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Proteomic analysis of tear fluid reveals disease-specific patterns in patients with Parkinson's disease – A pilot study

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ABSTRACT

Background: The diagnosis of Parkinson's disease (PD) is still challenging and biomarkers could contribute to an improved diagnostic accuracy. Tear fluid (TF) is an easily accessible body fluid reflecting pathophysiological changes in systemic and ocular diseases and is already used as a biomarker source for several ophthalmological disorders. Here, we analyzed the TF of patients with PD and controls (CTR) to describe disease-related changes in TF and identify putative biomarkers for the diagnosis of PD.

Methods: Unstimulated TF samples of a pilot cohort with 36 PD patients and 18 CTR were collected via Schirmer tear test strips and then analyzed via a Bottom-up liquid chromatography electrospray ionization tandem mass spectrometry (BULCMS) workflow, followed by functional analysis encompassing protein-protein interaction as well as cellular component and pathway analysis.

Results: BULCMS analysis lead to the identification of 571 tear proteins (false discovery rate, FDR < 1%), whereby 31 proteins were exclusively detected in the PD group and 7 only in the CTR group. Whereas 21 proteins were significantly increased in the PD versus CTR groups, 19 proteins were significantly decreased. Core networks of proteins involved in immune response, lipid metabolism and oxidative stress were distinctly altered in PD patients.

Conclusions: To our best knowledge, this is the first description of TF proteome in PD patients. Tear protein level alterations suggest the contribution of different disease-related mechanisms in ocular pathology in PD and propose candidate proteins to be validated as potential biomarkers in larger cohorts.

1. Introduction

Until today, the diagnosis of PD is mainly based on the evaluation of distinctive clinical features and the differentiation from atypical parkinsonian syndromes and PD mimics. The accuracy of diagnosis is limited [1]. Although several body fluids, e.g. blood and cerebrospinal fluid (CSF), have been intensively analyzed, a persuasive body fluid-derived biomarker has not yet been established for the diagnosis of PD [2].

Non-motor symptoms (e.g. hyposmia and REM sleep behavior disorder) appear many years before motor dysfunction in PD, and could thus be exploited as early diagnostic markers [3]. Significantly decreased tear volumes are a common finding in PD patients because of an exocrine tear glands dysfunction that is noticeably associated to disease stage and duration [4]. A reduced blink rate due to hypokinesia of the facial muscles [5] and a dysfunction of the lacrimal glands due to a lack of innervation likely contribute to this phenomenon [6,7]. In addition, as a result of their parasympathetic innervation by the superior salivatory nucleus, lacrimal glands have a close anatomical relationship to the brainstem, which is affected early in PD pathogenesis [6]. Recent

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reports also described alpha-synuclein (α-syn) pathology in salivary glands [8], which share a similar histopathological structure and innervation, raising the question whether early pathological changes could also be observed in the lacrimal glands.

Only little is known on the composition of tear fluid in neurodegenerative disorders: Elevated levels of tumor necrosis factor α have been described in a small PD cohort and were correlated to inflammation at the ocular surface [9]. A recent study in Alzheimer's disease (AD) patients identified a combination of proteins, which were able to discriminate between AD and controls with moderate sensitivity and specificity [10].

In this study, we analyzed TF from PD patients and controls (CTR) using mass spectrometry (MS)-based proteomics and identified several differentially regulated proteins, which could be validated as a biomarker for PD and shed more light on the role of the lacrimal glands in this disease.

2. Experimental procedures

2.1. Study cohort

Between July 2014 and May 2015, 36 patients with idiopathic Parkinson's disease (PD) (26 men, 10 women) and 18 control patients (CTR) (11 men, 7 women) met the inclusion criteria and were included in this pilot study. The CTR patients showed no sign of neurodegenerative, neuroinflammatory or acute ischemic central nervous diseases (e.g. headache, dizziness or functional disorders). Consecutively acquired TF samples were included, no specific method of patient randomization was employed. All donors were either in- or outpatients in the Department of Neurology at the University Medical Center Göttingen. The population is described in Table 1 and in more detail in the supplement.

