is an ATP-gated ion channel purinoreceptor that is broadly distributed in mammalian tissues and functions as a trimer composed of identical subunits [8]. The P2X2 receptor functions as a cation conducting ligand-gated ion channel, and plays a crucial role in ATP-mediated synaptic transmission between neurons and from neurons to smooth muscle [9,10]. In the inner ear, the P2X2 receptor is also expressed in the cochlea, particularly in hair cells, as well as in Reissner's membrane, which maintains the endolymphatic compartment as a distinct compartment [11–21]. When exposed to loud noise, ATP is released into the endolymphatic compartment. This is thought to activate P2X2 receptors in Reissner's membrane and increase cation permeability, thereby reducing the endolymphatic potential and driving force for sound transduction [16]. *P2x2*-knockout mice exhibit noise-induced HL caused by impaired adaption to loud noise and increased susceptibility to hair-cell overstimulation, leading to reduced noise-induced temporary threshold shifts and progressive noise-induced HL [8].

In clinical reports, *P2RX2* variants have typically been associated with post-lingual onset and progressive HL, and a history of noise exposure is associated with more severe HL [8,22–25]. Although *P2RX2* causes ADNSHL (DFNA41), only 14 variants have been reported in the Human Gene Mutation Database (accessed on 23 December, 2025) [26], and the detailed clinical characteristics remain unclear.

In this study, we performed next-generation DNA sequencing analysis for 18,475 patients with HL and identified 29 patients from 22 independent families with candidate *P2RX2* variants. This is the largest number of patients reported to date. Our findings further expanded our understanding of the clinical characteristics of *P2RX2*-associated HL. In addition, we performed haplotype analysis of the recurrent *P2RX2* variant c.1057G>A,

which we identified in this study, to determine whether this variant originated from a founder mutation.

2. Materials and Methods

2.1. Subjects

In this study, we enrolled 18,475 Japanese HL patients from 130 otolaryngology departments nationwide, as detailed in our previous paper [4]. We performed target resequencing analysis for 158 genes previously reported as causative of NSHL or syndromic HL. Among the 18,475 subjects, we identified those carrying candidate *P2RX2* variants. Written informed consent was obtained from all patients (or from their caretaker or guardian, in the case of children) prior to participating in this study. This study was approved by the Shinshu University Ethics Committee as well as the ethics committees of each participating institution. This study was performed in compliance with the principles outlined in the Declaration of Helsinki. The Ethics Committee of Shinshu University School of Medicine approved this study protocol [No. 387-4 (September 2012), No. 576-2 (May 2017), and No. 718-7 (March 2022)].

2.2. Variant Analysis

The Ion AmpliSeq Custom Panel (ThermoFisher Scientific, Waltham, MA, USA) and the Ion AmpliSeq Library Kit 2.0 (ThermoFisher Scientific) were used to create sequencing libraries for the 158 target genes, following the manufacturer's instructions. The detailed protocol was outlined in our previous report [27]. After sequencing library preparation, next-generation sequencing was conducted on the Ion S5 plus system with the Ion 540 Kit-Chef and Ion 540 Chip Kit (ThermoFisher Scientific) following the manufacturer's protocol.

The sequencing reads were aligned to the human reference genome (build GRCh37/hg19) using the Torrent Mapping Alignment Program. Variant calling was performed with the Torrent Variant Caller plug-in software version 5.16. After variant calling, variant annotation was performed using ANNOVAR software version 2020-06-08 [28]. Protein-affecting variants (including the missense, nonsense, insertion/deletion and splicing variants) with a minor allele frequency of less than 1% in the control databases (including the 1000 Genomes database, the Genome Aggregation Database, the Japanese genome variation database (ToMMo 60KJPN), and 333 in-house controls) were selected. All filtering procedures were performed using our original database software reported in our previous reports [29].

Sanger sequencing was conducted to confirm the identified variants and assess their segregation within families. We also performed copy number variation (CNV) analysis based on the read depth data as described in our previous reports [30]. The pathogenicity of the identified variants was assessed based on the American College of Medical Genetics (ACMG) standards and guidelines [31] incorporating the specification established by the ClinGen Hearing Loss Clinical Domain Working Group [32]. Candidate variants were selected based on the following criteria: (1) previously reported "pathogenic" or "likely pathogenic" variants; with no conflicting evidence, (2) novel variants predicted to be "pathogenic" or "likely pathogenic" and (3) a variant of uncertain significance (VUS) that remained the sole candidate after filtering, with no other candidate variants identified in the other 157 genes and no conflicting evidence regarding the VUS.

2.3. Clinical Evaluations

We collected information on age at genetic testing, HL onset age, HL progression, pedigree, use of hearing devices and outcomes, and episodes of vertigo from medical charts. Hearing thresholds were evaluated using pure-tone audiometry in patients aged 5 years and above, whereas auditory steady state response (ASSR), conditioned orientation response audiometry (COR), or play audiometry were performed for younger children and infants. The pure-tone average (PTA) was determined using the averaged hearing thresholds for four frequencies (500, 1000, 2000 and 4000 Hz). HL severity was classified into four categories: mild HL (>25 dB and ≤40 dB HL), moderate HL (>40 dB and ≤70 dB HL), severe HL (>70 dB and ≤90 dB HL) and profound HL (>90 dB HL). The audiometric configurations were categorized into four patterns: flat, low-frequency HL, mid-frequency HL, and high-frequency HL as previously described [33].

