Neurobiology of Aging xxx (2017) 1.e1–1.e6

Contents lists available at ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging

DLG2, but not *TMEM229B*, *GPNMB*, and *ITGA8* polymorphism, is associated with Parkinson's disease in a Taiwanese population

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ARTICLE INFO

Article history:
Received 21 June 2017
Received in revised form 30 October 2017
Accepted 30 November 2017

Keywords:
Transmembrane protein
TMEM229B
GPNMB
ITGA8
DLG2
Parkinson's disease
Polymorphism
Disease association

ABSTRACT

Transmembrane or membrane-associated protein dysfunction is increasingly recognized as an important mechanism of pathogenesis in Parkinson's disease (PD). Previous genome-wide association studies and their meta-analysis in PD genes have identified several risk foci in transmembrane protein-encoding genes. Herein, we investigated the effect of 4 such PD-associated genetic variants reported in Caucasians, including discs-large membrane-associated guanylate kinase scaffolding protein 2 (*DLG2* rs3793947), transmembrane protein 229B (*TMEM229B* rs1555399), glycoprotein nonmetastatic melanoma protein B (*GPNMB* rs199347), and integrin subunit alpha 8 (*ITGA8* rs7077361). A total of 1185 Taiwanese subjects comprising 592 PD patients and 593 unrelated age-matched controls were genotyped. *DLG2* rs3793947 AA genotype showed a significantly lower prevalence in female PD patients compared to the female controls ($p = 0.019$). The recessive model analysis also demonstrated a reduced PD risk for females in AA genotype (odds ratio = 0.573, 95% confidence interval: 0.379–0.868, $p = 0.008$). The frequencies of *TMEM229B* rs1555399 and *GPNMB* rs199347 genotypes and alleles were similar in PD patients and controls. *ITG8* rs7077361 was not polymorphic in all subjects of this study. These data suggested that *DLG2*, but not *TMEM229B*, *GPNMB*, and *ITGA8*, influenced the risk of PD in Taiwan.

1. Introduction

Compiling evidence supports a complex genetic and environmental contribution to Parkinson's disease (PD), with monogenetic forms of PD representing 5%–10% of cases in most populations (Gasser, 2001). Twenty years of genetic research in PD has identified mutations in several genes causing monogenetic forms of the disorder, namely *SNCA*, *LRRK2*, *PARKIN*, *PINK1*, *DJ-1*, *VPS35*, and *EIF4G1* (Puschmann, 2013). Genome-wide association study (GWAS) and subsequent meta-analysis have also identified at least 28 single-nucleotide polymorphism (SNP) variants that modify disease risk, with *SNCA*, *MAPT*, *GBA-SYT11*, *HLA-DQB1*, and *GAK-DGKQ* pointed as major risk foci for sporadic PD (International Parkinson Disease Genomics et al., 2011; Nalls et al., 2014; Pankratz et al., 2012). The majority of studies were conducted using samples from Caucasian populations (Do et al., 2011; Edwards et al., 2010; International Parkinson Disease Genomics et al., 2011; International Parkinson's Disease Genomics and Wellcome Trust Case; Control, 2011; Lill et al., 2012; Pankratz et al., 2012; Pihlstrom et al., 2013; Saad et al., 2011; Sharma et al., 2012; Simon-Sanchez et al., 2011). To date, only 2 Asian GWAS have been published (Foo et al., 2017; Satake et al., 2009). The Japanese GWAS identified *PARK16* and *BST1* as new loci and confirmed *SNCA* and *LRRK2* as risk loci (Satake et al., 2009). The East Asian GWAS did not identify Asian-specific loci, whereas it confirmed a strong association of *SNCA*, *LRRK2*, *MCCC1*, and 14 other previous reported loci with PD (Foo et al., 2017). These 2 Asian GWAS studies failed to demonstrate *MAPT* as risk loci and *MAPT* was nonpolymorphic in East Asian GWAS, indicating that some of the genetic loci for PD are ethnic specific.

