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Original Article

Genotype and age at Parkinson disease diagnosis

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Abstract: Parkinson disease (PD) is a degenerative movement disorder that results from the destruction of dopaminergic neurons in the midbrain substantia nigra. Both genetic and environmental factors contribute to PD risk, and likely to age at diagnosis. Among 258 newly diagnosed non-Hispanic Caucasian cases from Group Health Cooperative in western Washington State, we assessed whether diagnosis age was associated with 1,327 single nucleotide polymorphisms in genes related to central nervous system function, oxidative stress, inflammation or metal transport. We conducted linear regression to assess the age difference per variant allele while adjusting for sex and smoking. Of the polymorphisms associated with PD diagnosis age (p_{trend}<0.05), three demonstrated similar associations among 64 PD cases from the University of Washington Neurology Clinic, were not similarly associated ($p_{interaction}$<0.05) with age in general among 436 unrelated non-Hispanic Caucasian controls from the source population, and were predicted to be functional according to a public National Institute of Environmental Health Sciences polymorphism database. The most robust association was for rs10889162, a polymorphism in a predicted transcription factor binding site -582 bp from *CYP2J2* arachidonic acid epoxygenase. Each variant allele was associated with 5.04 years older diagnosis age (95% confidence interval 2.28-7.80, p=0.0003). This association did not vary by sex or smoking history. Polymorphisms in predicted microRNA binding sites in *GSTM5* and *SLC11A2* were also associated with >2-year differences in diagnosis age. These results await confirmation in other series of incident cases, but suggest that selected genes and environmental exposures may influence PD diagnosis age.

Keywords: Arachidonic acid, CYP2J2 protein, CYP3A7 protein, divalent metal transporter-1, GSTM5 protein, idiopathic Parkinson disease, linoleic acid, SLC11A2 protein

Introduction

Parkinson disease (PD, OMIM 168600) is a neurological disorder that affects approximately 2% of the population >65 years of age [1]. The incidence of PD differs by race and ethnicity, is greater in men than women, and increases markedly with age [2, 3]. Loss of dopaminergic neurons in the midbrain substantia nigra leads to the disease's characteristic signs: bradykinesia, muscle rigidity, tremor and gait disturbance. The underlying mechanisms of this neural loss remain poorly understood, but oxidative stress [4] and neuroinflammation [5] appear to play pathogenic roles.

Both genetic and environmental factors likely contribute to the development of PD. Eleven genetic loci are clearly associated with the occurrence of sporadic PD [6], including *SNCA* and *LRRK2*, which are also among the genes mutated in the less common (5-10%) familial form. *SNCA* codes for the major component of Lewy bodies, the pathogenic hallmark of PD [7]. The pathophysiological role of LRRK2 remains unknown [8], but may include effects on autophagy [9] or mitochondria [10]. Numerous additional genes remain to be confirmed or identified [11]. Suspected environmental risk factors for PD include various metals and pesticides, whereas cigarette smoking and coffee consumption are inversely associated with risk [12]. Notably, ever smokers have half the risk of PD relative to never smokers, making smoking the most prominent non-genetic correlate of PD beyond basic demographic characteristics.

One hypothesis of PD development is that loss of dopaminergic neurons occurs with aging – and that when a sufficient number are lost, the signs and symptoms of PD manifest. Identification of genes associated with age at onset (time of first signs and symptoms) or diagnosis may thus provide clues about what accelerates or delays neural loss. Because younger age at onset or diagnosis is associated with longer survival [3, 13-15], investigations of predictors of PD diagnosis age ideally should be restricted to incident or recently diagnosed cases, rather than prevalent cases of long duration, to avoid confounding by survival, which is also plausibly related to genotype, treatment and other exposures such as smoking. A demographically similar control group is also needed to verify that the observed associations do not simply reflect those in the source population [16, 17].

