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Haptoglobin Phenotype Modifies Serum Iron Levels and the Effect of Smoking on Parkinson Disease Risk

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Abstract

Introduction—Haptoglobin is a hemoglobin-binding protein that exists in three functionally different phenotypes, and haptoglobin phenotype 2-1 has previously been associated with Parkinson disease (PD) risk, with mechanisms not elucidated. Some evidence is emerging that low levels of serum iron may increase PD risk. In this study we investigated whether PD patients have lower serum iron and ferritin than controls, and whether this is dependent on haptoglobin phenotype. We also investigated the effect of Hp phenotype as a modifier of the effect of smoking on PD risk.

Methods—The study population consisted of 128 PD patients and 226 controls. Serum iron, ferritin, and haptoglobin phenotype were determined, and compared between PD cases and controls.

Stratified analysis by haptoglobin phenotype was performed to determine effect of haptoglobin phenotype on serum iron parameter differences between PD cases and controls and to investigate its role in the protective effect of smoking on PD risk.

Results—PD cases had lower serum iron than controls (83.28 ug/100ml vs 94.00 ug/100 ml, p 0.006), and in particular among subjects with phenotype 2-1. The protective effect of smoking on PD risk resulted stronger in subjects with phenotype 1-1 and 2-2, and weakest among subjects

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with phenotype 2-1. Ferritin levels were higher in PD cases than controls among subjects of White ethnicity.

Conclusions—Our results report for the first time that the haptoglobin phenotype may be a contributor of iron levels abnormalities in PD patients. The mechanisms for these haptoglobin-phenotype specific effects will have to be further elucidated.

Keywords

Idiopathic Parkinson Disease; tobacco smoking; serum iron; serum ferritin; haptoglobin phenotype

INTRODUCTION

One of the pathologic hallmarks of Parkinson's disease (PD) is increased nigral iron deposition [1]. Elevated levels of free iron in the brain can result in neurodegeneration through the formation of hydroxyl radicals and reactive oxygen species via the Fenton reaction [2]. The oxygen radical species generated by the Fenton reaction can initiate processes that ultimately lead to neuronal cell death [3], and intra-ventricular injections of iron chelators protect against the neurotoxic effect of 6-hydroxydopamine in rats [2]. In spite of the deleterious effect of high levels of iron in the brain, there is some evidence that the levels of iron in circulation may be lowered in PD patients as compared to controls [4,5,6,7,8], however with contradictory results in the literature [9,10]. Therefore, whether PD patients have reduced circulatory iron levels remains controversial.

Haptoglobin (Hp), is an acute phase serum protein that binds free hemoglobin (Hb) with very high affinity [11].

The Hb-Hp complexes are cleared by receptors on hepatocytes, and by endocytosis mediated by CD163 receptors on monocytes and macrophages [12].

Since Hb is the richest source of iron in the body and Hp strongly binds to Hb, Hp phenotype is involved in regulation of iron homeostasis and serum iron levels [13].

Haptoglobin consists of two distinct polypeptide chains, the alpha-chain, and the beta chain. The beta-chain has hemoglobin-binding capacity [11], and the gene encoding this chain is not polymorphic. In contrast, the gene encoding the alpha-chain has a common polymorphism, where alpha-1 and alpha-2 alleles have approximate frequencies of 0.4 and 0.6, respectively [14]. Thus, there are three major genotypes of Hp: Hp 1-1, Hp 2-1, and Hp 2-2, which exhibit profound structural and functional differences. The Hp 1-1 complexes are composed of two alpha-1 chains and two beta-chains and are the smallest type of haptoglobin, with molecular weight of 86 kDa. Hp 2-1 complexes include alpha 1 and alpha-2 chains in variable number and form high molecular weight polymers of 86–300 kDa (Hp 2-1). Hp 2-2 complexes consist of a variable number of alpha-2 chains that are bound to each other and with beta chains, and have the highest molecular weight, up to 900 kDa (Hp 2-2) [14]. These structural differences confer different functional properties to the different Hp variants, in fact Hp 2-2 has lower Hb-binding capacity than Hp 1-1 and Hp 2-1 [15]. Hp 2-2 also has lower Hb-scavenging power than Hp 2-1 and Hp 1-1 as a consequence of its

lower ability to reach extra-vascular fluids, which is due to its higher molecular mass [14,16]. In our previous study on PD patients and controls we found that Hp 2-1 phenotype was associated with significant increased risk of PD, while Hp 2-2 conferred a protective effect on PD risk [18].