2.4. SNPs Based Haplotype Analysis

The recurrent variant, c.1057G>A, was identified in 19 independent families. Haplotype analysis was conducted to investigate whether the recurrent occurrence of this variant was attributable to a mutational hotspot or a founder mutation. The haplotype patterns within the 1 Mbp region flanking the position of the c.1057G>A variant were characterized using 18 tag single nucleotide polymorphisms (SNPs) (9 sites upstream and 9 sites downstream of the variant). Tag SNPs were selected based on the minor allele frequency in the Japanese population (JPT) from the 1000 Genomes project using the LD Tag SNP Selection tool available on the SNPinfo Web Server (https://snpinfo.niehs.nih.gov).

3. Results

3.1. Identified Variants and Prevalence of P2RX2-Associated HL

We identified 29 patients from 22 independent families carrying candidate *P2RX2* variants in this study (Figure 1 and Table 1). The prevalence of *P2RX2*-associated HL in this large Japanese non-syndromic HL cohort was 0.12% (22/18,475). We identified only four variants in this study (Table 2), despite identifying 22 unrelated families with *P2RX2*-associated HL. Twenty families showed an autosomal dominant inheritance pattern, and two were sporadic cases (Figure 1 and Table 1). The most prevalent variant identified in this study was the c.1057G>A variant, which accounted for 86.4% (19/22) of the families with *P2RX2*-associated HL.

All four variants identified in this study were missense variants (Table 2). Among the four identified variants, the novel c.1057G>A variant was classified as "Pathogenic" (PS1 + PP1_Strong + PP3), whereas the two other novel variants were classified as variants of uncertain significance (Table 2). Interestingly, the novel and recurrent c.1057G>A variant identified in this study was located at the same position as the previously reported c.1057G>C variant, which was also identified from one family (OHL-3430). Although the two variants carry different base substitutions, they result in the same amino acid change (p.Gly353Arg).

Table 1. Clinical characteristics of *P2RX2*-associated hearing loss patients identified in this study.

Family Number	ID	Relationship	Base Change	AA Change	Inheritance	Onset	Age	Gender	Severity of HL at Genetic Testing	Type of HL	Progression	Vestibular Symptoms	Intervention After Hearing Loss Progression
1	JHLB-0894	Proband	c.1057G>A	p.Gly353Arg	AD	37	49	M	Severe	HF_gentle	Yes	No	Bilateral: HA
2	JHLB-1051	Proband	c.1057G>A	p.Gly353Arg	AD	35	43	M	Moderate	MF	Yes	Yes	Bilateral: CI
3	JHLB-1595	Proband	c.1057G>A	p.Gly353Arg	AD	35	54	F	Moderate	Flat	Yes	No	Right: N/A Left: CI
4	JHLB-1268	Proband	c.1057G>A	p.Gly353Arg	AD	30	38	F	Moderate	HF_gentle	Yes	No	Right: HA Left: CI
5	JHLB-1337	Proband	c.1057G>A	p.Gly353Arg	AD	50	69	F	Profound	HF_gentle	Yes	No	Right: N/A Left: CI
6	JHLB-2357	Proband	c.1057G>A	p.Gly353Arg	AD	6	46	F	Severe	HF_gentle	Yes	No	Right: N/A Left: EAS
	JHLB-2358	Father	c.1057G>A	p.Gly353Arg		N/A	77	M	Moderate	HF_gentle	N/A	N/A	Nothing
7	JHLB-2631	Proband	c.1057G>A	p.Gly353Arg	AD	N/A	54	F	Severe	HF_steeply	Yes	No	Right: EAS Left: HA
	JHBL-12278	Brother	c.1057G>A	p.Gly353Arg		13	64	M	Severe	HF_steeply	Yes	No	Right: EAS Left: HA
8	JHLB-4258	Proband	c.1057G>A	p.Gly353Arg	Sporadic	28	48	F	Severe	HF_steeply	Yes	No	Bilateral: HA
9	JHLB-4526	Proband	c.1057G>A	p.Gly353Arg	AD	40	66	F	Severe	HF_gentle	Yes	Yes	Bilateral: HA
10	JHLB-2947	Proband	c.1057G>A	p.Gly353Arg	AD	50	57	F	Moderate	MF	Yes	No	N/A
11	JHLB-6314	Proband	c.1057G>A	p.Gly353Arg	AD	38	54	F	Severe	HF_steeply	Yes	Yes	Right: CI Left: HA
12	JHLB-7176	Proband	c.1057G>A	p.Gly353Arg	AD	35	48	M	Severe	HF_steeply	Yes	No	Bilateral: EAS
13	JHLB-4351	Proband	c.1057G>A	p.Gly353Arg	AD	40	75	F	Profound	Flat	Yes	No	Right: Nothing Left: CI
	JHLB-4352	Sister	c.1057G>A	p.Gly353Arg		40	75	F	Profound	Flat	Yes	Yes	Right: Nothing Left: CI
	JHLB-4807	Son	c.1057G>A	p.Gly353Arg		N/A	41	M	Profound	Flat	N/A	N/A	Right: Nothing Left: CI
14	JHLB-15699	Proband	c.1057G>A	p.Gly353Arg	Sporadic	40	58	F	Severe	HF_steeply	Yes	No	Bilateral: HA
15	JHLB-16264	Proband	c.1057G>A	p.Gly353Arg	AD	40	44	F	Moderate	HF_gentle	No	No	Nothing

