

Non-Replication of Association for Six Polymorphisms From Meta-Analysis of Genome-Wide Association Studies of Parkinson's Disease: Large-Scale Collaborative Study

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Early genome-wide association (GWA) studies on Parkinson's disease (PD) have not been able to yield conclusive, replicable signals of association, perhaps due to limited sample size. We aimed to investigate whether association signals derived from the meta-analysis of the first two GWA investigations might be replicable in different populations. We examined six single-nucleotide polymorphisms (SNPs) (rs1000291, rs1865997, rs2241743, rs2282048, rs2313982, and rs3018626) that had reached nominal significance with at least two of three different strategies proposed in a previous analysis of the original GWA studies. Investigators from the "Genetic Epidemiology of Parkinson's Disease" (GEOPD) consortium were invited to join in this study. Ten teams contributed replication data from 3,458 PD cases and 3,719 controls. The data from the two previously published GWAs (599 PD cases, 592 controls and 443 sibling pairs) were considered as well. All data were synthesized using both fixed and random effects models. The summary allelic odds ratios were ranging from 0.97 to 1.09 by random effects, when all data were included. The summary estimates of the replication data sets (excluding the original GWA data) were very close to 1.00 (range 0.98–1.09) and none of the effects were nominally statistically significant. The replication data sets had significantly different results than the GWA data. Our data do not support evidence that any of these six SNPs reflect susceptibility markers for PD. Much stronger signals of statistical significance in GWA

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platforms are needed to have substantial chances of replication. Specifically in PD genetics, this would require much larger GWA studies and perhaps novel analytical techniques.

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INTRODUCTION

Genome-wide screening for genetic associations is a promising approach for the exploration of the relationship between the genetic determinants and common complex diseases [McCarthy et al., 2008]. Meta-analysis is a technique that entails the combination of different studies or data sets. Recently, it has become a powerful tool for the synthesis of different genome-wide association (GWA) studies for the same phenotype [Evangelou et al., 2007; Baum et al., 2008; Zeggini et al., 2008]. The first attempt to synthesize results from GWA studies has been performed for the genetics of Parkinson's disease (PD) [Evangelou et al., 2007]. The effect estimates from a two-tier design study (LEAPS) [Maraganore et al., 2005] and from a single-tier design (NINDS) [Fung et al., 2006] study were synthesized to identify potentially significant signals. Given the limited sample sizes of both combined GWA data sets, the attained statistical significance for the summary results were not impressive. Nevertheless, some tentative signals were noted and six single-nucleotide polymorphisms (SNPs) were found to be nominally significant at the $P < 0.05$ level even by random effects calculations with at least two of the three meta-analytic strategies that were investigated.

The replication of the findings from GWAs and the detection of loci with realistic effect sizes require the use of large sample sizes [Burton et al., 2008]. In order to assess if the signals from the meta-analysis reflect true findings, we co-ordinated an independent large-scale multicenter international replication effort. Our study incorporated also data from the previous published GWAs [Maraganore et al., 2005; Fung et al., 2006] along with the replication data. Meta-analytical techniques were applied for the combination of the available data.

METHODS

Study Population

Investigators consisting the "Genetic Epidemiology of Parkinson's Disease" (GEOPD) consortium were invited to join in this study.

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Ten teams participated in the replication study, contributing with individual-level data on PD cases and PD-free controls. Data from the two already published GWAs were also available for comparison with the replication data and for inclusion in the overall analysis.

Polymorphisms

The following six polymorphisms were evaluated: rs1000291, rs1865997, rs2241743, rs2282048, rs2313982, and rs3018626. These SNPs had been found to have nominally statistical significant association even by random effects calculations, in at least two of the three strategies used for the combination of data from the first two published GWA studies: enhancement of first-stage data, enhancement of replication data, and joint analysis of all available data sets [Evangelou et al., 2007]. Three of the six had nominally significant results with all three strategies and no or minimal heterogeneity (I^2 0–15%) across the combined data sets. The summary data from the combined GWA data sets for these six polymorphisms in the joint analysis varied from 0.76 to 0.81 (for protective effects) and 1.25 to 1.65 (for susceptibility effects) by fixed effects and from 0.76 to 0.80 and 1.25 to 1.67 by random effects analyses. P -values ranged from 0.0001 to 0.004 by fixed effects and from 0.0001 to 0.038 by random effects.

