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Pooled analysis of iron-related genes in Parkinson's disease: Association with transferrin

Shannon L. Rhodes [a,*], Daniel D. Buchanan [b,c,d], Ismaïl Ahmed [e,f], Kent D. Taylor [g], Marie-Anne Loriot [h,i], Janet S. Sinsheimer [j,k,l], Jeff M. Bronstein [m], Alexis Elbaz [n,o], George D. Mellick [p,q], Jerome I. Rotter [g], Beate Ritz [a,m,r]

[a] Department of Epidemiology, UCLA Fielding School of Public Health, 650 Charles E. Young Drive S, Los Angeles, CA 90095-1772, USA
[b] Cancer and Population Studies Group, Queensland Institute of Medical Research, 300 Herston Rd, Brisbane, QLD 4006, Australia
[c] University of Queensland, School of Medicine, Brisbane, Australia
[d] Princess Alexandra Hospital, Australia
[e] Centre for Research in Epidemiology and Population Health, Biostatistics team, INSERM U1018, F-94276 le Kremlin Bicêtre, France
[f] Univ Paris-Sud, UMRS 1018, F-94276 le Kremlin Bicêtre, France
[g] Institute for Translational Genomics and Population Sciences, Los Angeles Biomedical Research Institute, Harbor-UCLA Medical Center, 1124 West Carson, Bldg E5, Torrance, CA 90502, USA
[h] Sorbonne Paris Cité, Université Paris Descartes, INSERM UMR-S 775, France
[i] Assistance Publique Hôpitaux de Paris, Hôpital Européen Georges Pompidou, Service de Biochimie, Unité Fonctionnelle de Pharmacogénétique et Oncologie Moléculaire, France
[j] Department of Human Genetics, David Geffen School of Medicine at UCLA, 695 Charles E. Young Drive South, Box 708822, Los Angeles, CA 90095-7088, USA
[k] Department of Biomathematics, David Geffen School of Medicine at UCLA, Box 951766, Room 5303 Life Sciences, Los Angeles, CA 90095-1766, USA
[l] Department of Biostatistics, UCLA Fielding School of Public Health, 650 Charles E. Young Drive S, Los Angeles, CA 90095-1772, USA
[m] Department of Neurology, David Geffen School of Medicine at UCLA, 710 Westwood Plaza, Los Angeles, CA 90095-1769, USA
[n] Centre for Research in Epidemiology and Population Health, Social and Occupational Determinants of Health, INSERM U1018, F-94807 Villejuif, France
[o] Univ Versailles St-Quentin, UMRS 1018, F-94807, Villejuif France
[p] Eskitis Institute for Drug Discovery, Griffith University, Nathan 4111, Brisbane, Australia
[q] Department of Neurology, Princess Alexandra Hospital, Brisbane, Australia
[r] Department of Environmental Health Sciences, UCLA Fielding School of Public Health, 650 Charles E. Young Drive S, Los Angeles, CA 90095-1772, USA

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ABSTRACT

Pathologic features of Parkinson's disease (PD) include death of dopaminergic neurons in the substantia nigra, presence of α-synuclein containing Lewy bodies, and iron accumulation in PD-related brain regions. The observed iron accumulation may be contributing to PD etiology but it also may be a byproduct of cell death or cellular dysfunction. To elucidate the possible role of iron accumulation in PD, we investigated genetic variation in 16 genes related to iron homeostasis in three case–control studies from the United States, Australia, and France. After screening 90 haplotype tagging single nucleotide polymorphisms (SNPs) within the genes of interest in the US study population, we investigated the five most promising gene regions in two additional independent case–control studies. For the pooled data set (1289 cases, 1391 controls) we observed a protective association (OR = 0.83, 95% CI: 0.71–0.96) between PD and a haplotype composed of the A allele at rs1880669 and the T allele at rs1049296 in *transferrin* (*TF*; GeneID: 7018). Additionally, we observed a suggestive protective association (OR = 0.87, 95% CI: 0.74-1.02) between PD and a haplotype composed of the G allele at rs10247962 and the A allele at rs4434553 in *transferrin receptor 2* (*TFR2*; GeneID: 7036). We observed no associations in our pooled sample for haplotypes in *SLC40A1*, *CYB561*, or *HFE*. Taken together with previous findings in model systems, our results suggest that *TF* or a *TF-TFR2* complex may have a role in the etiology of PD, possibly through iron misregulation or mitochondrial dysfunction within dopaminergic neurons.

