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Association of the rs823144 variant of the *RAB29* gene with the activity of lysosomal hydrolases in blood cells and risk of Parkinson's disease

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Abstract. Recent genome-wide association studies have identified a link between the *RAB29* gene and Parkinson's disease (PD). The Rab29 protein encoded by *RAB29* regulates leucine-rich repeat kinase 2 (LRRK2). Mutations in the *LRRK2* gene increase its kinase activity and contribute to autosomal dominant forms of PD. Previous research has shown that altered LRRK2 kinase activity may correlate with the activity of lysosomal hydrolases and the concentration of sphingolipids. This study aimed to assess the association of the rs823144 variant in the promoter region of the *RAB29* gene with PD risk, and to evaluate *RAB29* expression, lysosomal hydrolase activity, and sphingolipid concentrations in the blood of PD patients. We screened the rs823144 variant of the *RAB29* gene in a cohort of PD patients ($N = 903$) and controls ($N = 618$) using next-generation sequencing (NGS) and polymerase chain reaction (PCR) followed by restriction fragment length polymorphism analysis. The expression of the *RAB29* gene was measured in peripheral blood mononuclear cells (PBMCs) using qPCR. We assessed the activities of lysosomal hydrolases (glucocerebrosidase (GCase), alpha-galactosidase (GLA), acid sphingomyelinase (ASMase), and galactosylcerebrosidase (GALC)) and the concentrations of sphingolipids (globotriaosylsphingosine (LysoGb3), sphingomyelin (LysoSM), and hexosylsphingosine (HexSph)) in blood using high-performance liquid chromatography with tandem mass spectrometry (HPLC-MS/MS). The *RAB29* rs823144 C allele was associated with a reduced risk of PD in the Northwestern Russian population (OR = 0.7806, 95 % CI: 0.6578–0.9263, $p = 0.0046$), which is consistent with global data. However, no significant association was observed between the rs823144 C allele and *RAB29* mRNA expression in PBMCs. Notably, the C allele was associated with increased GLA activity and decreased concentrations of LysoGb3 and LysoSM in the blood of PD patients. In conclusion, we demonstrate for the first time an association between the *RAB29* rs823144 C allele and a reduced risk of PD in the Northwestern Russian population. Moreover, the *RAB29* rs823144 C allele is associated with altered lysosomal enzyme activity and sphingolipid profiles, suggesting a potential role of *RAB29* in sphingolipid metabolism relevant to PD pathogenesis.

Key words: Parkinson's disease; *RAB29*; lysosomal hydrolases; lysosphingolipids; LRRK2

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Ассоциация варианта rs823144 гена *RAB29* с активностью лизосомных гидролаз в клетках крови и риском болезни Паркинсона

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Аннотация. Геномные исследования последних лет выявили ассоциацию гена *RAB29* с болезнью Паркинсона (БП). Белок Rab29, кодируемый геном *RAB29*, – один из регуляторов богатой лейциновыми повторами киназы 2 (LRRK2). Мутации в гене *LRRK2* ассоциированы с увеличением киназной активности LRRK2 и приводят к развитию аутосомно-доминантных форм БП. Недавно показано, что изменение киназной активности LRRK2 может быть связано с изменением активности лизосомных гидролаз и концентрации лизосфинголипидов. Цель данного исследования заключалась в оценке ассоциации rs823144 в промоторе гена *RAB29* с БП с экспрессией гена *RAB29*, активностью лизосомных гидролаз и концентрацией лизосфинголипидов в крови при БП. В ходе исследования проведены скрининг варианта rs823144 гена *RAB29* в группе пациентов с БП (N = 903) и в контроле (N = 618) с использованием методов массового параллельного секвенирования и полимеразная цепная реакция (ПЦР) с последующим рестрикционным анализом. Экспрессия гена *RAB29* оценивалась в мононуклеарах периферической крови методом ПЦР в режиме реального времени. Активности лизосомных гидролаз (глюкоцереброзидаза (GCase), альфа-галактозидаза (GLA), кислая сфингомиелиназа (ASMase), галактозилцереброзидаза (GALC)) и концентрации лизосфинголипидов (глоботриаозилсфингозин (LysoGb3), сфингомиелин (LysoSM), гексозилсфингозин (HexSph)) оценивались в крови методом высокоэффективной жидкостной хроматографии с тандемной масс-спектрометрией (ВЭЖХ-МС/МС). Аллель С rs823144 гена *RAB29* ассоциирован с пониженным риском БП в северо-западной популяции Российской Федерации (ОШ: 0.7806, 95 % ДИ: 0.6578–0.9263, p = 0.0046), что соответствует мировым данным. Однако в ходе работы не выявлено ассоциации аллеля С rs823144 гена *RAB29* с уровнем мРНК гена *RAB29* в мононуклеарах периферической крови. В то же время носительство аллеля С rs823144 было ассоциировано с повышенной активностью GLA и сниженной концентрацией LysoGb3 в крови при БП. Таким образом, нами впервые показана ассоциация аллеля С rs823144 гена *RAB29* с пониженным риском БП в северо-западной популяции Российской Федерации. Аллель С rs823144 ассоциирован с повышенной активностью GLA и сниженной концентрацией LysoGb3 в крови при БП. Полученные результаты позволяют предположить ассоциацию гена *RAB29* с метаболизмом сфинголипидов.

