

Nigral Iron in Parkinson's Disease Tracks Normative Mitochondrial Maps and Oxidative Stress Genes

Marta M. Pokotylo

University of Luebeck and University Hospital Schleswig-Holstein

Norman Griebner

University of Luebeck and University Hospital Schleswig-Holstein

Ylva Besler

University of Luebeck and University Hospital Schleswig-Holstein

Hanna Jaskolla

University of Luebeck and University Hospital Schleswig-Holstein

Norbert Brüggemann

`norbert.brueggemann@uni-luebeck.de`

University of Luebeck and University Hospital Schleswig-Holstein

Jannik Prasuhn

University of Luebeck and University Hospital Schleswig-Holstein

Article

Keywords: Parkinson's disease, quantitative susceptibility mapping, oxidative stress, mitochondrial dysfunction, iron accumulation, gene expression

Posted Date: October 18th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11207027/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: Competing interest reported. N.B. has received honoraria from AbbVie, Ipsen, Merz, Teva, and Zambon. N.B. has also received research grants from the German Research Foundation (FOR2488), the Michael J. Fox Foundation, the EU Joint Programme–Neurodegenerative Disease Research (JPND), BFMTR, the Parkinsonstiftung, and the Else Kröner-Fresenius-Stiftung (EKFS).

Abstract

Background

Iron dyshomeostasis and mitochondrial dysfunction are mechanistically linked contributors to Parkinson's disease (PD) pathogenesis. However, whether the iron homeostasis-mitochondrial coupling association is detectable *in vivo* is unknown.

Methods

We used quantitative susceptibility mapping (QSM) to identify regions with higher iron-related susceptibility in 46 patients with PD (PwPD) compared to 44 healthy controls (HC). We assessed spatial associations between PD-related QSM alterations and six mitochondrial feature maps: complexes I, II, and IV; mitochondrial density; mitochondrial respiratory capacity; and tissue respiratory capacity. We then used partial least squares regression with mitochondria-specific gene expression from the Allen Human Brain Atlas, informed by MitoCarta3.0, to characterize transcriptomic correlates of regional PD-related QSM alterations.

Results

Substantia nigra pars compacta showed the strongest iron homeostasis-mitochondrial coupling ($\rho = -0.570$, $p_{\text{FDR}} = 0.0104$), in addition to weaker associations in the globus pallidus internal segments and red nucleus ($\rho = 0.279-0.504$, $p_{\text{FDR}} < 0.0163$). We found no whole-brain associations. The first partial least squares component of mitochondrial gene expression profiles closely tracked PD-related QSM alterations, primarily characterized by genes involved in reactive oxygen species and glutathione metabolism ($\rho = -0.800$, $p_{\text{FDR}} = 0.0264$), which closely tracked subcortical PD-related alterations (77% variance explained; $\rho = 0.88$, $p < 0.0001$).

Conclusion

We identified iron dyshomeostasis-mitochondrial coupling in PD, which was regionally constrained to already-vulnerable subcortical regions. This coupling spatially converged onto gene expression related to oxidative stress and antioxidant defense pathways. In this study, we derived a spatially resolved and molecularly grounded framework for selective neuronal vulnerability in PD.

Introduction

Parkinson's disease is a progressive neurodegenerative disorder characterized by dopaminergic neuronal loss in the substantia nigra pars compacta (SNc), and its pathology extends beyond the

dopaminergic system¹. Dysregulated iron homeostasis is among the mechanisms believed to drive this neurodegeneration^{2,3}. Excessive iron accumulation promotes oxidative damage via Fenton chemistry, where Fe^{2+} catalyzes the conversion of hydrogen peroxide into highly reactive hydroxyl radicals⁴. The Haber-Weiss reaction further sustains this cycle by regenerating Fe^{2+} from Fe^{3+} , resulting in protein and DNA damage⁴. Therefore, the combination of iron dyshomeostasis and large axonal arborization⁵ makes dopaminergic neurons selectively vulnerable to cell death. Iron dysregulation is mechanistically intertwined with mitochondrial dysfunction, a process believed to be a central, convergent node in PD pathogenesis⁶. Protein complex I (CI) of the mitochondrial electron transport chain is iron-dependent, containing iron-sulfur clusters necessary for electron transport⁷. Impaired iron-sulfur cluster biogenesis reduces CI activity and simultaneously triggers a compensatory increase in cellular iron uptake, forming a self-reinforcing loop⁶. Consequently, well-established CI abnormalities in the substantia nigra (SN)⁸ result from and exacerbate abnormal iron homeostasis. This mechanistic link is further supported by evidence that neurotoxins that selectively inhibit CI, including 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and rotenone, cause nigrostriatal neurodegeneration and iron accumulation in animal models^{9,10}. Although substantial evidence links iron accumulation and mitochondrial function at the molecular level, we lack *in vivo* insight into whether regions with the greatest iron-level alterations in PD are spatially associated with normative mitochondrial architecture and function.

Quantitative susceptibility mapping (QSM) is a non-invasive magnetic resonance imaging (MRI) technique for quantifying magnetic susceptibility *in vivo*, which is substantially influenced by tissue iron content^{11,12}. Numerous studies have shown QSM's utility in detecting higher iron levels in patients with PD (PwPD) compared with healthy controls (HC). However, most studies used QSM as a descriptive or diagnostic biomarker and failed to link the identified macroscopic alterations to underlying molecular or cellular processes. Advances in baseline molecular and functional brain atlases and regional gene expression profiles make it possible to assess such spatial associations. A human brain atlas containing six maps of mitochondrial features, including maps of CI, complex II and IV (CI, CII, CIV), mitochondrial density (MitoD), mitochondrial respiratory capacity (MRC), and tissue respiratory capacity (TRC)¹⁶ allows assessment of the spatial relationship between iron level alterations and normative mitochondrial architecture. Additionally, an imaging transcriptomics approach using regional gene expression cataloged in the Allen Human Brain Atlas (AHBA) allows assessment of transcriptomic correlates of iron-level alterations in PD.

In this study, we combined a human brain mitochondrial atlas and mitochondrial-related gene expression to investigate their spatial relationship with PD-specific QSM alterations. We analyzed group differences in QSM between PwPD and HC and spatial associations across whole-brain gray matter, the disease-specific subcortical HybraPD atlas¹⁷, and the cortical human motor area template (HMAT)¹⁸. Additionally, we performed an exploratory analysis using the Jülich atlas, given the evidence of widespread cortical iron accumulation in PwPD¹⁹. We hypothesized that this coupling would be detectable *in vivo*, specifically that regions with greater PD-related QSM alterations would spatially

overlap with mitochondrial architecture and mitochondrial gene-expression modules involved in respiration and oxidative damage. Because SNc vulnerability is well established, we hypothesized that QSM-mitochondrial coupling would be more pronounced in the basal ganglia and subcortical regions of interest (ROIs) than in cortical ROIs. Additionally, we expected QSM-mitochondrial associations to be enriched in genes involved in oxidative stress and reactive oxygen species (ROS) metabolism, providing evidence of a link between regional iron levels, mitochondrial function, and oxidative injury.

