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ORIGINAL PAPER

Heat shock protein 70 gene polymorphisms are associated with paranoid schizophrenia in the Polish population

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Abstract HSP70 genes have been considered as promising schizophrenia candidate genes based on their protective role in the central nervous system under stress conditions. In this study, we analyzed the potential implication of *HSPA1A* +190G/C, *HSPA1B* +1267A/G, and *HSPA1L* +2437T/C polymorphisms in the susceptibility to paranoid schizophrenia in a homogenous Caucasian Polish population. In addition, we investigated the association of the polymorphisms with the clinical variables of the disease. Two hundred and three patients with paranoid schizophrenia and 243 healthy controls were enrolled in the study. Polymorphisms of *HSPA1A*, *-1B*, and *-1L* genes were genotyped using the PCR-RFLP technique. Analyses were conducted in entire groups and in subgroups that were stratified according to gender. There were significant differences in the genotype and allele frequencies of *HSPA1A* polymorphism between the patients and controls. The +190CC genotype and +190C allele were overrepresented in the patients and significantly increased the risk for developing schizophrenia (OR=3.45 and OR=1.61, respectively). Interestingly, such a risk was higher for females with the +190CC genotype than for males with the +190CC genotype (OR=5.78 vs. OR=2.76). We also identified the CGT haplotype as a risk haplotype for schizophrenia and demonstrated the effects of *HSPA1A* and *HSPA1B* genotypes on the psychopathology and age of onset. Our study provided the first evidence that the *HSPA1A* polymorphism may potentially increase the risk of developing paranoid schizophrenia. Further independent analyses in different populations to evaluate the role of gender are needed to replicate these results.

Keywords Association study · Paranoid schizophrenia · HSP70 gene · Single nucleotide polymorphism · Haplotype

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Introduction

Schizophrenia is a severe mental illness with a poorly defined etiology. Over the years, several hypotheses (neurodevelopmental, neurodegenerative, immunological, disruption of the neurotransmitter system) have been proposed to explain its pathogenesis (Leonard 2005; Fatemi and Folsom 2009; Howes and Kapur 2009) and to date more than 1,000 genes have been evaluated for their potential association with schizophrenia (www.schizophreniaforum.org/res/sczgene).

Heat shock proteins (HSPs) are a family of highly conserved proteins that are constitutively expressed or induced in response to various stressors. As molecular chaperones, HSPs promote the correct folding and assembly of polypeptides, prevent the aggregation of misfolded and denaturated proteins; their protective functions also include the inhibition of apoptosis, cytoskeletal protection, and immune modulation (Benarroch 2011). It is known that HSPs are expressed during development of the central nervous system (CNS) in a temporally and spatially controlled pattern that favors neuronal differentiation and survival (Reed-Herbert et al. 2006). Among several subfamilies of HSPs, proteins with molecular masses of 70 kDa (HSP70) seem to play a pivotal role in protection against damage to the CNS under stress conditions at vulnerable stages of embryonic development (Bates et al. 1996; Reed-Herbert et al. 2006). Expression of HSP70

in the CNS has been observed following a variety of stresses such as elevated temperature, hypoxia, ischemia, oxidative stress, or other pathological conditions that have also been indicated as risk factors for the development of schizophrenia (Bates et al. 1996; Kim et al. 2001). It has been hypothesized that the defective production or function of HSPs in response to embryonic insults may lead to the neurodevelopmental abnormalities that are observed in schizophrenic individuals (Bates et al. 1996). In fact, many experimental studies using both animal models and tissue culture systems have shown the neuroprotective effect of HSP70. It was demonstrated that the overexpression of HSP70 reduced ischemic injury and protected neurons against ischemic damage (Hoehn et al. 2001; van der Weerd et al. 2005; Terao et al. 2009) and also against excitotoxicity mediated by kainic acid (Tsuchiya et al. 2003) and glutamate (Sato and Matsuki 2002). Excitotoxic neuronal injury is a part of the neurodegenerative theory of schizophrenia (Csernansky 2007). Moreover, the selective overexpression of HSP70 was found to preserve synaptic function during times of stress (Bechtold et al. 2000; Karunanithi et al. 2002). Disturbed synaptic connectivity and aberrant neurotransmission systems are consistently reported in schizophrenia. Numerous studies in animal models of neurodegenerative diseases have provided further evidence for the protective effects of HSP70 (for a review, see Brown 2007; Benarroch 2011).

The presence of antibodies against HSP70 in the serum of schizophrenic individuals has been reported by several authors (Schwarz et al. 1999; Kim et al. 2001). Such elevated immunoreactivity to HSP70 supports the possibility of an immune mechanism in schizophrenia in which antibodies to HSP70 inhibit their neuroprotective functions (Kim et al. 2008).

