Published in final edited form as:

Neurobiol Aging. 2011 March ; 32(3): 548.e5–548.e7. doi:10.1016/j.neurobiolaging.2009.11.020.

Mutational analysis of *parkin* and *PINK1* in multiple system atrophy

Janet A. Brooks^{a,^}, Henry Houlden^{b,^}, Anna Melchers^b, Ansha J. Islam^a, Jinhui Ding^a, Abi Li^b, Reema Paudel^b, Tamas Revesz^b, Janice L. Holton^b, Nick Wood^b, Andrew Lees^b, Andrew B. Singleton^a, and Sonja W. Scholz^{a,b,*}

^aLaboratory of Neurogenetics, National Institute on Aging, National Institutes of Health, 35 Convent Drive, 20892 Bethesda, MD, USA

^bDepartment of Molecular Neuroscience and Reta Lila Weston Laboratories, Institute of Neurology, UCL, Queen Square House, London, WC1N 3BG, United Kingdom

Abstract

Multiple system atrophy (MSA) and Parkinson's disease (PD) are progressive neurodegenerative disorders with overlapping clinical, biochemical and genetic features. To test the hypothesis that the Parkinson's disease genes *parkin* and *PINK1* also play a role in the pathogenesis of MSA, we performed a mutational screening study involving 87 pathology-proven MSA cases. In *parkin* we identified eight sequence variants and four heterozygous deletions, and in *PINK1* we identified nine variants of which two silent mutations have not been previously reported (p.Gly189Gly and p.Arg337Arg). The frequencies of the observed variants were not significantly different from previously published control data and none of the possibly pathogenic variants were found in a homozygous state. Our results indicate that genetic variants at the *parkin* and *PINK1* loci do not play a critical role in the pathogenesis of MSA.

Keywords

multiple system atrophy; Parkinson's disease; *PINK1*; *parkin*

1. Introduction

Multiple system atrophy (MSA) and Parkinson's disease (PD) are progressive neurodegenerative disorders with largely unknown molecular pathogenesises. Similarities at the biochemical, clinical and genetic levels between both neurodegenerative diseases are increasingly appreciated. In both disorders deposition of abnormally phosphorylated,

*Corresponding author: Sonja W. Scholz, MD, Laboratory of Neurogenetics, National Institute on Aging, 35 Convent Drive, Room 1A-1014, 20892 Bethesda, MD, USA, Tel: +1 (301) 435 8772, Fax: +1 (301) 451 7295, scholz@mail.nih.gov.
^these authors contributed equally

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Disclosure statement

All authors have reviewed the contents of the manuscript being submitted, approve of its contents and validate the accuracy of the data. None of the authors has conflicts of interest including any financial, personal or other relationships with other people or organizations within three years of beginning the work submitted that could inappropriately influence (bias) their work. This research has not been previously published, has not been submitted elsewhere and will not be submitted elsewhere while under consideration at *Neurobiology of Aging*.

fibrillar α -synuclein can be found, - in PD typically in the form of neuronal aggregates called Lewy bodies and in MSA predominantly in the form of glial cytoplasmic inclusions (Spillantini, et al., 1998). MSA and PD present with similar clinical features that are often indistinguishable in early disease stages. Recently, we demonstrated that genetic risk variants at the *SNCA* locus, coding for α -synuclein, increase risk for both PD and MSA (Scholz, et al., 2008, Scholz, et al., 2009). Taken together, these observations indicate that other genes implicated in the pathogenesis of PD should also be considered as candidates risk genes for MSA.

Homozygous mutations in *parkin* and *PTEN-induced putative kinase 1 (PINK1)* are common causes for early-onset PD, and recent evidence suggests that heterozygous mutations may also be relevant in the pathogenesis of late-onset PD (Klein, et al., 2007). To test whether mutations in *parkin* and *PINK1* are involved in the pathogenesis of MSA, we performed mutational screening of *parkin* and *PINK1* in 87 autopsy-proven MSA samples.

