MitoPD Meeting 2017

Integrative analysis of mitochondrial molecular changes in Parkinson's disease

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Outline

- Mitochondrial gene collection and used omics datasets
- Meta-analyses of GWAS/Exome data and transcriptomic data from blood and brain (comparison to protein expression & qPCR data)
- Pathway and causal reasoning analysis of mitochondrial alterations
- Summary

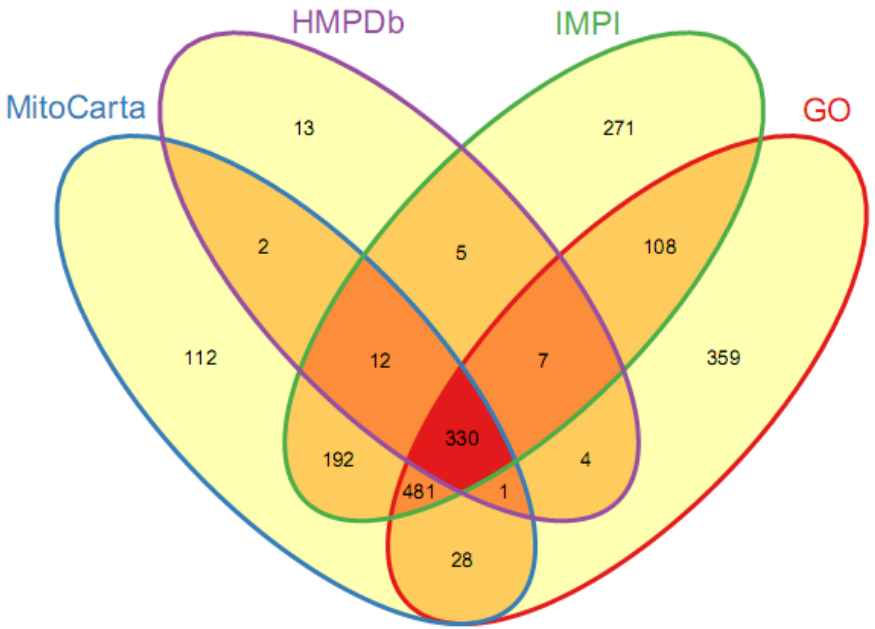
Updated collection of mitochondrial genes

→ Collect current genes from mitochondrial localization databases MitoCarta 2.0, IMPI (2016/2), HMPDb and MitoPhenome

→ Add genes from Gene Ontology term “Mitochondrion“

→ Convert identifiers to standard HGNC gene symbols

→ Retain origin annotations to enable post-filtering of likely false-positives



Transcriptomics datasets (Brain)

Datasets obtained using laser-capture microdissection (LCM):

Study	Cell type / stage	Conditions	Affymetrix chip
F. Simunovic et al., Brain, 2009	SN, post mortem	PD (10), control (9)	U133A
B. Zheng et al., Sci Transl Med, 2010	SN, post mortem	PD (10), control (8)	U133 Plus 2.0

Datasets obtained without LCM:

Study	Cell type / stage	Conditions	Affymetrix chip
Y. Zhang et al., Am J Med Genet B Neuropsychiatr Genet, 2005	multiple brain regions, post mortem	PD (40), control (53)	U133A
T. G. Lesnick et al., PloS Genet, 2007	SN, post mortem	PD (16), control (9)	U133 Plus 2.0
L. B. Moran et al., Neurogenetics, 2006	SN + frontal gyrus, post mortem	PD (29), control (18)	U133A
Roth et al., GEO dataset GSE7307	SN, post mortem	PD (6), control (9)	U133 Plus 2.0
B. Zheng et al., Sci Transl Med, 2010	SN, post mortem	3 datasets: PD (6), control (5); PD (8), control (9)	2x U133A

→ only samples from the substantia nigra (SN) are used for statistical analysis

→ LCM and non-LCM datasets displayed significant correlations and were thus both used for meta-analyses

Transcriptomics datasets (Blood)

Sporadic/idiopathic PD :