Methods

Experimental design and participants. We recruited participants from our inpatient and outpatient clinics at the Department of Neurology, University Medical Center Schleswig-Holstein (Campus Lübeck). We selected PwPD with a clinically confirmed diagnosis of PD using the Movement Disorders Society Clinical Diagnostic Criteria²⁰. We included participants aged between 50 and 80 years, with no concomitant neurological/psychiatric conditions, no MRI contraindications, no history of alcohol or substance abuse, no diagnosis of atypical or secondary Parkinsonism, and no history of structural brain diseases. Our study protocol included a clinical assessment using the Movement Disorders Unified Parkinson's Disease Rating Scale (subscore III, MDS-UPDRS-III) and an MRI session for PwPD, with an MRI session only for healthy controls. We adhered to the Declaration of Helsinki, and the local ethics committee (University of Lübeck, AZ-18-196) approved the study. All participants provided written informed consent before inclusion.

Image acquisition and image preprocessing. We performed MRI on a 3 Tesla Siemens Magnetom Skyra scanner equipped with a 64-channel head coil. Following the principal acquisition recommendations of the International Society for Magnetic Resonance in Medicine (ISMRM) Electro-Magnetic Tissue Properties Study Group²¹, we performed whole-brain imaging using a radiofrequency- and gradient-spoiled three-dimensional multi-echo gradient-recalled echo sequence (3D FLASH) with Cartesian k-space sampling and monopolar readout.

We acquired five echoes with a repetition time (TR) of 36.0 ms and echo times (TE) of 5.83, 12.02, 18.21, 24.40, and 30.59 ms. The flip angle was 15°, and the receiver bandwidth was 220 Hz/pixel. We acquired images in the transverse plane with right-to-left phase encoding. The field of view was 256 × 176 × 144 mm³ in the readout, phase-encoding, and partition directions. We used a base matrix of 256, 100% phase and partition resolution, and 144 partitions to provide a non-interpolated isotropic voxel size of 1.0 mm³. We performed parallel imaging in the phase-encoding direction using Generalized Autocalibrating Partial Parallel Acquisition (GRAPPA) with an acceleration factor of 2 and 24 reference lines. We applied no acceleration in the partition direction. We used a 7/8 partial Fourier factor in the partition direction and no partial Fourier sampling in the phase-encoding direction. We employed elliptical k-space sampling and 11.1% slab oversampling. We applied readout-direction flow compensation to the first echo. We acquired one signal average. We did not use fat or water suppression, and we disabled phase stabilization and image interpolation. We combined receive-coil signal online using the vendor-provided

Adaptive Combine method. We reconstructed magnitude and phase images for each echo and retained them for subsequent QSM processing. Our acquisition protocol was 7 min 12 s.

We processed magnitude and phase DICOM data using IronSmith (v1.4 beta) and incorporated the MEDI toolbox (version 01/15/2020). We performed phase unwrapping and combined multi-echo data before removing the background field. We generated an initial mask from root-sum-of-squares combined multi-echo magnitude data using FSL BET. We used the initial mask for background-field removal without further refinement. We removed background fields using projection onto dipole fields (PDF) with default parameters, followed by dipole inversion using MEDI_L1 ('lambda' = 1000, lambda_CSF' = 100, 'merit', 'smv' = 5). We reported susceptibility values in parts per million (ppm) relative to whole-brain cerebrospinal fluid within a FreeSurfer-derived bilateral lateral-ventricle reference mask, with positive values indicating greater and negative values indicating lower susceptibility relative to this reference. We subsequently non-linearly normalized QSM maps to the Montreal Neurological Institute (MNI)152 (1 mm) space using IronSmith, with AFNI 3dNwarpApply (windowed-sinc [wsinc5] interpolation) and no additional smoothing.

We assessed all original magnitude, phase, local-field, and susceptibility images for motion-related ringing or blurring, phase-unwrapping errors, signal dropouts, streaking artifacts, and major QSM reconstruction, segmentation, and registration issues. Additionally, IronSmith generated quality control measures such as signal-to-noise ratio and phase-artifact information. During quality control, we excluded 29 datasets, predominantly due to excessive motion artifacts, resulting in 64 PwPD and 44 HC being included in the final analysis. We applied all preprocessing transformations and interpolation steps after QSM reconstruction.

Group differences in quantitative susceptibility mapping. We assessed group differences between PwPD and HC using a two-sample t-test in Statistical Parametric Mapping (SPM25; Wellcome Centre for Human Neuroimaging, UCL, London, UK) within MATLAB (R2025b; The MathWorks Inc, Natick, MA, USA). We assessed a single contrast: higher QSM t-values in PwPD than in HC (PwPD > HC), with age and sex included as covariates. We assessed the PwPD > HC contrast because higher QSM values mainly reflect higher tissue iron content²², and iron accumulation is a recognized pathogenic mechanism in PD²³. The reverse contrast would yield the exact negative of the same t-statistic map, and therefore we did not analyze it separately. We performed each set of analyses separately using explicit masks, including the tissue probability mask for gray matter incorporated into the SPM25 environment and ROI-specific masks derived from the HybraPD, HMAT, and Jülich atlases^{17,18,24}. Overall, we assessed group differences across 73 ROIs, including whole-brain gray matter; 15 HybraPD-derived subcortical regions; 6 HMAT-derived cortical motor regions; and 51 Jülich-derived cortical regions.

Spatial correlation analyses. We performed spatial correlation analyses in R (v4.5.3; R Code Team, 2026; R Foundation for Statistical Computing, Vienna, Austria), using oro.nifti packages (v0.11.4), dplyr (v1.2.1), and modified.ttest (v0.4.1) packages. We derived mitochondrial maps of CI, CII, CIV, MitoD, MRC, and TRC from a previously published study¹⁶. We restricted the analysis to voxels with finite PwPD > HC

t-values and valid mitochondrial map t-values. We used Dutilleul's modified t-test, which corrects significance testing for spatial autocorrelation in the input map by estimating spatial autocorrelation from voxel-wise covariance and deriving a corrected effective sample size (ESS). For each analysis, we calculated and reported Spearman's correlation coefficient (ρ), ESS, 95% confidence intervals using ESS (CI_{lower} & CI_{upper}), degrees of freedom, and spatial autocorrelation-corrected p-value ($p_{spatial}$, Tables S1 and S2). We used the Benjamini-Hochberg false discovery rate (FDR) procedure to correct for multiple comparisons and report p_{FDR} , which we consider the primary significance metric. We created visual presentations in R (v4.5.3) using the ggplot2 package (v4.0.2) within RStudio (v2026.01.1 + 403; Posit Software, PBC, Boston, MA, USA).