We sought to identify functional genetic polymorphisms associated with age at PD diagnosis in a population-based case-control sample particularly well suited to this analysis. We included newly diagnosed PD cases identified at a single health maintenance organization to ensure that observed associations would not be biased by access to health care or survival. We assessed consistency of findings in a separate group of newly diagnosed PD cases from the University of Washington Neurology clinic. We also verified that the polymorphisms were not similarly associated with age in general among unrelated non-Hispanic Cancasian controls from the source population. In view of the central nervous system (CNS) pathophysiology associated with PD, we focused on genes coding for particular neurotransmitter and neurotransmitter receptor proteins, and genes involved in neurotransmitter synthesis and transport, especially dopamine. We also considered selected genes related to oxidative stress, inflammation and metal transport. Our hypothesis was that genes related to endogenous or xenobiotic factors that affect neural loss among PD patients would be associated with age at PD diagnosis.

Materials and methods

Study population

We enrolled 490 newly diagnosed idiopathic PD cases in a population-based case-control study [18, 19]. Of these, 387 were diagnosed in 1992-2008 at Group Health Cooperative (GHC), a health maintenance organization in the Seattle-Puget Sound region of Washington State. The remaining 103 cases were identified at the University of Washington (UW) Neurology Clinic in Seattle. All diagnoses were made by movement disorder specialists or were verified by study neurologists (GMF, WTL, PDS) via consensus chart reviews based on an *a priori* case definition. All cases had ≥2 of 4 cardinal signs of PD (bradykinesia, resting tremor, cogwheel rigidity, and postural reflex impairment). Excluded from this group were cases enrolled >4 years after diagnosis, with an established cause of secondary parkinsonism (e.g., stroke, brain tumor, selected medications), or with a Mini-Mental State Examination (MMSE) score <24. Controls were 644 GHC enrollees frequency-matched to GHC cases on sex, age, race/ethnicity, clinic and year of GHC enrollment. Controls were cognitively normal (MMSE ≥24) and without PD, Alzheimer disease, multiple sclerosis or other neurodegenerative disorder. We made no exclusions related to cardiovascular disease, cancer or other conditions affecting attained age. We obtained Human Subjects approval and written informed consent prior to study conduct.

Assessment of age at diagnosis

For the majority (72%) of cases we calculated age at diagnosis by subtracting the case's birth date from the neurologist-adjudicated date of PD diagnosis. Cases also were asked to self-report their age at diagnosis as part of a structured questionnaire administered in person. This value was used for the remainder of cases (28%), because among cases with both age at diagnosis variables, their correlation was excellent (r=0.99). We generated a reference age for each control by randomly subtracting up to 4 years from the interview age while ensuring that cases and controls in each age-sex stratum were comparable with respect to time between diagnosis/reference and interview. The questionnaire also ascertained detailed cigarette smoking histories up to the age at diagnosis/reference.

Genotyping

During the interviews we asked participants to provide a biospecimen; 471 (96%) of cases and

Table 1. Characteristics of non-Hispanic Caucasian Parkinson disease (PD) cases and controls with genotyping data, Group Health Cooperative (GHC) and University of Washington (UW), 1992-2008

	GHC cases N=258 n (%)	UW cases N=64 n (%)	Controls N=436 n (%)
Diagnosis/reference age (years)			
≥60	202 (78)	29 (45)	354 (81)
Range	39-88	28-80	43-85
Median	69	57	70
Mean (standard deviation)	67.2 (8.8)	56.1 (11.8)	68.0 (8.7)
Male	165 (64)	46 (72)	276 (63)
PD family history[a]	25 (12)	5 (9)	18 (5)
Ever smoked >100 cigarettes	119 (46)	25 (39)	243 (56)
Years between PD diagnosis and interview, mean (standard deviation)	0.73 (0.58)	0.92 (1.10)	--

[a]First degree relative; percent excludes 55 GHC cases, 11 UW cases and 99 controls with any missing data on PD family history.
Abbreviations: GHC, Group Health Cooperative; PD, Parkinson disease; UW, University of Washington.