The wide range of functions that are played by HSP70 in the CNS constitutes a strong basis for studying the influence of HSP70 gene polymorphisms on not only the risk of developing schizophrenia but also on the course and psychopathology of the disease. Although the human HSP70 family is composed of 13 unique gene products, excluding the many pseudogenes (Brocchieri et al. 2008), our study focused on only three genes: highly inducible *HSPA1A* (*HSP70-1*) and *HSPA1B* (*HSP70-2*) and constitutively expressed *HSPA1L* (*HSP70-hom*). These three genes are mapped to the major histocompatibility complex (MHC) class III region on chromosome 6p21.3 (Milner and Campbell 1990). Genome-wide association studies that have recently been conducted have shown the involvement of the MHC region in the susceptibility to schizophrenia (Purcell et al. 2009; Bergen et al. 2012; de Jong et al. 2012; Jia et al. 2012). However, association studies investigating HSP70 gene polymorphisms in schizophrenia are limited to only three analyses that were conducted exclusively on a Korean population (Pae et al. 2005; Kim et al. 2008; Pae et al. 2009).

In this study, we analyzed the potential implication of *HSPA1A* +190G/C, *HSPA1B* +1267A/G, and *HSPA1L* +2437T/C polymorphisms in the susceptibility to paranoid schizophrenia in a Caucasian Polish population. In addition, we investigated the association of the polymorphisms with the clinical variables of the disease.

Methods

Subjects

The study group consisted of 203 unrelated patients with a diagnosis of paranoid schizophrenia exclusively [90 (44.5 %) females and 113 (55.5 %) males; mean age±SD 41.9±12.7, range 18–70]. The patients were recruited from inpatients being treated at the Department and Clinic of Psychiatry, Medical University of Silesia in Katowice and the Neuropsychiatric Hospital in Lubliniec. All of the patients fulfilled the DSM-IV-TR (Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision) criteria for the paranoid type of schizophrenia. The final diagnosis was assigned by two experienced independent psychiatrists based on the Structured Clinical Interview for DSM-IV Axis I Disorders, Clinical Version (SCID-I-CV, First et al. 1997). Exclusion criteria for patients were: any other Axis I and Axis II diagnosis, neurological illness, endocrine disorders, and autoimmune diseases. The mean age of onset of schizophrenia was 25.8 years (SD=10.94, range 13–43). This information was acquired from patients' medical records. The age of onset of schizophrenia is defined as the age at which the first psychotic symptoms appeared. All of the patients were hospitalized because of an acute or chronic schizophrenic psychosis. The mean duration of schizophrenia was 16 years (SD=10.95, range 1–46). The Positive and Negative Syndrome Scale (PANSS, Kay et al. 1988) was assessed at the time of hospital admission. All of the patients were assessed to be capable of understanding the study and provided written consent before inclusion.

The control group was composed of 243 healthy, unrelated individuals [93 (38 %) females and 150 (62 %) males; mean age±SD 41.6±9.0, range 19–61] recruited from among the volunteer blood donors at the Regional Centre of Blood Donation and Treatment in Katowice. Exclusion criteria for controls were: current psychiatric problems, any other neurological disorders, and a family history of schizophrenia (verified during the interview), chronic and acute physical illness such as an infection, autoimmune, or allergic diseases.

All of the participants were of a Caucasian Polish origin living in the Silesia region. The study was approved by the Bioethics Committee of the Medical University of Silesia (No. NN-6501-213/II/04/06).

SNP choice

The three SNPs (rs1043618, rs1061581, and rs2227956) were selected according to their chromosomal location, heterozygosity (MAF≥10 %; information retrieved from a public database, the National Center for Biotechnology Information, dbSNP, http://www.ncbi.nlm.nih.gov/SNP/), assay availability and their potential effect on gene expression or protein function as confirmed by previous studies.

Genotyping with PCR-RFLP

The DNA was extracted from peripheral leukocytes using a Genomic Mini AX BLOOD kit (A&A Biotechnology, Poland) according to the manufacturer's instructions. The concentration of DNA was estimated spectrophotometrically using a BioPhotometer plus (Eppendorf AG, Hamburg).

