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Original Article

Association between Uncoupling Protein 2 Gene Ala55val Polymorphism and Sudden Sensorineural Hearing Loss

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OBJECTIVES: The pathology of sudden sensorineural hearing loss, which is known as sudden deafness (SD), remains unknown. The purpose of this study was to investigate the association between mitochondrial uncoupling protein 2 (UCP2) polymorphism and SD risk.

MATERIALS and METHODS: We compared 83 patients suffering from SD and 2048 controls who participated in the Longitudinal Study of Aging at the National Institute for Longevity Sciences. Multiple logistic regression was used to calculate the odds ratios (ORs) for SD with a polymorphism of the UCP2 (rs660339) gene.

RESULTS: Under the additive model of inheritance, UCP2 polymorphisms showed significant association with a SD risk. The OR was 1.468 (95% confidence interval, 1.056–2.040) with an adjustment for any past history, such as diabetes, dyslipidemia, or hypertension, and for age and sex.

CONCLUSION: Our results imply that the UCP2 (rs660339) polymorphism has a significant association with the risk of developing SD.

KEYWORDS: Genetic polymorphisms, sudden deafness, uncoupling protein 2

INTRODUCTION

The pathology underlying sudden sensorineural hearing loss, which is known as sudden deafness (SD), remains unknown. It affects the unilateral ear in most cases and is often accompanied by tinnitus and vertigo [1]. The hearing level can recover within the first 1 or 2 months, and after this period, the hearing level will be fixed. Several underlying pathologies, such as vascular disturbance and viral infection, are taken into consideration [2, 3].

Sudden deafness (SD) is thought to have multiple causes, including genetic and environmental factors. To date, some gene polymorphisms have been identified for the vasculature- or inflammation-related pathogenesis of SD, such as protein kinase C-eta 1425G/A, matrix metalloproteinase-1 1607G/2G, methylenetetrahydrofolate reductase 677C/T, prothrombin 20210G/A, platelet Gly IIIaA1/A2, factor V Leiden 1691G/A, interleukin-1A-889C/T, interleukin-6C 572C/G, complement factor H 402Y/H, and nitric oxide

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synthase 3 894G/T polymorphisms [4-13]. Environmental factors related to lifestyle, including short sleeping times, heavy smoking, alcohol abuse, and fatigue, have been found to be risk factors for SD [14, 15].

Uncoupling proteins (UCPs) are mitochondrial transporters located in the inner mitochondrial membrane and are able to uncouple adenosine triphosphate (ATP) from mitochondrial respiration. Among the five UCPs, UCP2 has a wide expression in almost all mammalian tissues, such as white adipose tissue, kidney tissue, and liver tissue [16]. The UCP2 mRNA transcripts have an abundant expression in the spiral and vestibular ganglia in the inner ear of rats [17, 18]. Sugiura et al. [19] have described that the UCP2 Ala55Val polymorphism (rs660339) was significantly associated with age-related hearing loss. The objective of the present study was to investigate the association between UCP2 polymorphism and SD.

MATERIALS and METHODS

Patients

In total, 83 patients (39 males and 44 females; mean age, 58.0±14.2 years; range, 22–86 years) with SD were enrolled at the Nagoya University Hospital between November 2007 and March 2011, and a genetic analysis was performed on these patients. SD was defined as a type of deafness or hearing loss with an unknown etiology and with a sudden onset complicated by no cranial nerve palsy other than the auditory nerve. We used the criteria established by the Sudden Deafness Research Committee Study Group of the Ministry of Health and Welfare (1973), Japan.

Controls

The controls population were recruited from the Longitudinal Study of Aging at the National Institute for Longevity Sciences (NILS-LSA). Entrants in the NILS-LSA hospital were sampled at random from resident registrations, and they were stratified by age and sex. The area for the NILS-LSA study was located within approximately 30 km of the Nagoya University hospital. The details of the NILS-LSA have been described previously [20]. The participants responded to a series of surveys helpful in collecting population statistics and clinical data, such as comorbidities. Those with a history of SD in the surveys were excluded. In total, 2048 participants (1033 males and 1015 females; mean age, 59.2±10.9 years) were included as controls. These subjects completed the first examination of NILS-LSA between November 1997 and April 2000, and the analyses of UCP2 rs660339 gene were sampled.