612 (95%) of controls agreed, with 85% of cases and 89% controls providing blood, and the remainder buccal specimens. DNA extraction and genotyping were performed blinded to age and case status at the Center for Ecogenetics and Environmental Health Functional Genomics Laboratory at the University of Washington (Seattle, WA). For non-Hispanic Caucasians with sufficient DNA for an Affymetrix custom array (Santa Clara, CA) at the time of analysis, we assessed single nucleotide polymorphisms (SNPs) in 120 genes related to CNS function, oxidative stress, inflammation or metal transport. We (TKB and FMF) selected SNPs using a TagSNP approach and the Genome Variation Server (http://gvs.gs.washington.edu), while enriching for SNPs reported in the literature as functional. Data were available for 322 cases (258 GHC, 64 UW) and 436 controls for 1,327 SNPs, after excluding participants with <90% individual call rate, and SNPs with <95% assay call rate or a lack of Hardy-Weinberg equilibrium ($p<0.001$) among controls.

Statistical analysis

We conducted multivariable linear regression to assess the association between age (continuous) at PD diagnosis among cases (or reference among controls) and genotype. Because we focused on functional SNPs, we modeled all SNPs linearly (log-additive), as in a recent study of PD age at onset [20]. We assessed functionality using a publicly available National Institute of Environmental Health Sciences database (http://snpinfo.niehs.nih.gov/snpinfo/snpfunc.htm, accessed November 16, 2012) [21]. We downloaded data for all 1,327 SNPs and classified a SNP as functional if the SNP results in a stop codon (1 SNP) or amino acid change (70 SNPs), is otherwise located in a splicing or splicing enhancer/silencing site (20 SNPs), or is located in a predicted binding site for a transcription factor (128 SNPs) or microRNA (miRNA, 44 SNPs). In total, 255 of the 1,327 SNPs met one or more of these criteria. We adjusted for sex and smoking (ever vs. never >100 cigarettes) in all models, which were restricted to non-Hispanic Caucasians. We used Stata version 11.1 (College Station, TX) to generate these models and to confirm the appropriateness of modeling genotype linearly. All reported β and 95% confidence intervals (CIs) indicate the sex- and smoking-adjusted difference in years per variant allele.

In the GHC case group we were able detect a clinically meaningful (>2 year) difference in age at PD diagnosis for SNPs with a minor allele frequency (MAF) as low as 0.05 given two-sided $\alpha=0.05$. For all SNPs with a p-value <0.05 in GHC cases, we repeated analyses in UW cases to assess consistency of the findings. Because statistical power was limited in the UW case group we determined whether the direction and magnitude of the gene-PD age association was similar, rather than requiring statistical significance. We then verified that the SNP was not similarly associated with attained age in the source population, that is, among controls. This general approach has been applied in a previous study of PD age at onset [16]. In our specific approach we constructed a linear regression model with all GHC cases and controls to determine the p-value of the product term between case status and linear genotype while including the respective main effect terms and

the covariates as above. This allowed us to formally test whether the gene-age association (β estimates) differed between GHC cases and controls. We report here all gene-PD age associations that were 1) statistically significant in GHC cases (uncorrected p<0.05), 2) of the same direction and of a clinically relevant magnitude (>2 years) in UW cases and 3) statistically significantly different in GHC cases than controls (uncorrected multiplicative interaction p-value<0.05). Because of these restrictive criteria and our exclusion of non-functional SNPs to reduce false positives, we did not correct p-values for multiple comparisons.

Results

Characteristics of cases and controls

All participants were non-Hispanic Caucasian, and a majority were male (**Table 1**). Most GHC cases (78%) had been diagnosed at ≥60 years of age, with a median age of 69 years. On average, UW cases were younger (median 57 years, p<0.001). GHC and UW cases were otherwise not markedly different. Relatively few (9-12%) had a family history of PD in a first-degree relative. The mean delay between PD diagnosis and interview was <1 year in each case group. Controls were similar in age to GHC cases (81% ≥60 years at reference, median 70 years). Proportionally more controls than cases had ever smoked >100 cigarettes (p<0.05 for each case group vs. controls).

