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LRRK2 gene G2019S mutation and SNPs [haplotypes] in subtypes of Parkinson's disease

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Abstract

Mutation within the Leucine-rich repeat kinase 2 (*LRRK2*) gene has been identified as a cause of autosomal dominant Parkinson's disease (PD). The purpose of this study was to determine the frequency of G2019S mutation and whether the differences in the allele and genotype distribution of six SNPs within *LRRK2* gene are associated with PD in an American non-Hispanic white population. The sample included 350 sporadic PD (SPD), 225 familial PD (FPD) patients and 186 controls of the same race and ethnicity. The frequency of *LRRK2* G2019S mutation in our total sample of PD (FPD and SPD) was 1.56%. The frequency of this mutation was 3.5% in the FPD and 0.3% in the SPD groups, respectively. Allele and genotype frequencies of six SNPs were compared between PD and control samples. In addition, PD groups were categorized by sporadic PD (no family history), familial PD (first degree relative with PD) and age of onset (AON, ≤50 or ≥51 years). The haplotypes of the six SNPs were also constructed for association analysis. After correction for multiple comparisons, there was no association between any SNPs (allele or genotype) and PD groups. One of the haplotypes was modestly associated with the combined PD (SPD & FPD) sample. There was also no association with age at onset of PD. Our study suggests that the *LRRK2* gene may be a risk factor or the cause for a very small fraction of PD in American white population.

Keywords

LRRK2; SNPs; Parkinson's disease; haplotype

Introduction

Parkinson's disease (PD) is one of the most common neurodegenerative diseases in the world, affecting nearly 1.8% of people above 65 years of age in Europe [1]. The major clinical symptoms of this disease are rigidity, bradykinesia, tremor, and postural instability caused by selective degeneration of dopaminergic neurons in the substantia nigra in the midbrain with

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proteinaceous Lewy body inclusions in surviving cells. Although PD is a sporadic disease in most cases, various hereditary forms have similar clinical phenotypes to those of sporadic PD. Genetic studies on these familial forms of PD have provided critical clues that enhance the understanding of the molecular mechanisms of selective degeneration of nigral dopaminergic neurons. At this point, mutations in eight genes (*SNCA, PRKN, PINK1, DJ-1, MAPT, UCH-L1, LRRK2* and *ATP13A2*) are identified to cause familial PD in different populations [2–7].

Mutations within Leucine-rich repeat kinase 2 (*LRRK2*) gene were identified as a potential risk factor for autosomal dominant, idiopathic and sporadic PD [8–10]. The *LRRK2* gene (PARK8; OMIM*609007) was mapped first in the Japanese "Sagamihara family" and it is localized to chromosome 12q12 [11]. *LRRK2* is encoded by 51 exons and seems to be expressed in most brain regions; the protein has a predicted molecular weight of 286 kDa. It is highly conserved among vertebrates and shares homology to the ROCO protein family [8,12,13].

The true prevalence of the *LRRK2* mutations in idiopathic PD, its penetrance, and the phenotypic heterogeneity of associated cases have important implications for genetic screening in the clinical field. The purpose of this study was to determine the frequency of G2019S mutation and whether the differences in the allele and genotype distribution of six SNPs within *LRRK2* gene are associated with PD in an American non-Hispanic white population. The task of mutation screening by direct sequencing of this gene in large samples would be too costly and time-consuming due to the size of the gene that includes 51 exons (7584 bp) and encodes 2,527 amino acids (AY792511). The attractive approach is to screen a large PD sample first with SNPs to establish the association. The individuals with associated haplotype could be selected for sequencing if the association is positive. To test this approach, we selected six SNPs in the *LRRK2* gene and screened a large sample of familial and sporadic PD and matched controls. Then, we evaluated any possible link between SNPs haplotypes and PD in the United States non-Hispanic white population.

