Published in final edited form as:

J Neural Transm (Vienna). 2016 November ; 123(11): 1319–1330. doi:10.1007/s00702-016-1590-x.

Smoking and haptoglobin phenotype modulate serum ferritin and haptoglobin levels in Parkinson disease

Paola Costa-Mallen¹, Cyrus P. Zabetian^{2,3}, Shu-Ching Hu³, Pinky Agarwal⁴, Dora Yearout², and Harvey Checkoway⁵

¹Bastyr University Research Institute, 14500 Juanita Drive NE, Kenmore, WA 98028, USA

²Veterans Affairs Puget Sound Health Care System, 1660 South Columbian Way, Seattle, WA 98108, USA

³Department of Neurology, University of Washington, 325 Ninth Avenue, 3EH70, Seattle, WA 98104, USA

⁴Booth Gardner Parkinson's Care Center, Evergreen Health, 12040 NE 128th Street, Mailstop 11, Kirkland, WA 98034, USA

⁵Department of Family and Public Health, University of California San Diego, 9500 Gilman Drive #0725, La Jolla, CA 92093, USA

Abstract

The phenotype Hp 2-1 of haptoglobin has been previously associated with increased risk of Parkinson disease (PD) and with serum iron abnormalities in PD patients. Tobacco smoking has been consistently observed in epidemiology studies to be inversely related to PD risk, with mechanisms that remain uncertain. We recently observed that the protective effect of smoking on PD risk is stronger among subjects of haptoglobin Hp 2-2 and Hp 1-1 phenotypes, and weaker among subjects of haptoglobin Hp 2-1 phenotype. In this PD case-control study, we investigated whether tobacco smoking was associated with changes in serum haptoglobin and ferritin concentration that depended on haptoglobin phenotype among 106 PD patients and 238 controls without PD or other neurodegenerative disorders. Serum ferritin concentration, serum haptoglobin concentration, haptoglobin phenotype, and smoking data information of cases and controls were

Paola Costa-Mallen, pcostamallen@bastyr.edu.

Conflict of interest Dr. Costa-Mallen reports no conflict of interest and has been funded by grants from NIH and Bastyr University. Dr. Zabetian reports no conflict of interest and has been funded by grants from the American Parkinson Disease Association, Department of Veterans Affairs, NIH, the Parkinson's Disease Foundation, and a gift from the Dolsen Foundation. Dr. Shu-Ching Hu reports no conflict of interest and has been funded by grants from NIH. Dr. Agarwal reports no conflict of interest, she reports consulting for Teva Pharmaceutical, Cynapsus, US WorldMeds, UCB, Lundbeck, Impax, and has received research funding from the following companies and institutions: AbbVie Inc., Adamas, Amaranthus Bioscience, Biotie, Astellas, Auspex, CHDI Foundation, Chelsea Therapeutic, Civitas, Cynapsus, EvergreenHealth, HSG, inVentive Health, Intec, Lunbeck, Massachusetts General Hospital, Merk & Co., Merz, NIH, Northwestern University, Omeros, Osmotica, Pfizer, PRA, PSG, Solstice, Teva, US WorldMeds. Dr. Checkoway reports no conflict of interest and has been consulting for Alcoa, Inc. (Pittsburgh), NIEHS, Exponent Corp (Los Angeles), Ohio State University, Electric Power Research Institute, (Palo Alto); he reports royalties from Elsevier Publishing Co. and Oxford University Press, and has been funded by grants from NIEHS and the National Multiple Sclerosis Society.

Compliance with ethical standards

Ethical approval All procedures performed in studies involved in human participants were in accordance with the ethical standards of the institution and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

obtained. Differences in haptoglobin and ferritin concentration by smoking status and pack-years of smoking were calculated as well as regression between pack-years and haptoglobin and ferritin concentration, and the effect of haptoglobin phenotype on these parameters. Tobacco smoking was associated with an elevation in serum haptoglobin concentration, especially among healthy controls of haptoglobin Hp 2-2 phenotype, and with an elevation in ferritin concentration especially among PD patients of haptoglobin Hp 2-1 phenotype. These findings suggest that an elevation in haptoglobin concentration, preferentially among subjects of haptoglobin Hp 2-2 phenotype, could be a contributing factor to the protective effect of smoking on PD risk.

Keywords

Tobacco smoking; Haptoglobin concentration; Ferritin concentration; Haptoglobin phenotype; Parkinson's disease

Introduction

Haptoglobin (Hp) is a serum protein that binds free hemoglobin and allows scavenging of free hemoglobin by macrophages (Bowman and Kurosky 1982; McCormick and Atassi 1990; Kristiansen et al. 2001). Because of a genetic polymorphism of the alpha chain of the Hp gene (Langlois and Delanghe 1996), there are three common Hp phenotypes in the population, Hp 1-1, Hp 2-1, and Hp 2-2, which exhibit profound structural and functional differences: Hp 1-1 molecules have the smallest molecular weight, and Hp 2-2 molecules have the highest molecular weight, up to 900 kDa (Langlois and Delanghe 1996). Hp 2-2 complexes have lower Hb-binding capacity than Hp 1-1 and Hp 2-1 (Javid 1965), and also have lower Hb-scavenging power than Hp 2-1 and Hp 1-1 as a consequence of their lower ability to reach extra-vascular fluids, due to their higher molecular mass (Langlois and Delanghe 1996; Melamed-Frank et al. 2001). Hp polymorphism also affects serum Hp concentration, since subjects with Hp 1-1 phenotype have the highest levels of serum Hp concentration, while subjects with Hp 2-2 phenotype have the lowest levels (Langlois and Delanghe 1996). Hp 2-1 phenotype was previously found to be associated with a significantly increased risk of Parkinson disease (Costa-Mallen et al. 2008). We also recently observed that abnormally low serum iron levels in PD patients are present predominantly among subjects of Hp 2-1 phenotype, while abnormalities in PD patients of Hp 2-2 and Hp 1-1 phenotype are less pronounced (Costa-Mallen et al. 2015).

