Published in final edited form as:
Mov Disord. 2010 September 15; 25(12): 1973–1976. doi:10.1002/mds.23163.

LRRK2 variation and Parkinson's disease in African Americans

Owen A. Ross[1,*], **Greggory J. Wilhoite**[1], **Justin A. Bacon**[1], **Alexandra Soto-Ortolaza**[1], **Jennifer Kachergus**[1], **Stephanie A. Cobb**[1], **Andreas Puschmann**[1], **Carles Vilariño-Güell**[1], **Matthew J. Farrer**[1], **Neill Graff-Radford**[2], **James F. Meschia**[2], and **Zbigniew K. Wszolek**[2]

[1]Department of Neuroscience, Mayo Clinic College of Medicine, Jacksonville, Florida, USA

[2]Department of Neurology, Mayo Clinic College of Medicine, Jacksonville, Florida, USA

Abstract

The global impact of *LRRK2* mutations is yet to be realized with a lack of studies in specific ethnic groups, including those of Asian and African descent. Herein we investigated the frequency of common *LRRK2* variants by complete exon sequencing in a series of publicly available African American Parkinson's disease patients. Our study identified three novel synonymous exonic

[*]Corresponding author: Owen A. Ross PhD Molecular Genetics Laboratory and Core, Morris K. Udall Parkinson's Disease Research Center of Excellence, Mayo Clinic, Department of Neuroscience, 4500 San Pablo Road, Jacksonville, FL 32224, Tel: (904)-953-0963, Fax: (904)-953-7370, ross.owen@mayo.edu.

Author contributions: Owen A. Ross: Funding, conception and organization of research project, drafting, review and critique of manuscript;
Greggory J. Wilhoite: Execution of research project, review and critique of manuscript;
Justin A. Bacon: Execution of research project, review and critique of manuscript;
Alexandra I. Soto-Ortolaza: Execution of research project, review and critique of manuscript;
Jennifer Kachergus: Execution of research project, review and critique of manuscript;
Stephanie A. Cobb: Execution of research project, review and critique of manuscript;
Andreas Puschmann: Execution of research project, review and critique of manuscript;
Carles Vilariño-Güell: Conception and organization of research project, review and critique of manuscript;
Matthew J. Farrer: Funding and conception of research project, review and critique of manuscript;
Neill Graff-Radford: Funding, sample collection, review and critique of manuscript;
James F. Meschia: Funding, sample collection, review and critique of manuscript;
Zbigniew K. Wszolek: Funding, conception and organization of research project, sample collection, review and critique of manuscript;
Financial Disclosures for the Past Year:
Owen A. Ross Stock Ownership in medically-related fields; Consultancies; Advisory Boards; Partnerships; Honoraria; Intellectual Property Rights; Expert Testimony; Contracts; Royalties: NONE
Employment: Mayo Clinic
Grants: Michael J. Fox Foundation and American Heart Association.
Greggory J. Wilhoite, Justin A. Bacon, Alexandra Soto-Ortolaza, Jennifer Kachergus, Stephanie A. Cobb, Andreas Puschmann, Carles Vilariño-Güell Stock Ownership in medically-related fields; Consultancies; Advisory Boards; Grants; hips; Honoraria; Intellectual Property Rights; Expert Testimony; Contracts; Royalties: NONE
Employment: Mayo Clinic
Matthew J. Farrer Dr. Farrer reports a US provisional patent application for a device that treats neurodegenerative diseases, which has been licensed to Alnylam Pharmaceuticals Inc. Dr Farrer also reports an honorarium for a seminar from H. Lundbeck A/S, GlaxoSmithKline, Elan Pharmaceuticals and Genzyme.
Advisory Boards: Michael J. Fox Foundation
Employment: Mayo Clinic
Grants: NIH and Michael J. Fox Foundation
James F. Meschia Stock Ownership in medically-related fields; Consultancies; Advisory Boards; Partnerships; Honoraria; Intellectual Property Rights; Expert Testimony; Contracts; Royalties: NONE
Employment: Mayo Clinic
Grants: NINDS and the Hanley Foundation (DC).
Neill Graff-Radford Stock Ownership in medically-related fields; Consultancies; Grants; Partnerships; Honoraria; Intellectual Property Rights; Expert Testimony; Contracts; Royalties: NONE
Employment: Mayo Clinic
Scientific Advisory Board: Codman
Zbigniew K. Wszolek Stock Ownership in medically-related fields; Consultancies; Advisory Boards; Partnerships; Honoraria; Intellectual Property Rights; Expert Testimony; Contracts; Royalties: NONE
Employment: Mayo Clinic
Grants: NIH and PARF

variants and thirteen known coding variations however, there did not appear to be any frequent (>5%) pathogenic mutations. Given the ethnic-specific *LRRK2* variation previously identified in PD further studies in under-represented populations are warranted.

