

Association between *GRN* rs5848 Polymorphism and Parkinson's Disease in Taiwanese Population

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Abstract

A single nucleotide polymorphism *GRN* rs5848 (3'UTR+78 C>T) was reported to alter the risk for frontotemporal lobar degeneration. Herein, we investigated the effect of *GRN* rs5848 on the risk of Parkinson's disease (PD) by genotyping 573 Taiwanese patients with PD and 490 age-matched control subjects. Compared to subjects with CC genotype, those with TT genotype had a 1.58-fold increased risk of PD (95% CI: 1.77~2.34, $P=0.021$). PD patients demonstrate a higher frequency of T allele (37.2%) than controls (32.2%; odds ratio [OR]= 1.24, 95% CI: 1.04~1.49, $P=0.017$). This susceptibility was particularly observed in female subjects, in which TT genotype had a 2.16-fold increased risk of PD as compared with controls (95% CI: 1.24~3.78, $P=0.006$). The frequency of T allele (39.3%) in female PD patients was higher than in female control subjects (31.1%; OR= 1.43, CI: 1.11~1.87, $P=0.007$). No association was observed between *GRN* rs5848 and susceptibility in male subjects. These findings show that the *GRN* rs5848 TT genotype and T allele are risk factors for female Taiwanese patients with PD.

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Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder worldwide [1]. It affects 1% of the population aged over sixty, and is characterized by a slowness of movement and a difficulty in initiating movement [1]. The pathogenesis of PD includes progressive degeneration of the dopaminergic neurons and the presence of Lewy bodies with enrichment of α -synuclein in the ventral midbrain [2]. This cell death results in a deficiency of dopamine in the striatum. When the level of striatal dopamine falls below 70~80%, the clinical presentations of PD are manifested [1].

The *GRN* gene (granulin, MIM#138945) encodes an 88-kDa secreted growth factor progranulin [3], which is involved in multiple physiological functions, including wound healing, tumor growth, and embryonic brain development [4,5,6]. A single nucleotide polymorphism (SNP) in the 3'-untranslated region of *GRN* (3'UTR+78C>T; rs5848) was reported to alter the risk for frontotemporal lobar degeneration (FTLD) [7]. Although *GRN* rs5848 polymorphism was not associated with the risk of PD in Caucasian populations [8], its effects in other ethnic genetic and environmental backgrounds is unknown. Therefore, we assessed whether *GRN* rs5848 polymorphism contributes to the genetic etiology of PD by using case-control analysis in 573 Taiwanese patients with PD and 490 control subjects.

Results

In total, 1063 subjects, including 573 patients (female/male: 253/320) with PD and 490 normal controls (female/male: 249/241, $P=0.030$), were recruited. Only 1 proband with familial PD in the same family was included to minimize the skew caused by the other family members carrying the same genetic polymorphism. *GRN* rs5848 TT genotype showed a higher prevalence in PD than CC genotype did (odds ratio [OR]= 1.63, 95% CI: 1.10 ~ 2.42, $P=0.015$, Table 1). This finding was also present in the recessive model on the borderline of statistical significance (OR= 1.51, 95% CI: 1.04 ~ 2.19, $P=0.031$). The T allele showed a greater frequency in PD than the C allele did (OR= 1.25, 95% CI: 1.05 ~ 1.50, $P=0.014$). These findings were absent in Caucasian populations [8]. The distributions of the genotypes and the minor allele frequency did not differ between Taiwanese and Caucasians in the control group.

The unequal gender distribution between PD and control groups may influence the prevalence between the *GRN* rs5848 genotype/allele and PD. Thus, we stratified our groups according to gender (Table 2). In female subjects, the TT genotype showed a significantly greater frequency in PD than did the CC genotype (OR= 2.99, 95% CI: 1.50 ~ 5.95, $P=0.002$). The recessive model represented this high frequency between TT genotype and PD (OR= 2.85, 95% CI: 1.48 ~ 5.48, $P=0.002$). The occurrence of the T allele in female PD patients was higher than that in female control subjects (OR= 1.59, 95% CI: 1.16 ~ 2.18, $P=0.004$). The distributions of the genotypes and the minor allele frequency did

Table 1. Frequency of genotype and allele polymorphisms of *GRN* rs5848 among Parkinson's disease (PD) patients and controls in Taiwanese and Caucasian.

