HSPA5 promoter polymorphisms and risk of Parkinson’s disease in Taiwan

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Abstract

Endoplasmic reticulum (ER) stress induced by misfolded proteins has been implicated in Parkinson’s disease (PD) pathogenesis. A malfunction of unfolded protein response (UPR) to ER stress can result in PD as well as other neurodegenerative diseases. Heat shock 70 kDa protein 5 (HSPA5) is one of the UPR chaperones reactive to ER stress to block the apoptotic process. HSPA5 promoter polymorphisms −415 G/A (rs391957), −370 C/T (rs17840761) and −180 del/G (rs3216733) and their derived haplotypes may affect promoter activity of the gene. This study examines whether these HSPA5 promoter polymorphisms are associated with the risk of Taiwanese PD and the age of disease onset using a case–control study.

Polymorphisms −415 G/A and −180 del/G were completely linked in our population (D′ = 1.00, Δ² = 1.00). The genotype or allele frequency distribution of each HSPA5 polymorphism was not significantly different between the controls (n = 341) and the PD patients (n = 393). Neither the linked −415 G/A and −180 del/G nor −370 C/T polymorphism influences PD onset age. Our data suggest that the HSPA5 −415 G/A, −370 C/T, and −180 del/G polymorphisms are unlikely to play a major role in risk of developing PD in Taiwan.

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Although the pathogenesis of Parkinson’s disease (PD) remains to be elucidated, interplay between environmental and genetic factors are thought to confer vulnerability to PD [25]. Various mutations in the genes for α-synuclein, parkin, ubiquitin carboxy-terminal hydrolase L1, DJ1, PTEN-induced kinase 1, and leucine-rich repeat kinase-2 can lead to familial PD [4,5], whereas the etiologies of sporadic cases remain mostly unknown. Accumulating evidence suggests that endoplasmic reticulum (ER) stress induced by aberrant protein degradation is associated with PD [24]. A malfunction of the ER stress response can result in PD as well as other neurodegenerative diseases such as Alzheimer’s and prion disease [26]. Parkin is the gene responsible for autosomal recessive juvenile parkinsonism (AR-JP)/PARK2. Parkin has been shown to protect cells from ER stress and oxidative stress, presumably due to its ubiquitin ligase (E3) activity that targets misfolded proteins derived from ER [10]. Loss of function of parkin causes ER stress with accumulation of cytotoxic fibrils and protein aggregates in cells [11]. α-Synuclein is a major component of Lewy bodies in sporadic PD, and mutations in α-synuclein cause autosomal-dominant hereditary PD. Induction of A53T α-synuclein increased ER stress and an ER stress inhibitor (salubrinal) partially protected against cell death and further reduced A53T toxicity [20]. These findings provide several lines of substantial evidence for the role of ER stress in pathogenesis of PD.

ER stress is caused by disturbances in the structure and function of the ER with the accumulation of misfolded proteins and alterations in the calcium homeostasis. The unfolded protein response (UPR) serves to protect the ER and restores function by inducing chaperons, blocking translation and increasing protein folding in the ER [15]. If the function of the ER is severely impaired, genes and pathway leading to cell death and/or inhibition of survival are also activated. Among the UPR, HSPA5 (heat shock 70 kDa protein 5) also referred to as GRP78 (glucose-regulated protein, 78 kDa) or BiP (immunoglobulin heavy chain-binding protein), is an important chaperon
involving in the folding and assembly of proteins in ER [14]. Disrupted function of HSPA5 through defect in its co-chaperon SIL1 causes Marinesco–Sjogren syndrome characterized by cerebellar ataxia, progressive myopathy and cataracts [18]. Increased expression of HSPA5 can have neuroprotection from ER stress by enhancing UPR [2].

HSPA5 promoter polymorphisms may affect the individual variability of ER stress response and has been reported to be a risk factor for bipolar disorder in a Japanese population [12]. Therefore, HSPA5 promoter polymorphisms may potentially confer a genetic risk factor to PD. We examined whether the HSPA5 single nucleotide polymorphisms (SNPs) (−415, −370, and −180) and haplotypes predispose to the risk of developing PD in Taiwan.

A total of 393 unrelated PD subjects (45.8% women, 54.2% men), all Taiwanese, diagnosed with idiopathic PD by two neurological specialists (C.M. Chen and Y.R. Wu), were recruited from the neurology clinics of Chang Gung Medical Center. All PD patients exhibited at least two of the four cardinal signs of PD: resting tremor, cogwheel rigidity, bradykinesia, and postural reflex impairment; all were diagnosed with probable idiopathic PD according to the published criteria [7]. Subjects with prior history of multiple cerebrovascular events or other causes of parkinsonian symptoms (e.g., brain injury or tumor,encephalitis, and antipsychotic medication) were excluded. The mean age at onset of PD was 63.6 ± 9.6 years, ranging between 39 and 85 years (10.4% of all PD cases had onset before age 50). The mean age at time of examination was 68.8 ± 9.6 years. A group of 341 normal control individuals without neurodegenerative diseases were recruited from the same ethnic community. Control subjects (49.6% women and 50.4% men) had mean age at examination of 62.5 ± 11.4 years, ranging between 39 and 94 years. All examinations were performed after obtaining informed consent from patients and control individuals.