METHODS

Patients

This study was approved by the Washington University Human Studies Committee and PD subjects were recruited from the Washington University Movement Disorder Center. We performed a Unified Parkinson's Disease Rating Scale-Motor Subsection (UPDRS; [14]) and Folstein Mini Mental State Examination [15] for each subject. The diagnosis of clinically definite PD patients was based upon criteria previously described [16]. Dementia was not an exclusion criterion if the onset of cognitive changes occurred after the first year of onset of parkinsonism that was otherwise typical of clinically definite idiopathic PD [17]. There were 225 FPD (124 males, 101 females; mean age of proband 67±13.7 (SD)), 350 SPD (208 males, 142 females; mean age of 68±11.7 years) patients and 186 controls (73 males, 113 females; mean age of 62±14 years) in our present investigation. The mean (SD) ages of onset of disease for SPD and FPD were 61.8 (11.8) and 57.6 (13.3) years, respectively. In the FPD group, the age at onset (AON) range was 22 – 81 years while for SPD the range was 30–82 years. A neurologist determined that all spouse controls had no evidence of parkinsonism. The remaining controls were selected from an epidemiological sample from Greater St. Louis area who received no diagnosis for parkinsonism. The gender distributions were not similar in both groups (PD: male 58%, controls: male 39%). All subjects are whites of non- Hispanic origin. To determine the frequency of G2019S in different races, we also screened a sample of 19 American Indian (AI; FPD=12, SPD=7) and 18 African American (AA; FPD= 5, SPD= 13). No mutation in *SNCA* and *Parkin* genes was detected in the patients and controls in these samples.

Genotyping of G2019S mutation and SNPs

Venous blood specimens were collected directly from all examined patients with familial or sporadic PD and controls. Genomic DNA was extracted using phenol chloroform extraction method [18]. Our samples were screened for G2019S mutation using the reported primers [19]. We also genotyped our samples with six SNPs (rs1388598, rs1491941, rs1491938, rs10506151, rs10784486 and rs1365763) [10] in the gene. We used HapMap which contains CEU sample (http://www.hapmap.org/cgi-perl/gbrowse/Density_test/) to identify tag SNPs. The LRRK2 gene spans 144.3kb. Three SNPs (rs1491938, rs10784486, and rs1365763) tag 74 SNPs in this gene. The coverage is impressive although they do not cover the whole region. Apart from rs10506151, the other two (rs1388598, rs1491941) were not listed. We assume they are new relative to HapMap. The information from ENSEMBL and HapMap are consistent. The genotyping reaction was composed of TaqMan universal PCR master mix, TaqMan SNP genotyping reagent which included forward and reverse primers and SNP specific probe labeled with VIC dye linked to the 5′ end of the allele 1 probes and FAM dye linked to the 5′ end of the allele 2 probes. Allelic discrimination was done automatically with 7900HT fast real-time PCR machine using SDS software (Applied Biosystem, Inc., Foster City, CA).

Statistical and Genetic Analysis

Data analysis was done with SAS/GENETICS v9.1.3 and R 2.3.1 (http://www.r-project.org). The comparison between FPD and controls, as well as between SPD and controls, was according to SAS CASECONTROL procedure, while the combined analysis treated FPD, SPD and controls in an ordinal logistic regression as implemented in SAS LOGISTIC procedure. For comparison, the ordinal logistic regression in R (with or without adding a dummy locus in order to use the haplo.score function in haplo.stats package [20] and lrm in Design package) was also used, which yielded comparable results. The details of these procedures are presented in Lake et al., [21], Zhao & Tan [22] and Barrett et al. [23]. The results of all tests were corrected according to Bonferroni's correction.

In the case of ordinal regression (control, sporadic, familial) for six-SNP haplotype analysis (via R haplo.stats library), assuming that the global association is comparable to that of a standard chi-squared statistic with value of 20.03, df =15, for a type-I error of 0.05 and a sample size of 684 used in the final analysis there would be 83% power. In case of the difference between the age of PD at diagnosis and controls, we don't expect an age-by-genotype interaction.

RESULTS

G2019S mutation frequency

The frequency of LRRK2 G2019S mutation in our total sample of PD (FPD and SPD) was 1.56% (9/575). The frequency of this mutation was 3.5% (8/225) in the FPD and 0.3% (1/350) in the SPD groups, respectively. There was a patient in FPD group with homozygous mutation (1/225 = 0.0044 (0.44%) or 1/575 = 0.0017 (0.17%) in total PD). Interestingly, there were two controls heterozygous for G2019S mutation (2/186 = 0.011 or 1.1%). The AON range in the FPD group with the mutation was from 43 to 68 years. There was no G2019S mutation in our AI or AA samples.