Ethics

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

Genotype Analysis

Genomic DNA was extracted from the lymphocytes isolated form peripheral blood using standard protocols, and polymerase chain reaction (PCR) amplification was performed. Genotyping using an allele-specific primer (ASP) method was also performed (Toyobo Gene Analysis, Tsuruga, Japan), as described previously [21]. The data of the primer sequences and PCR conditions are presented in Table 1.

Hearing Evaluation

We evaluated the hearing levels in patients with SD using the audiometer (Model AA-79S; Rion, Tokyo, Japan) in a silent chamber. The average hearing level was expressed as the average score recorded at five frequencies (250, 500, 1000, 2000, and 4000 Hz, respectively). The hearing level results of patients with SD were assessed against the criteria of the Ministry of Health and Welfare, Japan [22]. The recovery rate was divided into four classes: complete recovery (all frequencies on the final audiogram were ≤20 dB or improvement to the same degree of hearing level as in the other side of ear), remarkable improvement (improvement in the hearing level of ≥30 dB on an average), slight improvement (improvement in the hearing level of ≥10 dB but <30 dB on an average), and no change (improvement in the hearing level of <10 dB on an average). A good recovery comprises complete recovery and remarkable improvement. A poor recovery comprises slight improvement and no recovery. Because the possibility of recovery of hearing is extremely low at 1 month after the disease onset, to analyze the hearing recovery, SD patients who first visited our hospital within 1 month of disease onset were selected.

Statistical Analysis

Statistical analyses were conducted using the Statistical Analysis System software, version 9.1.3 (SAS institute, Cary, NC, USA), with significance achieved at a p value of <0.05. Univariate analyses of the categorical variables were performed using the chi-squared test. Student's t test was used to evaluate the differences in the continuous variables between the two groups. Multiple logistic regression was performed to calculate the odds ratios (ORs) for SD risk concerning the UCP2 polymorphism for the multivariate analysis. Genotypes were defined as major allele homozygotes (CC), heterozygotes (CT), and minor allele homozygotes (TT) in UCP polymorphism. The major allele was determined for practical reasons.

We used the additive genetic model for the analyses, and the minor allele frequency was compared between patients with SD and controls by allocating scores of 0, 1, and 2 to major allele homozygotes, heterozygotes, and minor allele homozygotes, respectively. We used

Table 1. PCR condition used in genotyping the gene

ASP method						
Gene	**rs No**	**Labeled primers**	**Sequence (5′→3′)**	**Amplicon (F1/R)/(F2/R) (bp)**	**Annealing temp.(°C)**	**Mg (mM)**
UCP2	rs660339	F1 (FITC)	CCAGTGCGCGCTACAxCC	65/72	65	2.5
		F2 (Texas Red)	CCAGTGCGCGCTACAxTC	55	67.5	4.5
		R (Biotin)	TCAGAATGGTGCCCATCACA			

PCR: polymerase chain reaction; ASP: allele-specific primer; UCP2: uncoupling protein 2; FITC: fluorescein isothiocyanate

Table 2. Characteristics of case and control groups

	SD group	Control group	p
No.	83 (3.9%)	2,048 (96.1%)	
Sex, % male	47.0	50.4	0.5375
Age (years)	58.0±14.2	59.2±10.9	0.3365

SD: sudden deafness

Table 3. Genotype distribution in SD cases and controls

Genotype	n (%)	Controls Total n=2048 n (%)	SD patients Total n=83 (vs controls)	p
UCP2 (rs660339)	CC	549 (26.81)	15 (18.07)	0.1571
	CT	1032 (50.39)	44 (53.01)	
	TT	467 (22.80)	24 (28.92)	