Transcriptomic analyses. We used partial least squares (PLS) regression to assess the relationship between QSM group differences and regional mitochondria-related gene expression. We performed transcriptomic analyses in Python (v3.13.9) using NiBabel (v.5.4.2), xlrld (v.2.0.2), openpyxl (v3.1.5), and Scikit-learn (PLSRegression, v1.9.0) libraries. We derived mitochondrial-associated genes from the Allen Human Brain Atlas (AHBA) using MitoCarta3.0²⁵. We summarized mean t-values from QSM group differences encoded in t-maps across all voxels within each ROI mask, then matched them to regional AHBA expression using PLS regression, fit with five components (LV1-5). We calculated the percentage of variance in QSM alterations explained by each PLS component (i) as:

$$\Delta R^2(i) = R_{cum}^2(i) - R_{cum}^2(i-1)$$

We performed this analysis across two atlas scenarios: disease-related (HybraPD and HMAT combined) and exploratory, cortical (Jülich atlas separately). For HybraPD-HMAT analysis, we excluded globus pallidus external and internal segments (GPe and GPi, respectively) and substantia nigra pars compacta and reticulata (SNc and SNr, respectively) because AHBA expression data were unavailable. We correlated regional first latent variable (LV1) scores with the atlas of origin using point-biserial correlation to ensure that LV1 was not driven by a cortical vs. subcortical mix. For HybraPD-HMAT analysis, we estimated LV significance against a non-spatial null model generated by randomly permuting the regional labels of QSM-derived t-values ($n = 10,000$), yielding p_{perm} per LV.

For Jülich atlas analyses, we estimated LV significance against two independent nulls: the same non-spatial permutation null ($n = 10,000$, p_{perm}) and a spatially constrained null generated using Moran Spectral Randomization ($n = 10,000$, $p_{spatial}$). We considered an LV if it survived dual-null testing, defined as $p_{combined}$, ($p_{combined} = \max(p_{perm}, p_{spatial})$). We created visual representations in Python (v3.13.9) using the Matplotlib (v.1.17.0) library.

Gene contribution and pathway-level analyses. We assessed gene-level contributions for LV1 via bootstrap resampling (5,000 resamples with replacement across ROIs), refitting a single-component PLS model on each resample and sign-aligning gene weights to the observed model. We computed a bootstrap ratio (BSR) per gene as the mean bootstrap weight divided by its standard deviation. We considered genes in the top and bottom 5th percentiles as the strongest contributors to LV1. We defined

MitoCarta3.0 pathway modules at two levels using the MitoPathways hierarchy: leaf-level modules (terminal categories with no child pathway) and broader mid-tier functional groups. We constructed mid-tier groups by pooling genes across all leaf-level descendants at a fixed depth in the hierarchy. We removed modules with fewer than five genes in the AHBA MitoCarta3.0 from testing. We calculated a regional pathway-expression score for each module as the mean z-scored expression across the module’s constituent genes. We also correlated z-scored expression with the regional t-value vector using Spearman’s correlation. We applied FDR correction across all module estimates within each pathway tier and atlas combination.

Results

Demographic and Clinical Assessment. After quality control, we included 46 PwPD and 44 HC (Table 1). Age did not differ between the groups (PwPD: 63.9 ± 8.7 ; HC: 63.7 ± 8.3 ; $U = 1360$, $p = 0.7660$). We observed a comparable sex distribution between the groups (PwPD: females: 21/46; HC: females: 19/44; $\chi^2 = 0.0556$, $df = 1$, $p = 0.8139$). The mean disease duration among PwPD was 6.6 ± 4.8 years. The severity of PD was assessed using the MDS-UPDRS-III (25.7 ± 9.5) and Hoehn and Yahr stage 2 (median [range]: 2 [1–2]), both measured in the ON state.

Table 1
Demographic and clinical characteristics.

number	PwPD	HC	p-value
	46	44	
male/female	25/21	25/19	0.8139 ¹
age (years)	63.9 ± 8.7	63.7 ± 8.2	0.7660 ²
MDS-UPDRS-III	25.7 ± 9.5	n.a.	
LEDD (mg/day)	584.6 ± 336.2	n.a.	
Disease duration (years)	6.6 ± 4.8	n.a.	
Hoehn and Yahr stage	2 [1–2]	n.a.	
Mean values and standard deviations are quoted. Values in square brackets represent the interquartile range. ¹ according to a χ^2 -test; ² Mann-Whitney test applied. HCs: healthy controls. LEDD: levodopa equivalent daily dose. MDS-UPDRS-III: Movement Disorders Society’s Unified Parkinson’s Disease Rating Scale (subscore III). n.a.: not available. PwPD: patients with Parkinson’s disease.			

PD-related QSM alterations were not associated with mitochondrial features at the whole brain level. We identified no spatially autocorrected significant QSM-mitochondrial associations at the whole-brain level ($p_{\text{spatial}} = 0.2478\text{--}0.7073$).

The substantia nigra pars compacta showed the strongest association between QSM and MitoD and MRC. Within the basal ganglia, SNc showed the strongest collective association magnitudes, with negative QSM-MitoD ($\rho = -0.570$, $p_{\text{FDR}} = 0.0104$) and positive QSM-MRS associations ($\rho = 0.318$, $p_{\text{FDR}} = 0.0009$; Fig. 1, Table S1). We observed similar patterns in the SN and SNr, with only QSM-MitoD in SN surviving FDR correction ($\rho = -0.423$, $p_{\text{FDR}} = 0.0043$). Globus pallidus internal segment (GPI) yielded the most QSM-mitochondrial associations, all negative ($\rho = -0.377$ to -0.287 , $p_{\text{FDR}} < 0.0163$). Among other basal ganglia structures, the subthalamic nucleus (STN) yielded positive QSM-CI and -CII associations ($\rho = 0.164$ & 0.280 , $p_{\text{FDR}} = 0.0133$ & 0.0105), and the caudate nucleus (CN) yielded a single negative QSM-CII association ($\rho = -0.148$, $p_{\text{FDR}} = 0.0011$).

Interestingly, among subcortical ROIs, only the red nucleus (RN) showed consistently positive associations across all mitochondrial features ($\rho = 0.279$ to 0.504 , $p_{\text{FDR}} < 0.0009$). Additionally, we identified a positive QSM-CIV association in the dentate nuclei ($\rho = 0.177$, $p_{\text{FDR}} = 0.0133$) and a QSM-CII association in the thalamus ($\rho = 0.108$, $p_{\text{FDR}} = 0.0304$).

We observed no FDR-significant QSM-mitochondrial associations in HMAT-derived ROIs.