A very consistent epidemiologic finding in PD research is a reduced risk of PD, by about 50 %, for smokers compared with non-smokers (Ritz et al. 2007). Lifetime cigarette smoking was also found to be associated with reduced Lewy body accumulation (Tsuang et al. 2010). Selective survival of non-smoking PD cases does not account for the protective effect of cigarette smoking (Morens et al. 1996), and several hypotheses have been advanced over the years to explain the strong protective effect of smoking on PD risk. For example, smoking causes inhibition of monoamine oxidase B, the main metabolizing enzyme of dopamine (Fowler et al. 1996). However, none of the current hypotheses are completely supported by epidemiological findings. Second-hand smoke was recently found to confer reduced PD risk with a magnitude similar to the effect of active smoking (Searles et al.

2012), suggesting a biological effect of cigarette smoke rather than a personality trait being predisposing both to smoking avoidance and PD risk. Haptoglobin phenotype is a modifying factor for the effect of smoking on PD risk, as tobacco smoking exerts a protective effect on PD risk for subjects of Hp 2-2 and Hp 1-1 phenotype, but not for subjects of Hp 2-1 phenotype (Costa-Mallen et al. 2015), with mechanisms not elucidated.

It is noteworthy that tobacco smoking causes an increase in hematocrit (Kurata 2006) and higher circulatory iron. This effect was postulated to be due to the induced hypoxia and to the increased red blood cell turnover (Young and Moss 1989; Northrop-Clewes and Thurnham 2007). Ghio et al. (2008) reported that serum iron and ferritin levels among smokers were higher compared with never smokers.

Excess of iron deposition in the brain has been shown to be able to cause oxidative stress and contribute to the neurodegenerative processes typical of PD (Halliwell 2001; Berg et al. 2002; Youdim et al. 2004). Changes in systemic iron metabolism could potentially affect the levels of brain iron. We, therefore, hypothesized that smoking-related changes in iron metabolism may underlie the inverse association of smoking with PD by ultimately leading to lower levels of iron in the brain.

In addition, haptoglobin can exert neuroprotection by scavenging free hemoglobin (Zhao et al. 2009), and an increase in haptoglobin concentration itself could potentially be neuroprotective by reducing oxidative stress (Alayash et al. 2013). We, therefore, explored whether tobacco smoking was associated with higher Hp concentration in PD cases and controls, and if this effect was present preferentially in subjects of Hp 2-2 phenotype.

Methods

Study participants

Informed consent was obtained from all individual participants included in the study. All study procedures were approved by the Institutional Review Boards of Bastyr University, the University of Washington, and the Veterans Affairs Puget Sound Health Care System (VAPSHCS). Study participants were recruited in the Puget Sound area of Washington State between 2010 and 2015. Recruitment sources for the PD patients were the Washington State Parkinson Disease Registry (WPDR), the Michael J. Fox Foundation Fox Trial Finder, Bastyr University campus, the Bastyr Center for Natural Health (BCNH), Senior Centers, and referral from collaborating Neurologists in the Seattle area.

Controls were recruited from the Bastyr University campus, the BCNH, Senior Centers, and advertisement over local websites. Seventeen PD cases and 12 controls for a total of 29 participants included in this study were enrolled in the Parkinson's Genetic Research Study (PaGeR) (Zabetian et al. 2005).

All PD cases recruited met the "UK Parkinson's Disease Society Brain Bank clinical diagnostic criteria" (UKBB) for PD (Gibb and Lees 1988). Only patients meeting UKBB clinical diagnostic criteria were included in the study.

For all PD cases, 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. In addition to the exclusion criteria as from the UKBB, the presence of more than one relative with PD also constituted exclusion criteria for the PD patients, as determined from a screening telephone interview before the study visit.

Controls were subjects free of PD or other neurodegenerative diseases, as determined from subjects' interviews and medical records reviews. For the 12 controls from the PaGeR study, no additional medical history exclusion criteria were imposed on control eligibility.

Exclusion criteria for the PD cases and controls recruited from all other sources other than the participants recruited from the PaGeR were: the presence of current active cancer under treatment, chronic hepatitis, and HIV sero-positivity, as determined from participants' interviews and medical records reviews. Participants who had an acute illness or fever at the time of the visit were rescheduled for a different date when the acute illness had subsided.

The control group was frequency matched to cases by age in 10-year categories.

Data collection

Blood samples were collected by venipuncture from each study participant; serum was separated, split in different aliquots, and frozen at -70°C . One aliquot was submitted to the LabCorp Clinical laboratory for tests of serum haptoglobin (Hp) concentration and ferritin concentration. Hp concentration was expressed as mg/dl, and ferritin concentration was expressed as ng/ml. Hp phenotyping was performed on serum samples for all subjects, with a method as previously described (Costa-Mallen et al. 2008). Briefly, each serum sample was mixed with purified hemoglobin (Hb) solution, allowing Hp in the serum sample to bind to Hb. The Hp-Hb complexes were subjected to non-denaturing acrylamide gel electrophoresis on 4–20 % gradient gels at 70 V for 16 h, followed by peroxidase staining for bands visualization.

For the 29 participants from the PaGeR study, ferritin concentration could be determined for all participants, whereas Hp concentration could only be determined for 7 participants, because of lack of availability of sufficient sample for Hp concentration tests; as a result, ferritin concentration was collected for a total of 344 participants, 106 PD cases and 238 controls, while Hp concentration was collected for a total of 322 participants, 91 PD cases and 231 controls.

Demographic and smoking history information was obtained from all study participants, including age, gender, race, number of cigarettes a day smoked, and years smoked. Participants were considered “ever smokers” if they had smoked at least 100 cigarettes during their lifetime. Participants were considered as “past” smokers if they were ever smokers but were not currently smokers at the time of the visit. Participants who were not current smokers but smoked at least 100 cigarettes in the past were considered as past smokers. Pack-years of smoking were calculated by multiplying packs/day of cigarettes smoked by the number of years smoked.

Data analysis

Age- and gender-adjusted differences in mean serum Haptoglobin and serum ferritin were calculated between never smokers and ever smokers, and among never smokers, past smokers, and current smokers 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 PD case/control status.

Linear regression was used to test the relation between pack-years of smoking and serum Hp concentration, and serum ferritin concentration, in models where age and gender were included as covariates. Standardized beta (B) coefficients of linear regression between pack-years of smoking and Hp concentration and ferritin concentration were calculated. Linear regression was used also to test for interaction between never/past/current smoking status and Hp phenotype on ferritin concentration and Hp concentration levels, in models where case/control status, gender, and Hp phenotype were used as fixed factors, age was used as covariate, and either serum ferritin or serum Hp concentration was the dependent variable.

