Heat Shock Protein 70 Gene Polymorphisms in Sudden Sensorineural Hearing Loss

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Key Words

Genetic association • rs2075800 • Sudden deafness • HSP70

Abstract

Background: Heat shock proteins protect cells and tissues against different types of damage. Previous studies have revealed that the serum level of heat shock protein 70 (HSP70) increases in sudden sensorineural hearing loss (SSNHL) patients. We hypothesized that genetic variants of the *HSP70* gene are associated with susceptibility to SSNHL. **Methods:** We conducted a case-control study with 160 SSNHL cases and 178 controls. Three tagging single nucleotide polymorphisms (SNPs) were selected. The genotypes were determined using TaqMan technology. Hardy-Weinberg equilibrium was tested for each SNP, and genetic effects were evaluated according to three inheritance modes. A haplotype analysis was also performed. **Results:** All three SNPs were in Hardy-Weinberg equilibrium. The CT genotype of rs2075800 exhibited an adjusted odds ratio of 0.59 (95% confidence interval 0.37–0.94; $p = 0.027$). The T allele of SNP rs2075800 was associated with SSNHL under the dominant model ($p = 0.019$; odds ratio 0.59). Haplotype analysis of the three SNPs demonstrated that the haplotype TGC (rs2075800/rs1043618/

rs2763979) was statistically significant ($p = 0.0137$). **Conclusions:** These results suggest that *HSP70* gene polymorphisms influence the susceptibility to the development of SSNHL in the Taiwanese population.

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Introduction

Sudden sensorineural hearing loss (SSNHL) is defined as a loss of at least 30 dB in 3 contiguous frequencies over a period of 3 days or less [Wu et al., 2006]. The incidence rates per 100,000 people in the Taiwanese population are 8.85 for men and 7.79 for women [Wu et al., 2006]. The etiology and pathogenesis of SSNHL are not known, but the proposed primary causes include viral infection, vascular disease, and autoimmunity [Wu et al., 2006]. SSNHL is a complex multifactorial disease that includes genetic factors, such as prothrombin G20210A, factor V Leiden G1691A, and methylene-tetrahydrofolate reductase (MTHFR) C677T [Schreiber et al., 2010].

The heat shock proteins are classified by molecular weight and grouped into families of different sizes [Macario and Conway de Macario, 2005]. These proteins

function as intracellular chaperones which control protein folding and unfolding, and translocation under physiological conditions and following physical or chemical stress [Matouschek, 2003]. Extracellular heat shock proteins process and present immunogenic peptides to cytotoxic T cells, activate the innate immune system, and induce proinflammatory cytokine secretion from monocytes [Asea et al., 2000; Srivastava et al., 1994]. Heat shock protein 70 (HSP70) molecules play a key role in the immune response by functioning both as chaperones and inducers of proinflammatory cytokine secretion [Spagnolo et al., 2007].

SSNHL patients exhibit significantly higher levels of serum anti-HSP70 antibodies compared to normal controls [Park et al., 2006]. A previous study revealed a positive association between anti-HSP70 antibodies and positive outcomes in SSNHL [Gross et al., 2008]. Three members of the *HSP70* gene family have been mapped on 6p21.3 in the human leukocyte antigen (HLA) class III region [Milner and Campbell, 1990]. The human *HSP70* gene family consists of HSPA1L (HSP70-Hom), HSPA1A (HSP70-1), and HSPA1B (HSP70-2) [Milner and Campbell, 1990]. The expression of HSPA1A and HSPA1B is heat-inducible, and these two genes encode an identical 641 amino acid protein. HSPA1L is not heat-inducible, and it encodes a protein that is highly related to HSPA1A [Milner and Campbell, 1990].

HSP70 gene polymorphisms may play a role in the pathogenesis of SSNHL. No data have been published on the relationship between *HSP70* genetic polymorphisms and the risk of SSNHL. This study investigated whether *HSP70* gene polymorphisms are risk factors for SSNHL in Taiwanese subjects. We conducted a case-control study to systematically investigate the role of *HSP70* tagging single nucleotide polymorphisms (tSNPs) in the development of SSNHL in the Taiwanese population.

Materials and Methods

Subjects

We recruited 160 patients with the SSNHL and 178 controls at the Kaohsiung Medical University Hospital between October 2010 and November 2011. The diagnostic criteria of SSNHL included sensorineural hearing loss of at least 30 dB in 3 contiguous frequencies using a pure tone audiogram with an onset within 3 days [Wu et al., 2006]. All the patients had no treatment for hearing loss or impairment before taking the hearing test. The exclusion criteria were hearing losses caused by acoustic neuroma, central lesions, Ménière's disease, multiple sclerosis, infectious diseases, drug-induced and noise-induced hearing losses, or hearing losses due to prior ear surgery.

