Eur Arch Psychiatry Clin Neurosci (2008) 258:239–244 DOI 10.1007/s00406-007-0791-6

ORIGINAL PAPER

Jung Jin Kim · Laura Mandelli · Suzy Lim · Hyun Kook Lim · Oh Joo Kwon · Chi Un Pae · Alessandro Serretti · Vishwajit L. Nimgaonkar · In-Ho Paik · Tae-Youn Jun

Association analysis of heat shock protein 70 gene polymorphisms in schizophrenia

Received: 28 June 2007 / Accepted: 5 November 2007 / Published online: 25 February 2008

Abstract *Objectives* Heat shock proteins (HSPs) are a promising candidate gene in schizophrenia as they are believed to play a protective role in the central nervous system. An alteration in the titers of antibodies to the HSPs in schizophrenia patients has been suggested. Association between the three polymorphisms of HSP70-1 (HSPA1A), HSP70-hom (HSPA1L) and HSP70-2 (HSPA1B) and schizophrenia has been reported. Therefore, this study investigated the association between an enlarged set of SNPs at HSP70 gene and schizophrenia. *Methods* Two hundred and ninety-four patients with schizophrenia and 287 controls were enrolled in the study. Genotypings of 5 SNPs of HSP70 were performed using pyrosequencing method. Haploview 3.2 was used to generate a linkage disequilibrium map and to test for Hardy–Weinberg equilibrium. Single locus and haplotype-based associations were tested. Tests for associations using and multi-marker haplotypes were performed by using a COCAPHASE v2.403. Association of SNP markers and clinical variables were analyzed by analysis of variance. *Results* Significant association was detected at rs2075799 (allele A, $\chi^2 = 8.03$, $df = 1$, $P = 0.0046$), but not at rs2227956 ($P = 0.28$), rs1043618 ($P = 0.88$), rs562047 ($P = 0.47$) or rs539689 ($P = 0.32$). In fact, the rs2075799*G/A genotype was more represented in patients with schizophrenia than in controls ($\chi^2 = 8.23$, $df = 1$, $P = 0.0041$). Haplotype based associations were also detected (global P value 0.000003); the T-A-C-C-G haplotype was more prevalent among the patients (odds ratio, OR 5.95). Sliding windows analysis revealed a major contribution from rs2227956 and rs2075799 (global-P value 0.0075), with T-A haplotype significantly associated with schizophrenia. There was no evidence of an association between the clinical variables and schizophrenia across the genotypes. *Conclusion* Our results raise the possibility that HSP70 gene (i.e., haplotypes of rs2075799) might be implicated in the development of schizophrenia, although limited by rare haplotypic association with the disease. Hence further studies from different ethnics should be performed to confirm these results.

Key words schizophrenia · heat shock protein 70 · polymorphism · association · haplotype

J.J. Kim · H.K. Lim · C.U. Pae (✉) · I.-H. Paik · T.-Y. Jun
Department of Psychiatry
The Catholic University of Korea College of Medicine
Kangnam St Mary's Hospital
505 Banpo-dong, Seocho-gu
Seoul 137-701, Republic of Korea
Tel.: +82-2-590/2780
Fax: +82-2-536/8744
E-Mail: pae@catholic.ac.kr

L. Mandelli · A. Serretti
Institute of Psychiatry
University of Bologna
Bologna, Italy

S. Lim
University of Pittsburgh, School of Medicine
Pittsburgh, PA, USA

V.L. Nimgaonkar
Department of Psychiatry
University of Pittsburgh School of Medicine
Western Psychiatric Institute and Clinic
3811 O'Hara Street
Pittsburgh, PA, 15213 USA

O.J. Kwon
Department of Biochemistry
The Catholic University of Korea College of Medicine
Seoul, Republic of Korea

Introduction

Genes encoding heat shock proteins (HSPs) might be promising candidates for schizophrenia as they have been considered having a protective role in the central nervous system, in particular being molecular chap-

erones responsible for the repair, maintenance, and removal of cellular proteins damaged form various stress condition [16, 19, 28].

It is also worth noting that neurodevelopmental abnormalities have been consistently suggested in patients with schizophrenia [2, 10, 15, 27]. In this context HSPs are known to have a pivotal role in normal growth and differentiation of a mammalian cell and they are observed in a stage-dependent manner during normal development of neuron [26, 29]. It might be postulated that stress-inducing agents such as HSPs acting upon a compromised cellular system resulting from abnormal plasma membrane lipids could affect the neuronal abnormalities observed in schizophrenia [14].

