HHS Public Access

Author manuscript

Neurobiol Aging. Author manuscript; available in PMC 2018 February 01.

Published in final edited form as:

Neurobiol Aging. 2017 February ; 50: 39–46. doi:10.1016/j.neurobiolaging.2016.10.006.

Aging Modifies the Effect of GCH1 RS11158026 on DAT Uptake and Parkinson's Disease Clinical Severity

Joseph Webb, B.S.[A] and **Auriel A. Willette, Ph.D., M.S.**[A,B,C,D]

[A]Department of Food Science and Human Nutrition, Iowa State University, Ames, IA

[B]Neuroscience Graduate Program, Iowa State University, Ames, IA

[C]Department of Psychology, Iowa State University, Ames, IA

[D]Department of Neurology, University of Iowa, Iowa City, IA

Abstract

Novel single nucleotide polymorphisms within Parkinson's disease (PD) can predict disease risk, but their influence on clinical, cognitive and neurobiological indices remain unexplored. We investigated differences between functional polymorphisms at RS11158026 coding for guanosine triphosphate cyclohydrolase-1 (GCH1), an essential enzyme for dopamine production in nigrostriatal cells. Among newly diagnosed, untreated PD subjects and age-matched controls from the Parkinson's Progression Markers Initiative, T allele carriers showed higher PD risk (Odds Ratio=1.23, P=0.048), earlier age of onset by 5 years (P=0.003), and lower striatal DAT uptake (P=0.003). Carriers also had increased CSF α-synuclein (P=0.016), worse motor function (P=0.041), anxiety (P=0.038), and executive function (P<0.001). Strikingly, these effects were only in younger T carriers (<50 years), where aging quells the effects of these genetic factors. This suggests GCH1 variants affect early PD risk through altered dopamine uptake, and aging alters how genetic factors contribute to disease development. Future studies should investigate how aging modifies genotypes contributions on PD risk and sequelae.

Keywords

Parkinson's disease; Guanosine Cyclohydrolase 1 (GCH1); Age; DAT-SPECT; Parkinson's Progression Marker Initiative (PPMI)

Introduction

Parkinson's disease (PD) is an age-related disorder resulting from degeneration of nigrostriatal dopaminergic neurons. PD is believed to be due to α-synuclein aggregation (Allen Reish and Standaert, 2015), decreasing dopamine production whereby many genetic

Corresponding Author: Auriel A. Willette, 224A MacKay Hall, Ames, IA 50011, Phone: (515) 294-3110, Fax: (515) 294-6193, awillett@iastate.edu; Statistical analysis conducted by Joseph Webb, Iowa State University, Joseph Webb, jlwebb@iastate.edu.

risk factors act individually or in concert to impact disease pathogenesis (Lesage and Brice, 2009). Guanosine triphosphate cyclohydrolase-1 (GCH1; OMIM 600225), located on chromosome 14q22.1-q22.2, encodes the rate-limiting enzyme catalyzing the first step in tetrahydrobiopterin (BH4) synthesis. BH4 is a required cofactor for tyrosine hydroxylase, ultimately leading downstream to production of dopamine (Kurian, et al., 2011).

Recently, a large-scale meta-analysis of genome-wide association study (GWAS) data identified new risk loci for the development of PD(Nalls, et al., 2014), including a novel variant at RS11158026, hereafter RS026, for GCH1. No studies have investigated how RS026 variants affect clinical characteristics in PD in populations of European descent. Furthermore, it is unknown how the RS026 T or C alleles may impact neural, cognitive, or biomarker sequelae relevant to PD or dopamine transport uptake, such as α-synuclein(Goedert, 2001), tau species(Kang, et al., 2016), as well as metabolic factors like cholesterol that may affect dopamine uptake(Jones, et al., 2012).

This study explored relationships between markers of PD with a GCH1 mutation, to determine if mutation carriers of European origin are at increased risk of developing PD. We describe the clinical, neuropsychological, volumetric imaging and dopaminergic imaging findings of GCH1 variants in a cross-sectional cohort of newly diagnosed, untreated individuals with PD and healthy, age-matched controls. We also wished to explore how RS026 impacted processes among young and older subjects, as aging is the greatest PD risk factor (Rodriguez, et al., 2015) and may impact the effect of RS026.

Methods

Setting

The Parkinson's Progression Markers Initiative (PPMI) is described at www.ppmi-info.org. PPMI is a public–private partnership funded by the Michael J Fox Foundation for Parkinson's Research and funding partners listed at www.ppmi-info.org/fundingpartners. PPMI is an observational, multicenter longitudinal case-control study designed to identify PD biomarkers (The Lancet, 2010).

Participants

PPMI data was obtained from 289 de novo PD and 233 aged healthy controls, including: 1) demographic data 2) genetic data; 3) serum and CSF biomarkers; 4) structural Magnetic Resonance Imaging (MRI) scans; 5) Unified Parkinson's Disease Rating Scale (UPDRS) scores and neuropsychological performance; and 6) Single Photo Emission Computed Tomography (SPECT) dopamine reuptake transporter (DAT) scans. Participants were clinically diagnosed at every visit based on standardized criteria (Emre, et al., 2007). All subjects enrolled in the PPMI study diagnosed with PD had received a diagnosis of PD for two years or less and were not taking PD medication while enrolled in the study. Data were downloaded on May 15th, 2016. Participants with genotype data available at baseline were categorized as carriers of only the major allele (C/C; n=237) versus participants who had at least one minor T allele (C/T or T/T; n=185), based on previous reports showing evidence for autosomal dominance inheritance (Hwu, et al., 2000).

Standard Protocol Approvals, Registrations, and Patient Consent

The PPMI study is registered with ClinicalTrials.gov (NCT01141023). All PPMI sites received approval from an ethics committee on human experimentation before study initiation. Written informed consent for research was obtained from all individuals participating in the study.

Genotyping and SNP Selection

All PPMI samples were genotyped using the Illumina Immunochip and NeuroX. Before association analyses. All samples and genotypes underwent stringent quality controls (QC). The NeuroX array underwent the same QC steps as the Immunochip but removed variants based on the Minor Allele Frequency (MAF)[11]. For SNP selection, we extracted *GCH1* RS026 genotype data (C/C, C/T, T/T).

