Identification of novel autoantibody signatures related to non-motor symptoms in individuals with high-risk of Parkinson's Disease, using KREX-based functional protein microarrays. A cross-sectional study.

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Summary

We used protein microarrays with 1600+ correctly folded proteins to identify an autoantibody signature (sensitivity = 0.905, specificity = 0.706) that can identify which high risk patients that are likely to phenoconvert to Parkinson's Disease.

Background

Parkinson's disease (PD) is a devastating neurodegenerative disease with unknown etiopathogenesis, characterized by the degeneration of dopaminergic neurons in the substantia nigra. There is currently no established molecular test available to diagnose PD. A panel of diagnostic biomarkers would allow for effective detection, classification, and treatment of PD before irreversible damage.

Current research indicates a potential autoimmune component in PD. Accumulating evidence suggests that PD begins years before clinical motor symptoms are detectable. Autoantibodies are being investigated as potential biomarkers for diagnosis and prognosis of disease including in neurological disorders (Prüss, 2021). As autoantibodies can often be detected years before clinical manifestations appear, they show great promise as useful biomarkers.

The PARS cohort used in this study consists of plasma samples from at risk and no risk individuals at the prodromal stage which was recruited based on the smell identification (olfactory dysfunction) and DAT imaging testing. These individuals were evaluated longitudinally with annual clinical examination and biannual DAT imaging over the span of 4 years (Jennings et al, 2017). We identified potential autoantibody biomarkers for the early diagnosis of PD from 145 samples consisting of hyposmic (HYP) and normosmic participants (NRM), further classified based on dopamine transporter (DAT) deficit with or without phenoconversion to PD. Serum samples were assayed to detect autoantibodies with the KREX-based i-Ome Protein Microarray, which contains 1600+ natively folded proteins for highly specific antibody binding.

Machine learning-based classification identified 116 autoantibodies biomarkers for stratifying the groups within the PARS cohort. The predictive value of the biomarkers was evaluated using ROC curve analysis. Consequently, a 22-biomarker signature was found to identify the HYP, DAT deficit group that phenoconverted to PD with a sensitivity of 90.5% and specificity of 70.6%. Amongst the identified biomarkers are proteins involved in immune regulation, protein degradation (RNF7), cell death (RIPK1) and vitamin D regulation (VDR), all potentially involved in the progression of PD.

With a complex, heterogenous disease such as PD, it is unlikely that an individual biomarker with sufficient predictive value will be found. We have discovered a biomarker signatures that are able to identify early PD in high-risk patients, thereby opening the possibility for early treatment and monitoring disease prognosis. With a larger sample pool and longitudinal monitoring, we hope to further validate and refine these signatures.

Study Design

We analyzed 145 samples from the PARS cohort. No participants had dementia at the time of enrolment. In the hyposmic/ DAT deficit cohort, 17 patients phenoconverted to PD within 4 years. In the normosmic cohort, none of the participants exhibited a DAT deficit or went on to phenoconvert to PD. Four comparisons were analyzed as outlined in Figure 1 and Table 1. Seven pooled normal samples were included as technical replicates (not shown).

Figure 1

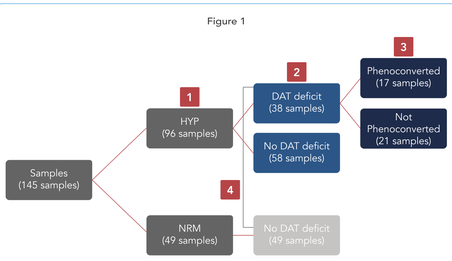

Figure 1. Flow chart showing the subsets of 145 PARS cohort patients. Machine learning analysis comparisons are labeled 1-4. HYP: hyposmic, NRM: normosmic, DAT: dopamine transporter

Table 1. Table detailing machine learning analysis comparisons with designated cases and controls.

Groups of Comparison		
1	HYP **vs.** NRM	96 (cases) **vs.** 49 (controls)
2	HYP: DAT Deficit **vs.** HYP: No DAT Deficit	38 (cases) **vs.** 58 (controls)
3	HYP: DAT Deficit: Phenoconverted to PD **vs.** Not Phenoconverted to PD	17 (cases) **vs.** 21 (controls)
4	HYP: DAT Deficit **vs.** NRM, No DAT Deficit	38 (cases) **vs.** 49 (controls)

Methods

We assayed 145 plasma samples from the PARS cohort to detect autoantibodies on the i-Ome protein microarray, which contains more than 1600 full-length human antigens. Seven pooled normal serum samples were included as a technical control.