HSP is associated with immune responses and thereby it helps the endogenous antigen process, which guide antigenic peptides to immunorecognition [25]. In particular, HSP70, a multigene family encoding several 70 kDa proteins, were found to be implicated in both pathophysiology and treatment of schizophrenia (i.e., higher levels of anti-HSP70 antibodies patients with schizophrenia) [12, 24].

In addition, schizophrenia shares several features such as stress-related exacerbation of symptoms with autoimmune diseases [13, 14]. This increased immunoreactivity to HSP70 supports the possibility of an autoimmune mechanism in schizophrenia, where antibodies to HSP70 inhibit the neuroprotective action of HSP70 [12, 24]. After 6 weeks of anytipychotic treatment, the percentage of patients with schizophrenia with high levels of anti-HSP70 antibody was decreased [12]. Similarly, antipsychotics such as abaperidone and clozapine were found to reduce basal HSP70 mRNA expression in rat prefrontal cortex [7].

Taken together, schizophrenia might be associated with the defective production of HSPs caused by environmental stresses in the neuronal growth and development and immunological process [3].

While there are numerous HSP70 loci in the human genome, this study focused on HSP70 genes located in the class III region of the major histocompatibility complex (MHC) on 6p21.3, a region that has been implicated in the susceptibility to schizophrenia [8, 23, 30]. This location contains three homologs of the HSP70 gene: HSPA1A, HSPA1B, HSPA1L [17, 22].

Three polymorphisms of HSPA1A, HSPA1B, and HSPA1L in the MHC class III region [18, 22] were genotyped in-patient with schizophrenia by a recent study [20]. In this study, we analyzed five SNPS of HSP70 genes to extend and confirm the previous findings of associations between schizophrenia and SNPs HSP 70 in larger samples.

Methods

Subjects

The patient group consisted of 294 inpatients with schizophrenia. One hundred and fifty-seven were male and 137 were female, with mean age were 34.8 ± 12.4 years. Mean age of onset was 23.3 ± 6.4 years and mean duration of illness was 13.7 ± 13.4. Some subjects participated in our previous study (see [20]). The diagnosis was based on the consensus between two psychiatrists (H.K.L.; C.U.L.), according to the DSM-IV criteria [1]. Subjects with neurological illnesses or autoimmune diseases were excluded.

Two hundred and eighty-eight voluntary controls were recruited from the personnel and medical students of the Kangnam St Mary's Hospital (male 182; female106, mean age 49.6 ± 10.1 years). A semi-structured interview was given to determine whether the control subjects had current psychiatric problem, or had history of psychiatric or neurological illness, prior to sampling.

All the subjects were given information about the study, and written informed consents were obtained. The Ethics Committee of the Kangnam St Mary's Hospital, The Catholic University of Korea, approved this study.

SNP selection

SNP information was retrieved from public database (National Center for Biotechnology Information, dbSNP, http://www.ncbi.nlm.nih.gov/SNP/). A total of five SNPS (rs2227956 C/T, rs2075799 A/G, rs1043618 C/G, rs562047 C/G, rs539689 C/G) were selected according to chromosomal location and heterozygosity. Characteristics and locations of the 5 SNPs are shown in Table 1 and Fig. 1, respectively.

PCR

DNA was extracted from whole blood of the subjects using AccuPrep Genomic DNA Extraction Kit (Bioneer, Korea) and amplified using PCR primers (Bioneer, Korea) designed by using the Pyrosequencing™ Assay Design Software (Biotage AB, Sweden) in PTC 200 thermal cycler (Bio-Rad Laboratories, USA). Information of PCR primers and sequencing primers of five SNPS are listed in Table 2. One primer per primer set was biotinylated.

