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Interaction between SNCA, LRRK2 and GAK increases susceptibility to Parkinson's disease in a Chinese population

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ABSTRACT

PD is a complex disease, and may result from gene–gene and gene–environment interactions. There are limited studies on gene–gene interactions in PD. We and others have previously shown that SNCA rs356219, LRRK2 (rs2046932 and rs7304279) and GAK (rs1564282) are risk factors in sporadic PD. Since the expression of SNCA and neurotoxicity of alpha-synuclein are affected by LRRK2 and GAK, we hypothesize that their genetic risk variants may interact with each other. Here we investigated the interaction of SNCA rs356219, LRRK2rs7304279 and rs2046932 and GAK rs1564282 using the Multifactor Dimensionality Reduction (MDR) in a Chinese PD patient–control series (534 patients and 435 controls) and the cumulative risk effect of SNCA, LRRK2 and GAK. The MDR analysis showed a significant gene–gene interaction between the rs356219 of SNCA, rs2046932 of LRRK2 and rs1564282 of GAK. Moreover, individuals with increasing numbers of variants had an increasing likelihood of having PD, compared with those carrying none of the variants. The estimated OR for developing PD in individuals carrying 3 variants was 5.89. We demonstrated for the first time that SNPs in SNCA, LRRK2 and GAK interacted with each other to confer an increased risk of PD. In addition, PD risk increased cumulatively with the increasing number of variants.

1. Introduction

Parkinson's disease (PD; OMIM #168600) is one of the most common neurodegenerative diseases, affecting about 2% of people over the age of 65 [1]. The disease is characterized by resting tremor, rigidity, bradykinesia and postural instability, and associated with selective loss of dopamine neurons (DA) and formation of Lewy bodies (LBs) [2]. About 90% of cases are apparently sporadic, monogenetic mutations are now estimated to cause about 10% of PD cases [1]. Over the past two decades, numerous studies have been conducted to explore the genetic basis of PD. A recent large-scale meta-analysis of genome-wide association data in Europe has replicated 22 loci for Parkinson's disease (including SNCA, MAPT, LRRK2, BST1, GAK), while it has also reported 6 new risk loci (SIPA1L2, INPP5F, MIR4697, GCH1, VPS13C and DDRGK1) [3]. However, most reports just analyzed the association between single nucleotide polymorphism (SNP) and sporadic PD with small attributable risk. To date, few studies have evaluated gene–gene and gene–environment interactions.

SNCA is one of the causative genes of PD associated with alpha-synuclein in Lewy bodies [4,5]. Alpha-synuclein, encoded by SNCA, is the major fibrillar component of Lewy bodies. It is a presynaptic phosphoprotein with an intrinsic propensity to aggregate and has been demonstrated to have a role in both inherited and idiopathic PD [6]. Based on the critical role that alpha-synuclein may play in PD and functional connections with other identified genes, some efforts have been made in exploring the gene–gene interactions focusing on SNCA [7–10].

Besides alpha-synuclein, the LRRK2 proteins have also been identified in the Lewy bodies by immunohistochemical studies [11]. Mutations in LRRK2 are generally considered the most common genetic determinant of familial and sporadic PD. A recent study reported a strong interaction between LRRK2 and alpha-synuclein at both over-expressed and endogenous levels [12]. Moreover, LRRK2, which is thought to be an upstream factor in the neurodegenerative pathway, have also been shown to enhance alpha-synuclein-mediated cytotoxicity [13]. MAPT and SNCA may modify LRRK2-related risk for PD [14].

Another newly identified gene, named GAK, was one of the 137 genes differentially expressed in the substantia nigra pars compacta of PD patients when compared with controls, with a 1.56-fold change [15]. GAK was recently reported to have a modification on alpha-synuclein expression and toxicity [16]. In addition, GAK and two other proteins have been identified as binding partners of LRRK2 and these

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proteins form a complex that promotes clearance of Golgi-derived vesicles through the autophagy–lysosome system both in vitro and in vivo [17]. Based on these biological evidences, we hypothesize that the genetic risk variants of these genes may interact with each other.

In 2001, Ritchie et al. first developed a multifactor dimensionality reduction (MDR) method for detecting and characterizing high-order gene–gene and gene–environment interactions in case–control and discordant-sib-pair studies with relatively small samples [18]. The MDR method is nonparametric (i.e., no hypothesis about the value of a statistical parameter is made), is model-free (i.e., it assumes no particular inheritance model), and is directly applicable to case–control and discordant-sib-pair studies 3. So far, this new method has been successfully used for genetic studies of common complex multifactorial diseases [19].

In our previous studies, we have demonstrated that SNCA rs356219, LRRK2 (rs7304279 and rs2046932) and GAK rs1564282 increased the risk of sporadic PD in a Han population from mainland China [20–22]. Here we examined the interaction using the MDR method and cumulative risk effect of SNCA, LRRK2 and GAK. To our knowledge, this is the first attempt to explore the interactions between SNCA, LRRK2 and GAK.