The genotypes for polymorphisms +190G/C in the *HSPA1A* (rs1043618), +1267A/G in the *HSPA1B* (rs1061581) and +2437T/C in the *HSPA1L* (rs2227956) were determined by a PCR-restriction fragment length polymorphism (PCR-RFLP) assay under modified conditions according to a previously described method (Vinasco et al. 1997). Briefly, the regions spanning polymorphisms were amplified using the following primers: forward: 5′-TCCGGCGTCCGGAAGGACC-3′, reverse: 5′-TGCGGCCAATCAGGCGCTT-3′ (*HSPA1A*); forward: 5′-CATCGACTTCTACACGTCCA-3′, reverse: 5′-CA AAGTCCTTGAGTCCCAAC-3′ (*HSPA1B*); forward: 5′-GG ACAAGTCTGAGAAGGTACAG-3′, reverse: 5′-GTAACTT AGATTCAGGTCTGG-3′ (*HSPA1L*). PCR conditions were an initial denaturation step at 94 °C for 5 min followed by 35 cycles under the following conditions: 94 °C for 30 s, 56 °C (*HSPA1B*), 57 °C (*HSPA1L*) or 58 °C (*HSPA1A*) for 1 min and 72 °C for 1 min (*HSPA1A* and *HSPA1L*) or 72 °C for 1.20 min (*HSPA1B*) with a final elongation at 72 °C for 10 min. Amplification was performed using a G-Storm GS1 thermal cycler (Gene Technologies LTD, Essex, UK) with a Taq polymerase (Epicentre, Biotechnologies) according to the manufacturer's instructions in a reaction mix with a total volume of 25 μl. For RFLP detection, amplified PCR products were digested with the appropriate restriction enzymes (Fermentas) and subsequently separated on 2–3 % agarose gels stained with ethidium bromide. The *HSPA1A* +190G/C PCR products (325 bp) were digested with *Bsr*BI. The restriction fragments that were observed were 241 bp and 84 bp for the C allele and 171 bp, 84 bp and 70 bp for the G allele. The *HSPA1B* +1267A/G PCR products (1,117 bp) were digested with *Pst*I. An undigested product of 1,117 bp for the A allele and two products of 936 bp and 181 bp for the G allele were observed. The *HSPA1L* +2437T/C PCR products (878 bp) were digested with *Nco*I. An uncut product of 878 bp for the C allele and two fragments of 551 bp and 327 bp for the T allele were visible.

Statistical analysis

The results are presented as the mean±standard deviation. Qualitative data are expressed as percentage values. Differences in the allele, genotype and haplotype frequencies of the *HSPA1A*, *HSPA1B,* and *HSPA1L* polymorphisms between the groups were calculated using the χ^2 test, the maximum likelihood χ^2 test or the Fischer's exact test. The Shapiro–Wilk test was used to estimate the normality of the data. The Hardy–Weinberg equilibrium at each polymorphism was examined based on the inbreeding coefficient using the Fischer's exact test. The extent of the linkage disequilibrium (LD) expressed in terms of the D' and r^2 coefficients and haplotypes were estimated using the SNPStats. The odds ratio (OR) with a 95 % confidence interval was used as the measure of the strength of the association between allele, genotype and haplotype frequencies, and paranoid schizophrenia. The association between the genotypes, sex and PANSS subscales, and age of onset was calculated using two-way ANOVA with Duncan's post-hoc test. The homogeneity of variance was assessed with the Levene test. p values less than 0.05 were considered statistically significant. All of the statistical calculations were performed using Statistica 8.0 software (www.statsoft.com), MS Office Excel and SNPStats (bioinfo.inconcologia.net).

Results

Comparison of genotype and allele distributions between patients and controls

There was a difference in age between males and females (38.5±12.2 vs. 46.2±12.2, $p<0.0001$) in the study group. There was no difference in age between males and females among the controls (42.2±8.8 vs. 41.2±9.2, $p=0.3977$). There was no difference in the percentage of females between study and control groups ($\chi^2=1.68$, $p=0.1948$).

A marginal deviation from Hardy–Weinberg Equilibrium (HWE) was observed for +190G/C polymorphism ($p<0.05$) in the schizophrenia samples, while the other study polymorphisms were in HWE [+1267A/G ($p=0.4229$), +2437T/C ($p=0.6623$)]. All of the polymorphisms were in HWE [+190G/C ($p=0.7982$), +1267A/G ($p=0.4036$), +2437T/C ($p=0.7496$)] in the controls.

There were no statistically significant differences in the genotype and allele frequencies between the schizophrenics and controls for *HSPA1B* and *HSPA1L* polymorphisms in either the entire sample or after stratification according to sex (Table 1). However, there were significant differences in the genotype and allele frequencies of the *HSPA1A* polymorphic site between the patient and control groups. The +190CC genotype and +190C allele were more represented among the patients than among the control subjects (Table 1). The +190CC genotype showed an OR

Table 1 Genotype and allele distributions for the *HSPA1A* (+190G/C), *HSPA1B* (+1267A/G), and *HSPA1L* (+2437T/C) polymorphisms in paranoid schizophrenia patients (n=203) and control subjects (n=243)

