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SCARB2 variants and glucocerebrosidase activity in Parkinson’s disease

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Mutations in glucocerebrosidase (GBA) are a common risk factor for Parkinson’s disease (PD). The scavenger receptor class B member 2 (SCARB2) gene encodes a protein, lysosome membrane protein 2 (LIMP-2), that transports β-glucocerebrosidase (GCase) from the endoplasmic reticulum through the Golgi apparatus and endosomes to the lysosome. Homozygous mutations in SCARB2 cause a rare form of progressive myoclonic epilepsy, action myoclonus-renal failure. Affected patients have significantly reduced GCase activity (7 nmol/mg protein per h compared with 15 nmol/mg protein per h in controls), which is in the range observed for carriers of Gaucher disease (GBA heterozygotes). Two common single-nucleotide polymorphisms (SNPs), rs6812193 (refs 5, 6) and rs6825004 (intron in SCARB2), and rs68250047,8 (intron in SCARB2), have been shown to be associated with PD5–7,9 and Dementia with Lewy Bodies6 in several genetic studies, including large genome-wide association studies. Furthermore, given that homozygous mutations in SCARB2 are associated with reduced GCase activity,2 it is possible that these SNPs modify the risk for PD via modulation of GCase activity. In this study, we tested the hypotheses that (1) these SNPs would be associated with PD in a New York PD cohort; and (2) that protective variants are associated with higher GCase enzymatic activity as measured in dried blood spots when compared with carriers of the non-protective variant (e.g., if indeed rs6812193 is associated with lower PD risk via SCARB2 then carriers of the protective nucleotide, T, will have higher GCase activity than carriers of the C allele).

RESULTS

Eight hundred and twenty participants were genotyped for the SCARB2 SNPs, including 548 PD cases and 272 controls. Demographics and GBA and LRRK2 mutation status are presented in Table 1. The genotype and allele frequencies of rs6812193 and rs6825004 are presented in Table 2. Genotype at rs6825004 was not associated with PD status in our cohort, nor was it associated with GCase enzymatic activity (C/C genotype 11.75 μmol/l/h; C/T: 11.80 μmol/l/h; T/T: 12.02 μmol/l/h; P = 0.867). Genotype at rs6825004 was not associated with either PD status or GCase activity. In conclusion, our results support an association between SCARB2 genotype at rs6812193 and PD, but suggest that the increased risk is not mediated by GCase activity.

npj Parkinson’s Disease (2016) 2, 16004; doi:10.1038/npjparkd.2016.4; published online 10 March 2016

INTRODUCTION

Heterozygous mutations and variants in glucocerebrosidase (GBA) are present in 3–5% of individuals with Parkinson’s disease (PD).1,2 The scavenger receptor class B member 2 (SCARB2) gene encodes a protein, lysosome membrane protein 2 (LIMP-2), that transports β-glucocerebrosidase (GCase) to the lysosome. Two common SNPs in linkage disequilibrium with SCARB2, rs6812193 and rs6825004, have been associated with PD and Lewy Body Disease in genome-wide association studies. Whether these SNPs are associated with altered glucocerebrosidase enzymatic activity is unknown. Our objective was to determine whether SCARB2 SNPs are associated with PD and with reduced GCase activity. The GBA gene was fully sequenced, and the LRRK2 G2019S and SCARB2 rs6812193 and rs6825004 SNPs were genotyped in 548 PD patients and 272 controls. GCase activity in dried blood spots was measured by tandem mass spectrometry. We tested the association between SCARB2 genotypes and PD risk in regression models adjusted for gender, age, and LRRK2 G2019S and GBA mutation status. We compared GCase activity between participants with different genotypes at rs6812193 and rs6825004. Genotype at rs6812193 was associated with PD status. PD cases were less likely to carry the T allele than the C allele (OR = 0.71; P = 0.004), but GCase enzymatic activity was similar across rs6812193 genotypes (C/C: 11.88 μmol/l/h; C/T: 11.80 μmol/l/h; T/T: 12.02 μmol/l/h; P = 0.703). This result suggests that the protective variant associated with lower PD risk via SCARB2 then carriers of the protective nucleotide, T, will have higher GCase activity than carriers of the C allele.

Received 23 October 2015; accepted 9 January 2016
differential GCase activity (C/C: 11.88 μmol/l/h; C/T: 11.80 μmol/l/h; T/T: 12.02 μmol/l/h; P = 0.867. This comparison included PD cases and controls and excluded GBA and LRRK2 p.G2019S mutation carriers).