Among the environmental factors that affect risk of PD, tobacco smoking has been consistently found to confer a protective effect, with a reduction of PD risk by about 50%, among cigarette smokers as compared to non-smokers [18]. Smoking has also been associated with reduced Lewy body accumulation [19]. Selective survival of non-smoking PD cases does not account for the seemingly protective effect of cigarette smoking.

In this study we tested whether Hp phenotype had an effect on differences in iron parameters between PD patients and controls. We also tested for the presence of a modifying effect of Hp phenotype on the association of smoking with PD.

METHODS

Study participants

All study participants provided written consent. All study procedures were approved by Institutional Review Boards of Bastyr University, the University of Washington, and the Veteran Administration Puget Sound Health Care System (VAPSHCS). Study participants were recruited in the Puget Sound area of Washington State between 2007 and 2014. Recruitment sources were: 99 PD patients and 40 controls enrolled in the Parkinson's Genetic Research Study (PaGeR) [20]; 11 PD patients and 28 controls recruited from the University of Washington Medical Center (UWMC) and the Group Health Cooperative (GHC) in Seattle, WA; 18 PD patients and 158 controls from Bastyr University, including the Washington Parkinson's Disease Registry (WPDR), Bastyr University campus, Bastyr Center for Natural Health (BCNH), and Senior Centers.

All PD patients recruited from PaGeR and Bastyr University met "UK Parkinson's Disease Society Brain Bank clinical diagnostic criteria" (UKBB) for PD [21]. Only patients meeting UKBB clinical criteria of diagnosis were included in the study.

For patients recruited from the WPDR, and for patients not directly referred to the study by a neurologist, medical records were obtained from each patient's neurologist and were reviewed by a second neurologist (S-C Hu) to determine whether each patient met UKBB criteria.

For the PD patients recruited from the UWMC and GHC, PD cases had a neurologist-confirmed diagnosis based on the presence of 2 or more cardinal signs (bradykinesia, resting tremor, rigidity, postural reflex impairment), and only patients with diagnosis of PD were included. Exclusion criteria for the PD patients were: the presence of a medical condition that could mimic PD symptoms, such as the use, during the 12 months preceding onset of PD symptoms, of certain medications (e.g., phenothiazines, haloperidol), whose side effects include parkinsonism signs and symptoms; prior history of 2 or more cerebrovascular events; or another explanation for parkinsonism symptoms (e.g. brain injury, brain tumor),

as previously described [17]. These criteria were evaluated by the neurologists during the diagnosis of PD.

Controls were subjects free of PD or other neurodegenerative diseases, as determined from chart reviews and subject interviews. The control group was frequency-matched to cases by age in 10-year categories. For the controls from PaGeR and GHC, no additional medical history exclusion criteria were imposed on control eligibility. Controls recruited from Bastyr University, exclusion criteria also included, in addition to the criteria described above, the presence of current active cancer under treatment, chronic hepatitis, and HIV sero-positivity, as determined from participants' interviews and charts reviews.

Data collection

Blood samples were collected by venipuncture from each study participant, serum was separated, split in different aliquots and frozen at −70°C, and submitted to the UW Clinical Chemistry laboratory and the LabCorp Clinical laboratory for tests of serum iron and serum ferritin. Hp phenotype was determined with method as previously described [17].

Aliquots of frozen serum samples were available for total serum iron tests for all 354 participants. Serum samples for ferritin tests were available for 206 samples, since ferritin tests need to be performed for optimal results within 1 month of blood collection, and 148 of the samples had been stored frozen for more than 1 month. Hp phenotype could be determined for 345 participants.