Allele and Genotype frequencies of SNPs

The allele and genotype distributions of the six SNPs in FPD, SPD, and control groups are presented in Tables 1 and 2. All the SNPs genotype data in all groups were in Hardy Weinberg Equilibrium except SNP rs10784486 in SPD (HWE p= 0.04). However, this HWE p value was

not significant after Bonferroni's correction for multiple comparisons. Using the chromosome counting method allele frequencies for all SNPs (rs10784486, rs1388598, rs1491941, rs1491938, rs10506151 and rs1365763) were not significantly different in SPD, FPD, or controls.

Association Analysis

All p values calculated were based on trend tests and logistic regressions and were non-significant for alleles of rs1388598, rs1491941, rs1491938, rs10506151 and rs1365763 in SPD, FPD, or controls. Only SNP rs10784486 resulted in p= 0.07 value calculated based on trend tests and rs1388598 with p= 0.08 calculated based on ordinal logistic regressions with adjustment for sex. Gender had no influence as we found that in general there were no substantial changes in p value when sex was included in the regression model as covariate (Table 3).

Haplotype analysis

We measured the strength of LD between the six SNPs in control sample (Figure 1). There was evidence of marker-marker association according to control data (global test statistic = 1196, p<0.0001) but not in FPD/control (χ^2= 25.24, p=0.99) and SPD/control (χ^2=35.72, p=0.94) samples. For the combined (FPD + SPD + controls) analysis using haplo.score [20], the statistical significance of specific haplotype was at most borderline (p=0.03, simulated p=0.053) for haplotype 122221.

Age of Onset (AON)

For G2019S mutation, the AON range in the FPD group with the mutation was from 43 to 68 years. There was no correlation with AON. In association study**, t**he PD affected individuals were categorized based on AON of ≤50 and ≥51 years. There was no association between any AON group and individual SNPs or haplotypes of the six SNPs.

Discussion

Several studies have reported that LRRK2 mutations may contribute to more typical idiopathic PD and one such mutation (G2019S) has been reported in PD patients of all ages. A clinic-based case control study indicated that the G2019S mutation in exon 41 of the LRRK2 gene might be a common cause of PD but the frequency of this mutation was only 1.6% [24]. These data do not confirm the important contribution of this mutation in LRRK2 to PD susceptibility in a clinic-based population. However, the reported frequency of this mutation by Zabetian, et al., [24] is very similar to ours for total PD sample (1.56%). They also identified only two of 586 Japanese patients with PD heterozygous for the G2019S mutation. Their finding suggests that G2019S originated from separate founders in Europe and Japan and is more widely dispersed than previously recognized [25]. Paisan-Ruiz et al [26] screened all the exons of *LRRK2* gene in a sample of 23 patients, substantially smaller than that from the other studies [24,27]. They identified two probands with G2019S heterozygous mutations and one with a novel I1371V substitution that had been identified in white patients of Canadian origin. However, there was no association between PD and any of the four polymorphisms at the allelic or genotypic levels (p <0.17) [26].

Overall, the frequency of *LRRK2* G2019S mutation was reported to be 1.3% among 297 sporadic patients of European ancestry [24], similar to the 1.6% reported in 482 UK PD patients [9], 1% reported by Toft et al [28] and 1.56% in present study. Mutation screening from 629 probands from Italy, including early (<50 years) and late onset, familial and sporadic PD revealed that 13 (2.1%) were heterozygous carriers of the G2019S mutation. The mutation frequency was higher among familial (5.1%, 9/177) than among sporadic probands (0.9%,

4/452; p<0.002), and highest among probands with one affected parent (8.7%, 6/69; p<0.001). There was no difference in the frequency of the G2019S mutation in probands with early vs. late onset disease [29]. This study is very similar to our study especially by categorizing the PD sample based on family history and AON. It is very interesting that the results are also very similar (e.g. for FPD: 5.1% and 3.5%, respectively). Surprisingly, the frequency of G2019S mutation found in North African families (7/17, 41%) was greater than those from Europe (5/174, 2.9%) [30]. Their North African sample is very small; however, w**e** didn't detect G2019S mutation in our AI or AA samples. The difference in the frequency of G2019S mutation in African populations may indicate the founder effect. However, the heterogeneity observed in American white population is due to the admixture.