Nevertheless, analyzing individual susceptibility variants is of limited value in explaining the mechanistic steps for complex diseases like PD wherein a combination of variants in key genes and pathways may influence fundamental disease processes. By combining pathways approaching for both GWAS and gene expression results, Edwards et al. identified 3 pathways potentially important for the pathogenesis of PD, including focal adhesion, axonal guidance, and calcium signaling (Edwards et al., 2011). These pathways are regulated largely by transmembrane proteins. More

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https://doi.org/10.1016/j.neurobiolaging.2017.11.016

than 6000 SNPs have been identified in *DLG2* that encodes a transmembrane protein as PD risk loci, and SNP rs3793947 is listed as one of the top results in Caucasian populations (http://www.pdgene.org/top_results). Along with 3 other PD SNPs in genes encoding transmembrane or membrane-associated proteins (*TMEM229B*, *GPNMB*, *ITGA8*), we sought to investigate whether transmembrane protein dysfunction could modulate the risk of developing PD in Taiwanese population.

2. Material and methods

2.1. Subjects

This study included a total of 1185 Taiwanese subjects comprising 592 PD patients and 593 controls, all of whom were enrolled from the Neurological clinics of Chang Gung Memorial Hospital-Linkou Medical Centre. The diagnosis of PD was made in accordance with the U.K. Parkinson's Disease Society Brain Bank clinical diagnostic criteria by 2 neurologists specialized in movement disorders (Y.-R.W and C.-M.C). Healthy control individuals of similar ethnic, gender, and age were from the same region as the PD patients. The mean age at onset of PD symptoms was 62.7 ± 11.1 years. The mean age of recruitment was 60.0 ± 12.7 years for controls. This study was conducted under a protocol proved by the institutional review boards of Chang Gung Memorial Hospital (ethical license number: 102-5614A3). All examinations were performed after obtaining written informed consents.

2.2. Genetic analysis

Four genetic loci (*DLG2* rs3793947, *TMEM229B* rs1555399, *GPNMB* rs199347, and *ITGA8* rs7077361) related to transmembrane proteins or membrane-associated proteins were selected from the risk foci identified by GWAS meta-analysis in PDGene database (http://www.pdgene.org/gwas). The SNP genotyping was performed by Agena MassARRAY platform with iPLEX gold chemistry (Agena, San Diego, CA, USA) following the manufacturer's protocol. The sequences of specific polymerase chain reaction (PCR) primers and extension primers (Supplementary Table S1) were designed with Assay Designer software package (v.4.0).

One μL of genomic DNA sample (10 ng/μL) was added to mutiplex PCR reaction in 5 μL containing 1 unit of Taq polymerase, 500 nmol of each PCR primer mix, and 2.5 mM of each deoxynucleotide (Agena, PCR accessory and Enzyme kit). Thermocycling was set at 94 °C for 4 minutes, followed by 45 cycles of 94 °C for 20 seconds, 56 °C for 30 seconds, and 72 °C for 1 minute, and 72 °C for 3 minutes. Unincorporated deoxynucleotides were deactivated using 0.3 units of shrimp alkaline phosphatase. The single base extension reaction was using iPLEX enzyme, terminator mix, and extension primer mix with thermocycling set up at 94 °C for 30 seconds, followed by 40 cycles of 94 °C for 5 seconds, and 5 inner cycle of 56 °C for 5 seconds, and 80 °C for 5 seconds, then 72 °C for 3 minutes (Agena, iPLEX gold kit). Following the addition of a cation exchange resin to remove residual salt from the reactions, 7 nL of the purified primer extension reaction was loaded onto a matrix pad of a SpectroCHIP (Agena). SpectroCHIPs were analyzed using a MassARRAY Analyzer 4 and the calling by clustering analysis with TYPER 4.0 software.