Positive QSM-mitochondrial associations were mainly located in the frontal and parietal lobes. The analysis of Jülich-derived ROIs yielded the strongest magnitude associations in the intra-parietal sulcus 2 and amygdala centromedial group, which, however, did not survive FDR correction (Fig. 2, Table S2). Primary auditory cortex TE1.0 was the ROI with FDR significance, highest magnitude, and negative QSM-mitochondrial associations ($\rho = -0.321$ to -0.276 , $p_{\text{FDR}} < 0.0001$). Broadman's areas 44 (BA44) and 45 (BA45) showed convergent, positive QSM-mitochondrial associations (BA44: $\rho = 0.204$ to 0.317 , $p_{\text{FDR}} < 0.0001$; BA45: $\rho = 0.194$ to 0.211 , $p_{\text{FDR}} < 0.0009$). Additionally, superior parietal lobule 5L (SPL-5L) and 7A (SPL-7A) showed positive associations between QSM and nearly all mitochondrial associations (SPL-5L: $\rho = 0.119$ to 0.250 , $p_{\text{FDR}} < 0.0019$; SPL-7A: $\rho = 0.131$ to 0.196 , $p_{\text{FDR}} < 0.0088$). Interestingly, we observed sparse low-magnitude associations across motor cortical regions, including a positive QSM-MRC association in the primary motor cortex BA4a (BA4a, $\rho = 0.160$, $p_{\text{FDR}} = 0.0179$) and a negative QSM-CI association in the premotor cortex BA6 (BA6, $\rho = -0.107$, $p_{\text{FDR}} = 0.0354$).

Glutathione peroxidase and oxidative stress-related pathways were significantly linked to PD-related QSM alterations. We performed a partial least squares regression analysis to identify a mitochondrial gene-expression signature associated with regional PD-related QSM changes across subcortical and motor cortical regions. Latent variable 1 (LV1) explained most of the variance, accounting for 77% ($p = 0.0297$, Fig. 3A). As we expected, LV1 scores were strongly correlated with regional QSM t-values ($\rho = 0.88$, $p < 0.0001$, Fig. 3B), consistent with LV1 capturing most of the modeled variance.

Several mitochondrial functional categories are associated with LV1 (Fig. 3C), including mitochondrial DNA nucleoid ($\rho = 0.60$, $p = 0.0172$), pyruvate metabolism ($\rho = 0.58$, $p = 0.0218$), and EF hand motif ($\rho = 0.53$, $p = 0.0356$). Associations between LV1 and GABA ($\rho = -0.53$, $p = 0.0428$) and proline

metabolism ($\rho = -0.54$, $p = 0.0380$), complex III assembly ($\rho = -0.54$, $p = 0.0365$), import motor ($\rho = -0.57$, $p = 0.0249$), xenobiotic metabolism ($\rho = -0.64$, $p = 0.0102$), urea cycle ($\rho = -0.73$, $p = 0.0021$) were negative, with only negative associations between LV1 and reactive oxygen species and glutathione metabolism surviving the FDR correction across mitochondrial categories ($\rho = -0.80$, $p < 0.0001$, $p_{\text{FDR}} = 0.0264$), highlighting oxidative stress as a central process underlying this signature.

Analysis of individual genes using BSR revealed genes whose normative expression contributes most strongly to LV1. For example, normative expression of genes involved in mitochondrial protein import and pyruvate dehydrogenase activity genes, such as *TIMM17B* (BSR = 6.07), *SLC25A27* (BSR = 5.21), and *PDHA1* (BSR = 5.20), positively co-varied with the LV1 QSM pattern. Meanwhile, genes such as *GPX1* (BSR = -6.00), *MTPAP* (BSR = -5.60), and *CCDC90B* (BSR = -5.50) negatively co-varied with LV1 QSM pattern (Fig. 3D). Interestingly, *GPX1* encodes the glutathione peroxidase enzyme, which is consistent with pathway-level findings of a negative association between ROS metabolism and LV1.

Mitochondrial bioenergetic signatures correlated with PD-related QSM alterations in the Jülich atlas, with no FDR significance. Within the Jülich atlas showed that mitochondrial gene expression was not significantly associated with PD-related QSM alterations (LV1: 63% of covariance; $p_{\text{perm}} = 0.5180$, $p_{\text{spatial}} = 0.6834$, $p_{\text{FDR}} = 0.6834$, Figure S1). Nevertheless, LV1 scores were negatively correlated with regional QSM t-values ($\rho = -0.90$, $p < 0.0001$), consistent with LV1 capturing the modeled variance.

At the mitochondrial pathway level, tricarboxylic acid (TCA) cycle ($\rho = 0.34$, $p = 0.0190$), proteases ($\rho = 0.34$, $p = 0.0190$), MICOS complex ($\rho = 0.31$, $p = 0.0303$), and copper metabolism ($\rho = 0.30$, $p = 0.0350$) were positively associated with LV1. Mitochondrial fission ($\rho = -0.30$, $p = 0.0361$) was negatively associated with LV1 scores. No identified pathways reached statistical significance after FDR correction in this analysis.

At the gene level, *ACSM2A* (BSR = 3.68), *SLC25A13* (BSR = 3.64), *HADHA* (BSR = 3.60), and *NDUFB1* (BSR = 3.54) were the strongest positive contributors to LV1, while *ECHDC2* (BSR = -3.53), *D2HGDH* (BSR = -2.99), *PMAIP1* (BSR = -2.98) were the strongest negative contributors. Interestingly, mitochondrial carrier and complex I subunit genes were among the top contributors, suggesting that a broader mitochondrial bioenergetic signature underlies regional QSM alterations.

Discussion

We combined PD-related QSM alterations with baseline mitochondrial architecture and mitochondria-related gene expression to assess whether an established iron dysregulation-mitochondrial coupling in PD is spatially detectable *in vivo*. We observed no global, whole-brain iron dysregulation-mitochondria coupling; instead, it was regionally specific, with the strongest associations in the SNc. At the transcriptomics level, genes involved in ROS and glutathione metabolism closely tracked regional PD-related QSM alterations across subcortical regions.

Associations in the SNc were divergent, with iron-related changes in PD co-localizing with lower baseline mitochondrial density but higher baseline respiratory, consistent with disproportionately higher metabolic demand relative to mitochondrial reserve in the SNc. Additionally, we identified weaker patterns in SNr, GPi, and RN, demonstrating that iron dysregulation-mitochondrial coupling may extend to a broader basal ganglia and brainstem network, rather than being confined to dopaminergic neurons only. The negative associations in SNc extend to all mitochondrial features in GPi, suggesting these structures may share a vulnerability mechanism in which regions with low mitochondrial architecture are less able to manage iron-related oxidative damage. Meanwhile, RN yielded all positive associations, unlike SNc/GPi profiles, suggesting the observed coupling may reflect a physiological relationship between mitochondrial and iron levels rather than a pathological process²⁶. This divergence suggests that the nature of QSM-mitochondrial association could help distinguish regions with pathological iron accumulation from those with normal tissue composition; however, extensive histopathological validation is needed.

Across imaging transcriptomics analyses, we derived converging molecular-level evidence that supports the imaging findings. Our model explained 77% of the variance in QSM across subcortical and motor cortical regions. Across mitochondrial functional categories, only the ROS and glutathione metabolism module survived FDR correction, with the *GPX1* gene encoding glutathione peroxidase emerging as the top negative contributor. This evidence indicates that regions with elevated QSM in PD tend to show lower baseline expression of antioxidant-dense machinery, which is less equipped to buffer iron-related oxidative damage via Fenton chemistry^{27,28}. The lack of whole-brain QSM-mitochondrial associations suggests that iron accumulation-mitochondrial coupling is constrained to specific regions, potentially within already-vulnerable networks. This observation is consistent with the literature, suggesting that PD-related iron alterations are neither uniform nor proportional^{29,30} and highlighting the value of regional assessment in this context.

