

RESEARCH NOTE

Exploration of genetic susceptibility factors for Parkinson's disease in a South American sample

BRUNO A. BENITEZ^{1,5†}, DIEGO A. FORERO^{1,5,6†}, GONZALO H. ARBOLEDA^{1,4,5}, LUIS A. GRANADOS^{1,2}, JUAN J. YUNIS^{1,4,5}, WILLIAM FERNANDEZ^{1,2,5} and HUMBERTO ARBOLEDA^{1,3,5*}

¹Neurosciences Research Group, School of Medicine and Institute of Genetics, Universidad Nacional de Colombia, Bogotá, Colombia

²Department of Internal Medicine, ³Department of Pediatrics, ⁴Department of Pathology, School of Medicine, Universidad Nacional de Colombia, Bogotá, Colombia

⁵MSc Program in Neurosciences, Universidad Nacional de Colombia, Bogotá, Colombia

⁶Present address: Biomedical Science Research Group, School of Medicine, Universidad Antonio Nariño, Bogotá, Colombia

Introduction

The genetic susceptibility factors for Parkinson's disease (PD) in non-European populations, including those from Latin American countries, are unknown (Thomas and Beal 2007). The objective of this study was to explore the possible association of polymorphisms in eight candidate genes with PD in a Colombian sample. We analysed common polymorphisms in eight candidate genes (*A2M*, *ACE*, *BDNF*, *COMT*, *MAPT*, *SLC6A3*, *SLC6A4* and *UCHL1*) in a clinic-based sample of 104 PD patients and 136 controls from Colombia, South America. We did not find significant allele or genotype associations for single markers. We found significant evidence of gene–gene interactions for the markers in the *A2M*, *SLC6A4* and *UCHL1* genes in our PD sample. This is the first systematic study of multiple common genetic markers for PD in Latin America. Further exploration of additional candidate genes will be helpful to identify the precise variants related to PD in non-European populations (Thomas and Beal 2007).

Parkinson's disease is a common neurodegenerative disorder, clinically characterized by tremor, bradykinesia, rigidity and postural instability, and neuropathologically by the loss of dopaminergic neurons in the substantia nigra and the presence of Lewy bodies (Thomas and Beal 2007). In recent

years, several genes responsible for familial forms of the disease have been discovered (*LRRK2*, *PARK2*, *PARK7*, *PINK1* and *SNCA*) and a large number of studies have been carried out to explore genetic factors associated with non-familial forms of PD (Thomas and Beal 2007). These studies have been focussed in the analysis of a number of candidate genes through case–control association studies, mainly in populations of European descent (Thomas and Beal 2007).

In the current work, we report the results of a systematic analysis of eight candidate genetic polymorphisms for PD in a South American population. We selected eight candidate genes that are related to different pathophysiological hypotheses of PD (Thomas and Beal 2007): alpha-2-macroglobulin (*A2M*, 12p13.3-p12.3), angiotensin I converting enzyme (*ACE*, 17q23.3), brain-derived neurotrophic factor (*BDNF*, 11p13), catechol-O-methyl transferase (*COMT*, 22q11.21), microtubule associated protein tau (*MAPT*, 17q21.1), dopamine transporter (*SLC6A3*, 5p15.3), serotonin transporter (*SLC6A4*, 17q11.1-q12) and ubiquitin carboxyl-terminal esterase L1 (*UCHL1*, 4p14).

Materials and methods

Subjects

Hundred and four PD patients (age at evaluation, 60.1 ±12.6 years; age of onset, 53.7 ±13.4 years; % early onset PD, 40.3; % male, 50.9) and 136 unaffected controls (age at evaluation, 62.4 ±9.5 years; % male, 46.6) were included in the present study ($P > 0.05$ for differences in age and sex distributions between patients and controls). Patients were evaluated by an interdisciplinary team led by a neurologist (WF) with advanced expertise in movement disorders

*For correspondence. E-mail: harboledag@unal.edu.co.

†These authors contributed equally to this work.

The authors reported no biomedical financial interests or potential conflicts of interest.