Magnetic Resonance Imaging (MRI) Acquisition and Pre-Processing

T1-weighted MRI scans were acquired from September 2013 to January 2014. Acquisition parameters are detailed in the data set (http://www.ppmi-info.org/wpcontent/uploads/2015/03/PPMI-MRI-Operations-Manual-V7-0-20JAN2015-FINAL.pdf). As described (Willette, et al., 2013), baseline T1-weighted images were preprocessed using the SPM12 "New Segmentation" tool to extract modulated gray matter (GM) volume maps, followed by smoothing maps with a 8mm Gaussian kernel. GM images were subsequently used in voxel-wise analyses.

CSF collection and biomarker measurement

CSF and serum sample collection and processing are described in the PPMI protocol manual (http://www.ppmi-info.org/). Baseline CSF samples were obtained after an overnight fast.

DAT Scan

123I-ioflupane SPECT DAT scans were acquired from September 2013 to January 2014. The regional 123I-Ioflupane binding values for bilateral putamen and bilateral caudate regions were derived for PD patients and controls.

Clinical, Cognitive and Neuropsychological Assessments

All clinical assessments were conducted at the PPMI screening visit. As described(The Lancet, 2011), various motor, neuropsychological, and cognitive tests were performed, including the: Movement Disorders Society-UPDRS (MDS-UPDRS) to measure both non-motor and motor activities of daily living (Goetz, et al., 2007); the Symbol Digit Modalities Test (SDMT)(Sheridan, et al., 2006) to gauge attention, visual scanning, and motor speed; and the State-Trait Anxiety Inventory (STAI)(CD, 1983) to measure emotional state anxiety, which is a common PD phenotype (Richard, et al., 1996).

Statistical Analysis

All analyses were conducted using SPSS 23 (IBM Corp., Armonk, NY) or SPM12 (http://www.fil.ion.ucl.ac.uk/spm/software/spm12/). We assessed the frequencies of the alleles among individuals with PD compared to controls using $\chi 2$ and calculated odds ratios with

logistic regression models. For all other non-voxelwise analyses, linear mixed effects models tested the association between RS026 genotype, baseline diagnosis, and their interaction on outcomes of interest. In separate exploratory analyses, we also examined how age modified these associations using genotype x age interactions. To restrict type 1 error, the main effect and interaction were tested in a single model. Covariates included age at baseline, sex, body mass index (BMI), and education. Outcomes included PD risk and age of diagnosis; bilateral DAT uptake in caudate and putamen; MDS-UPDRS scores; neuropsychological performance; CSF biomarkers including α-synuclein, Aβ1-42, t-tau and p-tau181; and metabolic factors including LDL cholesterol and total cholesterol, which exert roles in DAT uptake (Jones, et al., 2012). All indices included in these analyses were tested for the statistical differences between genotypes. A two-tailed t-test was performed between the data from the groups. Alpha was set at .05 for all tests.

To correct for type 1 error in non-voxelwise analyses, as described previously (Willette, et al., 2015b), Holm-Bonferroni correction (Holland and Copenhaver, 1987) was used for each set of analyses. This closed test procedure maintains a family-wise Alpha = 0.05 by requiring unadjusted P values of 0.05 divided by x, x being the number of null hypotheses tested. For 4 cognitive tests, for example, P values of .0125, .025, .0375, and .050 are successively needed among any test to proceed with testing in the closed set. For sets that were in part or completely not robust to Holm-Bonferroni correction, a less strict form of correction was used. Specifically, omnibus testing using MANCOVA (Wilkinson, 1975) incorporating all dependent variables of the set was conducted, where genotype main effect or Genotype by diagnosis interaction significance allowed further testing of all outcomes as follow-up tests. Follow up included Age, Sex, Education and BMI as covariates. Follow up analyses included a covariate to capture the time of disease progression for each subjects before they were examined at the baseline/screening visit which are presented in Supplemental Table 2. A Family-wise Alpha of .05 is maintained using such an approach (Wilkinson, 1975).

For voxel-wise analysis, 2nd-level mixed models tested main effects and the interaction of RS026 genotype and PD diagnosis on regional GM volume, controlling for age, sex, BMI, and education. The voxel and cluster thresholds were set at $P < .005$ (uncorrected) and $P < .05$ (corrected). Results were considered significant at the cluster level. We minimized type 1 error by first using a GM threshold of 0.2 to ensure that voxels with <20% likelihood of being GM were not analyzed. Next, Monte Carlo simulations in AlphaSim (http://afni.nimh.nih.gov/afni/doc/manual/alphasim) were used to estimate that 352 contiguous voxels were needed for such a cluster to occur at $P < 0.05$. Previous reports have similarly examined voxel-wise associations with metabolic factors and regional GM atrophy (Willette, et al., 2015a).

Results

Data Summary

Clinical, demographic, and CSF data are presented in Table 1. There were no differences between genotypes in BMI, years of education, age at baseline or the percentage of individuals diagnosed with PD. The two subjects with the youngest age of PD diagnosis (16

& 24) both were carriers for a T allele. We did not observe any significant difference in genotype distribution between PD patients and controls even after stratifying by early PD onset (<50 years) versus late PD (>50) (Schrag and Schott, 2006). As expected, there were significant differences between genotypes in baseline MDS-UPDRS scores. Genotype distributions are in Figure 1C.

Age of Onset and Risk for PD

Having at least one risk T allele was associated with earlier age of PD diagnosis (P=0.003). Specifically, the mean age at PD onset of individuals with 1 or more T alleles was 47.2 ± 3.4 years, while C allele homozygotes had a mean age of onset at 52.1 ± 1.7 years (Figure 1 A). Allelic frequencies did not differ among PD patients and controls. However, regression models showed that individuals with a T allele were at a 23% increased risk of developing PD (Wald=4.224, P=0.048).

Dopamine imaging

In the 325 subjects with DAT imaging, those with at least 1 T allele showed significantly lower (F=11.27, P < 0.001) dopamine uptake in both bilateral caudate and putamen compared to the CC genotype. Figure 2 illustrates a gene-dose association (CC, CT, TT) corresponding to less striatal dopamine uptake in bilateral putamen (F=9.325, P=0.002) and caudate (F=13.239, P<0.001). Correlation analyses revealed significant correlations between dopamine uptake and UPDRS I (R= -0.38, P<0.001) and UPDRS II (R= -0.63 P<0.001) scores (Supplementary Figure 1).