Pyrosequencing

Biotinylated PCR products were immoblized onto streptavidin-coated beads (37 μl of binding buffer, 3 μl of streptavidin sepharose beads, Amersham, Sweden) and incubating at room

Table 1 Characteristics of five SNPs of heat shock protein (HSP) 70 gene

Gene name	Gene size	Chromosomal position of gene	SNP name	Chromosomal position of SNP	Alleles	Heterozygosity
HSPA1L	5411pb	31885375–31890786	rs2227956	31886251	C/T	0.238
			rs2075799	31886508	A/G	0.24
HSPA1A	2382bp	31891316–31893698	rs1043618	31891486	C/G	0.497
			rs562047	31891842	C/G	0.074
HSPA1B	2507bp	31903503–3190610	rs539689	31905566	C/G	0.497

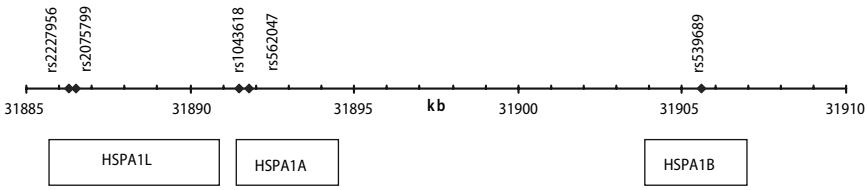

Fig. 1 Map of five SNPs of heat shock protein (HSP) 70 genes

temperature for 10 min. The beads were transferred to a filter plate and liquid was removed by vaccum filtration (Biotage AB, Sweden). DNA was purified by washing in ethanol for 5 s, denatured in 0.2 M NaOH for 5 s, and washing with washing buffer (10 mM Tris-acetate) for 5 s. The immobilized template was washed, then transferred to a PSQ 96 Plate and annealed with the sequencing primer using 0.4 μl of sequencing primer and 100 μl of annealing buffer (20 mM Tris-acetate, 2 mM Mg-Acetate) and incubating at 90°C for 2 min. Substrates, enzymes, and dNTPs from the SNP Reagent Kits (Biotage AB, Sweden) were added to a cartridge according to amounts specified by Pyrosequencing Assay Design Software (Biotage AB, Sweden). The five SNPs were genotyped using PSQ 96MA Pyrosequencer (Biotage AB, Sweden) and SNP Software (Biotage AB, Sweden). The genotypes were determined by the two independent researchers (C.U.P.; S.J.L.) who were blind to the patients' information.

Statistical analysis

Tests for single marker and multi-marker haplotypes were performed using "R" (http://www.R-project.org), package "haplo.score". Permutation (50,000 permutations) was also used to estimate the global significance of the results for haplotype analyses to confirm the expectation-maximization values. Single locus allele tests were also performed. Haploview 3.2 was used to generate a linkage disequilibrium (LD) map and to test for Hardy–Weinberg equilibrium. We calculated the power of our sample with a conservative alpha level of 0.01. For single marker analyses (minor allele frequency 0.25) in our sample we had a power of 0.80 to detect a small effect size of $w = 0.14$, corresponding to a difference of approximately 14% between two genotypes (Odds Ratio = 1.77). For haplotype analyses, considering a frequency of the disease allele of 0.4, disease prevalence of 0.01, phenocopy rate of 0.1, penetrance of 0.8, a co-dominant transmission model, strong LD ($D' = 0.8$) between disease and marker; we had a sufficient power (0.80) to detect a genotypic relative risk of 1.78 (Aa) and 3.07 (AA) [21].

Results

In the controls, we observed a marginal deviation from Hardy–Weinberg Equilibrium (HWE) regarding rs2227956 ($P = 0.03$), while other polymorphisms were all in HWE [rs2075799 ($P = 1.0$), rs1043618 ($P = 1.0$), rs562047 ($P = 0.8$), rs539689 ($P = 0.5$)]. In the schizophrenia sample, rs2227956 showed a marginal deviation ($P = 0.06$) while other SNPs were in HWE [rs2075799 ($P = 1.0$); rs1043618 ($P = 0.15$); rs562047 ($P = 0.93$); rs539689 ($P = 0.74$)].

In the whole sample all markers were in moderate-strong linkage disequilibrium (Fig. 2), while in controls, moderate-strong linkage disequilibrium (LD) was observed between rs562047, rs539689 and rs1043618, rs2227956. No LD was observed between rs2227956 and rs2075799, rs562047, rs539689 and rs2075799. Rs2227956 and rs2075799, rs2075799 and rs1043618, rs1043618 and rs562047 showed strong LD, but not significant (LOD scores, respectively of 1.84, 0.23, 1.95) (Fig. 3). A slightly different pattern was observed in the schizophrenia sample: rs2227956, rs2075799 were in moderate linkage, rs2075799 and rs562047 were in strong linkage. Rs1043618 and rs539689 were in full linkage (Fig. 4).