Demographic information including age, gender, and race, and data on number of cigarettes a day smoked, and years smoked was obtained from all 354 study participants. Participants were considered "ever-smokers" if they had smoked at least 100 cigarettes during their lifetimes. Pack-years of smoking were calculated by multiplying packs/day by number of years smoked.

Data analysis

Age- adjusted differences in mean serum iron and serum ferritin were compared between PD cases and controls by one-way Analysis of Covariance (ANCOVA). This analysis was performed for the overall study population and separately for the three Hp phenotype groups. Separate analyses were also performed by gender.

The effect of ever/never smoking status on PD risk was calculated by logistic regression to estimate OR and 95% CI, in models that adjusted for age. The effect of smoking on PD risk was also evaluated in terms of pack-years of smoking in a logistic regression. Differences in Hp phenotype frequencies between PD patients and controls were tested by Chi-square, Odds Ratios (OR) and 95% Confidence Intervals (95%CI). Logistic regression was used to determine age-adjusted p-values for association of Hp phenotypes with PD in models that adjusted for age. The interaction between smoking and Hp phenotype on PD risk was also tested applying logistic regression.

All data analysis was performed with SPSS 19.0 software.

RESULTS

The study population consisted of 354 subjects, 128 PD patients (mean age 69.01 years, 40 women, 88 men), and 226 controls (mean age 62.57 years, 122 women and 104 men) for whom serum iron levels were tested and smoking status information was collected. Ethnicity distribution of study participants is 88.70% White, 4.24% Multiethnic, 3.39% African American, 2.26% Asian, 1.13% Hispanic, 0.28% Native American (Table 1).

Overall, PD patients had significantly lower serum iron than controls (83.28 ug/100ml vs. 94.00 ug/100ml), this difference was highly significant (p=0.006; Table 2). When stratifying by gender, the difference in serum iron levels between PD cases and controls was significant among men, but not among women. Interestingly, serum iron was particularly low in PD cases as compared to controls among subjects of Hp 2-1 phenotype (79.13 ug/100ml vs 94.51 ug/100 ml, p = 0.015). While serum iron was lower in PD cases than controls for all Hp phenotype groups, the difference between PD cases and controls was much less pronounced for subjects of Hp 1-1 phenotype and Hp 2-2 phenotype (91 ug/100 ml vs 94.66 ug/100 ml, and 84.54 ug/100 ml vs 91.60 ug/100 ml, respectively) (Table 2). In particular, among men of Hp 2-1 phenotype group serum iron was 79.23 ug/100 ml in PD cases and 107.91 ug/100 ml in Controls, p = 0.002, while the difference in serum iron between PD cases and Control among in Hp 2-1 women was not statistically significant.

Ferritin was significantly higher among PD cases than controls among subjects of White ethnicity (133.94 ng/ml vs 88.95 ng/ml). The differences between PD cases and controls were not statistically significant when including all ethnicities, or after stratification by gender and Hp phenotype (Table 2).

As shown in Table 3, the protective effect of cigarette smoking on iPD risk was greatest among subjects of Hp 1-1 (OR = 0.13, 95% CI = 0.03–0.66) and Hp 2-2 phenotype (OR = 0.41, 95% CI = 0.19–0.87). For the subjects of Hp 2-1 phenotype, the effect of smoking was much less prominent (OR = 0.83, 95% CI= 0.44–1.56). Notably, in the Hp 1-1 phenotype group, a subject resulted 7.69 times more likely to develop PD if never-smoker than if ever-smoker, and in the Hp 2-2 phenotype group a subject resulted 2.43 times more likely to develop PD if neversmoker. However, in the Hp 2-1 phenotype group, the increase risk for PD for never-smokers was only 1.20, or 20%, and non-significant. The Hp phenotype*ever/never smoking interaction with respect to PD risk resulted statistically significant, p = 0.023.

