Polymorphisms in Genes Involved in Oxidative Stress Response in Patients with Sudden Sensorineural Hearing Loss and Ménière's Disease in a Japanese Population

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The etiologies of idiopathic sudden sensorineural hearing loss (SSNHL) and Ménière's disease remain unclear. Recently, accumulating evidence has demonstrated that oxidative stress is related to the pathology of inner ear disease. Because genetic factors may contribute partly to the etiologies of SSNHL and Ménière's disease, we investigated the associations between genetic polymorphisms located in oxidative stress response genes and susceptibility to SSNHL and Ménière's disease. We compared 84 patients affected by SSNHL, 82 patients affected by Ménière's disease, and 2107 adults (1056 men and 1051 women; mean age, 59.2 years; range, 40–79 years) who participated in the National Institute for Longevity Sciences, Longitudinal Study of Aging. Multiple logistic regression was used to calculate odds ratios for SSNHL and Ménière's disease in individuals with polymorphisms in the genes glutathione peroxidase 1 (*GPX1*) (Pro198Leu, rs1050450), paraoxonase 1 (*PON1*) (Gln192Arg, rs662; and Met55Leu, rs854560), *PON2* (Ser311Cys, rs7493), and superoxide dismutase 2 (*SOD2*) (Val16Ala, rs4880), with adjustment for age and gender. No significant differences in the distribution of the genotypes at these polymorphisms were observed among individuals with SSNHL and Ménière's disease and controls. No significant risk for SSNHL and Ménière's disease was observed in the additive genetic model, regardless of moderating variables. The C allele of *SOD2* (rs4880) was more frequent in Ménière's disease cases with a hearing level over 50 dB compared with cases with a hearing level below 50 dB, suggesting that this polymorphism is associated with progression of a hearing loss in Ménière's disease. In conclusion, no significant associations between the polymorphisms of *GPX1*, *PON1*, *PON2*, and *SOD2* and risk of SSNHL and Ménière's disease were observed in this Japanese case–control study.

Introduction

SUDDEN SENSORINEURAL HEARING LOSS (SSNHL) is usually unilateral and can be associated with tinnitus and vertigo. In most cases, the cause is not identified and the condition is termed idiopathic SSNHL (Schreiber *et al.*, 2010). Complete, partial, or no hearing recovery can occur within 1 or 2 months after the onset of the hearing loss. After this critical period, hearing becomes stable and no further recovery can be expected. The etiology of idiopathic SSNHL remains unclear. Proposed pathologies include viral infections, circulatory disturbance, and membrane breakage in the cochlea (Merchant *et al.*, 2005). Among these, impaired inner ear perfusion and ischemic vascular damage of

the cochlea are widely recognized as possible pathogenic mechanisms.

Ménière's disease is a syndrome characterized by repeated vertiginous spells accompanied by a fluctuating hearing loss and aural fullness. Almost simultaneously, but independently, Hallpike and Cairns (1938) and Yamakawa (1938) reported the presence of an enlarged endolymphatic space in the temporal bones of patients with Ménière's disease, demonstrating that endolymphatic hydrops is its principal underlying pathology. Thus, the clinical symptoms of SSNHL and Ménière's disease are different. However, some patients were diagnosed as having SSNHL first, and then diagnosed as having Ménière's disease because of subsequent hearing fluctuation with vertigo. SSNHL and

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Ménière's disease are the most important inner ear diseases (Hallpike and Cairns, 1938; Yamakawa, 1938).