Single marker analysis

Schizophrenia was not associated with rs2227956 ($P = 0.28$), rs1043618 ($P = 0.88$), rs562047 ($P = 0.47$)

Table 2 Primer sequences and PCR conditions for pyrosequencing of five SNPs of heat shock protein 70 gene

SNP name	Primer sequences	Contents of PCR reaction	PCR conditions
rs2227956 (C/T)	Forward: 5′-AGGGGAGTTCCCTTCAGATCGA-3′ Reverse: 5′-Biotin-AACCATGCGCTCAATCTCCTC-3′ Sequencing primer: 5′-TTCTCAATGTCACAGCCA-3′	DyeMix DNA polymerase (Biostream, Suwon, Korea) 10 μl, forward and reverse primers 0.5 μl each, water 8.5 μl, DNA template 0.5 μl	94°C 5 min, 94°C 30 s, 67°C 30 s, 72°C 30 s, 72°C 7 min (40 cycles)
rs2075799 (A/G)	Forward: 5′-CTGATGGGGGACAAGTCTGAGA-3′ Reverse: 5′-Biotin-TGGGGATGGTGGAGTTGC-3′ Sequencing primer: 5′-GTCCCTGGGGCTGGAG-3′	Dye mix 10 μl, forward and reverse primers 0.25 μl each, water 9.0 μl, DNA template 0.5 μl	94°C 5 min, 94°C 30 s, 65°C 30 s, 72°C 30 s, 72°C 7 min (40 cycles)
rs1043618 (C/G)	Forward: 5′-TCTTCTCGCGGATCCAGTGTT-3′ Reverse: 5′-Biotin-TGGTCGTTGGCGATGATCT-3′ Sequencing primer: 5′-AGCCCCCAATCTCAG-3′	Dye mix 10 μl, forward and reverse primers 0.5 μl each, water 8.5 μl, DNA template 0.5 μl	94°C 5 min, 94°C 30 s, 67°C 30 s, 72°C 30 s, 72°C 7 min (40 cycles)
rs562047 (C/G)	Forward: 5′-AGATCTCCTCGGGGTAGAATGC-3′ Reverse: 5′-Biotin-GCTGATCGGCCGCAAGTT-3′ Sequencing primer: 5′-CGGGGTAGAATGCCTTG-3′	Dye mix 10 μl, forward and reverse primers 0.5 μl each, water 8.5 μl, DNA template 0.5 μl	94°C 5 min, 94°C 30 s, 67°C 30 s, 72°C 30 s, 72°C 7 min (40 cycles)
rs539689 (C/G)	Forward: 5′-AACAAAGGCCCCTAATCCAC-3′ Reverse: 5′-Biotin-TCATCAGCGGACTGTACCAG-3′ Sequencing primer: 5′-CCTGAGCCCCGAAGCC-3′	Dye mix 10 μl, forward and reverse primers 0.25 μl each, water 9.0 μl, DNA template 0.5 μl	94°C 5 min, 94°C 30 s, 65°C 30 s, 72°C 30 s, 72°C 7 min (40 cycles)

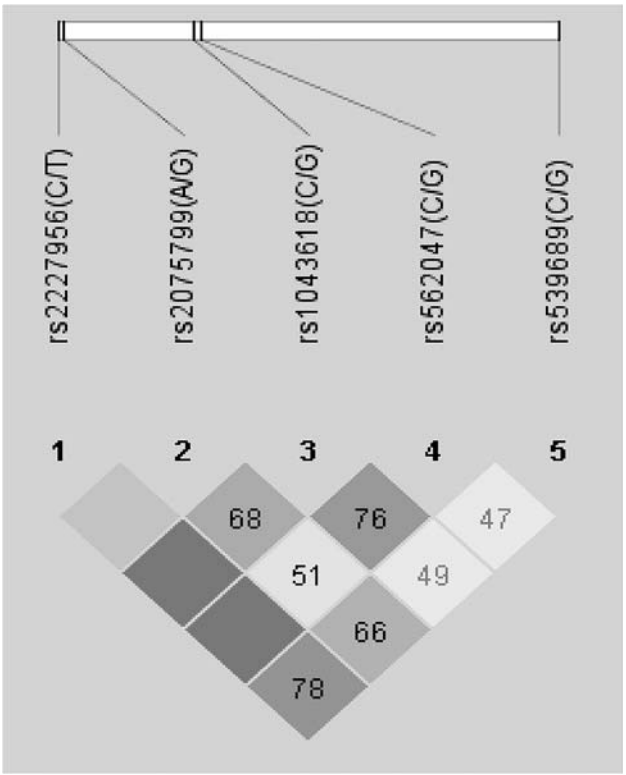

Fig. 2 Linkage disequilibrium of five SNPs of heat shock protein 70 gene on chromosome 6p 21.3 in the whole sample (schizophrenic patients and controls together)