Ключевые слова: болезнь Паркинсона; *RAB29*; лизосомные гидролазы; лизосфинголипиды; LRRK2

Introduction

Parkinson's disease (PD) is a common, slowly progressive neurodegenerative disorder characterized by the loss of dopaminergic neurons in the substantia nigra (SN) of the brain (Lill, 2016). A central pathological mechanism in PD pathogenesis is the accumulation and aggregation of the alpha-synuclein protein in the SN. Although PD is primarily sporadic, approximately 15 % of patients report a positive family history. The molecular mechanisms underlying PD remain largely unclear; however, increasing evidence implicates lysosomal dysfunction as a key contributor to disease pathogenesis (Nechushtai et al., 2023). In particular, our group and others have demonstrated reduced lysosomal hydrolase activity and altered sphingolipid levels in the peripheral fluids of patients with idiopathic PD (Alcalay et al., 2015; Galper et al., 2022; Usenko et al., 2022). Additionally, changes in lysosomal enzyme activity and sphingolipid concentrations have been observed in PD cases associated with mutations in the *LRRK2* gene, one of the most common monogenic forms of the disease (Alcalay et al., 2015; Usenko et al., 2023, 2024).

The *LRRK2* gene encodes leucine-rich repeat kinase 2 (LRRK2), a multidomain protein implicated in Parkinson's disease pathogenesis (Zimprich et al., 2004). A key group of LRRK2 substrates comprises small Rab GTPases, which are critical regulators of vesicular trafficking, particularly within the endolysosomal system (Steger et al., 2016; Wang et al., 2014). Dysregulated LRRK2 kinase activity disrupts the trafficking of lysosomal hydrolases to their proper destinations, thereby impairing lysosomal function (MacLeod et al., 2013; Ysselstein et al., 2019; Rivero-Ríos et al., 2020; Kedariti et al., 2022).

Among the LRRK2 substrates, Rab29 – encoded by the *RAB29* gene – has attracted particular interest (Steger et al., 2016). Rab29 has been identified as a key upstream regulator of LRRK2, responsible for its activation (Liu et al., 2018; Madero-Pérez et al., 2018; Purlyte et al., 2018; Kuwahara,

Iwatsubo, 2020). Rab29 localizes to the membranes of lysosomes and the Golgi apparatus, where it recruits inactive cytoplasmic LRRK2 monomers and promotes their oligomerization into active dimers or tetramers (Purlyte et al., 2018; Zhu et al., 2023). The *RAB29* gene is located within the PARK16 locus, which has previously been associated with reduced PD risk (Satake et al., 2009; Pihlstrøm et al., 2015; Nalls et al., 2019). A recent multi-trait analysis of genome-wide association studies (MTAG) further confirmed the association between *RAB29* and PD at both the transcriptomic and proteomic levels (Shi et al., 2024).

Several studies have identified variants in the promoter region of *RAB29* that are associated with a reduced risk of Parkinson's disease. These variants are thought to influence *RAB29* gene expression levels (Gan-Or et al., 2012; Khaligh et al., 2017; Sun et al., 2021), potentially modulating the activation of LRRK2 and thereby affecting lysosomal hydrolase activity in PD.