All analyses were performed in the overall study population independently of ethnicity. In separate analyses limited to non-Hispanic white subjects, the results did not change materially, and thus, only data for the entire study population are presented.

All data analysis was performed with SPSS 19.0 software.

Results

The study population consisted of 344 subjects, 106 PD cases and 238 controls, for whom ferritin concentration and Hp phenotype were determined and smoking status information was collected.

Hp concentration could be measured for a total of 322 participants, of which 91 PD cases and 231 controls. The demographics and smoking status of the participants are shown in Table 1.

Serum ferritin concentration results

Serum ferritin was overall significantly higher in men than women (men = 141.94 ng/ml, and women = 81.03 ng/ml, $p < 0.001$), as expected (Cook et al. 1976).

PD cases had overall slightly higher serum ferritin concentration than controls (PD = 120.22 ng/ml, controls = 106.31 ng/ml); however, this difference was not statistically significant (Table 2). The difference in serum ferritin between PD cases and controls was not significant also when considering men and women separately, or after stratification for Hp phenotype groups (Table 3).

Ferritin was higher in subjects of Hp 2-1 phenotype as compared with subjects of Hp 1-1 and Hp 2-2 phenotype overall (Hp 1-1 = 91.20 ng/ml, Hp 2-1 = 124.40 ng/ml, Hp 2-2 = 99.57 ng/ml, age-, gender-, smoking status-adjusted p value = 0.003). To test whether the higher ferritin levels in subjects of Hp 2-1 phenotype were due to an effect of smoking, we stratified by ever/never smoking status, and the higher levels of serum ferritin in subjects of

Hp 2-1 phenotype was present among subjects who were ever smokers (Hp 1-1 = 88.04 ng/ml, Hp 2-1 = 139.34 ng/ml, Hp 2-2 = 89.25 ng/ml, $p = 0.025$), but not among subjects who were never smokers (Hp 1-1 = 93.93 ng/ml, Hp 2-1 = 110.30 ng/ml, Hp 2-2 = 110.78 ng/ml, $p = 0.713$). Tobacco smoking was associated with an elevation of serum ferritin in subjects of Hp 2-1 phenotype, but not among subjects of Hp 1-1 or Hp 2-2 phenotype. In fact, as shown in Table 3), while there were no significant differences in serum ferritin levels among never–past–current smokers overall, after stratifying by Hp phenotype, serum ferritin levels resulted to be progressively higher from never smokers to past smokers to current smokers among subjects of Hp 2-1 phenotype, especially among PD cases. On the other hand, serum ferritin levels resulted progressively lower from never smokers to past smokers to current smokers among subjects of Hp 2-2 phenotype (Table 3); this interaction between Hp phenotype and never/past/current smoker status was statistically significant ($p = 0.036$). The three way-interaction between Hp phenotype, never-/past/current smoker status, and PD case/control status, was also statistically significant ($p = 0.041$).

The interaction between never/past/current smoker status, Hp phenotype, and gender was not statistically significant ($p = 0.136$).

We tested the correlation between pack-years of smoking and serum ferritin concentration (Table 4) and observed a significant increase in serum ferritin levels with increase in pack-years of smoking among PD cases (standardized beta coefficient = 0.289, $p = 0.005$). When stratifying by Hp phenotype, pack-years of smoking was correlated with an increase in serum ferritin among PD cases of Hp 2-1 phenotype (standardized beta coefficient = 0.392, $p = 0.008$), but not among PD cases of Hp 2-2 phenotype, similar to what observed for the association between ferritin and never–past–current smoking status. The interaction between pack-years of smoking and Hp phenotype was statistically significant ($p = 0.003$).

Among controls, the correlation between pack-years of smoking and serum ferritin levels resulted non-significant overall, as well as for the individual Hp phenotype groups (Table 4).

Serum Hp concentration results

Serum Hp concentration was similar in men and women (Hp in men = 126.86 mg/dl, Hp in women 135.03 mg/dl, $p = 0.165$). As shown in Table 5), serum Hp concentration was not significantly different in PD cases and controls overall, or after stratification by gender and Hp phenotype.

Consistently with what was previously shown (Langlois and Delanghe 1996), serum Hp concentration was higher for subjects of Hp 1-1 phenotype, intermediate for subjects of Hp 2-1 phenotype, and lowest for subjects of Hp 2-2 phenotype (serum Hp in Hp 1-1 = 161.59 mg/dl, in Hp 2-1 = 140.67 mg/dl, in Hp 2-2 = 103.54 mg/dl, $p < 0.001$).

Serum Hp concentration was not different between PD cases and controls overall (PD = 126.69 mg/dl, and controls = 133.11 mg/dl, age- and gender-adjusted $p = 0.435$) (Table 5). No significant differences in Hp concentration between PD cases and controls were present also after stratification by gender or Hp phenotype (Table 5).

Hp concentration resulted lower in PD patients than controls among subjects who were ever smokers (PD = 112.64 mg/dl, controls = 139.12 mg/dl, age- and gender-adjusted p value = 0.032). Among never smokers, instead, serum Hp concentration was not different between PD cases and controls (PD = 132.94 mg/dl, controls = 125.10 mg/dl, age- and gender-adjusted p value = 0.294). The interaction between case/control status and ever/never smoking status on Hp concentration was statistically significant ($p = 0.016$).

Hp concentration resulted significantly higher among current smokers as compared with past smokers and never smokers in the overall study population ($p = 0.016$) (Table 6). The difference in Hp concentration between never/past/current smokers was strongest among subjects of Hp 2-2 phenotype ($p = 0.001$) as compared with subjects of Hp 2-1 and Hp 1-1 phenotype. The interaction between Hp phenotype and never/past/current smoking status on Hp concentration levels was statistically significant ($p < 0.001$).

Among control subjects, Hp concentration resulted progressively higher from never smokers, to past smokers to current smokers ($p = 0.005$), and this increase in Hp concentration associated with smoking was strongest among subjects with Hp 2-2 phenotype ($p < 0.001$), while it was not present among subjects of Hp 2-1 phenotype (Table 6). Among PD cases, the differences in Hp concentration between never/past/current smokers were overall less pronounced than in controls, and not statistically significant, either as a main effect or for the different Hp phenotype groups.