Interestingly, a prior study reported that cortical PD-related QSM alterations were associated with genes involved in metal detoxification and synaptic signaling, with no identified associations with genes in mitochondrial- or autophagy-related gene modules¹⁹. To our knowledge, this is the first study to combine PD-related QSM alterations with an independent human brain mitochondrial atlas and pathway-targeted mitochondrial gene expression, identifying a robust regional QSM and ROS/glutathione metabolism association, which may be more readily detectable using a pathway-targeted approach or may be more prominent in subcortical rather than cortical regions.

The convergence of imaging and transcriptomic findings on ROS and glutathione metabolism suggests that antioxidant-targeted or iron-chelation therapies could yield differential efficacy due to the underlying region's baseline mitochondrial and antioxidant-related gene expression profiles^{31–33}. For example, administering the iron chelator deferiprone to treatment-naïve PwPD reduced nigrostriatal iron content but was associated with greater worsening of MDS-UPDRS scores than placebo and a higher rate of dopaminergic therapy initiation². Lowering iron content does not necessarily translate into clinical

benefit, as iron is essential for dopamine synthesis; non-selective chelation may impair dopaminergic neurotransmission and exacerbate PD. If regional gene expression shapes vulnerability to iron-associated oxidative damage, it could explain why clinical trials of antioxidant and iron-targeted therapies in PD have demonstrated heterogeneous outcomes to date, potentially highlighting the value of stratification by regional or biomarker-defined profiles³⁴. However, our study provides only hypothesis-generating evidence and would require direct testing, such as relating QSM to mitochondria-specific module expression and treatment response in an interventional cohort, prior to its use to inform trial design or clinical decision-making. The well-documented heterogeneity among PwPD highlights the value of the methodology presented here, which combines regional imaging with baseline molecular profiling and represents a promising direction for future work.

Several limitations should be discussed. First, the cross-sectional design limits the assessment of causal inference regarding the direction and temporal dynamics of iron homeostasis-mitochondrial coupling, necessitating longitudinal assessment. Second, our sample included a modest number of early- and middle-stage PwPD, suggesting our observations may not generalize to more advanced stages of PD, and larger, more heterogeneous cohorts are needed. Third, QSM measurements in regions adjacent to air-tissue-bone interfaces are susceptible to field-mapping artifacts, resulting in increases and decreases in apparent susceptibility unrelated to tissue iron content, which we could not fully exclude in the presented analyses. Additionally, we used group-level mitochondrial and transcriptomics atlases rather than patient-specific molecular data, as no currently available technique allows these measures to be obtained *in vivo*. Therefore, the identified spatial associations reflect cohort-average correlations rather than each participant's own molecular profile. The identified associations also do not account for potential PD-related alterations in mitochondrial function or gene expression themselves, which could independently influence iron-related alterations.

To conclude, we provide *in vivo* evidence that PD-related iron accumulation is spatially coupled with baseline mitochondrial architecture and mitochondria-related gene expression. We observed region-specific circuit vulnerability, most prominent in the SNc, that converged with gene expression involved in oxidative stress and glutathione metabolism, providing a mechanistic link between iron dyshomeostasis and mitochondrial dysregulation and offering a framework for selective vulnerability in PD.

Declarations

Competing interests statement

N.B. has received honoraria from AbbVie, Ipsen, Merz, Teva, and Zambon. N.B. has also received research grants from the German Research Foundation (FOR2488), the Michael J. Fox Foundation, the EU Joint Programme–Neurodegenerative Disease Research (JPND), BFMTR, the Parkinsonstiftung, and the Else Kröner-Fresenius-Stiftung (EKFS).

Funding declaration

The author JP gratefully acknowledges support from the Detlef Zillikens Clinician Scientist Academy of Precision Health in Schleswig-Holstein (PHSH), the Clinician Scientist Academy Lübeck, the Michael J. Fox Foundation for Parkinson's Research (MJFF-025746, -028069, and - 028070), and the Else Kröner-Fresenius-Stiftung (2023_EKEA. 168). NB acknowledges support from the EU Joint Programme–Neurodegenerative Disease Research (JPND) (Control-PD).

Author Contribution

MMP: Conceptualization, Methodology, Data curation, Formal analysis, Visualization, Writing the original draft. NG: Investigation, Data curation, Formal analysis, Writing review and editing. YB: Investigation, Data curation, Writing, review and editing. HJ: Investigation, Data curation, Writing, review and editing. NB: Supervision, Project administration, Writing, review and editing. JP: Conceptualization, Methodology, Supervision, Writing, review and editing.

Acknowledgement

Not applicable

Data Availability

All data supporting the findings of this study are available from the corresponding author upon reasonable request

References

1. Poewe, W. *et al.* Parkinson disease. *Nat. Rev. Dis. Primer* 3, 17013 (2017).
2. Dexter, D. T. *et al.* INCREASED NIGRAL IRON CONTENT IN POSTMORTEM PARKINSONIAN BRAIN. *The Lancet* 330, 1219–1220 (1987).
3. Sofic, E. *et al.* Increased iron (III) and total iron content in post mortem substantia nigra of parkinsonian brain. *J. Neural Transm.* 74, 199–205 (1988).
4. Gerlach, M., Ben-Shachar, D., Riederer, P. & Youdim, M. B. H. Altered Brain Metabolism of Iron as a Cause of Neurodegenerative Diseases? *J. Neurochem.* 63, 793–807 (1994).
5. Pissadaki, E. K. & Bolam, J. P. The energy cost of action potential propagation in dopamine neurons: clues to susceptibility in Parkinson's disease. *Front. Comput. Neurosci.* 7, (2013).
6. Muñoz, Y., Carrasco, C. M., Campos, J. D., Aguirre, P. & Núñez, M. T. Parkinson's Disease: The Mitochondria-Iron Link. *Park. Dis.* 2016, 1–21 (2016).