The overall Hp phenotype frequencies were not significantly different between PD cases than controls. However, after stratification by ever/never smoking status, Hp 2-1 phenotype was significantly associated with PD in the ever-smokers group, but not in the never-smokers. Among ever-smokers, the O.R. for PD risk for Hp 2-1 vs (Hp 2-2 + Hp 1-1) was = 1.98 (95% CI = 1.01–3.89, p=0.045). O.R for PD risk for Hp 2-1 vs Hp 2-2 was = 1.61 (0.79–3.26, p=0.187), and OR for PD for Hp 2-1 vs Hp 1-1 was = 5.18 (1.13–23.80, p=0.012) (Table 4). Among eversmokers, very few of the Hp 1-1 and Hp 2-2 subject had PD (Table 3), confirming the presence of a protective effect of smoking especially for Hp 1-1 and Hp 2-2, rather than Hp 2-1 carriers, result that is presented in the previous analysis of Table 3, due to the Hp phenotype ever/never smoking interaction on PD risk.

DISCUSSION

In this study we observed that PD patients had significantly lower serum iron than controls. This difference was statistically significant for men but not for women. The results of a lower level of serum iron among PD patients are consistent with previous studies that showed lower values of circulatory iron, ferritin and transferrin saturation in PD [4], and with the study of Savica et al. [5], that reported that anemia in early life precedes IPD.

In support of an increased risk of PD associated with lower circulatory iron is also the report of Forte et al. [6], who observed significantly lower levels of iron in the hair of PD patients compared with controls, and an elevated risk of PD among men who reported recent multiple blood donations [7]. Hemochromatosis gene markers related to elevated serum iron have been associated with reduced risk of PD [8]. In contrast, there were no significant differences in serum iron between PD patients and controls in two other studies [9,10].

Notably, in our study, we observed that among men, PD patients with Hp 2-1 Hp phenotype, the Hp phenotype that had been previously associated with PD risk, have particularly low serum iron as compared to controls. Subjects with Hp 2-1 phenotype did not result having lower serum iron that subjects Hp 1-1 or Hp 2-2 among controls. The factors that cause a decrease in serum iron among PD patients of Hp 2-1 phenotype, but not controls, need further investigation, to determine whether alteration of iron metabolism have a role in the etiology of PD.

The results of differences in serum iron between PD patients and Controls among subjects of non-White ethnicity were not statistically significant, however these results cannot be considered conclusive, due to the small number of study participants in this group.

For what concerns gender differences, our results point toward the presence of a stronger abnormality in serum iron levels in PD patients among men rather than women, especially if of Hp 2-1 phenotype. We have a smaller number of women participants than men, which may contribute to the lower statistical significance of the results among women. However, the absolute values of difference in mean serum iron between PD patients and controls are larger among men than women, which represents a real gender-difference or results. The physiological basis for the gender-difference in results in currently not known. Serum ferritin resulted significantly higher in PD patients than controls among the subjects of White ethnicity, but the difference was not statistically significant when considering the whole study population that included different ethnicities.

Therefore, in this study PD patients resulted having lower serum iron than controls, but higher ferritin than controls, at least among the group of White ethnicity. The discrepancy between serum iron and serum ferritin levels can be explained since serum ferritin is primarily derived from macrophage secretion, and is composed of high levels of apo-ferritin, and contains low amounts of iron [22]. Typically, serum ferritin correlates with body iron status and its levels increase in case of body iron overload [23]. However, serum ferritin is also increased in case of inflammation in absence of body iron overload [24], and in some inflammatory conditions serum ferritin levels are increased but there is systemic iron

deficiency, due to macrophages iron retention [22]. Whether similar abnormalities are present in PD patients remains to be elucidated.

Our study population of ethnicity different than White was very small, and further studies will have to be done to establish whether differences in ferritin between PD patients and controls exist for subjects of non-White ethnicity. Logroscino et al., (1997) [4] previously observed that both serum iron and serum ferritin were lower in PD patients than controls: it is possible that different ethnic composition may account for the discrepancy of our results with those of Logroscino et al. (1997). Studies in larger populations and meta-analyses of several studies will be needed to establish whether serum ferritin is consistently elevated in PD patients.