CSF Biomarkers

We next examined the effect of GCH1 genotype in 334 subjects with CSF data on α-synuclein and tau species. For α-synuclein, patients with the T/C or T/T genotype had significantly higher CSF concentrations (P=0.016) than patients homozygous for the common C genotype. Median CSF α-synuclein levels in CC genotypes were 80% compared with patients having two T risk alleles. Furthermore, there was a striking gene-dose (CC, CT, TT) increase in CSF α-synuclein in controls (Figure 3A –1898 ± 520) and PD (Figure 3B - 2016 ± 791). This result was unexpected, given the gene-dose effect on lower striatal DAT uptake and that α-synuclein degrades nigrostriatal cells (Goedert, 2001).

We likewise assessed the relationship between RS026 genotype with tau protein concentrations. While genotype main effects were non-significant, a genotype by PD diagnosis interaction (F=5.312, P=0.029) showed that controls carrying T alleles had higher total tau (P=0.034) and phosphorylated tau (P=0.019), whereas individuals with PD did not demonstrate any difference between risk allele status and tau species (Figure 3 C & D).

Neuropsychological and UPDRS scores

Among neuropsychological indices (Figure 5), a main effect for the multivariate omnibus [F=8.697, P=0.001] with follow-up tests showed that T carriers performed significantly worse on: the SDM (P<0.001), LNS (P=0.003), and both STAI (P=0.038) and the MDS-UPDRS I anxiety sub-score (P=0.042), which assess processing speed, verbal and visuo-spatial working memory, and anxiety. A similar omnibus main effect [F=5.25, P= 0.002] was

found for UPDRS I and II. For motor (P=0.046) and non-motor (P=0.04) activities of daily living, as assessed by the MDS-UPDRS II, RS026 T allele genotype was related to worse motor function (P=0.046, Supplemental Figure 1). Supplemental analyses including disease duration as a covariate strengthen these results, revealing that carries perform worse on UPDRS III when accounting for this difference in time with disease which is presented in Supplemental Figure 2.

Metabolic Biomarkers

In 278 subjects, based on a main effect for the multivariate omnibus test [F=4.43, P=0.005], minor allele carriers had higher total serum cholesterol (P=0.042) and LDL cholesterol (P=0.029) (Supplementary Figure 2). Total cholesterol levels correlated with striatal dopamine uptake (p=0.022) but mediation analyses revealed that total cholesterol only mediated the genotype effects on DAT in PD subjects but not controls.

Regional grey matter volume

Finally, voxel-based morphometry was used to regress RS026 genotype against regional GM at baseline in all subjects. There was no significant RS026 genotype main effect or interaction with diagnosis on regional GM volume. Restriction to striatum with a very liberal voxel threshold ($p < .05$) also yielded no significant clusters.

Aging Interactions with RS026 Genotype

Across all subjects, models testing Age by GCH1 genotype interaction effects on each of the main outcomes demonstrated differences among risk allele carriers according to their age. A genotype by age interaction (P=0.041, F= 2.783) indicated differences among Age of Onset based on age. Splitting the cohort to compare subjects under 50 (N=156) to subjects above age 50 (n=266) indicated that individuals younger than 50 drove effects on PD risk and Age of Onset. Specifically, regression models showed that T allele carries under 50 were at a 46% increased risk of developing PD (Wald=5.134, P=0.002) but those over the age of 50 were not at an increased risk (P=0.83). Carriers of a T allele under 50 showed a mean age of onset at 33.8 ± 5.97 years, and non-carriers had a mean age of onset at 45.78 ± 4.37 years (Figure 1 B).

An exploratory analysis to view gene-dose effects, showed carriers of two risk alleles (N=6) were compared to those with 1 risk allele (n=56) and non-carriers (n=54). Carriers of two risk alleles showed marked decreases in age of onset compared to the other two groups at 28.9 ± 6.38 years. We warrant caution regarding the allele-dose effect, due to sample size. An age by GCH1 interaction (P=0.004, F =7.83) indicated differences among T allele carriers according to their age on DAT scans. Figure 4 A indicates across all subjects that carriers of a risk allele initially have lower dopamine uptake than non-carriers, and Figure 4 B indicates difference became more significant for subjects under 50 years old. Another age by GCH1 interaction (F=5.332, P=0. 0.011) indicated differences among T allele carriers according to their age on UPDRS I scores. The same effects were noted for UPDRS II (F=2.973, P=0. 0.047) and UPDRS Total scores (F=3.111, P=0. 0.0.43).

Splitting the cohort to compare subjects under 50 (N=116) to subjects above age 50 (n=306) again indicated the interaction effects were driven by individuals younger than 50. Carriers under 50 showed increased UPDRS I scores (P=0.002, F = 13.68), UPDRS II (P<0.001, F=15.43) & UPDRS Total scores, (P<0.001, F= 14.98). In those above 50 years of age, no significant associations were found for UPDRS total score, while UPDRS I and II were still significant (p=0.049, F=1.167).

Age by GCH1 genotype interaction effects on regional grey matter volume showed decreases in grey matter in three small clusters in the left and right postcentral sulcus and right temporal middle (Supplemental Table 1). Cholesterol showed a significant age by GCH1 genotype interaction (P=0.035, F=3.075), whereby splitting the cohort showed again younger carriers had higher total cholesterol (P=0.024, F= 3.678) compared to non-carriers. Carriers above 50 also had significantly higher cholesterol than non-carriers (F=2.173, P=0.046). Neuropsychological factors and CSF Biomarkers were similarly tested and did not show any significant interaction effects. Follow up analyses including an additional covariate to take into account the time subjects were diagnosed with PD before enrolling are presented in Supplemental table 2.

Discussion

Several studies examining dopamine dysregulation in PD and DRD (Lewthwaite, et al., 2015, Mencacci, et al., 2014) suggest GCH1 deficiency may contribute to the pathologic features of PD through dopamine deficiency. This is the first study to assess whether *GCH1* variants explained PD age of onset, striatal DAT uptake, and both clinical and cognitive markers of PD. Our main findings include a gene-dose response of the minor allele contributing to an earlier age of onset, markedly lower DAT uptake, and worse motor performance and increased α-synuclein. This is also the first study examining the effects of RS026 in subjects of European descent, which comprise the majority of Americans who develop PD.