Introduction

Brain iron accumulation, beyond that seen in age-matched controls, is frequently observed in Parkinson's disease (PD) affected brains (reviewed in (Gerlach et al., 2006)). Histochemical comparisons of post-mortem brain tissue have found increased levels of iron deposits in the substantia nigra (SN) of Parkinsonian brains (Dexter et al., 1987; Sofic

* Corresponding author.
E-mail address: shannon.rhodes@ucla.edu (S.L. Rhodes).
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et al., 1991) and specifically in neurons of the SN (Oakley et al., 2007). These pathologic findings are further supported by magnetic resonance imaging (Bartzokis et al., 1999; Gorell et al., 1995; Martin et al., 2008; Michaeli et al., 2007) and transcranial ultrasound (Becker et al., 1995; Mehnert et al., 2010; Walter et al., 2002) studies that observed iron deposits in the SN in living idiopathic PD patients.

It is debatable whether this accumulation of iron is a cause, co-factor, or consequence of the dopaminergic (DA) cell death in PD. Iron is a very potent oxidation–reduction agent that can create oxidative stress in cells and prior work suggests that neurons may be more sensitive to alterations in iron (LaVaute et al., 2001; Moos et al., 1998) than other cell types in the brain. Iron is also hypothesized to aggravate some key pathogenic processes related to PD including alpha-synuclein fibril formation (Olivares et al., 2009; Uversky et al., 2001) and mitochondrial dysfunction (Horowitz and Greenamyre, 2010; Lin et al., 2001). Finally, iron may simply be a remnant of neuronal cell death (He et al., 2003).

Animal models provide some potential insight. Genetic knockouts of some iron-related genes, when not lethal, produce altered brain iron levels (LaVaute et al., 2001; Moos and Morgan, 2004; Patel et al., 2002). Iron administration to unaltered animals has resulted in brain iron accumulation (Sengstock et al., 1993) or iron accumulation with a decrease in dopamine levels (Sziraki et al., 1998; Wesemann et al., 1994). Iron feeding studies in animals have also observed decreased dopamine levels with a large excess of dietary iron (Kaur et al., 2007) and with iron in combination with toxins (Levenson et al., 2004; Peng et al., 2007). These observations suggest that iron is either a cause or a co-factor, not a consequence, of dopaminergic cells damage, possibly due to the role iron plays in the synthesis of tyrosine hydroxylase (Snyder and Connor, 2009). Therefore, we hypothesize that small imbalances in ferrous or ferric brain iron contribute to one or more of the pathogenic processes contributing to neurodegeneration in PD and propose to investigate this hypothesis by evaluating the associations between PD and iron-related genes.

To date, epidemiologic studies investigating associations between occupational or dietary iron exposure and PD have been unpersuasive, and the few reports of iron-related genes and PD have been predominantly in small studies, investigating rare exonic SNPs, and inconclusive (reviewed in (Rhodes and Ritz, 2008)). Nevertheless, studies in experimental models of PD support a role for iron in the etiology or progression of PD (e.g. (Ben-Shachar and Youdim, 1991; Fredriksson et al., 2001; Kaur et al., 2003)). To facilitate the investigation of the iron-PD hypothesis, we pooled data from three independent case–control studies: one each from the United States (US), Australia (AU), and France (FR). All studies were designed for the investigation of genetic variation; the US and FR studies were also designed to investigate the influence of pesticide exposure on PD etiology and, therefore, include subjects with an increased likelihood of exposure to pesticides. In this report, we present the results from our two-phase study of promoter region, intronic, and exonic SNPs specifically selected to span each of 16 iron-related candidate genes and their associations with PD in a pooled sample of 1286 idiopathic PD cases and 1391 controls.