Similar to what we observed for ever/past/current smoking status, serum Hp concentration was significant correlated with pack-years of smoking in the overall population (standardized beta coefficient = 0.157, $p = 0.003$) (Table 7). When stratifying by Hp phenotype, the correlation between Hp concentration and pack-years of smoking was stronger for subjects of Hp 2-2 phenotype (standardized beta coefficient = 0.324, $p = 0.001$), and non-significant for subjects of Hp 2-1 and Hp 1-1 phenotype (Table 7).

The correlation between pack-years of smoking and Hp concentration was significant among control participants overall (standardized beta coefficient = 0.188, $p = 0.002$), and in particular for control subjects of Hp 2-2 phenotype (standardized beta coefficient = 0.446, $p < 0.001$), but not among PD cases (Table 7), consistently to what observed for the association between Hp concentration and never/-past/current smoking status.

Discussion

In this case-control study of PD, we investigated possible effects of tobacco smoking that could be at the base of the consistently observed inverse association between smoking and PD risk (Morens et al. 1996; Ritz et al. 2007; Tsuang et al. 2010; Searles et al. 2012). Accordingly, we tested whether tobacco smoking was associated with changes in serum proteins that are implicated in regulation of iron metabolism and protection from oxidative stress, and tested serum ferritin and serum Hp concentration in PD cases and controls.

Our results for ferritin are consistent to those previously reported by Ghio et al. (2008), that smoking was associated with elevated serum ferritin concentration. We observed that the elevation in serum ferritin associated with pack-years of smoking was significant among PD

patients, and not significant among controls. Notably, the association between higher serum ferritin levels and smoking was present among PD cases of Hp 2-1 phenotype, but not PD cases of Hp 2-2 phenotype. In fact, among patients of Hp 2-2 phenotype, smoking was associated with an opposite effect, lower levels of serum ferritin. The interaction between Hp phenotype and smoking on serum ferritin levels was significant, indicating that the Hp phenotype of a subject influences the effect of tobacco smoking on ferritin levels.

In spite of ferritin levels being higher among men than women, as expected, there were no significant differences by gender on the effect of smoking on ferritin levels, and no significant interaction between gender, Hp phenotype, and smoking status on ferritin levels.

Serum ferritin levels usually correlate with body iron status (Jacobs and Worwood 1975), but in conditions of inflammation, ferritin levels can also increase in the absence of iron overload (Torti and Torti 1994; Cohen et al. 2010).

The main change in brain ferritin in PD, especially in the early stages of the disease, consists in a decrease in the concentration of L-ferritin, while an increase in H-ferritin occurs in later stage of PD progression (Koziorowski et al. 2007; Connor et al. 1995). The decrease in L-ferritin in the SN of PD patients suggests that the process of degeneration starts with a higher availability of free iron, which is released from the ferritin shell. An increase in brain non-ferritin labile iron may be responsible to neurodegenerative processes in PD patients (Wypijewska et al. 2010). How serum ferritin correlates with brain iron levels in general has still not been completely elucidated. In the study of Walter et al. (2012), serum iron was inversely correlated with brain iron in PD patients. It is still not known whether serum ferritin levels may affect brain iron binding proteins and iron metabolism to affect brain levels of L-ferritin and H-ferritin.

If indeed elevated serum ferritin levels were a contributing factor to increased brain free iron and brain oxidative stress, the fact that smoking was associated with higher serum ferritin levels among PD cases with Hp 2-1 phenotype, but not Hp 2-2 phenotype, may also contribute to the lack of protective effect of smoking on PD risk for subjects with Hp 2-1 phenotype that we have previously described (Costa-Mallen et al. 2015).

Serum Hp concentration in the study subjects with Hp 1-1 phenotype was the highest, while subjects with Hp 2-1 phenotype had intermediate Hp levels, and subjects with Hp 2-2 phenotype had the lowest levels: this is a well-known functional effect of the haptoglobin polymorphism, and has been consistently observed (Langlois and Delanghe 1996). We identified an effect of tobacco smoking on serum Hp concentration, as pack-years of smoking was significantly correlated with an increase in serum Hp concentration in the overall study population. To the best of our knowledge, this is the first time that Hp concentration is observed to directly correlate with pack-years of smoking.

Our results also demonstrate that Hp phenotype modifies the effect of smoking on serum Hp concentration. In fact, tobacco smoking resulted in an elevation in serum Hp concentration in subjects of Hp 2-2 phenotype, but not Hp 2-1 phenotype.

Hp has hemoglobin-binding and hemoglobin-scavenging activity (Javid 1965; Langlois and Delanghe 1996; Melamed-Frank et al. 2001; Zhao et al. 2009; Alayash et al. 2013), and an increase in serum Hp concentration could lead to better removal and scavenging of hemoglobin and potentially contribute to lower oxidative stress and PD risk. For these reasons, we believe that a smoking-induced increase in Hp concentration over the lifetime could potentially be a contributing factor to the protective effect of smoking on PD risk that has been consistently observed in epidemiology studies. Since tobacco smoking in this study resulted associated with an elevation in serum Hp concentration among subjects with Hp 2-2, but not Hp 2-1 phenotype, this could potentially be an explanation of the lower risk of PD for subjects with Hp 2-2 phenotype that has been previously observed (Costa-Mallen et al. 2008).

Serum Hp concentration was only slightly and non-significantly lower in PD cases than controls in this study, overall, but it was significantly lower in PD patients than controls among ever smokers: this was because of the association of smoking with an elevation of Hp concentration that occurs specifically among controls, especially of Hp 2-2 phenotype. The correlation between smoking and Hp concentration was much stronger for controls than PD patients. In this respect, we need to consider that a higher percentage of current-smokers were present among controls than PD patients, and this may be a contributing factor to the fact that the elevation of Hp concentration associated with smoking was not statistically significant among PD patients. Further studies on larger populations of PD patients and controls will be needed to determine if the effect of smoking on Hp concentration is present more strongly among healthy control subjects than PD patients.

