Letter to the Editor

Am. J. Hum. Genet. 78:1088, 2006

No Evidence for Association with Parkinson Disease for 13 Single-Nucleotide Polymorphisms Identified by Whole-Genome Association Screening

To the Editor:

The 13 SNPs identified by Maraganore et al.[1] as being potentially associated with Parkinson disease (PD [MIM 168600]) represent some of the first fruit produced by the whole-genome association screening era and are clearly worthy of follow-up. To further explore these exciting candidates, we typed each SNP in 538 patients with idiopathic PD and in 516 control individuals from the United Kingdom. Cases included 160 patients involved in a community-based epidemiological study of incident PD and 378 consecutive patients with prevalent PD attending our research clinic. All cases met United Kingdom Parkinson's Disease Society Brain Bank criteria for the diagnosis of PD. The mean age at disease onset was 63 years (range 25–91 years); 2% of patients had early-onset disease ($\leqslant$40 years), and 14% of patients reported a family history of one or more first-degree relatives with parkinsonian symptoms or tremor. The control group consisted of 146 spouses of patients with PD and 370 blood donors. All individuals were white, except for four patients and one spouse. All gave written informed consent and a blood sample from which DNA was extracted using standard methods. Genotyping was performed using Taqman Assay-on-Demand (*rs2245218*) and Assays-by-Design products on a 7900HT Sequence Detection System (Applied Biosystems). Only samples that typed successfully for at least one-third of markers were included in the analysis (520 cases and 499 controls). Genotyping success rates were all $\geqslant$97%, and no marker showed evidence of deviation from Hardy-Weinberg equilibrium. Two pairs of SNPs (*rs2313982* and *rs1509269*; *rs682705* and *rs7520966*) were found to be in strong linkage disequilibrium ($D' = 1.0$, $r^2 > 0.69$), which reduced the number of independent tests to 11. Allele frequencies in cases and controls were compared using the COCAPHASE program in the UNPHASED package.[2] Our study provides, on average, 85% power (range 68%–96%) to detect the case-control differences averaged over tier 1 and tier 2, as observed by Maraganore et al.[1]

In our data set, none of the 13 SNPs showed any evidence of association, all P values being >.25, even without correction for multiple testing (tables 1 and 2). Fewer than half of the SNPs (46%) showed allele frequency differences between cases and controls in the same direction as that reported by Maraganore et al.[1] The combination of our data with those from the original report, with the use of the Mantel-Haenszel test statistic (Statsdirect) and correction for the 11 independent tests performed, revealed that only three markers (*rs10200894, ss46548856,* and *rs7702187*) retain any evidence of significance at the 5% level in the total data (table 1). In summary, our study suggests that none of the 13 markers identified by Maraganore et al.[1] is associated with PD.

Under the null hypothesis that there are no genes influencing susceptibility to PD, a follow-up of 1.4% (2,734) of the 198,345 markers included in the screening stage, as performed by Maraganore et al.,[1] would be expected to identify 27–28 markers showing $P < .01$ in the replication stage, with half of these—that is, 13–14—showing an allele frequency difference in the same direction as that seen in the screening stage. The number of markers identified by Maraganore et al.[1] is, thus, in keeping with that expected under the null hypothesis. However, since such screens are not intended to identify all susceptibility genes and, indeed, would be considered successful if they identified even a single such locus, we would not expect to see a striking excess of markers above the predicted 13. In short, it could be anticipated that most of the 13 markers identified by Maraganore et al.[1] would be false positives. However, our failure to replicate results for any of the 13 markers identified by Maraganore et al.[1] suggests that their screen lacked power in one or more critical dimensions. Although typing 200,000 markers in 450 cases and controls is a substantial effort, it is clear that this will adequately interrogate only a part of the common variation in the genome. Increasing the density of markers and the number of samples studied would be the most effective way to increase the power of the study but, in practice, would be the most difficult. It must remain possible that a more generous threshold (such as $P < .1$) would have captured relevant loci currently lying high in the ranking of mark-

Table 1

Thirteen SNPs Reported by Maraganore et al.,[1] Ranked in Accordance with Evidence for Association in a Meta-Analysis Combined with Data from This Study

dbSNP Accession Number	Gene	Chromosome	Position	Control MAF[a]	Case MAF	OR (95% CI)	*P*	
							This Study[b]	Meta-Analysis[c]
rs10200894	...	2q36	228642637	.09	.08	.91 (.67–1.24)	.53	.01
ss46548856[d]	...	10q21	58986929	.10	.09	.92 (.68–1.24)	.58	.02
rs7702187	*SEMA5A*	5p15	9385281	.16	.16	.97 (.76–1.23)	.81	.02
rs17329669	...	7p14	36625169	.13	.13	1.04 (.80–1.35)	.79	.06
rs7723605	...	5p15	5407615	.13	.14	1.07 (.83–1.39)	.59	.06
rs7878232	*PASD1*	Xq28	150516943	.23	.23	.99 (.78–1.26)	.95	.11
rs682705	*LOC200008*	1p32	54349438	.26	.28	1.08 (.89–1.31)	.44	.20
rs7520966	*LOC200008*	1p32	54357283	.26	.28	1.07 (.88–1.30)	.51	.22
rs2245218	*PRDM2*	1p36	13885132	.16	.14	.89 (.70–1.14)	.36	.28
rs2313982	...	4q31	139145665	.09	.08	.83 (.61–1.14)	.26	.33
rs1509269	...	4q31	139111329	.12	.12	.92 (.70–1.20)	.53	.41
rs11737074	...	4q27	125438978	.23	.21	.90 (.73–1.11)	.32	.86
rs16851009	*GALNT3*	2q24	166456214	.10	.09	.86 (.64–1.16)	.33	.94

[a] Minor-allele frequency.

[b] *P* value for comparison of case and control allele frequencies with the use of UNPHASED.[2]

[c] *P* value corresponding to Mantel-Haenszel test statistic for association, with data from this study and that from Maraganore et al.,[1] after correction for the number of independent tests.

[d] Perlegen Sciences internal SNP identifier, as used by Maraganore et al.[1]

Table 2

Genotype Counts for 13 SNPs Studied

The table is available in its entirety in the online edition of *The American Journal of Human Genetics*.

ers provided by the screening stage performed by Maraganore et al.[1] but falling outside their stringent threshold. On the downside, this approach would greatly increase the number of markers requiring follow-up, generating a list of nearly 1,000 instead of just 13 potentially associated loci.

Various strategies for multistage whole-genome association studies have been proposed,[3–6] and the importance of setting an appropriate threshold for following up first-stage results has been stressed. We feel that the present observations, regarding one of the first whole-genome association screens performed, strengthen the importance of these theoretical recommendations. To ensure that replication and follow-up phases are not overwhelmingly large, it is essential to ensure high power in the screening phase. If thresholds as stringent as $P < .01$ are to be used, the screening phase in future PD screens will need to be very much larger than that performed by Maraganore et al.[1]

Acknowledgments

This work was supported by the Medical Research Council and the Parkinson's Disease Society. A.G. is a postdoctoral fellow of the Research Foundation of Flanders (FWO Vlaanderen). C.H.W.G. is a Patrick Berthoud clinical research fellow and holds a Raymond and Beverley Sackler scholarship.

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Web Resources

The URLs for data presented herein are as follows:

dbSNP, http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?CMD=search&DB=snp

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for PD)

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 0002-9297/2006/7806-0022$15.00