Figure 2

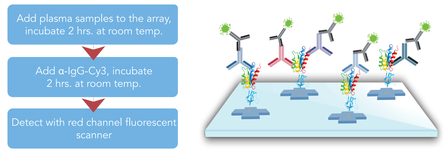

Figure 2. Graphic outlining the experimental steps of the autoantibody profiling assay conducted on the i-Ome protein microarray.

Figure 3

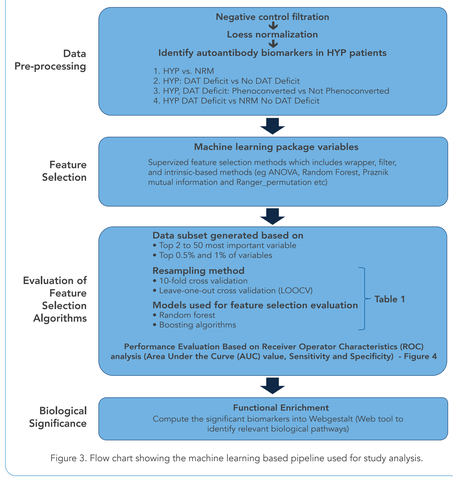

Figure 3. Flow chart showing the machine learning based pipeline used for study analysis.

Results

Whole Model Performance (WMP) - ROC curves showed high diagnostic accuracy

Following model-based evaluation, the best panels of features for stratifying between the control and pre-symptomatic groups were selected based on the highest diagnostic accuracy (represented by Area Under the Curve (AUC), sensitivity (Sens), and specificity (Spec)).

Figure 4

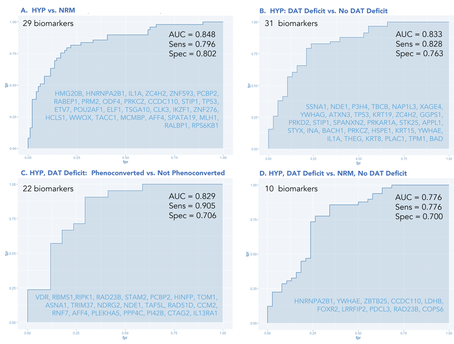

Figure 4 A-D. ROC curve for each of the 4 comparisons. AUC, sensitivity and specificity values are listed along with a list of biomarkers.

Summary of ML Analysis - Consistent diagnostic accuracy observed.

A total of 116 non-redundant biomarkers were identified from the aforementioned analyses. Comparison number 5 was added to compare the non-DAT deficient patients who are and are not hyposmic(true negative).

Table 2. Summary of machine learning analysis of 5 comparison groups. The number of biomarkers, AUC, Sens & Spec are listed for each analysis type.

#	Subgroup Comparison	Model	# of Biomarkers	AUC	Sens	Spec
1	HYP vs NRM n = 96 vs. n = 49	Model based (LogitBoost/10CV/Ranger impurity)	29	0.657	0.740	0.555
		ROC WMP (All biomarkers)	29	0.848	0.796	0.802
		ROC WMP (Elevated in Cases)	11	0.643	0.714	0.51
2	HYP: DAT Deficit vs. No DAT Deficit n = 38 vs. n = 58	Model based (LogitBoost/10CV/Praznik JMI)	31	0.736	0.533	0.843
		ROC WMP (All biomarkers)	31	0.833	0.828	0.763
		ROC WMP (Elevated in Cases)	29	0.826	0.793	0.763
		WMP (Significant biomarkers) – p-value ≤0.05	14	0.732	0.776	0.658
3	HYP, DAT Deficit: Phenoconverted vs. Non-Phenoconverted n = 17 vs. n = 21	Model based (Random Forest/10CV/Praznik MIM)	22	0.8583	0.8333	0.800
		ROC WMP (All biomarkers)	22	0.829	0.905	0.706
		ROC WMP (Elevated in Cases)	2	0.507	0.524	0.529
4	HYP, DAT Deficit vs. NRM, No DAT Deficit n = 38 vs. n = 49	Model based (LogitBoost/LOOCV/Ranger impurity)	10	0.709	0.632	0.796
		ROC WMP (All biomarkers)	10	0.776	0.776	0.7
		ROC WMP (Elevated in Cases)	7	0.653	0.673	0.658
5	HYP, No DAT Deficit vs. NRM, No DAT Deficit n = 58 vs. n = 49	Model based (LogitBoost/10CV/party_cforest.importance)	49	0.736	0.770	0.715
		ROC WMP (All biomarkers)	49	0.955	0.939	0.897
		ROC WMP (Elevated in Cases)	12	0.668	0.592	0.724
			116			