2.2. Tear fluid collection

After assessment of the anterior ocular status of each patient, unstimulated TF was collected from the inferior meniscus of both eyes by insertion of a standard Schirmer filter paper strip. A more detailed description is found in the supplement. After 5 min, the length of the moist part of the strip was measured. The collected tear samples were immediately frozen and stored at −80 °C until further analysis.

2.3. Bottom-up LC ESI MS/MS proteomics, bioinformatics and statistics

Briefly, protein was extracted from Schirmer tear test strips, and the protein content recorded by use of a bicinchoninic acid assay. In PD and CTR, four identical group corresponding pool replicates, each containing 50 µg of total tear protein content were prepared for BULCMS analysis.

The detected proteins were then identified using MaxQuant. LFQ normalized peak intensities were used for statistical analysis of protein levels by the use of Statistica. The results of differential protein abundance were annotated, visualized and an analysis of protein-protein interaction by Cytoscape was performed. A more detailed description is given in the supplement.

3. Results

3.1. Assessment of basic tear secretion and total tear protein concentration

Secreted tear volume is a known factor influencing tear protein concentration. PD patients were previously shown to display altered tear secretion [4]. We therefore first assessed basic tear secretion (BST) and total tear protein concentration (TPC): BST of tear volumes showed a trend for reduced running lengths in the PD group with a mean length of 11.31 ± 9.33 mm/5min compared to 17.11 ± 12.06 mm/5min in the CTR group (p = 0.092) (Fig. 1A). In the PD cohort, 49 out of 70 (70%) Schirmer tear test strips showed a running length of < 15 mm/5min (defined as pathologically reduced run length), while only 16 out of 34 (47.06%) test strips in the CTR group were below the normal range (p = 0.03) (Fig. 1B). There were no significant differences in TPC between both cohorts, measured with BCA assay (Fig. 1C). The correlation analysis displayed strong positive correlation of BST to TPC values in the PD subgroup ($r_{Pearson(P)} = 0.71$, $p < 0.0001$) and a trend for a moderate correlation in CTR patients ($r_P = 0.42$, $p = 0.0826$) (Fig. 1D and E).

We next analyzed the correlations between BST or TPC to multiple demographic and clinical parameters. Expectedly, we observed significant positive correlations between disease duration and UPDRS part III score ($r_P = 0.56$, $p < 0.001$), disease duration and mHY scale ($\tau = 0.40$, $p = 0.003$) as well as UPDRS part III and mHY ($\tau = 0.5$, $p < 0.001$). There was a trend for a moderate negative correlation of mean BST and PD-NMS ($r_P = -0.31$, $p = 0.071$). BST and TPC did not significantly correlate to other clinical or demographic parameters (Supplementary Fig. S1). Interestingly, there were no significant correlations between age, BST or TPC in the CTR group (Supplementary

Table 1
Demographic data and clinical characteristics of the cohort.

	Parkinson's disease patients	Control patients	*p*-value
Number	36	18	
Gender, Male (%)	26 (72.2%)	11 (61.1%)	ns (0.536[b])
Age			
Mean ± SD, years	66.3 ± 10.1	63.4 ± 12.5	ns (0.401[c])
Median (Min. - Max.)	68 (33–85)	61.5 (45–84)	
Disease duration, years			
Mean ± SD	5.6 ± 4.4	NA	
Median (Min. - Max.)	4.5 (1–23)	NA	
Modified Hoehn and Yahr scale			
Mean ± SD	2.0 ± 0.6	NA	
Median (Min.-Max.)	2 (1–3)	NA	
Stage 1	7 (19.4%)	NA	
Stage 1,5	0	NA	
Stage 2	20 (55.6%)	NA	
Stage 2,5	6 (16.7%)	NA	
Stage 3	3 (8.3%)	NA	
Stage 4	0	NA	
Stage 5	0	NA	
UPDRS part III score, points			
Mean ± SD	25.1 ± 10.5	NA	
Median (Min. - Max.)	25 (8–53)	NA	
PD-NMS questionnaire, points			
Mean ± SD	8.4 ± 5.4	NA	
Median (Min. - Max.)	8.5 (0–24)	NA	
Ophthalmological comorbidities			
Dry eyes	1 (2.8%)	0	ns (1[b])
Glaucoma	2 (5.6%)	2 (11.1%)	ns (0.593[b])
Cataract (not operated)	1 (2.8%)	1 (5.6%)	ns (1[b])
Cataract (operated)	1 (2.8%)	1 (5.6%)	ns (1[b])
Macular degeneration	1 (2.8%)	0	ns (1[b])
Medication with possible effects on the lacrimal functional unit[a]			
Beta blocker (local)	0	1 (5.6%)	ns (1[b])
Beta blocker (systemic)	9 (25.0%)	8 (44.4%)	ns (0.215[b])
Diuretics	6 (16.7%)	2 (11.1%)	ns (0.704[b])
Corticosteroids	0	2 (11.1%)	ns (0.107[b])
Prostaglandin analogues	1 (2.8%)	1 (5.6%)	ns (1[b])
Carbonic anhydrase inhibitors	1 (2.8%)	0	ns (1[b])
Anticholinergics	1 (2.8%)	0	ns (1[b])
Antidepressants	3 (8.3%)	3 (16.7%)	ns (0.388[b])
Neuroleptics	2 (5.6%)	0	ns (0.547[b])