Genotyping of DNA samples was undertaken either on-site or through commercial contract. Methods that were used was fluorescence polarization single-base extension (Wirdefeldt), allelic discrimination (real-time PCR) (Tan), mass spectrometry (van Broeckhoven), TaqMan assay (Hadjigeorgiou, Ferrarese, Annesi), Illumina genotyping chips (Illumina Humanhap550) (Sharma), Sequenom Platform (Mellick), direct sequencing (Brice), and Illumina Golden Gate assay (Elbaz).

For teams where the genotyping call rate was $< 95\%$, we requested a second effort to genotype the missing samples, so that the 95% threshold could be exceeded for completeness of genotyping information. We also used an exact test to examine whether there is a deviation from the Hardy–Weinberg equilibrium (HWE) among controls in each team. Deviation for HWE was deemed significant

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for P -value <0.05 . In the presence of significant deviation from HWE, the specific teams were also asked to consider re-genotyping their samples. We specified upfront that we would perform for each SNP sensitivity analyses that would exclude data from teams where the missing rate was $>5\%$ or there was nominally significant deviation from HWE in the controls.

Data Synthesis

The natural log odds ratio and their respective standard errors were calculated for each evaluated SNP within each team. For consistency, we computed all the effect estimates based on the major versus minor allele contrast. Assignment of minor allele was based on the allele frequencies of the control samples in the NINDS GWA study. Then the log ORs were synthesized using both fixed [Lau et al., 1997] and random effects models [DerSimonian and Laird, 1986]. In fixed effects models, it is assumed that the risk of the alleles is always the same across the comparisons. In random effects models, the risk is varying around an overall average. Thus, random effects models can be considered more conservative. In absence of heterogeneity, summary effects obtained from fixed and random effects models coincide. We tested the presence of between-study heterogeneity using Cochran's Q test and we calculated the extent of inconsistency using I^2 , which ranges from 0% to 100% [Higgins and Thompson, 2002; Higgins et al., 2003]. We also calculated 95% confidence intervals for I^2 [Ioannidis et al., 2007].

In the main analysis, all the effect estimates from the replication effort and the published GWA studies were considered. Another analysis was also undertaken where summary effects were provided separately for replication and already published data. Even for genuine associations, it is expected that effect sizes may be inflated in the discovery (GWA) phase [Zollner and Pritchard, 2007] [Ioannidis, 2008b] and this may result in heterogeneity in the data when both the GWA and replication data are considered.

As above, in a sensitivity analysis we excluded studies that deviated from HWE and studies that had genotyping call rate $<95\%$ in cases or in controls groups only.

We calculated the Bayes factor for nominally significant associations (uncorrected for multiple comparisons), considering a spike and smear prior [Ioannidis, 2008a] and assuming that average effect sizes for associations in PD may reflect odds ratios of 1.3. Bayes factor evaluates if a nominally statistically significant result increases the credibility of a postulated association.

The six evaluated SNPs were very common with minor allele frequencies ranging from 22.7% to 49.3% in the combined GWA data sets, with one exception (rs2313982) where the minor allele frequency was only 9.4%. For the lowest minor allele frequency among them, odds ratios of 1.25 would require almost 6,800 cases and controls to have 80% power to replicate at $\alpha = 0.05$, and about 10,450 cases and controls to have 80% power to replicate at $\alpha = 0.05/6 = 0.0083$, assuming 1:1 allocation and homogeneous effects. For minor allele frequencies of 22.7%, the respective numbers are 3,400 and 5,240 cases and controls for odds ratios of 1.25 for $P = 0.05$ and 0.0083, respectively. For odds ratios of 0.8, the respective numbers are 3,840 cases and controls for $P = 0.05$ and 5,920 cases and controls for $P = 0.0083$.

All analyses were performed with Intercooled Stata 10 (College Station, TX).

