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Variants in the *SNCA* gene associate with motor progression while variants in the *MAPT* gene associate with the severity of Parkinson's disease

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ABSTRACT

Introduction: It is well known that α-synuclein (*SNCA*) and microtubule associated protein (*MAPT*) genes predispose individuals to develop Parkinson's disease (PD). However, whether these genes contribute to differences in the variable progression observed in PD is obscure. This study aims to evaluate the association of common variants in *SNCA* (rs11931074, rs894278) and *MAPT* (rs242557_H1c haplotype, rs3744456) genes with the severity and duration of motor and cognitive performance.
Methods: 296 Chinese patients with PD were recruited from Shanghai Ruijin Hospital. Motor performance was assessed using the Unified Parkinson's Disease Rating Scale (UPDRS-III) and Hoehn &Yahar (H&Y) stages and cognitive performance using the Mini-Mental Status Examination (MMSE). Genetic associations were analysed using general linear modelling for severity and Cox regression analysis for duration to motor (UPDRS-III≥36 or H&Y ≥ 3, average duration 13 years) and cognitive (MMSE<27, average duration 8 years) cutoffs, covarying for age and gender.
Results: The severity of motor function associated with synergic interaction of *SNCA* (rs11931074) and *MAPT* (rs3744456) ($p \leq 0.05$) while longer survival to the motor cutoff associated with *SNCA* (rs11931074/T, HR = 0.4, $p = 0.03$). Increased severity of cognitive function associated with *MAPT* (H1c haplotype, $p = 0.05$) with none of the risk alleles chosen associated with survival to the cognitive cutoff ($p > 0.05$).
Conclusion: Our findings add further data showing that common variants in *SNCA* and *MAPT* genes contribute to variability in progression of PD, with *SNCA* variants associating with motor progression while *MAPT* variants associated with clinical severity.

1. Introduction

Both α-synuclein (*SNCA*) and microtubule associated tau (*MAPT*) genes contribute to susceptible for Parkinson's disease (PD) [1–4]. On average, patients with duplication of the *SNCA* gene have a relatively later age of PD onset and more benign disease course [5] compared with patients with *SNCA* triplication who have a more rapid disease course with cognitive impairment [6]. This suggests that variation of gene dosage in *SNCA* influences the progression of PD.

Racial genetic variation is known to influence disease risk and presentation. In Caucasian populations, the *SNCA* Rep1 polymorphism has been associated with different motor outcomes [7–9], consistent with its associations to increased PD risk [10] and variation in PD onset [11,12]. In Caucasians, the H1 haplotype of *MAPT* has been associated with dementia onset in PD [9] with the H1c-specifiers 242557/A allele having higher *MAPT* promoter activity [13]. By contrast, in Chinese PD populations, genome-wide association studies (GWAS) have identified the rs894278 and rs11931074 SNPs in *SNCA* as conferring the strongest PD risk [4] with the rs11931074/T and rs894278/G alleles of *SNCA* increasing PD risk [4,14] and earlier PD onset [15]. In Chinese, the rs3744456 SNP of *MAPT* has been associated with modifying PD risk [16] with

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its C allele up-regulating the expression of *MAPT* [17].

Motor disability and cognitive decline have a major influence on quality of life in patients with PD [18]. After contracting PD it is difficult to predict the progression of these factors or disease duration in general [19,20]. The progression of motor deterioration varies significantly in PD patients [8] with dementia becoming a significant feature over time [21–23]. Prognostic information on these factors for patients with PD is important for their disease management and the development of therapies targeting their underlying mechanisms. There is limited biological information regarding factors that influence these motor and cognitive outcomes in PD.

To date there have been no studies evaluating the influence of *SNCA* and *MAPT* gene variants to PD motor and cognitive phenotypes (both severity and progression) in Chinese, although *SNCA* rather than *MAPT* has been associated with variation in PD onset [15]. Therefore, the present study aims to investigate whether known risk alleles in the *SNCA* and *MAPT* genes associate with the phenotypes (severity and duration) of both motor and cognitive performance in Chinese PD patients.