The aim of this study was to investigate the association of the rs823144 single nucleotide polymorphism (SNP), located in the promoter region of *RAB29*, with PD risk, *RAB29* gene expression, lysosomal hydrolase activity – including glucocerebrosidase (GCase), α-galactosidase (GLA), galactocerebrosidase (GALC), and acid sphingomyelinase (ASMase) – and the concentrations of lysosphingolipids in the blood. The lysosphingolipids analyzed included hexosylsphingosine (HexSph), a mixture of glucosylsphingosine (GlcSph) and galactosylsphingosine (GalSph); lysosphingomyelin (LysoSM); and lysoglobotriaosylsphingosine (LysoGb3). These parameters were evaluated in both PD patients and healthy control subjects.

Materials and methods

Characteristics of the study groups. The study included 903 patients with sporadic PD and 618 control individuals matched for age and gender. All patients were recruited from the clinic of the N.P. Bechtereva Institute of the Human Brain of the Russian Academy of Sciences. The control group comprised individuals seen at the consultative and diagnostic center of the First St. Petersburg State Medical University named after academician I.P. Pavlov. To exclude PD and other neurodegenerative disorders, all control participants underwent neurological examination. Clinical and demographic characteristics of the study groups are presented in Table 1. No significant differences in age or gender distribution were observed between the groups ($p > 0.05$).

All procedures involving human participants were conducted in accordance with the ethical standards of the National Research Ethics Committee and the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. Written informed consent was obtained from each participant prior to inclusion in the study. The study protocol was approved by the Ethics Committee of the First St. Petersburg State Medical University named after academician I.P. Pavlov (Protocol No. 275, dated September 04, 2023).

Genetic analysis. Two methods were used to genotype the rs823144 variant in the *RAB29* gene: massively parallel sequencing (next-generation sequencing, NGS) and polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP) analysis. Peripheral blood samples were collected from all study participants, and genomic DNA was extracted using phenol-chloroform extraction, as previously described (Maniatis et al., 1994).

NGS genotyping of the rs823144 variant in the *RAB29* gene was performed in a subset of 521 PD patients and 420 control individuals using molecular inversion probes, as previously described (Rudakou et al., 2021). Sequencing was conducted on the Illumina NovaSeq 6000 SP PE100 platform. Sequence alignment was performed using the Burrows–Wheeler Aligner (BWA) with the hg19 human genome reference (Li, Durbin, 2009). Variant calling and post-alignment quality control were carried out using the Genome Analysis Toolkit (GATK, v3.8) (McKenna et al., 2010). Variants were filtered based on coverage depth and quality metrics; only those with a minimum read depth >30 and a quality score >20 were included in the analysis.

An additional 473 PD patients and 384 controls were screened for rs823144 using PCR followed by restriction analysis. Primer sequences were designed using Primer3 v. 0.4.0 (http://bioinfo.ut.ee/primer3-0.4.0/) (FOR: 5′-CCCTGCACGTGACGCTTG-3′, REV: 5′-GAATCCCAGTCAGCTCCTTACA-3′). Restriction enzyme selection was performed using the NEBCutter tool (Vincze et al., 2003), and BstAC I was chosen for RFLP analysis (Fig. 1).

A total of 91 PD patients and 186 control individuals were genotyped for the rs823144 variant in the *RAB29* gene using both PCR with restriction analysis and NGS. These overlaps were accounted for in subsequent statistical analyses.

To validate the results obtained from both methods, a subset of samples was confirmed by Sanger sequencing using a Nanofor-05 genetic analyzer (Synthol, Russia). The Sanger sequencing data were visualized and analyzed using Tracy software (Rausch et al., 2020) (Fig. 2).

Evaluation of the relative expression level of the *RAB29* gene in peripheral blood mononuclear cells of PD patients and controls. Peripheral venous blood samples were collected from PD patients (N = 30) and control individuals (N = 43). Peripheral blood mononuclear cells (PBMCs) were

Table 1. Clinical and demographic characteristics of the study groups

Group	Sex (male:female)	Age, years	Age at onset, years	Duration of the disease, years
PD (N = 903)	378:525	65 (25–90)	59 (20–88)	3 (1–36)
Controls (N = 618)	228:390	64 (40–96)	–	–

Note. PD – Parkinson's disease; data are presented as median (min–max).