Abbreviations: NA, not applicable; SD, standard deviation; UPDRS, Unified Parkinson's disease rating scale; PD-NMS questionnaire, Parkinson's Disease Non-motor symptoms questionnaire.

[a] Some patients took more than one medication.

[b] Fisher's exact test.

[c] Student's t-test.

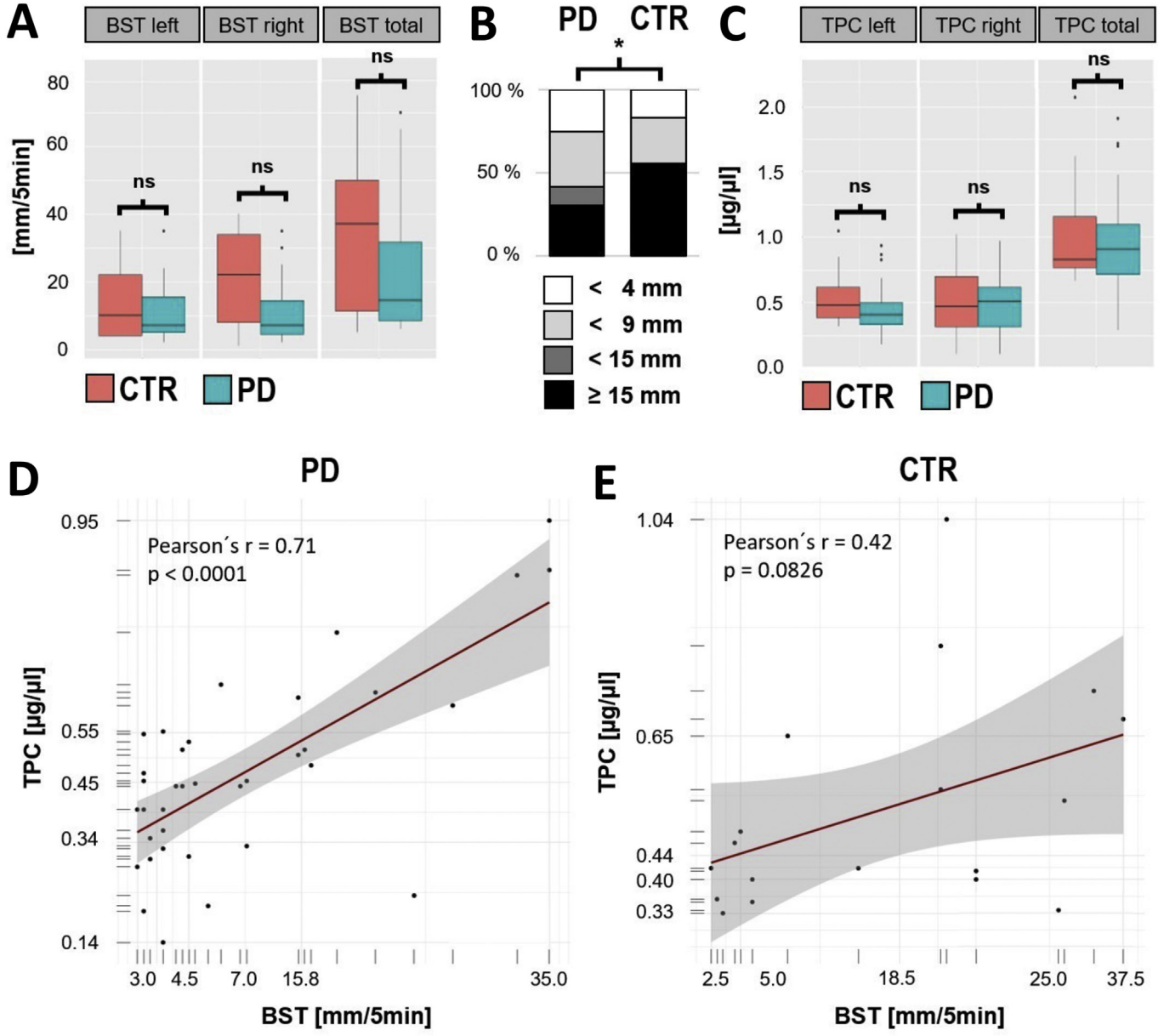

Fig. 1. A. Basic secretion testing (BST) shows no significant differences between Parkinson's disease (PD) and controls (CTR), but a clear trend to reduced tear volumes in PD patient cohort (ns, not significant, Student's *t*-test). **B.** Schirmer tear test values in PD and CTR shows a moderate (< 15 mm) to severe (< 5 mm) decline in tear volume indicated by reduce running length in 70% of PD patients (*p < 0.05, Fisher's exact test). **C.** Results in total tear protein concentration (TPC) shows no significant alterations in PD vs. CTR (ns, not significant, Student's *t*-test). **D.** Correlation analysis showed a significant large positive correlation of BST to TPC values in PD patient cohort (Pearson's r = 0.71, p < 0.0001). **E.** A trend for a medium positive correlation of BST to TPC values in CTR subjects could be detected (Pearson's r = 0.42, p < 0.0826).

Fig. S2).

3.2. Bottom up LC-MS/MS analysis

In order to reveal the protein composition, we performed BULCMS analysis using pooled TF samples of PD and CTR patients. BULCMS corresponding initial 1D SDS-PAGE resulted in high qualitative tear protein migration pattern (Supplementary Fig. S3).

In this pilot study, a total of 571 tear proteins could be identified (FDR < 1%), whereby a high proteomic congruency with previous tear proteomic studies could be demonstrated [11]. Using univariate statistics, several group-specific tear proteomic alterations could be observed considering both groups: a sum of 31 proteins were exclusively identified in the PD group while 7 proteins could not be identified in the PD group but were detected exclusively in the CTR group (Supplementary Tbl. S4).

21 proteins were significantly increased in PD versus CTR (p < 0.05), while 11 proteins were increased by trend (p < 0.10). In contrast, 19 proteins were significantly decreased in PD versus CTR (p < 0.05) (Supplementary Tbl. S5, Supplementary Fig. S6).

3.3. Functional gene ontology annotation and protein-protein interaction analysis

To understand the role of the differentially regulated proteins in a biological context, we performed functional annotation analyses to different gene ontology (GO) terms using Cytoscape and DAVID. For analysis and visualization of protein-protein interaction (PPI) networks the STRING platform was used.