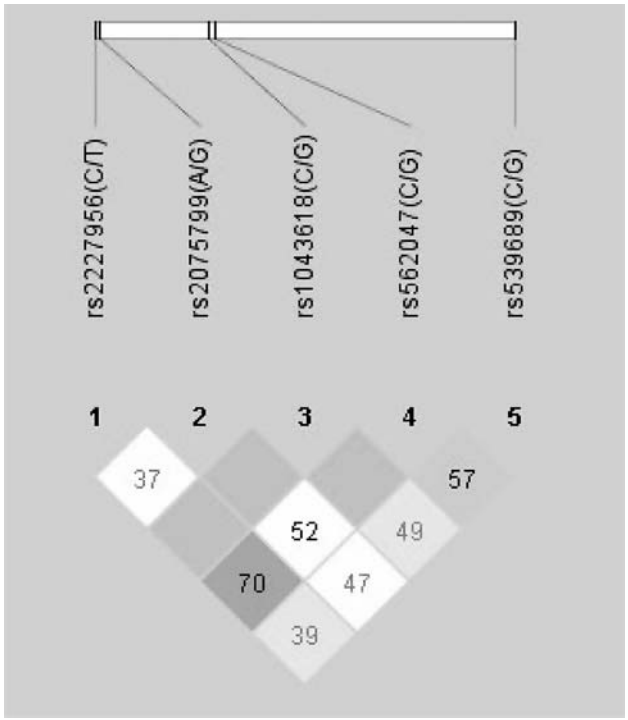

Fig. 3 Linkage disequilibrium of five SNPs of heat shock protein 70 gene on chromosome 6p 21.3 in control subjects

and rs539689 ($P = 0.32$), but strongly with the rare rs2075799*A allele ($\chi^2 = 8.03$, $df = 1$, $P = 0.0046$). None of the patients and controls carried the rs2075799*A/A genotype, being the *A allele very rare; nevertheless, the rs2075799*G/A genotype was more represented in patients with schizophrenia than in controls ($\chi^2 = 8.23$, $df = 1$, $P = 0.0041$).

Haplotype analysis

Haplotypes were significantly associated with schizophrenia (global-stat. = 48.11, $df = 12$, $P = 0.000003$); the rare T-A-C-C-G haplotype showed the higher odd ratio (OR) for schizophrenia (Table 3). At the opposite, some haplotypes showed protective effects, being more frequent among controls than in patients: this is the case, in particular, of the T-G-G-C-G and the C-G-G-C-C haplotypes. Given that rs2227956 was not in Hardy–Weinberg equilibrium in controls, we also performed a four-markers analysis excluding rs2227956. The model was still significant (global stat. = 25.59, $df = 9$, $P = 0.0016$), with a significant effect exerted by the A-C-C-G haplotype ($P = 0.012$) and a significant protective effect exerted by the G-G-G-C haplotype ($P = 0.007$).

Sliding windows analysis revealed a major contribution from rs2227956 and rs2075799 (global-stat. = 9.79, $df = 2$, $P = 0.0075$), with T-A haplotype, included in the T-A-C-C-G risk combination, significantly associated with schizophrenia (Table 4). The more common T-G haplotype, included in the T-G-G-