RESULTS

Characteristics of Participating Studies

Ten replicating teams contributed 3,458 cases and 3,719 controls. Characteristics of these 10 study populations are shown in Table I. The previously published GWAs included (a) 443 sibling pairs (family-based design) (cases were enrolled at the Department of Neurology of the Mayo Clinic in Rochester, MN, and their siblings were contacted, if permitted, in order to exclude Parkinsonism via a validated tool) and 332 matched case-unrelated control pairs (LEAPS, two-tier design) [Maraganore et al., 2005] and (b) 267 publicly available samples from a cohort of PD patients and 270 neurologically normal controls (NINDS, single-tier design) with publicly available samples [Fung et al., 2006].

Analysis Including All Data

Allele frequencies per team and per SNP of interest are shown in Table II. The meta-analysis of these data did not reveal any nominally statistically significant associations by random effects with the exception of rs3018626. The summary OR for this SNP was 1.09 (95% CI 1.01–1.17) with $P = 0.037$ uncorrected for multiple comparisons (Table II). With fixed effects analysis the summary estimate for the specific SNP was also nominally statistically significant ($P = 0.032$ uncorrected) and low heterogeneity was observed ($I^2 = 6\%$) (Table II).

In two cases, one study deviated from HWE equilibrium in the control group (Brice for rs3018982 and Annesi for rs2282048 [$P < 0.0001$ and 0.02, respectively]). Moreover, in six studies even though the overall genotyping call rate was $>95\%$, more than 5% missing genotypes existed in the control group. We performed a sensitivity analysis excluding these studies and the results were essentially unaltered (Table III). For rs2313982, a nominally statistically significant association was detected (OR = 1.15) (95% CI 1.02–1.31) ($P = 0.026$, uncorrected for multiple corrections), but moderate heterogeneity was observed. The observed association was not nominally significant with random effects analysis (Table II).

None of the aforementioned nominally significant associations revealed with either main or sensitivity analyses would remain significant after adjusting even for six tested SNPs.

Analysis Including Only Replication Studies

All summary effects obtained from the replication effort were very close to unity (Fig. 1) with estimated summary ORs ranging from 0.98 to 1.09 with random effects analyses. None of the effects were nominally statistically significant. Also, no heterogeneity was observed in three SNPs (rs2241743, rs2313982, and rs3018626 with I^2 ranging from 0% to 6%), whereas moderate to large heterogeneity was observed for the other three SNPs under study (rs1000291, rs1865997, and rs2241743) with I^2 ranging from 49% to 67%.

TABLE I. Characteristics of the Participating Teams Evaluating the Replication of the Six SNPs

Team	Location	Cases (N)	Controls (N)	Age at onset, (SD)		Age at study, (SD)		Gender, females (%)	Familial PD ^a (%)	Diagnostic criteria	Genotyping methods
				cases mean	controls mean	cases mean	controls mean				
Annesi	Italy	200	200	61.2 (10.5)	67.0 (9.2)	44.7 (15.5)	49.25	NA	UKPDBB	TaqMan assay	
Brice	France	291	250	47.6 (10.1)	57.8 (11.5)	57.8 (11.9)	40.7	0	At least two of the Parkinsonian triad of signs (bradykinesia, rigidity, rest tremor), at least 30% improvement after L-dopa therapy and absence of other PD case among first-degree relatives	Direct sequencing	
Elbaz	France	209	501	63.5 (7.3)	67.0 (7.0)	66.9 (7.2)	41.55	6.5	Bower	Illumina Golden Gate assay	
Ferrarese	Italy	100	100	58.8 (8.2)	65.5 (7.0)	65.0 (6.9)	44.5	NA	Gelb	TaqMan assay	
Hadjigeorgiou	Greece	300	300	64.4 (9.7)	69.5 (9.4)	70.0 (8.4)	42.64	0	Bower	TaqMan assay	
Mellick	Australia	1,013	681	59.4 (11.5)	72.3 (10.7)	71.2 (10.9)	46.93	24.6	UKPDBB	Sequenom Platform	
Sharma	Germany	742	944	NA	NA	NA	NA	NA	UKPDBB	Illumina Humanhap 550	
Tan	Singapore	203	201	61.5 (10.0)	66.3 (10.0)	53.5 (11.0)	48.76	5.9	UKPDBB	Allelic discrimination (real-time PCR)	
Van Broeckhoven	Belgium	305	364	NA	60.3 (11.3)	60.8 (14.4)	51.7	NA	Pals/Engelborghs	Mass spectrometry	
Wirdefeldt	Sweden	95	179	65.9 (10.9)	75.7 (8.6)	74.9 (9.1)	48.18	25.2	Gelb	Fluorescence polarization single-base extension	

UKPDBB, United Kingdom Parkinson's Disease Brain Bank.
^aProportion of PD cases that have family history of PD.