7. Ohnishi, T., Blum, H., Galante, Y. M. & Hatefi, Y. Iron-sulfur N-1 clusters studied in NADH-ubiquinone oxidoreductase and in soluble NADH dehydrogenase. *J. Biol. Chem.* 256, 9216–9220 (1981).
8. Schapira, A. H. V. et al. Mitochondrial Complex I Deficiency in Parkinson's Disease. *J. Neurochem.* 54, 823–827 (1990).
9. Langston, J. W., Ballard, P., Tetrud, J. W. & Irwin, I. Chronic Parkinsonism in Humans Due to a Product of Meperidine-Analog Synthesis. *Science* 219, 979–980 (1983).
10. Betarbet, R. et al. Chronic systemic pesticide exposure reproduces features of Parkinson's disease. *Nat. Neurosci.* 3, 1301–1306 (2000).
11. De Rochefort, L., Brown, R., Prince, M. R. & Wang, Y. Quantitative MR susceptibility mapping using piece-wise constant regularized inversion of the magnetic field. *Magn. Reson. Med.* 60, 1003–1009 (2008).
12. Shmueli, K. et al. Magnetic susceptibility mapping of brain tissue in vivo using MRI phase data. *Magn. Reson. Med.* 62, 1510–1522 (2009).
13. Lotfipour, A. K. et al. High resolution magnetic susceptibility mapping of the substantia nigra in Parkinson's disease. *J. Magn. Reson. Imaging* 35, 48–55 (2012).
14. He, N. et al. Region-specific disturbed iron distribution in early idiopathic Parkinson's disease measured by quantitative susceptibility mapping. *Hum. Brain Mapp.* 36, 4407–4420 (2015).
15. Pyatigorskaya, N. et al. Iron Imaging as a Diagnostic Tool for Parkinson's Disease: A Systematic Review and Meta-Analysis. *Front. Neurol.* 11, 366 (2020).
16. Mosharov, E. V. et al. A human brain map of mitochondrial respiratory capacity and diversity. *Nature* 641, 749–758 (2025).
17. Yu, B. et al. HybraPD atlas: Towards precise subcortical nuclei segmentation using multimodality medical images in patients with Parkinson disease. *Hum. Brain Mapp.* 42, 4399–4421 (2021).
18. Mayka, M. A., Corcos, D. M., Leurgans, S. E. & Vaillancourt, D. E. Three-dimensional locations and boundaries of motor and premotor cortices as defined by functional brain imaging: A meta-analysis. *NeuroImage* 31, 1453–1474 (2006).
19. Thomas, G. E. C. et al. Regional brain iron and gene expression provide insights into neurodegeneration in Parkinson's disease. *Brain* 144, 1787–1798 (2021).
20. Postuma, R. B. et al. MDS clinical diagnostic criteria for Parkinson's disease: MDS-PD Clinical Diagnostic Criteria. *Mov. Disord.* 30, 1591–1601 (2015).
21. QSM Consensus Organization Committee et al. Recommended implementation of quantitative susceptibility mapping for clinical research in the brain: A consensus of the ISMRM electro-magnetic tissue properties study group. *Magn. Reson. Med.* 91, 1834–1862 (2024).
22. Langkammer, C. et al. Quantitative susceptibility mapping (QSM) as a means to measure brain iron? A post mortem validation study. *NeuroImage* 62, 1593–1599 (2012).
23. Ward, R. J., Zucca, F. A., Duyn, J. H., Crichton, R. R. & Zecca, L. The role of iron in brain ageing and neurodegenerative disorders. *Lancet Neurol.* 13, 1045–1060 (2014).

24. Eickhoff, S. B. *et al.* A new SPM toolbox for combining probabilistic cytoarchitectonic maps and functional imaging data. *NeuroImage* 25, 1325–1335 (2005).
25. Rath, S. *et al.* MitoCarta3.0: an updated mitochondrial proteome now with sub-organelle localization and pathway annotations. *Nucleic Acids Res.* 49, D1541–D1547 (2021).
26. Johnson, M. L. *et al.* Neuropathological findings and in vivo imaging correlates of the red nucleus compared to those of the substantia nigra pars compacta in parkinsonisms. *Parkinsonism Relat. Disord.* 125, 107043 (2024).
27. Gardaneh, M., Gholami, M. & Maghsoudi, N. Synergy between glutathione peroxidase-1 and astrocytic growth factors suppresses free radical generation and protects dopaminergic neurons against 6-hydroxydopamine. *Rejuvenation Res.* 14, 195–204 (2011).
28. Bai, X. *et al.* Neurochemical and motor changes in mice with combined mutations linked to Parkinson's disease. *Pathobiol. Aging Age-Relat. Dis.* 7, 1267855 (2017).
29. Fu, X. *et al.* Time-Specific Pattern of Iron Deposition in Different Regions in Parkinson's Disease Measured by Quantitative Susceptibility Mapping. *Front. Neurol.* 12, 631210 (2021).
30. Chen, Y. *et al.* Differential brain iron deposition in Parkinson's disease subtypes: a comparative quantitative susceptibility mapping study of tremor-dominant versus postural instability/gait difficulty phenotypes. *Front. Neurol.* 17, 1788931 (2026).
31. Martin-Bastida, A. *et al.* Brain iron chelation by deferiprone in a phase 2 randomised double-blinded placebo controlled clinical trial in Parkinson's disease. *Sci. Rep.* 7, 1398 (2017).
32. Devos, D. *et al.* Trial of Deferiprone in Parkinson's Disease. *N. Engl. J. Med.* 387, 2045–2055 (2022).
33. Mohammed, I. *et al.* The role of N-acetylcysteine and glutathione in the management of Parkinson's disease: a systematic review of oxidative biomarkers and clinical outcomes. *Amino Acids* 58, 26 (2026).
34. Pokotylo, M. M., Brüggemann, N. & Prasuhn, J. Biological heterogeneity in Parkinson's disease: implications for MRI-based patient stratification. *Npj Park. Dis.* 12, 207 (2026).
35. Yaghmaie, N. *et al.* QSMART: Quantitative susceptibility mapping artifact reduction technique. *NeuroImage* 231, 117701 (2021).

