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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/100 ml 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 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.

1. 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 [4–8], 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

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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.

2. Methods

2.1. 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.

2.2. 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.

2.3. 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.

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