Oxidative stress, which is defined as an excess of pro-oxidant species that are not counterbalanced by an adequate endogenous and exogenous antioxidant defense system, has been proposed as a risk factor for microvascular damage. It is also involved in the development of endolymphatic hydrops. In addition, cellular damage and apoptotic cell death associated with oxidative stress might contribute to the sensorineural hearing loss found in later stages of Ménière's disease (Labbe *et al.*, 2005). Furthermore, the serum concentration of reactive oxygen species (ROS; as measured using spectrophotometric methods) and a global oxidative stress index, which reflect oxidative and antioxidant counterparts, respectively, were significantly higher in patients with SSNHL than they were in controls (Capaccio *et al.*, 2011). Increased levels of protein carbonyls and 4-hydroxynonenal, which are markers of protein oxidation, have been detected in the lymphocytes of patients with Ménière's disease compared with controls, with a significant decrease in the ratio of reduced glutathione versus oxidized glutathione both in the plasma and in lymphocytes (Calabrese *et al.*, 2010). The results of these previous reports imply that the oxidative stress response is involved in the pathophysiology of both SSNHL and Ménière's disease.

Genetic factors may contribute partly to the etiologies of SSNHL and Ménière's disease. In SSNHL, significant associations have been reported mainly for polymorphisms in genes related to blood vessels, circulation, or inflammation, including protein kinase C ϵ type (1425G/A), matrix metalloproteinase-1 (–1607G/2G), interleukin 1A (–889C/T), interleukin 6 (–572C/G), methylenetetrahydrofolate reductase (C677T), prothrombin (G20210A), platelet Gly IIIaA1/A2, and factor V Leiden (Capaccio *et al.*, 2005a, 2005b, 2007, 2009; Uchida *et al.*, 2010, 2011; Furuta *et al.*, 2011; Nam *et al.*, 2011; Hiramatsu *et al.* 2012). In Ménière's disease, significant associations have been reported for genetic polymorphisms, such as those in the *KCNE* potassium channel genes (in the Japanese population, but not in Caucasians), adducin 1 (Gly460Trp), heat-shock protein 70-1 (190G/C), and interleukin 1A (–889C/T) (Doi *et al.*, 2005; Kawaguchi *et al.*, 2008; Teggi *et al.*, 2008; Campbell *et al.*, 2010; Furuta *et al.*, 2011). To date, there has been a limited number of papers on the relationship between genetic polymorphisms involved in oxidative stress response and SSNHL and Ménière's disease, with the exception of the two reports mentioned previously, which revealed an absence of significant associations between polymorphisms of glutathione S-transferase T1 (*GSTT1*) and *GSTM1* and SSNHL (Cadoni *et al.*, 2006; Um *et al.*, 2011).

Thus, in the present study, we aimed to assess the effects of five additional polymorphisms of genes involved in the oxidative stress response on the risk of susceptibility to SSNHL and Ménière's disease.

Materials and Methods

Patients

Eighty-four patients (38 men, 46 women; mean age $\pm$ standard deviation, 58.2 $\pm$ 14.3 years; range, 22–86 years) affected by SSNHL, and 82 patients (32 men, 50 women; mean age $\pm$ standard deviation, 54.4 $\pm$ 15.3 years; range, 20–79 years) affected by Ménière's disease, who visited the Department of

Otorhinolaryngology of the Nagoya University Hospital between November 2007 and March 2011 were enrolled in the study. The patients were recruited consecutively. Idiopathic SSNHL was defined as a sensorineural hearing loss of sudden onset with unknown etiology accompanied by no cranial nerve symptoms other than those from the VIII nerve, according to the criteria established by the Sudden Deafness Study Group of the Ministry of Health and Welfare (1973) and revised by the Acute Severe Hearing Loss Study Group of the Ministry of Health, Labor and Welfare (2011), Japan. Among the 84 patients with SSNHL, 66 (78.6%) had tinnitus and 32 (38.1%) had vertigo. Ménière's disease was diagnosed according to the criteria of the 1995 American Academy of Otolaryngology, Head and Neck Surgery (AAO-HNS, 1995). Among the 82 patients with Ménière's disease, 41 were classified as having definite Ménière's disease, 6 were classified as having probable Ménière's disease, and 35 were classified as having possible Ménière's disease, according to AAO-HNS criteria. Demographic data were recorded via medical interviews or self-reporting.