TABLE II. Allele Counts for Each of the Six SNPs in Each of the Replication Teams

Team	N	Cases (major allele/minor allele)						Controls (major allele/minor allele)					
		rs1000291, A/G	rs1865997, A/G	rs2241743, T/C	rs2282048, A/G	rs3313982, C/T	rs3018626, G/T	rs1000291, A/G	rs1865997, A/G	rs2241743, T/C	rs2282048, A/G	rs3313982, C/T	rs3018626, G/T
Annesi	200	216/184	253/147	237/163	219/181	376/24	308/92	205/195	243/157	259/141	205/195	365/35	314/86
		0.54/0.46	0.63/0.37	0.59/0.41	0.55/0.45	0.94/0.06	0.77/0.23	0.51/0.49	0.61/0.39	0.65/0.35	0.51/0.49	0.91/0.09	0.79/0.21
Brice	291	295/287	372/210	339/239	239/237	521/59	444/134	252/248	308/192	298/202	305/195	463/37	367/133
		0.51/0.49	0.64/0.36	0.58/0.42	0.59/0.41	0.90/0.10	0.77/0.13	0.505/0.495	0.62/0.38	0.60/0.40	0.61/0.39	0.93/0.07	0.73/0.27
Elbaz	209	219/197	248/166	256/156	258/140	390/24	317/99	543/453	615/377	604/390	582/362	934/64	780/216
		0.53/0.47	0.60/0.40	0.62/0.38	0.65/0.35	0.94/0.06	0.76/0.24	0.55/0.45	0.62/0.38	0.61/0.39	0.62/0.38	0.94/0.06	0.78/0.22
Ferrarese	100	105/95	113/87	124/76	108/92	190/10	158/42	123/77	116/82	116/84	93/107	187/13	152/48
		0.53/0.47	0.57/0.43	0.62/0.38	0.54/0.46	0.95/0.05	0.79/21	0.62/0.38	0.59/0.41	0.58/0.42	0.47/0.53	0.94/0.06	0.76/0.24
Hadjigeorgiou	300	287/289	387/183	336/232	307/273	546/36	441/135	333/239	354/238	345/239	293/307	570/26	451/145
		0.50/0.50	0.68/0.32	0.59/0.41	0.53/0.47	0.94/0.06	0.77/0.23	0.58/0.42	0.60/0.40	0.59/0.41	0.49/0.51	0.96/0.04	0.76/0.24
Mellick	1,013	1,018/972	1,129/871	1,090/890	1,236/768	1,869/147	1,489/469	723/613	793/553	751/577	822/528	1,263/95	996/324
		0.51/0.49	0.56/0.44	0.55/0.45	0.62/0.38	0.93/0.07	0.76/0.24	0.54/0.46	0.59/0.41	0.57/0.43	0.61/0.39	0.93/0.07	0.75/0.25
Sharma	742	778/706	NA	NA	NA	1,372/112	1,159/323	1,026/862	NA	NA	NA	1,727/159	1,456/430
		0.52/0.48				0.92/0.08	0.78/0.22	0.54/0.46				0.92/0.08	0.77/0.23
Tan	203	86/318	251/147	154/252	154/248	384/20	337/65	108/294	225/163	138/262	129/265	379/23	333/65
		0.21/0.79	0.63/0.37	0.38/0.62	0.38/0.62	0.95/0.05	0.84/0.16	0.27/0.73	0.58/0.42	0.35/0.65	0.33/0.67	0.94/0.06	0.84/0.16
Van Broeckhoven	305	317/283	367/237	358/242	391/211	549/49	462/128	343/337	385/305	403/283	415/273	625/63	542/158
		0.53/0.47	0.61/0.39	0.60/0.40	0.65/0.35	0.92/0.08	0.78/0.22	0.505/0.495	0.56/0.44	0.59/0.41	0.60/0.40	0.91/0.09	0.77/0.23
Wirdefeldt	95	107/83	114/76	112/78	105/83	165/25	NA	198/158	207/147	199/157	221/131	318/38	NA
		0.56/0.44	0.60/0.40	0.59/0.41	0.56/0.44	0.87/0.13		0.56/0.44	0.58/0.42	0.56/0.44	0.63/0.37	0.89/0.11	
Total	3,458	3,719											

NA, not available.
Some genotypes may be missing in some cases and controls groups.

