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Sequencing of the *GBA* co-activator, Saposin C, in Parkinson disease

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Abstract

Saposin C (SapC), encoded by *PSAP*, is required for the activity of glucocerebrosidase, encoded by *GBA*. While *GBA* mutations have been studied thoroughly in Parkinson disease (PD), genetic studies on SapC, are still lacking. Full sequencing of *PSAP* was performed in 1,123 PD patients and 1,153 controls, using targeted sequencing. Regression models adjusted for age and sex, and optimal sequence Kernel association test (SKAT-O) were performed. After correction for multiple comparisons, *PSAP* variants were not associated with PD. Two variants located within the SapC

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sequence were found in one PD patient each. SKAT-O analysis of SapC had nominal statistical significance (p=0.027), but not after correction for multiple comparisons. Combining data with a published dataset revealed that a total of four out of 2,290 PD patients (0.2%) had variants (p.G365C or p.T363M) within the binding and activation site with glucocerebrosidase, versus zero out of 2,838 controls (0%), but this was not statistically significant. Larger studies are necessary to examine the role of very rare SapC variants in PD.

Introduction

Glucocerebrosidase (GCase), encoded by *GBA*, is a lysosomal enzyme which degrades glucocerebrosides to glucose and ceramide (Hruska et al., 2008). For GCase to properly function, an acidic environment is required, as well as activation by Saposin C (SapC), which directly binds GCase. SapC is encoded by *PSAP*, which encodes a prosaposin protein that degrades into four active Saposins, A-D. The SapC protein is a short, 80 amino acid-protein, derived from amino acids 311–390 of the prosaposin protein. The SapC binding site with glucocerebrosidase is located within amino acids 351–390, and a 16-amino acid sequence (357–372) is important for glucocerebrosidase activation (Lieberman, 2011).

Biallelic *GBA* mutations may cause Gaucher disease (GD), a lysosomal storage disorder, and in rare cases, biallelic *PSAP* mutations may also cause GD type 1 or type 3 (Motta et al., 2014). *GBA* variants are among the most common genetic risk factors for Parkinson disease (PD), found in 3–20% of PD patients from different populations (Gan-Or et al., 2015; Sidransky et al., 2009). Some of the *GBA* variants that were reported in PD, such as p.T369M, p.N370S and p.L444P, are located at or near the binding site of SapC (Lieberman, 2011). This may suggest that disrupted binding of SapC to GCase may be underlying the reduced GCase activity in these carriers and the increased risk for PD.

Therefore, it is possible that *PSAP* variants that affect SapC sequence or function may also lead to PD. However, little is known about the potential role of *PSAP* variants in PD. In the current study, we fully sequenced the *PSAP* gene in PD patients and controls, and examined the association between common and rare variants and PD, with specific focus of the SapC sequence of *PSAP.*

Methods

Population

The study included a total of 1,123 consecutively recruited PD patients and 1,153 controls from two cohorts: a French-Canadian/French (FCFR) cohort and a New York (NY) cohort. French-Canadian patients and controls were recruited at the Centre Hospitalier Universitaire de Quebec and at the Montreal Neurological Institute. French patients and controls were collected at Montpellier, France, and could be combined with French-Canadian due to the shared ancestry and genetic similarity (based on unpublished GWAS data that we have for both populations). The NY cohort was recruited at the Center for Parkinson's Disease at Columbia University Medical Center in New York, as previously described (Alcalay et al., 2015). The diagnosis of PD was performed according to the UK brain bank criteria (Hughes

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et al., 1992) (without excluding patients with family history of PD) by a specialist in movement disorders. The FCFR cohort included 544 PD patients (male:female ratio of 1.7:1, average age 65.7 ± 9.9 years, data on sex were not available for 2 patients and data on age was not available for 12 patients). The FCFR control group included 869 individuals (male:female ratio of 1.06:1), and was composed of two sub-groups: 263 elderly controls (average age 60.02±7.9) and 606 young controls (average age 34.4±4.8, data on age was not available for 11 controls). There were no differences in the frequencies of common or rare *PSAP* variants between the elderly and young control groups, allowing us to combine the two for the analysis. The NY cohort included 579 patients (male:female ratio of 1.7:1, average age 66±11) and 284 controls (male:female ratio of 0.5:1, average age 65±10). In both cohorts, adjustment for age and sex was performed in the regression models and burden analyses. All patients have signed informed consent prior to enrollment, and the institutional review boards have approved the studies.