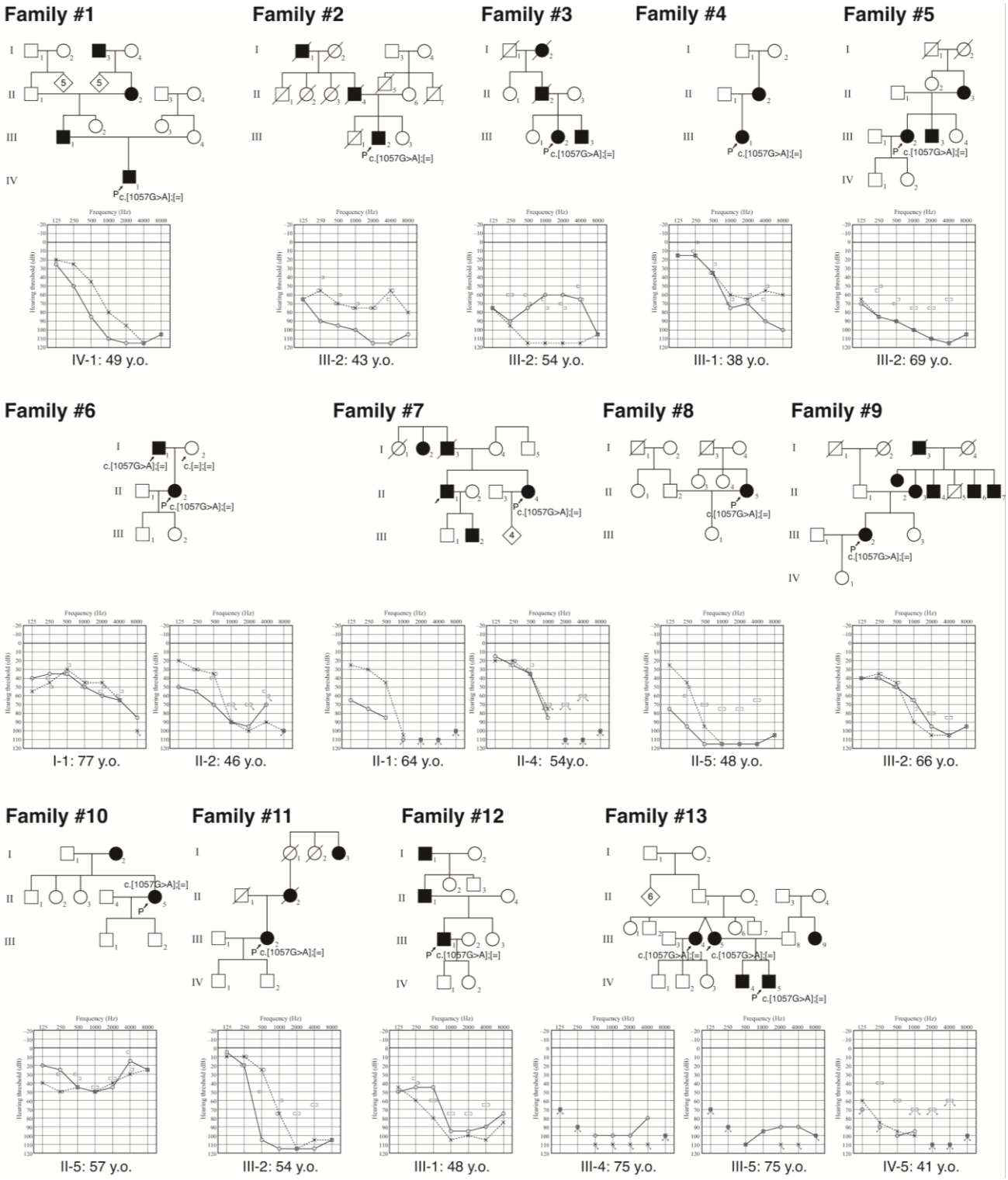
16	JHLB-17059	Proband	c.1057G>A	p.Gly353Arg	AD	42	68	M	Severe	HF_gentle	Yes	No	Right: CI Left: HA
17	JHLB-17729	Proband	c.1057G>A	p.Gly353Arg	AD	35	59	M	Severe	HF_gentle	Yes	No	Right: CI Left: HA
18	JHLB-18225	Proband	c.1057G>A	p.Gly353Arg	AD	58	58	F	Moderate	HF_gentle	Yes	No	Bilateral: HA
19	HL-17430	Proband	c.1057G>A	p.Gly353Arg	AD	40	54	F	Profound	Flat	Yes	No	Right: CI Left: HA
20	OHL-3428	Proband	c.1057G>C	p.Gly353Arg	AD	32	43	F	Profound	Flat	Yes	Yes	Right: N/A Left: CI
	OHL-3429	Daughter	c.1057G>C	p.Gly353Arg		7	10	F	Moderate	HF_gentle	Yes	N/A	Right: CI Left: HA
	OHL-3430	Daughter	c.1057G>C	p.Gly353Arg		N/A	7	F	Normal	Normal	N/A	No	Bilateral: CI
21	JHLB-4085	Proband	c.380A>C	p.Glu127Ala	AD	2	19	F	Profound	Flat	No	N/A	Bilateral: HA
	JHLB-4086	Mother	c.380A>C	p.Glu127Ala		14	48	F	Profound	Flat	Yes	N/A	N/A
22	JHLB-8560	Proband	c.600G>C	p.Lys200Asn	AD	20	41	M	Moderate	HF_gentle	Yes	Yes	Bilateral: HA

AD: autosomal dominant, M: male, F: female, HF: high frequency, MF: mid frequency, N/A: data not available, CI: cochlear implantation.

Table 2. *P2RX2* variants identified in this study.