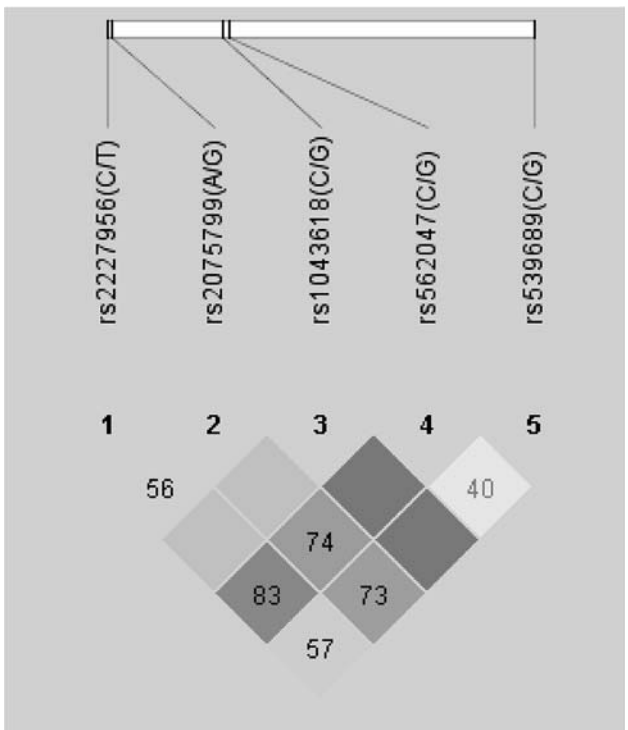

Fig. 4 Linkage disequilibrium of 5 SNPs of heat shock protein 70 gene on chromosome 6p 21.3 in patients with schizophrenia

Table 3 Haplotype analysis of five SNPs (rs2227956 C/T, rs2075799 A/G, rs1043618 C/G, rs562047 C/G, rs539689 C/G) of heat shock protein 70 gene

Haplotypes	Frequency	Controls	Cases	Global statistics	*P* value	Odd ratio
T-G-G-C-C	0.533	0.512	0.556	0.96	0.34	1.00
T-G-C-C-G	0.095	0.116	0.076	−1.97	0.049	0.57
T-G-C-G-G	0.081	0.091	0.072	−1.13	0.26	0.76
C-G-G-C-G	0.069	0.048	0.087	2.29	0.022	1.78
T-G-C-C-C	0.068	0.052	0.084	1.83	0.067	1.43
T-G-G-C-G	0.056	0.079	0.034	−2.90	0.004	0.42
T-G-C-G-C	0.035	0.032	0.036	0.34	0.73	1.29
T-G-G-G-C	0.016	0.031	0.002	−2.43	0.015	0.14
C-G-G-C-C	0.012	0.025	0	−2.68	0.007	<0.01
T-A-C-G-G	0.009	0.005	0.013	1.71	0.087	2.49
T-G-G-G-G	0.008	0.002	0.012	0.69	0.49	4.11
T-A-C-C-G	0.008	0.003	0.013	2.01	0.044	5.95

Table 4 Sliding windows analysis of two SNPs (rs2227956 C/T, rs2075799 A/G) of heat shock protein 70 gene

Haplotypes	Frequency	Controls	Cases	Global statistics	*P* value	Odd ratio
T-G	0.893	0.915	0.872	−2.48	0.013	1.00
C-G	0.082	0.073	0.090	1.11	0.27	1.37
T-A	0.025	0.012	0.037	2.80	0.005	3.42

C-G protective combination, showed instead a small protective effect.

Discussion

This study was aimed to analyze five SNPS of HSP70 genes in order to find out the association of schizophrenia with HSP70 gene. Rs2075799*G/A genotype was more represented in patients with schizophrenia than in controls and T-A haplotype of rs2227956 and rs2075799 was significantly associated with schizophrenia.

The results of this study confirmed the previous finding of our group of associations between schizophrenia and polymorphisms of HSP 70 genes [20]. In this study, the samples were larger than those of previous study and included the samples of previous study, though SNPs investigated in each study was not concordant. In this study, rs2227956 C/T and rs2075799 A/G in HSP1L, rs1043618 C/G and rs562047 C/G in HSP1A, and rs539689 C/G in HSP1B were selected. On the other hand, rs2227956 in HSP1L, rs562047 in HSP1A, and rs1061581 in HSP1B, were selected in previous study. Unlike previous study [20], rs2075799 C/T in HSP 1 L showed strong association and a power of distribution to schizophrenia and rs2227956 and rs2075799 had a major contribution in haplotype analysis. In previous study, rs2227956 in HSP1L was not associated with schizophrenia. Because rs2227956 and rs2075799 are in moderate linkage in patients with schizophrenia, the discrepancy between two results should be evaluated in future study. However, instead of RFLP method of previous study, more accurate genotyping method (Pyrosequencing) was used. On the other hand, the T-A combination that we found conferring a higher risk for Schizophrenia was characterized by a low frequency in the overall sample, as compared with the other haplotypes. Thus, considering larger sample of this study, it is possible that the results of this study can be more reliable.

