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Genomewide linkage study of modifiers of *LRRK2*-related Parkinson's disease

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Abstract

Background—Mutations in the leucine-rich repeat kinase 2 gene (*LRRK2*), located at 12q12, are the most common known genetic causes of Parkinson's disease (PD). Studies of *LRRK2* mutation carriers have shown incomplete and age-dependent penetrance and previous studies have suggested that inherited susceptibility factors may modify the penetrance of *LRRK2* mutations.

Methods—Genomewide linkage to age of onset of *LRRK2*-related PD was evaluated in a sample of 113 *LRRK2* mutation carriers from 64 families using single nucleotide polymorphism data from the Illumina HumanCNV370 genotyping array. Association between onset age and SNPs located under suggestive linkage peaks was also evaluated.

Results—The top LOD-score for onset age (LOD-score=2.43) was located in the chromosome 1q32.1 region. Moderate linkage to onset was also identified at 16q12.1 (LOD-score=1.58). Examination of single nucleotide polymorphism association to PD onset under the linkage peaks revealed no statistically significant SNP associations.

Conclusions—The two novel genomic regions identified may harbor modifiers of *LRRK2*-related PD onset age or penetrance and further study of these regions may provide important insight into *LRRK2*-related PD.

Keywords

Parkinson's Disease; LRRK2; Linkage

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INTRODUCTION

Parkinson's disease (PD) is a neurodegenerative disorder affecting approximately 1.8% of individuals over the age of 65 [1]. While some cases of PD have a known genetic or environmental cause, most appear to be due to complex interactions among unidentified genetic and environmental susceptibility factors. Mutations in the leucine-rich repeat kinase 2 gene (*LRRK2*) are the most common known genetic cause of PD, with the most frequent *LRRK2* mutation, G2019S, estimated to be associated with 5% to 6% of familial PD and 1% to 2% of idiopathic cases in populations of European descent [2-5]. However, studies of the G2019S mutation have reported a wide range of penetrance estimates. Early studies performed in large families with multiple affected members reported high lifetime penetrance for *LRRK2* mutations, ranging from 70% [6] to 100% [7] and further examination of the age-dependent penetrance in families with multiple affected members ranged from 17-33% penetrance at age 50 increasing to 85-100% at ages above 70 [8, 9]. However, more recent studies examining cohorts of PD patients not ascertained for familial history of the disease have generally reported both lower lifetime penetrance estimates (22-32%) [10-12] and lower age-dependent estimates (2% at age 50 to 33% at age 80) [13].

While the early familial penetrance estimates may have been upwardly biased due to ascertainment strategies, we have recently shown in an unbiased analysis of unascertained parents of PD affected siblings, that the penetrance of *LRRK2* mutations in families with multiple affected members may be substantially higher than in randomly ascertained idiopathic PD cases (67% versus 33% at age 85) [13, 14]. This suggests the presence of additional genetic susceptibility factors influencing the risk of *LRRK2*-related PD.

While younger onset of symptoms is commonly observed with some other PD related genes including *PARK1* (*SNCA*) and *PARK2* (parkin) [15-17], PD associated with *LRRK2* mutations presents an onset distribution very similar to that seen in idiopathic PD [4, 13, 18]. The range in onset age and clear age-dependent penetrance of *LRRK2* mutations suggest that genetic variants associated with onset age of *LRRK2*-related PD may represent a primary mechanism by which penetrance is modified. These genetic modifiers are potential targets not only for treatment, but also for prevention of *LRRK2*-related PD.

We have therefore undertaken the first genomewide linkage and association studies aimed at identifying modifiers of penetrance in *LRRK2*-related PD.

METHODS

Ethics Statement

These studies were approved by the Institutional Review Boards of Boston University and Indiana University. Appropriate written informed consent was obtained for all participants included in this study.

Sample

A sample of 113 *LRRK2* mutation carriers from 64 families was identified from two ongoing studies of familial PD, the GenePD study and the PROGENI study, and was included in a genomewide association study (GWAS) with a large additional sample of familial PD cases that did not carry any known pathogenic *LRRK2* mutations and healthy controls. Most of the *LRRK2* mutation carriers were ascertained in pedigrees with multiple members (90 carriers from 41 pedigrees), but 23 singletons with familial PD were also studied. Four of the *LRRK2* carriers showed no signs of PD, though they had multiple relatives with PD; the remaining 109 carriers were verified PD cases. PD cases underwent a uniform neurological evaluation that employed PD diagnostic criteria based upon a modified

version of the United Kingdom PD Society Brain Bank Criteria [19]. Onset age of *LRRK2*-related PD was determined by interview and reflected the age of first PD symptom, which commonly preceded age of physician diagnosis.