Figures

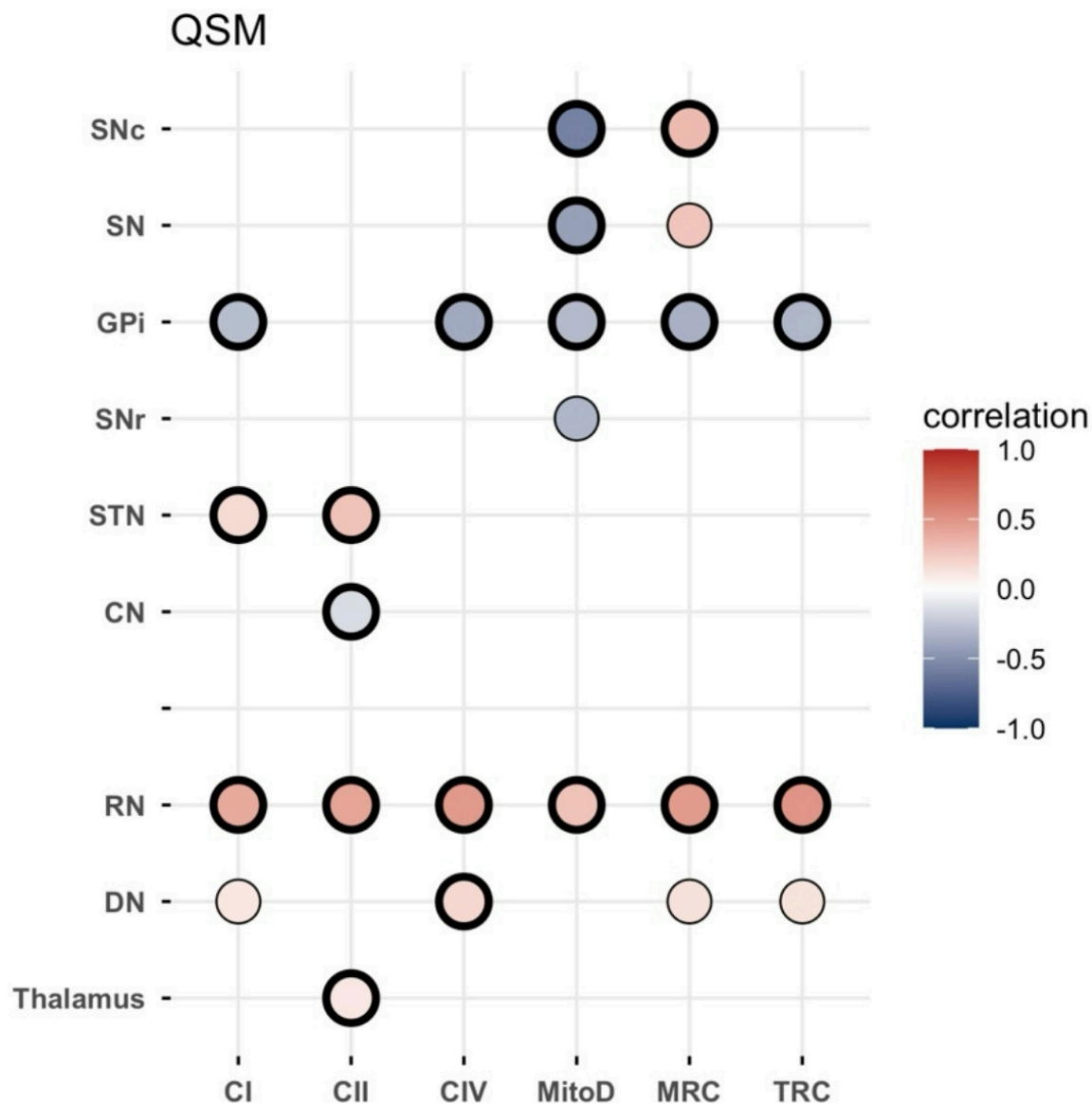


Figure 1

Regional spatial association between PD-derived QSM measures and mitochondrial features within the subcortical HybraPD-derived ROIs. Each row corresponds to a ROI, and each column corresponds to a mitochondrial feature. Within each anatomical section, regions are ordered from bottom to top based on the cumulative strength of associations across all mitochondrial features. Missing values represent associations that did not survive the spatial autocorrelation-corrected threshold ($p_{\text{spatial}} < 0.05$). Bold-bordered cells represent associations that survived the false-discovery rate (FDR) corrected threshold ($p_{\text{FDR}} < 0.05$). CI, CII, CIV: mitochondrial electron transport chain protein complexes I, II, and IV. CN:

caudate nucleus. DN: dentate gyrus. GPi: globus pallidus internal segment. MitoD: mitochondrial density. MRC: mitochondrial respiratory capacity. PD: Parkinson's disease. QSM: quantitative susceptibility mapping. RN: red nucleus. ROI(s): regions of interest. SNc/SNr: substantia nigra pars compacta/reticulata. STN: subthalamic nucleus. TRC: tissue respiratory capacity.