Study	Cell type, target	Conditions (*drug-naive)	Platform
DeNoPa	Whole blood, mRNA	PD* (50), control (50)	Affy U133A 2.0
GENEPARK	Whole blood, mRNA	PD (50), control (50)	Affy U133A 2.0
C. R. Scherzer et al., PNAS, 2011	Whole blood, mRNA	PD (50), control (21), other neurodeg. disorders (33)	Affy U133A
Calligaris et al., 2015	Whole blood, mRNA	PD* (40), control (19)	Affy U133A 2.0
L. Soreq et al., PLoS Comp. Biol., 2014	Leukocytes, mRNA	PD (3), control (3)	ABI SOLiD 3.0, RNAseq

LRRK2 and Parkin patients:

Study	Cell type, target	Conditions	Platform
MEFOPA	Whole blood, mRNA	LRRK2 (40), control (23)	Affy HG U219
MEFOPA	Whole blood, mRNA	PARK2 (28), control (23)	Affy HG U219
Chikina et al., 2014 (GSE62469)	Whole blood, mRNA	LRRK2 (34), control (32)	NanoString nCounter

Overview: Genetic datasets

GWAS data:

Study	Conditions	Platform
TREND	PD (1298), control (883)	NeuroX chip
LANDSCAPE (Dutch cohort)	PD (772), control (2024)	Illumina Human660W-Quad beadchip
PPMI	PD (383), SWEDD (58), control (178)	NeuroX chip
Nalls et al., Nat. Genet., 2014 (pre-processed data from 15 independent GWAS datasets of European descent)	PD (13708), control (95282)	Multiple Illumina GWAS platforms

Exome sequencing data:

Study	Conditions	Platform
PPMI	PD (391), SWEDD (60), control (178)	Illumina

Mitochondrial genes in the meta-analysis of GWAS data

Determine mitochondrial genes containing SNPs with putative PD associations from the meta-analysis of GWAS datasets (significance threshold: p < 1E-05)

Gene Symbol	Description	MitoFDR
MCCC1*†	methylcrotonoyl-CoA carboxylase 1 (alpha)	0
BCKDK*†	branched chain ketoacid dehydrogenase kinase	0
MALSU1	mitochondrial assembly of ribosomal large subunit 1	0
SPATA19	spermatogenesis associated 19	0.01
PCBD2	pterin-4 alpha-carbinolamine dehydratase/dimerization cofactor of hepatocyte nuclear factor 1 alpha (TCF1) 2	0.01
NDUFAF2	NADH dehydrogenase (ubiquinone) complex I, assembly factor 2	0.01
CHCHD5	coiled-coil-helix-coiled-coil-helix domain containing 5	0.01
ABCB9†	ATP-binding cassette, sub-family B (MDR/TAP), member	0.03
VARS	valyl-tRNA synthetase	0.239
GRN	granulin	0.708
SLC44A1	solute carrier family 44 (choline transporter), member 1	0.767
CSNK2B	Casein Kinase 2 Beta	0.826
CCAR2	cell cycle and apoptosis regulator 2	0.831
GRAMD4‡	GRAM Domain Containing 4	0.887

* previously shown to contain genome-wide significant SNPs, Nalls et al., 2014
Differentially expressed in transcriptomics meta-analysis: †whole-blood, ‡brain

Mitochondrial genes in the analysis of Exome seq. data

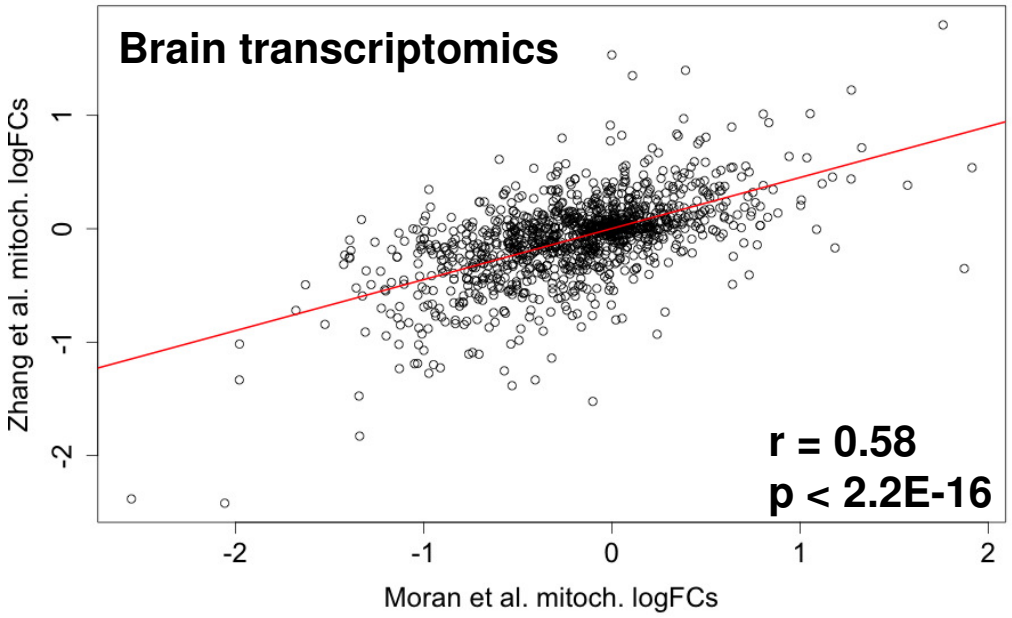
Apply multiple variant association tests to find case/control differences in mitochondrial genes → limited power: need to use high p-value cut-off ($p < 0.01$)