TABLE III. Summary Odds Ratios and 95% Confidence Intervals for the Six SNPs Including Both the Original GWA and Replication Data

dbSNP id	No. studies	No. ^a cases/controls	Fixed effects OR (95% CI)	P-value	Random effects OR (95% CI)	P-value	I ² (%) (95% CI)
rs1000291							
Overall	13	4,020/4,257	1.03 [0.97–1.10]	NS	1.05 [0.94–1.18]	NS	67 [40–81]
rs1865997							
Overall	12	3,278/3,322	0.98 [0.92–1.05]	NS	0.99 [0.87–1.12]	NS	67 [39–82]
Sensitivity analysis	11	2,976/2,977	0.96 [0.90–1.03]	NS	0.97 [0.85–1.10]	NS	65 [34–82]
rs2241743							
Overall	12	3,266/3,316	1.05 [0.98–1.12]	NS	1.06 [0.97–1.16]	NS	32 [0–66]
Sensitivity analysis	10	2,682/2,681	1.06 [0.98–1.14]	NS	1.08 [0.97–1.20]	NS	44 [0–73]
rs2282048							
Overall	12	3,224/2,714	0.99 [0.93–1.07]	NS	0.98 [0.86–1.13]	NS	71 [48–84]
Sensitivity analysis	9	2,524/2,290	0.94 [0.87–1.02]	NS	0.93 [0.79–1.09]	NS	74 [50–87]
rs2313982							
Overall	13	4,301/4,552	1.11 [0.99–1.25]	NS	1.13 [0.95–1.34]	NS	49 [4–73]
Sensitivity analysis	11	3,712/3,952	1.15 [1.02–1.31]	0.026	1.18 [0.98–1.43]	NS	48 [0–74]
rs3018626							
Overall	12	3,900/4,090	1.08 [1.01–1.17]	0.032	1.09 [1.01–1.17]	0.037	6 [0–61]

CI, confidence interval; NS, not nominally significant ($P > 0.05$); OR, odds ratio.

Sensitivity analysis: analysis excluding studies with $>5\%$ missing genotype rate or significant deviation from Hardy–Weinberg equilibrium.

^aFour hundred forty-nine sibling pairs were evaluated in all analyses.

The summary effects obtained from the replication data were statistically significantly different from the results of the synthesis of the GWA data. Four out of six SNPs (rs1000291, rs1865997, rs2241743, and rs2282048) had summary effects that were even in the opposite direction compared to summary effects obtained from the original GWA data.

Bayes Factor and Credibility of the Association

We calculated the Bayes factors for the association between rs3018626 and PD that was nominally statistically significant when all data were included. For an expected OR = 1.3, the $-\log_{10}$ Bayes factor is 0.01. This means that the probability of the association being true does not increase given the observed data, compared with what we thought before obtaining these data.

DISCUSSION

Our analysis of six SNPs suggested by a meta-analysis of two GWA data sets does not show any convincing associations with PD. None of the SNPs under study reached even nominal significance in the replication data sets and inclusion of all data (including the GWA discovery data) yielded only one SNP with a P -value of 0.037, which should be dismissed given the extreme multiplicity of the agnostic discovery approach in the GWA setting. The Bayes factor for the combined data was not supportive of an association being present.