2. Methods

We studied 87 Caucasian MSA cases from the Queen Square Brain Bank for Neurological Disorders, London, UK. Control subjects consisted of 276 neurologically normal Caucasian controls in which we have previously screened both *parkin* and *PINK1* (Brooks, et al., 2009). In this control cohort, we observed 15 sequence variants, two heterozygous deletions and one heterozygous duplication in *parkin* and seven sequence variants in *PINK1*. None of the control subjects carried a copy number variant in *PINK1*. In all 87 MSA cases we sequenced the entire coding sequence and the flanking exon-intron boundaries of *parkin* (NM_004562.1) and *PINK1* (NM_0032409.2) using standard methods described elsewhere (Brooks, et al., 2009). Gene dosage for *parkin* exons 1–12 was determined using 5'-FAM fluorescently labeled probes (Applied Biosystems, CA, USA) on an ABI Prism 7900 Sequence Detection System (Applied Biosystems). The dosage of each *parkin* exon relative to β -globin and normalized to the control DNA was determined using the $2^{-\Delta\Delta C_t}$ method (Livak and Schmittgen, 2001). We did not screen *PINK1* for copy number mutations. Fisher's exact tests on allelic association of *parkin* and *PINK1* variants between cases and controls were calculated using PLINK software (version 1.04) (Purcell, et al., 2007). A *p* value of <0.0029 was considered statistically significant (two-sided α of 0.05 divided by 17 tests). The number of copy number changes in MSA cases and in controls was compared using a chi-squared test. The study was approved by the appropriate institutional review boards and written informed consent was obtained for each patient.

3. Results

In this study, we screened *parkin* and *PINK1* in 87 neuropathologically confirmed MSA patients (the results are summarized in the Table and a schematic illustration is shown in Supplementary Fig. 1). No clearly pathogenic homozygous mutations, homozygous copy number changes or compound heterozygous mutations were found in the cases. In *parkin* we identified eight sequence variants and four heterozygous deletions, and in *PINK1* we identified nine sequence variants of which two silent mutations have not been previously reported (c.661C>A = p.Gly189Gly and c.1105C>T = p.Arg337Arg; chromatograms are shown in Supplementary Fig. 2). None of the identified variants were associated with disease after Bonferroni correction for multiple testing. Copy number changes in *parkin* were not significantly overrepresented in cases versus controls (*p* value = 0.08).

4. Discussion

We report the first comprehensive mutation screening investigating the role of genetic variants in *parkin* and *PINK1* in pathology-proven MSA cases. Clearly pathogenic homozygous mutations were not identified. Although heterozygous variants were relatively common, these alleles/copy number variants were not statistically significantly associated with disease, and their pathogenicity is unproven (Table). We conclude that genetic variants in *parkin* and *PINK1* are not commonly associated with MSA.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank the patients and their families for supporting our research. DNA panels from the NINDS Human Genetics Resource Center DNA and Cell Line Repository (<http://ccr.coriell.org/ninds>) were used in this study, as well as clinical data. We would like to thank the following submitters that contributed samples to these DNA panels: Drs. Russell Buono, Petra Kaufman, Eric Sorenson, Catherine Lomen-Hoerth, David Simon, John Hardy, Robert Brown, Okun Mandel, Micheline Gravel, Peter Bosch, Paul Gordon, Dennis Dickson, Zbigniew Wszolek, Matthew Farrer, Daniel Newman, Laura Sams, Angela Gresham, Edward Kasarskis, Kapil Sethi, Frederick Wooten, Anthony Crawley, Nickolas Maragakis, Robert Miller, Robert Hamill, Jayaraman Rao, Burton Scott, Ray Watts, and Kevin Boylan. This research was funded in part by the Intramural Research Program of the National Institute on Aging and the National Institute on Neurological Disorders and Stroke, Department of Health and Human Services; project numbers Z01-AG000957-06 (J.A.B., A.J.I., J.D., S.W.S., A.B.S.). We gratefully acknowledge support by the Medical Research Council (MRC) (H.H. G108/638 Clinician Scientist Fellowship; N.W., J.A.H.), the Michael J. Fox Foundation (H.H., J.A.H.), the Reta Lila Weston Institute for Neurological Studies (J.A.H., T.R., A.L., J.H.), the Sarah Matheson Trust for Multiple System Atrophy (H.H., T.R., J.H.), the Alzheimer Research Trust (J.A.H.), the National Organization for Rare Disorders (NORD) (H.H.), Ataxia UK (H.H.) and the Progressive Supranuclear Palsy (Europe) Association (J.A.H., T.R., J.H., A.L.).