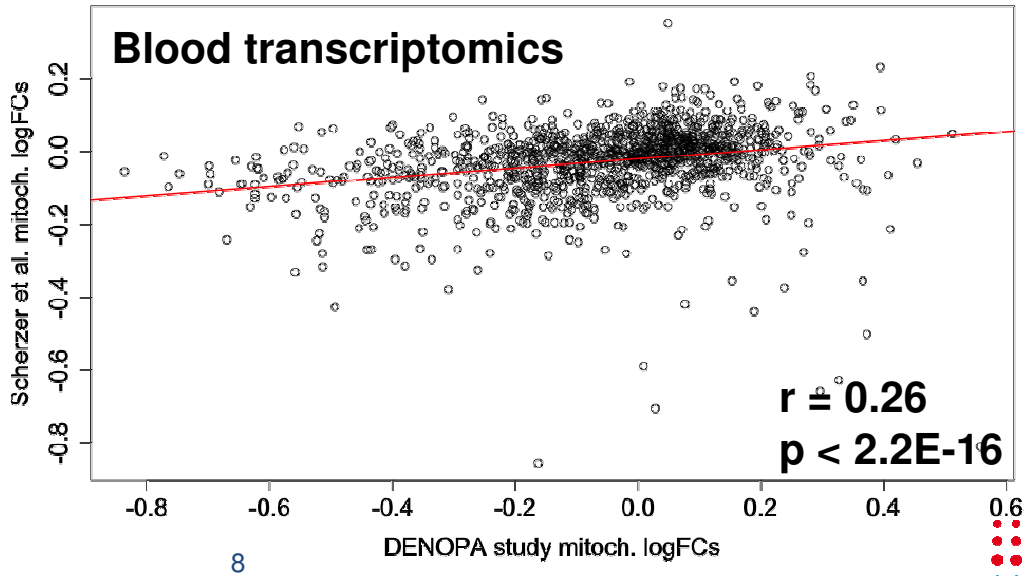
Statistic	Candidate Genes (identified at least 3 times across different tests)
Burden test	MRPL53, SIRT3, (not in MitoCarta: YWHAH†)
C-alpha test	SIRT3, ATAD3B, ATP5J, BNIP3, CMC1, CMPK2, DIABLO‡, FASTKD2, GRSF1, LAMC1, LAP3, LETM1, LYRM1, LYRM9‡, MFN1, MRPS11‡, NDUFA9‡, PMPCB, PYCR1, RECQL4, SDHAF2, SETD9, TRMU, (not in MitoCarta: ATP6V1E1, DAO, ERBB4‡, GLUL, GLYATL3, GSK3B, HSD3B2, KRT71, LRRC24, MAATS1, MARCKS, NPC1, PI4K2A‡, RAD51C)
FRQWT test	MRPL53, (not in MitoCarta: YWHAH†)
SUMSTAT test	ATAD3B, ATP5J, BNIP3, CMC1, CMPK2, DIABLO‡, FASTKD2, GRSF1, LAMC1, LAP3, LETM1, LYRM1, LYRM9‡, MFN1, MRPS11‡, NDUFA9‡, PMPCB, PYCR1, RECQL4, SDHAF2, SETD9, TRMU (not in MitoCarta: ATP6V1E1, DAO, ERBB4‡, GLUL, GLYATL3, GSK3B, HSD3B2, KRT71, LRRC24, MAATS1, MARCKS, NPC1, PI4K2A, RAD51C)
UNIQ test	MRPL53, (not in MitoCarta: YWHAH†)
VT test	MRPL53, SIRT3, (not in MitoCarta: YWHAH†)
SKAT test	SIRT3, ATAD3B, ATP5J, BNIP3, CMC1, CMPK2, DIABLO‡, FASTKD2, GRSF1, LAMC1, LAP3, LETM1, LYRM1, LYRM9‡, MFN1, MRPS11‡, NDUFA9‡, PMPCB, PYCR1, RECQL4, SDHAF2, SETD9, TRMU (not in MitoCarta: ATP6V1E1, DAO, ERBB4‡, GLUL, GLYATL3, GSK3B, HSD3B2, KRT71, LRRC24, MAATS1, MARCKS, NPC1, PI4K2A‡, RAD51C)