We postulate that the increase in red blood cells turnover due to hypoxia in smokers (Young and Moss 1989; Northrop-Clewes and Thurnham 2007) may be responsible for an increase in hemoglobin release in the bloodstream, that may ultimately lead to the elevation of Hp and ferritin levels. In our study, smoking was associated with an elevation in Hp concentration especially in controls of Hp 2-2 phenotype and an elevation in ferritin especially in PD patients of Hp 2-1 phenotype, indicating that Hp phenotype influences the effect of smoking on these serum proteins levels.

The heterozygous Hp 2-1 phenotype conferred features that were different than both homozygous Hp 1-1 and Hp 2-2 phenotypes with respect to the effect of smoking on Hp concentration and ferritin concentration. Similarly, we had previously observed that the Hp 2-1 phenotype conferred association with PD (Costa-Mallen et al. 2008), and lower levels of serum iron in PD (Costa-Mallen et al. 2015). The phenomenon where the heterozygous phenotype behaves differently than both homozygous phenotypes is known as heterosis. Heterosis has been observed for association of Hp phenotype with other diseases, such as motor neuron disease, in addition to PD (Fröhlander and Forsgren 1988). In fact an excess of the Hp 2-1 phenotype as compared with both Hp 1-1 and Hp 2-2 phenotypes was observed among motor neuron disease patients (Fröhlander and Forsgren 1988). At the molecular level, it is not known how the heterozygous Hp 2-1 confers features that are different from both homozygous phenotypes. Interestingly, many other human polymorphic genes display heterosis, for example, the dopamine D2 receptor (DRD2) gene, where carriers of the

heterozygous TaqIA 12 genotype display lower DRD2 receptor density than carriers of both homozygous TaqIA 11 and TaqIA 22 genotypes (Comings and MacMurray 2000).

In conclusion, our study identifies associations between tobacco smoking and serum haptoglobin levels and ferritin levels that depend on the haptoglobin phenotype. Further studies will be needed to determine the mechanisms of the observed associations, as well as the temporal patterns of effects after smoking cessation, and the possible role of an elevation of haptoglobin levels in protection from PD risk.

Acknowledgments

The authors would like to thank all the individual study participants. This study was funded by grants from NIH (Grants # R21 NS070202, P50 NS062684, R01 NS065070), the Department of Veteran Affairs (Grant # 1101BX000531), and Bastyr University (Faculty Seed Grants # 3 and # 4 2009-2011).

References

- Alayash AI, Andersen CB, Moestrup SK, Bülow L. Haptoglobin: the hemoglobin detoxifier in plasma. *Trends Biotechnol.* 2013; 31:2–3. doi: 10.1016/j.tibtech.2012.10.003. [PubMed: 23140673]
- Berg D, Roggendorf W, Schröder U, Klein R, Tatschner T, Benz P, Tucha O, Preier M, Lange KW, Reiners K, Gerlach M, Becker G. Echogenicity of the substantia nigra: association with increased iron content and marker for susceptibility to nigrostriatal injury. *Arch Neurol.* 2002; 59:999–1005. [PubMed: 12056937]
- Bowman BH, Kurosky A. Haptoglobin: the evolutionary product of duplication, unequal crossing over, and point mutation. *Adv Hum Genet.* 1982; 12:189–261. [PubMed: 6751044]
- Cohen LA, Gutierrez L, Weiss A, Leichtmann-Bardoogo Y, Zhang DL, Crooks DR, Sougrat R, Morgenstern A, Galy B, Hentze MW, Lazaro FJ, Rouault TA, Meyron-Holtz EG. Serum ferritin is derived primarily from macrophages through a nonclassical secretory pathway. *Blood.* 2010; 116:1574–1584. doi: 10.1182/blood-2009-11-253815. [PubMed: 20472835]
- Comings DE, MacMurray JP. Molecular heterosis: a review. *Mol Genet Metab.* 2000; 71:19–31. [PubMed: 11001792]
- Connor JR, Snyder BS, Arosio P, Loeffler DA, LeWitt P. A quantitative analysis of iso-ferritins in select regions of aged, parkinsonian, and Alzheimer's diseased brains. *J Neurochem.* 1995; 65:717–724. [PubMed: 7616228]
- Cook JD, Finch CA, Smith NJ. Evaluation of the iron status of a population. *Blood.* 1976; 48:449–455. [PubMed: 953365]
- Costa-Mallen P, Checkoway H, Zabeti A, Edenfield MJ, Swanson PD, Longstreth WT Jr, Franklin GM, Smith-Weller T, Sadrzadeh SM. The functional polymorphism of the hemoglobin-binding protein haptoglobin influences susceptibility to idiopathic Parkinson's disease. *Am J Med Genet B Neuropsychiatr Genet.* 2008; 147B:216–222. [PubMed: 17918239]
- Costa-Mallen P, Zabetian CP, Agarwal P, Hu SC, Yearout D, Samii A, Leverenz JB, Roberts JW, Checkoway H. Haptoglobin phenotype modifies serum iron levels and the effect of smoking on Parkinson disease risk. *Parkinsonism Relat Disord.* 2015; 21:1087–1092. doi: 10.1016/j.parkreldis.2015.07.006. [PubMed: 26228081]
- Fowler JS, Volkow ND, Wang G-J, Pappas N, Logan J, MacGregor R, Alexoff D, Shea C, Schlyer D, Wolf AP, Warner D, Zezulova I, Cilento R. Inhibition of monoamine oxidase B in the brains of smokers. *Nature.* 1996; 379:733–766. [PubMed: 8602220]
- Fröhlander N, Forsgren L. Haptoglobin phenotype in motor neuron disease. *J Neurol Neurosurg Psychiatry.* 1988; 51:440–442. [PubMed: 3361338]
- Ghio AJ, Hilborn ED, Stonehuerner JG, Dailey LA, Carter JD, Richards JH, Crissman KM, Foronjy RF, Uyeminami DL, Pinkerton KE. Particulate matter in cigarette smoke alters iron homeostasis to produce a biological effect. *Am J Respir Crit Care Med.* 2008; 178:1130–1138. doi: 10.1164/rccm.200802-334OC. [PubMed: 18723436]