The collaborative analysis has sufficient power to detect modestly small associations for five of the six tested SNPs. However, this would not be the case for very subtle associations. For example, effects in the range of odds ratios of <1.12 may have been missed, and experience from meta-analysis of GWA in type 2 diabetes (T2D) suggests that such effects cannot be excluded [Zeggini et al.,

2008]. On the other hand, we managed to assemble one of the largest studies in the history of PD research. Perusal of very subtle effects, if any such exist, would really require full exploitation of all available samples from multiple consortia [Seminara et al., 2007]. Moreover,

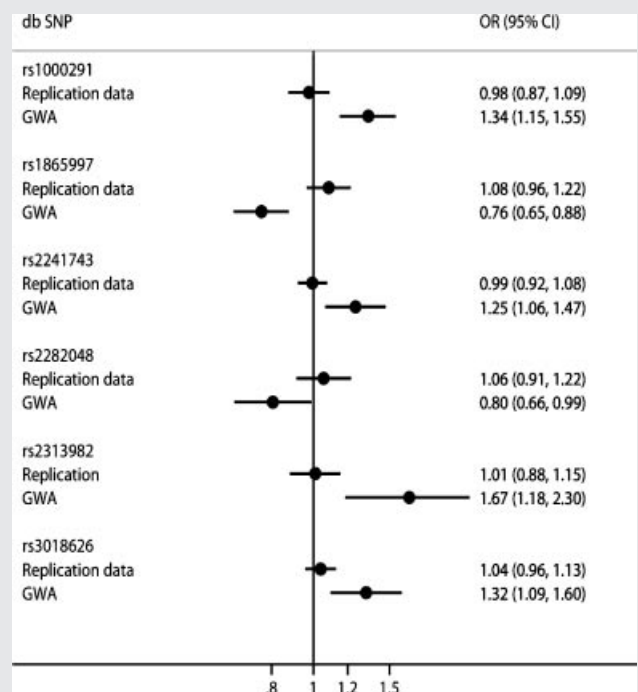


FIG. 1. Forest plot of subgroup summary effects estimates presenting ORs and 95% CIs computed by random effects for the replication data and for the original meta-analysis of GWA data.

if diagnostic criteria and patient characteristics create genuine heterogeneity in the genetic effects, replication would be difficult or even impossible even with extremely large sample sizes [Mooneshinghe et al., 2008]. Power may also be diluted by genotyping errors and unfortunately we were not able to perform central genotyping or central assessment/quality control for the assessment of genotyping errors. Nevertheless, while genuine heterogeneity and genotyping errors can never be totally excluded, we doubt that they primarily explain the non-replication in the observed results.

The most likely explanation for our findings is that none of these six SNPs has a genuine association with PD risk. Even when combined, the two GWAs remain underpowered to reveal efficiently true markers with modest effects. Moreover, testing only six SNPs is unlikely to have hit genuine markers for PD susceptibility. Due to resource constraints, we could not evaluate a wider range of SNPs that might have been more informative. It is expected that genuine associations are not on the very top of the list in the data that arise from GWA studies, regardless of the selection analytical strategy [McCarthy et al., 2008]. Moreover, we should acknowledge that one SNP (rs2313982) has been further evaluated with published data before, and this information is widely available at www.pdgene.org. This database provides a comprehensive assessment of all prior genetic association studies in PD. Analysis of this SNP shows nominally statistically significant random effects odds ratios in the PDGene meta-analyses after exclusion of the initial study ($P=0.012$). However, both main and sensitivity analyses by random effects did not show any statistical significant results in our sample. An informal meta-analysis of previous data (excluding the first data) plus our new data (excluding previously published information) by random effects would give an odds ratio of 1.08 (95% CI 1.01–1.17), which is not nominally significant ($P=0.055$, no between-study heterogeneity, $I^2=0$, 95% CI 0–45%).

Given our current experience, and the recurrent inability to replicate top hits derived from modest size GWA data sets [Elbaz et al., 2006], we believe that a more extensive list of candidate markers for replication should await the accumulation of much larger sample sizes from GWA platforms. Additional GWA studies are currently being completed on PD and their data would be very useful to combine with the existing information. Given that different platforms are used in these GWA investigations, imputation of polymorphisms that are not directly typed [Marchini et al., 2007] can enhance also the genome coverage compared with the first meta-analysis of GWA data that was limited only to common, overlapping polymorphisms across Perlegen and Illumina platforms [Evangelou et al., 2007]. Concurrently, novel analytical approaches, such as pathways analyses [Lesnick et al., 2007; Wang et al., 2007; Li et al., 2008; Srinivasan et al., 2008], and extension to other kinds of genetic variation, such as copy number variation or rare variants [Jakobsson et al., 2008] may be helpful in determining whether we can expand our knowledge base about genetic determinants of PD.

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