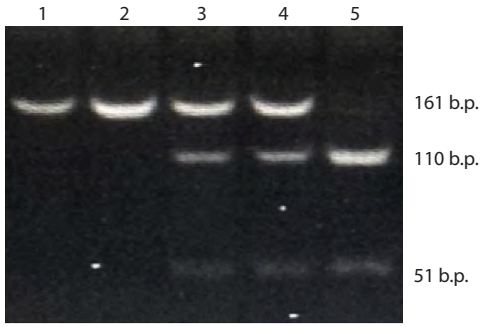

Fig. 1. Electropherogram showing the results of genotyping of the rs823144 variant in the *RAB29* gene.

1, 2 – homozygote for the A allele (genotype AA), 3, 4 – heterozygote (genotype AC), 5 – homozygote for the C allele (genotype CC).

isolated by density gradient centrifugation using Ficoll-Paque PLUS (GE Healthcare) at 400*g* for 40 minutes, as described by Böyum (1968). The resulting mononuclear fraction was washed twice with phosphate-buffered saline (PBS; Biolot, St. Petersburg) and centrifuged at 3,000 rpm for 10 minutes. Total RNA was extracted from PBMCs using the RNeasy Mini Kit (Qiagen, 74104, USA). Complementary DNA (cDNA) was synthesized via reverse transcription using the RevertAid First Strand cDNA Synthesis Kit (K1622, Thermo Scientific, Lithuania).

The relative expression level of the *RAB29* gene in PBMCs of PD patients (N = 30) and control individuals (N = 43) was quantified by real-time PCR using SYBR Green I as the intercalating dye. The housekeeping genes *RPLP0* and *GAPDH*, which are constitutively expressed in PBMCs, were used as internal reference genes for normalization. Primer sequences were designed using the Primer3 v. 0.4.0 program (https://bioinfo.ut.ee/primer3-0.4.0) (FOR: 5′-CGGTTTCACAGGTTGGACAG-3′, REV: 5′-CCCTTGGGTGGACAAAGACA-3′). The relative mRNA level for each gene was calculated by comparing the threshold amplification levels ΔΔCt (Livak, Schmittgen, 2001).

Evaluation of lysosomal hydrolase activities and lysosphingolipid concentrations in peripheral blood of PD patients and controls. Peripheral venous blood samples were collected from PD patients and controls into EDTA tubes. To obtain dried blood spots, 40 µl of whole blood was applied to each spot on a filter paper test blank, after which the spots were allowed to air dry at room temperature for 2 hours and were then stored at +4 °C until extraction. Activities of four lysosomal hydrolases: glucocerebrosidase (GCase), α-galactosidase (GLA), galactocerebrosidase (GALC), and sphingomyelinase (ASMase), as well as the concentration of three lysosphingolipids: hexazyl sphingosine (HexSph) (a mixture of glycosyl sphingosine (GlcSph) and galactosyl sphingosine (GalSph)), lysosphingomyelin (LysoSM) and lysoglobotriaosyl sphingosine (LysoGb3) were estimated by high-performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS) according to our previously published protocol (Pchelina et al., 2018).

Statistical data processing. Statistical analyses were performed using the R programming environment (version 4.0.5). Odds ratios (OR) with 95 % confidence intervals (CIs) were calculated using logistic regression models adjusted for age and gender. The nonparametric Mann–Whitney U-test was applied to compare the relative expression levels of the *RAB29* gene, lysosomal hydrolase activities, and lysosphingolipid concentrations between study groups. To evaluate the association between the rs823144 variant of the *RAB29* gene and lysosomal hydrolase activity, multiple linear regression analysis was conducted, adjusting for age, gender, and disease duration. Statistical significance was set at $p < 0.05$. Data are presented as median (min–max).