Genes identified at least 4 times are highlighted in matching colors
Differentially expressed in transcriptomics meta-analysis: †whole-blood, ‡brain

Correlation between mitochondrial changes across studies

- Significant correlations of PD vs. control log. fold changes of mitochondrial genes across transcript-omics studies for the same tissue/body fluid
- Correlations between brain (substantia nigra) datasets higher than between whole-blood datasets
- Correlations between brain and blood datasets not significant





Top-ranked genes from brain/blood meta-analysis

- Determine genes with significant differential expression in the meta-analyses (Marot et al., 2011) for brain (substantia nigra) and whole-blood (FDR < 0.05)
- All genes have a MitoCarta FDR-score ≤ 0.03 for mitochondrial localization

Gene symbol	Description	Brain Z-score	Brain FDR	Blood Z-score	Blood FDR
AFG3L2	AFG3 like matrix AAA peptidase subunit 2	-2.7	0.027	-3.2	0.042
MCCC2	methylcrotonoyl-CoA carboxylase 2	-3.1	0.013	-3.8	0.022
NNT	nicotinamide nucleotide transhydrogenase	-3.0	0.015	-3.8	0.023
SLC25A17	solute carrier family 25 member 17	-4.4	<0.001	-3.2	0.041
TFB1M	transcription factor B1, mitochondrial	-3.1	0.012	-3.4	0.041
LRPPRC	leucine rich pentatricopeptide repeat containing	-3.5	0.004	-3.3	0.041
NGRN	neugrin, neurite outgrowth associated	-2.8	0.025	-3.4	0.038
PDSS2	prenyl (decaprenyl) diphosphate synthase, subunit 2	-4.1	0.001	-4.0	0.019

Relevant information in the literature for top-ranked genes

1) **AFG3L2:** Subunit of a metalloprotease involved in maintenance of the mitochondrial proteome; loss of AFG3L2 causes complex I deficiency and increased sensitivity to oxidative stress in hereditary spastic paraplegia (Atorino & Casari, 2003)

2) **MCCC2:** Subunit in carboxylase protein complex with MCCC1, a gene with a known PD risk factor mutation (rs12637471, PDGENE Meta-P: 5.4E-22, Nalls et al., 2014)

3) **NTT:** Encodes integral protein of inner mitochondrial membrane, couples hydride transfer between NAD(H) and NADP(+) to proton translocation across the membrane.