RS026 T allele carriers developed PD 5 years earlier, in concert with the T allele predicting a 23% increased risk of developing PD where these results were stronger in younger subjects.

Here, the minor allele carriers had robustly lower DAT uptake in both caudate and putamen, similar to other studies comparing dopaminergic findings at other SNPs in GCH1 variants (Furukawa, et al., 1998, Takahashi, et al., 1994). While the exact mechanism is still unknown, our data show that GCH1 variants alter several biological indices which may increase the vulnerability of the neurons to ageing, resulting in lower striatal dopamine levels in GCH1 mutation carriers and establishing a lower threshold of nigral cell loss to induce clinical symptoms. Recent evidence with in vitro studies show α-synuclein regulates DAT function, specifically controlling the rapid shuttling of the transporter to and from the cell membrane and its availability to take up dopamine (Fountaine and Wade-Martins, 2007). This SNP appears to exert a functional influence on dopamine uptake in striatum, which may be explained by the striatum's dense population of dopamine receptors (Gerfen, et al., 1995). In concert with cognitive deficits seen in minor allele carriers, changes in dopamine

signaling may affect cognitive function in PD due to dopamine regulating cognitive functions in prefrontal cortex (PFC)(Alexander, et al., 1986),(Nieoullon, 2002). Similar signaling dysfunction of dopamine may also explain greater anxiousness and mood perturbations (Hosenbocus and Chahal, 2012).

Unexpectedly, subjects with one or more copies of the T risk allele showed higher CSF α-synuclein concentrations, which could point to increased α-synuclein in the CSF of patients with PD (Hansson, et al. 2014). Since α-synuclein plays a role in the regulation of dopamine biosynthesis (Perez, et al., 2002), it may also induce selective damage in dopamine neurons of PD patients (Lee, et al., 2011). The lack of differences in regional gray matter between GCH1 genotypes, even after very liberal thresholding, points to a possible alternative mechanism whereby reactive oxygen species (ROS) potentiate dopaminergic neuronal loss in the PD brain, which results from disrupted oxidation homeostasis impacting dopamine metabolism (Jenner and Olanow, 2006).

C/T and T/T carriers had worse motor speed and output, as well as well worse performance on tests of visuo-spatial working memory, set-shifting, attention and tracking, and cognitive flexibility. Other studies administering BH4 showed subjects had improved working memory in individuals with PKU (Christ, et al., 2013), where our study provides evidence that a decrease in metabolic BH4 through less GCH1 activity could also lead to a decrease in working memory. Tanaka et al. noted that individuals who received BH4 supplementation earlier in life performed better on executive function tasks than individuals who received treatment later in life, pointing to BH4's role in executive function (Tanaka, et al., 2007). As minor allele carriers also have lower dopamine uptake, these results are in line with other studies showing how dopamine impacts cognition and executive function (Zhang, et al., 2015).

GCH1 variants in PD patients showed increased cholesterol in a striking allele-dependent manner from CC, CT and TT genotypes, pointing to possible vascular differences between genotypes. Antoniades et al. examined how low levels of circulating plasma BH4 disrupted systemic oxidative homeostasis through increased oxidation of LDL particles (Antoniades, et al., 2008) and higher concentrations of superoxide in the vasculature. These values were still well within the normal ranges but could affect the maintenance of redox potential in the neurochemical environment. The interrelationship between these various mechanisms and neurodegeneration in PD could be interpreted as a feed-forward scenario where primary insults lead to increased oxidative stress, which damages key cellular pathogenic proteins and disrupts lipid membranes that in turn cause more ROS production.

The noteworthy interaction of these effects with age provides evidence supporting the role for GCH1 as a risk factor for PD, specifically early onset PD. Importantly, that these decreases in the dopamine-synthesizing enzyme system in patients with PD could be due to GCH1 deficiency, predisposing people to degeneration and cell death in the substantia nigra (Parker Jr, et al., 2008). While the mechanism is uncertain, biochemical evidence of GCH1 deficiency and reduced dopamine production has been reported in asymptomatic carriers of GCH1 mutations (Furukawa, et al., 1998, Takahashi, et al., 1994). We speculate that GCH1 deficiency and the consequent dopamine deficiency could directly facilitate nigral cell death,

whereby normal levels of BH4 and dopamine may exert a protective role on the survival of nigral neurons. It is also possible that the reduced striatal basal dopamine levels found in GCH1 mutation carriers may lower the threshold of nigral cell loss before parkinsonian symptoms are exhibited or even increase nigral cell vulnerability to environmental and other genetic factors, favoring degeneration over time.

Limitations of our study should be noted. The study sample size was modest, and the research was designed to evaluate differences among genotypes which would benefit from a larger sample. Since this is a cross-sectional study using baseline data from the initial 342 subjects from the PPMI study cohort, this limited our ability to determine the relationships between these genotypes on longitudinal CSF biomarkers and disease progression. One limitation of this dataset is the variation in time subjects have been diagnosed with PD before enrolling in the study, which was accounted for in supplemental analyses. Additionally, another limitation is our study did not evaluate other genetic factors which have yet to be identified, or histological samples from these patients. However, these limitations will be resolved in future PPMI analyses These results provide evidence for GCH1's role in early onset PD development, especially when considering the clinical applicability of determining who may be at risk for developing PD, but may have limited applicability to older Caucasian PD cases. Lastly, we cannot exclude that other yet unrecognized cellular pathways, not related to dopamine synthesis, may be disrupted by *GCH1* and BH4 deficiency influencing PD progression.