DNA was extracted from leukocytes using the standard protocols. The polymorphisms of −415 G/A (rs391957) and −370 C/T (rs17840761) were determined using the polymerase chain reaction (PCR)-restriction fragment length polymorphism method. Sequences of primers and polymorphic change (underlined) in the enzyme recognition site were as follows: 5′-TCAGAGACTGGATGGAACCTG-3′ (forward primer), 5′-TGGCTGCTATTGCTTCTAAAG-3′ (reverse primer), GAANNNNTTC (XmnI, −415 G/A) and RGATCCY (BstYI, −370 C/T). To genotype −180 del/G (rs3216733), primers were 5′-hex-CGGGTCAGAAGTCGCAGGAGAT-3′ and 5′-CGTGGAGCCGCTTCTATTGG-3′ and the length of the amplified products (212 and/or 213 bp) was determined by electrophoresis in a linear polyacrylamide gel on an automated MegaBACE Analyzer (Molecular Dynamics, Division of Amersham Pharmacia Biotech). In addition, aliquots of the amplified products were mixed with an equal volume of 95% formamide buffer and subjected to single-strand conformation polymorphism (SSCP) analysis using GeneGel Excel gels as recommended by the manufacturer (Pharmacia Biotech). Alleles del and G were confirmed by DNA sequencing.

Genotype and allele frequencies for each polymorphic site were calculated, and the differences between patients and controls were tested by the χ²-test of association and the Fisher’s exact test where appropriate. The χ²-test was used to test Hardy–Weinberg equilibrium for each polymorphic site. The SNPSpD method [17] was used to generate an adjusted significance threshold for correction of multiple SNP testing. The experiment-wide significance threshold of 0.031 was required to keep the type I error rate at 5%. Measures of pairwise linkage disequilibrium (LD) between SNPs, including Lewontin’s standardized disequilibrium coefficients (D′), the squared pairwise correlations (Δ²), and eigenvalues (λs) were computed with the LDMAX software-part of the GOLD Command Line Tools package [1]. PHASE, Version 2.1 was used to reconstruct the HSPA5 gene haplotypes [21,22]. The HSPA5 pairwise haplotype frequencies were computed and Chi-square tested for significance. One-way analysis of variance was used to test between-group differences in age of disease onset. Odds ratios (ORs) with 95% confidence intervals (95% CI) were calculated to test association between genotype/allele/haplotype and disease. Using the current sample size (393 PD patients) in our study, we evaluated the ability to detect an association between a SNP and PD by power calculation implemented in QUANTO, Version 1.0 [6]. For genetic effect >1.4, we had enough power (0.88) to identify the association under additive model with disease allele frequency greater than 0.3.

The genotype distributions in PD and controls did not deviate significantly from Hardy–Weinberg equilibrium for all the polymorphisms examined (data not shown). The modified SNPSpD method was employed for correction of multiple SNP testing. SNPSpD output of three λs was shown in Table 1. As described by Cheverud [3], high correlation among variables leads to high λs. In this case, the first λ (1.62) is less than 2 (the number of variables in the correlation matrix), suggesting that not all variables are completely correlated. The magnitude of pairwise LD was quantified by the metrics D′ and Δ². The D′ coefficient of −415 G/A and −180 del/G was equal to 1 (D′ = 1.0), strongly suggesting that there has been no recombination in the region over time, and a very strong LD was observed between −415 G/A and −180 del/G sites (Δ² = 1.0). SNP −415 G/A and SNP −180 del/G were completely linked.