Polymorphisms	*N* (%)								
	Genotype					Allele			
HSPA1A (rs1043618)	GG	GC	CC	χ^2	p	G	C	χ^2	p
Patients	94 (46.3)	76 (37.4)	33 (16.3)	13.45	*<0.01*	264 (65.0)	142 (35.0)	10.35	*<0.01*
Control	135 (55.5)	94 (38.7)	14 (5.8)			364 (75.0)	122 (25.0)		
Male patients	52 (46.0)	42 (37.2)	19 (16.8)	6.17	*<0.05*	146 (64.6)	80 (35.4)	5.42	*<0.05*
Male controls	83 (55.4)	56 (37.3)	11 (7.3)			222 (74.0)	78 (26.0)		
Female patients	42 (46.7)	34 (37.8)	14 (15.5)	8.36	*<0.05*	118 (65.6)	62 (34.4)	5.18	*<0.05*
Female controls	52 (55.9)	38 (40.9)	3 (3.2)			142 (76.3)	44 (23.7)		
HSPA1B (rs1061581)	AA	AG	GG	χ^2	p	A	G	χ^2	p
Patients	84 (41.4)	89 (43.8)	30 (14.8.)	0.01	0.9999	257 (63.3)	149 (36.7)	0.01	0.9999
Control	100 (41.2)	107 (44.0)	36 (14.8)			307 (63.2)	179 (36.8)		
Male patients	47 (41.6)	48 (42.5)	18 (15.9)	0.33	0.8479	142 (62.8)	84 (37.2)	0.07	0.7913
Male controls	58 (38.7)	69 (46.0)	23 (15.3)			185 (61.7)	115 (38.3)		
Female patients	37 (41.1)	41 (45.6)	12 (13.3)	0.42	0.8106	115 (63.9)	65 (36.1)	0.12	0.7290
Female controls	42 (45.1)	38 (40.9)	13 (14.0)			122 (65.6)	64 (34.4)		
HSPA1L (rs2227956)	TT	TC	CC	χ^2	p	T	C	χ^2	p
Patients	139 (68.5)	59 (29.0)	5 (2.5)	0.53	0.7672	337 (83.0)	69 (17.0)	0.47	0.5214
Control	174 (71.6)	64 (26.3)	5 (2.1)			412 (84.8)	74 (15.2)		
Male patients	76 (67.2)	35 (31.0)	2 (1.8)	–	0.5327	187 (82.7)	39 (17.3)	0.84	0.3594
Male controls	108 (72.0)	41 (27.3)	1 (0.7)			257 (85.7)	43 (14.3)		
Female patients	63 (70.0)	24 (26.7)	3 (3.3)	–	0.9250	150 (83.3)	30 (16.7)	0	1
Female controls	66 (71.0)	23 (24.7)	4 (4.3)			155 (83.3)	31 (16.7)		

All data in italics are statistically significant

for the development of schizophrenia of 3.45 after comparison with the +190GG genotype (Table 2). Similarly, the odds ratio of the +190C allele was 1.61 when using the +190G allele as a reference (Table 3). After the stratification according to gender, significant differences in the genotype and allelic distributions were still observed both between the schizophrenics and the control males and females (Table 1). Interestingly, female patients with the +190CC genotype have more than a two-fold greater risk of developing paranoid schizophrenia than male patients with the +190CC genotype compared to the +190GG female and male carriers, respectively [OR=5.78 vs. OR=2.76, Table 2]. The odds ratio of the +190C allele was 1.69 for female carriers and 1.56 for male carriers (Table 3).

Haplotype analysis

The linkage disequilibrium analysis showed a strong LD in every pair of the markers: *HSPA1A* and *HSPA1B* (D'=0.9153, r^2=0.6056, p<0.0001), *HSPA1A* and *HSPA1L* (D'=0.9985, r^2=0.0800, p<0.0001), *HSPA1B* and *HSPA1L* (D'=0.9988, r^2=0.1107, p<0.0001).

The results of the three-marker haplotype association tests in the entire group are summarized in Table 4. The CGT haplotype (over-represented among the patients), which showed an odds ratio of 1.55, was found to be significantly associated with schizophrenia when using the most frequent GAT haplotype as a reference. In contrast, the GGT haplotype (over-represented among the controls), which showed an odds ratio of 0.23, was found to exert a significant protective effect in comparison to the GAT haplotype. However, a significant association only remained for the GGT haplotype after the subjects were stratified by sex [females: OR=0.26 (95 % CI=0.10–0.68, p<0.05) vs. males: OR=0.29 (95 % CI=0.12–0.63, p<0.05)].

Correlation between sex, genotypes, and clinical presentation of the disease

The results of the ANOVA that examined the impact of sex, genotype, and sex-genotype interaction on PANSS and age of onset are shown in Table 5.