Genotype and age at PD diagnosis

Three of the 255 functional SNPs in the 120 targeted genes were associated with age at PD diagnosis (**Table 2**). The most robust association was seen for *CYP2J2* rs10889162. This SNP was the one most strongly associated with PD age at diagnosis in GHC cases, and was also of borderline statistical significance in UW cases. In the pooled analysis each *CYP2J2* rs10889162 variant (T) allele was associated with 5.04 years later occurrence of PD (95% CI 2.28-7.80 years older, p=0.0003, **Table 3**). Each variant allele of two other SNPs, *GSTM5* rs11807 and *SLC11A2* rs150909, was associated with a >2 year difference in PD occurrence in both case groups, although confidence intervals were very wide for UW cases (**Table 2**). With the two case groups combined, each *GSTM5* rs11807 variant (G) allele was associated with 2.37 (95% CI 0.52-4.21) years younger age at PD diagnosis, and each *SLC11A2* rs150909 variant (C) allele was associated with 3.09 (95% CI 0.13-6.06) years older age at PD diagnosis (**Table 3**). These associations did not vary markedly by sex or smoking. None of these three SNPs was assessed in the only publicly available genome-wide data well-suited to obtaining unbiased results, that is, with newly diagnosed cases and the expected strong inverse association between PD and smoking [22].

When we examined whether other available SNPs in these genes (13 *CYP2J2*, 5 *GSTM5*, 7 *SLC11A2*) were additionally informative *CYP2J2* rs11572285 was marginally associated with diagnosis age, and adjustment for it strengthened the association between *CYP2J2* rs10889162 and diagnosis age (**Table 4**). We constructed rs10889162-rs11572285 haplotypes, but they provided no information beyond the simpler model. Consideration of the often-studied [23] rs890293 (*CYP2J2* G-50T (*7), as assessed by rs11572321) also did not improve the model. Relaxing our definition of SNP functionality to include SNPs with regulatory potential or evolutionary conservation scores >0 did not uncover additional SNPs associated with PD diagnosis age. When we removed the functionality criterion entirely, only one SNP, *GRIN2B* rs2058878, met all remaining criteria for being associated with PD diagnosis age (β=2.47, 95% CI 1.01-3.93, p=0.001). We also conducted sensitivity analyses to assess the effect of starting with the UW cases instead of the GHC cases, and again only identified one additional SNP possibly associated with PD diagnosis age, *CYP3A7* rs10211 (β=3.39, 95% CI 0.73-6.06, p=0.01).

Discussion

We used a well-characterized population-based study with newly-diagnosed PD cases to explore whether selected genes pertinent to oxidative stress, inflammation, metal transport or CNS function in general might influence age at PD diagnosis. We identified potentially functional SNPs that appear to be associated with age at diagnosis, most clearly *CYP2J2* rs10889162. *CYP2J2* (chromosome 1p31) codes for the cytochrome P450 enzyme (EC 1.14.14.1) known as arachidonic acid epoxygenase. It metabolizes arachidonic acid to epoxyeicosatrienoic acids

Table 2. Genotype and difference in age at Parkinson disease diagnosis, Group Health Cooperative (GHC) and University of Washington (UW), 1992-2008

Gene (chromosome)	Gene Product	SNP	SNP function (location)	GHC cases N=258		UW cases N=64		Controls[a] N=436	
				MAF	Years per variant allele[b]	MAF	Years per variant allele[b]	MAF	Years per variant allele[a,b]
CYP2J2 (1p31)	Cytochrome P450 2J2 (Arachidonic acid epoxygenase)	rs10889162	Transcription factor binding site (-582 bp 5')	0.08 (T)	4.69 (1.67, 7.71) p=0.002	0.07 (T)	6.23 (-0.55, 13.02) p=0.07	0.08 (T)	-0.24 (-2.41, 1.93) p=0.83
GSTM5 (1p13)	Glutathione S-transferase Mu 5	rs11807[c]	miRNA binding site (3' UTR)	0.19 (G)	-2.43 (-4.34, -0.51) p=0.01	0.16 (G)	-2.46 (-7.93, 3.00) p=0.37	0.19 (G)	0.45 (-1.03, 1.93) p=0.55
SLC11A2 (12q13)	Divalent metal transporter 1	rs150909	miRNA binding site (3' UTR)	0.07 (C)	3.44 (0.28, 6.59) p=0.03	0.07 (C)	2.50 (-5.52, 10.52) p=0.54	0.07 (C)	-1.03 (-3.33, 1.27) p=0.38