Results

Association between rs823144 of the *RAB29* gene and PD risk

Genotyping of the rs823144 variant in the *RAB29* gene among PD patients and controls from the Northwestern Russian population revealed that the major allele is A. Hardy–Weinberg equilibrium (HWE) analysis confirmed that the genotype

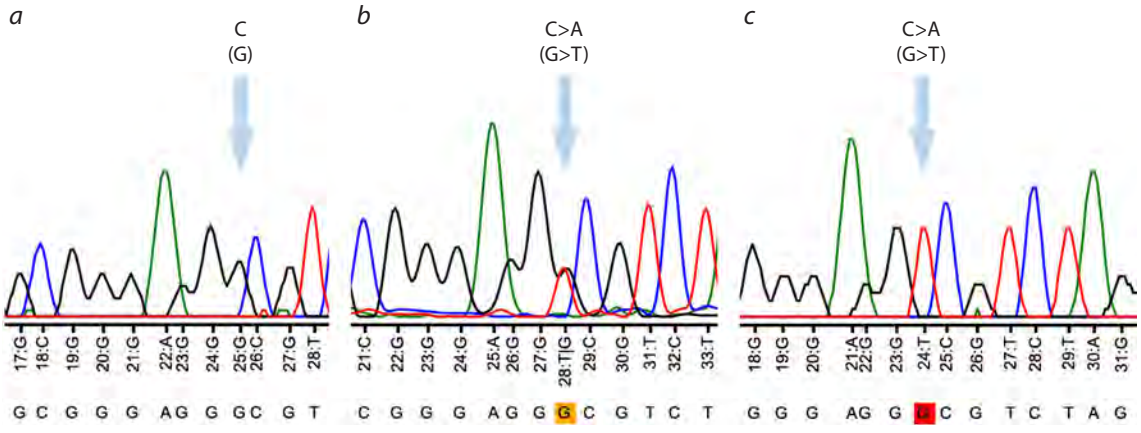

Fig. 2. Graphical representation of Sanger sequencing results (electropherogram displaying the results of genotyping of the rs823144 variant of the *RAB29* gene).

a – homozygote for the C allele (genotype CC); *b* – heterozygote (genotype AC); *c* – homozygote for the A allele (genotype AA).

distributions in both groups were in equilibrium ($p > 0.05$). The genotype frequencies are summarized in Table 2. Logistic regression analysis demonstrated that the rs823144 variant is significantly associated with a reduced risk of Parkinson's disease in this population (OR: 0.7806, 95 % CI: 0.6578–0.9263, $p = 0.0046$).

Evaluation of the relative level of *RAB29* gene mRNA in the mononuclear fraction of peripheral blood cells of PD patients and controls

This study assessed for the first time the association between the rs823144 variant of the *RAB29* gene and the relative expression level of *RAB29* mRNA in peripheral blood mononuclear cells (PBMCs) from PD patients and controls. The median relative expression of *RAB29* mRNA in PBMCs was 1.00 (0.22–1.75) in PD patients and 0.96 (0.13–1.79) in controls, with no statistically significant difference between groups ($p > 0.05$). When stratified by rs823144 genotype, the relative *RAB29* mRNA levels were as follows: in PD patients, AA genotype carriers had a median expression of 1.09 (0.–1.68), while carriers of AC and CC genotypes combined showed 1.13 (0.36–1.75); in controls, AA carriers had 1.05 (0.58–1.28) and AC + CC carriers had 0.97 (0.55–1.33). No significant association was found between the rs823144 genotype and *RAB29* mRNA levels in either group ($p > 0.05$).

Association of the rs823144 variant of the *RAB29* gene with the activity of lysosomal hydrolases and the concentration of lysosphingolipids in the peripheral blood of patients with PD and controls

This study is the first to investigate the association of the rs823144 variant of the *RAB29* gene with lysosomal hydrolase activities and lysosphingolipid concentrations in the blood of PD patients and controls. Patients with PD exhibited significantly increased GALC activity and decreased LysoSM concentrations compared to controls ($p = 0.008$ and $p = 0.01$, respectively) (Table 3). Stratification by rs823144 genotype revealed that carriage of the C allele of the rs823144 variant in the *RAB29* gene was associated with increased GLA activity and decreased levels of LysoGb3 – a substrate of GLA – in PD patients ($p = 0.038$ and $p = 0.022$, respectively) (Table 3). These associations were confirmed by multiple linear regression adjusted for gender, age, and disease duration (GLA: $\beta = 1.11$, $p = 0.024$; LysoGb3: $\beta = –0.23$, $p = 0.015$) (Table 4). No significant differences were observed in GCase, GLA and ASMase activities, or HexSph and LysoGb3 concentrations when analyzing the combined groups of PD patients and controls, regardless of rs823144 genotype. Similarly, no genotype-dependent differences were detected in GCase, ASMase, GALC activities, or HexSph concentrations.