It is important to note that several studies have detected the G2019S mutation in a few controls [31–35]. We also detected two controls with heterozygous genotype for this mutation. These two individuals are in their late 70's with no sign of Parkinsonism. The very low frequency of G2019S mutation in PD and the detection of this mutation in controls raise several questions regarding the role of this gene in the development of PD. In addition, there is no difference in the diagnosis of PD symptoms between carriers and non-carriers of this mutation even in form of homozygous. In our sample of FPD, there was one patient with homozygous G2019S mutation. The diagnosis of this patient is typical idiopathic PD. These findings indicate that the G2019S mutation in LRRK2 gene may play a minor role in the development of PD in a very small portion of PD population.

Studies on variation in LRRK2 gene and its contribution to the development or risk of PD have not been evaluated in details in the white population (non- Hispanic origin) of the United States [10,26,36]. Previous studies demonstrate a limitation of population specific haplotype in disease association investigations [10,36]. It is also established that a human genome as a colorful mosaic of haplotype blocks delimited by recombination hotspots [36]. Differences in haplotype distribution leads to differences in association among different population as found in Chinese [10] and Greek [36] population but no association reported in German population [37–38]. This concept is also reflected in our study that is very similar to the findings of Skipper et al [10] and Paisan-Ruiz et al [36]. Skipper et al [10] reported that the tSNP rs10506151 dramatically increases PD risk in a Chinese population who were homozygous for the minor allele (rs10506151 A) under both recessive and additive models. Although in the United States white population this SNP is in very low LD with the other five that we tested, there were no statistical significant differences in allelic distribution among SPD, FPD and control. From our study it is clear that the haplotype block for LRRK2 gene among Chinese and United States white populations are different.

In our SNP genotyping assay, we included 575 patients with PD (SPD & FPD) and 186 matched normal controls for analysis with the six SNPs within LRRK2. None of the six SNPs was significantly associated with FPD or SPD when analysis was performed based on trend tests and logistic regressions with and without adjustment for gender. There is a possibility that these six SNPs are involved with other risk factors for the development of PD or they are in LD with other mutation or variations in the gene. Smith et al. [13] reported that kinase activity of mutant LRRK2 is critical for neuronal toxicity of the pathogenesis of LRRK2-linked PD, potentially may illuminate mechanism of the development of sporadic PD. Therefore, it is possible that a very small fraction of susceptibility to SPD is associated with variation within LRRK2 gene in addition to other endogenous and exogenous factors. Paisan-Ruiz et al. [26] found no association between PD (early onset and positive family history) and four SNPs in a smaller sample (N=121). The findings from a small sample may not generalize to larger samples. Also, different populations may have different recombination hotspots and differences in haplotype frequency. Paisan-Ruiz et al. [36] also did a comprehensive analysis of 31 SNPs including five potentially functional SNPs in diverse human populations. While

they found marginal association between PD and SNPs rs10878258 and rs2723264 only in the Greek cohort. Their study cannot, however, exclude the possibility that low frequency alleles within LRRK2 may exert an effect on lifetime risk for PD. As they [36] did not have a sufficiently large sample to study or they also not included SNPs that have found to be significant in other populations [10], we can not reject the possibility that other SNPs are in LD within their PD sample. When we did the combined (FPD, SPD & controls) analysis with the specific haplotypes, only the haplotype 122221 reached borderline significance (p=0.03, simulated p=0.053). These findings suggest that the LRRK2 six SNPs halpotype may contribute to PD risk in the United State white population. Of course, other genetic and non genetic risk factors must be explored.