[a]GHC enrollees without Parkinson disease or other neurological disorder, age at reference used in lieu of age at diagnosis. [b]Linear regression β (years) and 95% confidence interval, adjusted for sex and smoking; all non-Hispanic Caucasians. [c]Excludes 17 cases and 18 controls without genotyping data for rs11807. Abbreviations: GHC, Group Health Cooperative; MAF, minor allele frequency; miRNA, microRNA; SNP, single nucleotide polymorphism; UTR, untranslated region; UW, University of Washington.

Table 3. Genotype and difference in age at Parkinson disease diagnosis, overall and by smoking and sex, Group Health Cooperative (GHC) and University of Washington (UW), 1992-2008

	Years per variant allele[a]				
		Smoked[b]		Sex	
	All cases N=322	Never N=178	Ever N=144	Men N=211	Women N=111
CYP2J2 rs10889162	5.04 (2.28, 7.80) p=0.0003	4.95 (0.91, 8.98) p=0.02	5.19 (1.40, 8.97) p=0.008	5.95 (2.53, 9.36) p=0.001	3.51 (-1.19, 8.21) p=0.14
GSTM5 rs11807[c]	-2.37 (-4.21, -0.52) p=0.01	-2.86 (-5.16, -0.56) p=0.02	-1.60 (-4.61, 1.42) p=0.30	-2.28 (-4.51, -0.05) p=0.05	-2.89 (-6.21, 0.43) p=0.09
SLC11A2 rs150909	3.09 (0.13, 6.06) p=0.04	2.90 (-1.37, 7.17) p=0.18	3.62 (-0.50, 7.74) p=0.09	4.64 (0.53, 8.75) p=0.03	1.90 (-2.50, 6.30) p=0.40

[a]Linear regression β (years) and 95% confidence interval, adjusted for case group, and adjusted for or stratified by smoking and sex; all non-Hispanic Caucasians. [b]Ever >100 cigarettes. [c]Excludes 17 cases and 18 controls without genotyping data for rs11807. Abbreviations: GHC, Group Health Cooperative; UW, University of Washington.

Table 4. *CYP2J2* genotype and difference in age at Parkinson disease diagnosis, overall and by group, Group Health Cooperative (GHC) and University of Washington (UW), 1992-2008

	Years per variant allele[a]		
		Case group	
	All cases N=322	GHC N=258	UW N=64
rs10889162	7.93 (3.89, 11.97) p=0.0001	7.76 (3.50, 12.02) p=0.0003	9.01 (-3.15, 21.18) p=0.14
rs11572285	-3.48 (-7.04, 0.08) p=0.06	-3.64 (-7.22, -0.06) p=0.05	-3.46 (-16.00, 9.08) p=0.58
Both SNPs[b]	p=0.0003	p=0.001	p=0.15
Both SNPs (haplotype)[c]	p=0.0009	p=0.004	p=0.28

[a]Linear regression β (years) and 95% confidence interval, adjusted for the other *CYP2J2* SNP, smoking and sex, and adjusted for or stratified by case group. [b]p-value from likelihood ratio test (model with both SNPs modeled linearly vs. model without either SNP). [c]p-value from likelihood ratio test (model with all haplotypes modeled linearly vs. model without haplotype terms). Abbreviations: GHC, Group Health Cooperative; SNP, single nucleotide polymorphism; UW, University of Washington.