LRRK2 mutations were identified through the genotyping of known mutations (G2019S, R1441C, R1441H, Y1699C, I1192V, L1795F, Q1111H, E334K and I2020T) using TaqMan technology implemented on either the ABI PRISM® 7900HT or the ABI PRISM® 7300 Sequence Detection system [4, 5, 18]. The majority of the *LRRK2* carriers identified were G2019S carriers (99 carriers from 57 families). In addition we identified 7 families with mutations at either I1192V(1 family, 2 carriers), L1795F (1 family, 2 carriers), Q1111H (1 family, 2 carriers), E334K (1 family, 2 carriers) and R1441C (3 families, 6 carriers).

Microarray Genotyping and Quality Assessment

Genotyping was performed by the Center for Inherited Disease Research (CIDR) using the Illumina HumanCNV370 version1_C BeadChips (Illumina, San Diego, CA, USA) and the Illumina Infinium II assay protocol [20] as part of a larger study which also included 895 control and 935 non-*LRRK2* PD cases [21, 22]. SNP quality control, cryptic relatedness and population stratification were evaluated in the entire sample of genotyped cases and controls as describe in previous manuscripts [21, 22]. One *LRRK2*-related PD case was removed due to low call rate, leaving 108 *LRRK2*-related PD cases for study. All samples were self-reported as white, non-hispanic, however, seven *LRRK2*-related PD cases, from four families, were flagged for non-Caucasian ancestry during population stratification analyses and were removed from subsequent association analyses, but not linkage analysis.

Statistical Methods

In order to identify modifiers of age-dependent penetrance of *LRRK2* mutations, linkage and association analyses of PD onset age were conducted. For linkage analyses, 23 singletons, two sib-pairs discordant on *LRRK2* carrier status (case-phenocopy pairs) and one pedigree with only parent offspring pairs were excluded, leaving 85 individuals from 38 pedigrees for analysis (Table 1).

Distinct exclusion criteria, appropriate for association analysis, were applied in order to generate the final sample used in the association analyses. The seven *LRRK2*-related PD cases (from four families) who were flagged during the initial population stratification analyses as having Hispanic or Asian ancestry were excluded from the association analyses (described previously)[21]. In addition, two cases were removed due to a lack of known age of onset, leaving 99 cases from 59 families.

Principal Component Analysis

As described above, extreme population outliers representing individuals of likely Hispanic, Asian, or African descent were identified in population stratification analyses during our initial sample QC [21, 22]. To identify any additional population stratification related to onset age in LRRK2 cases, principal components were generated from the final set of 99 *LRRK2*-related PD cases only. The region surrounding the *LRRK2* locus on chromosome 12 was excluded from the calculations of the principal components. All principal components were generated using Eigenstrat [23] and only one member per family was included in the analysis. Eigenstrat was then used to apply principal components to the remaining family members who were not originally used to define the components. Principal components identified to be associated with age of onset with a p-value less than 0.10 were included as covariates in the regression analyses where appropriate.

Linkage Analysis

A subset of the 328,189 SNPs passing all quality control measures was selected for the linkage analyses to avoid inflation of LOD scores caused by linkage disequilibrium between SNPs [24]. Plink [25]was used to select markers with MAF>0.20, and pairwise correlation < 0.04, identifying 10,129 informative and independent markers for use in the linkage analyses. Information content across the chromosomes was calculated using Merlin.

Estimated heritability of onset age for *LRRK2*-related PD was obtained for this sample using SOLAR[26]. Linkage analysis to onset age was performed using two methods: (1) multipoint non-parametric quantitative trait linkage (QTL) implemented in Merlin [27] and (2) a robust score statistic based on variance components (RSS) [28] implemented in R.

Association Analysis

Two principal components were identified to be associated with onset age ($p < 0.10$) in the association sample, and thus were included in subsequent analyses. Association to onset age was tested under an additive mode of inheritance for SNPs with a MAF greater than 0.2 and a dominant model for SNPs with a MAF less than or equal to 0.2. A linear mixed effects model using the kinship coefficient matrix (implemented using the kinship package in R) was used to account for the familial relationships. These analyses were also repeated in the subset of cases with G2019S mutations only.

To test whether there were significant associations in the linkage regions, the p-values for SNPs in each region were adjusted for multiple comparisons using a Bonferroni correction for the number of SNPs under the peak. The boundaries of the linkage peaks were defined as the positions where the LOD score dropped to one-half of the peak LOD score in that region. To test for significant SNP associations genomewide, the commonly accepted criterion for genomewide significance of $p < 5\times10^{-8}$ [29], based on recent estimates of independent genomewide sequence variation to maintain 5% genomewide type I error rate [30, 31] was used.