Table 1. Baseline demographics of subjects

	Case (n = 160)	Control (n = 178)	p value
Age, years	51.6 ± 15.0	51.7 ± 8.0	0.917 ^a
Sex, n (%)			
Male	86 (54)	95 (53)	0.944 ^b
Female	74 (46)	83 (47)	

^a Independent t test. ^b χ^2 test.

Healthy volunteers without a history of hearing loss or any ear disorders were enrolled as controls. Information on demographic characteristics was collected. Audiometric tests, including pure tone audiometry, tympanometry, stapedial reflexes, auditory brainstem-evoked responses, and computer tomography or magnetic resonance imaging (to exclude acoustic neuroma) were performed in SSNHL patients. The Institutional Review Board of the Kaohsiung Medical University Hospital approved this study (approval No. KMH-IRB-990243), and informed consent was obtained from all subjects or their guardian if the participant was under 18 years old.

SNP Selection and Genotyping

Genomic DNA was extracted from peripheral blood using a standard method. Three tSNPs were selected from the HapMap Project (International HapMap Consortium) using the following criteria: (1) a minor allele frequency $\geq 30\%$ in the Han Chinese population and (2) a linkage disequilibrium value of (r^2) ≥ 0.8 . The three tSNPs included the nonsynonymous SNP rs2075800 (Glu/Lys) at exon 2 in the HSPA1L (*HSP70-Hom*) gene, rs1043618 at the 5' untranslated region in the HSPA1A (*HSP70-1*) gene and rs2763979 at 936 bp upstream of the 5' end of the HSPA1B (*HSP70-2*) gene.

Genotyping was performed using TaqMan technology (7500 Real Time PCR System, Applied Biosystems, Foster City, Calif., USA), and reactions were performed in 96-well microplates in ABI 9700 thermal cyclers (Applied Biosystems). Fluorescence was measured using an ABI 7500 Real Time PCR System and analyzed with System SDS software version 1.2.3. Each subject was typed for all SNPs.

Statistical Analysis

Continuous variables were analyzed using independent t tests, and the results are presented as means $\pm$ SD. The allele frequency was obtained using direct gene counting. The Hardy-Weinberg equilibrium was examined in controls using the χ^2 test. Multiple logistic regression analysis was performed to adjust for the effects of age and sex while assessing the genetic effects. We examined the effect of the minor allele of each SNP in three genetic models: dominant, additive, and recessive. Linkage disequilibrium was assessed for pairs of SNPs, and haplotype blocks were defined using the default setting in the Haploview software [Barrett et al., 2005]. The Hap-Clustering program [Tzeng et al., 2006] evaluated the haplotype-phenotype association. SPSS 13.0 version for Windows was used for statistical analysis. Two-tailed p values < 0.05 were considered significant.

Table 2. Distribution of *HSP70* genotype and allele frequencies, ORs, and 95% CI of SSNHL susceptibility and control groups

SNP	SSNHL group n (%)	Control group n (%)	Total n (%)	p value ^a	OR (95% CI)	p value	Adjusted OR (95% CI) ^b	p value
<i>rs2075800</i>								
Genotypes								
CC	68 (43)	54 (30)	122 (36)	0.067	1.00 ^c		1.00 ^c	
CT	74 (46)	99 (56)	173 (51)		0.59 (0.37–0.95)	0.029*	0.59 (0.37–0.94)	0.027*
TT	18 (11)	25 (14)	43 (13)		0.57 (0.28–1.16)	0.119	0.57 (0.28–1.15)	0.117
Alleles								
C	210 (66)	207 (58)	417 (62)	0.046*	NA			
T	110 (34)	149 (42)	259 (38)					
<i>rs1043618</i>								
Genotypes								
GG	75 (47)	82 (46)	157 (46)	0.178	1.00 ^c		1.00 ^c	
GC	59 (37)	78 (44)	137 (41)		0.83 (0.52–1.31)	0.419	0.82 (0.52–1.31)	0.408
CC	26 (16)	18 (10)	44 (13)		1.58 (0.80–3.11)	0.186	1.60 (0.81–3.17)	0.178
Alleles								
G	209 (65)	242 (68)	451 (67)	0.463	NA			
C	111 (35)	114 (32)	225 (33)					
<i>rs2763979</i>								
Genotypes								
CC	80 (50)	101 (57)	181 (54)	0.399	1.00 ^c		1.00 ^c	
CT	69 (43)	64 (36)	133 (39)		1.36 (0.87–2.13)	0.179	1.37 (0.87–2.14)	0.175
TT	11 (7)	13 (7)	24 (7)		1.07 (0.45–2.51)	0.880	1.07 (0.46–2.52)	0.878
Alleles								
C	229 (72)	266 (75)	495 (73)	0.355	NA			
T	91 (28)	90 (25)	181 (27)					

* $p < 0.05$. ^a χ^2 test. ^b Adjusted for age and sex (male, female). ^c Reference group.