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References

1. De Rijk MC, Launer LJ, Berger K, Breteler MM, Dartigues JF, Baldereschi M, et al. Prevalence of Parkinson's disease in Europe: a collaborative study of population based cohorts. Neurologic Diseases in the Elderly Research Group. Neurology 2000;54:S21–23. [PubMed: 10854357]
2. Parsian AJ, Racette BA, Zhao JH, Sinha R, Patra B, Perlmutter JS, et al. Association of α-Synuclein gene haplotypes with Parkinson's disease. Parkinsonism & Related Disorders 2007;13:343–347. [PubMed: 17292657]
3. Zarranz JJ, Alegre J, Gomez-Esteban JC, Lezcano E, Ros R, Ampuero I, et al. The new mutation, E46K, of alpha-synuclein causes Parkinson and Lewy body dementia. Ann Neurol 2004;55:164–173. [PubMed: 14755719]
4. Chartier-Harlin M, Kachergus J, Roumier C, Mouroux V, Douay X, Lincoln S, et al. Alpha-synuclein locus duplication as a cause of familial Parkinson's disease. The Lancet 2004;364:1167–1169.
5. Pals P, Lincoln S, Manning J, Heckman M, Skipper L, Hulihan M, et al. Alpha- Synuclein promoter confers susceptibility to Parkinson's disease. Ann Neurol 2004;56:591–595. [PubMed: 15455394]
6. Leroy E, Boyer R, Auburger G, Leube B, Ulm G, Mezey E, et al. The ubiquitin pathway in Parkinson's disease. Nature 1998;395:451–452. [PubMed: 9774100]
7. Ramirez A, Heimbach A, Grundemann J, Stiller B, Hampshire D, Cid LP, et al. Hereditary parkinsonism with dementia is caused by mutations in ATP13A2, encoding a lysosomal type 5 P-type ATPase. Nature Genetics 2006;38:1184–1191. [PubMed: 16964263]
8. Zimprich A, Biskup S, Leitner P, Lichtner P, Farrer M, Lincoln S, et al. Mutations in LRRK2 cause autosomal-dominant parkinsonism with pleomorphic pathology. Neuron 2004;44:601–607. [PubMed: 15541309]
9. Gilks WP, Abou-Sleiman PM, Gandhi S, Jain S, Singleton A, Lees AJ, et al. A common LRRK2 mutation in idiopathic Parkinson's disease. Lancet 2005;365:415–416. [PubMed: 15680457]
10. Skipper L, Li Y, Bonnard C, Pavanni R, Yih Y, Chua E, et al. Comprehensive evaluation of common genetic variation within LRRK2 reveals evidence for association with sporadic Parkinson's disease. Hum Mol Genet 2005;14:3549–3556. [PubMed: 16269443]
11. Funayama M, Hasegawa K, Kowa H, Saito M, Tsuji S, Obata F. A new locus for Parkinson's disease (PARK8) maps to chromosome 12p11.2-q13.1. Ann Neurol 2002;51:296–301. [PubMed: 11891824]
12. Bosgraaf L, Van Haastert PJ. Roc, a Ras/GTPase domain in complex proteins. Biochim Biophys Acta 2003;1643:5–10. [PubMed: 14654223]
13. Smith WW, Pei Z, Jiang H, Dawson VL, Dawson TM, Ross CA. Kinase activity of mutant LRRK2 mediates neuronal toxicity. Nat Neurosci 2006;9:1231–1233. [PubMed: 16980962]