Keywords

Parkinsonism; Leucine-rich repeat kinase 2; genetics

Introduction

Leucine-rich repeat kinase 2 (*LRRK2*) mutations are recognized as the most frequent genetic cause of Parkinson's disease (PD) identified to date. Although only a small number of the variations within the gene are known to affect disease risk (n=8), the pathogenicity of many others remains equivocal. *LRRK2* mutations highlight many of the phenomena associated with late-onset sporadic PD including pleomorphism in phenotypic presentation and a large variation in the disease penetrance. This has been exemplified by carriers of Lrrk2 p.R1441C and p.G2019S with a diverse range in age-at-onset of PD[1, 2]. Another prominent feature of *LRRK2* parkinsonism is the relatively high frequency of specific mutations observed in ethnically distinct populations[3–6].

Mutations in *LRRK2* are distributed across the gene and affect different domains of the protein, which makes screening an expensive and time-consuming proposal. For this reason the great majority of samples sequenced for the full 51 exon *LRRK2* gene have been Caucasian samples from Western Europe or North America. Only a relatively small number of samples have been sequenced in African or Asian populations although the latter have been fruitful identifying two common risk factors (Lrrk2 p.R1628P and p.G2385R)[3, 6, 7]. Genetic studies of PD in sub-Saharan Africa are rare[8–11], however a number of studies have recently been published regarding the observed high frequency of Lrrk2 p.G2019S (30–40% of patients) in the Maghreb region of North Africa[2, 4, 12, 13].

To assess the full impact of *LRRK2* mutations on the global PD community it is crucial that full gene sequencing is performed on different ethnic groups. In the present study we set out to sequence subjects of African American declared ethnicity with PD to assess the frequency of common *LRRK2* variation in this population.

Subjects and Methods

DNA samples from African American patients with PD were obtained from the Coriell Cell Repository collection (Coriell Institute for Medical Research, Camden, NJ) under the appropriate ethical approval. Our series contained 22 patients (12 female and 10 male) with an average age-at-onset of 50.6 years (range 27–65 years) and an average age-at-study of 58.7 years (range 40–72 years). A family history of parkinsonism was reported in 6 patients (27%). Unfortunately given the nature of samples (Coriell Cell Repository) the clinical records are limited. To establish allele frequencies we screened 92 African American control subjects (67 female and 25 male) obtained through the Coriell Repository or collected at Mayo Clinic Florida with an average age-at-study of 41.9 years (range 20–71 years). In addition, a series of 420 US Caucasians controls collected at Mayo Clinic Florida (196 female and 224 male) were genotyped to provide allelic frequencies, this series had an average age-at-study of 73.4 years (range 34–93 years).

Primers were designed for sequencing of the entire coding region of the *LRRK2* gene (51 exons) and exon-intron boundaries (Available on request). PCR products were generated on

384 well plates using standard protocols. A Biomek FX robot allows highly efficient, easily automated magnetic-bead based PCR purification system (AMPure beads, Agencourt, Beverly, MA) that requires no sample transfer, centrifugation or filtration steps. The same bead technology (CleanSEQ beads, Agencourt) allows for sequencing reactions to be performed in 10μl volumes containing 5μl of clean PCR product, 1:32 dilution of BigDye Terminator and 3.2pmol of primer. After the removal of dye-terminator using the Biomek FX robot and magnetic-bead technology, sequencing products are electrophorized on an ABI 3730 capillary array and the data analyzed with SeqScape software (ABI).

Results

We identified 16 exonic variants in the 22 African American PD patients, three novel synonymous changes (p.A75A, p.V674V and p.S1721S) were observed and are shaded in Table 1. The three common variants highlighted with an asterisk represent a switch of the minor and major allele designations from that observed in Caucasian samples. Control frequencies are provided for a series of 92 African American and 420 Caucasian US subjects. Linkage disequilibrium measures are provided as r^2-values within the African American subjects (Figure 1)[14]. In addition, there was no evidence of any intron-exon boundary variants that would result in alternate splicing.