Base Change	AA Change	Exon	SIFT	PP2	MutTaster	REVEL	CADD Phred	ToMMo 60KJPN	gnomAD All	Pathogenicity	Reference
c.380A>C	p.Glu127Ala	exon3	D	D	D	0.269	24.2	-	-	VUS	This study
c.600G>C	p.Lys200Asn	exon6	D	D	D	0.416	28.8	5.80×10^{-05}	6.84×10^{-07}	VUS	This study
c.1057G>A	p.Gly353Arg	exon10	D	D	D	0.805	26.3	3.30×10^{-05}	1.06×10^{-05}	Pathogenic	This study
c.1057G>C	p.Gly353Arg	exon10	D	D	D	0.806	25.7	-	-	Pathogenic	Faletra et al., 2014 [22]

All variants are indicated on NM_174873.3. AA: amino acid, PP2: PolyPhen2, MutTaster: Mutation Taster, D (in SIFT): deleterious, D (in PP2): probably damaging, D (in MutTaster): disease causing, VUS: variant of uncertain significance.



https://doi.org/10.3390/genes17111397

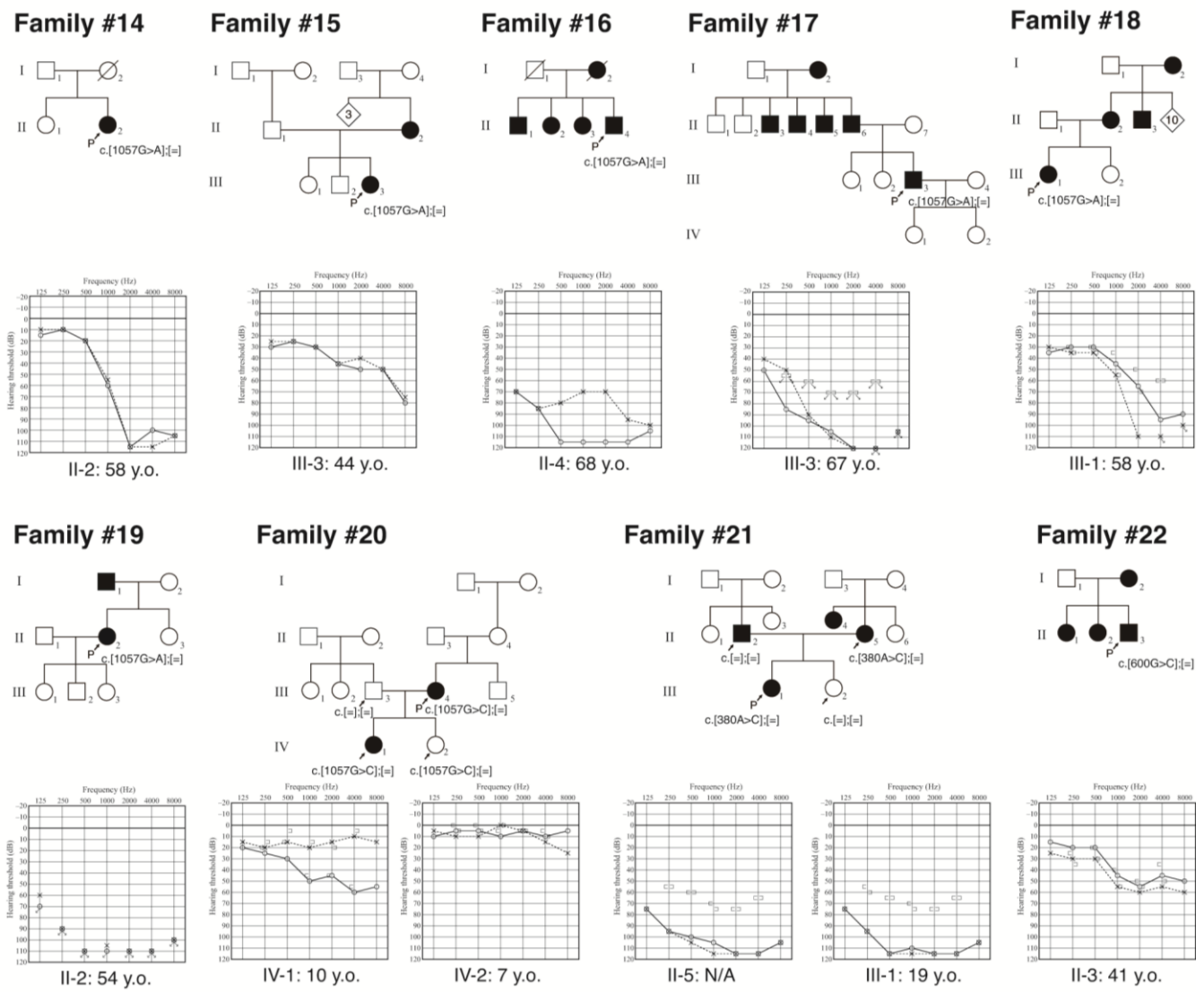


Figure 1. Pedigrees and audiograms of *P2RX2*-associated HL identified in this study.

3.2. Clinical Characteristics of P2RX2-Associated HL

Table 1 summarizes the clinical characteristics of 29 patients from 22 families with HL caused by *P2RX2* variants. The average age at onset of HL was 32.3 years, ranging from 2 to 58 years. Most patients exhibited post-lingual onset HL. The severity of HL varied from normal to profound at the time of genetic testing. Nine cases had moderate HL, eleven cases had severe HL, eight cases had profound HL, and one younger patient (aged 7 y.o.) had normal hearing at the time of genetic testing. Most individuals exhibited progressive HL (92.3%, 24/26). Regarding the audiometric configuration, the majority of cases (18 out of 29) exhibited high-frequency HL (twelve cases had high-frequency gently sloping HL, and six cases had high-frequency steeply sloping HL), eight cases had flat-type HL and two cases had mid-frequency HL at the time of genetic testing. It is noteworthy that, even among individuals carrying the same c.1057G>A variant, hearing levels varied. In addition, some cases showed asymmetrical HL, with differences of 15 dB or more between the hearing thresholds of the left and right ears (see Family #1: JHLB-0894, Family #2: JHLB-1051, Family #3: JHLB-1595, Family #6: JHLB-2357, Family #7: JHBL-12278, Family #8: JHLB-4258, Family #11: JHLB-6314, Family #16: JHLB-17059, Family #20: OHL-3429 in Figure 1). Vestibular symptoms were relatively rare, and were reported in six out of 24 cases.