Results

Study Participants

Table 1 presents the baseline characteristics of the subjects. The mean age of the 160 cases (86 male and 74 female) was 51.6 ± 15.0 years, and the mean age of the 178 control volunteers (95 male and 83 female) was 51.7 ± 8.0 years. The sex distribution was 1.2:1 (male to female). The age and sex characteristics were not significantly different between the two groups.

Single SNP Results

The distribution of *HSP70* genotypes was in Hardy-Weinberg equilibrium among control subjects using $p = 0.05$ as the cutoff point. The distributions of genotypes and allele frequencies of the *HSP70* SNPs are listed in table 2. The SNP rs2075800 allele T was significantly higher in the control group compared to SSNHL patients (42 vs. 34%; $p = 0.046$; table 2). None of the SNPs rs1043618 and rs2763979 exhibited significant p values

(table 2). The crude and adjusted odds ratios (ORs) were computed for the association analyses between *HSP70* genotypes and SSNHL. The multivariate logistic regression model yielded an adjusted OR of 0.59 (95% confidence interval (CI) 0.37–0.94; $p = 0.027$) for the genotype CT versus CC of rs2075800 after adjustment for age and sex (table 2). A significant result was observed under the dominant model, which yielded an adjusted OR of 0.59 (95% CI 0.37–0.92; $p = 0.019$) for T carrier (TT + CT) versus CC of rs2075800 after adjustment for age and sex (table 3). No significant results for SNPs rs1043618 and rs2763979 were observed in any of the three genetic models.

Linkage Disequilibrium and Haplotype Analysis

The three SNPs formed one haplotype block. Haplotype analyses demonstrated a global p value of 0.1298. The distribution of haplotypes between the SSNHL and control groups is listed in table 4. One of these haplotypes, TGC (rs2075800/rs1043618/rs2763979), was signif-

Table 3. Three SNPs and their relationships with SSNHL under three genetic models (dominant, additive, and recessive)

SNP	SSNHL	Genotype (n)			p value		
					dominant	additive	recessive
rs2075800	Case	CC (68)	CT (74)	TT (18)	0.019*	0.066	0.443
HSPA1L	Control	CC (54)	CT (99)	TT (25)			
rs1043618	Case	GG (75)	GC (59)	CC (26)	0.878	0.173	0.092
HSPA1A	Control	GG (82)	GC (78)	CC (18)			
rs2763979	Case	CC (80)	CT (69)	TT (11)	0.212	0.394	0.878
HSPA1B	Control	CC (101)	CT (64)	TT (13)			

Dominant, additive, or recessive model: the rare allele had a dominant, additive, or recessive effect. All p values were adjusted by age and sex. * $p < 0.05$.

Table 4. Results from haplotype analysis which yielded a global p value of 0.1298

Haplotype ^a	Case, %	Control, %	p value ^b
CGC	24	18	0.1194
CGT	11	8	0.2784
CCC	17	15	0.5977
CCT	16	17	0.9574
TGC	32	42	0.0137*

^a The allele order is rs2075800 (*HSPA1L*)/rs1043618 (*HSPA1A*)/rs2763979 (*HSPA1B*) from left to right. ^b Omnibus as the reference group. * $p < 0.05$.

icantly associated with a decreased SSNHL susceptibility compared to all other haplotypes ($p = 0.0137$). The haplotype TGC (rs2075800/rs1043618/rs2763979) exhibited a frequency of 32% in cases and 42% in controls. The other haplotypes did not yield significant results.

Discussion

We systematically investigated the relationship between three SNPs in the *HSP70* gene and SSNHL in the Taiwanese population. Our results revealed that one SNP was associated with SSNHL susceptibility. This significant result stemmed from the *HSPA1L* (*HSP70-Hom*; rs2075800) polymorphism of the *HSP70* gene, which indicates that the rare T allele significantly decreases the risk for SSNHL. The logistic regression model yielded an adjusted OR of 0.59 ($p = 0.027$) for the genotype CT versus CC of rs2075800 after adjustment for age and sex. Subjects with the heterogeneous CT genotype in our

study exhibited a lower incidence of SSNHL than subjects with the homogeneous CC genotype of SNP rs2075800.