(EETs) [23], and is highly expressed in the substantia nigra [24]. EETs have anti-inflammatory properties [23] and inhibit apoptosis [25]. In addition, arachidonic acid affects the phosphorylation of microtubule-associated protein tau [26] coded by an established PD gene [6]. *CYP2J2* rs10889162 is located in a predicted transcription factor binding site [21] 582bp upstream from the gene's transcription start site, and therefore may affect transcriptional regulation. This SNP potentially affects binding of some of the same transcription factors [21] influenced by a functional SNP in the proximal promoter [23] that is responsive to nitrosative stress [27]. The protective variant rs10889162 T allele also disrupts a 9bp AGC triplet repeat. Intronic *CYP2J2* rs11572285 does not appear to be functional [21], but is in weak linkage disequilibrium (Genome Variation Server, http://gvs.gs.washington.edu, accessed December 21, 2012) with several functional [21] *CYP2J2* SNPs. It is also in strong linkage disequilibrium with an evolutionarily conserved SNP [21] in *HOOK1*, whose product binds to microtubules and is involved in endocytosis, a process previously suggested to influence PD onset age [16].

The other functional SNPs associated with PD diagnosis age, *GSTM5* rs11807 and *SLC11A2* rs150909 (as well as *CYP3A7* rs10211 identified only in sensitivity analyses) are each located in the 3' untranslated region of the respective gene and are predicted to alter miRNA binding [21]. miRNAs are small (≈22 nucleotide) non-coding RNA molecules that affect gene expression by binding to the 3' untranslated region in messenger RNA, resulting in gene silencing via translational repression or target degradation. With respect to PD, the potential relevance of miRNAs [28-30] and genetic polymorphisms that affect their binding [31] has been recognized.

GSTM5 is a member of the prominent glutathione S-transferase (GST, EC 2.5.1.18) mu enzyme family located at chromosome 1p13. GST mu 5 is found in the brain and metabolizes a variety of exogenous and endogenous chemicals [32]. These include trichloroethylene (http://www.genome.jp/kegg, accessed December 21, 2012), a solvent previously associated with PD [33, 34]. While GST mu 5 has not been investigated for its enzymatic capacity to detoxify dopaminochrome, a product of dopamine oxidation, two closely related GST mu enzymes are efficient at this detoxification [35].

SLC11A2 (chromosome 12q13) codes for the divalent metal transporter 1 that is involved in the *in vivo* distribution of some metals including iron and manganese [36], which have been implicated in some epidemiologic studies of PD [12, 37], along with *SLC11A2* itself [38]. However, *SLC11A2* rs150909 is in linkage disequilibrium with polymorphisms in *LETMD1*, a mitochondrial outer membrane protein, and in genes that encode transcription factors, including CP2. Binding of CP2 appears to be affected [21] by *CYP2J2**7 and rs10889162. Thus, the finding for *SLC11A2* may be related to that for *CYP2J2*. The same is true for *CYP3A7*. CYP2J2 and CYP3A7 are among the few human cytochrome P450 enzymes involved in the metabolism of linoleic acid (http://www.genome.jp/kegg, accessed December 21, 2012), an essential fatty acid that can be converted to arachidonic acid.

We may have missed some associations due to the focus on functional SNPs, the small size of our secondary case group, and the requirement that the gene-age association be statistically significantly different in cases than controls. However, only one non-functional SNP met all other criteria (0.09% vs. 1.2% of functional SNPs, Fisher's exact p=0.02). In addition, the importance of verifying that genotype-age associations do not simply reflect associations in the general population has been recognized [16, 17], and our approach was objective.

Prior genome-wide studies have not identified *CYP2J2*, *GSTM5* or *SLC11A2* as associated with age at PD onset or diagnosis. One possible explanation is that reports focused on PD onset or diagnosis age and including data from across the genome have been restricted to or dominated by familial cases [16, 39-41], whereas the present study contained few cases with a first degree relative with PD. *CYP2J2* and *SLC11A2* are in or near chromosomal regions previously associated with age at PD onset (the *PARK10* locus (OMIM 606852, 1p32) [40, 42, 43], *LRRK2* (12q12) [44] and *VDR* (vitamin D receptor, 12q13) [45]), but barring long-range linkage disequilibrium, these signals are presumably independent from our findings.

Confirmation of our results in other samples of incident PD cases will be needed to strengthen inferences regarding underlying relations with diagnosis age. Studies powered to also consider biologically plausible gene-gene and gene-environment interactions may also improve our understanding of PD.

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Conflicts of interest

The authors have no conflicts of interest to declare.

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