To estimate the rate of hearing deterioration for *P2RX2*-associated HL, we performed scatter plotting and regression analysis of the pure-tone average (the average at 500 Hz, 1000 Hz, 2000 Hz, and 4000 Hz) and age. Audiometric data from the same individual were connected by lines (Figure 2). Linear regression lines fitted to all data points are shown in the graph. As seen in Figure 2, pure-tone average hearing thresholds deteriorated with increasing age; however, substantial variability in the clinical course was observed among individual patients. Notably, several patients exhibited rapid hearing deterioration from mild or even normal hearing to severe-to-profound HL within a few years. For example, one patient (OHL-3430) initially had normal hearing but progressed to profound HL within 11 years.

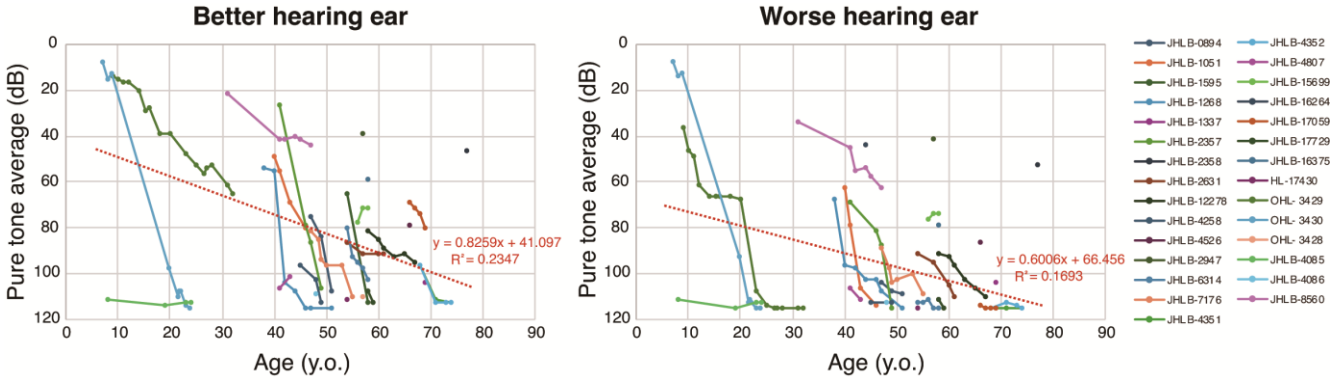


Figure 2. Hearing deterioration of *P2RX2*-associated HL for better hearing ears and worse hearing ears.

Figure 3 shows the progression of HL in each ear of individual patients. Many patients identified in this study exhibited asymmetric progression of HL (JHLB-0894, JHLB-1051, JHLB-1268, JHLB-2357, JHLB-2631, JHLB-12278, JHLB-4258, JHLB-6314, JHLB-17059, OHL-3429 and JHLB-8560). Despite this asymmetric progression of HL, hearing in both ears appeared to eventually progress to severe-to-profound HL (for example, JHLB-1268, JHLB-2357, and JHLB-4258 showed asymmetric progression, but hearing in both ears eventually deteriorated to the severe-to-profound range).

As interventions for HL, 13 patients had undergone cochlear implantation, seven patients were using hearing aids, and nine patients had no device or their status was unknown at the time of genetic testing. As HL progressed, the number of patients who underwent cochlear implantation increased to 18 (Table 1). Most patients who underwent cochlear implantation achieved favorable postoperative hearing thresholds and speech perception outcomes (Figure 4). The monosyllable score significantly improved after cochlear implantation ($p < 0.01$).