Controls

The controls used in this study were derived from the National Institute for Longevity Sciences, Longitudinal Study of Aging (NLS-LSA), which is an ongoing population-based biennial survey. Participants in the NLS-LSA were selected randomly from resident registrations and were stratified according to both age and gender. The NLS-LSA study area is located within 30 km of the Nagoya University Hospital. Details of the NLS-LSA have been described elsewhere (Uchida *et al.*, 2005). Overall, 2107 participants (1056 men and 1051 women; mean age $\pm$ standard deviation, 59.2 $\pm$ 10.9 years; range, 40–79 years) were selected. These individuals completed the first wave of NLS-LSA examinations between November 1997 and April 2000 and provided samples for the six single-nucleotide polymorphism (SNP) genotype analyses.

Ethics

The study protocol was reviewed and approved by the ethics committees of the Nagoya University (370-4) and the National Center for Geriatrics and Gerontology (#14, #52, and #74), and written informed consent was obtained from all subjects.

Genotype analysis

Five polymorphisms in genes encoding mediators involved in oxidative stress response glutathione peroxidase 1 (*GPX1*; Pro198Leu, rs1050450); paraoxonase 1 (*PON1*; Gln192Arg, rs662; and Met55Leu, rs854560); *PON2* (Ser311Cys, rs7493); and superoxide dismutase 2 (*SOD2*; Val16Ala, rs4880) were investigated. Genomic DNA was extracted from peripheral blood lymphocytes using standard procedures, and polymerase chain reaction (PCR) amplification was performed. Genotyping was carried out using an allele-specific primer method and an intercalator-mediated fluorescence resonance energy transfer probe method, as described previously (Yamada *et al.*, 2003; Hirota *et al.*, 2007). Details of primer sequences and PCR conditions are listed in Table 1.

TABLE 1. POLYMERASE CHAIN REACTION CONDITIONS USED IN GENOTYPING THE POLYMORPHISMS OF GENES INVOLVED IN OXIDATIVE STRESS

Allele-specific primer (ASP) method						
Gene	rs No.	Labeled primers	Sequence (5' → 3')	Amplicon (F1/R)/(F2/R)(bp)	Annealing temperature (°C)	Mg (mM)
Glutathione peroxidase 1	rs1050450	F1 (FITC)	GCGCCCTAGGCACAGCTxAG	100/100	65.0	2.5
		F2 (Texas Red) R (Biotin)	GCGCCCTAGGCACAGCTxGG GTGTGCCCTACGCAGGTACA			
Paraoxonase 1 (Met55Leu)	rs854560	F1 (FITC)	TCCATTAGGCAGTATCTCCXGT	48/48	60.0	2.5
		F2 (Texas Red) R (Biotin)	TCCATTAGGCAGTATCTCCXAG TTTCTGGCAGAAACTGGCTC			
Paraoxonase 2	rs7493	F1 (FITC)	CGCATCCAGAAACATTCTAxGT	51/52	60.0	1.8
		F2 (Texas Red) R (Biotin)	CCGCATCCAGAAACATTCTAXCT GGCATAAACTGTAGTCACTGTAGGC			
Superoxide dismutase 2	rs4880	F1 (FITC)	AGGCAGCTGGCTCCGXCT	44/44	62.5	2.5
		F2 (Texas Red) R (Biotin)	AGGCAGCTGGCTCCGXTT TTCTGCCTGGAGCCCCAGATA			
Intercalator-mediated fluorescence resonance energy transfer probe (IFP) method						
Gene	rs No.	Primers	Sequence (5' → 3')	Amplicon (bp)	Annealing temperature (°C)	Mg (mM)
Paraoxonase 1 (Gln192Arg)	rs662	F R	TGAGCACTTTTATGGCACAAATGATCAC CGACCACGCTAAACCCAAATACATCTC	85	65.0	2.0
						TTGACCCCTACTTACGATCCTG

TABLE 2. GENOTYPE DISTRIBUTIONS OF OXIDATIVE STRESS-RELATED GENE POLYMORPHISMS IN SUDDEN SENSORINEURAL HEARING LOSS PATIENTS, MÉNIÈRE'S DISEASE PATIENTS, AND CONTROLS