2. Methods

2.1. Clinical subjects and assessments

296 Chinese patients with PD were consecutively recruited from the Neurology department of Ruijin Hospital, affiliated with Shanghai Jiaotong University for this study. This study was approved by the ethics committee of the faculty of medicine, Shanghai Jiaotong University. All patients satisfied the Queen Square Brain Bank Criteria for clinical diagnosis of PD [24] and signed the informed consent. Demographic data, including age at enrolment, age at onset, gender, education, family history, medical history and current medication use were recorded. Motor function for each patient was assessed using the Unified Parkinson's Disease Rating Scale (UPDRS-III) and Hoehn &Yahar (H&Y) stages. Cognitive function was assessed using Mini-Mental Status Examination (MMSE), composed of five cognitive domains, ie. orientation (time and location, total 10 points), memory (registration and delayed recall, total 6 points), attention and calculation (total 5 points), language (naming and repetitive, total 3 points), and complex commands (total 6 points). Performances on each cognitive domain as well as total MMSE score were recorded (Supplementary Table 1). A small subset of 22 patients was available to return for longitudinal follow-up using the same tests after 4 years. The median time taken from H&Y stage 1 to 2, 2 to 2.5, 2.5 to 3, 3 to 4, and 4 to 5 was 20, 62, 25, 24, and 26 months, respectively. Patients who progressed through H&Y stages with less than half of median time were categorized as fast progressors [25].

2.2. Genetic analysis

To exclude genetic forms of PD in patients ≤40 years old, exonal mutations in Parkin and PINK1 genes were assessed using sequencing methods, and deletions and multiplications of Parkin gene detected using quantitative PCR according to the established protocols [26,27].

Genomic DNA samples were extracted from peripheral blood using conventional phenol/chloroform extraction method. *MAPT* SNP rs3744456 and *SNCA*SNP rs894278 were genotyped using direct sequencing (3730xl DNA analyser, Applied Biosystems, Foster City, CA, USA). DNA fragments of *MAPT* covering rs11931074 (F: ACCTATCTATTCCGCCCATCC, R: TAGCCAAATCTATAAGAGCAACAC) or rs2425577 (F: GACACTAATAAGGGAAAATCTC R: GACTGTGGAAGGCTCTGA) were amplified and the PCR products digested using the restriction enzymes BsrI (invitrogen) and APaLI (invitrogen) respectively. The rate of genotype calls was ≥95% for all SNPs. For variants identified by genotyping, further confirmation via direct PCR product sequencing was performed on random samples.

2.3. Statistics

All statistical analyses were performed using SPSS software (IBM SPSS statistics 22, 32-bit edition) with a p-value of ≤0.05 considered as significant. Two primary genetic association outcomes were assessed.

Associations to the severity of motor and cognitive performance were assessed using univariate general linear regression modelling. The univariate dependent variables were the severity scores (UPDRS-III, H&Y stages or MMSE scores), the fixed factor was the SNP variability, and the covariates were gender, years of education, age at assessment, and disease duration. To determine whether any SNPs associated with a particular cognitive domain, multivariate general linear regression modelling was used with the multivariate variables the cognitive domain scores using the same fixed factors and covariates.

To assess associations to disease progression, survival to motor and cognitive cut off scores were assessed using Cox regression modelling and bias corrected accelerated boostrapping. For the Cox regression analysis, cut off status for motor performance was defined as UPDRS-III ≥ 36 and/or H&Y stage ≥3 (15% of the cohort) and for cognitive performance a MMSE score <27, while time-to-event was defined as disease duration (age at enrolment minus age at onset). *SNCA* and *MAPT* genetic information was used as covariates in the first block, while gender and age at recruitment (and years of education for cognitive outcome) were used as covariates in block 2. Hazard ratios (HR) for genetic variation were determined and the associations visualised using Kaplan–Meier survival plots with postoc Log rank, Breslow and Tarone–Ware tests for confirmation of significance.

To validate predictions of genetic associations to disease progression, sensitivity, specificity and positive and negative predictive values were determined for dichotomised SNP variability versus faster disease progression as assessed longitudinally.

3. Results

3.1. Demographic and clinical characteristics

At enrolment, 72.3% of the PD patients had been diagnosed within 5 years (average ± SD disease duration 4.7 ± 4.2 years, range 2 months–28 years). 3.4% of PD patients (10) had disease onset ≤ 40 years, with 22.7% having onset ≤ 50 years and 88.2% ≤ 70 years. Neither Parkin gene deletions or multiplications, nor homogenous Parkin or PINK1 gene mutations were identified. About 30% had tertiary education, 15% had a UPDRS-III score ≥36, 26% had a H&Y stage ≥3 and 61.4% had a MMSE score ≥27 (Supplementary Table 1). The most common cognitive domain impairment (below 2 points of norms) was in attention/calculation (20.6%), followed by memory (19.1%), complex commands (9.2%), and then orientation (3.8%, Supplementary Table 2).

3.2. SNCA and MAPT SNP association with motor severity and progression

Both the T allele of rs11931074 *SNCA* SNP and G allele of rs3744456 *MAPT* SNP related to motor severity scores using univariate analyses, although this association was not significant for either gene independently (Table 1). There was an increase in