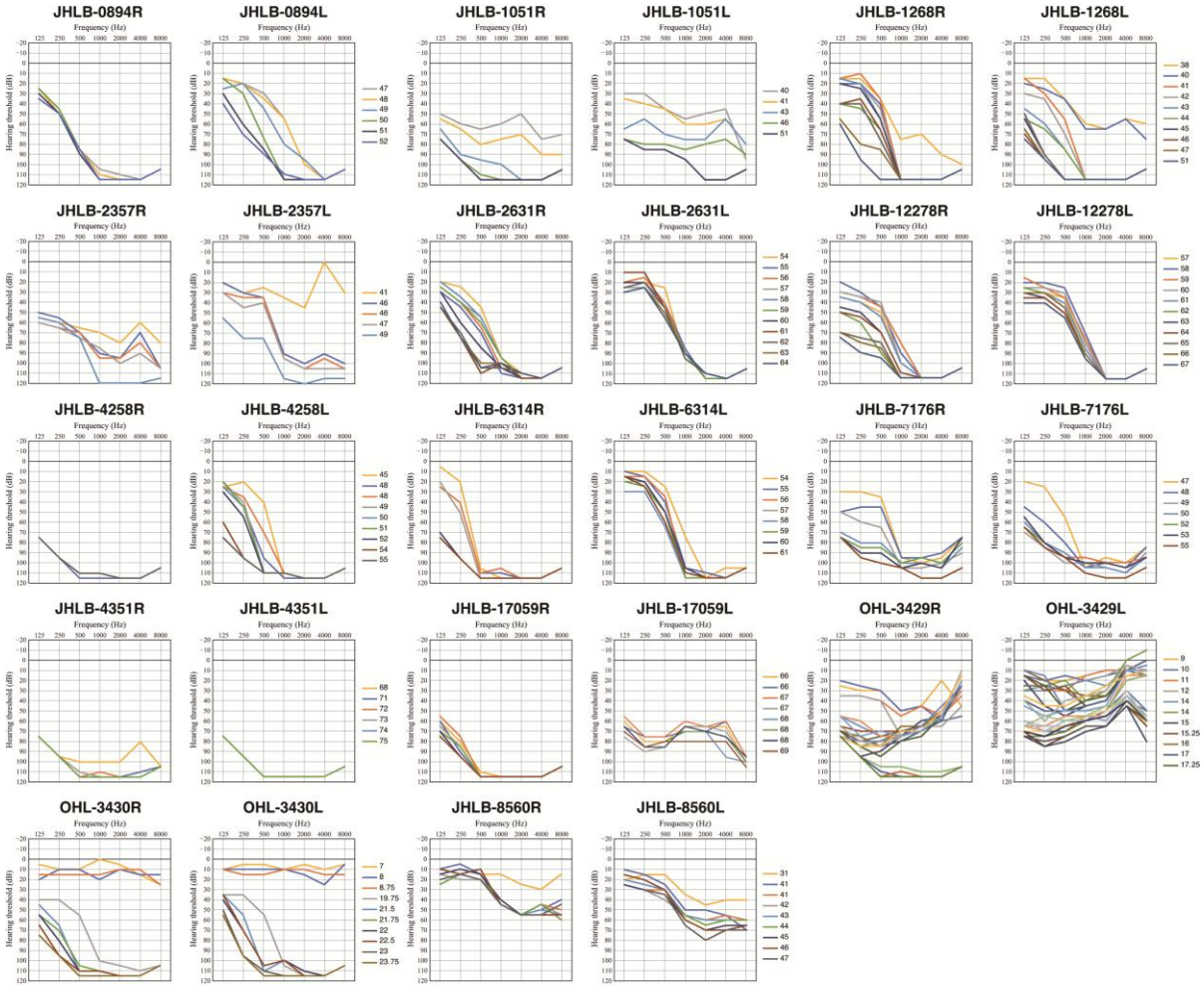


Figure 3. Hearing deterioration in each ear of individual *P2RX2*-associated HL patients.

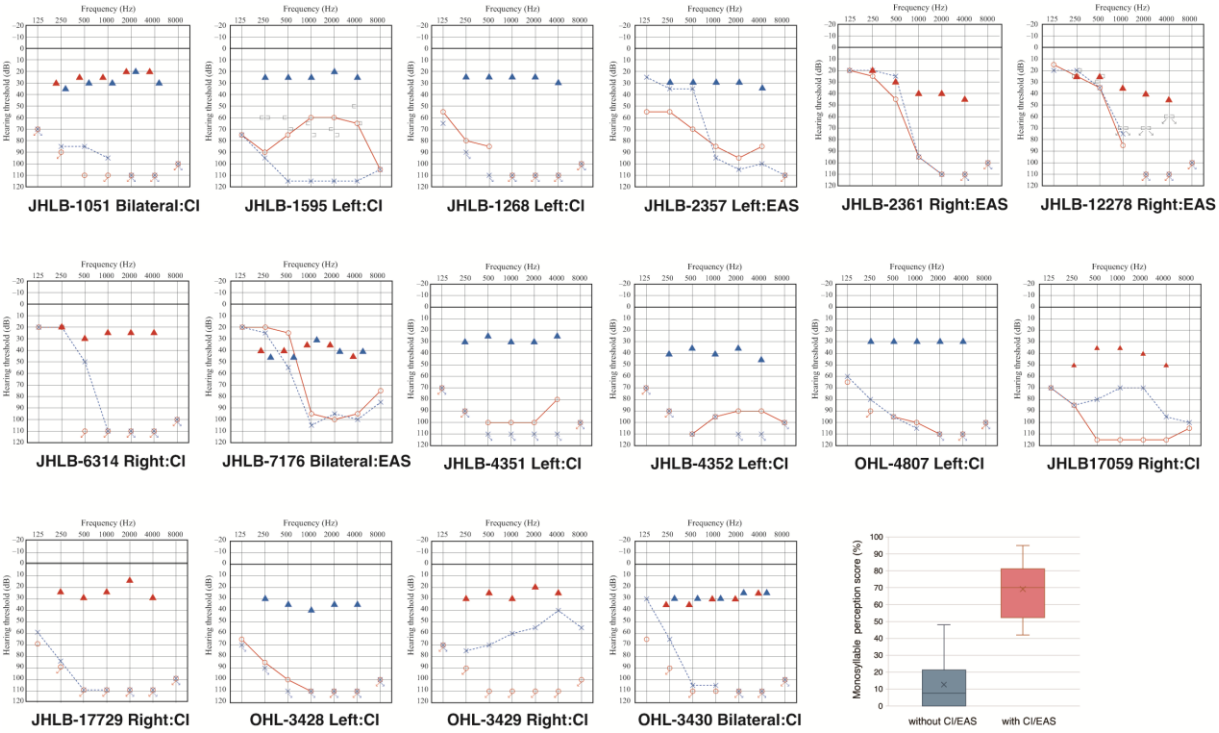


Figure 4. Hearing thresholds and monosyllable perception scores with CI/EAS in patients with *P2RX2*-associated HL. Red and blue indicate hearing thresholds for the right and left ears,

respectively. Triangles indicate hearing thresholds measured with cochlear implants. The panel in the bottom right showed monosyllable perception scores with and without cochlear implantation. The cross symbols indicate the mean values.

