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Synaptotagmin XI in Parkinson's disease: New evidence from an association study in Spain and Mexico

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ABSTRACT

Introduction: The pathophysiology of PD (Parkinson's disease) has been related to the ubiquitin proteasome system and oxidative stress. Parkin acts as ubiquitin ligase on several substrates. Because genetic variants often have different frequencies across populations, population specific analyses are necessary to complement and validate results from genome-wide association studies.
Methods: We carried out an association study with genes coding for parkin substrates and cellular stress components in the Galician population (Northern Spain). *SNCA* and *MAPT* SNPs were also analyzed. We studied 75 SNPs in a discovery sample of 268 PD patients and 265 controls from Galicia. A replication sample of 271 patients and 260 controls was recruited from Mexico City.
Results: We observed significant association between PD and SNPs in *MAPT*. Nominal p-values < 0.05 were obtained in the Galician cohort for SNPs in *SYT11*, coding for synaptotagmin XI. These results were replicated in the Mexican sample.
Discussion: The associated markers lie within a ~140 kb strong linkage disequilibrium segment that harbors several candidate genes, including *SYT11*. SNPs from the *GBA-SYT11-RAB25* region have been previously associated with PD, however the functionally relevant variants remain unknown. Our data support a likely role of genetic factors within 1q22 in PD susceptibility.

1. Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder after Alzheimer's. Around 1–2% of subjects over 60 years in European populations suffer from PD. There is increasing evidence that genetic factors contribute to sporadic PD [16]. Although sample sets from large consortia assure statistical power, independent replication studies are important to validate risk variants identified in genome-wide association studies (GWAS). Additional questions are how Mendelian genes or their interactors act on the sporadic forms of a disease, and what gene is truly relevant within an associated genetic region.

While mutations in *PARK2* account for up to 77% early-onset PD [10], its function in late-onset PD is unclear. A ubiquitin ligase (E3) role has been attributed to parkin, the protein product of *PARK2*, as part of the ubiquitin proteasome system (UPS) [10]. Some known or alleged substrates for parkin are synphilin 1, septin 5, Pael-R, synaptotagmin XI (SYT11) or cyclin E [21]. The association between *SYT11* in 1q22 and PD, recently suggested, needs confirmation [17]. Our aim was to carry

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out a candidate-gene association study in PD with our patient population including parkin substrates and some genes previously associated to PD.

2. Methods

2.1. Recruitment of study subjects

The target of this study was the population of Galicia, a 29,574.4 km^2 territory in Northern Spain with 2,731,406 people as of January 2015 (http://ine.es). We recruited 279 unrelated patients and 288 control individuals of Caucasian origin, with the following criteria: 1) diagnosis of idiopathic PD according to Gelb criteria [4], 2) at least three grandparents of Galician origin, and 3) Galician independent controls older than 60, neurologically intact and without a family history suggestive of a neurodegenerative disorder. 36 patients (13.1%) reported possible PD or other movement disorder (e.g. tremor, unsteady gait, cognitive decline) in a relative. PD patients with early onset disease or with a family history indicating Mendelian inheritance were not included. All patients with a family history were tested for the most frequent *LRRK2* mutations (exons 31, 35 and 41) and positive cases were excluded from further analysis.

We obtained a replication sample from 271 Mexican patients diagnosed according to the UK PD Society Brain Bank criteria [9]. Control subjects were 260, all of them over 40 with no family history of movement disorders. Patients and controls were Mexican Mestizo for at least three generations, and all of them were natural from the central area of Mexico. Both Galician and Mexican local institutional review boards approved the study and all participants signed written informed consent.

2.2. Genotyping of single nucleotide polymorphisms (SNPs) in candidate genes

We carried out a genetic association analysis of eight candidate genes that are substrates of parkin and participate in the stress response pathway: *CCNE1*, *GPR37*, *SEPT5*, *SNCAIP*, *SYT11*, *CRYAB*, *NOB1*, and *SKP1*. We also included the genes *PRDM2* and *SEMA5A*, previously associated to PD in a GWAS [12], as well as *SNCA* and *MAPT*, both consistently identified as susceptibility factors in PD (all genes and polymorphisms analyzed are listed in Supplementary material). We used CEU HapMap database and Haploview 4.2 software to select 115 tag SNPs (single nucleotide polymorphisms) with $r^2 \leq 0.85$ and minor allele frequency (MAF) > 0.10.