Genotypes/ minor-allele frequencies	Controls	SSNHL patients	MD patients	p-Value (versus controls)
	n=2107 No. (%)	n=84 No. (%)	n=82 No. (%)	
GPX1 (rs1050450)				
CC	1782 (84.6)	71 (84.5) ^a	72 (87.8) ^b	0.4351 ^a
CT	318 (15.1)	12 (14.3) ^a	10 (12.2) ^b	
TT	7 (0.3)	1 (1.2) ^a	0 (0) ^b	0.6664 ^b
PON1 (rs662)				
GG	928 (44.0)	34 (40.5) ^a	28 (34.2) ^b	0.3891 ^a
GA	952 (45.2)	37 (44.1) ^a	44 (53.7) ^b	
AA	227 (10.8)	13 (15.5) ^a	10 (12.2) ^b	0.2056 ^b
PON1 (rs854560)				
AA	1820 (86.4)	78 (92.9) ^a	69 (84.2) ^b	0.1615 ^a
AT	271 (12.9)	5 (6.0) ^a	13 (15.9) ^b	
TT	16 (0.8)	1 (1.2) ^a	0 (0) ^b	0.5453 ^b
PON2 (rs7493)				
CC	1375 (65.3)	59 (70.2) ^a	57 (69.5) ^b	0.4596 ^a
CG	631 (30.0)	20 (23.8) ^a	21 (25.6) ^b	
GG	101 (4.8)	5 (6.0) ^a	4 (4.9) ^b	0.6983 ^b
SOD2 (rs4880)				
TT	1595 (75.7)	60 (71.4) ^a	64 (78.1) ^b	0.3929 ^a
TC	460 (21.8)	23 (27.4) ^a	15 (18.3) ^b	
CC	52 (2.5)	1 (1.2) ^a	3 (3.7) ^b	0.6193 ^b

^aComparison between SSNHL patients and controls.

^bComparison between MD patients and controls.

SSNHL, sudden sensorineural hearing loss; MD, Ménière's disease; GPX1, glutathione peroxidase 1; PON1, paraoxonase 1; SOD2, superoxide dismutase 2.

Statistical analysis

Statistical analyses were conducted using Statistical Analysis System version 9.1.3 (SAS Institute, Cary, NC) with significance accepted at $p < 0.05$. Univariate analyses of categorical variables were performed using the chi-squared test or the Fisher's exact test. The Student's t test was used to assess differences in continuous variables between two groups. For multivariate analysis, multiple logistic regression was performed to obtain the odds ratios (ORs) for the risk of SSNHL in individuals with polymorphisms. Genotypes were coded as major-allele homozygous, heterozygous, and minor-allele homozygous. The major alleles of five SNPs were determined in analyses, as follows: C in rs1050450 of GPX1; A in rs1695 of GSTP1; G in rs662 of PON1; T in rs3202100 of PON1; C in rs6954345 of PON2; and T in rs1799725 of SOD2. The additive genetic model, which is the prevailing analytical model in genetic epidemiology, assumes that there is a linear gradient in risk with an increasing number of mutated alleles. The ORs for SSNHL and Ménière's disease were determined using the additive genetic model of six polymorphisms. The minor allele was compared between cases and controls by assigning scores of 0, 1, and 2 to major-allele homozygotes, heterozygotes, and minor-allele homozygotes, respectively. In this analysis, we used two models with and without moderator variables. Model 1 was not adjusted for moderator variables, whereas age and gender were used as moderator variables in model 2.