3.3. Haplotype Analysis

In this study, we identified 23 cases from 19 unrelated families carrying the same c.1057G>A variant. This variant was a novel variant identified in this study, but a different base substitution at the same base position, c.1057G>C, has been reported as a genetic cause of HL in an Italian family with *P2RX2*-associated HL. Therefore, this recurrent variant, c.1057G>A, may be attributable to either a mutational hotspot or a founder mutation. To clarify this, we performed haplotype analysis within the 1 Mbp region surrounding the c.1057G>A variant to characterize the region using a set of 18 single nucleotide polymorphisms (SNPs) (9 sites upstream and 9 sites downstream). Results showed that all affected individuals from the two unrelated families with the c.1057G>A variant shared an identical pattern in the region closest to c.1057G>A, although the other allele showed a variety of haplotype patterns (Figure 5). Thus, we speculated that this variant originated from a founder mutation and spread into the Japanese population.

SNP No.	Distance from the c.1057G>A mutation (bp)	Family #6						Family #13			
		JHLB-2357 (Proband / Affected)		JHLB-2358 (Father / Affected)		JHLB-2359 (Mother / Unaffected)		JHLB-4351 (Proband / Affected)		JHLB-4351 (Sister / Affected)	
		Affected	Unaffected	Affected	Unaffected	Unaffected	Unaffected	Affected	Unaffected	Affected	Unaffected
1	−595,997	G	A	G	A	A	G	G	G	G	G
2	−503,311	C	G	C	C	G	G	C	C	C	C
3	−360,187	G	G	G	T	G	G	T	T	T	T
4	−235,712	A	C	A	A	C	A	A	C	A	C
5	−128,056	G	G	G	G	G	G	G	G	G	G
6	−82,966	C	C	C	C	C	T	C	T	C	T
7	−41,920	C	T	C	C	T	C	C	C	C	C
8	−12,324	C	T	C	C	T	T	C	T	C	T
9	−3,086	A	A	A	A	A	A	A	A	A	A
	c.1057G>A	A	G	A	G	G	G	A	G	A	G
10	3,783	C	C	C	C	C	C	C	C	C	C
11	25,271	T	C	T	T	C	C	T	C	T	C
12	54,335	C	C	C	C	C	C	C	C	C	C
13	78,063	T	C	T	T	C	C	T	C	T	C
14	182,127	G	G	G	G	G	G	G	A	G	A
15	229,811	G	G	G	G	G	G	G	A	G	A
16	313,134	A	A	A	A	A	G	A	A	A	A
17	485,407	T	T	T	A	T	A	T	A	T	A
18	518,983	G	G	G	A	G	A	G	A	G	A

Figure 5. Haplotype analysis for 2 unrelated families with the c.1057G>A variant. Pink indicates the haplotype shared among affected individuals.

4. Discussion

P2RX2 encodes the ATP-gated ion channel P2X2 receptor, which is particularly expressed in cochlear hair cells and Reissner's membrane, and plays a crucial role in temporary threshold shift after loud noise exposure [18]. In *P2rx2*-deficient mice, no temporary threshold shift occurs, and irreversible threshold elevation develops after noise exposure [8]. Furthermore, knockout models display abnormal ribbon synapse localization and suppressed inner hair cell development, supporting an essential role of *P2RX2* in maintaining cochlear homeostasis [11–21].

P2RX2 is a rare causative gene for hereditary HL, and only 14 variants have been reported to cause autosomal dominant HL [8,22,25,26,34–43]. Thus, the detailed clinical

phenotypes remain unclear. In this study, we performed a comprehensive genetic analysis of 158 genes previously reported to cause non-syndromic HL and syndromic HL in 18,475 Japanese patients with HL, and identified 29 patients from 22 families with *P2RX2*-associated HL. To our knowledge, this study reports the largest number of families with *P2RX2*-associated HL to date. Among the four variants identified in this study, two novel missense variants, c.380A>C and c.600G>C, were classified as variants of uncertain significance. Further studies including family segregation analysis and/or functional characterization using ATP-evoked current measurements will be needed to clarify their pathogenicity.

The average age at onset of HL was 32.3 years, ranging from 2 to 58 years, and most individuals developed HL in their 4th decade in this study. Li et al. summarized six previously reported papers on *P2RX2*-associated HL and reported that the age at onset of *P2RX2*-associated HL ranged from the 1st to 2nd decade of life [23]. The age at onset in our cohort was broadly consistent with that reported in previous studies, suggesting that late-onset HL, rather than congenital HL, may be a clinical feature for *P2RX2*-associated HL.

Progressive hearing deterioration was observed in most cases (92.3%, 24/26), which was consistent with previous studies [23]. Regarding the audiometric configuration, most cases identified in this study showed high-frequency HL and progressed to a flat audiogram (Table 1 and Figure 1). In a previous report, Liu et al., noted sex-related phenotypic differences in *P2RX2*-associated HL in a family carrying the p.Val60Leu variant. Females in this family exhibited low-frequency HL, whereas males showed high-frequency HL that flattened over time [22]. On the other hand, Faletra et al. reported a family carrying the p.Gly355Arg variant, and almost all cases showed high-frequency HL [20]. Based on our results and previous reports, high-frequency HL may be a typical audiometric pattern for *P2RX2*-associated HL, but even among patients carrying the same variant, the audiometric configuration varied.