Targeted next generation sequencing and validation of SapC variants

The entire sequence of the *PSAP* gene including 5′ and 3′ untranslated regions (UTRs) and exon-intron boundaries (±50bps) were targeted using Molecular Inversion Probes (MIPs). Using an online MIP design tool (http://krishna.gs.washington.edu/mip_pipeline), 43 molecular inversion probes (MIPs) spanning the 14 exons of *PSAP* gene, were designed (Supplementary Table 1). Sequencing and analysis were performed as previously described (Ross et al., 2016), and the full protocol is available upon request. To validate the *PSAP* variants in the SapC sequence, two pairs of primers were designed using Batch primers3 (https://probes.pw.usda.gov/batchprimer3/): F1 (5′ – CCAGCCTTGGCATACTTCAT - 3′), R1 (5′ – ATTTTCAGAATGTCCCCCAAC - 3′) and F2 (5′ – CCCTCCCAGACCCAAGAG - 3′), R2 (5′ – CCCACCATTGACTCATTTCC - 3′). PCR was performed using Taq DNA polymerase (Qiagen, Valencia, CA) as per manufacturer's guidelines. PCR Products were sequenced using 3730XL DNA Analyser. Mutation Surveyor Version 5.0 (Softgenetics, State College, PA, USA) was used for mutation detection analysis.

Statistical analysis

To test for the association of common *PSAP* variants with PD, binary logistic regression adjusted for age and sex was performed using PLINK v1.90. To examine the association and burden of rare variants, optimal Sequence Kernel Association Test (SKAT-O) was performed using R (Lee et al., 2012). SKAT-O was performed on several levels: the entire gene (including coding and non-coding variants, analyzed together and separately), domain-by-domain (i.e. separately for variants in the different Saposin A-D domains) and exon-by-exon. Synonymous, nonsynonymous, stop, frameshift and splicing variants were analyzed together and separately of the synonymous variants. Bonferroni correction for multiple comparisons was performed when performing the domain-by-domain and exon-by-exon analysis according to the number of domains and exons. To examine the tolerability of *PSAP* for loss of function mutations, we used data from the Residual Variation Intolerance Score (RVIS) database (Petrovski et al., 2013) and from the public database of the Exome Aggregation Consortium (ExAC) (Lek et al., 2016).

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Results

Sequencing quality control and data summary

In the FCFR cohort, the average coverage of the *PSAP* gene, including 5′ and 3′ UTRs, was 582X, with 95.1% of nucleotides covered by >10X, 91.7% with >20X and 84% with >50X. In the NY cohort, the average coverage was 538X, with 93.6% of nucleotides covered by >10X, 89.3% with >20X and 82.9% with >50X. We performed the analysis twice, using a conservative minimal coverage of 50X (i.e., to call a variant, a coverage of at least 50X was necessary, and variants with lower coverage were excluded), and a less conservative minimal coverage of 15X. To avoid biases due to missing variant calls, we performed the analysis using variants that were called in >90% of samples. After coverage and genotype filtrations, 18 samples were excluded from the analysis due to missing genotypes and/or low coverage, 10 from the FCFR cohort and eight from the NY cohort.

SapC variants are rare, and *PSAP* has no major role in PD

In the FCFR cohort, a total of 73 *PSAP* variants with a minimal coverage of 15X were identified, including 10 nonsynonymous variants, one frameshift deletion, eight synonymous variants, 42 intronic variants and 12 3′ UTR variants (Table 1 details the coding variants, Supplementary Table 2 detail all variants). When considering variants with a minimal coverage of 50X, a total of 20 variants were identified, including five nonsynonymous variants, three synonymous variants and 12 intronic variants (Supplementary Table 3). In the age- and sex-adjusted regression model, none of the variants were associated with PD in both the 50X and 15X minimal coverage data (Supplementary Tables 2 and 3). Two variants were identified within the SapC sequence, p.G365C (rs747170456) and p.L372L (rs745951723) in the 15X analysis, both validated by Sanger sequencing. SKAT-O analysis (Table 2) identified a nominal association of exon 10 and the SapC domain (p=0.027 for both, Table 2), but these associations were not statistically significant after correction for multiple comparisons.