There were statistically significant differences in the mean PANSS positive (P) scores between the *HSPA1A* +190G/C genotypes and the *HSPA1B* +1267A/G genotypes. Patients with the +190GC and +1267AG genotypes, respectively, have significantly higher mean scores for positive symptoms than those with the +190CC and the +1267GG genotypes (Duncan post-hoc test: GC vs. CC, p<0.05; AG vs. GG, p<0.05). A tendency to a statistical difference between the PANSS general (G) mean scores and the +2437T/C genotypes was found for the *HSPA1L* polymorphic site.

Table 2 The association between the genotype for *HSPA1A* (+190G/C, rs1043618) polymorphism and the development of paranoid schizophrenia using the odds ratio (OR)

Genotype	*N* (%)			
	Control	Schizophrenia	OR (95 % CI)	*p*
Total				
GG	135 (55.5)	94 (46.3)	1.00	
GC	94 (38.7)	76 (37.4)	1.16 (0.77–1.73)	
CC	14 (5 8)	33 (16.3)	*3.45* (1.75–6.81)	*<0.0001*
Females				
GG	52 (55.9)	42 (46.7)	1.00	
GC	38 (40.9)	34 (37.8)	1.11 (0.60–2.05)	
CC	3 (3.2)	14 (15.6)	*5.78* (1.56–21.45)	*<0.05*
Males				
GG	83 (55.3)	52 (46.0)	1.00	
GC	56 (37.3)	42 (37.2)	1.20 (0.71–2.03)	
CC	11 (7.3)	19 (16.8)	*2.76* (1.21–6.26)	*<0.05*

All data in italics are statistically significant

CI confidence interval

Significant differences were also observed in the age of onset between males and females among the *HSPA1A* genotypes (Duncan post-hoc test: GG males vs. GG females, $p<0.01$; GG females vs. CC females, $p<0.05$). Females carrying the +190CC genotype had the earliest age of onset, while those with the +190GG genotype had the latest age of onset (mean age of onset: GG—28.8, GC—27.2, CC—25.3 years). In contrast, males carrying the +190GG genotype had their first episode at a younger age than males with the other genotypes (GG—23.3, GC—24.2, CC—26.7; note there was a later age of onset in males with the +190CC genotype than in females with the +190CC genotype). Similar gender differences in the age of onset were also detected across the *HSPA1B* genotypes, but the differences failed to reach statistical significance.

Discussion

In the study presented, we explored the possibility that *HSPA1A*, *HSPA1B*, and *HSPA1L* gene polymorphisms might be involved in the susceptibility to paranoid schizophrenia and the clinical presentation of the disease in the Polish population. To the best of our knowledge, this is the first study that investigated the association between HSP70 gene polymorphisms and schizophrenia in Caucasian individuals. Moreover, in contrast to previous reports, our study group was homogenous and was composed of patients with only a diagnosis of paranoid schizophrenia. Taking into account that particular schizophrenia subtypes are characterized by different clinical pictures, the homogeneity of the samples can increase the value of study, especially when the impact of polymorphisms on the psychopathology of schizophrenia is being assessed. In addition, all of the analyses that were performed were stratified according to gender. Gender differences in the age of onset, the course of the illness or the clinical symptoms in schizophrenia are well-described in the research literature (Grossman et al. 2006; Strkalj Ivezić and John 2009) and were also confirmed in this study. Many factors including sex steroid hormones may account for these differences between the genders. The implication of genetic factors has also been suggested. Gender-specific genetic associations with schizophrenia have been reported in several

Table 3 The association between the allele for *HSPA1A* (+190G/C, rs1043618) polymorphism and the development of paranoid schizophrenia using the odds ratio (OR)

Allele	*N* (%)			
	Control	Schizophrenia	OR (95 % CI)	*p*
Total				
G	364 (74.9)	264 (65.0)	1.00	
C	122 (25.1)	142 (35.0)	*1.61* (1.20–2.15)	*<0.05*
Females				
G	142 (76.3)	118 (65.6)	1.00	
C	44 (23.7)	62 (34.4)	*1.69* (1.07–2.68)	*<0.05*
Males				
G	222 (74.0)	146 (64.6)	1.00	
C	78 (26.0)	80 (35.4)	*1.56* (1.07–2.30)	*<0.05*

All data in italics are statistically significant

CI confidence interval

Table 4 Haplotype analysis of three SNPs: *HSPA1A* +190G/C (rs1043618), *HSPA1B* +1267A/G (rs1061581), *HSPA1L* +2437T/C (rs2227956) in patients with paranoid schizophrenia and control subjects

Haplotype	Frequency (%)			
	Total	Control	Cases	OR (95 % CI)
GAT	46.12	46.49	45.73	1.00
*CGT**	27.99	23.65	33.20	*1.55* (1.12–2.14)
GAC	15.51	15.23	15.80	1.42 (0.92–2.18)
*GGT**	8.25	13.18	2.30	*0.23* (0.10–0.53)
rare	2.13	1.45	2.98	1.48 (0.50–4.45)

All data in italics are statistically significant

CI confidence interval

*$p<0.05$

genes such as *ZNF804A* (Zhang et al. 2011a), the myelin transcription factor 1-like (*MYT1L*) (Li et al. 2012), the synapse-associated protein 97 gene (*SAP97*) (Uezato et al. 2012), disrupted-in-schizophrenia-1 (*DISC1*) (Schumacher et al. 2009), reelin (*RELN*) (Shifman et al. 2008), and interferon γ (*IFN-γ*) (Paul-Samojedny et al. 2011).

