

## Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Ozelius LJ, Senthil G, Saunders-Pullman, et al. *LRRK2* G2019S as a cause of Parkinson's disease in Ashkenazi Jews. *N Engl J Med* 2006;354:424-5.

## **Supplementary Material**

### *Methods*

DNA was extracted from white blood or buccal cells using the Puregene procedure (Gentra Systems Inc, Minneapolis, MN). The G2019S mutation in LRRK2 (G6055A SNP in exon 41) along with two other coding SNPs, rs1427263 and rs11564148, were amplified by PCR using standard conditions. The primers used for amplification and sequencing are shown in Table 1 and were designed using PSQ version 1.0.6 software (Biotage). Each amplification set contained a biotin labeled primer. Genotyping was performed using a Pyrosequencing PSQ HS 96A system 1.2 (Biotage).

Five microsatellite markers spanning the LRRK2 gene including, D12S2194, D12S2515, D12S2516, D12S2519 and D12S2521, were amplified using the primers and conditions previously reported<sup>6</sup>. All primer sets contain a fluorescent tag at the 5' end of the sense primer. The amplified PCR products were resolved on the ABI 3730 automated DNA sequencer and the results analyzed using the GeneMapper 3.5 software (Applied Biosystems). The presence of significant allelic association was tested using a Chi square test.

Table 1. Primer sequences used for LRRK2 mutation and SNP genotyping.

SNP	Primer sequence	Amplicon length	Mg+2, Temp
G2019S	F-CCTGTGCATTTTCTGGCAGATA	171 bp	1.5 mM
	*R-CCTCTGATGTTTTTATCCCCATTC		61°C
	S-TGCAAAGATTGCTGAC		
rs1427263	*F-TCTCCTATTGGCAAAGCAATC	180 bp	1.5 mM
	R-GACTCGAATCTTTCAGATTTTGAC		60°C
	S-TGTCCAAAACACCCTAA		
rs11564148	*F-AGGAAATTTCCAAAGAACTACATG	98 bp	1.5 mM
	R-CTTGGAACCAGCAAATATTCTTCT		60°C
	S-TAGGAGCTTAAAATACTGTG		

F-forward primer; R-reverse primer; S-Pyrosequencing primer

\* 5' Biotinylated primer