Methods

For our two-phase design we screened a larger number of genetic variants in an initial study, the US Study described below, followed by genotyping of selected variants in two additional studies based on findings from the initial study. All three studies (Supplemental Table 1) were pooled for the final analysis.

Studies and subjects

US Study

The Parkinson's Environment and Genes (PEG) Study enrolled incident PD patients with movement disorder specialist confirmed idiopathic PD and population-based controls between 2001 and 2007 from three counties in the highly agricultural central California valley of the United States. The recruitment strategy and case definition criteria have been described in detail elsewhere (Costello et al., 2009; Jacob et al., 2010). Participants provided either a blood or saliva sample and DNA was extracted by standard methods. This study was approved by the UCLA Institutional Review Board. At the time of this investigation, the US Study had enrolled a total of 373 PD cases, but reclassified 13 PD cases as not idiopathic PD during a follow-up study (Ritz et al., 2012), resulting in a total of 360 idiopathic PD cases and 403 population-based controls available for this investigation.

AU Study

The Australian case–control sample is a subset of the larger Queensland Parkinson's Project (QPP) Cohort of over 4000 community dwelling individuals recruited to participate in research into Parkinsonism and related disorders. The recruitment strategy and inclusion criteria are detailed elsewhere (Sutherland et al., 2009). Only Caucasian subjects were included in the study. Patients with PD meeting standard criteria (Gelb et al., 1999) were recruited from two private and two public movement disorder clinics in Brisbane, Australia. Control subjects consisted of unaffected electoral roll volunteers, patient spouses, and community-dwelling unaffected volunteers collected from community groups and patient neighborhoods. Participants provided blood samples and genomic DNA was extracted according to standard methods. The study was approved by human research ethics committees at the Princess Alexandra Hospital, University of Queensland, Royal Brisbane and Women's Hospital and Griffith University. At the time of this investigation, the AU Study had enrolled 1035 PD cases and 774 controls.

FR Study

This French population-based case–control study was conducted among subjects enrolled in the Mutualité Sociale Agricole (MSA), the organization responsible for the reimbursement of health-related expenses to workers in agriculture and has been described previously (Dutheil et al., 2010; Elbaz et al., 2004, 2009). Patients in 62 French districts fulfilling standard criteria (Bower et al., 1999), applying for free health care for PD for the first time between February 1998 and August 1999, and aged 18–75 years old were enrolled in the study. Population-based controls were recruited among all the MSA affiliates who requested reimbursement of health expenses between February 1998 and February 2000. A maximum of three controls was matched to each case on age (±2 years), sex, and region of residency. Participants provided blood samples and genomic DNA was extracted from peripheral blood leukocytes. The research protocol was approved by the ethics committee of Hôpital du Kremlin-Bicêtre, and all subjects signed an informed consent. At the time of this investigation, the FR Study had enrolled a total of 209 PD cases and 501 population-based controls.

Selection of genes and SNPs

Candidate genes were selected based on (i) prior reports in studies of PD, (ii) reported associations from iron-related disorders, and (iii) biologic support for a role in brain iron homeostasis. Genes were selected prior to publication of the genome-wide association studies (GWAS) so certain new candidate genes for iron metabolism (e.g. TMPRSS6 (Tanaka et al., 2010)) were not investigated. The 16 selected genes and their role in iron homeostasis are listed in Table 1. Candidate SNPs were selected from the literature and haplotype tagging SNPs sufficient to capture the majority of genetic variation in each gene were selected using Haploview (Barrett et al., 2005). Some candidate SNPs, e.g. H63D in *HFE*, were not available on the platform used for the initial phase of genotyping and therefore were not investigated. In total 90 SNPs in 16 genes were genotyped in the US Study.