Notably, PD patients carrying the C allele exhibited decreased blood concentrations of LysoGb3 and LysoSM compared to the combined control group ($p = 0.045$ and $p = 0.015$, respectively), whereas PD patients with the AA genotype showed increased LysoSM levels relative to controls ($p = 0.022$). The increase in GLA activity and corresponding decrease in its substrate LysoGb3 were specific to carriers of C allele of the rs823144 variant in the *RAB29* gene within the PD group and were not observed in controls.

Discussion

The *RAB29* gene is one of five genes located within the PARK16 locus on chromosome 1q32, previously implicated in Parkinson's disease risk (Simón-Sánchez et al., 2009; Tucci et al., 2010). Multiple association studies have identified the minor allele C of rs823144, positioned in the promoter region of *RAB29*, as protective against PD across various populations (Gan-Or et al., 2012; Xia et al., 2015; Khaligh et al., 2017; Sun et al., 2021). It has been hypothesized that this allele modulates transcription factor binding (Gan-Or et al., 2012; Khaligh et al., 2017).

In silico analyses suggest that the C allele of the rs823144 variant in the *RAB29* gene abolishes the binding site for c-Ets-1 and introduces binding sites for p300, GATA-1, and Sp1, potentially enhancing *RAB29* expression (Gan-Or et al., 2012). However, data from the GTEx database (https://www.gtexportal.org/) indicate that the CC genotype of rs823144 correlates with reduced *RAB29* expression in whole blood and brain tissues ($p < 0.0001$). In turn, increased expression of the *RAB29* gene, encoding one of the main regulators of LRRK2 kinase, may potentially lead to impaired activation of this kinase. LRRK2, by phosphorylating Rab family proteins, regulates endolysosomal transport (Reczek et al., 2007; Wei et al., 2023). Dysregulation of LRRK2 activation can impair lysosomal hydrolase transport and function, contributing to PD pathogenesis.

Table 2. Frequencies of rs823144 genotypes and alleles in the study groups

Genotypes and alleles	PD, % (*N* = 903)	Controls, % (*N* = 618)	OR (95 % CI), *p*-value
AA	63.3 (*N* = 572)	57 (*N* = 352)	AC+CC vs AA: 0.7658 (0.6213–0.9438), *p* = 0.0123; CC vs AA+CC: 0.6671 (0.4392–1.0132), *p* = 0.0576
AC	31.5 (*N* = 284)	35.4 (*N* = 219)	
CC	5.2 (*N* = 47)	7.6 (*N* = 47)	
AC+CC	36.7 (*N* = 331)	43 (*N* = 266)	
AA+AC	94.8 (*N* = 856)	92.4 (*N* = 571)	
A	79.1 (*N* = 1428)	74.7 (*N* = 923)	C vs A: 0.7806 (0.6578–0.9263), *p* = 0.0046
C	20.9 (*N* = 378)	25.3 (*N* = 313)	

Note. PD – Parkinson's disease; OR – odds ratio.

Table 3. Lysosomal hydrolase activity and lysosphingolipid levels in the peripheral blood of PD patients and controls

Genotypes	Lysosomal hydrolase activity, mM/L/hour				Lysosphingolipid concentration, ng/ml		
	GCase	GLA	ASMase	GALC	HexSph	LysoGb3	LysoSM
Combined groups							
PD ($N = 211$)	6.78 (2.07–23.08)	4.8 (1.33–36.39)	4.54 (1.53–13.25)	2.13 (0.21–12.68) *$p = 0.008$	2.63 (0.49–13.23)	0.8 (0.04–40.77)	3.62 (0.72–16.08) *$p = 0.01$
Controls ($N = 179$)	6.29 (1.55–32.13)	4.18 (1.03–14.81)	4.14 (1.4–12.39)	1.86 (0.24–9.35)	2.97 (0.57–15.36)	0.78 (0.03–2.31)	3.98 (0.59–11.6)
Groups divided by genotypes of the rs823144 variant of the *RAB29* gene							
PD AA ($N = 91$)	5.67 (2.07–19.52)	4.05 (1.2–13.93)	4.28 (1.67–11.83)	2.17 (0.12–12.68)	2.55 (0.5–13.81)	0.93 (0.02–2.49)	3.84 (1.65–16.08) *$p = 0.022$
PD AC + CC ($N = 55$)	5.78 (2.42–23.08)	5.39 (1.61–13.72) **$p = 0.038$	4.76 (1.53–9.31)	2.2 (0.52–7.28)	2.89 (0.87–13.08)	0.61 (0.04–3.73) *$p = 0.045$ **$p = 0.022$	3.45 (0.72–15.34) *$p = 0.015$
Controls AA ($N = 52$)	6.92 (1.55–32.13)	4.23 (1.86–12.94)	4.35 (1.5–10.99)	2.19 (0.58–9.35)	2.37 (0.57–12.11)	0.77 (0.16–2.27)	4.37 (0.59–14.87)
Controls AC + CC ($N = 40$)	8.11 (3.9–17.23)	4.42 (2.17–12.6)	4.4 (1.82–12.03)	2.19 (0.96–8.37)	2.54 (0.69–9.87)	0.86 (0.03–2.31)	4.44 (2.03–11.75)