Conclusion

This is the first study to demonstrate a significant association of GCH1 RS026 with factors of PD in a European population, whose results would contribute significantly to the future meta-analyses evaluating PD development utilizing genetic loci. Overall, this PPMI data suggests that GHC1 functional variants represent rare variant risk factors for clinical PD that whose effects are significantly affected by aging. Given GCH1's reported function in BH4 and dopamine synthesis, it is likely for GCH1 variants to play a role in dopamine uptake and motor output while also affecting cognitive function and affect like anxiousness. These genetic variants could lead to changes in systemic oxidation, potentially harming downstream regulators of PD development. This article suggests that RS11158026 affects early PD risk through altered dopamine uptake but aging abates these effects. Future studies should screen for GCH1 variants in subjects with familial PD, and in younger subjects with parkinsonism features. Future post-mortem histological data on affected individuals, particularly those with the PD phenotype, may shed more light on the mechanisms by which GCH1 carriers develop PD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This study was funded by Iowa State University and NIH AG047282. Neither funding source had any involvement in the report. Data used in the preparation of this article were obtained from the Parkinsons Progression Markers

Initiative (PPMI) database (www.ppmi-info.org/data), a public-private partnership funded by the Michael J. Fox Foundation for Parkinsons Research and funding partners, including Abbott, Avid Radiopharmaceuticals, Biogen Idec, Bristol-Myers Squibb, Covance, lan, GE Healthcare, Genentech, GSK-GlaxoSmithKline, Lilly, Merck, MSD-Meso Scale Discovery, Pfizer, Roche, and UCB (www.ppmi-info.org/fundingpartners

References

1. Allen Reish HE, Standaert DG. Role of α-synuclein in inducing innate and adaptive immunity in Parkinson disease. Journal of Parkinson's disease. 2015; 5:1–19.
2. Lesage S, Brice A. Parkinson's disease: from monogenic forms to genetic susceptibility factors. Hum Mol Genet. 2009; 18:R48–59. [PubMed: 19297401]
3. Kurian MA, Gissen P, Smith M, Heales S Jr, Clayton PT. The monoamine neurotransmitter disorders: an expanding range of neurological syndromes. Lancet Neurol. 2011; 10:721–733. [PubMed: 21777827]
4. Nalls MA, Pankratz N, Lill CM, et al. Large-scale meta-analysis of genome-wide association data identifies six new risk loci for Parkinson's disease. Nat Genet. 2014; 46:989–993. [PubMed: 25064009]
5. Goedert M. Alpha-synuclein and neurodegenerative diseases. Nat Rev Neurosci. 2001; 2:492–501. [PubMed: 11433374]
6. Kang JH, Mollenhauer B, Coffey CS, et al. CSF biomarkers associated with disease heterogeneity in early Parkinson's disease: the Parkinson's Progression Markers Initiative study. Acta Neuropathol. 2016; 131:935–949. [PubMed: 27021906]
7. Jones KT, Zhen J, Reith ME. Importance of cholesterol in dopamine transporter function. J Neurochem. 2012; 123:700–715. [PubMed: 22957537]
8. The Lancet N. Biomarker promise for Parkinson's disease. Lancet Neurol England. 2010:1139.
9. Emre M, Aarsland D, Brown R, et al. Clinical diagnostic criteria for dementia associated with Parkinson's disease. Movement Disorders. 2007; 22:1689–1707. [PubMed: 17542011]
10. Hwu WL, Chiou YW, Lai SY, Lee YM. Dopa-responsive dystonia is induced by a dominant-negative mechanism. Ann Neurol. 2000; 48:609–613. [PubMed: 11026444]
11. The Parkinson Progression Marker Initiative (PPMI). Prog Neurobiol. 2011; 95:629–635. [PubMed: 21930184]
12. Willette AA, Xu G, Johnson SC, et al. Insulin Resistance, Brain Atrophy, and Cognitive Performance in Late Middle–Aged Adults. Diabetes Care. 2013; 36:443–449. [PubMed: 23069842]
13. Goetz CG, Fahn S, Martinez-Martin P, et al. Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS): Process, format, and clinimetric testing plan. Mov Disord. 2007; 22:41–47. [PubMed: 17115387]
14. Sheridan LK, Fitzgerald HE, Adams KM, et al. Normative Symbol Digit Modalities Test performance in a community-based sample. Archives of Clinical Neuropsychology. 2006; 21:23–28. [PubMed: 16139470]
15. CD, S. Manual for the State-Trait Anxiety Inventory. Gorsuch, RLLR.; Vagg, PR.; Jacobs, GA., editors. Consulting Psychologists Press, Inc; Palo Alto, CA: 1983.
16. Richard IH, Schiffer RB, Kurlan R. Anxiety and Parkinson's disease. J Neuropsychiatry Clin Neurosci. 1996; 8:383–392. [PubMed: 9116473]
17. Willette AA, Modanlo N, Kapogiannis D. Insulin resistance predicts medial temporal hypermetabolism in mild cognitive impairment conversion to Alzheimer disease. Diabetes. 2015; 64:1933–1940. [PubMed: 25576061]
18. Holland BS, Copenhaver MD. An Improved Sequentially Rejective Bonferroni Test Procedure. Biometrics. 1987; 43:417–423.
19. Wilkinson L. Response variable hypotheses in the multivariate analysis of variance. Psychological Bulletin. 1975; 82:408–412.
20. Willette AA, Bendlin BB, Starks EJ, et al. ASsociation of insulin resistance with cerebral glucose uptake in late middle–aged adults at risk for alzheimer disease. JAMA Neurology. 2015; 72:1013–1020. [PubMed: 26214150]