Also, although Pae et al. [20] reported that rs1061581 in HSP 1B has been associated with schizophrenia, rs539689 C/G in HSP1B was not associated with schizophrenia in this study. Considering there is no linkage disequibrium between rs1061581 and rs539689 (LD, $D' = 0.1944$; $r = 0.0232 \times 2 = 1.3428$; $P = 0.2456$), it cannot be neglected that rs1061581 is associated with schizophrenia. Therefore, it is needed to confirm the findings in different population.

The HSP70 genes on chromosome 6p21.3 are located adjacent to the tumor necrosis factor (TNF) genes in the MHC class III region [17, 22]. Recent studies reported the positive association of TNF-α and -β gene polymorphisms with schizophrenia [4, 11]. Because the TNF- gene is located at the 250-kb telomeric of hsp70 genes, it should be considered that the possibility that the association found between schizophrenia and HSP70 genetic polymorphism could be due to linkage disequilibrium with a neighboring TNF allele. Further research of HSP70 in conjunction with TNF genes in schizophrenia is highly suggested. In the context of possible role of HSP 70 genes in immunogenetics, interaction studies with other promising immune system-related candidate genes in schizophrenia [9].

HSP70-1 and HSP70-2 genes code for identical, heat-inducible proteins, and HSP70-hom gene encodes a protein that is highly related to HSP70-1, but which is not heat-inducible [18]. Hsp70-Hom is capable of binding peptides, with substrate specificity similar to other mammalian Hsp70s [6] and exhibits low but constitutive RNA expression [17]. However, exact function and substrate specificity of HSP70 genes has not been identified yet. If the correlation between HSP 70 expression and the genotypes of HSP 70 genes can be discovered more accurately, the meaning of the association between schizophrenia and HSP 70 genes will be more valuable.

In this study, five SNPs of HSP70 genes were selected. The distance between those SNPs were not evenly distributed and not enough to cover the whole genetic variation. Therefore, it cannot be confirmed which SNPs have a critical role in the pathogenesis of schizophrenia by the result of this study. The main drawback is the small sample size, which was less than the size needed in most case-control association studies. However, our sample had a statistical power (0.80) to detect an OR as high as 1.77 with a conservative *P* value of 0.01. Reports of positive associations that are not subsequently replicated raise the issue of

false positive findings in terms of the sample size or the LD. More SNPs on HSP70 genes in larger sample are needed. Finally, ethnic origin may lead to stratification bias, however, Korean population is considered genetically homogenous [5] and no patients from other regions were included in the study.

According to this study expanding the previous one, the role of HSP70 gene in schizophrenia becomes more valuable. However, it is needed to be confirmed and validated which polymorphisms of HSP70 are associated with schizophrenia and the functions of HSP70 in schizophrenia should be speculated.

Acknowledgements This study was supported by a Grant (KRF-2004-003-E00145) from Korea Research Foundation. The authors would like to express a sincere gratitude to all the patients participated in the study.