RESULTS

For the linkage analyses, sib-pairs concordant for *LRRK2* mutation status were included providing 85 individuals from 38 pedigrees for analysis (Table 1). Four non-penetrant siblings were observed, all of whom were female, and had an average age of exam similar to the affected group (actual ages 52, 54, 67, 71).

Principal Components Analysis

For the association study, extreme population outliers identified in population stratification analyses were excluded. A second set of principal components recalculated using only the *LRRK2* case sample was tested for association to onset age. PC 2 and 10 were significantly associated with onset age of PD and were included in the onset-age association analyses as covariates ($p = 0.002$, $p = 0.06$ respectively).

Linkage Analysis

The estimated heritability of onset age in these families was 60.6% and was found to be significantly different than zero ($p=0.02$; 95% CI 8%-100%), supporting the hypothesis that genetic modifiers play an important role in onset and penetrance for *LRRK2* carriers in PD.

The maximum LOD score for onset age observed using the QTL method was 2.43 and the maximum score using the RSS method was 1.94, both located in the chromosome 1q32.1 region at 199 cM (Figure 1A). Moderate linkage peaks were also identified at 16q12.1 using

both methods (QTL LOD=1.58 at 63 cM, RSS LOD=1.10 at 60 cM) (Figure 1B). No linkage to onset age was observed in the area of the *LRRK2* locus on Chromosome 12.

Association Analysis

Association to onset age was examined in the regions showing evidence of linkage. After Bonferroni correction for multiple comparisons under the peaks (onset: comparisons=3101, $\alpha=1.6 \times 10^{-5}$), no statistically significant SNP associations were observed.

All associations with onset age yielding a p-value less than 0.005, in either the full sample or in the subset of G2019S carriers, under the chromosome 1 and chromosome 16 peaks are shown in Table 2A and 2B, respectively.

In the genomewide association analyses for onset age, no SNPs reached a genomewide level of significance ($p< 5\times10^{-8}$). Supplementary Table S1 lists all SNP associations to onset age with p<0.005. We reviewed the results for SNPs located within 500kb of the LRRK2 gene to see if there were any trans- allele influence on age of onset of LRRK2 related PD, however no SNPs within this region demonstrated association to PD at even a nominal level ($p\leq0.05$).

DISCUSSION

This study represents the first genomewide linkage scan and association study for modifiers of *LRRK2*-related PD. We have demonstrated significant heritability of onset age of *LRRK2*-related PD and identified two chromosomal regions with suggestive evidence of linkage to *LRRK2* onset age. Association analysis of SNPs under these peaks showed no statistically significant associations after correction for multiple comparisons. We also observed no evidence that additional variability in the *LRRK2* locus influences age of onset of *LRRK2*-related PD.

The methods used to define the linkage peaks allows the peaks to encompass a broad range, with 121 and 185 annotated genes in the linked regions on chromosomes 1 and 16, respectively. Using an inclusive definition to define the linkage region results in a larger interval and may detract from our power to detect association in these regions. However, this approach was appropriate due to the imprecision of linkage mapping.

An earlier study of onset age in 44 *LRRK2*-related PD cases [32] that focused on the previously identified PD related genes *SNCA* and *MAPT*, identified a SNP located in *MAPT* that showed a significant increase in age of onset in LRRK2 positive subjects who carried the minor allele. This SNP (rs2435207) was not genotyped in our GWAS platform. A nearby SNP in strong LD with it (rs2435211, $R^2=0.97$ from HapMap CEU [33]) was genotyped, but was not significantly associated with onset age in our study (p=0.10) and the estimate was not in the same direction of effect as the prior study. No *MAPT* or *SNCA* SNPs genotyped in this study showed significant association to onset age.

In the genomewide SNP association analyses, no SNPs reached the commonly accepted level of genomewide significance of 5×10^{-8}. The 99 *LRRK2*-related PD cases (Table 1) included were the only cases available for study; therefore, the genomewide association studies were underpowered. Nevertheless, genomewide SNP association results in this well characterized sample may provide a valuable resource for researchers who have other LRRK2 cohorts to evaluate whether these same regions are implicated in their samples. These results may also provide important prioritization information for use in other gene characterization experiments. Therefore, an extended list of the top association study results (p<0.005) are provided in the supplementary tables S1.