21. Mencacci NE, Isaias IU, Reich MM, et al. Parkinson's disease in GTP cyclohydrolase 1 mutation carriers. Brain. 2014; 137:2480–2492. [PubMed: 24993959]
22. Lewthwaite AJ, Lambert TD, Rolfe EB, et al. Novel GCH1 variant in Dopa-responsive dystonia and Parkinson's disease. Parkinsonism & Related Disorders. 2015; 21:394–397. [PubMed: 25634433]
23. Furukawa Y, Lang AE, Trugman JM, et al. Gender-related penetrance and de novo GTP-cyclohydrolase I gene mutations in dopa-responsive dystonia. Neurology. 1998; 50:1015–1020. [PubMed: 9566388]
24. Takahashi H, Levine RA, Galloway MP, Snow BJ, Calne DB, Nygaard TG. Biochemical and fluorodopa positron emission tomographic findings in an asymptomatic carrier of the gene for dopa-responsive dystonia. Ann Neurol. 1994; 35:354–356. [PubMed: 8122887]
25. Fountaine TM, Wade-Martins R. RNA interference-mediated knockdown of alpha-synuclein protects human dopaminergic neuroblastoma cells from MPP(+) toxicity and reduces dopamine transport. J Neurosci Res. 2007; 85:351–363. [PubMed: 17131421]
26. Gerfen CR, Keefe KA, Gauda EB. D1 and D2 dopamine receptor function in the striatum: coactivation of D1- and D2-dopamine receptors on separate populations of neurons results in potentiated immediate early gene response in D1-containing neurons. J Neurosci. 1995; 15:8167–8176. [PubMed: 8613751]
27. Alexander GE, DeLong MR, Strick PL. Parallel organization of functionally segregated circuits linking basal ganglia and cortex. Annu Rev Neurosci. 1986; 9:357–381. [PubMed: 3085570]
28. Nieoullon A. Dopamine and the regulation of cognition and attention. Prog Neurobiol. 2002; 67:53–83. [PubMed: 12126656]
29. Hosenbocus S, Chahal R. A review of executive function deficits and pharmacological management in children and adolescents. J Can Acad Child Adolesc Psychiatry. 2012; 21:223–229. [PubMed: 22876270]
30. Hansson O, Hall S, Öhrfelt A, et al. Levels of cerebrospinal fluid α-synuclein oligomers are increased in Parkinson's disease with dementia and dementia with Lewy bodies compared to Alzheimer's disease. Alzheimer's Research & Therapy. 2014; 6(3):25.
31. Perez RG, Waymire JC, Lin E, Liu JJ, Guo F, Zigmond MJ. A role for alpha-synuclein in the regulation of dopamine biosynthesis. J Neurosci. 2002; 22:3090–3099. [PubMed: 11943812]
32. Lee H-J, Baek SM, Ho D-H, Suk J-E, Cho E-D, Lee S-J. Dopamine promotes formation and secretion of non-fibrillar alpha-synuclein oligomers. Exp Mol Med. 2011; 43:216–222. [PubMed: 21415592]
33. Jenner P, Olanow CW. The pathogenesis of cell death in Parkinson's disease. Neurology. 2006; 66:S24–36. [PubMed: 16717250]
34. Christ SE, Moffitt AJ, Peck D, White DA. The effects of tetrahydrobiopterin (BH4) treatment on brain function in individuals with phenylketonuria. Neuroimage Clin. 2013; 3:539–547. [PubMed: 24371792]
35. Tanaka Y, Kato M, Muramatsu T, et al. Early initiation of L-dopa therapy enables stable development of executive function in tetrahydrobiopterin (BH4) deficiency. Developmental Medicine & Child Neurology. 2007; 49:372–376. [PubMed: 17489812]
36. Zhang T, Mou D, Wang C, et al. Dopamine and executive function: Increased spontaneous eye blink rates correlate with better set-shifting and inhibition, but poorer updating. Int J Psychophysiol. 2015; 96:155–161. [PubMed: 25913095]
37. Mattsson N, Insel P, Tosun D, et al. Effects of Baseline CSF α-Synuclein on Regional Brain Atrophy Rates in Healthy Elders, Mild Cognitive Impairment and Alzheimer's Disease. PLoS ONE. 2014; 8:e85443.
38. Antoniades C, Shirodaria C, Van Assche T, et al. GCH1 Haplotype Determines Vascular and Plasma Biopterin Availability in Coronary Artery Disease: Effects on Vascular Superoxide Production and Endothelial Function. Journal of the American College of Cardiology. 2008; 52:158–165. [PubMed: 18598896]
39. Parker WD Jr, Parks JK, Swerdlow RH. Complex I deficiency in Parkinson's disease frontal cortex. Brain Research. 2008; 1189:215–218. [PubMed: 18061150]

Author Contributions

Joseph Webb researched data, wrote manuscript, reviewed/edited manuscript, contributed to discussion.

Auriel Willette reviewed/edited manuscript, contributed to discussion.

Auriel Willette takes full responsibility for the data, the analyses and interpretation, and the conduct of the research, that the author has full access to all of the data, and that the author has the right to publish any and all data separate and apart from any sponsor.

Author Disclosures

Auriel Willette - Reports no disclosures

Joseph Webb - Reports no disclosures

Appendix

1. All authors must disclose

(a) Any actual or potential conflicts of interest including any financial, personal or other relationships with other people or organizations within three years of beginning the work submitted that could inappropriately influence (bias) their work. Examples of potential conflicts of interest which should be disclosed include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/ registrations, and grants or other funding. If there are no actual or potential conflicts of interest, please state this. Should a significant conflict of interest be present, the Editors reserve the right to reject the article on that basis.

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(b)Whether any author's institution has contracts relating to this research through which it or any other organization may stand to gain financially now or in the future.

There is no contract relating to this research and no other organization may stand to gain financially now or in the future through this research.

(c) Any other agreements of authors or their institutions that could be seen as involving a financial interest in this work.

Auriel Willette - Reports no disclosures

Joseph Webb - Reports no disclosures

2. Please disclose sources of financial support related to the manuscript being submitted

This study was funded by Iowa State University and NIH AG047282. Neither funding source had any involvement in the report. Data used in the preparation of this article were obtained from the Parkinsons Progression Markers Initiative (PPMI) database (www.ppmi-info.org/data), a public-private partnership funded by the Michael J. Fox Foundation for Parkinsons Research and funding partners, including Abbott, Avid Radiopharmaceuticals, Biogen Idec, Bristol-Myers Squibb, Covance, lan, GE Healthcare, Genentech, GSK-GlaxoSmithKline, Lilly, Merck, MSD-Meso Scale Discovery, Pfizer, Roche, and UCB (www.ppmi-info.org/fundingpartners).

3. Please verify that the data contained in the manuscript being submitted have not been previously published, have not been submitted elsewhere and will not be submitted elsewhere while under consideration at Neurobiology of Aging

The data contained in the manuscript being submitted have not been previously published, as there have only been 2 studies looking at this SNP in Chinese and Tiawanese cohorts. This data has not been submitted elsewhere and will not be submitted elsewhere while under consideration at Neurobiology of Aging.

4. When applicable, provide statements verifying that appropriate approval and procedures were used concerning human subjects and animals

The PPMI study is registered with ClinicalTrials.gov (NCT01141023). All PPMI sites received approval from an ethics committee on human experimentation before study initiation. Written informed consent for research was obtained from all individuals participating in the study.