Wypijewska et al., (2010) [25] demonstrated that differences in concentration of iron in the Substantia Nigra (SN) between PD patients and controls concern only the non-ferritin labile iron, which is in concentrations that are 2000 times smaller than the total SN iron concentration. In this respect, further research will be needed to understand whether lower levels of serum iron, as we observed in PD patients in this study, and Hp phenotype, may affect brain iron-binding proteins and concentrations of brain non-ferritin labile iron.

Ward et al., [26] suggested that low levels of iron may increase the risk of PD due to insufficient supply of iron for the synthesis of dopamine in the brain, since iron is a co-factor of tyrosine hydroxylase, an enzyme of dopamine biosynthesis. Some previous studies report an increase in ferritin-iron in the SN of PD patients, while others have reported a decrease of the specific form L (light chain) of ferritin in parkinsonian SN [27]. Human serum ferritin consists predominantly of L ferritin with only trace amounts of H ferritin, since L ferritin is glycosylated and easily secreted [28]. In this study we have not measured the relative amounts of L and H (heavy chain) in serum ferritin, however it would be interesting to determine in future studies whether different forms than the typical L ferritin are present in PD patients, in circulation.

In this study we observed that the protective effect of smoking on PD risk was stronger for subjects of Hp 1-1 and Hp 2-2 phenotype than subjects with Hp 2-1 phenotype.

Since Hp 2-1 molecular complexes have higher efficiency in hemoglobin-binding and hemoglobin-scavenging activity than Hp 2-2 molecules [14,15,16], these functional differences of Hp phenotypes may underlie the observed Hp-phenotype specific differences in serum iron levels between PD cases and controls and interaction smoking/Hp phenotype on PD risk.

The similar findings for homozygous phenotypes (Hp 2-2 and Hp 1-1) compared with findings for heterozygous phenotype Hp 2-1 could be due to heterosis, which is a rather common occurrence among human polymorphic traits, as has been for the genetic polymorphism that determines dopamine D2 receptor density [29].

Data on dietary habits was not collected in this study, and it cannot be excluded that differences in nutritional status between PD patients and controls might contribute to the lower levels of serum iron in PD patients here observed. The fact that the difference in

serum iron between PD patients and controls was stronger among subjects of Hp 2-1 phenotype represents a genetic effect that is not likely due to dietary factors, since dietary patterns are most likely not dependent on the Hp phenotype of the subject.

In conclusion, our study suggests a possible role of haptoglobin phenotype on iron metabolism abnormalities that are observed in PD patients. Further studies will be needed to characterize the role of systemic iron abnormalities in PD and the interactive effect of additional environmental and genetic factors on these parameters.

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- Serum iron is significantly lower in PD patients as compared to controls
- PD patients with Hp 2-1 phenotype have particularly low serum iron
- Smoking decreases PD risk for Hp 1-1 and Hp 2-2, but not Hp 2-1 carriers

Table 1

Demographics of the study population

	Idiopathic PD		Controls		Total	
Total participants	128		226		354	
Mean age	69.01 y/o		62.57 y/o		64.89 y/o	
Women	40		122		165	
Mean age	68.85 y/o		61.73 y/o		63.49 y/o	
Men	88		104		194	
Mean age	69.07 y/o		63.53 y/o		66.07 y/o	
Tobacco smoking						
Never smokers	75 (58.59%)		99 (43.81%)		174 (49.15%)	
Ever smokers	50 (39.07%)		127 (56.19%)		177 (50.00%)	
Not known	3 (2.34%)		0 (0.00%)		3 (0.85 %)	
Total	128 (100%)		226 (100%)		354 (100%)	
Ethnicity						
White	121	(90.63%)	198	87.61%	314	88.70%
African American	1	(0.78%)	11	4.87%	12	3.39%
Hispanic	3	(2.34%)	1	0.44%	4	1.13%
Asian	4	(3.13%)	4	1.77%	8	2.26%
Native American	1	(0.78%)	0	0.00%	1	0.28%
Multiethnic	3	(2.34%)	12	5.31%	15	4.24%
Total	133	(100.00%)	226	100.00%	354	100.00%