14. Fahn, S.; Elton, RL. Members of the UPDRS Development Committee. Unified Parkinson's disease rating scale. In: Fahn, S.; Marsden, CD.; Goldstein, M.; Calne, DB., editors. A Recent Developments in Parkinson's Disease. New York: Macmillan; 1987. p. 153-163.
15. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state": a practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975;12:189–198. [PubMed: 1202204]
16. Racette BA, Rundle M, Parsian A, Perlmutter JS. Evaluation of a screening questionnaire for genetic studies of Parkinson's disease. Am J Med Genet 1999;88:539–543. [PubMed: 10490713]
17. Hughes AJ, Daniel SE, Kilford L, Lees AJ. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: A clinico-pathological study of 100 cases. J Neurol Neurosurg Psychiatry 1992;55:181–184. [PubMed: 1564476]
18. Parsian A, Racette B, Zhang ZH, Chakraverty S, Rundle M, Goate A, et al. Mutation sequence analysis and association studies of α-synuclein in Parkinson,s disease. Neurology 1998;51:1757–1759. [PubMed: 9855543]
19. Nichols WC, Pankratz N, Hernandez D, Paisán-Ruíz C, Jain S, Halter CA, et al. Genetic screening for a single common LRRK2 mutation in familial Parkinson's disease. Lancet 2005;365:410–412. [PubMed: 15680455]
20. Schaid DJ, Rowland CM, Tines DE, Jacobson RM, Poland GA. Score tests for association of traits with haplotypes when linkage phase is ambiguous. Am J Hum Genet 2002;70:425–34. [PubMed: 11791212]
21. Lake S, Lyon H, Tantisira K, Silverman EK, Weiss ST, Laird NM, et al. Estimation and tests of haplotype-environment interaction when linkage phase is ambiguous. Hum Hered 2003;55:56–65. [PubMed: 12890927]
22. Zhao JH, Tan Q. Genetic dissection of complex traits in silico: approaches, problems, and solutions. Curr Bioinformat 2006;1:359–369.
23. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005;21:263–265. [PubMed: 15297300]
24. Zabetian CP, Samii A, Mosley AD, Roberts JW, Leis BC, Yearout D, et al. A clinic-based study of the LRRK2 gene in Parkinson disease yields new mutations. Neurology 2005;65:741–744. [PubMed: 16157909]
25. Zabetian CP, Morino H, Ujike H, Yamamoto M, Oda M, Maruyama H, et al. Identification and haplotype analysis of LRRK2 G2019S in Japanese patients with Parkinson disease. Neurology 2006;67:697–9. [PubMed: 16728648]
26. Paisan-Ruiz C, Lang AE, Kawarai T, Sato C, Salehi-Rad S, Fisman GK, et al. LRRK2 gene in Parkinson disease: mutation analysis and case control association study. Neurology 2005;65:696–700. [PubMed: 16157901]
27. Bialecka M, Hui S, Klodowska-Duda G, Opala G, Tan EK, Drozdzik M. Analysis of LRRK2 G2019S and I2020T mutations in Parkinson's disease. Neuroscience Letters 2005;390:1–3. [PubMed: 16115731]
28. Toft M, Mata IF, Kachergus JM, Ross OA, Farrer MJ. *LRRK2* mutations and parkinsonism. Lancet 2005;365:1229–1230. [PubMed: 15811454]
29. Goldwurm S, Di Fonzo A, Simons EJ, Rohé CF, Zini M, Canesi M, et al. The G6055A (G2019S) mutation in LRRK2 is frequent in both early and late onset Parkinson's disease and originates from a common ancestor. J Med Genet 2005;42:e65. [PubMed: 16272257]
30. Lesage S, Ibanez P, Lohmann E, Pollak P, Tison F, Tazir M, et al. French Parkinson's Disease Genetics Study Group. G2019S LRRK2 mutation in French and North African families with Parkinson's disease. Ann Neurol 2005;58:784–787. [PubMed: 16240353]
31. Clark LN, Wang Y, Karlins E, Saito L, Mejia-Santana H, Harris J, et al. Frequency of LRRK2 mutations in early- and late-onset Parkinson's disease. Neurology 2006;67:1786–1791. [PubMed: 17050822]
32. Farrer M, Stone J, Mata IF, Lincoln S, Kachergus J, Hulihan M, et al. LRRK2 mutations in Parkinson's disease. Neurology 2005;65:738–740. [PubMed: 16157908]
33. Ishihara L, Warren L, Gibson R, Amouri R, Lesage S, Dürr A, et al. Clinical features of Parkinson disease patients with homozygous Leucine-Rich Repeat Kinase 2 G2019S mutations. Arch Neurol 2006;63:1250–1254. [PubMed: 16966502]

34. Papapetropoulos S, Singer C, Ross OA, Toft M, Johnson JL, Farrer MJ, et al. Clinical heterogeneity of the *LRRK2* G2019S mutation. Arch Neurol 2006;63:1242–1246. [PubMed: 16966501]

35. Cossua G, van Doeselaarb M, Deriu M, Melis M, Molari A, Di Fonzo A, et al. LRRK2 mutations and Parkinson's disease in Sardinia—A Mediterranean genetic isolate. Parkinsonism and Related Disorders 2007;13:17–21. [PubMed: 17064949]