Discussion

Ongoing studies by our group and others are attempting to resolve the pathogenicity, prevalence and penetrance of *LRRK2* coding variants in PD. There is a gap in our knowledge regarding the frequency and impact of *LRRK2* mutations on PD in distinct ethnic groups of people. To this goal we performed sequencing of 22 African American PD patients for the entire *LRRK2* gene identifying a number of known and novel exonic variations (Table 1). There was neither evidence of any common (>5%) pathogenic mutations, nor of known variants of high (e.g. p.G2019S) or low penetrance (e.g. p.R1628P) in these samples from African American PD patients[2, 6].

This study does not rule out a role for *LRRK2* mutations in PD patients of African descent but does show that unlike some populations from North Africa there is no highly prevalent pathogenic mutation. Our sample size although relatively small (n=22) reflects the comprehensive sequencing approach of the all 51 exons of the *LRRK2* gene and represents a large body of data. However this study does not account for possible variation in non-coding regions of the gene which may account for disease risk. Gene sequencing studies are important as we examine different ethnic groups for *LRRK2* variants given the knowledge of the ethnic frequency differences that already exists.

These results represent our preliminary studies to characterize the frequency of *LRRK2* variants in the African American population. Differences are observed for the minor allele frequencies of a number of *LRRK2* variants between African American and Caucasian subjects which will be an important consideration for future association studies (Table 1). To date, over 75 non-synonymous substitutions have been reported with the pathogenicity and frequency of these yet to be established in a number of different populations. The novel variants identified in the present study in these African American patients will be included in our future *LRRK2* genetic studies.

Acknowledgments

We would like to thank all those who have contributed to our research, particularly Jill A Searcy for assistance. Mayo Clinic Jacksonville is a Morris K. Udall Parkinson's Disease Research Center of Excellence (NINDS P50 #NS40256) and is supported by a grant from the Michael J. Fox foundation. ZKW is also partially funded by P01

AG017216, R01 NS057567, R01 AG015866, CIHR 121849, and PARF C06-01. This study used samples from the NINDS Human Genetics Resource Center DNA and Cell Line Repository (http://ccr.coriell.org/ninds), as well as clinical data. NINDS Repository sample numbers corresponding to the samples used are: ND04381; ND05017; ND00165; ND20308; ND01131; ND01078; ND02892; ND00079; ND05171; ND14044; ND06940; ND01137; ND05602; ND06487; ND14506; ND14907; ND01985; ND14630; ND05460; ND06105; ND01177; ND04160.

Financial Disclosures: Mayo Clinic Jacksonville is a Morris K. Udall Parkinson's Disease Research Center of Excellence (NINDS P50 #NS40256). MJF and ZKW are also supported by a Pacific Alzheimer Research Foundation (PARF) grant C06-01. ZKW is partially funded by NIH P01 AG017216, R01 NS057567, R01 AG015866 and CIHR 121849. JFM is funded by NINDS R01 NS39987. MJF and OAR are supported by the Michael J. Fox Foundation.