5. Please verify that all authors have reviewed the contents of the manuscript being submitted, approve of its contents and validate the accuracy of the data

Joseph Webb researched data, wrote manuscript, reviewed/edited manuscript, contributed to discussion. Auriel Willette reviewed/edited manuscript, contributed to discussion. Auriel Willette takes full responsibility for the data, the analyses and interpretation, and the conduct of the research, that the author has full access to all of the data, and that the author has the right to publish any and all data separate and apart from any sponsor. All authors have reviewed the contents of the manuscript being submitted, approve of its contents and validate the accuracy of the data

Highlights

- Age alters the impact of GCH1 mutation carriers on clinical Parkinsonian features.
- Carriers show increased risk, earlier age of onset and lower striatal DAT uptake.
- Carriers showed worse anxiety, executive function and motor function.
- Carriers <50 years old had increased risk where those >50 had no increased risk.
- Mutations in GCH1 may contribute to multiple pathologic features of PD

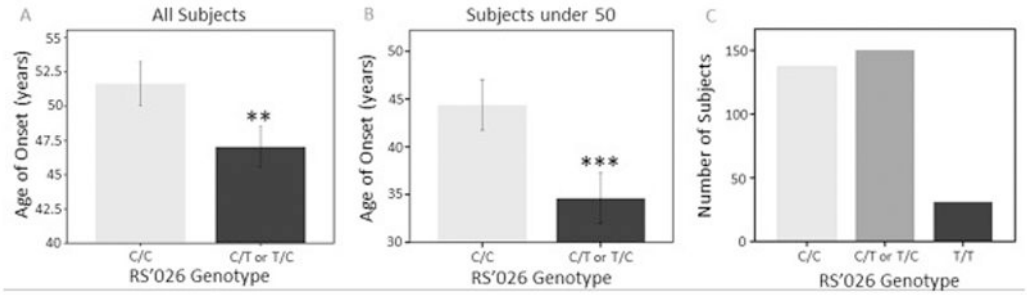

Figure 1.

The relationship between RS026 genotype and mean age of Parkinson's disease onset in A) All subjects B) Subjects under 50 years of age. **=P<0.01 C) Genotype distribution across all subjects

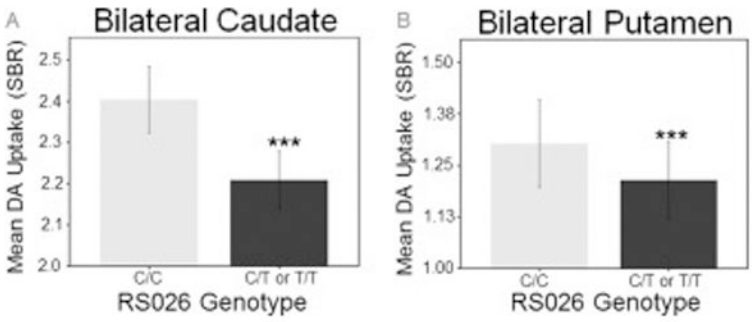

Figure 2.

The relationship between RS026 genotype and bilateral dopamine uptake in A) caudate; and B) putamen, as indicated by DAT-SPECT imaging. DA = Dopamine, SBR = Specific Binding Ratio. ***=P<0.001.

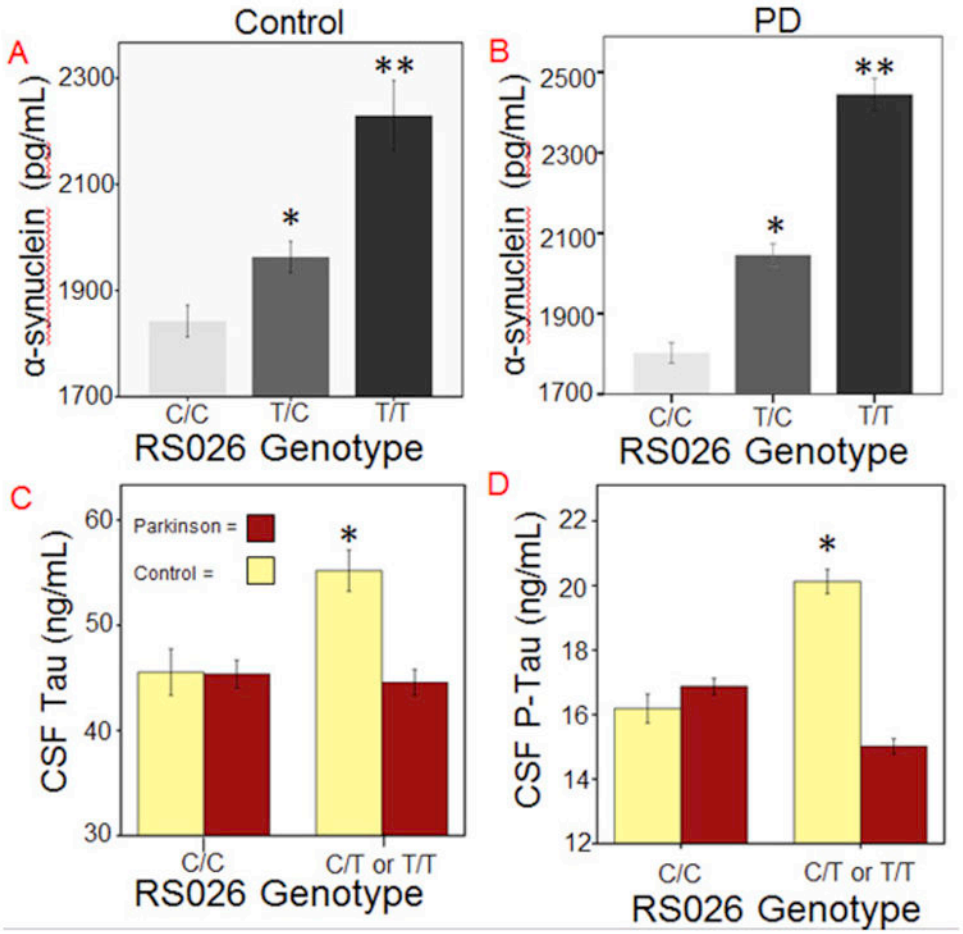

Figure 3.

The relationship between RS026 genotype and A) α-synuclein in Controls; B) α-synuclein in PD C) CSF Tau; and D) Phosphorylated tau; the "red" color indicates PD subjects and the "yellow" color indicates healthy controls in C & D. *=P<0.05.