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Table 2

Differences in serum iron and serum ferritin between PD Cases and Controls, stratified by gender, ethnicity, and Hp phenotype.

		iPD Cases		Controls		p-value	age-adjusted p-value
Serum iron ug/100 ml	**Total**	**n.**	**Mean (SD)**	**n.**	**Mean (SD)**		
Total	**354**	**128**	**83.28 (29.46)**	**226**	**94.00 (34.14)**	**0.003**	**0.006**
Women	162	40	79.28 (30.45)	122	88.23 (29.61)	0.102	0.103
Men	192	88	85.10 (28.52	104	100.77 (37.82)	0.002	0.004
Ethnicity							
Non-hispanic White	314	121	84.05 (28.77)	198	93.72 (31.38)	0.007	0.006
African American	12	1	59.00	11	82.36 (27.41)	0.434	0.394
Hispanic	4	3	54.33 (20.21)	1	154.00	0.051	0.346
Asian	8	4	102.25 (23.48)	4	90.50 (7.76)	0.379	0.785
Native American	1	1	60.00	0	-	-	-
Multiethnic	15	3	73.00 (59.75)	12	105.42 (69.98)	0.477	0.734
Hp phenotype							
Hp 1-1	51	16	91.75 (37.42)	35	94.66 (32.47)	0.779	0.719
Hp 2-1	164	62	79.13 (29.41)	102	94.51 (39.11)	0.008	0.015
Hp 2-2	130	46	84.54 (26.10)	84	91.60 (27.07)	0.153	0.211
Women							
Hp 1-1	19	4	79.50 (43.47)	15	96.13 (30.65)	0.387	0.426
Hp 2-1	80	23	78.96 (33.03)	57	83.94 (31.00)	0.525	0.596
Hp 2-2	60	13	79.77 (25.07)	47	90.09 (26.50)	0.214	0.150
Men							
Hp 1-1	32	12	95.83 (36.35)	20	93.55 (34.50)	0.860	0.950
Hp 2-1	84	39	79.23 (27.52)	45	107.91 (44.26)	0.001	0.002
Hp 2-2	70	33	86.42 (26.63)	37	93.51 (28.02)	0.283	0.450
Serum ferritin ng/ml							
Total	**206**	**36**	**128.03 (172.29)**	**170**	**101.46 (99.24)**	**0.210**	**0.154**
Women	115	11	45.09 (32.74)	104	77.45 (59.22)	0.078	0.125

		iPD Cases		Controls		p-value	age-adjusted p-value
Serum iron ug/100 ml	**Total**	**n.**	**Mean (SD)**	**n.**	**Mean (SD)**		
Men	91	25	164.52 (195.72)	66	139.29 (132.93)	0.483	0.371
Ethnicity *							
Non-hispanic White	176	34	133.94 (175.59)	142	88.95 (66.11)	0.016	0.014
All other Ethnicities	30	2	27.50 (9.19)	28	164.88 (183.90)	0.308	0.276
Hp phenotype							
Hp 1-1	30	4	160.25 (207.65)	26	87.31 (56.78)	0.128	0.181
Hp 2-1	95	17	157.17 (218.75)	78	114.51 (118.11)	0.260	0.139
Hp 2-2	77	15	86.40 (84.28)	62	93.58 (88.60)	0.777	0.705
Women							
Hp 1-1	14	1	120	13	70.92 (38.53)	0.243	0.124
Hp 2-1	54	7	36.42 (23.04)	47	79.42 (64.81)	0.090	0.116
Hp 2-2	44	3	40.33 (25.77)	41	79.21 (60.56)	0.280	0.385
Men							
Hp 1-1	16	3	173.66 (252.19)	13	103.69 (68.19)	0.356	0.381
Hp 2-1	41	10	241.70 (255.80)	31	167.70 (156.56)	0.276	0.109
Hp 2-2	33	12	97.91 (90.53)	21	121.61 (123.92)	0.567	0.411

*Because numbers were too small for separate analyses of ferritin by all different ethnicities, the study population was split in two ethnic groups: Non-hispanic Whites, and All other Ethnicities.