- Gibb WR, Lees AJ. A comparison of clinical and pathological features of young- and old-onset Parkinson's disease. *Neurology*. 1988; 38:1402–1406. [PubMed: 3412587]
- Halliwel B. Role of free radicals in the neurodegenerative diseases: therapeutic implications for antioxidant treatment. *Drugs Aging*. 2001; 18:685–716. [PubMed: 11599635]
- Jacobs A, Worwood F. Ferritin in serum. Clinical and biochemical implications. *N Engl J Med*. 1975; 292:951–956. [PubMed: 1090831]
- Javid J. The effect of haptoglobin-polymer size on hemoglobin binding capacity. *Vox Sang*. 1965; 10:320–325. [PubMed: 14332932]
- Koziorowski D, Friedman A, Arosio P, Santambrogio P, Dziewulska D. ELISA reveals a difference in the structure of substantia nigra ferritin in Parkinson's disease and incidental Lewy body compared to control. *Parkinsonism Relat Disord*. 2007; 13:214–218. [PubMed: 17275395]
- Kristiansen M, Graversen JH, Jacobsen C, Sonne O, Hoffman HJ, Law SK, Moestrup SK. Identification of the haemoglobin scavenger receptor. *Nature*. 2001; 409:198–201. [PubMed: 11196644]
- Kurata C. Medical check-up findings characteristic of smokers: aimed at improving smoking cessation interventions by physicians. *Intern Med*. 2006; 45:1027–1032. [PubMed: 17043372]
- Langlois MR, Delanghe JR. Biological and clinical significance of haptoglobin polymorphism in humans. *Clin Chem*. 1996; 42:1589–1600. [PubMed: 8855140]
- McCormick DJ, Atassi MZ. Hemoglobin binding with haptoglobin: delineation of the haptoglobin binding site on the alpha-chain of human hemoglobin. *J Protein Chem*. 1990; 9:735–742. [PubMed: 2073325]
- Melamed-Frank M, Lache O, Enav BI, Szafronek T, Levy NS, Ricklis RM, Levy AP. Structure-function analysis of the antioxidant properties of haptoglobin. *Blood*. 2001; 98:3693–3698. [PubMed: 11739174]
- Morens DM, Grandinetti A, Davis JW, Ross GW, White LR, Reed D. Evidence against the operation of selective mortality in explaining the association between cigarette smoking and reduced occurrence of idiopathic Parkinson's disease. *Am J Epidemiol*. 1996; 144(4):400–404. [PubMed: 8712197]
- Northrop-Clewes CA, Thurnham DI. Monitoring micronutrients in cigarette smokers. *Clin Chim Acta*. 2007; 377:14–38. [PubMed: 17045981]
- Ritz B, Ascherio A, Checkoway H, Marder KS, Nelson LM, Rocca WA, Ross GW, Strickland D, Van Den Eeden SK, Gorell J. Pooled analysis of tobacco use and risk of Parkinson disease. *Arch Neurol*. 2007; 64:990–997. [PubMed: 17620489]
- Searles Nielsen S, Gallagher LG, Lundin JI, Longstreth WT Jr, Smith-Weller T, Franklin GM, Swanson PD, Checkoway H. Environmental tobacco smoke and Parkinson's disease. *Mov Disord*. 2012; 27:293–296. doi:10.1002/mds.24012. [PubMed: 22095755]
- Torti SV, Torti FM. Iron and ferritin in inflammation and cancer. *Adv Inorg Biochem*. 1994; 10:119–137. [PubMed: 8203285]
- Tsuang D, Larson EB, Li G, Shofer JB, Montine KS, Thompson ML, Sonnen JA, Crane PK, Leverenz JB, Montine TJ. Association between lifetime cigarette smoking and Lewy body accumulation. *Brain Pathol*. 2010; 20:412–418. doi: 10.1111/j.1750-3639.2009.0029.00296.x. [PubMed: 19531100]
- Walter U, Witt R, Wolters A, Wittstock M, Benecke R. Substantia nigra echogenicity in Parkinson's disease: relation to serum iron and C-reactive protein. *J Neural Transm*. 2012; 119:53–57. doi: 10.1007/s00702-011-0664-z. [PubMed: 21626410]
- Wypijewska A, Galazka-Friedman J, Bauminger ER, Wszolek ZK, Schweitzer KJ, Dickson DW, Jaklewicz A, Elbaum D, Friedman A. Iron and reactive oxygen species activity in parkinsonian substantia nigra. *Parkinsonism Relat Disord*. 2010; 16:329–333. doi:10.1016/j.parkreldis.2010.02.007. [PubMed: 20219408]
- Youdim MBH, Stephenson G, Ben Shachar D. Ironing out iron in Parkinson's disease and other neurodegenerative diseases with iron chelators. A lesson from 6-hydroxydopamine and iron chelators desferal and VK-28. *Ann NY Acad Sci*. 2004; 1012:306–326. [PubMed: 15105275]
- Young CJ, Moss J. Smoke inhalation: diagnosis and treatment. *J Clin Anesth*. 1989; 1:377–386. [PubMed: 2697244]

- Zabetian CP, Samii A, Mosley AD, Roberts JW, Leis BC, Yearout D, Raskind WH, Griffith A. A clinic-based study of the LRRK2 gene in Parkinson disease yields new mutations. *Neurology*. 2005; 65:741–744. [PubMed: 16157909]
- Zhao X, Song S, Sun G, Strong R, Zhang J, Grotta JC, Aronowski J. Neuroprotective role of haptoglobin after intracerebral hemorrhage. *J Neurosci*. 2009; 29:15819–15827. doi:10.1523/JNEUROSCI.3776-09.2009. [PubMed: 20016097]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table 1