[Benitez B. A., Forero D. A., Arboleda G. H., Granados L. A., Yunis J. J., Fernandez W. and Arboleda H. 2010 Exploration of genetic susceptibility factors for Parkinson's disease in a South American sample. *J. Genet.* **89**, 229–232]

Keywords. human genetics; candidate genes; Latin America; Parkinson's disease; polymorphisms.

(Jenkins *et al.* 1992); final diagnoses were carried out with basis on the United Kingdom Parkinson's Disease Society brain bank diagnostic criteria (Jenkins *et al.* 1992). Control subjects were evaluated by general physicians and did not have personal or family history of neurodegenerative disorders. Patients and controls were living in Bogotá, the capital city of Colombia (around eight million inhabitants in 2007); a population that is composed of a southern European genetic background with historical admixture with Amerindians (Yunis *et al.* 2005). All subjects, or their healthy relatives, signed an informed consent following international recommendations (Forero *et al.* 2006a) and the project was approved by the respective institutional research and ethics committees.

Genotyping

The polymorphisms analysed in this study are: a non-synonymous SNP in exon 24 of *A2M* (Val1000Ile; rs669), an insertion/deletion polymorphism in intron 15 of *ACE* (rs1799752), a non-synonymous SNP in exon 2 of *BDNF* (Val66Met; rs6265), a non-synonymous SNP in exon 4 of *COMT* (Val158Met; rs4680), an insertion/deletion polymorphism in intron 10 of *MAPT* (*MAPT* indel), a *VNTR* in exon 15 of *SLC6A3* (rs28363170), a *VNTR* in the promoter region of *SLC6A4* (rs4795541) and a non-synonymous SNP in the exon 3 of *UCHL1* (Ser18Tyr; rs5030732).

Genomic DNA was extracted from peripheral blood using a modified salting-out protocol (Forero *et al.* 2006a). Genotyping protocols used in our laboratory for the analysis of rs1799752 (*ACE*; PCR, electrophoresis), rs6265 (*BDNF*; PCR, RFLP), rs4680 (*COMT*; Bi, PASA), rs4795541 (*SLC6A4*; PCR, electrophoresis) and rs5030732 (*UCHL1*; PCR, RFLP) polymorphisms have been described in our previous publications (Forero *et al.* 2006a,b,c). Details about genotyping protocols for rs669 (*A2M*; PCR, RFLP),

MAPT indel (*MAPT*; PCR, electrophoresis) and rs28363170 (*SLC6A3*; PCR, electrophoresis) polymorphisms have been described elsewhere (Cook *et al.* 1995; Liao *et al.* 1998). A random subsample of patients and controls were reanalysed to confirm the consistency of the genotyping procedures.

Statistical analysis

Comparisons were carried out for allelic and genotype frequencies between patients and controls using the Fisher's exact and χ^2 tests; *P* values <0.05 were considered as statistically significant. Hardy-Weinberg equilibrium (HWE) was calculated for both groups. SNPStats and GraphPad Instat programs (Sole *et al.* 2006) were used for these statistical analyses. MDR program (Hahn *et al.* 2003) was used for the exploration of gene-gene interactions: genotype data for all eight polymorphisms for patients and controls were entered in the program and the 'exhaustive' search method was selected (attribute count range: 1-3). Calculations of statistical power were carried out with the Genetic Power Calculator tool (Purcell *et al.* 2003). An in-house developed program for genetic analysis (written in Python 2.5) was also used as an additional tool to integrate and automate calculation of relevant parameters. Information for available meta analysis for the set of candidate markers was retrieved from PDGene database (Bagade *et al.* 2009), a useful resource for neuropsychiatric genetics (Bertram *et al.* 2007).

Results

Allele and genotype frequency comparisons between patients and controls are shown in table 1. All markers were in HWE in patients and controls. We did not find statistically significant differences for allele or genotypes for any of these eight candidate polymorphisms. The current sample size provides a power of 0.7 (considering the MAFs found in our control

Table 1. Genotype and allele frequencies of eight candidate polymorphisms in PD cases and healthy controls in a Colombian sample.