**DISCUSSION**

Our findings support previous reports that the genotype at rs6812193 SNP is associated with PD. This SNP, which is in linkage disequilibrium with the SCARB2 gene, was found in association with PD in two large multi-ethnic genome-wide association studies (OR = 0.84; P = 7.6 × 10^{-10} and OR = 0.907; P = 2.95 × 10^{-11})\(^5,^9\) and in a German association study (OR = 0.86; P = 0.02)\(^6\). However, the association was not replicated in a Chinese\(^10\) or a Greek cohort\(^11\), likely due to either population differences or insufficient power. Here, we confirm the association in a clinic based New York cohort, which consists of 40% AJ PD patients. An association between the SCARB2 gene and PD was hypothesized to be due to differential trafficking of GCase to the lysosome.\(^4\) In the current study we hypothesized that if indeed rs6812193 is associated with PD risk via SCARB2, then carriers of the protective nucleotide (T) should have higher GCase activity than carriers of the PD susceptibility allele (C). We have previously shown in dried blood spots that heterozygous GBA carriers have lower GCase activity than non-carriers (by ~ 33%), and that idiopathic PD cases have slightly lower GCase activity (by ~ 5%) than controls.\(^12\) Our current findings do not support the link between rs6812193 and PD through a mechanism of reduced

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**Table 1. Demographics, GBA and LRRK2 mutation status in PD cases and controls**

| Status | Controls (n = 272) | PD Cases (n = 548) | P-value for PD risk | OR for PD (95% CI)** |
|--------|-------------------|-------------------|---------------------|----------------------|
|        | N (%)             | GCase enzymatic activity μmol/l/h (s.d.) | N (%)             | GCase enzymatic activity μmol/l/h (s.d.) |                        |
| rs6812193 |                   |                   |                     |                      |                        |
| C/C    | 115 (42.3%)       | 11.97 (3.31)      | 273 (49.8%)         | 11.82 (3.16)         | 0.057 Reference        |
| C/T    | 123 (45.2%)       | 11.96 (2.90)      | 229 (41.8%)         | 11.70 (3.09)         | 0.52 (0.30–0.89); P = 0.018 |
| T/T    | 34 (12.5%)        | 12.07 (4.43)      | 46 (8.4%)           | 11.98 (3.10)         | 0.62 (0.36–1.07); P = 0.086 |
| C      | 353 (64.8%)       | 775 (70.7%)       | 0.0175              | 0.71 (0.56–0.90); P = 0.004** |
| T      | 191 (35.2%)       | 321 (29.3%)       |                      |                      |                        |
| rs6825004 |                 |                   |                     |                      |                        |
| C/C    | 125 (46.1%)       | 11.94 (3.35)      | 243 (44.4%)         | 11.63 (2.88)         | 0.562 Reference        |
| C/G    | 115 (42.4%)       | 11.90 (3.22)      | 229 (41.9%)         | 11.97 (3.42)         | 1.03 (0.74–1.45); P = 0.833 |
| G/G    | 31 (11.4%)        | 12.42 (3.33)      | 75 (13.7%)          | 11.76 (3.04)         | 0.79 (0.48–1.30); P = 0.788 |
| C      | 365 (67.3%)       | 715 (65.3%)       | 0.438               | 1.17 (0.93–1.47); P = 0.182** |
| G      | 177 (32.7%)       | 379 (34.7%)       |                      |                      |                        |

Abbreviations: CI, confidence interval; OR, odds ratio; PD, Parkinson’s disease.

**Table 2. Association between rs6812193 and rs6825004 genotypes and Parkinson’s disease**

- **OR calculated in models adjusted for age, gender, LRRK2 and GBA mutation status.**
- **There was no difference in GCase activity among the genotypes, either in controls or in PD cases. Values presented here after excluding GBA and LRRK2 p.G2019S carriers.**
- **Model included sex, age, rs6812193 genotype, and rs6825004 genotype.**
- **rs6825004 was missing on one PD and one control participant.**

Abbreviations: OR, odds ratio; PD, Parkinson’s disease.
GCase activity (at least not as measured in dried blood spots). There are several possible explanations for our findings. First, rs6812193 is associated with PD via a different gene/mechanism not linked to SCARB2 or GCase. This explanation is supported by a prior study showing that variants in this SNP do not correlate with LIMP-2 messenger RNA or protein levels in leukocytes from a small sample of controls.13 Second, rs6812193 is associated with PD through SCARB2, but its biological effect is not mediated by GCase activity. Finally, rs6812193 is associated with PD through SCARB2 and GCase pathways but this effect is either present in selected tissues (e.g., brain) and not leukocytes, or it involves altered intracellular routing and localization of GCase that is not apparent in the DBS assay.

In addition, we were not able to replicate the association between rs6825004 and PD. This association was reported in a Greek cohort7 and was also reported in a Dementia with Lewy Body association study, but not in other PD genome-wide studies.8 It is possible that we did not observe the association because our cohort did not include Dementia with Lewy Body cases.

In summary, we provide evidence that SCARB2 is associated with PD although the mechanism remains unknown. Full sequencing of SCARB2 in PD cohorts and correlation of PD risk with GCase activity may help to clarify these associations further.