We obtained DNA from whole blood using the Wizard® Genomic DNA Purification Kit (Promega Corporation, Madison, WI). Genotyping was carried out with the SNPLex® platform for 115 SNPs in the Galician sample and with MassArray (Sequenom®) for 3 SNPs in the replication sample.

2.3. Data analysis

We calculated statistical power using Quanto v.1.2.4 software. We carefully checked population ancestry. All patients and controls of the discovery sample were from Galicia, North-West Spain. Genotyping quality control followed recommendations for candidate gene association studies [1]. We applied exact test to detect differences in genotyping rates between cases and controls. We excluded from further analysis samples with call rate < 0.80, SNPs with call rate < 0.95 and those deviating from Hardy Weinberg (HW) equilibrium in controls ($p < 0.01$).

We performed association analysis with the SNPassoc R package using logistic regression under a log-additive model. We determined statistical significance with likelihood ratio test. False Discovery Rate (FDR) correction for multiple testing was done with the q-value R package. Linkage disequilibrium (LD) and haplotype analysis both in the study sample and 1000 Genomes Project data (Phase 3 1000 Genome Project variant set, 20130502, EUR set) were obtained with Haploview and vcftools v0.1.12. For haplotype definition, we only considered phased SNPs with MAF ≥ 0.10. We applied the solid spine of LD algorithm.

For those genes with SNPs showing nominal association ($p < 0.05$), imputation of additional variants was performed using IMPUTE v2.2 and a multi-population reference panel [8]. The software automatically selects a reference panel for each individual being imputed. Markers that met the quality control criteria were used for imputation. Association analysis of both imputed and genotyped- variants was carried out with SNPTESTv2 [13] and the results were compared to PDGene meta-analysis values and previously reported associations in Spanish population.

Those genes with nominally significant associations and not yet validated in Spanish samples were subsequently analyzed in the replication sample. Association analysis of genotyped and imputed variants as described above was also carried out for the replication sample.

3. Results

Out of the initial 115 SNPs, we dismissed 40 for final analysis (27 due to a call rate under 95%, 10 had a MAF under 10%, 2 were not in HW equilibrium in control subjects and for 1 the genotyping rate in cases and controls was significantly different). After exclusion of samples with a genotype call rate under 80%, the final analysis set in the discovery sample consisted of 75 SNPs genotyped in 268 patients (mean onset age: 60.7 years, 45.9% female) and 265 controls (mean age: 72.2 years, 56.2% female).

Under a log-additive model, our study had 80% power to detect effect sizes (OR ≥ 1.7) for SNPs with MAF ≥ 0.10. We found nominally significant association for polymorphisms in *MAPT*, *SNCA* and *SYT11*. Only the association with rs2435207 in *MAPT* remained significant after FDR (Table 1). In the discovery sample, two *SYT11* SNPs (rs3820594 and rs729022) showed nominal association with PD, whereas a third one (rs12563627) fell short of reaching significance. Imputation of the reported PD-associated *SYT11-RAB25* variant rs34372695 yielded no significant results.

The replication sample from Mexico was then analyzed to verify association with *SYT11* SNPs. The same genotyping criteria described above were applied to the Mexican sample. Due to assay design constraints, rs3820594 was replaced by the proxy variant rs822508 (distance: 21 kb, $r^2 = 0.97$). All three SNPs in *SYT11*, which are in complete LD ($r^2 \geq 0.98$), showed nominally significant association with PD. In this sample, genotype imputation of rs34372695 also showed significant association (Table 1). The LD map of this region, including SNPs reported here as well as in previous studies, is shown in Fig. 1.

Table 2 shows the comparison of the results obtained for both genotyped and imputed markers with PDGene meta-analysis values and previously reported associations in Spanish population.

4. Discussion

Herein we report a candidate gene association study with PD carried out in two genetically different populations (Galician and Mexican-Mestizo). We focused our analysis in substrates of parkin, a ubiquitin-ligase that participates in the ubiquitin-proteasome degradation system [6], as well as NOB1, also related with the degradation of ubiquitinylated proteins. Four genes that had been previously associated with PD but not studied in the Galician population – *SNCA*, *MAPT*, *PRDM2* and *SEMA5A* – were also included in our study. We found association of *MAPT* and *SNCA* markers to PD in the discovery sample. Association between these genes and PD has been widely validated elsewhere, including Asturias, a Spanish region close to Galicia [14]. The significant association observed in our study with rs2435207 in *MAPT* even after