Demographics and characteristics of the study population

	Total	PD cases	Controls
Number, <i>n</i>	344	106	238
Men, <i>n</i> (%)	167 (48.55 %)	68 (64.15 %)	99 (41.60 %)
Women, <i>n</i> (%)	177 (51.45 %)	38 (35.85 %)	139 (58.40 %)
Age, mean (SD)	64.18 (10.93)	67.05 (9.98)	62.90 (11.10)
Men, mean age (SD)	64.77 (11.72)	67.40 (9.94)	62.96 (12.53)
Women, mean age (SD)	63.62 (10.12)	66.42 (10.42)	62.86 (10.01)
Hoehn and Yahr PD stage (SD)		2.15 (0.69)	0
Men		2.25 (0.69)	0
Women		1.95 (0.66)	0
PD duration (years since diagnosis)		5.51 (5.28)	
Men		4.92 (4.94)	
Women		6.45 (5.71)	
Smoking status			
Never smokers (%)	174 (50.6 %)	70 (66.0 %)	104 (43.7 %)
Ever smokers (%)	170 (49.4 %)	36 (34.0 %)	134 (56.3 %)
Past smokers (%)	95 (27.6 %)	31 (29.2 %)	64 (26.9 %)
Current smokers (%)	75 (21.8 %)	5 (4.7 %)	70 (29.4 %)
Years smoked, mean (SD)	11.43 (15.20 %)	6.33 (11.46)	13.70 (16.10)
Packs/day, mean (SD)	0.38 (0.50)	0.24 (0.47)	0.44 (0.50)
Pack-years, mean (SD)	9.89 (16.75)	5.57 (14.77)	11.82 (17.24)
Pack-years past smokers	14.92 (16.56)	17.74 (23.10)	13.55 (12.23)
Pack-years current smokers	26.49 (20.25)	8.05 (5.57)	27.81 (20.29)
Years since quitting for past smokers (SD)	29.23 (13.54)	32.35 (13.04)	27.79 (13.64)
Race			
White	307 (89.2 %)	100 (94.3 %)	207 (87.0 %)
African American	16 (4.7 %)	2 (1.9 %)	14 (5.9 %)
Asian	7 (2.0 %)	2 (1.9 %)	5 (2.1 %)
Native American	1 (0.3 %)	1 (0.9 %)	0 (0.0 %)
More than 1 race	13 (3.8 %)	1 (1.0 %)	12 (5.0 %)
Serum ferritin ^a (ng/ml), mean (SD)	110.60 (110.61)	120.22 (124.44)	106.31 (103.86)
Min–max (ng/ml)	3–868	3–790	9–868
Men, ferritin (ng/ml), mean (SD)	141.94 (135.81)	148.47 (145.14)	137.45 (129.58)
Min–max (ng/ml)	3–868	3–790	9–868
Women, ferritin (ng/ml), mean (SD)	81.03 (68.109)	69.68 (41.35)	84.13 (73.56)
Min–max (ng/ml)	7–634	7–176	11–634
Serum Hp ^b (mg/dl), mean (SD)	131.30 (52.57)	126.69 (54.70)	133.11 (51.71)
Min–max (mg/dl)	4–332	4–326	25–332
Men, Hp (mg/dl), mean (SD)	126.86 (55.13)	124.81 (60.08)	128.04 (52.35)
Min–max (mg/dl)	4–326	4–326	33–322

	Total	PD cases	Controls
Women, Hp (mg/dl), mean (SD)	135.03 (50.17)	129.43 (46.41)	136.53 (51.19)
Min–max (mg/dl)	25–332	31–215	25–332

^aFerritin concentration was measured for all the participants, 106 PD cases and 238 controls

^bHp concentration was measured for 91 PD cases and 231 controls. For 22 participants, 15 PD cases and 7 controls, Hp concentration could not be measured. Demographic characteristics of the PD cases and controls for whom Hp concentration was measured were very similar to the ones for the overall study population

Table 2

Mean ferritin in PD cases and controls and stratified by gender and Hp phenotype groups

	PD case/control status	<i>n</i>	Mean ferritin concentration (ng/ml)	Standard error	Age-adjusted <i>p</i> value	Age- and gender-adjusted <i>p</i> value
Total subjects	PD cases	106	120.22	12.087	0.237	0.995
	Controls	238	106.31	6.732		
	Total	344	110.60	5.964		
Women	PD cases	38	69.68	6.709	0.461	–
	Controls	139	84.13	6.240		
	Total	177	81.03	5.119		
Men	PD cases	68	148.47	17.601	0.310	–
	Controls	99	137.45	13.023		
	Total	167	141.94	10.509		
Hp 1-1	PD cases	17	111.05	26.043	0.259	0.238
	Controls	37	82.08	8.587		
	Total	54	91.20	10.109		
Hp 2-1	PD cases	50	129.86	21.17	0.487	0.849
	Controls	121	122.14	11.31		
	Total	171	124.40	10.09		
Hp 2-2	PD cases	39	111.87	15.012	0.281	0.880
	Controls	80	93.57	9.194		
	Total	119	99.57	7.903		

Table 3

Mean ferritin in never smokers, past smokers, and current smokers stratified by Hp phenotype and PD case/control status

Case/control status and Hp phenotype	Smoking status	<i>n</i>	Mean ferritin concentration (ng/ml)	Standard error	Age-adjusted <i>p</i> value	Age- and gender-adjusted <i>p</i> value
Total subjects	Never smokers	174	107.73	7.480	0.914	0.997
	Past smokers	95	116.08	12.232		
	Current smokers	75	110.30	14.533		
Hp 1-1	Never smokers	29	93.93	15.793	0.641	0.644
	Past smokers	15	90.66	18.571		
	Current smokers	10	84.10	13.663		
Hp 2-1	Never smokers	88	110.30	11.141	0.233	0.383
	Past smokers	53	131.00	19.125		
	Current smokers	30	154.10	33.140		
Hp 2-2	Never smokers	57	110.78	12.823	0.088	0.111
	Past smokers	28	99.67	17.411		
	Current smokers	34	80.67	9.616		
Total PD cases	Never smokers	70	117.18	12.172	0.569	0.530
	Past smokers	31	120.22	26.848		
	Current smokers	5	162.80	107.302		
PD Hp 1-1	Never smokers	14	119.07	30.236	0.566	0.651
	Past smokers	3	73.66	46.548		
	Current smokers	0	–	–		
PD Hp 2-1	Never smokers	32	101.93	16.610	0.010	0.031
	Past smokers	17	155.35	46.072		
	Current smokers	1	590.00	–		
PD Hp 2-2	Never smokers	24	136.41	21.671	0.032	0.088
	Past smokers	11	78.63	17.456		
	Current smokers	4	56.00	13.391		
Total controls	Never smokers	104	101.37	9.458	0.932	0.771
	Past smokers	64	114.07	12.842		
	Current smokers	70	106.55	13.855		
Controls Hp1-1	Never smokers	15	70.46	9.524	0.149	0.163
	Past smokers	12	94.91	21.023		
	Current smokers	10	84.10	13.663		
Controls Hp 2-1	Never smokers	56	115.08	14.783	0.812	0.911
	Past smokers	35	121.02	18.628		
	Current smokers	30	136.63	29.635		
Controls Hp 2-2	Never smokers	33	92.15	15.025	0.922	0.771
	Past smokers	17	113.29	26.264		
	Current smokers	30	83.96	10.685		