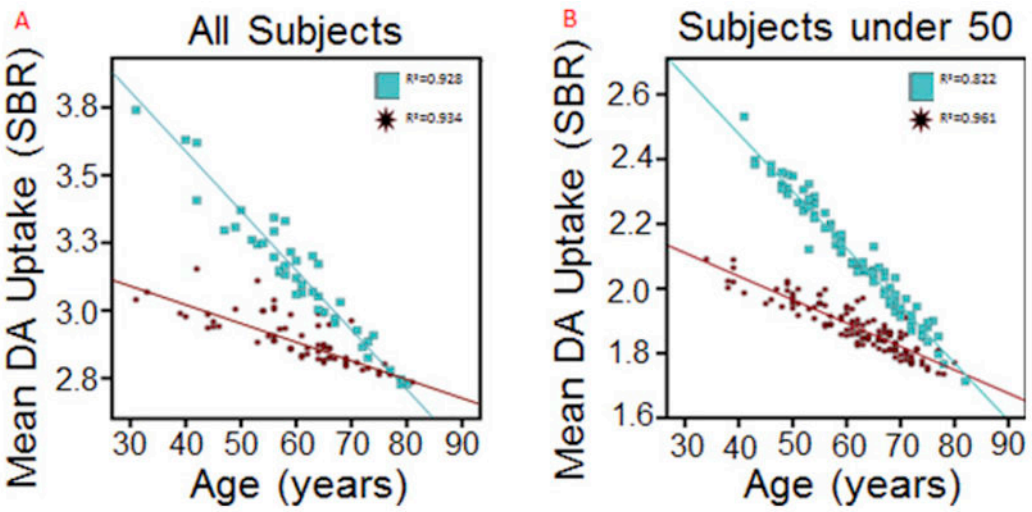

Figure 4.

The interaction between RS026 genotype and age plotted against dopamine uptake in both controls and PD for: A) All Subjects B) Subjects under 50. SBR = signal binding ratio

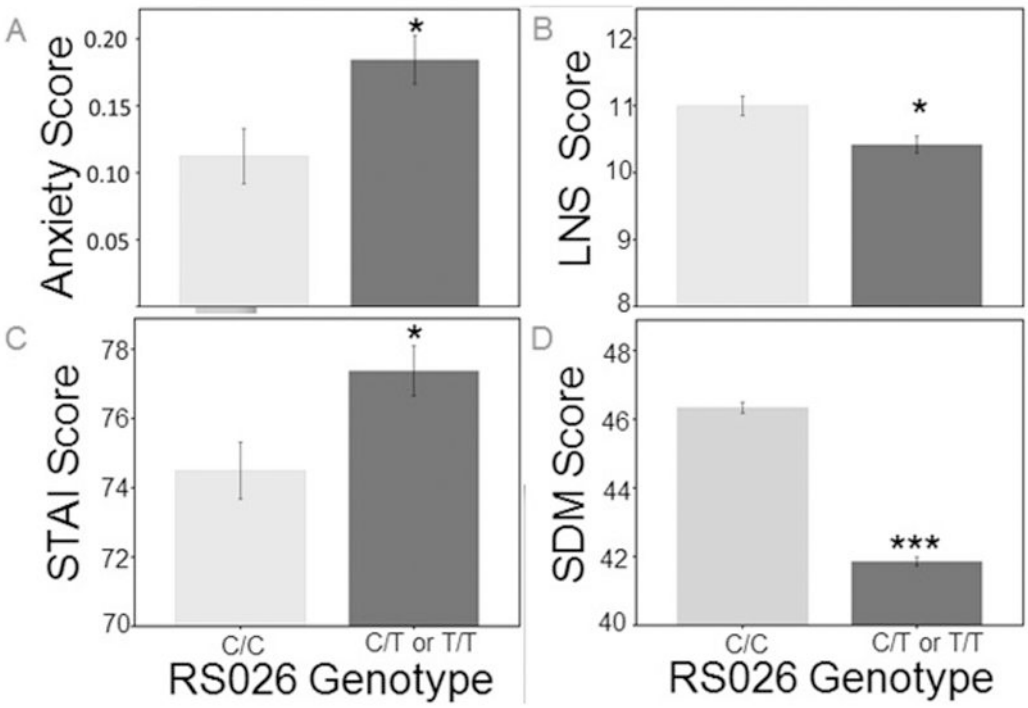

Figure 5.

The relationship between RS026 genotype and neuropsychological scores. A) Anxiety Scores; B) Letter Number Sequencing Scores; C) State Trait Anxiety Inventory Score; D) Symbol Digit Modality Score. *, **, ***=P<0.05, 0.01, or 0.001.

Table 1

	C/C (n=137)	T/C or T/T (n=185)
Age at baseline (years)	61.2(9.8)	61.3 (12.2)
Gender (% male)	69%	65%
Education (years)	15.5 ± 2.8	15.8 ± 2.9
BMI	26.6 ± 5.1	27.4 ± 4.7
Parkinsons disease %	68%	69%
Mean Bilateral Putamen DAT	1.3 ± 0.16	1.1 ± 0.12***
Mean Bilateral Cuadate DAT	2.4 ± 0.7	2.2 ± 0.7***
α-Syn (ng/ml)	1883.6 ± 74.4	2103.1 ± 93.2***
Apathy Score	0.09 ± 0.3	0.18 ± 0.4
Anxiety Sub Score	0.11 ± 0.04	0.19 ± 0.03*
UPDRS - I Score	1.85 ± 0.1	2.33 ± 0.3*
UPDRS - II Score	4.77 ± 1.1	6.25 ± 1.5*
UPDRS - III Score	14.2 ± 11.9	14.2 ± 12.0
STAI Score	73.51 ± 1.1	77.39 ± 1.3*
LNS Score	10.96 ± 0.1	10.37 ± 0.05**
SDM Score	46.2 ± 0.6	41.9 ± 0.5***

DAT, Dopamine Transporter Uptake; BMI, Body Mass Index; STAI, State-Trait Anxiety Inventory; α-Syn, Alpha Synuclein; LNS, Letter Number Sequencing; SDM, Symbol Digit Modality; UPDRS, Unified Parkinson's Disease Rating Scale. Values are Mean± SD.

* P < 0.05

** P < .01

*** P < .001.