The genotype and allele frequency distributions of the polymorphisms in PD patients and controls are displayed in Table 2. Neither the genotypic (P = 0.117) nor the allelic frequencies (P = 0.494) of −415 G/A (−180 del/G) polymorphism were statistically different between the PD and the control groups.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Pairwise linkage disequilibrium measures4 for HSPA5 SNPs</th>
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<tr>
<td></td>
<td>−415 G/A</td>
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<tr>
<td>−415 G/A</td>
<td>1.00</td>
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<tr>
<td>−370 C/T</td>
<td>0.38</td>
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<tr>
<td>−180 del/G</td>
<td>1.00</td>
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4 Lewontin’s standardized disequilibrium coefficients (D′) are given above the diagonal and the squared pairwise correlations (Δ²) are given below the diagonal; the eigenvalues (λs) associated with the LD correlation matrix are given along the diagonal (bold and italic).
The genotypic and the allelic frequencies of \(-370\ C/T\) polymorphism were also similar between the PD patients and the controls (\(P = 0.809\) for genotype; \(P = 0.580\) for allele). For PD patients, the mean ages at onset for the \(-415\ G/A\) \((-180\ del/G)\) genotype-carrying groups (in years) were \(64.7 \pm 11.0\) for AA, \(63.0 \pm 9.1\) for GA, \(64.0 \pm 9.8\) for GG, and \(63.6 \pm 9.6\) for overall. The mean ages at onset for the \(-370\ C/T\) genotype-carrying groups (in years) were \(63.7 \pm 9.9\) for CC, \(63.5 \pm 9.4\) for CT, and \(63.8 \pm 10.0\) for TT. The ages of onset were not significantly different when comparing the three \(-415\ G/A\) \((-180\ del/G)\) genotype groups (\(P = 0.263\)) and when comparing the three \(-370\ C/T\) genotype groups (\(P = 0.940\)). We further analyzed the \(HSPA5\) polymorphisms and PD risk for the group of early onset of PD (age onset \(<50\) years, \(n = 41\)). The genotypic frequencies of \(-415\ G/A\) \((-180\ del/G)\) and \(-370\ C/T\) polymorphisms were not significantly different between the early onset of PD patients and the controls (\(P = 0.609\) for \(-415\ G/A\) and \(P = 0.808\) for \(-370\ C/T\)).

Pairwise haplotype analysis of the three polymorphisms showed that none of the haplotype frequencies were different between the controls and the PD (\(P = 0.783\) (Table 2). ORs of the at-risk genotype, allele or haplotype were calculated by comparing each value to the common genotype, allele or haplotype (Table 2). None of the genotypes, alleles or haplotypes of the \(HSPA5\) gene polymorphisms confers an increased risk to PD (\(P = 0.110–0.918\)).

The UPR is activated in PD and that UPR activation is closely associated with the accumulation and aggregation of \(\alpha\)-synuclein [8]. Loss of parkin function and mutation of \(\alpha\)-synuclein can increase ER stress and then lead to neuronal loss in PD, if UPR is not adequately activated [11,20]. Similarly, neuronal death can be induced by ER in Alzheimer’s disease (AD) [13] and ER chaperones can protect against AD by inhibiting the production of amyloid-\(\beta\) peptide [9]. Among UPR, \(HSPA5\) is one of the chaperons to protect cell from pro-apoptotic pathways [14]. Recently, polymorphisms and haplotypes in \(HSPA5\) promoter was found to affect the basal activity of \(HSPA5\) promoter and haplotypes carrying high activity of promoter were found to be a risk factor for bipolar disorder in a Japanese population [12]. Although how the haplotypes carrying high promoter activity increase the disease risk is not clear, the biological function makes \(HSPA5\) a good candidate for the risk study of PD. Given that multi-factors involving both genetics and environment are implicated in the PD pathogenesis, we examined if the \(HSPA5\) polymorphisms influence risk of PD. However, our study did not demonstrate an association between the \(HSPA5\) polymorphisms and haplotypes with PD in our Taiwanese population. In addition, the onset age of Taiwanese PD is not influenced by the \(HSPA5\) polymorphisms. When the analysis was performed separately for the group of early onset PD (onset before age 50), the \(HSPA5\) polymorphisms did not confer an increased risk to young onset PD.

We have examined the limitations and advantages of our study. Although our study cohort includes 393 unrelated PD subjects and 341 normal controls, which may still be unable to detect an uncommon disease locus with a small effect. Secondly, environmental agent exposure and its interaction with \(HSPA5\) promoter were not assessed, and these factors may be relevant in the context of this particular gene. Furthermore, as false positive and negative results are common in genetic association studies [23], replication studies with independent sample sets are needed to exclude the association of genetic loci with the disease [19]. However, our study have also two strengths: (1) laboratory genotyping was performed blind to case–control status, minimizing experimental bias and genotyping was rechecked if there is ambiguity; (2) all PD patients and control subjects had
a similar ethnic background, limiting the possible confounding effect of population stratification.

As locus heterogeneity for the pathogenesis of PD in different genetic backgrounds has been indicated [16], our negative results do not rule out an association between HSPA5 and PD in other ethnic populations. While our results do not exclude a possible association of other genetic variants within the HSPA5 gene in PD, we conclude that the HSPA5 −415 G/A (−180 del/G) and −370 C/T polymorphisms are unlikely to play a major role in our population.

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