Gene (Allele 1/allele 2)	<i>A2M</i> (A/G)	<i>ACE</i> (Ins/del)	<i>BDNF</i> G/A	<i>COMT</i> G/A	<i>MAPT</i> (Ins/del)	<i>SLC6A3</i> (10/9)	<i>SLC6A4</i> (s/l)	<i>UCHL1</i> (C/A)
Genotype 1/1 cases	31 (31.6)	28 (26.9)	68 (68.0)	47 (45.6)	77 (74.8)	65 (65.7)	26 (25.0)	45 (43.7)
Genotype 1/2 cases	48 (49.0)	48 (46.2)	26 (26.0)	39 (37.9)	23 (22.3)	31 (31.3)	53 (51.0)	49 (47.6)
Genotype 2/2 cases	19 (19.4)	28 (26.9)	6 (6.0)	17 (16.5)	3 (2.9)	3 (3.0)	25 (24.0)	9 (8.7)
Allele 1 cases	110 (56.2)	104 (50.0)	162 (81.0)	133 (64.6)	177 (85.9)	161 (81.3)	105 (50.5)	139 (67.5)
Allele 2 cases	86 (43.8)	104 (50.0)	38 (19.0)	73 (35.4)	29 (14.1)	37 (18.7)	103 (49.5)	67 (32.5)
Genotype 1/1 controls	40 (35.7)	45 (33.1)	97 (71.3)	52 (39.1)	94 (70.7)	77 (58.8)	41 (30.4)	66 (52.8)
Genotype 1/2 controls	48 (42.9)	58 (42.6)	36 (26.5)	68 (51.1)	31 (23.3)	49 (37.4)	67 (49.6)	48 (38.4)
Genotype 2/2 controls	24 (21.4)	33 (24.3)	3 (2.2)	13 (9.8)	8 (6.0)	5 (3.8)	27 (20.0)	11 (8.8)
Allele 1 controls	128 (57.1)	148 (54.5)	230 (84.5)	172 (64.6)	219 (82.3)	203 (77.5)	149 (55.2)	180 (72.0)
Allele 2 controls	96 (42.9)	124 (45.5)	42 (15.5)	94 (35.3)	47 (17.7)	59 (22.5)	121 (44.8)	70 (28.0)
<i>P</i> allele	0.84	0.35	0.32	1.00	0.31	0.35	0.31	0.30
<i>P</i> genotype	0.67	0.58	0.32	0.08	0.50	0.56	0.58	0.35

Relative frequencies (%) are given in parentheses.

subjects) to detect markers with effects characterized by ORs from 2.0 (*BDNF*) to 2.3 (*ACE*). We explored the potential existence of gene–gene interactions in our samples (using the MDR program) and found a statistically significant evidence of interaction between the polymorphisms in *A2M*, *SLC6A4* and *UCHL1* genes (global $P = 0.0107$, for the best model) and the risk for PD. We found that a specific combination of genotypes for the three markers are significantly more frequent in patients than in controls ($P = 0.04$) (table 2).

Table 2. Significant results from the analysis of gene–gene interactions. The composition of the significant combination of genotypes for the three markers are given, including the respective relative frequencies in patients and controls and its P value.

<i>A2M</i> (rs669)	<i>SLC6A4</i> (rs4795541)	<i>UCHL1</i> (rs5030732)	% Patients	% Controls	P value
AG	sl	AA	6.18	0.90	0.04

Discussion

Molecular genetics of PD has been largely explored in populations of European descent (Thomas and Beal 2007; Okubadejo 2008); the possible role of these candidate genes for PD in developing regions is largely unknown. This contrasts with the fact that the impact of PD, and other age-related diseases, is predicted to be much higher in developing countries in future years (Dorsey *et al.* 2007). In the current work, we report the initial results of a multidisciplinary effort aimed to the analysis of multiple genetic risk factors for PD in a case control sample in Colombia, a South American population; being the first systematic study of several common genetic markers for PD in Latin America. A previous paper reported an association with a single polymorphism in *APOE* gene in a PD sample from Mexico (López *et al.* 2007).