In the NY cohort, a total of 62 variants with a minimal coverage of 15X were identified, including six nonsynonymous variants, one frameshift deletion, eight synonymous variants, 29 intronic variants, seven 3′ UTR, six 5′ UTR and five upstream variants (Supplementary Table 4). When considering variants with a minimal coverage of 50X, a total of 20 variants were identified, including three nonsynonymous variants, seven synonymous variants, nine intronic variants and one 3′ UTR variant (Supplementary Table 5). None of these variants was within the SapC sequence, and both age- and sex-adjusted regression (Supplementary Table 5) and SKAT-O analyses did not identify statistically significant associations.

The p.G365C variant is found within the binding and activation sites (amino acids 351–390 and 357–372, respectively), and re-analysis of data from a previous study (Robak et al., 2017) identified three additional patients (out of 1,167 patients) with a mutation in this domain, p.T363M, which was not found in controls (n=1,685). Overall, four (0.2%) PD patients carried a mutation in this domain versus 0 (0%) controls, but this difference was not statistically significant. The allele frequency of p.G365C in ExAC is 0.000008319 and of p.T363M is 0.0003493. Of note, *PSAP* is among the top 8.5% intolerant genes for variations

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based on the RVIS, with an FDR adjusted *p* value of 0.0004 when comparing the expected number of loss of function variants (n=18) with the observed number (n=2) in the ExAc server.

Discussion

Given the important role of GCase and its encoding gene, *GBA*, in PD, the current study aimed to examine the role of genetic variants in the activator of GCase, SapC, in PD. Our results do not support a role for *PSAP* or SapC variants in PD. Only two variants were identified in SapC in two PD patients in our cohorts, and the burden analysis was not statistically significant after correction for multiple comparisons. Furthermore, one of these variants was a synonymous variant, p.L372L, and therefore is less likely to have a deleterious effect on SapC. While we cannot rule out that the *PSAP* p.G365C variant, located within the SapC sequence, contributed to the development of PD in the specific patient who carried this variant, the overall picture does not support the involvement of *PSAP* or SapC in PD. It is important to note that we did not identify any of the very rare *PSAP* mutations that cause GD (Diaz-Font et al., 2005; Motta et al., 2014; Rafi et al., 1993; Schnabel et al., 1991; Tylki-Szymanska et al., 2007; Tylki-Szymanska et al., 2011; Vaccaro et al., 2010).

To further examine this hypothesis, we extracted published data from a recent study on 1,167 PD patients and 1,685 controls which examined the burden of variants in lysosomal storage disorders-related genes (Robak et al., 2017). This study focused on the gene level only, and did not examine whether *PSAP* variants located specifically within the SapC sequence are associated with PD. Similar to what we have reported here, there was no burden of *PSAP* variants in PD patients vs. controls. Furthermore, the authors identified three variants in the SapC sequence, p.T344S (found in one patient and four controls), p.M345I (found in one control) and p.T363M (found in three patients). Interestingly, the p.T363M found in the previous study in three patients is very close to the variant found in one patient in our study, p.G365C, and they are both found within the 16 amino acids that are important for glucocerebrosidase activation (amino acids 47–62 of SapC, corresponding to amino acids 357–372 of the full prosaposin sequence) (Lieberman, 2011). While this is an interesting observation, identifying four out of a total of 2,290 PD patients (0.2%) with mutations in this specific domain, versus zero out of 2,838 controls (0%), could still be due to chance alone, and is not statistically significant. As our study has limited power (power of ~65%–70% to detect the observed frequencies with uncorrected $p<0.05$), larger studies in other populations and meta-analysis are required to examine whether mutations within the binding and activation sites of SapC are associated with PD.