Functional Enrichment

The Uniprot ID for each of the 116 significant biomarkers was used as an input into WebGestalt, a web-based application that identifies classes of relevant bioentities (including functionalities, gene ontology and biological pathways) associated with genes or proteins (Thanati et al., 2021). The collection of 1609 proteins on the i-Ome Protein Microarray was used as the reference list for this analysis. Top 5 pathways (by p-value) and 5 neurologically (p-value and keyword based) are shown in Table 3.

Figure 5

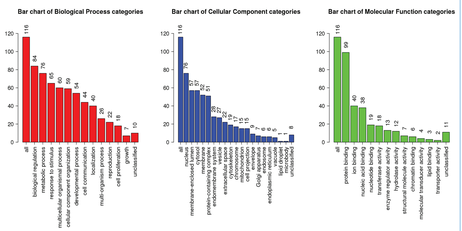

Figure 5. The top biological processes, cellular compartments, and molecular functions of the 116 proteins identified in the cohort comparison analyses are shown. The figure was generated with WebGestalt and the numbers above the bars represent the number of biomarkers found in each category.

Table 3. List of neurologically related pathways found in the functional enrichment analysis of the 116 biomarkers.

Top 5 pathways (ranked based on p-value)

No	Description	Size	Overlap	Expect	Enrichment Ratio	P-value	Database	Protein ID
1	Loss of Nlp from mitotic centrosomes	16	6	1.15	5.20	0.0005	Pathway_Reactome	HSP90AA1;NDE1; SDCCAG8;SSNA1;YWHAE; YWHAG
2	Loss of proteins required for interphase microtubule organization from the centrosome	16	6	1.15	5.20	0.0005	Pathway_Reactome	HSP90AA1;NDE1; SDCCAG8;SSNA1;YWHAE; YWHAG
3	Organelle assembly	103	17	7.43	2.29	0.0007	Geneontology_ Biological_Process	HSP90AA1;KRT19;KRT8; MAPK9;MLH1;NDE1; PIP4K2B;PRKAA2;PRKAR1A; RFX3;SDCCAG8;SSNA1; STAM2;TPM1;TRIM37; YWHAE;YWHAG
4	Sarcomere organization	7	4	0.51	7.92	0.0008	Geneontology_ Biological_Process	KRT19;KRT8;PRKAR1A; TPM1
5	AURKA Activation by TPX2	17	6	1.23	4.89	0.0008	Pathway_Reactome	HSP90AA1;NDE1; SDCCAG8;SSNA1;YWHAE; YWHAG

Top 5 pathways (keyword based, p-value ranked)

No	Description	Size	Overlap	Expect	Enrichment Ratio	P-value	Database	Protein ID
1	Myelin sheath	36	6		2.3095	0.0404	Cellular component	ENO2;HSP90AA1;INA; PRKCZ;STIP1;YWHAG
2	Substantia nigra development	12	3		3.4643	0.0498	Biological process	INA;NDRG2;YWHAE
3	Neuronal cell body	39	6		2.1319	0.0568	Cellular component	DNAJB1;ENO2;HOMER2; HSP90AA1;PRKAA2;PRKCZ
4	Regulation of postsynaptic membrane neurotransmitter receptor levels	7	2		3.9592	0.0854	Biological process	PRKCZ;YWHAE
5	Long-term memory	7	2		3.9592	0.0854	Biological process	PRKCZ;RPS6KB1

Disease Association

A list of Parkinson's disease related genes was retrieved from https://www.genecards.org and was used as a reference for disease association. Sixty of the 116 biomarkers were found to be related to genes tied to Parkinson's disease.

Figure 6

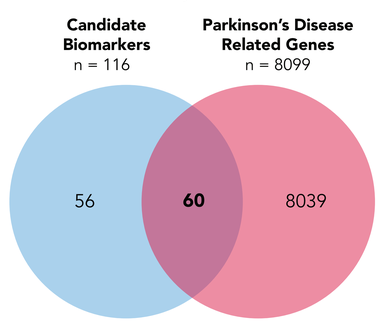

Figure 6. Venn diagram highlighting the overlap of biomarker genes found within the Genecards collection of Parkinson's disease related genes.