Note. PD – Parkinson's disease; GCase – glucocerebrosidase; GLA – α-galactosidase; GALC – galactocerebrosidase; ASMase – sphingomyelinase; HexSph – hexasylsphingosine; LysoSM – lysosphingomyelin; LysoGb3 – lysoglobotriaosylsphingosine.

* Compared with the combined control group; ** compared with PD patients with the AA genotype. Data are presented as median (min–max).

In our study of the Northwestern Russian population, we confirmed the association of the C allele of rs823144 with a reduced risk of PD, which is consistent with global findings (Gan-Or et al., 2012; Khaligh et al., 2017; Sun et al., 2021). Notably, we did not observe any association between the C allele of rs823144 genotype and *RAB29* mRNA levels in peripheral blood mononuclear cells from either PD patients or controls.

Lysosomal dysfunction is widely recognized as a central mechanism in PD. Previous work by our group and others has demonstrated altered lysosomal hydrolase activity and sphingolipid metabolism in peripheral fluids and postmortem brain regions of PD patients (Alcalay et al., 2018; Nelson et al., 2018; Huebecker et al., 2019; Chang et al., 2022; Usenko et al., 2022, 2024). Specifically, we reported increased GALC activity and decreased LysoSM – a substrate of acid ASMase – concentration in PD peripheral blood (Usenko et al., 2022, 2024). In turn, disruption of lysosphingolipid metabolism, including through altered lysosomal hydrolase activity, may contribute to the aggregation of α-synuclein (Mazzulli et al., 2011; Marie et al., 2015).

Accumulating lysosphingolipids in neurons can stabilize neurotoxic alpha-synuclein oligomers (Battis et al., 2023). Prior brain autopsy studies revealed correlations between LysoGb3 isoform concentrations and pathological phosphorylated alpha-synuclein as well as an negative correlation of GLA activity with levels of α-synuclein phosphorylated at serine 129 – the pathological form of the protein that predominates in aggregates in PD (Nelson et al., 2018). Our novel findings linking the C allele of rs823144 to increased GLA activity and decreased LysoGb3 concentration, alongside elevated LysoSM levels in AA genotype carriers, underscore a potential role for *RAB29* in sphingolipid metabolism in PD.

It should be noted that a marked reduction in GLA activity due to mutations in the *GLA* gene leads to a rare lysosomal storage disorder, Fabry disease. Notably, elevated LysoGb3 concentration is a risk factor for white matter lesions in Fabry disease. (Rombach et al., 2010). We have also observed LysoGb3 accumulation in neuronopathic mucopolysaccharidoses (Baydakova et al., 2020), suggesting that LysoGb3 elevation may not be exclusive to Fabry disease. Therefore, the association of the *RAB29* rs823144 C allele with increased GLA activity and decreased LysoGb3 in PD patients may influence disease progression and clinical phenotype.

The present study has several limitations. The sample sizes of PD patients and the control group included in the experiment assessing the association of the *RAB29* rs823144 variant with *RAB29* mRNA levels were limited. The compared groups were population-wise heterogeneous. Furthermore, we did not directly assess the impact of rs823144 on LRRK2 kinase activity, which warrants investigation in future studies.