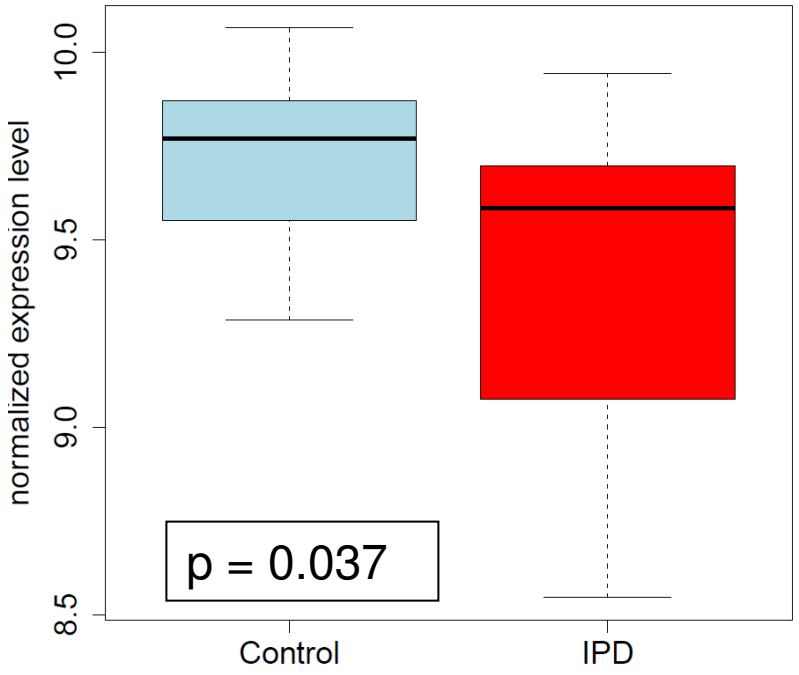
4) **TFB1M:** Encodes a dimethyltransferase that methylates the conserved stem loop of mitochondrial 12S rRNA (necessary for mtDNA transcription, Falkenberg et al., 2002)

5) **LRPPRC**: transcriptional regulator of both nuclear and mitochondrial genes

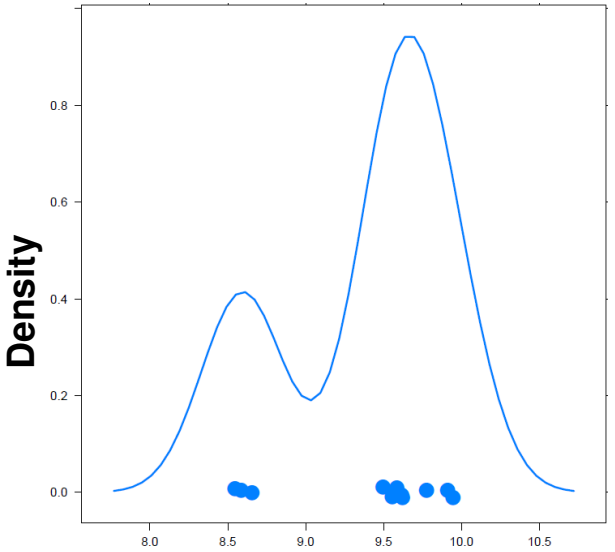
6) **PDSS2**: synthesizes prenyl side-chain of coenzyme Q10 (CoQ) in the respiratory chain; defects in this gene are a cause of CoQ deficiency (Lopez et al., 2012)

qPCR validation and check for multimodal distribution in PD

Validate top-ranked differentially expressed genes from the meta-analysis of microarray datasets for whole-blood samples using qRT-PCR (11 patients and 11 age- and gender-matched controls → validated qualitative changes for 26 out of 36 genes, **most significant mitochondrial gene: MRPL42**