36. Paisan-Ruiz C, Evans EW, Jain S, Xiromerisiou G, Gibbs JR, Eerola J, et al. Testing association between LRRK2 and Parkinson's disease and investigating linkage disequilibrium. J Med Genet 2006;43:e9. [PubMed: 16467219]

37. Biskup S, Mueller JC, Sharma M, Lichtner P, Zimprich A, Berg D, et al. Common variants of LRRK2 are not associated with sporadic Parkinson's disease. Ann Neurol 2005;58:905–908. [PubMed: 16254973]

38. Zhang K, Sun F. Assessing the power of tag SNPs in the mapping of quantitative trait loci (QTL) with extremal and random samples. BMC Genet 2005;6:51. [PubMed: 16236175]

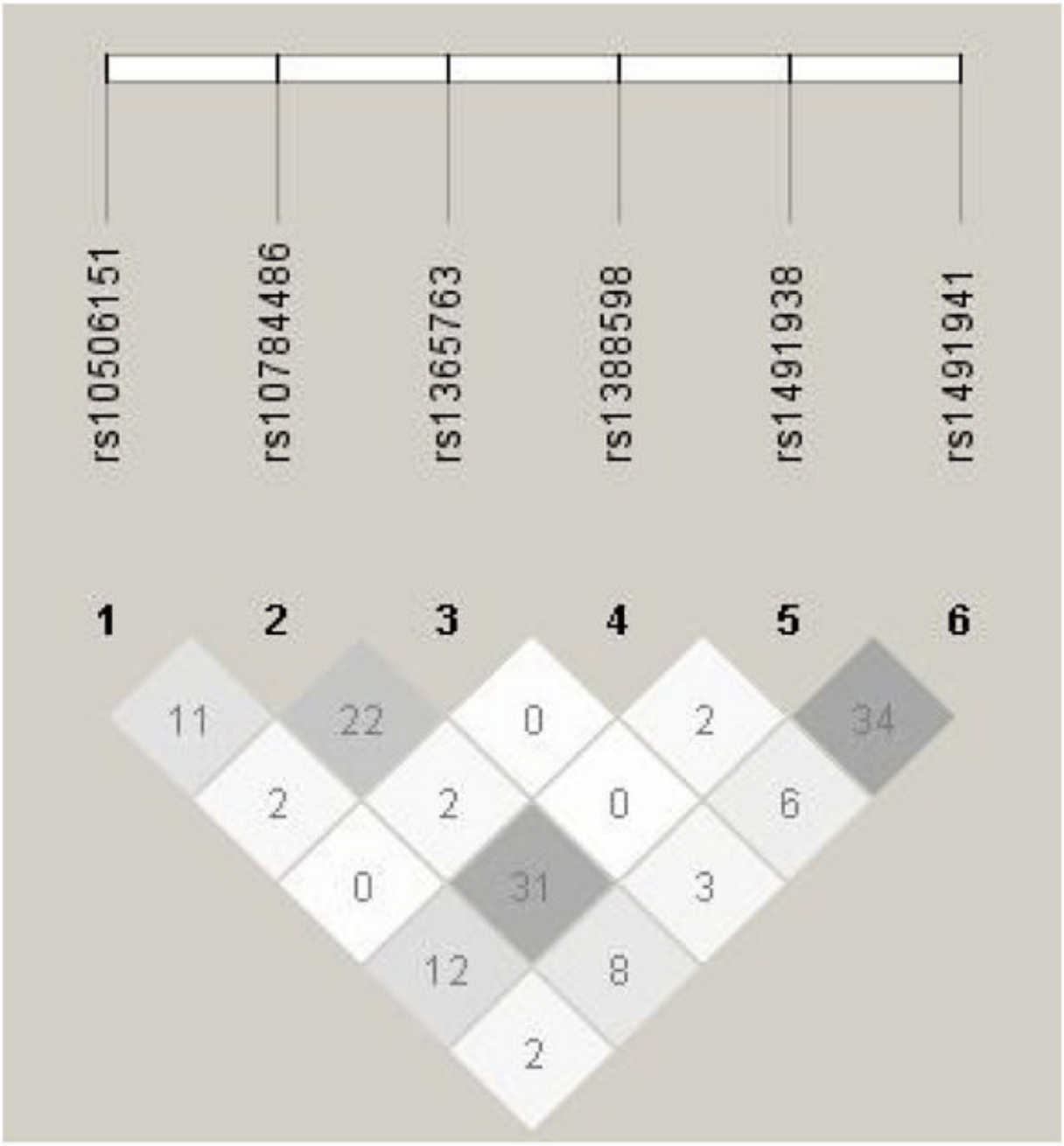

Figure 1.
Physical distance among six SNPs presented in the diagram by specifying unit distances (the quantities shown are in r^2).