According to Cytoscape GO cellular compartment analysis 93% of

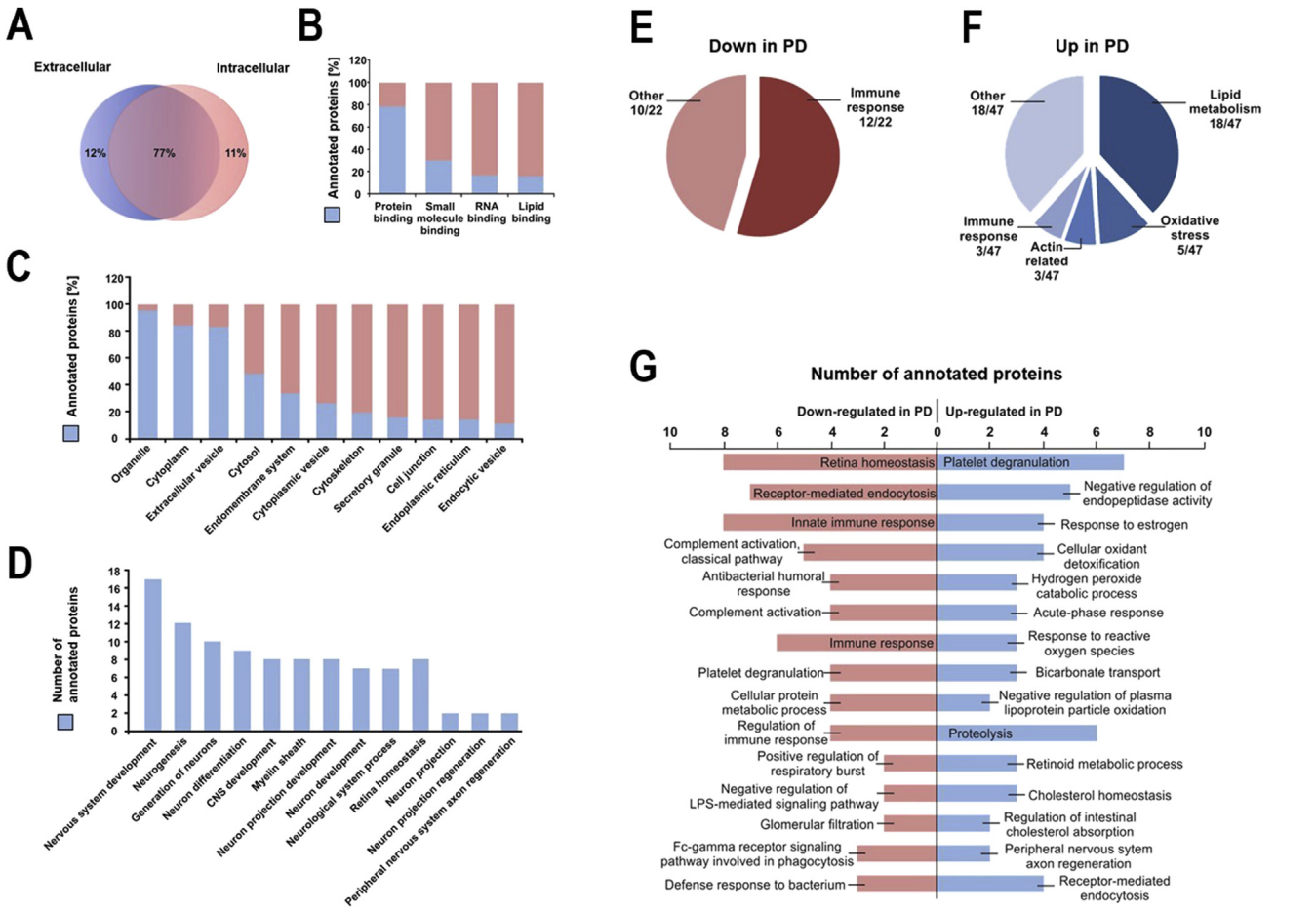

Fig. 2. Functional annotation to different gene ontology (GO) terms of differentially expressed proteins in tear fluid of Parkinson's disease (PD) patients. **A.** Venn diagram showing annotation of regulated proteins to the extracellular and intracellular compartment. **B.** Annotated GO terms for molecular function of the differentially expressed proteins. **C.** Annotation to GO terms for cellular compartment. **D.** 40% of proteins dysregulated in PD patients are associated with functions in the nervous system, including the retina. Annotation of differentially regulated proteins in tear fluid of PD patients to GO biological process terms. **E, F.** Most represented GO biological process categories in the annotation of decreased (**E**) and increased (**F**) proteins in PD tear fluid. **G.** Top 15 annotated GO biological process terms in decreased (red) and increased (blue) proteins in tear fluid of PD patients.

the differentially regulated proteins (i.a. absent, exclusive or dysregulated proteins in the PD group) were annotated to different cellular compartment terms. With 77% the vast majority of differentially expressed proteins could be located in both, the extra- and intracellular compartments, whereas 12% of the proteins were exclusively found in the extracellular compartment and 11% exclusively correspond to the intracellular compartment (Fig. 2A).