2. Subjects and methods

2.1. Subjects

A total of 969 ethnic Han Chinese study subjects comprising 534 independent sporadic PD patients and 435 neurologically healthy control individuals were recruited from the Department of Neurology at West China Hospital. All patients (the mean age 58.30 ± 11.05, range from 30 to 86, 41.1% women) were examined and evaluated longitudinally by two movement disorders neurologists and diagnosed with idiopathic PD according to the UK Parkinson's Disease Society Brain Bank Clinical Diagnostic Criteria [23]. None of the patients had a positive family history. All control individuals (the mean age 52.30 ± 14.11, range from 30 to 91, 43.9% women) were healthy volunteers without any neurological or psychiatric diseases and were recruited from the same ethnic group. Written informed consent was obtained from all participants. DNA was extracted from blood leukocytes by standard procedures. This study was approved by the Ethics Committee of Sichuan University.

2.2. Genetic analysis

All participants were genotyped by using a matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry on a MassArray system (Sequenom, San Diego, USA). Approximately 15 ng of genomic DNA was used to genotype each sample. Locus-specific polymerase chain reaction (PCR) and detection primers were designed using the MassArray Assay Design 3.0 software (Sequenom, San Diego, USA). The sample DNAs were amplified by primers flanking the targeted sequence, followed by dephosphorylation and allele-specific primer extension. Cleaned extension products were loaded into a 384-format Spectro-Chip, and subjected to MALDI-TOF mass spectrometry. The resultant data were analyzed by the Sequenom MassArray Typer software (Sequenom, San Diego, USA). The methods were carried out in accordance with the approved guidelines.

2.3. Statistical analysis

We assessed Hardy–Weinberg equilibrium (HWE) in cases and controls with a Fisher's exact test. The frequencies of the alleles and genotypes in the patients and control groups were analyzed using the Chi-square test. The clinical data were analyzed by student T test or Mann–Whitney U test. A two-tailed P-value ≤ 0.05 was considered statistically significant. Statistical analysis was performed using the Statistical Package for the Social Sciences version 16.0 (SPSS, Chicago, IL, USA) for Windows.

We used the MDR to analyze the gene–gene interactions between SNCA, LRRK2 and GAK. MDR is a powerful method for analyzing interactions. It pools genotypes into "high-risk" and "low-risk" groups to reduce multidimensional data into one-dimension [18]. The specific methods and procedures of MDR are introduced by Ritchie et al. [18]. The classification errors and the prediction errors are estimated by 10-fold cross-validation. Additionally, 10-fold cross-validation consistency (CVC) and permutation test are used to select the final best model.

Chi-square test was used to compare the clinical manifestations with different numbers of associated variants.

3. Results

3.1. Characteristics of participants

Data from a total of 969 individuals including 534 sporadic PD patients and 435 healthy controls were analyzed. All polymorphisms were in Hardy–Weinberg equilibrium for PD patients and controls. The mean age of PD patients, which consisted 41.1% women, is 58.30 ± 11.05 years, ranging from 20 to 80. The mean age of the healthy controls is 52.30 ± 14.11 years, a little younger than the PD groups.

3.2. Associations between each single nucleotide polymorphism with PD susceptibility

Some of the data for each single nucleotide polymorphism (rs356219, rs7304279, rs2046932 and rs1564282) have been reported in our previous papers which showed that these are risk variants [20–22]. Here, we included 969 subjects who have full genotype information of the four SNPS, to do the gene–gene interaction analysis. Subjects with GG + GA genotypes of rs356219 have an increased risk compared to those with AA genotype. So did the TT + CT genotypes of rs7304279, TT + TC genotypes of rs2046932 and TT + TC genotypes of rs1546282 (Table A.1).

3.3. MDR analysis of gene–gene interactions between SNCA (rs356219), LRRK2 (rs7304279, rs2046932) and GAK (rs1564282)

Gene–gene interactions were investigated for PD using MDR method, and two significant interactions were found (Table A.2). Compared with the other models, a two-locus model incorporating rs356219 and rs2046932 was the best with the maximum testing accuracy of 0.6091 and cross-validation consistency of 10 out of 10. However, the best three-locus model including rs356219, rs2046932 and rs1564282 also had a maximum cross-validation consistency of 10 and higher testing accuracy of 0.6059, secondary to the best two-locus model. The best two- and three-locus models were both significant at $P < 0.05$. Though subtle difference existed, to pinpoint the polymorphisms that are of particular interest, the three-locus model was regarded as the overall best MDR model in our study.

3.4. Logistic regression analysis

We have also explored a combination of the three interactive SNPs (SNCArs356219, LRRK2rs2046932 and GAKrs1564282) with the risk of having PD. Compared with those carrying none of the variants, individuals carrying one variant (OR = 2.40, 95% CI = 1.46, 3.94, $P = 4.05 \times 10^{-4}$), carrying two variants (OR = 3.52, 95% CI = 2.06, 6.02, $P = 2.0 \times 10^{-6}$) and carrying three variants (OR = 5.89, 95% CI = 2.08, 16.68, $P = 3.86 \times 10^{-4}$) increased the risk of sporadic PD (Table A.3). The estimated OR for developing PD in individuals carrying 2 variants was 3.52. The estimated OR for developing PD in individuals carrying 3 variants was 5.89.