References

- Brooks J, Ding J, Simon-Sanchez J, Paisan-Ruiz C, Singleton AB, Scholz SW. *Parkin* and *PINK1* mutations in early-onset Parkinson's disease: comprehensive screening in publicly available cases and control. *J Med Genet*. 2009; 46(6):375–381. [PubMed: 19351622]
- Klein C, Lohmann-Hedrich K, Rogaeva E, Schlossmacher MG, Lang AE. Deciphering the role of heterozygous mutations in genes associated with parkinsonism. *Lancet Neurol*. 2007; 6(7):652–662. [PubMed: 17582365]
- Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2⁻(Delta Delta C(T)) Method. *Methods*. 2001; 25(4):402–408. [PubMed: 11846609]
- Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet*. 2007; 81(3):559–575. [PubMed: 17701901]
- Scholz S, Schulte C. Genome-Wide Association Study in Parkinson's Disease: Identification of Strong Associations with SNCA and MAPT. Presented at the annual meeting of The American Society of Human Genetics; November 13, 2008; Philadelphia, Pennsylvania. 2008. [Abstract program number 90]
- Scholz SW, Houlden H, Schulte C, Sharma M, Li A, Berg D, Melchers A, Paudel R, Gibbs JR, Simon-Sanchez J, Paisan-Ruiz C, Bras J, Ding J, Chen H, Traynor BJ, Arepalli S, Zonozi RR, Revesz T, Holton J, Wood N, Lees A, Oertel W, Wullner U, Goldwurm S, Pellecchia MT, Illig T, Riess O, Fernandez HH, Rodriguez RL, Okun MS, Poewe W, Wenning GK, Hardy JA, Singleton AB, Gasser T. SNCA variants are associated with increased risk for multiple system atrophy. *Ann Neurol*. 2009; 65(5):610–614. [PubMed: 19475667]
- Spillantini MG, Crowther RA, Jakes R, Cairns NJ, Lantos PL, Goedert M. Filamentous alpha-synuclein inclusions link multiple system atrophy with Parkinson's disease and dementia with Lewy bodies. *Neurosci Lett*. 1998; 251(3):205–208. [PubMed: 9726379]

Table

Sequence variants at the *parkin* and *PINK1* loci in 87 MSA cases

Nucleotide Change	Amino Acid Change	Location	Mutation Type	Alleles in Cases	Alleles in Controls [†]	p value
<i>parkin</i>						
c.258C>T	p.Arg42Cys	exon 2	missense	1 / 175	0 / 552	0.242
c.272+25C	-	intron 2	intronic	34 / 142	115 / 437	0.747
c.514-20T	-	intron 3	intronic	13 / 163	44 / 508	0.873
c.601G>A	p.Ser167Asn	exon 4	missense	2 / 174	9 / 543	1
c.835G>A	p.Arg234Gln	exon 6	missense	1 / 175	0 / 552	0.242
c.1239G>C	p.Val380Leu	exon 10	missense	33 / 143	99 / 453	0.823
c.1281G>A	p.Asp394Asn	exon 11	missense	12 / 162	14 / 538	0.016
c.1444C>T	p.Pro437Leu	exon 12	missense	1 / 175	0 / 552	0.242
<i>PINK1</i>						
c.283C>T	p.Leu63Leu	exon 1	silent	36 / 130	88 / 464	0.101
c.438A>T	p.Gln115Leu	exon 1	missense	12 / 154	32 / 520	0.467
c.482-7A	-	intron 1	intronic	21 / 147	69 / 483	1
c.661C>A	p.Gly189Gly	exon 2	silent	1 / 167	0 / 552	0.233
c.1054-5G	-	intron 4	intronic	20 / 156	67 / 485	0.894
c.1105C>T	p.Arg337Arg	exon 5	silent	1 / 175	0 / 552	0.242
c.1112G>A	p.Ala340Thr	exon 5	missense	9 / 167	18 / 534	0.257
c.1189C>T	p.Ser365Ser	exon 5	silent	1 / 175	0 / 552	0.242
c.1656A>C	p.Asn521Thr	exon 8	missense	53 / 121	152 / 400	0.499

[†] mutational screening results of normal controls have been published elsewhere (see Brooks et al., 2009).