2.3. Statistical analysis

Hardy-Weinberg equilibrium for genotype frequencies of the patients and controls were assessed with an exact test. The Pearson's χ^2 test was used to compare the frequencies of genotypes and alleles between PD patients and controls. To account for the multiple comparisons, the 2-tailed p values <0.0125 were considered statistically significant using Bonferroni method. For the recessive model, we had a power greater than 0.8 to identify an association when the odds ratio was less than 0.6.

3. Results

A total of 1185 subjects were recruited in this study, including 592 patients with PD (female/male: 269/323) and 593 normal controls (female/male: 312/281). Only 1 proband in the 2-generation PD family was included to minimize the skew caused by the other family member carrying the same genetic polymorphism. The mean age of PD symptoms onset was 62.7 ± 11.1 years and that of controls upon recruitment was 60.1 ± 12.7 years. The distributions of *DLG2* rs3793947 AA genotype showed a trend of lower prevalence in the PD patients compared to the controls (19.6% vs. 23.1%) (Table 1). Further analysis separating all subjects by different genders revealed that the AA genotype is significantly less in female PD patients ($p = 0.019$), which became highly significant in the recessive model (odds ratio = 0.573, 95% confidence interval: 0.379–0.868, $p = 0.008$). The distributions of *DLG2* genotypes and the allele frequency did not differ between PD and controls in male subjects. The frequencies of *TMEM229B* rs1555399 (Table 2) and *GPNMB* rs199347 (Table 3) genotypes and alleles were similar in PD patients and controls. *ITG8* rs7077361 was not polymorphic in all subjects of this study (data not shown).

4. Discussion

This study shows that *DLG2* rs3793947 polymorphism AA genotype has a significant protective effect for PD. We failed to replicate the association of *GPNMB* and *TMEM229B* with PD shown by previous study in Caucasians (Nalls et al., 2014). It has been shown that *ITGA8* rs7077361 polymorphism, although associated with PD in Caucasians (Simon-Sanchez et al., 2009), is very rare in Chinese Han population (Fang et al., 2016). This study further suggested that *ITGA8* might not be polymorphic in Taiwanese population.

The relationship between *DLG2* and PD has been implicated in few GWAS reports (Foo et al., 2017; Fung et al., 2006; Nalls et al., 2014). Fung et al. suggested a trend that *DLG2* rs10501570 polymorphism reduced PD risk in Caucasians (Fung et al., 2006). Despite including Fung's data in their GWAS meta-analysis, Nalls et al. demonstrated the trend of protective effect for another *DLG2* polymorphism (rs3793947) in Caucasian PD patients (Nalls et al., 2014) (Table 4). A recent Asian PD GWAS demonstrated a consistent association at *DLG2* rs7479949, which was close to genome-wide significance (Foo et al., 2017). However, the association of *DLG2* rs3793947 with PD was not confidently imputed in this Asian GWAS. Our study further shows a strong protective effect of *DLG2* rs3793947 AA genotype in female Asian population. This is, to our best knowledge, the first report that *DLG2* rs3793947 minor allele is protective in female Asian PD patients.

DLG2 encodes a membrane-associated protein that belongs to a membrane-associated guanylate kinase protein family. *DLG2* has been shown to interact with glutamate receptors such as α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (Elias et al., 2006; Kruger et al., 2013) and Fyn-dependent tyrosine phosphorylation of NR2 subunits of *N*-methyl-D-aspartate receptors (Brenman et al., 1996; Chen and Roche, 2007; Levy et al., 2015; Tao et al., 2003). Glutamate-mediated excitotoxicity has been implicated as a pathogenic mechanism in PD. An increase in intracellular calcium levels by the excessive activation of *N*-methyl-D-aspartate receptors can activate cell death pathways and lead to apoptosis (Mody and MacDonald, 1995). Vaarmann et al. suggested that the absence of dopamine (DA) caused by striatal DA depletion in PD may result in an