Mean life expectancy for Colombia was 72.3 years in 2005 (Forero *et al.* 2006b). A previous population-based study showed that the crude overall prevalence of parkinsonism in Colombian population is around 101/100,000 inhabitants (Pradilla *et al.* 2003); this translates in an estimated total number of around 198,000 patients in the country. There is a high variability in the prevalence rates reported for parkinsonism in different populations around the world, such as those found in other developing countries (50/100,000 in Bolivia) (Dorsey *et al.* 2007) and in developed regions (168/100,000 in Italy and 128/100,000 in United Kingdom) (Dorsey *et al.* 2007).

We have analysed eight genetic markers that have been shown as potential risk factors for PD in different populations (Thomas and Beal 2007). All eight polymorphisms are common variants (all of them have MAF >0.15 in our sample) and six of them are known to have allele-specific functional effects (Forero *et al.* 2006a,d). There are 88 published studies analysing these eight markers, 57 of them (65.0%) were carried out in populations of European descent (Bagade *et al.* 2009). Two of the markers analysed in the current work

(*MAPT* and *UCHL1*) appear as two of the top 17 genes for PD in the PDGene database with ORs of 0.74 for *MAPT* (20 studies) and 0.93 for *UCHL1* (12 studies) in samples from Caucasian populations.

Using single marker analysis, we did not find significant differences in allele or genotype frequencies for any of these eight polymorphisms in the current PD sample. We found statistical evidence of gene–gene interaction for the polymorphisms in the *A2M*, *SLC6A4* and *UCHL1* genes ($P = 0.01$), the combination of genotypes AG/sl/AA for these three markers was more frequent in patients than in controls (6.18 versus 0.9%). Gene–gene interactions are supposed to be underlying a major part of the genetic risk for complex disorders; however, their complete identification in PD, as in other diseases, remains elusive (Thomas and Beal 2007). It is important to highlight that two of these three polymorphisms (rs4795541 in *SLC6A4* and rs5030732 in *UCHL1*) are known to have allele-dependent effects on gene function (Forero *et al.* 2006a,d).

The current sample size provides a power of 0.7 (considering the MAFs found in our control subjects) to detect markers with effects characterized by ORs from 2.0 (*BDNF*) to 2.3 (*ACE*); as a reference point, markers in the *GBA* and *LRRK2* genes (two of the top candidates in the PDGene database) have pooled ORs of 3.07 and 2.42, respectively.

Heritability of PD has been estimated to be around 0.18 (Thomas and Beal 2007), which is much lower than those found for other neurodegenerative disorders (0.58 for Alzheimer's disease (AD), for example) (Forero *et al.* 2006d). These findings can explain the relative failure to identify major susceptibility factors for non-Mendelian forms of PD (Thomas and Beal 2007). In this context, we have described significant associations of several markers with AD in our population (Arboleda *et al.* 2001). Further exploration of common (HapMap-based analysis) and rare (mutational analysis) variants (Bodmer and Bonilla 2008) in additional candidate genes will be helpful to identify the precise molecular susceptibility factors for PD in developing regions around the world.

Acknowledgements

This work was supported by grants from Fundación Banco de la Republica, COLCIENCIAS and DIB-UNAL.

References

- Arboleda G. H., Yunis J. J., Pardo R., Gómez C. M., Hedmont D., Arango G. *et al.* 2001 Apolipoprotein E genotyping in a sample of Colombian patients with Alzheimer's disease. *Neurosci. Lett.* **305**, 135–138.
- Bagade S., Allen N. C., Tanzi R., Bertram L., Kavvoura F., Schjeide B. *et al.* 2009 The PDGene Database. *Alzheimer. Res.* (<http://www.pdgene.org>).
- Bertram L., McQueen M. B., Mullin K., Blacker D. and Tanzi R. 2007 Systematic meta-analyses of Alzheimer disease genetic association studies: the AlzGene database. *Nat. Genet.* **39**, 17–23.