While the association analyses in this study were unable to provide additional support for more localized regions underneath either of the linkage peaks, recent reports from two large GWAS of idiopathic PD implicate a region on chromosome 1q32, overlapping the onset age linkage peak observed in this study [34, 35]. The observation of association to PD risk in the same region as the linkage to age of onset in *LRRK2*-related PD supports the hypothesis that genetic modifiers of penetrance of known disese-causing genes may also be involved in the disease process of idiopathic PD. Furthermore, this convergence suggests that continued study of these genes in the less heterogeneous *LRRK2*-related PD cohort may provide a powerful method for the functional characterization of this region.

In conclusion, we have identified two genomic regions that may harbor modifiers of *LRRK2*-related PD onset age or penetrance. Further study of these regions may provide important insight into the function and etiology of *LRRK2*-related PD, as well as potential therapeutic targets for the treatment and possible prevention of this form of PD. Such insights would undoubtedly affect the understanding of idiopathic PD as well.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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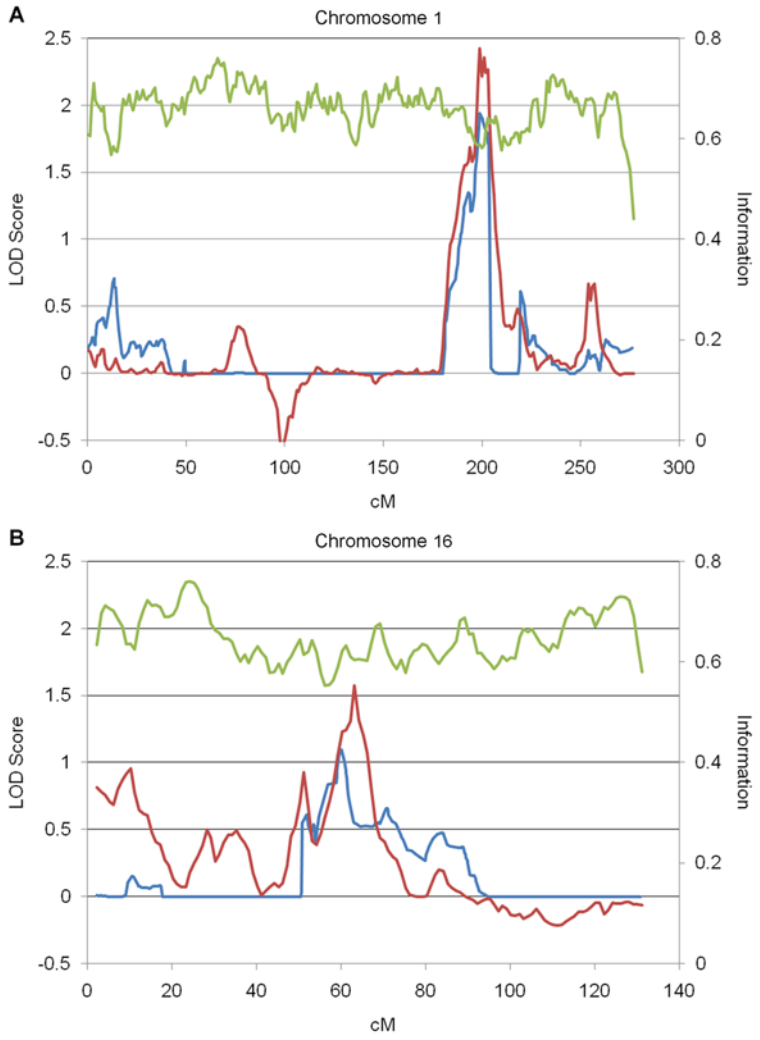

Figure 1.
Linkage to LRRK2-related PD onset age. Quantitative trait linkage (red) and Robust score statistic (blue) LOD scores for onset age and information content (green) are shown for A. chromosome 1 and B. chromosome 16.

Table 1

Description of linkage and association study samples

Genotyped Sample		**Individuals**	**Families**	**PD affected carriers**	**Non-penetrant**
	total	113	64	109	4
	In Families	92	41	86	4
	Singletons	23	23	23	0
Analysis Subsets		Individuals	Families	average age (SD) of PD onset or unaffected enrollment	%Male
Linkage	Families	85	38	-	-
	PD affected carriers	81		61 (10.15)	53%
	Non-penetrant carriers	4		61 (9.42)	0%
Association	PD affected carriers	99	59	62.6 (9.8)	54.5%

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Table 2

A: SNP Association to onset age under linkage peaks on Chromosomes 1.