The functional significance of the selected polymorphisms has previously been analyzed. Although Wu et al. (2004) did not observe the influence of +190G/C SNP in the *HSPA1A* gene on transcriptional activity, it was found that this SNP through its impact on translation efficiency or post-trancriptional regulation may affect the synthesis level of the HSP70-1A protein, which is lower for the +190C allele (He et al. 2009). Recently, Dulin et al. (2012) found a significant decrease in the intragranulocytic HSP70-1A concentration, which is associated with the presence of the +190CC genotype. In contrast to the lower expression of intracellular HSP70, Zhang et al. (2011b) reported a more elevated plasma level of extracellular HSP70 in healthy individuals with the +190CC genotype. However, the authors postulated that the plasma HSP70 level might not be necessarily reflective of the intracellular level of HSP70. The association of a lower mRNA expression with the +1267G allele of the *HSPA1B* +1267A/G SNP has also been reported, which suggests that the inter-individual differences in HSP70 expression could be related to regulatory mechanisms that are distinct from transcriptional regulation (Pociot et al. 1993, Wu et al. 2004). Taking into account that HSP70-1A and HSP70-1B are major stress-inducible family members, we hypothesized that a decreased synthesis of those proteins in patients with the homozygous genotypes +190CC or +1267GG may impair their protective functions under stress conditions and be one of the factors that contribute to a predisposition to schizophrenia. The *HSPA1L* +2437T/C SNP that result in a Met→Thr amino acid substitution at position 493 in the peptide-binding domain may affect the substrate specificity and chaperone activity of the HSP70-1L protein (Milner and Campbell 1992).

A marginal deviation from HWE was observed for +190G/C polymorphism in the schizophrenia group. Because the proportion of affected subjects in a general population is small, the deviation from HWE can be expected in cases, and may indicate a genetic association (Ziegler et al. 2011), as in our study. Surprisingly, when the frequency of the minor allele (C) for the +190G/C SNP were compared with data available in dbSNP for Caucasian (European) populations, it was similar to that observed in the patients, and clearly lower to that found in the controls. This discrepancy may result from a larger size of a control population in our study (243 vs. 120 individuals). It may also be explained by the genetic diversity between European populations. A recent analysis demonstrated significant differences in SNP allele frequencies within five European populations (Scotland, Ireland, Sweden, Bulgaria, and Portugal). The HLA region on chromosome 6 (containing *HSPA1A*, *HSPA1B*, and *HSPA1L* genes) was among the regions with the highest differences between the populations studied (Moskvina et al. 2010).

We found that the *HSPA1A* +190CC genotype and the +190C allele (connected with lower protein synthesis) were over-represented among patients and significantly increased the risk of developing paranoid schizophrenia in the study population. The association between this polymorphism and schizophrenia was significant in both male and female subjects even when analyzed separately according to gender. However, females with the +190CC genotype have two-fold greater risk of developing schizophrenia than males carrying the +190CC genotype (OR=5.78 vs. OR=2.76). These findings suggest that in the case of *HSPA1A* polymorphism, sex may modulate the risk of paranoid schizophrenia. In contrast to our results, studies in a Korean population showed no impact of *HSPA1A* gene polymorphism on the susceptibility to schizophrenia (Pae et al. 2005; Kim et al. 2008). However, a significant association of the *HSPA1B* +1267A allele with schizophrenia in Koreans has been reported (Pae et al. 2005). Kim et al. (2008) also found a strong association between the rare A allele of the *HSPA1L* polymorphism (rs2075799, not

Table 5 The results of two-way ANOVA (sex and genotype distributions) for the PANSS subscales and age of onset of paranoid schizophrenia