Table 4. List of the 60 biomarkers found within the Genecards collection of Parkinson's disease related genes.

Parkinson's Disease Associated Biomarker				
AFF4	FTH1	LDHB	PYCR1	SUB1
APPL1	GAGE1	MAPK9	RABEP1	TARDBP
ATXN3	HMG20B	MCMBP	RAD23B	TBCB
BACH1	HNRNPA2B1	MLH1	RALBP1	TMEFF2
BAD	HOMER2	MLX	RFX3	TOM1
CDK16	HSP90AA1	NDE1	RIPK1	TP53
CD01	HSPE1	NDRG2	RPS6KB1	TPM1
CLK3	IL13RA1	ODF4	SDCCAG8	VDR
DFFA	IL1A	PCBP2	SPATA19	WWOX
DMPK	INA	PRKAA2	STIP1	YWOX
DNAJB1	KRT15	PRKCZ	STK25	YWHAG
ENO2	KRT8	PSME3	STYX	ZSCAN18

Biomarkers Elevated in Control Samples

The same analysis pipeline as previously described was conducted on the unfiltered dataset (n=1609 antigens) for downstream machine learning analysis following data pre-processing. Interestingly, we identified 23 biomarkers significantly elevated in controls.

Table 5. List of the 23 biomarkers found to be elevated in control samples.

Comparison Analysis	Biomarker	p-value Range
HYP vs NRM	AK9, CDK8, PTGER3, UPT1	0.0075 – 0.041
HYP: DAT Deficit vs No DAT Deficit	FAM50B, KAT2A, NDE1, CYCS, MLPH, ELOA, FES, FGFR1, MAP3K14, MAP4K5, PTPN6, STAT4, TRAF2, NEDD9, XBP1	0.00076-0.041
HYP, DAT Deficit: Phenoconverted vs Not Phenoconverted	DSTYK	0.048
HYP, DAT Deficit vs NRM, No DAT Deficit	CCDC110, CXCR6, ZNF19	0.0054-0.018

Discussion and Conclusions

We identified 22 biomarkers that can stratify between patients with hyposmia and a DAT Deficit who phenoconvert to PD and those who do not phenoconvert with a sensitivity of 90.5 % and specificity 70.6 %. In the 4 comparisons performed, we identified 116 non-redundant features, each group with a unique set of features.

We explored the biological significance of these PD related features and found that immune response mediator components were prominent in the top biological pathways identified including those involved in substantia nigra development. Additionally, we have also identified proteins that are involved in other neurological processes including long-term memory, regulation of postsynaptic membrane neurotransmitter receptor levels, regulation of the neuronal cell body, neuron projection and regulation of myelination.

Naturally occurring autoantibodies may act as a clearance or blocking mechanism to pathogenic proteins that contribute to progressive brain disorders such as PD and MSA and that the decline of autoantibodies was detected in plasma from PD patients, relative to healthy controls (Brudek et al., 2017). Similarly, the autoantibody response to 23 antigens found elevated in control relative to cases suggest a potential protective function of autoantibodies in asymptomatic individuals. This may provide insight towards immunotherapeutic strategies.

Autoantibodies are detectable many years before disease symptoms are observed. There is an ever-growing list of disease areas including lupus (Yaniv et al., 2015), rheumatoid arthritis (Burska et al., 2014), Graves' disease (Chazenbalk et al., 2002), Sjogren's syndrome (Fayyaz et al., 2016) and autoimmune hepatitis (Zachou et al., 2004) where potential autoantibody biomarkers have been identified. Screening for autoantibody biomarkers of PD from liquid biopsies has the potential to enable new approaches to early disease diagnosis, drug response monitoring and identification of novel therapeutic targets.

The performance of the 116 identified biomarkers will be further validated experimentally in an independent PD cohort from samples collected at longitudinal timepoints. The next phase of this study will focus on profiling autoantibody responses in samples from patients with progressive PD using a custom protein microarray consisting of the 161 features identified from this study. This array may enable enrichment and stratification of PD patients prior to enrolment in clinical trials, as a route to more quickly prioritize, fail, or re-route drug candidates based on small initial cohorts. In addition, this array may allow for further enrichment of clinical trials by identifying autoantibody signatures that correspond to responder and non-responder phenotypes, as well as to the risk of developing adverse drug reactions.

References

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