Table 3

Odds Ratios for PD risk for ever-smokers versus never-smokers in the overall population, and stratified by Hp phenotype groups, gender, and ethnicity

Hp phenotype	Ever/Never smoking status	Controls	iPD	Odds Ratio	95% Confidence Interval	p-value	Age adjusted p-value
Overall population	Never smoked	99	75				
	Ever smoked	127	50	0.52	(0.33–0.81)	0.004	0.050
Hp phenotype:							
	Never smoked	16	13				
Hp 1-1	Ever smoked	19	2	0.13	(0.03–0.66)	0.007	0.043
	Never smoked	47	31				
Hp 2-1	Ever smoked	55	30	0.83	(0.44–1.56)	0.558	0.940
	Never smoked	34	28				
Hp 2-2	Ever smoked	50	17	0.41	(0.19–0.87)	0.018	0.057
Gender:							
Females	Never smoked	59	27				
	Ever smoked	63	13	0.45	(0.21–0.96)	0.034	0.378
Hp 1-1	Never smoked	8	4				
	Ever smoked	7	0	0.53	(0.33–0.85)	0.086	0.999
Hp 2-1	Never smoked	27	17				
	Ever smoked	30	6	0.32	(0.11–0.92)	0.031	0.266
Hp 2-2	Never smoked	22	6				
	Ever smoked	25	7	1.02	(0.30–3.52)	0.967	0.675
Males	Never smoked	40	48				
	Ever smoked	64	37	0.48	(0.27–0.86)	0.013	0.028
Hp 1-1	Never smoked	8	9				
	Ever smoked	12	2	0.15	(0.02–0.87)	0.025	0.048
Hp 2-1	Never smoked	20	14				
	Ever smoked	25	24	1.37	(0.56–3.31)	0.483	0.491
Hp 2-2	Never smoked	12	22	0.22	(0.08–0.60)	0.003	0.007

Hp phenotype	Ever/Never smoking status	Controls	iPD	Odds Ratio	95% Confidence Interval	p-value	Age adjusted p-value
	Ever smoked	25	10				
Ethnicity:							
Non-hispanic White	Never smoked	91	71	0.50	(0.31–0.81)	0.004	0.027
	Ever smoked	107	42				
All other Ethnicities	Never smoked	8	4	0.80	(0.18–3.42)	0.765	0.714
	Ever smoked	20	8				

Table 4

Odds Ratios and 95% Confidence Intervals for PD risk for Hp phenotypes and stratified by ever/never smoking status.

	OR	(95% CI)	p-value	Age-adjusted p-value
Total				
Hp 2-1 vs (Hp 11+Hp 22)	1.16	(0.75–1.81)	0.493	0.322
Hp 2-1 vs Hp 22	1.11	(0.69–1.79)	0.669	0.846
Hp 2-1 vs Hp 11	1.33	(0.68–2.60)	0.400	0.264
Never-smokers				
Hp 2-1 vs (Hp 11+Hp 22)	0.80	(0.44–1.48)	0.486	0.580
Hp 2-1 vs Hp 22	0.80	(0.41–1.57)	0.519	0.550
Hp 2-1 vs Hp 11	0.81	(0.34–1.92)	0.664	0.844
Ever-smokers				
Hp 2-1 vs (Hp 11+Hp 22)	1.98	(1.01–3.89)	0.046	0.028
Hp 2-1 vs Hp 22	1.61	(0.79–3.25)	0.187	0.113
Hp 2-1 vs Hp 11	5.18	(1.13–23.80)	0.031	0.029