	PD (%)	Controls (%)	OR (95% CI)	P value
Taiwan				
Genotype frequency				
CC	231 (40.3%)	226 (46.1%)	1.00	
CT	258 (45.0%)	212 (43.3%)	1.17 (0.91 ~ 1.52)	0.227 [#]
TT	84 (14.7%)	52 (10.6%)	1.63 (1.10 ~ 2.42)	0.015 [#]
Dominant model				
CC	231 (40.3%)	226 (46.1%)	1.00	
CT+TT	342 (59.7%)	264 (53.9%)	1.26 (0.99 ~ 1.62)	0.062 [#]
Recessive model				
CT+CC	489 (85.7%)	438 (89.4%)	1.00	
TT	84 (14.7%)	52 (10.6%)	1.51 (1.04 ~ 2.19)	0.031 [#]
Allele frequency				
Major allele (C)	720 (62.8%)	664 (67.8%)	1.00	
Minor allele (T)	426 (37.2%)	316 (32.2%)	1.25 (1.05 ~ 1.50)	0.014 [#]
Caucasian*				
Genotype frequency				
CC	361 (46.8%)	312 (48.6%)	1.00	
CT	324 (42.0%)	263 (41.0%)	1.07 (0.85 ~ 1.33)	0.580 [‡]
TT	86 (11.2%)	67 (10.4%)	1.11 (0.78 ~ 1.58)	0.565 [‡]
Dominant model				
CC	361 (46.8%)	312 (48.6%)	1.00	
CT+TT	410 (53.2%)	330 (51.4%)	1.07 (0.84 ~ 1.32)	0.506 [‡]
Recessive model				
CT+CC	685 (88.8%)	575 (89.4%)	1.00	
TT	86 (11.2%)	67 (10.4%)	1.08 (0.77 ~ 1.51)	0.665 [‡]
Allele frequency				
Major allele (C)	1046 (67.8%)	887 (69.1%)	1.00	
Minor allele (T)	496 (32.2%)	397 (30.9%)	1.06 (0.90 ~ 1.24)	0.478 [‡]

OR: odds ratio.

*Genotype and allele frequencies of *GRN* rs5848 are from Jasinska-Myga et al. (2009) [8].[#]P value of binary logistic regression with adjustment of age and gender.[‡]P values of Chi square test.

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not differ between PD and controls in male subjects. Both female and male control subjects displayed similar distributions of genotypes and the minor allele.

Discussion

The present study showed that the *GRN* rs5848 SNP affects the risk of developing PD in Taiwanese population. Our results differ from those of Jasinska-Myga et al. (2009), who reported a lack of association between PD and the *GRN* rs5848 T allele and TT genotype in patients with PD in the US and Poland [9]. This discrepancy demonstrates the differential effect of *GRN* rs5848 on PD risk between Eastern and Western populations.

A number of genetic variants exert population-specific influences on the risk of developing PD. For example, *LRRK2* G2835R and R1628P are common polymorphisms in Taiwan and Singapore [9,10,11]. By contrast, these associations were not observed in Caucasian populations [12,13]. SNPs rs11931532,

Table 2. Frequency of genotype and allele polymorphisms of *GRN* rs5848 among Parkinson's disease (PD) patients and controls in female and male patients.

	PD (%)	Controls (%)	OR (95% CI)	P value
Female				
Genotype frequency				
CC	99 (39.1%)	119 (47.8%)	1.00	
CT	109 (43.1%)	105 (42.2%)	1.11 (0.71 ~ 1.76)	0.643
TT	45 (17.8%)	25 (10.0%)	2.99 (1.50 ~ 5.95)	0.002
Dominant model				
CC	99 (39.1%)	119 (47.8%)	1.00	
CT+TT	154 (60.9%)	130 (52.2%)	1.42 (0.93 ~ 2.17)	0.103
Recessive model				
CT+CC	208 (82.2%)	224 (90.0%)	1.00	
TT	45 (17.8%)	25 (10.0%)	2.85 (1.48 ~ 5.48)	0.002
Allele frequency				
Major allele (C)	307 (60.7%)	343 (68.9%)	1.00	
Minor allele (T)	199 (39.3%)	155 (31.1%)	1.59 (1.16 ~ 2.18)	0.004
Male				
Genotype frequency				
CC	132 (41.3%)	107 (44.4%)	1.00	
CT	149 (46.5%)	107 (44.4%)	1.24 (0.86 ~ 1.20)	0.251
TT	39 (12.2%)	27 (11.2%)	1.14 (0.64 ~ 2.03)	0.649
Dominant model				
CC	132 (41.3%)	107 (44.4%)	1.00	
CT+TT	188 (58.7%)	134 (55.6%)	1.22 (0.86 ~ 1.74)	0.265
Recessive model				
CT+CC	281 (87.8%)	214 (88.80%)	1.00	
TT	39 (12.2%)	27 (11.2%)	1.02(0.59 ~ 1.76)	0.941
Allele frequency				
Major allele (C)	413 (64.5%)	321 (66.6%)	1.00	
Minor allele (T)	227 (35.5%)	161 (33.4%)	1.12 (0.86 ~ 1.45)	0.395

OR: odds ratio.

P value of logistic regression with adjustment of age.

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rs12645693, rs4698412, and rs4538475 of *BST1* are identified as risk factors for PD in a Japanese population [14], while this association has not been described in Chinese populations [15]. To further understand the role of *GRN* rs5848 in determining PD in ethnology, more genetic epidemiological studies should be performed in other races.