Audiological testing

Hearing levels were evaluated in patients with SSNHL and Ménière's disease using an audiometer (Model AA-79S; Rion, Tokyo, Japan) in a sound-insulated chamber. The average hearing level was expressed as the average score at five frequencies (250, 500, 1000, 2000, and 4000 Hz). If the patient did not respond to the maximum sound level produced by the audiometer, we defined the threshold as 5 dB above the maximum level. For patients with SSNHL, the initial

TABLE 3. MULTIVARIABLE LOGISTIC REGRESSION ANALYSIS OF OXIDATIVE STRESS-RELATED GENE POLYMORPHISMS AS RISK FACTORS FOR SUDDEN SENSORINEURAL HEARING LOSS AND MÉNIÈRE'S DISEASE

Mode of inheritance	Crude: model 1			Adjusted: model 2		
	Odds ratio	95% CI	p-Value	Odds ratio	95% CI	p-Value
GPX1 (rs1050450)						
SSNHL	1.065	0.603–1.883	0.8272	0.916	0.498–1.683	0.7774
MD	0.752	0.389–1.454	0.3968	0.735	0.379–1.425	0.3618
PON1 (rs662)						
SSNHL	1.201	0.871–1.656	0.2630	1.216	0.879–1.681	0.2383
MD	1.285	0.930–1.777	0.1290	1.272	0.918–1.762	0.1480
PON1 (rs854560)						
SSNHL	0.572	0.268–1.218	0.1473	0.588	0.276–1.252	0.1684
MD	1.108	0.627–1.956	0.7249	1.131	0.640–1.999	0.6708
PON2 (rs7493)						
SSNHL	0.889	0.601–1.314	0.5536	0.915	0.618–1.354	0.6563
MD	0.878	0.590–1.307	0.5224	0.871	0.586–1.296	0.4971
SOD2 (rs4880)						
SSNHL	1.124	0.737–1.715	0.5868	1.105	0.719–1.700	0.6477
MD	0.953	0.606–1.499	0.8357	0.934	0.594–1.467	0.7666

Moderating variables: model 1, no variables; model 2, age and sex.
CI, confidence interval.

audiograms were obtained at the first visit and the final audiograms were taken at least 2 months after the onset of deafness, with the exception of patients who recovered completely within this period. The outcome of SSNHL was evaluated using the criteria of the Ministry of Health and Welfare, Japan (Nakashima *et al.*, 1989). Recovery was ranked as follows: no change (improvement in hearing of less than 10 dB on average); slight improvement (improvement in hearing of 10 dB or more, but less than 30 dB on average); remarkable improvement (improvement in hearing of 30 dB or more on average); and complete recovery (the hearing levels in all five frequencies were 20 dB or less or there was improvement to the same degree of hearing as that found in the contralateral ear). Complete recovery and remarkable improvement were defined as good recovery. Slight improvement and no recovery were defined as poor recovery. To analyze hearing recovery, subjects with SSNHL whose first visit to our hospital was within 1 month after onset were selected.

For patients with Ménière's disease, the worst hearing level at attack periods was evaluated using the average score at five frequencies described above. According to the average hearing level at the five frequencies, we divided these patients into two groups for further analysis: in one group, hearing level exceeded 50 dB, and in the other, hearing level was below 50 dB.

Results

The genotype distributions and allele frequencies of the polymorphisms of *GPX1* (Pro198Leu, rs1050450), *PON1* (Gln192Arg, rs662; and Met55Leu, rs854560), *PON2* (Ser311-

Cys, rs7493), and *SOD2* (Val16Ala, rs4880) in patients with SSNHL and Ménière's disease are described in Table 2. No significant differences in the distribution of genotypes and allele frequency of the polymorphisms described above were observed among SSNHL patients, Ménière's disease patients, and controls.

Table 3 shows the results of the multivariable logistic regression analysis. No significant risk of SSNHL and Ménière's disease were observed for the five polymorphisms in the additive genetic model, regardless of adjustments for age and gender.

Table 4 shows the relationship between recovery of hearing loss and the five oxidative stress-related polymorphisms in SSNHL patients. The T allele of *PON1* (rs854560) was more frequent in SSNHL cases with good recovery compared with those with poor recovery. The allele frequencies of the remaining four polymorphisms were not significantly different between patients with SSNHL exhibiting good or poor recovery. Baseline clinical characteristics, such as age, initial hearing level, period from onset to first visit to our hospital, and vertigo, which can affect the prognosis of hearing recovery, were not significantly different between patients with good recovery and those with poor recovery.