SD: sudden deafness; UCP2: uncoupling protein 2

Table 4. Risk of SD associated with the additive model

	Crude: model 1			Adjusted: model 2		
Mode of inheritance	OR	95% CI	p value	OR	95% CI	p
UCP2(rs660339)	1.352	0.987–1.851	0.0601	1.468	1.056–2.040	0.0222

SD: sudden deafness; UCP2: uncoupling protein 2; OR: odds ratio; CI: confidence interval

Table 5. Allele frequency in SD case with good and poor recovery

		SD cases with good recovery	SD cases with poor recovery	p
Number of cases		22	38	
UCP2 (rs660339)	C allele, T allele	20, 24	33, 43	0.8289
Age (years)		57.2±12.1	59.5±15.8	0.5701
Average hearing level (dB)		74.7±19.6	68.2±25.7	0.3071
Period from onset to first visit (days)		4.6±4.6	7.8±8.8	0.1178

SD: sudden deafness; UCP2: uncoupling protein 2

two models in this analysis, in the presence and absence of moderator variables, respectively; the former is the crude model, while the latter is the adjusted model in which age; sex; and a history of hypertension, diabetes, or dyslipidemia, were taken as moderator variables.

RESULTS

The characteristics of patients with SD and controls are summarized in Table 2. No significant difference in sex or age was found between patients with SD and controls. The genotype distributions are shown in Table 3. No significant difference in the genotype distributions was observed between the patients with SD and controls based on the chi-squared test.

The results of multiple logistic regression are shown in Table 4. A significant difference in SD risk was observed between patients with SD and controls in terms of UCP2 (rs660339) polymorphisms following adjustments for age, sex, and diseases, and the OR for SD risk was 1.468 (95% confidence interval, 1.056-2.040).

Allele frequency was compared between the good recovery group and poor recovery groups among patients with SD. No significant difference was found between the good and poor recovery groups (Table 5).

DISCUSSION

We conducted a case-control retrospective study in which the OR analysis was mainly used to predict disease susceptibility. This study demonstrated that the UCP2 polymorphism (rs 660339) has a significant association with SD risk. UCPs are the members of the anion carrier protein family and are located in the inner mitochondrial membrane. UCP inhibits insulin secretion and may be related to obesity, β cell damage, and diabetes. UCP exerts a protective effect against free radicals, and it is related to thermogenesis [23]. Previous studies have investigated the association between UCP2 gene polymorphisms and the presence of diabetic complications. The polymorphisms of the UCP2 gene have been reported to be significantly associated with a risk of proliferative diabetic retinopathy in Brazilian patients with type 1 and type 2 diabetes mellitus [24]. The mRNA expression of UCP3, UCP4, and especially UCP2 is upregulated after a unilateral labyrinthectomy in the inner ear of rats. Furthermore, the expression of UCP2, 3, and 4 is upregulated after a systemic administration of kanamycin in the mouse inner ear [17, 18]. Sugiura et al. [19] have described that the UCP2 T allele (rs660339) was also associated with presbycusis as in SD. The neuroprotective part against oxidative stress may be associated with the hearing function. Conversely, it is assumed that the UCP2 C allele may have a protective role to prevent sensorineural hearing loss by strengthening the antioxidative function. This was suggested in the present study of SD as well as another study of presbycusis. Manche et al. [25] have also reported that UCP2 (G-866 A)—another common polymorphism of this gene–was associated with presbycusis.

CONCLUSION

The present study demonstrates that UCP2 polymorphisms may be associated with the risk of developing SD, as suggested by the results of our multivariate analysis with moderating variables. Future studies including more cases are warranted.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committees of Nagoya University (370-4) and by the National Center for Geriatrics and Gerontology (nos.14, 52, and 74).

Informed Consent: Written informed consent was obtained from all subjects who participated in this study.

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Conflict of Interest: The authors have no conflict of interest to declare.

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