Several studies have shown that cerebral spinal fluid, serum, and plasma progranulin levels are significantly lower in *GRN* mutation carriers than in non-carriers [16,17,18]. Reduced levels of progranulin could affect both neuronal survival and CNS inflammatory processes [19,20], which leads to loss of neurons. To date, more than 80 mutations of *GRN* have been found in neurodegenerative diseases [http://www.hgmd.cf.ac.uk/ac/gene.php?gene=GRN]. Similar to our results, patients homozygous for the *GRN* rs5848 T allele are more prone to developing FTLD than are homozygous C-allele carriers [7]. The *GRN* rs5848 SNP is predicted to create a micro-RNA 659 (miR-659) binding site [7]. Specifically, *GRN* rs5848 T increases the binding of miR-659 compared with the C allele, which thereafter increases transla-

tional suppression of *GRN* [7]. Reduced serum level of the progranulin was identified in homozygous *GRN* rs5848 T-allele carriers [21], supporting the hypothesis that *GRN* rs5848 affects the risk of neurodegenerative diseases by regulating *GRN* expression.

In elderly populations, men are approximately 2 times more likely to develop PD than are women [22,23]. The factors contributing to the male prevalence of PD are unknown. Environmental factors, such as work-related exposure to toxins or heavy metals and pesticides, may be present at lower levels in the female living environment, which may result in the lower prevalence of PD in female populations [22]. Women may also benefit from putative protective factors, such as early life exposures to endogenous estrogen [24,25,26]. Further, there may also be gender-related differences in expression of genes related to signaling pathways associated with PD [27,28]. Although the present study showed that the *GRN* rs5848 SNP modifies the risk of PD, particularly in the female populations of Taiwan, a large-scale study on female PD patients should be carried out to consolidate this gender-specific association.

An association between PD and *GRN* has not been described in published PD- genome-wide association studies (PD-GWAS) in North American, European, and Asian populations [14,29,30,31,32,33,34,35,36]. This is because PD is probably a multifactorial disorder for which a number of modest risk factors, over a lifespan, may contribute to ethnic or geographic differences of genetic susceptibility in PD patients. In support of this is the observation of an interaction between coffee and the glutamate receptor gene, *GRIN2A*, in PD [37]. Thus it is more likely that *GRN* rs5848 plays the role of a PD risk modifier by interaction with unknown environmental factors in Taiwan, which may explain the negative results in the available PD-GWAS.

Although our result is significant, there are limitations in this study. The single SNP analysis does not clarify the association between other regions around or within the gene and PD. The role of gene-gene or gene-environmental interaction has not been evaluated. In addition, the sample size in our study may not be able to identify an association when the genetic effect of the allele is less than 1.5. Nevertheless, our finding indicates the potential of *GRN* rs5848 T allele as a genetic risk factor in PD patients, particularly in the female population. More research into the influence of SNPs in *GRN* on PD onset or progression is clearly warranted.

Subjects and Methods

Ethics Statement

This study was performed according to a protocol approved by the institutional review boards of Chang Gung Memorial Hospital

(ethical license No: 98-3980A3), and all examinations were performed after obtaining written informed consents.

Patient Population

Patients diagnosed with PD were recruited from the neurology clinics of Chang Gung Memorial Hospital. The diagnosis of PD was based on the UK PD Society Brain Bank clinical diagnostic criteria by 2 neurologists specialized in movement disorders (YR Wu and CM Chen) [38]. Unrelated healthy adult volunteers matched for age, gender, ethnic origin, and area of residences were recruited as controls.

Genetic Analysis

Genomic DNA was isolated from peripheral leukocytes by using a DNA Extraction Kit (Stratagene). *GRN* rs5848 polymorphism was determined using a pre-designed custom TaqMan SNP genotyping assay (assay ID: C7452046_20) on an ABI 7000 Real Time PCR system according to the manufacturer's protocol (Applied Biosystems, Foster City, CA, USA). Briefly, each reaction included 20 ng of DNA, 0.9 μ M of each primer, 0.2 μ M of probe (probe sequence: TCTGCTCAGGCCTCCCTAGCACCTC[C/T]CCCTAACCAAATTCCTCCCTGGACCC), and Universal PCR Master Mix (Applied Biosystems). PCR parameters were 50°C for 2 min, 95°C for 10 min, 40 cycles of 95°C for 15 sec, and 60°C for 1 min. The genotyping results were analyzed using SDS software version 1.1 (Applied Biosystems).

Statistical Analysis

The genotypes of the patients and controls followed the Hardy-Weinberg equilibrium. Logistic regression analysis was carried out with PD as the dependent variable, and age, gender, and GRN genotypes as the independent variables. All *P* values were two-tailed. *P* values <0.025 were considered statistically significant to account for the multiple comparisons. Given the observed allele frequency in the present case-control study at a 0.025 significant level, we had power greater than 0.8 to identify an association when the per-allele genetic effect was greater than an odds ratio of 1.5 and 1.7 under a dominant and a recessive genetic model, respectively.

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Author Contributions

Conceived and designed the experiments: YRW GJLC. Performed the experiments: YCH HCH. Analyzed the data: KHC YRW GJLC. Contributed reagents/materials/analysis tools: YRW CMC YCC CCH HCK KHC. Wrote the paper: KHC YRW GJLC.

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