Conclusion

This study demonstrates for the first time worldwide that the C allele of the rs823144 variant in the *RAB29* gene, previously identified as protective against PD in other populations,

Table 4. Lysosomal hydrolase activities and lysosphingolipid concentrations in the peripheral blood of patients with PD (regression analysis)

Lysosomal hydrolases/lysosphingolipids	Genotype/ other parameters	PD			Controls		
		β	95 % CI	*p*-value	β	95 % CI	*p*-value
GCase	AC+CC	–0.95	–2.32–0.43	0.17	1.44	–0.25–3.14	0.09
	Sex (male:female)	0.55	–0.81–1.90	0.43	0.12	–1.61–1.85	0.89
	Age at onset, years	–0.03	–0.094–0.028	0.29	–0.06	–0.17–0.05	0.30
	Duration of the disease, years	0.004	–0.042–0.049	0.87	–	–	–
GLA	AC+CC	**1.11**	**0.15–2.07**	**0.02**	–0.29	–1.52–0.95	0.64
	Sex (male:female)	0.03	–0.93–0.98	0.96	0.93	–0.33–2.18	0.14
	Age at onset, years	–0.03	–0.07–0.01	0.16	–0.00001	–0.080–0.080	1
	Duration of the disease, years	–0.002	–0.03–0.03	0.93	–	–	–
ASMase	AC+CC	0.23	–0.53–0.98	0.55	0.31	–0.85–1.46	0.60
	Sex (male:female)	0.05	–0.69–0.80	0.87	–0.20	–1.38–0.97	0.73
	Age at onset, years	**0.06**	**0.03–0.09**	**0.0005**	–0.021	–0.096–0.054	0.58
	Duration of the disease, years	**0.025**	**0.0005–0.050**	**0.046**	–	–	–
GALC	AC+CC	0.080	–0.57–0.72	0.81	0.07	–0.64–0.77	0.85
	Sex (male:female)	0.16	–0.48–0.80	0.62	–0.15	–0.87–0.57	0.67
	Age at onset, years	–0.017	–0.046–0.012	0.25	–0.011	–0.057–0.035	0.63
	Duration of the disease, years	–0.0051	–0.027–0.016	0.64	–	–	–
HexSph	AC+CC	–0.014	–0.92–0.90	0.97	0.12	–0.80–1.04	0.80
	Sex (male:female)	0.35	–0.55–1.24	0.45	0.49	–0.44–1.43	0.30
	Age at onset, years	–0.028	–0.069–0.013	0.18	0.011	–0.048–0.071	0.71
	Duration of the disease, years	–0.028	–0.058–0.0022	0.069	–	–	–
LysoGb3	AC+CC	–0.23	**–0.41–0.044**	**0.015**	0.039	–0.16–0.24	0.70
	Sex (male:female)	–0.08	–0.26–0.10	0.39	–0.043	–0.25–0.16	0.67
	Age at onset, years	–0.007	–0.015–0.0016	0.11	–0.0039	–0.017–0.009	0.55
	Duration of the disease, years	–0.001	–0.007–0.004	0.66	–	–	–
LysoSM	AC+CC	–0.17	–1.18–0.84	0.74	–0.25	–1.51–1.014	0.70
	Sex (male:female)	–0.82	–1.82–0.17	0.10	–0.63	–1.91–0.65	0.33
	Age at onset, years	–0.064	–0.11–0.018	**0.006**	0.070	–0.011–0.15	0.089
	Duration of the disease, years	–0.028	–0.061–0.0056	0.10	–	–	–

Note. PD – Parkinson's disease; GCase – glucocerebrosidase; GLA – α-galactosidase; GALC – galactocerebrosidase; ASMase – sphingomyelinase; HexSph – hexasylsphingosine; LysoSM – lysosphingomyelin; LysoGb3 – lysoglobotriaosylsphingosine.

is associated with a reduced risk of PD in the North-West region of Russia. Additionally, we report for the first time that carriage of the C allele correlates with increased GLA activity and decreased LysoGb3 concentration in the blood of PD patients.

These findings suggest a potential role for *RAB29* in lysosphingolipid metabolism and imply that the rs823144 variant may influence the clinical course of PD. Further research is warranted to elucidate the relationship between the PARK16 locus, *RAB29* gene, lysosphingolipid metabolism, and PD progression.

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