MATERIALS AND METHODS

Participants and clinical evaluation

Participants in the ‘SPOT’ study included PD patients and genetically unrelated controls (mostly spouses) from the Center for Parkinson’s Disease at Columbia University Medical Center in New York, NY, recruited between 2010 and 2014.14 The cohort has been previously described.12 In brief, a blood sample and the data on demographics, medical history, medication, PD family history,15 the Unified Parkinson’s Disease Rating Scale (UPDRS) in the ‘on’ state and the Montreal Cognitive Assessment (MoCA)16 were collected from consecutive PD cases, as defined by the United Kingdom PD brain bank criteria (except that we did not exclude cases with a family history of PD).17 A blood sample was collected from genetically unrelated control individuals, mostly spouses. All study procedures were approved by the Columbia University IRB, and all participants signed informed consent.

Genotyping of GBA and SCARB2

All study participants were fully sequenced for GBA mutations and genotyped for the LRRK2 p.G2019S mutation as previously described.12 Two SCARB2 SNPs were genotyped by TaqMan SNP genotyping assays, rs6812193 and rs6825004 (assay IDs: C_xylene_379_10 and C_xylene_379_20, respectively) following the manufacturer’s instructions, and the genotypes were called using QuantStudio™ 7 Flex Real-Time PCR System and Software (Applied Biosystems, Foster City, CA, USA).

GCase activity assay

Dried blood spots were obtained as previously described.12,18 In brief, 75 µl of blood was ‘spotted’ on a filter paper and dried at room temperature for at least 4 h. GCase activity was measured at Sano Genzyme laboratories using a previously published protocol as part of a multiplex assay together with four additional lysosomal enzymes.19 Activity was expressed as micromoles of product per liter of whole blood per hour (µmol/l/h). All Sano Genzyme scientists were blinded to PD and genetic status.

Statistical analysis

Demographics, frequency of GBA variants, LRRK2 p.G2019S mutations, rs6812193 and rs6825004 genotypes were compared between PD cases and controls using the Student t-test for continuous variables, and the chi-square and Fisher’s exact tests for categorical variables. We used logistic regression analyses to test whether the SNPs (predictors) are associated with PD status (outcome) in models adjusted for age, gender, and GBA and LRRK2 mutation status.

To test the association between GCase activity and the rs6812193 and rs6825004 genotypes, we first compared GCase enzymatic activity by genotypes in the entire sample (including PD cases and controls), and then in PD cases and controls separately. Analyses of GCase activity were repeated excluding all carriers of GBA variants previously associated with reduced GCase activity and the LRRK2 p.G2019S mutation which was previously shown to be associated with increased GCase activity.12 Analyses were performed using SPSS Statistics version 21.0 software (Chicago, IL, USA).

ACKNOWLEDGMENTS

Funding for this study provided by the Parkinson’s Disease Foundation, the National Institutes of Health (K02NS080915 and UL1 TR000040, formerly the National Center for Research Resources, Grant Number UL1 RR024156) and the Brookdale Foundation. The authors would like to thank Ms Judy Hull for coordinating the collaboration and Mr. Pauciulo and Dr Nichols for LRRK2 genotyping.

COMPETING INTERESTS

R.N. is supported by the Parkinson’s Disease Foundation, the National Institutes of Health (K02NS080915, and UL1 TR000040, formerly the National Center for Research Resources, Grant Number UL1 RR024156), the Smart Foundation and the Brookdale Foundation. He received consultation fees from Genzyme/Sanoﬁ and Prophage. Ms P.W. and Drs P.O. and X.K.Z. are employees of Genzyme/Sanoﬁ. Dr S.F. reports Consulting and Advisory Board Membership with honoraria: Merz Pharma, Genervon Biotechnology; PixarioBio; Lundbeck Pharma. Grants/Research Support: 69Genervon Biotechnology; U.K. reports research grants from NIH, the Michael J Fox Foundation, and the Parkinson Disease Foundation; thebrookdaleFoundation, not related to the submitted work. He consults for Caremark/CVS. S.K. has received funding from the National Institutes of Health: NINDS #K08 NS083738 (principal investigator), the Louis V. Gerstner Jr. Scholar Award, the Parkinson’s Disease Foundation, the American Parkinson’s Disease Association, the International Essential Tremor Foundation, NEIHS Pilot Grant ES009089 (principal investigator), and the Smart Foundation. K.S.M. reports grants from NIH (#NS036630 (PI), UL1 RR024156-01(Director PCIR), PO412196-G(Co-I), and PO412196-G(Co-I)), grants from steering committee for U01NS052592, grants from the Parkinson’s Disease Foundation, and grants from Michael J Fox Foundation, outside the submitted work. The remaining authors declare no conﬂict of interest.

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