References

1. American Psychiatric Association (1994) Diagnostic and Statistical Manual of Mental Disorders (DSM-IV), 4th edn. Washington
2. Andreasen N, Nasrallah HA, Dunn V, Olson SC, Grove WM, Ehrhardt JC, Coffman JA, Crossett JH (1986) Structural abnormalities in the frontal system in schizophrenia. A magnetic resonance imaging study. Arch Gen Psychiatry 43:136–144
3. Bates PR, Hawkins A, Mahadik SP, McGrath JJ (1996) Heat stress lipids and schizophrenia. Prostaglandins Leukot Essent Fatty Acids 55:101–107
4. Boin F, Zanardini R, Pioli R, Altamura CA, Maes M, Gennarelli M (2001) Association between -G308A tumor necrosis factor alpha gene polymorphism and schizophrenia. Mol Psychiatry 6:79–82
5. Cavalli Sforza L (1994) The history and geography of human genes. Princeton University Press, Princeton
6. Fourie AM, Peterson PA, Yang Y (2001) Characterization and regulation of the major histocompatibility complex-encoded proteins Hsp70-Hom and Hsp70-1/2. Cell Stress Chaperones 6:282–295
7. Garcia-Osta A, Frechilla D, Del Rio J (2003) Reduced basal and phencyclidine-induced expression of heat shock protein-70 in rat prefrontal cortex by the atypical antipsychotic abaperidone. Prog Neuropsychopharmacol Biol Psychiatry 27:31–36
8. Goate AM, Cooper DN, Hall C, Leung TK, Solomon E, Lim L (1987) Localization of a human heat-shock HSP 70 gene sequence to chromosome 6 and detection of two other loci by somatic-cell hybrid and restriction fragment length polymorphism analysis. Hum Genet 75:123–128
9. Hanninen K, Katila H, Saarela M, Rontu R, Mattila KM, Fan M, Hurme M, Lehtimaki T (2007) Interleukin-1 beta gene polymorphism and its interactions with neuregulin-1 gene polymorphism are associated with schizophrenia. Eur Arch Psychiatry Clin Neurosci (in press)
10. Huttunen MO, Machon RA, Mednick SA (1994) Prenatal factors in the pathogenesis of schizophrenia. Br J Psychiatry Suppl 15–19
11. Jun TY, Pae CU, Chae JH, Bahk WM, Kim KS, Han H, Serretti A (2003) TNFB polymorphism may be associated with schizophrenia in the Korean population. Schizophr Res 61:39–45
12. Kim JJ, Lee SJ, Toh KY, Lee CU, Lee C, Paik IH (2001) Identification of antibodies to heat shock proteins 90 and 70 kDa in patients with schizophrenia. Schizophr Res 52:127–135
13. Knight JG (1984) Is schizophrenia an autoimmune disease? A review. Methods Find Exp Clin Pharmacol 6:395–403
14. Knight JG, Menkes DB, Highton J, Adams DD (2007) Rationale for a trial of immunosuppressive therapy in acute schizophrenia. Mol Psychiatry
15. Lewis SW, Murray RM (1987) Obstetric complications, neurodevelopmental deviance, and risk of schizophrenia. J Psychiatr Res 21:413–421
16. Lindquist S (1992) Heat-shock proteins and stress tolerance in microorganisms. Curr Opin Genet Dev 2:748–755
17. Milner CM, Campbell RD (1990) Structure and expression of the three MHC-linked HSP70 genes. Immunogenetics 32:242–251
18. Milner CM, Campbell RD (1992) Polymorphic analysis of the three MHC-linked HSP70 genes. Immunogenetics 36:357–362
19. Nagao RT, Kimpel JA, Key JL (1990) Molecular and cellular biology of the heat-shock response. Adv Genet 28:235–274
20. Pae CU, Kim TS, Kwon OJ, Artioli P, Serretti A, Lee CU, Lee SJ, Lee C, Paik IH, Kim JJ (2005) Polymorphisms of heat shock protein 70 gene (HSPA1A, HSPA1B and HSPA1L) and schizophrenia. Neurosci Res 53:8–13
21. Purcell S, Cherny SS, Sham PC (2003) Genetic power calculator: design of linkage and association genetic mapping studies of complex traits. Bioinformatics 19:149–150
22. Sargent CA, Dunham I, Trowsdale J, Campbell RD (1989) Human major histocompatibility complex contains genes for the major heat shock protein HSP70. Proc Natl Acad Sci USA 86:1968–1972
23. Schwab SG, Hallmayer J, Freimann J, Lerer B, Albus M, Borrmann-Hassenbach M, Segman RH, Trixler M, Rietschel M, Maier W, Wildenauer DB (2002) Investigation of linkage and association/linkage disequilibrium of HLA A-, DQA1-, DQB1-, and DRB1-alleles in 69 sib-pair- and 89 trio-families with schizophrenia. Am J Med Genet 114:315–320
24. Schwarz MJ, Riedel M, Gruber R, Ackenheil M, Muller N (1999) Antibodies to heat shock proteins in schizophrenic patients: implications for the mechanism of the disease. Am J Psychiatry 156:1103–1104
25. Suto R, Srivastava PK (1995) A mechanism for the specific immunogenicity of heat shock protein-chaperoned peptides. Science 269:1585–1588
26. Walsh D, Li K, Crowther C, Marsh D, Edwards M (1991) Thermotolerance and heat shock response during early development of the mammalian embryo. Results Probl Cell Differ 17:58–70
27. Weinberger DR (1987) Implications of normal brain development for the pathogenesis of schizophrenia. Arch Gen Psychiatry 44:660–669
28. Welch WJ (1991) The role of heat-shock proteins as molecular chaperones. Curr Opin Cell Biol 3:1033–1038
29. Wolgemuth DJ, Gruppi CM (1991) Heat shock gene expression during mammalian gametogenesis and early embryogenesis. Results Probl Cell Differ 17:138–152
30. Wright P, Nimgaonkar VL, Donaldson PT, Murray RM (2001) Schizophrenia and HLA: a review. Schizophr Res 47:1–12