	Effect	Females			Males			Sex		Genotype		Sex × genotype	
		GG	GC	CC	GG	GC	CC	F	*p*	F	*p*	F	*p*
HSPA1A rs1043618 (+190G/C)	PANSS positive	21.28±5.54	23.29±5.54	21.07±4.61	23.02±6.01	25.09±5.38	22.31±5.59	3.32	*0.0699*	3.58	*<0.05*	0.03	0.9703
	PANSS negative	25.26±6.37	26.06±6.31	24.86±6.04	26.58±5.90	25.17±6.14	27.63±6.53	1.21	0.2727	0.12	0.8828	1.18	0.3101
	PANSS general	43.48±8.37	44.53±7.41	41.50±7.13	46.25±8.67	45.83±8.04	46.79±8.46	5.96	*<0.05*	0.18	0.8348	0.68	0.5085
	Age of onset (years)	28.86±6.50	27.20±8.32	25.36±6.98	23.29±4.98	24.21±5.56	26.74±6.08	4.95	*<0.05*	0.19	0.8268	4.23	*<0.05*
		AA	AG	GG	AA	AG	GG						
HSPA1B rs1061581 (+1267A/G)	PANSS positive	21.38±5.58	23.02±5.45	20.50±4.76	22.79±5.93	24.91±5.56	22.67±5.54	4.11	*<0.05*	3.28	*<0.05*	0.06	0.9369
	PANSS negative	26.30±6.87	25.32±5.83	23.67±5.61	26.85±5.97	25.38±6.53	26.89±5.36	1.66	0.1995	0.98	0.3782	0.72	0.4885
	PANSS general	44.40±8.52	43.70±7.45	40.50±6.50	45.89±8.78	46.17±7.93	47.00±8.62	7.07	*<0.01*	0.32	0.7237	1.02	0.3622
	Age of onset (years)	29.00±6.38	27.15±8.00	25.50±7.55	23.53±5.19	24.44±6.14	25.39±4.28	6.02	*<0.05*	0.31	0.7344	2.81	*0.0625*
		TT	TC	CC	TT	TC	CC						
HSPA1L rs2227956 (+2437T/C)	PANSS positive	22.44±5.57	21.25±5.13	19.00±5.29	23.81±5.71	23.59±6.01	19.00±2.83	0.46	0.4969	1.45	0.2369	0.20	0.8214
	PANSS negative	26.17±6.31	24.50±5.87	19.33±4.93	26.45±6.18	25.68±6.15	28.00±4.24	2.89	*0.0907*	1.11	0.3313	1.13	0.3257
	PANSS general	44.60±7.66	41.54±8.10	38.00±3.60	46.68±7.94	45.35±9.37	41.50±2.12	1.45	0.2306	2.46	*0.0876*	0.24	0.7897
	Age of onset (years)	27.63±7.74	27.75±6.71	28.33±4.16	24.65±5.79	23.32±4.82	22.50±2.12	4.54	*<0.05*	0.11	0.8939	0.48	0.6187

All data in italics are statistically significant

F Fisher–Snedecor test in multivariate analysis

analyzed in this study). The discrepancies between our results and published data may reflect the population differences that exist, but also may result from different inclusion criteria (mixed paranoid schizophrenia with other subtypes).

Haplotype analysis showed that the CGT haplotype was associated with a significantly increased risk of developing schizophrenia (OR=1.55), while the GGT haplotype was associated with a significantly decreased risk of developing schizophrenia (OR=0.23). The fact that the coincidence of the +190C and the +1267G alleles increases the susceptibility to the development of schizophrenia is not surprising because these alleles contribute to a low synthesis of HSP70-1A and HSP70-1B proteins, which play a protective role during times of stress. A similar haplotype analysis in a Korean population failed to find any schizophrenia-associated haplotypes (Pae et al. 2005).

Two polymorphisms, +190G/C and +1267A/G, were found to be significantly associated with positive PANSS scores. Heterozygous genotypes were associated with the highest scores in both sexes, whereas homozygous genotypes for the minor allele (+190CC and +1267GG) were associated with the lowest scores. Recently, Pae et al. (2009) studied the influence of the five SNPs of HSP70 genes on clinical presentation and drug response in schizophrenic patients. They found that rs539689 SNP in the *HSPA1B* gene (not analyzed in this study) was marginally associated with positive PANSS scores at discharge and that two haplotypes were significantly associated with the difference in total and negative PANSS subscales from baseline to discharge. The same authors speculated that HSP gene polymorphisms may differentially influence negative and positive phenotypes. On the one hand, disturbed HSP activities may lead to reduced viability and synaptic functions that are associated with negative symptoms while on the other hand, impairment of neuroprotective HSP functions may have an impact on the neurotransmitter system. An imbalance of neurotransmitters in the brain (dopamine, serotonin, glutamate) has been indicated as a direct cause that leads to the development of characteristic symptoms of schizophrenia. An association between a pentanucleotide insertion/deletion (A1/A2) polymorphism of the *HSPA1B* gene and overexpression of noncognitive symptoms has also been reported in Alzheimer's disease (Clarimon et al. 2003).