- Bodmer W. and Bonilla C. 2008 Common and rare variants in multifactorial susceptibility to common diseases. *Nat. Genet.* **40**, 695–701.
- Cook E. H., Stein M. A., Krasowski M. D., Cox N. J., Olkon D. M., Kieffer J. E. et al. 1995 Association of attention-deficit disorder and the dopamine transporter gene. *Am. J. Hum. Genet.* **56**, 993–998.
- Dorsey E. R., Constantinescu R., Thompson J. P., Biglan K. M., Holloway R. G., Kieburtz K. et al. 2007 Projected number of people with Parkinson disease in the most populous nations, 2005 through 2030. *Neurology* **68**, 384–386.
- Forero D. A., Benitez B., Arboleda G., Yunis J. J., Pardo R. and Arboleda H. 2006a Analysis of functional polymorphisms in three synaptic plasticity-related genes (BDNF, COMT AND UCHL1) in Alzheimer's disease in Colombia. *Neurosci. Res.* **55**, 334–341.
- Forero D. A., Pinzón J., Arboleda G. H., Yunis J. J., Alvarez C., Cataño N. et al. 2006b Analysis of common polymorphisms in angiotensin-converting enzyme and apolipoprotein E genes and human longevity in Colombia. *Arch. Med. Res.* **37**, 890–894.
- Forero D. A., Arboleda G., Yunis J. J., Pardo R. and Arboleda H. 2006c Association study of polymorphisms in LRP1, tau and 5-HTT genes and Alzheimer's disease in a sample of Colombian patients. *J. Neural. Transm.* **113**, 1253–1262.
- Forero D. A., Casadesus G., Perry G. and Arboleda H. 2006d Synaptic dysfunction and oxidative stress in Alzheimer's disease: emerging mechanisms. *J. Cell Mol. Med.* **10**, 796–805.
- Hahn L. W., Ritchie M. D. and Moore J. H. 2003 Multifactor dimensionality reduction software for detecting gene-gene and gene-environment interactions. *Bioinformatics* **19**, 376–382.
- Jenkins I. H., Fernandez W., Playford E. D., Lees A. J., Frackowiak R. S., Passingham R. E. et al. 1992 Impaired activation of the supplementary motor area in Parkinson's disease is reversed when akinesia is treated with apomorphine. *Ann. Neurol.* **32**, 749–757.
- Liao A., Nitsch R. M., Greenberg S. M., Finckh U., Blacker D., Albert M. et al. 1998 Genetic association of an alpha2-macroglobulin (Val1000Ile) polymorphism and Alzheimer's disease. *Hum. Mol. Genet.* **7**, 1953–1956.
- López M., Guerrero J., Yescas P., Boll M. C., Familiar I., Ochoa A. et al. 2007 Apolipoprotein E epsilon4 allele is associated with Parkinson disease risk in a Mexican Mestizo population. *Mov. Disorders* **22**, 417–420.
- Okubadejo N. U. 2008 An analysis of genetic studies of Parkinson's disease in Africa. *Parkinsonism Rel. Disord.* **14**, 177–182.
- Pradilla A. G., Vesga A. B. and Leon-Sarmiento F. E. 2003 National neuroepidemiological study in Colombia (EPINEURO). *Rev. Panam. Salud Publica* **14**, 104–111.
- Purcell S., Cherny S. S. and Sham P. C. 2003 Genetic power calculator: design of linkage and association genetic mapping studies of complex traits. *Bioinformatics* **19**, 149–150.
- Sole X., Guino E., Valls J., Iniesta R. and Moreno V. 2006 SNPStats: a web tool for the analysis of association studies. *Bioinformatics* **22**, 1928–1929.
- Thomas B. and Beal M. F. 2007 Parkinson's disease. *Hum. Mol. Genet.* **16**, R183–R194.
- Yunis J. J., Garcia O., Cuervo A. G., Guio E., Pineda C. R. and Yunis E. J. 2005 Population data for PowerPlex 16 in thirteen departments and the capital city of Colombia. *J. Forensic Sci.* **50**, 685–702.

Received 28 September 2009; accepted 7 January 2010

Published on the Web: 10 June 2010