The SNP rs2075800 of the *HSPA1L* (*HSP70-Hom*) gene is a nonsynonymous variation. Thymine is substituted for cytosine in the *HSPA1L* polymorphism Glu602Lys (C > T transition), which causes a change from glutamic acid to lysine at position 602 in the C-terminal domain of HSP70-Hom [Jenkins et al., 2000]. The SNP rs2075800 changes a negatively charged amino acid (Glu) to a positively charged amino acid (Lys). This charge shift may influence the 3D structure of HSP70 protein and altered protein folding. Further studies are needed to address the molecular mechanisms by which the HSP70 gene is involved in the development of SSNHL. The associations of the *HSPA1L* (*HSP70-Hom*) SNP rs2075800 with the predisposition to some autoimmune diseases has been documented previously [Jenkins et al., 2000; Bogunia-Kubik et al., 2006; Spagnolo et al., 2007]. The *HSPA1L* (*HSP70-Hom*) rs2075800 T allele is associated with rheumatoid arthritis [Jenkins et al., 2000], and the *HSPA1L* SNP rs2075800 C allele is associated with the susceptibility to sarcoidosis and Lofgren's syndrome [Bogunia-Kubik et al., 2006]. A strong association between *HSPA1L* SNP rs2075800 and the increased risk of uveitis has been reported in patients with sarcoidosis, which suggests that the C allele of this SNP is a risk factor of sarcoidosis-related uveitis [Spagnolo et al., 2007]. Spagnolo et al. [2007] postulated that the altered expression of *HSPA1L* influences the ability of the cell to withstand inflammation or alters the apoptotic threshold within the eye. However, SNP rs2075800 has not been associated with hearing impairment. Our previous study revealed an association between HSP70 polymorphism and noise-induced hearing loss in the SNP rs1043618 of gene *HSPA1A* (*HSP70-1*), but not in rs2075800 of *HSPA1L* (*HSP70-Hom*) or rs2763979 of *HSPA1B* (*HSP70-2*) [Chang et al., 2011].

Furthermore, significant results were observed under dominant models in this study, which yielded an adjusted OR of 0.59 ($p = 0.019$) for T carrier (TT + CT) versus CC of rs2075800 after adjustment for age and sex. This result revealed the tendency for the T-carrier subjects (TT + CT) to exhibit a lower incidence of SSNHL than subjects with the CC genotype. This result indicated that the T carrier is a protective factor for SSNHL.

The multivariate logistic regression model yielded an adjusted OR of 0.57 (95% CI 0.28~1.15; $p = 0.117$) for the genotype TT versus CC of rs2075800 after adjustment for age and sex (table 2). However, no significant differences between the SSNHL and control groups at SNP rs2075800 genotype TT versus CC were observed. SNP rs2075800 genotype TT was higher in the control group compared to SSNHL patients (14 vs. 11%; table 2). The small number of subjects in the SSNHL group may have contributed to this result. An increase in the number of SSNHL subjects may reveal a significant difference.

This study analyzed the association of haplotypes with SSNHL susceptibility. Five common haplotypes were identified in our population, and the haplotype TGC (rs2075800/rs1043618/rs2763979) was significantly associated with resistance to SSNHL. The haplotype analysis results provided stronger statistical support for *HSP70* as a genetic marker. The current data suggest that *HSP70* polymorphisms influence the susceptibility to the development of SSNHL. This investigation is the first report of a potential contribution of the *HSP70* genetic variants to the susceptibility of SSNHL.

Three SNPs that are located in 3 genes of the *HSP70* family in an Asian population were analyzed. Our results support the association of genetic polymorphisms in the *HSP70* genes with an individual's susceptibility to SSNHL. The *HSP70* family may play a key role in the development of SSNHL. The major limitation of this study is the small sample size. Further studies to replicate our results are necessary, and further functional analyses of SNP rs2075800 in the *HSP70* gene in SSNHL are warranted.

Conclusions

The results of this study support the influence of genetic polymorphisms of the *HSP70* gene on the risk of SSNHL in a Taiwanese population. Furthermore, the T allele of the nonsynonymous SNP rs2075800 may be a protective allele for SSNHL. However, the functional role of SNP rs2075800 in SSNHL requires further investigation.

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