Table 1

Distribution of alleles in Parkinson's and controls

	FPD	SPD	CONTROL
rs10506151			
1	63 (0.16)	94 (0.16)	55 (0.15)
2	321 (0.84)	508 (0.84)	317 (0.85)
rs10784486			
1	135 (0.35)	200 (0.33)	143 (0.39)
2	247 (0.65)	408 (0.67)	227 (0.61)
rs1365763			
1	63 (0.16)	90 (0.15)	63 (0.17)
2	325 (0.84)	516 (0.85)	309 (0.83)
rs1388598			
1	10 (0.03)	30 (0.05)	11 (0.03)
2	374 (0.97)	574 (0.95)	355 (0.97)
rs1491938			
1	157 (0.41)	251 (0.42)	155 (0.42)
2	229 (0.59)	353 (0.58)	213 (0.58)
rs1491941			
1	105 (0.27)	184 (0.31)	123 (0.33)
2	281 (0.73)	420 (0.69)	247 (0.67)

RS10506151: 1=A, 2=C; RS1365763: 1=T, 2=C; RS1491938: 1=C, 2=T; RS10784486: 1=A, 2=C; RS1388598: 1=C, 2=T and RS1491941: 1=C, 2=T.

Table 2

The distribution of genotypes in Parkinson's and controls

	FPD	SPD	CONTROL
rs10506151			
1/1	4(0.02)	9(0.03)	3(0.02)
1/2	55(0.29)	76(0.25)	49(0.26)
2/2	133(0.69)	216(0.72)	134(0.72)
HWE *p*	0.53	0.47	0.54
rs10784486			
1/1	26(0.14)	25(0.08)	30(0.16)
1/2	83(0.43)	150(0.49)	83(0.45)
2/2	82(0.43)	129(0.42)	72(0.39)
HWE *p*	0.50	**0.04***	0.46
rs1365763			
1/1	4(0.02)	9(0.03)	7(0.04)
1/2	55(0.28)	72(0.24)	49(0.26)
2/2	135(0.70)	222(0.73)	130(0.70)
HWE *p*	0.56	0.29	0.39
rs1388598			
1/1	0(0.00)	2(0.01)	0(0.00)
1/2	10(0.05)	26(0.08)	11(0.04)
2/2	182(0.95)	274(0.91)	172(0.96)
HWE *p*	0.71	0.13	0.68
rs1491938			
1/1	33(0.17)	51((0.17)	37(0.20)
1/2	91(0.47)	149(0.49)	81(0.44)
2/2	69(0.36)	102(0.34)	66(0.36)
HWE *p*	0.75	0.78	0.19
rs1491941			
1/1	15(0.08)	25(0.08)	20(0.11)
1/2	75(0.39)	134(0.44)	83(0.45)
2/2	103(0.53)	143(0.47)	82(0.44)
HWE *p*	0.79	0.41	0.88

* Not significant after correction for multiple testing

Table 3

The results for analysis involving case-control data, where gender was also used as a covariate in the combined analysis.

	FPD	SPD	All	With adjustment for sex
rs10506151	0.54	0.78	0.54	0.67
rs10784486	0.37	0.07	0.33	0.42
rs1365763	0.84	0.42	0.81	0.54
rs1388598	0.82	0.20	0.77	0.08
rs1491938	0.72	0.89	0.69	0.75
rs1491941	0.09	0.38	0.07	0.25

All p values calculated were based on trend tests and logistic regressions and were non-significant. The second and third columns are based on trend tests of FPD and SPD with controls, respectively, while the last two columns are based on ordinal logistic regressions without or with adjustment for gender.