Statistically significant values at $p = 0.05$ are shown in bold

Table 4

Correlation between pack-years of smoking and ferritin concentration in the overall study population and stratified by Hp phenotype and PD case/control status

Subjects	<i>n</i>	Standardized beta coefficient	<i>p</i> value	Age-adjusted <i>p</i> value	Age- and gender-adjusted <i>p</i> value
Total subjects	344	0.110	0.041	0.045	0.130
Hp 1-1	54	0.053	0.706	0.616	0.769
Hp 2-1	171	0.192	0.012	0.015	0.062
Hp 2-2	119	−0.055	0.554	0.557	0.527
PD cases	106	0.289	0.003	0.002	0.005
Hp 1-1	17	0.067	0.799	0.886	0.873
Hp 2-1	50	0.392	0.005	0.002	0.008
Hp 2-2	39	−0.110	0.505	0.489	0.401
Controls	238	0.049	0.455	0.514	0.924
Hp 1-1	37	0.149	0.378	0.301	0.434
Hp 2-1	121	0.084	0.359	0.486	0.787
Hp 2-2	80	−0.006	0.958	0.994	0.824

Statistically significant values at *p* 0.05 are shown in bold

Table 5

Mean Hp concentration in PD cases and controls, and stratified by gender and Hp phenotype groups

	PD case/control status	<i>n</i>	Mean Hp concentration (mg/dl)	Standard error	Age-adjusted <i>p</i> value	Age- and gender-adjusted <i>p</i> value
Total subjects	PD cases	91	126.69	5.735	0.322	0.435
	Controls	231	133.11	3.403		
	Total	322	131.30	2.930		
Women	PD cases	37	129.43	7.631	0.559	–
	Controls	138	136.53	4.358		
	Total	175	135.03	3.793		
Men	PD cases	54	124.81	8.177	0.597	–
	Controls	93	128.04	5.429		
	Total	147	126.86	4.548		
Hp 1-1	PD cases	16	165.88	9.773	0.792	0.713
	Controls	35	159.63	6.468		
	Total	51	161.59	5.355		
Hp 2-1	PD cases	44	133.36	6.509	0.309	0.389
	Controls	117	143.42	4.705		
	Total	161	140.67	3.860		
Hp 2-2	PD cases	31	97.00	10.996	0.411	0.583
	Controls	79	106.10	5.257		
	Total	110	103.54	4.872		

Table 6

Mean Hp concentration in never smokers, past smokers, and current smokers, for the overall study population, and stratified by Hp phenotype and PD case/control status

Case/control status and Hp phenotype	Smoking status	<i>n</i>	Mean Hp concentration (mg/100 ml)	Standard error	Age-adjusted <i>p</i> value	Age- and gender-adjusted <i>p</i> value
Total subjects	Never smokers	162	128.15	4.198	0.018	0.016
	Past smokers	88	123.86	5.300		
	Current smokers	72	147.47	6.053		
Hp 1-1	Never smokers	27	156.30	7.542	0.068	0.065
	Past smokers	14	166.07	9.576		
	Current smokers	10	169.60	12.837		
Hp 2-1	Never smokers	83	141.08	5.413	0.532	0.459
	Past smokers	48	130.94	6.719		
	Current smokers	30	155.10	9.083		
Hp 2-2	Never smokers	52	92.88	6.735	0.001	0.001
	Past smokers	26	88.08	7.430		
	Current smokers	32	133.41	9.404		
Total PD cases	Never smokers	63	132.94	7.305	0.215	0.212
	Past smokers	26	109.23	8.163		
	Current smokers	2	157.00	52.000		
PD Hp 1-1	Never smokers	14	164.71	10.899	0.690	0.357
	Past smokers	2	174.00	24.000		
	Current smokers	0	–	–		
PD Hp 2-1	Never smokers	29	138.62	9.046	0.284	0.279
	Past smokers	15	123.20	7.352		
	Current smokers	0	–	–		
PD Hp 2-2	Never smokers	20	102.45	15.105	0.940	0.994
	Past smokers	9	71.56	9.660		
	Current smokers	2	157.00	52.000		
Total controls	Never smokers	99	125.10	5.064	0.008	0.005
	Past smokers	62	130.00	6.582		
	Current smokers	70	147.20	6.134		
Controls Hp1-1	Never smokers	13	147.23	10.206	0.063	0.071
	Past smokers	12	164.75	10.798		
	Current smokers	10	169.60	12.837		
Controls Hp 2-1	Never smokers	54	142.41	6.809	0.396	0.319
	Past smokers	33	134.45	9.190		
	Current smokers	30	155.10	9.083		
Controls Hp 2-2	Never smokers	32	86.91	5.555	<0.001	<0.001
	Past smokers	17	96.82	9.665		
	Current smokers	30	131.83	9.657		

Statistically significant values at $p = 0.05$ are shown in bold

Table 7

Correlation between pack-years of smoking and Hp concentration in the overall study population and stratified by Hp phenotype and case/control status

Subjects	<i>n</i>	Standardized beta coefficient	<i>p</i> value	Age-adjusted <i>p</i> value	Age- and gender-adjusted <i>p</i> value
Total subjects	322	0.157	0.005	0.005	0.003
Hp 1-1	51	0.144	0.312	0.279	0.380
Hp 2-1	161	0.134	0.091	0.104	0.064
Hp 2-2	110	0.324	0.001	0.001	0.001
PD cases	91	0.019	0.855	0.887	0.928
Hp 1-1	16	−0.063	0.818	0.394	0.631
Hp 2-1	44	0.074	0.633	0.541	0.522
Hp 2-2	31	−0.113	0.544	0.555	0.574
Controls	231	0.188	0.004	0.005	0.002
Hp 1-1	35	0.254	0.141	0.130	0.181
Hp 2-1	117	0.136	0.145	0.155	0.101
Hp 2-2	79	0.446	<0.001	<0.001	<0.001

Statistically significant values at *p* = 0.05 are shown in bold