To analyze the molecular function of regulated proteins we performed Cytoscape GO molecular function annotation. 92% of the differentially expressed proteins could be annotated to GO molecular function terms, whereof most are annotated to “protein binding” functions (Fig. 2B).

The annotation of the differentially regulated proteins to cellular compartment terms revealed an origin from cytoplasmic organelles for most of the proteins, whereas > 80% were associated with extracellular vesicles (Fig. 2C). Interestingly, ~40% of proteins dysregulated in PD could be related to neuronal functions (Fig. 2D). Of those, 7 proteins (Proline-rich protein 4 [PRR4], Transferrin [TF], Immunoglobulin kappa constant [IGKC], Zymogen granule protein 16 homolog B [ZG16B], Beta-2 microglobulin [B2M], Ig alpha-2 chain C region [IGHA2] and Lactotransferrin [LTF]) were associated to retina homoeostasis and 8 proteins (Alpha-centractin [ACTR1A], Heat shock protein HSP 90-alpha member A1 [HSP90AA1], Gelsolin [GSN], WD repeat-containing protein 1 [WDR1], Carbonic anhydrase II [CA2], Carbonic anhydrase 13 [CA13], Myosin-14 [MYH14] and Ezrin [EZR]) were related to the myelin sheath. Interestingly, the PD-related Protein deglycase DJ-1 [PARK7] could be recovered from CTR tears and with numerically higher levels in PD tear samples.

To gain deeper insight into the biological processes that might be affected by dysregulated protein levels we annotated the dysregulated proteins in PD tears to GO biological process terms using DAVID. 95.2% (25 out of 26) of the diminished proteins could be annotated to 22 GO biological process terms, whereas 12 of these 22 GO biological process terms were related to *immune response* category (Fig. 2E), indicating that proteins in PD tears that are involved in immune system function are diminished. On the other side, among proteins that display increased levels in PD tears, 94.1% (48 out of 51) were annotated to 47 different GO biological process terms. Here, the most represented biological process categories were *lipid metabolism* (18 out of 47) and *oxidative stress* (5 out of 47) (Fig. 2F). The top 15 GO biological process terms for decreased and increased proteins and the number of proteins annotated to them was identified (Fig. 2G). For proteins decreased in PD tears, the overrepresentation of processes related to *immune function* is obvious. For proteins showing increased levels within the tears of PD patients their involvement in processes related to *oxidative stress* and *lipid metabolism* is demonstrated by the high ranking of these terms in the GO biological process analysis.

The STRING PPI network analysis for the increased proteins in the tears of PD patients revealed significantly more interactions than expected (p = 6.55e-15), indicating that these increased proteins are at