SapC deficiency mutations can cause rare forms of GD, with both GD type 1 and type 3 described with *PSAP* mutations (Tamargo et al., 2012). Some of these mutations are found within the binding site of SapC to glucocerebrosidase (e.g. p.C382G, p.C382F and p.F385C), others may cause early termination of the protein, lack of translation or RNA mediated decay (e.g. p.M1L, p.M1V, p.Q430* and p.342_348FDKMCSKdel), but there are yet other mutations found outside of the binding site (e.g. p.C315S and p.L349P) (Motta et al., 2014; Tamargo et al., 2012). The latter, however, may affect the folding of SapC and

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affect its binding to glucocerebrosidase indirectly. The two variants that were found solely in PD patients, p.T363M and p.G365C, were not reported in GD due to *PSAP* mutations.

Overall, our results suggest no or a very minor role for *PSAP*/SapC variants in PD. There is some evidence that variants in the binding and activation site of SapC might be involved in PD, however, this should be considered with caution, and larger studies in different populations are required to further examine this hypothesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

***PSAP* coding variants**

Including variants with >15X and >90% genotype calls

Exon	Variant	AF PD	AF Controls	*p* value[a]
FCFR cohort				
2	c.C78T:p.T26T	0.000958	0	NA
2	c.96delG:p.W32fs	0.000945	0	NA
2	c.C113T:p.T38M	0.000945	0.000577	0.4136
3	c.C189T:p.C63C	0.004708	0.002309	0.1173
3	c.C204T:p.D68D	0.003766	0.009815	0.06804
4	c.T268C:p.L90L	0.001894	0.000579	0.463
4	c.G294A:p.P98P	0.000947	0.000578	0.512
5	c.A488T:p.D163V	0.000951	0.001159	0.7086
6	c.A578G:p.D193G	0	0.000577	NA
6	c.C621T:p.D207D	0	0.000577	NA
6	c.A644G:p.N215S	0	0.000577	NA
6	c.G695T:p.R232L	0.000945	0.000577	0.4301
7	c.A763G:p.M255V	0	0.000577	NA
8	c.G837A:p.M279I	0	0.001155	NA
10	c.G1093T:p.G365C	0.000977	0	NA
10	c.C1114T:p.L372L	0.000979	0	NA
11	c.G1228C:p.E410Q	0.000945	0	NA
13	c.C1515T:p.N505N	0	0.000647	NA
13	c.C1523T:p.T508I	0.00098	0	NA
NY cohort				
1	c.C19T:p.L7L	0	0.001908	NA
2	c.G88T:p.A30S	0.001764	0.001825	0.8404
2	c.A112G:p.T38A	0	0.001812	NA
2	c.C120T:p.S40S	0.000874	0	NA
2	c.C126T:p.C42C	0	0.001812	NA
3	c.C189T:p.C63C	0	0.001812	NA
3	c.C204T:p.D68D	0.009615	0.005435	0.2863
5	c.A470G:p.N157S	0.000885	0	NA
6	c.A578G:p.D193G	0.000876	0	NA
6	c.C588T:p.D196D	0.000876	0	NA
6	c.C621T:p.D207D	0.000874	0	NA
6	c.C697T:p.L233L	0	0.001812	NA
8	c.C797T:p.A266V	0	0.001792	NA
12	c.1376delA:p.E459fs	0	0.001818	NA

AF, allele frequency; PD, Parkinson disease; NA, not applicable

[a] adjusted for age and sex

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Table 2

SKAT-O burden analysis of *PSAP* variants in FCFR cohort

Including variants >15X with >90% genotype calls.

Set ID	N	*p* value
All	72	0.5317312
Intronic, exonic, UTR3 and UTR5		
exonic	19	0.1819121
intronic	42	0.7253546
UTR3	11	1.0000000
Non-synonymous, Synonymous, frameshift and splicing		
Non-synonymous, frameshift and splicing	11	0.8520893
Synonymous	8	0.1360483
Exon by exon		
exon2	3	0.17166347
exon3	2	0.10985978
exon4	2	0.48619802
exon5	1	0.79526831
exon6	4	0.64413288
exon7	1	0.78289658
exon8	1	0.39407910
exon10	2	0.02792887
exon11	1	0.10309443
exon13	2	0.50855618
Domain by domain		
Propeptide	8	0.88714782
Saposin_A	4	0.13286855
Saposin_B	4	0.64150983
Saposin_C	2	0.02765281
Saposin_D	1	0.10309443

N, number of variants