Table 5 shows the relationship between the severity of hearing loss in Ménière's disease and the five oxidative stress-related polymorphisms. The C allele of *SOD2* (rs4880) was more frequent in Ménière's disease cases with a hearing level over 50 dB compared with those with a hearing level below 50 dB. The allele frequencies of the remaining four polymorphisms were not significantly different between patients with Ménière's disease with a hearing level over 50 dB or below 50 dB. The characteristics of age and duration of the disease did not differ between patients with Ménière's disease with a hearing level over 50 dB or below 50 dB.

TABLE 4. HEARING RECOVERY AND ALLELE FREQUENCY OF OXIDATIVE STRESS-RELATED POLYMORPHISMS IN SUDDEN SENSORINEURAL HEARING LOSS

	SSNHL cases with good recovery	SSNHL cases with poor recovery	p Value
Number of cases	22	39	
<i>GPX1</i> (rs1050450); C allele, T allele	41, 3	76, 2	0.248
<i>PON1</i> (rs662); G allele, A allele	27, 17	48, 30	0.985
<i>PON1</i> (rs854560); A allele, T allele	40, 4	78, 0	0.015
<i>PON2</i> (rs7493); C allele, G allele	34, 10	68, 10	0.156
<i>SOD2</i> (rs4880); T allele, C allele	36, 8	66, 12	0.689
Age (years)	57.3±12.1	59.7±15.6	0.533
Average initial hearing level (dB)	74.7±19.6	67.8±25.5	0.273
Period from onset to first visit (days)	4.6±4.6	7.9±8.7	0.110
Number of patients with vertigo	8	15	0.871

Average initial hearing level: average hearing level at five frequencies (250, 500, 1000, 2000, and 4000 Hz); period from onset to first visit: period from onset of SSNHL to the date at which the patients visited our hospital for the first time.

Values for age, average initial hearing level, and period from onset to first visit represent the average±standard deviation.

TABLE 5. HEARING LEVEL AND ALLELE FREQUENCY OF OXIDATIVE STRESS-RELATED POLYMORPHISMS IN MÉNIÈRE'S DISEASE

	MD cases with hearing level below 50 dB	MD cases with hearing level over 50 dB	p Value
Number of cases	47	35	
<i>GPX1</i> (rs1050450); C allele, T allele	89, 5	65, 5	0.878
<i>PON1</i> (rs662); G allele, A allele	62, 32	38, 32	0.130
<i>PON1</i> (rs854560); A allele, T allele	89, 5	62, 8	0.152
<i>PON2</i> (rs7493); C allele, G allele	78, 16	57, 13	0.797
<i>SOD2</i> (rs4880); T allele, C allele	88, 6	55, 15	0.004
Age	51.7±15.2	58.6±16.0	0.051
Average hearing level (dB)	29.5±11.1	69.8±14.6	<0.001
Duration of the disease (years)	4.1±6.2	6.2±8.3	0.194

Average hearing level: average hearing level at five frequencies (250, 500, 1000, 2000, and 4000 Hz).

The values for age, average hearing level, and duration of the disease represent the average±standard deviation.

Discussion

In the present study, we aimed to investigate, for the first time, the association between the risk of SSNHL and Ménière's disease and five oxidative stress-related genetic polymorphisms using a case-control study. No significant differences in the distribution of genotypes and allele frequencies of the polymorphisms described above were observed among SSNHL patients, Ménière's disease patients, and controls. No significant risk of SSNHL and Ménière's disease was observed for the five polymorphisms.

Recently, it was reported that the blood-labyrinth barrier is disrupted in at least one-third of patients with SSNHL, because gadolinium contrast agents injected intravenously appeared in the affected inner ear on magnetic resonance imaging (MRI) (Yoshida *et al.*, 2008). MRI also revealed a weak blood-labyrinth barrier in ears affected by Ménière's disease compared with asymptomatic contralateral ears (Nakashima *et al.*, 2010; Tagaya *et al.*, 2011). As disruption of the blood-labyrinth barrier is associated with increased permeability of blood vessels in the inner ear and endothelium tight junctions, which leads to the disruption of the permeability of blood by ROS (Grammas *et al.*, 2011), ROS may be involved in the pathology of SSNHL and Ménière's disease.