We also observed a significant effect of the interaction between sex and the *HSPA1A* genotypes on the age of onset. The presence of the +190C allele was connected with an increase in the age of onset in males, but a decrease in the age of onset in females and female carriers of the +190CC genotype had an earlier age of onset than males with the same genotype. Interestingly, in males the +190CC genotype was associated with increased risk for schizophrenia on one hand, but on the other with a later age of onset in comparison with the +190GG and +190GC genotypes. Saccheti et al. (2007) found that the A allele of the -G308A polymorphism in the tumor necrosis factor-α (*TNF*-α) gene increased the susceptibility for schizophrenia only in males, and that the association became more specific when only patients of the paranoid subtype were compared to the controls. At the same time, the presence of the A allele was also associated with a later age of onset of schizophrenia in the entire sample.

Estrogen is well known to have diverse neuroprotective properties against injury from excitotoxicity, oxidative stress, ischemia, and apoptosis. Many studies have also confirmed estrogen's influence on the central neurotransmitter systems, thus indicating that it may have an antipsychotic activity (for a review see Hayes et al. 2012). This corresponds to the lower PANSS scores in females than in males that were observed in our study group. Despite the many potential benefits of estrogen, female carriers of the +190CC genotype were found to be not only at a greater risk for developing schizophrenia than male carriers, but that they also had the first episode of disease earlier than males. Lu et al. (2002) investigated the molecular mechanism for estradiol protection against cerebral ischemia in rats and indicated that the acute neuroprotection afforded by estrogen may be partly due to the induction of an HSP response. Zhang et al. (2004) postulated that estrogen protects human neurons against intracellular amyloid β toxicity by increasing the levels of HSP70 in neurons. If HSP induction is one of the mechanisms by which estrogens exert their neuroprotective effects, the presence of the *HSPA1A* +190CC genotype (associated with a lower protein synthesis) may partly reduce estrogen-mediated neuroprotection and lead to an increased susceptibility to schizophrenia.

The *HSPA1A*, *HSPA1L*, and *HSPA1B* genes are located 250 kb from the *TNF* gene cluster in the MHC class III region on chromosome 6p21.3 (Milner and Campbell 1990). The MHC region has an extremely high gene density and low recombination rates (long-range LD blocks) (Traherne 2008). Therefore, it should be considered that the association found between the *HSPA1A* polymorphism and schizophrenia may be due to LD with a *TNF*-α or other adjacent genes (e.g., HLA genes). Schroeder et al. (1999) noted the LD between *TNF* locus and *HSPA1B*. The strong association between HLA-DR3 and polymorphisms in the regulatory region of the *HSPA1A* gene was also reported (Cascino et al. 1993). Several studies have shown an association between HLA alleles and schizophrenia (Schwab et al. 2002; Wright et al. 2001) and *TNF*-α, -β gene polymorphisms and schizophrenia (Jun et al. 2003; Sacchetti et al. 2007). Kim et al. (2008) proposed that in the context of the role of HSP70 in the immune response (e.g., HSPs bind and deliver antigenic peptides to MHC class), interaction studies of HSP70 genes with other promising immune system-related candidate genes in schizophrenia (*TNF*-α, genes encoding HLA antigens system) could be interesting. A recently conducted study in transgenic hsp70-overexpresing mice showed that the MHC haplotype determined the effectiveness of HSP70 protection against the measles virus neurovirulence (Carsillo et al. 2009).

In view of the protective role of HSP70 in the CNS, it would also be interesting to study the interaction between HSP70 gene polymorphisms and the polymorphisms of other genes that have protective functions, such as the genes encoding neurotrophins (e.g., the nerve growth factor, NGF; the brain-derived neurotrophic factor, BDNF) or Neuregulin-1. These proteins regulate neuronal development and survival, synaptogenesis, and synaptic plasticity (Otnæss et al. 2009; Feng et al. 2010). Himeda et al. (2007) investigated the immunohistochemical alterations of BDNF, NGF, HSP70, and Ubiquitin in the gerbil hippocampus after transient cerebral ischemia and found that the expression of stress proteins may play a key role in providing protection against neuronal damage to the hippocampal CA1 after ischemia in comparison with the expression of neurotrophins. Taking into account that schizophrenia is a polygenic disease, studying the combined effect of polymorphisms of many potential candidate genes that have overlapping functions might lead to a better understanding of the pathogenic mechanisms of schizophrenia.

In summary, we analyzed *HSPA1A*, *HSPA1B*, and *HSPA1L* polymorphism in a highly homogenous population with respect to ethnicity, geographic region, and schizophrenia subtype. Our case–control study provided the first evidence that the *HSPA1A* +190CC genotype and the +190C allele may potentially be associated with an increased risk for developing paranoid schizophrenia in Caucasian Polish individuals. We also identified the CGT haplotype as a risk haplotype for schizophrenia and demonstrated the effects of the *HSPA1A* and *HSPA1B* genotypes on the psychopathology and age of onset. Due to the relatively small sample size, further independent analyses performed with larger samples are needed to replicate these results.

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