MRPL42



Normalized gene expression in PD

Dip test: p = 0.14

MRPL42

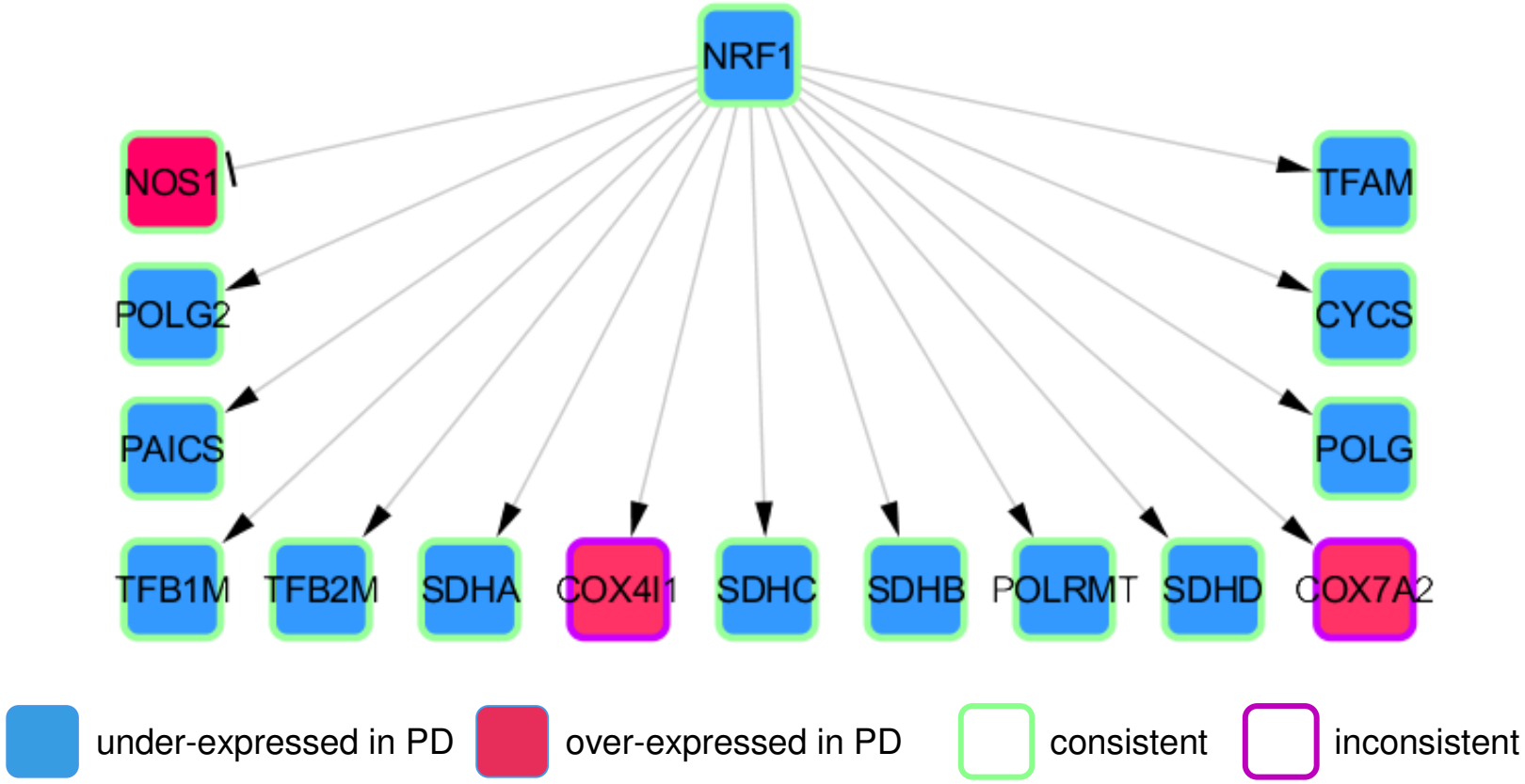
Alterations in mitochondrial processes and complexes

Identify mitochondrial processes and complexes (Gene Ontology, CORUM) with differential median expression in whole-blood transcriptomics data from IPD patients & controls (only processes with min. of 10 mapped genes):

Mitochondrial process / complex / GO term	**logFC**	**FDR**
regulation of mitochondrial translation	-0.95	2.0E-09
mitochondrion morphogenesis	-0.58	1.1E-05
mitochondrial calcium ion homeostasis	-0.78	2.4E-05
establishment of mitochondrion localization	-0.46	7.2E-04
F1F0-ATP synthase, mitochondrial	-0.36	3.4E-03
mitochondrion distribution	0.50	5.5E-03
mitochondrial protein complex	-0.25	5.5E-03
mitochondrial electron transport nadh to ubiquinone	-0.29	5.7E-03
mitochondrial envelope	-0.19	5.7E-03
protein targeting to mitochondrion	-0.26	7.0E-03
inner mitochondrial membrane protein complex	-0.28	7.0E-03

Causal reasoning analysis of mitochondrial transcriptomics

Use causal reasoning analysis (Chindelevitch, 2012) of differentially expressed mitochondrial genes from the blood transcriptomics meta-analysis to identify alterations in regulators that can explain the observed downstream changes.



Summary

- Joint analysis of GWAS/exome and transcriptomics data identifies mitochondrial genes with putative PD-associated SNPs and significant alterations in the transcriptomics meta-analyses

- Mitochondrial genes with shared expression alterations in meta-analyses for blood and brain transcriptomics data were determined and compared to representative qPCR and protein expression measurements to find the most robust changes

- Mitochondrial processes with differential median gene expression were ranked and a causal reasoning analysis identified changes in NRF1 expression as a potential key regulatory event behind downstream mitochondrial alterations in PD

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