SOD enzymes catalyze the conversion of superoxide radicals to hydrogen peroxide. The SOD2 enzyme protects against damage caused by free radicals (Fortunato *et al.*, 2004). It has been reported that Ala16Ala homozygotes may have higher SOD2 activity than Val16Val homozygotes (Sutton *et al.*, 2003). The SOD2 Ala16Ala genotype is associated with an increased breast cancer risk, a high degree of carotid atherosclerosis, and exudative age-related macular degeneration in Japanese individuals, in whom allele Ala occurs less frequently than it does in Caucasians (Ambrosone *et al.*, 1999; Kimura *et al.*, 2000; Kakko *et al.*, 2003). Hearing loss in Ménière's disease is associated with loss of spiral ganglion neurons and hair cells. In a guinea pig model of endolymphatic hydrops, oxidative stress mediated the loss of spiral ganglion neurons (Labbe *et al.*, 2005). In the present study, Ménière's disease patients with a hearing level over 50 dB exhibited a higher minor-allele frequency compared with patients with a hearing level below 50 dB, suggesting that the SOD2 Val16Ala (rs4880) polymorphism is associated with progression of hearing loss in Ménière's disease.

Paraoxonases exert antioxidant activity and may protect against diseases, such as atherosclerosis, diabetes, Alzheimer dementia, and Parkinson disease (Mackness *et al.*, 2000; Akhmedova *et al.*, 2001; Janka *et al.*, 2002; Fortunato *et al.*, 2003). The PON gene family consists of PON1, PON2, and PON3, which are located on chromosome 7q21-q22 (Primo-Parmo *et al.*, 1996). The PON1 55Leu, PON1 192Arg, and, more recently, PON2 311Cys variants have been implicated in the oxidative damage associated with the pathogenesis of neurodegenerative diseases, such as Alzheimer disease and Parkinson disease (Carminc *et al.*, 2002; Shi *et al.*, 2004). The C allele of the PON2 Ser311Cys polymorphism is associated with a noise-induced hearing loss (Fortunato *et al.*, 2004). A higher frequency of the minor allele of the PON1 Met55Leu polymorphism was observed in SSNHL cases with good recovery compared with those with poor recovery. However, as the frequency of the minor allele of this polymorphism is very small, that is, only four, further investigations using

larger case samples are required to confirm the association between this polymorphism and hearing recovery in SSNHL.

Via its action in regulating the cellular levels of peroxide, GPX plays a critical role in minimizing the production of hydroxyl radical. Although there may be species differences, GPX appears to be expressed at comparatively high levels in the cochlea. GPX1 knockout mice exhibited auditory brainstem response thresholds that were up to 16 dB higher before noise exposure, and up to 15 dB greater noise-induced hearing loss, depending on test frequency, compared with controls (Ohlemiller *et al.*, 2000). However, no association was found between the GPX Pro198Leu polymorphism and the risk of SSNHL and Ménière's disease.

Generally, statistical power depends on the sample size, the significance criterion, and the effect size (Cohen, 1992). The power level in the comparison of genotype distribution in each polymorphism in the present study varied between 0.019 and 1.0 and was below 0.8, with the exception of the cases of PON2 Ser311Cys and SOD2 Val16Ala. The observation that the genotype distribution for each polymorphism between controls and SSNHL or Ménière's disease was similar in this negative study may lower the effect size, leading to a low power level. This is the weakness of our study and these results need to be confirmed in larger series of patients in future studies.

Conclusion

In conclusion, no significant associations were observed between the polymorphisms of GPX1 (Pro198Leu, rs1050450), PON1 (Gln192Arg, rs662; and Met55Leu, rs3202100), PON2 (Ser311Cys, rs6954345), and SOD2 (Val16Ala, rs1799725) and the risk of SSNHL and Ménière's disease in this Japanese case-control study.

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Disclosure Statement

No competing financial interests exist.

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