SNP	ref allele	chr	BP position	cM position	gene symbol	Control MAF	LRRK2 MAF	All effect estimate	All p-value	G2019S only effect estimate	G2019S only p-value
rs2186024	C	1	190,696,708	190.66	RGS1	0.45	0.43	-4.60	0.00068	-3.13	0.03575
rs2494354	G	1	192,721,321	193.26		0.10	0.06	-8.31	0.0047	-6.30	0.0759
rs2494312	C	1	192,763,213	193.28		0.11	0.07	-7.85	0.0043	-6.40	0.0521
rs927724	C	1	194,441,413	193.87	KCNT2	0.12	0.11	7.13	0.0030	7.06	0.0059
rs599779	A	1	197,604,771	195.46		0.40	0.39	4.52	0.0023	4.16	0.0109
rs1898240	G	1	197,613,703	195.47		0.20	0.15	5.85	0.0046	5.24	0.0148
rs487359	A	1	197,648,234	195.49		0.37	0.31	4.58	0.0015	4.65	0.0032
rs1890133	T	1	197,721,501	195.54		0.24	0.23	-4.40	0.0048	-2.49	0.1374
rs10919967	T	1	198,769,294	196.41		0.19	0.15	1.82	0.4443	7.93	0.0022
rs1400875	G	1	200,088,066	199.67	IPO9	0.32	0.29	-4.40	0.0043	-2.65	0.1394
rs2820312	T	1	200,135,880	199.86	LMOD1	0.32	0.29	-4.40	0.0043	-2.65	0.1394
rs3087949	C	1	201,314,244	202.52	PPFIA4	0.29	0.32	-2.99	0.0395	-4.57	0.0024
rs2050935	C	1	201,598,170	203.24	FMOD	0.20	0.18	-5.80	0.0037	-5.13	0.0244
rs4950978	T	1	203,291,196	205.91	CNTN2	0.23	0.23	-4.63	0.0028	-3.74	0.0211
rs1470637	T	1	203,299,906	205.92	CNTN2	0.34	0.36	-3.73	0.0045	-2.85	0.0367
B: SNP Association to onset age under linkage peaks on Chromosomes 16.											
rs7200879	G	16	30,855,073	56.80	FBXL19	0.23	0.23	3.44	0.04620	5.29	0.00485
rs1344528	G	16	48,099,429	59.05	ZNF423	0.45	0.43	-3.69	0.0080	-4.58	0.0025
rs7204293	A	16	48,585,606	59.77	TMEM188	0.08	0.09	-8.27	0.0025	-7.28	0.0188
rs1861662	T	16	48,664,884	59.84	HEATR3	0.08	0.08	-7.52	0.0045	-6.55	0.0319
rs4785239	T	16	49,758,032	61.67	SALL1	0.15	0.14	6.57	0.0013	6.70	0.0034
rs11645369	C	16	51,507,565	65.31		0.25	0.20	-6.12	0.0017	-5.12	0.0216
rs3095635	G	16	52,098,599	66.77	AKTIP	0.18	0.10	-6.69	0.0031	-6.52	0.0128
rs1075440	C	16	52,348,407	67.17	FTO	0.32	0.24	-4.53	0.0017	-3.03	0.0848
rs17224310	A	16	52,548,454	67.49	FTO	0.17	0.20	4.52	0.0107	5.22	0.0043
rs7206456	A	16	52,562,990	67.51	FTO	0.29	0.33	-3.77	0.0048	-3.96	0.0047
rs4784384	T	16	53,029,562	68.25		0.32	0.35	4.34	0.0022	4.51	0.0033
rs893265	G	16	53,864,590	70.30	IRX6	0.37	0.38	-3.72	0.0025	-4.11	0.0026

SNP	ref allele	chr	BP position	cM position	gene symbol	Control MAF	LRRK2 MAF	All effect estimate	All p-value	G2019S only effect estimate	G2019S only p-value
rs2397442	T	16	53,866,761	70.31	IRX6	0.50	0.44	4.43	0.0004	4.06	0.0038
rs30935	C	16	53,878,002	70.37	IRX6	0.43	0.42	5.11	0.0001	4.67	0.0010
rs733140	C	16	53,881,595	70.39	IRX6	0.23	0.20	-3.73	0.0105	-4.63	0.0028
rs11860394	G	16	53,884,305	70.40	IRX6	0.35	0.35	-3.87	0.0037	-3.04	0.0420
rs10521323	A	16	54,038,722	70.70	MMP2	0.25	0.28	4.58	0.0005	4.15	0.0033
rs1420228	C	16	54,042,674	70.70	MMP2	0.11	0.18	-5.83	0.0022	-5.23	0.0109
rs243842	C	16	54,084,923	70.70	MMP2	0.39	0.37	4.52	0.0010	4.34	0.0029
rs899228	G	16	54,944,789	71.69	GNAO1	0.22	0.20	4.84	0.0041	4.69	0.0078

[a]Nearest genes within 100kb of SNP