References

1. Haugarvoll K, Rademakers R, Kachergus JM, et al. Lrrk2 R1441C parkinsonism is clinically similar to sporadic Parkinson disease. Neurology. 2008; 70(16 Pt 2):1456–1460. [PubMed: 18337586]
2. Hulihan MM, Ishihara-Paul L, Kachergus J, et al. LRRK2 Gly2019Ser penetrance in Arab-Berber patients from Tunisia: a case-control genetic study. Lancet Neurol. 2008; 7(7):591–594. [PubMed: 18539535]
3. Di Fonzo A, Wu-Chou YH, Lu CS, et al. A common missense variant in the LRRK2 gene, Gly2385Arg, associated with Parkinson's disease risk in Taiwan. Neurogenetics. 2006; 7(3):133–138. [PubMed: 16633828]
4. Lesage S, Durr A, Tazir M, et al. LRRK2 G2019S as a cause of Parkinson's disease in North African Arabs. N Engl J Med. 2006; 354(4):422–423. [PubMed: 16436781]
5. Ozelius LJ, Senthil G, Saunders-Pullman R, et al. LRRK2 G2019S as a cause of Parkinson's disease in Ashkenazi Jews. N Engl J Med. 2006; 354(4):424–425. [PubMed: 16436782]
6. Ross OA, Wu YR, Lee MC, et al. Analysis of Lrrk2 R1628P as a risk factor for Parkinson's disease. Ann Neurol. 2008; 64(1):88–92. [PubMed: 18412265]
7. Mata IF, Kachergus JM, Taylor JP, et al. Lrrk2 pathogenic substitutions in Parkinson's disease. Neurogenetics. 2005; 6(4):171–177. [PubMed: 16172858]
8. Atadzhanov M, Zumla A, Mwaba P. Study of familial Parkinson's disease in Russia, Uzbekistan, and Zambia. Postgrad Med J. 2005; 81(952):117–121. [PubMed: 15701745]
9. Okubadejo N, Britton A, Crews C, et al. Analysis of Nigerians with apparently sporadic Parkinson disease for mutations in LRRK2, PRKN and ATXN3. PLoS ONE. 2008; 3(10):e3421. [PubMed: 18927607]
10. Okubadejo NU. An analysis of genetic studies of Parkinson's disease in Africa. Parkinsonism Relat Disord. 2008; 14(3):177–182. [PubMed: 17881276]
11. Okubadejo NU, Bower JH, Rocca WA, Maraganore DM. Parkinson's disease in Africa: A systematic review of epidemiologic and genetic studies. Mov Disord. 2006; 21(12):2150–2156. [PubMed: 17044056]
12. Ishihara L, Gibson RA, Warren L, et al. Screening for Lrrk2 G2019S and clinical comparison of Tunisian and North American Caucasian Parkinson's disease families. Mov Disord. 2007; 22(1): 55–61. [PubMed: 17115391]
13. Ishihara L, Warren L, Gibson R, et al. Clinical features of Parkinson disease patients with homozygous leucine-rich repeat kinase 2 G2019S mutations. Arch Neurol. 2006; 63(9):1250–1254. [PubMed: 16966502]
14. Barrett JC. Haploview: visualization and analysis of SNP genotype data. CSH Protoc. 2009; 2009(10) pdb ip71.

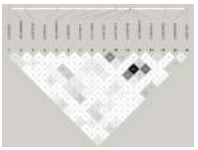

Figure 1.
The linkage disequilibrium between the sixteen *LRRK2* variants observed within the African American samples is highlighted in a Haploview plot with r^2-values shown and represented in shading[14]. All African American patients and controls were used to generate the plot.

Table 1

Observed sixteen exonic variants in the *LRRK2* sequencing of 22 African American PD patients

Exon	SNP	c.LRRK2	p.Lrrk2	Carriers (n=22)	MAF in AA PD (n=44)	MAF in AA controls (n=184)	MAF in Caucasian US controls (n=840)
Exon 1	rs2256408	149A>G	H50R	5	0.11	0.09	0.001
Exon 2	ss142460309	225G>A	A75A	1	0.02	0.01	0.00
Exon 5	rs10878245	457T>C	L153L	17	0.39	0.43	0.64
Exon 14	rs7308720	1653C>G	N551K	9	0.20	0.12	0.07
Exon 17	rs72546319	2022A>C	V674V	1	0.02	0.00	0.00
Exon 22	rs7966550	2857T>C	L953L	1	0.02	0.02	0.12
Exon 30	rs7133914	4193G>A	R1398H	6	0.14	0.12	0.07
Exon 30	rs11175964	4269G>A	K1423K	2	0.05	0.03	0.06
Exon 34	*rs1427263	4872A>C	G1624G	9	0.20	0.24	0.33
Exon 34	rs11176013	4911A>G	K1637K	21	0.48	0.59	0.57
Exon 34	rs11564148	4939T>A	S1647T	7	0.16	0.19	0.31
Exon 35	ss142460319	5163A>G	S1721S	1	0.02	0.00	0.004
Exon 37	rs10878371	5457T>C	G1819G	20	0.45	0.61	0.43
Exon 43	rs10878405	6324G>A	E2108E	6	0.14	0.16	0.30
Exon 48	rs33962975	7155A>G	G2385G	4	0.09	0.05	0.14
Exon 49	*rs3761863	7190C>T	T2397M	18	0.41	0.46	0.34

All sixteen exonic variants observed in the 22 African American PD patients are shown. Novel synonymous variants identified in the present study are shaded.

MAF, minor allele frequency; AA African American. The MAF for each variant is shown for the 22 African American patients, 92 African American controls and 420 Caucasian controls (all subjects are US citizens).

*The minor and major allele designations have switched from that normally observed in Caucasians.

Mov Disord. Author manuscript; available in PMC 2011 September 15.