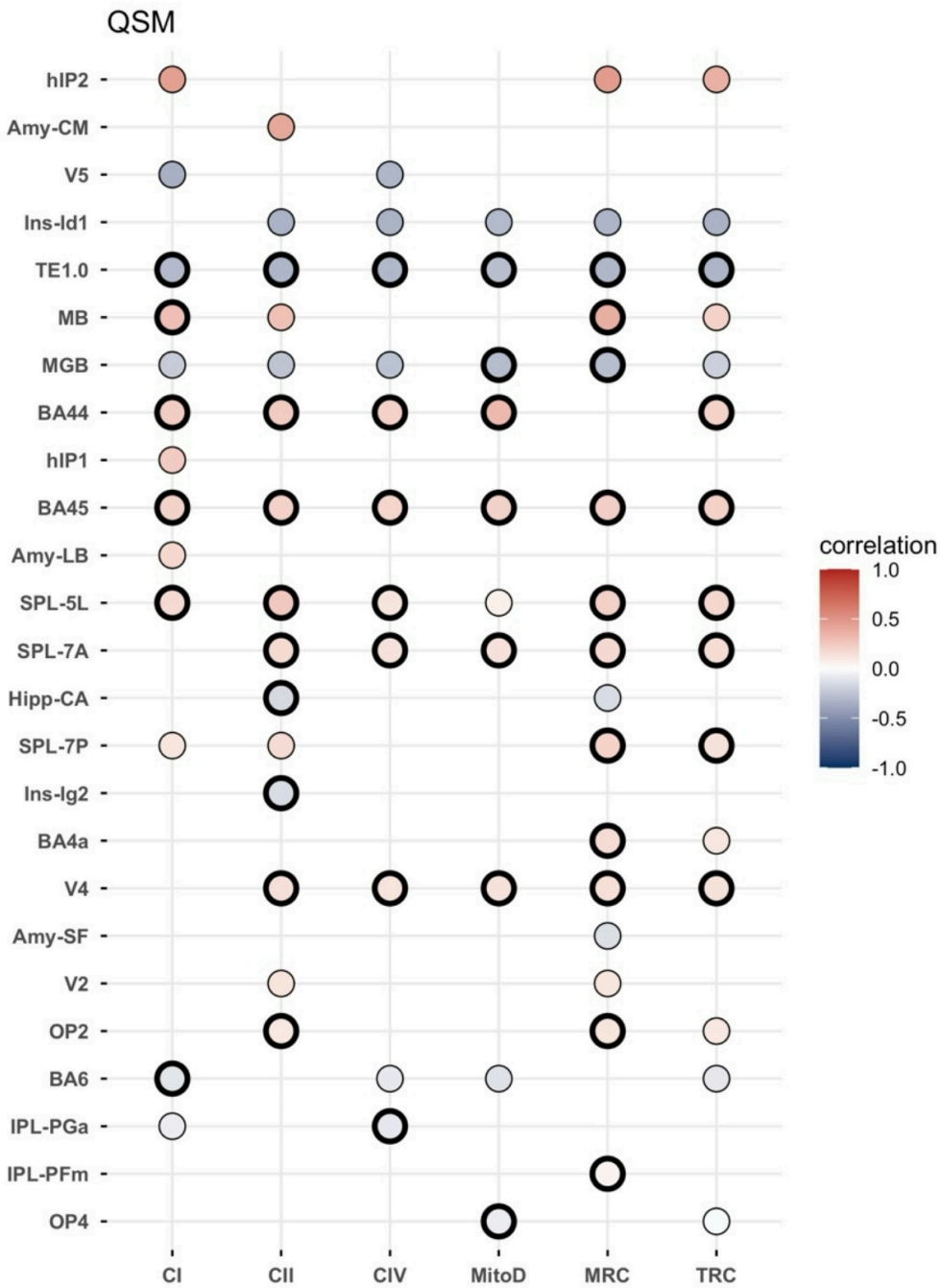


Figure 2

Regional spatial association between PD-derived QSM measures and mitochondrial features within the cortical Jülich-derived ROI(s). Each row corresponds to a ROI, and each column corresponds to a mitochondrial feature. Within each anatomical section, regions are ordered from bottom to top based on the cumulative strength of associations across all mitochondrial features. Missing values represent associations that did not survive the spatial autocorrelation-corrected threshold ($p_{\text{spatial}} < 0.05$). Bold-bordered cells represent associations that survived the false-discovery rate (FDR) corrected threshold ($p_{\text{FDR}} < 0.05$). Amy-CM: amygdala centromedial group. Amy-LB: amygdala laterobasal group. Amy-SF: amygdala superficial group. BA44: Broca's area 44. BA4a: primary motor area BA4a. BA45: Broca's area 45. BA6: premotor cortex BA6. CI, CII, and CIV: electron transport chain protein complexes I, II, and IV. hIP1/hIP2: anterior intra-parietal sulcus 1/2. Hipp-CA: hippocampus cornu ammonis. Ins-Id1: dysgranular insular area 1. Ins-Ig2: granular insular area 2. IPL-PFm: inferior parietal lobule PFm. IPL-PGa: inferior parietal lobule PGa. MB: mammillary body. MGB: medial geniculate body. MitoD: mitochondrial density. MRC: mitochondrial respiratory capacity. OP2/OP4: secondary somatosensory cortex 2/4. PD: Parkinson's disease. PwPD: patients with Parkinson's disease. QSM: quantitative susceptibility mapping. SPL-5L: superior parietal lobule 5L. SPL-7A/SPL-7P: superior parietal lobule 7A/7P. TE1.0: primary auditory cortex TE1.0. TRC: tissue respiratory capacity. V2: visual cortex V2, Brodmann's area 18. V4/V5: visual cortex V4/V5.

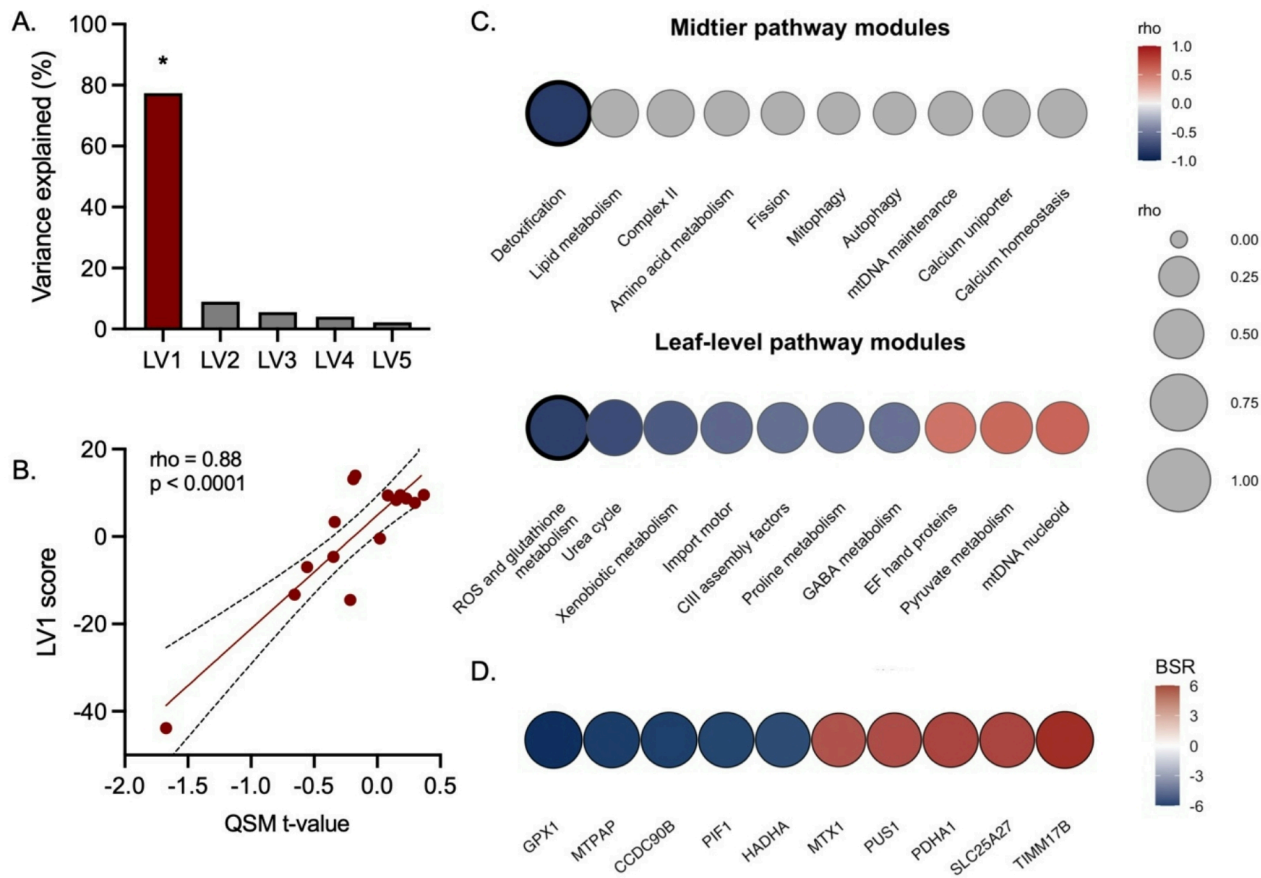


Figure 3

Regional spatial associations between PD-related QSM alterations and mitochondrial gene expression across PD-related HybraPD- and HMAT-derived ROIs. **A.** Percentage of variance explained by each latent variable (LV1-5) from partial least squares regression analysis. **B.** Correlation between LV1 scores and QSM-derived t-values across HybraPD- and HMAT-derived ROIs. Linear fit is indicated by the red line, and the 95% confidence interval is indicated by dashed lines. **C.** Top 10 correlations between mitochondrial pathway module expression and LV1, presented at the midtier and leaf-level of the MitoCarta3.0 functional hierarchy. Correlations are presented from most negative (blue) to most positive (red). Colored dots represent modules at nominal significance ($p < 0.05$), and bold-border dots represent modules at FDR-corrected significance ($p_{\text{FDR}} < 0.05$). Grey-colored dots represent modules with no statistical significance. Dot size represents the magnitude of ρ . **D.** Bootstrap ratio scores (BSR) for the top 10 genes negatively (blue) and positively (red) contributing to LV1, ranked by magnitude. Numerical values represent each gene's BSR. HMAT: Human Motor Template Area. mtDNA: mitochondrial DNA. PD: Parkinson's disease. QSM: quantitative susceptibility mapping. ρ : Spearman's correlation coefficient. ROIs: regions of interest. ROS: reactive oxygen species.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Supplementarymaterial.docx](#)