In addition, as shown in Figure 2 and Figure 3, asymmetrical hearing deterioration may also be a unique feature of *P2RX2*-associated HL. Many cases identified in this study showed asymmetrical HL although hearing eventually deteriorated to bilateral severe-to-profound HL. Asymmetrical hearing deterioration was clearly shown to occur in patients with *P2RX2*-associated HL (Figure 2). Although genetic HL is generally described as bilateral and symmetrical, one plausible explanation for the asymmetrical HL observed in *P2RX2*-associated HL is differences in noise exposure between the ears. Yan et al., reported that *P2RX2* variants cause progressive HL and increased susceptibility to noise [6]. Noise exposure often affects one ear more than the other; therefore, the asymmetric HL may reflect gene–environment interactions. Asymmetric HL is unusual in cases of genetic HL and may be a distinctive clinical feature of *P2RX2*-associated HL. We attempted to obtain information on the noise exposure history for 29 patients. Of these, four patients reported a history of noise exposure, while nine patients reported no history of noise exposure, and information was unavailable for the remaining 16 patients. Due to the limited availability of noise exposure data, we were unable to conclude whether noise exposure was associated with asymmetrical hearing deterioration. Further study will be needed to clarify this.

Regarding the rate of hearing deterioration, linear regression analysis of the entire cohort estimated a progression rate of approximately 0.60–0.83 dB/year for *P2RX2*-associated HL. This rate is comparable to those reported for *MYO6*-associated HL (approximately 0.56 dB/year) [44], *ACTG1*-associated HL (approximately 1.1 to 2.1 dB/year) [45] and *KCNQ4*-associated HL (approximately 0.89 dB/year) [46]. Nevertheless, considerable interindividual variability in the rate of disease progression was observed. Notably, some patients exhibited rapid deterioration in hearing thresholds, reaching approximately 10

dB per year. These findings suggest that *P2RX2*-associated HL may represent one of the more rapidly progressive forms of hereditary HL.

More than half of the cases underwent cochlear implantation and achieved favorable auditory outcomes. These findings indicate that although *P2RX2* dysfunction contributes to cochlear pathology, cochlear implants can effectively restore auditory input when severe-to-profound HL develops. Li et al. also reported favorable auditory outcomes after cochlear implantation for *P2RX2*-associated HL [25].

We identified a recurrent variant (c.1057G>A) that was identified in 19 out of 22 families with *P2RX2*-associated HL included in this study. This variant was a novel variant identified in this study, but a different base substitution at the same position (c.1057G>C) has been reported as a genetic cause of HL in an Italian family with *P2RX2*-associated HL. To determine whether this recurrent variant originated as a hotspot mutation or a founder mutation, we performed haplotype analysis of the 1M base region surrounding the variant. As shown in Figure 5, the same haplotype was observed even among unrelated families, suggesting that a founder mutation occurred in a common ancestor and subsequently spread into the Japanese population. Such a founder mutation is relatively rare in autosomal dominant HL, but has been reported for the *KCNQ4* c.211del variant [47].

However, the c.1057G>A variant is also observed in gnomAD non-Finnish European and Finnish populations. In addition, the c.1057G>C variant identified in this study and that identified in an Italian family may have arisen through a mutational hotspot. Based on these findings, this region may represent a mutational hotspot, with base substitutions occurring spontaneously in many ethnic populations; however, in the Japanese population, it may have spread from a common ancestor. Supporting this hypothesis, all patients with the c.1057G>A variant identified in this study were from the main island of Japan and this variant was not identified in patients from Hokkaido in the north or Kyushu in the south. This uneven geographic distribution also supports the common ancestor hypothesis for the c.1057G>A variant in the Japanese population.

5. Conclusions

In conclusion, this study expands our knowledge of the clinical features of *P2RX2*-associated HL. Our findings highlight late-onset, asymmetric, progressive hearing deterioration as a potentially distinctive feature of *P2RX2*-associated HL, which may be associated with responses to noise exposure. Our findings may help to further clarify the natural history of the condition and optimize management strategies for *P2RX2*-related HL.

Author Contributions: Conceptualization, S.O. and S.-i.U.; methodology, S.O. and S.-y.N.; software, S.-y.N.; validation, S.O. and S.-y.N.; investigation, S.-i.U.; resources, S.O., T.Y., H.N., K.O., Y.H., M.M., T.I., N.S., K.K., M.K., H.M., Y.A., H.Y., J.N., T.N., A.M. and C.M.; data curation, S.-y.N.; writing—original draft preparation, S.O.; writing—review and editing, S.-y.N. and S.-i.U.; visualization, S.O. and S.-y.N.; project administration, S.-i.U.; funding acquisition, S.-i.U. All authors have read and agreed to the published version of the manuscript.

Funding: This study was funded by a Health and Labor Sciences Research Grant for Research on Rare and Intractable Diseases and Comprehensive Research on Disability Health and Welfare from the Ministry of Health, Labor and Welfare of Japan (S.U. H29-Nanchitou(Nan)-Ippan-031, 20FC10148, 23FC10149); and Grants-in-Aid from the Japan Agency for Medical Research and Development (AMED) (S.U. JP16kk0205010, JP18ek0109363, JP21ek0109542, JP24ek0109741).

Institutional Review Board Statement: This study was conducted in accordance with the guidelines of the Declaration of Helsinki, and was approved by the Institutional Ethics Committee of Shinshu University School of Medicine [No. 387 (September 4th, 2012), No. 576 (May 2nd, 2017), and No. 718 (March 7th, 2022)].

Informed Consent Statement: Written informed consent was obtained from all patients (or from their next of kin, caretaker, or legal guardian, in the cases of minors or children).

Data Availability Statement: The datasets used during the current study are available from the corresponding author on reasonable request.

Acknowledgments: We thank all participants in the present study. We also thank Fumiko Tomioka and Sachiko Matsuda for their technical assistance with this research.

Conflicts of Interest: All authors declare no conflicts of interest in this study.

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