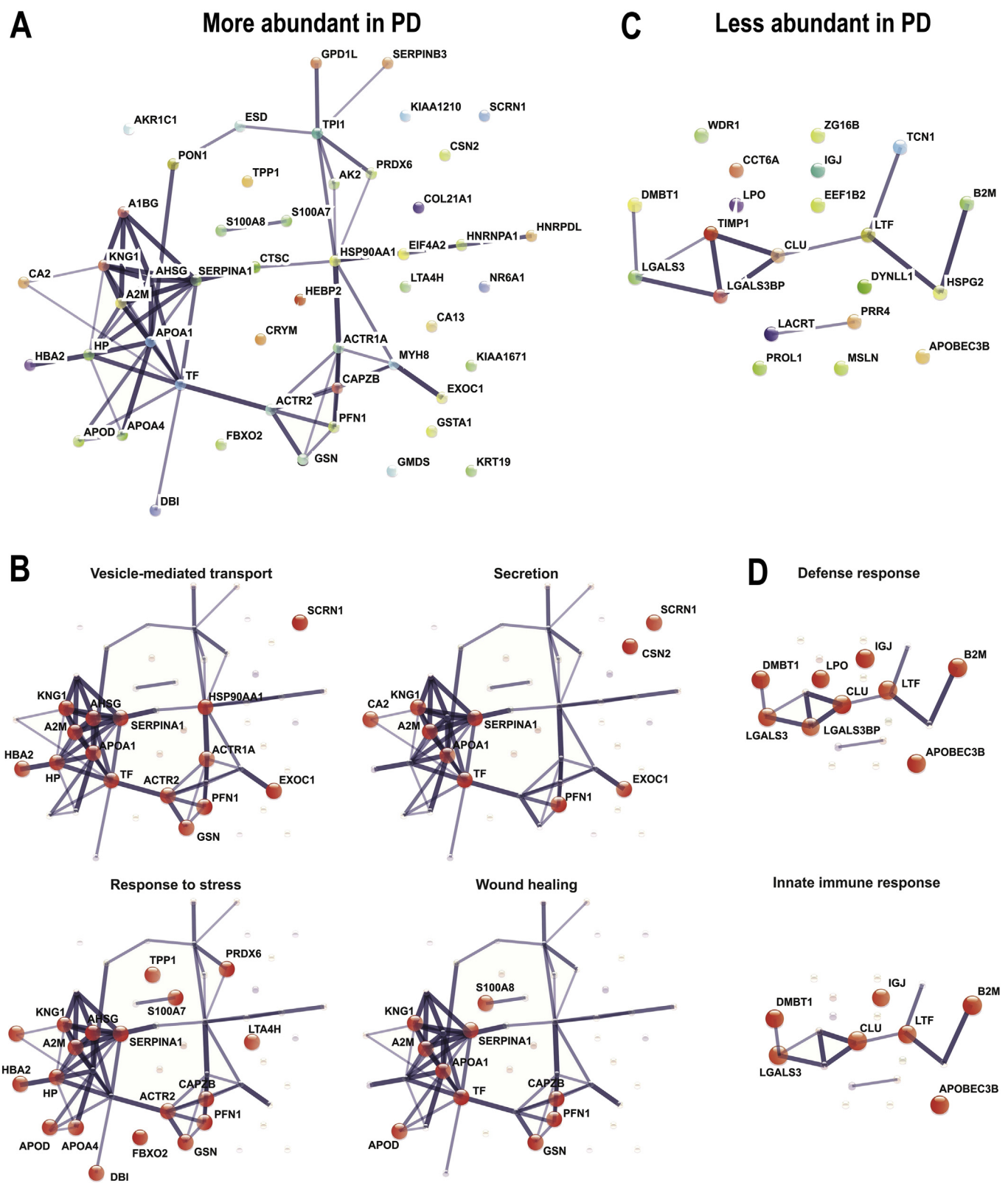

Fig. 3. STRING network analysis of tear fluid proteins. **A.** Complete Protein-protein interaction (PPI) network of proteins with increased abundancies in Parkinson's disease (PD) patients, including proteins exclusively found in the PD group (node colors were randomly assigned). **B.** Core networks derived from **A** for selected gene ontology (GO) biological process terms. **C.** Complete PPI network of proteins decreased in tear fluid of PD patients, including proteins exclusively found in the age-matched control group (node colors were randomly assigned). **D.** Core networks derived from **C** for different GO biological process terms.

least partially biologically connected (Fig. 3). The PPI network (Fig. 3A) showed different subnetworks associated to the GO biological process terms *vesicle-mediated transport*, *secretion*, *response to stress* and *wound healing* (Fig. 3B) that share some of their interacting proteins. The strong interaction in this data set and the enriched biological process terms indicate a possible relevance for the pathomechanism of PD. Also, for the set of proteins with diminished abundancies in PD tears, the STRING network analysis showed a significantly higher number of interactions than would be expected (p = 4.20e-07). In this network (Fig. 3C) the GO biological process terms *defense response* and *innate immune response* were enriched and could be displayed in subnetworks (Fig. 3D), confirming the results of the DAVID GO analysis (Fig. 2).

4. Discussion

In this pilot study, we provide a first description of the tear fluid proteome in a cohort of PD patients and CTR subjects. A significantly higher number of pathologically reduced tear volumes was observed in the PD group, which was previously described in comparable cohorts with even shorter run lengths in more advanced disease stages [12]. Our data thus supports the evidence that PD patients more often suffer from Dry eye syndrome (DES) than their age-matched peers.

In correlation analysis, a trend for a moderate negative correlation of the run length and the PD-NMS score was observed. Although our pilot group size is too small to demonstrate a significant correlation here, it suggests that the reduced rate of tear production in PD patients may represent another non-motor symptom correlating with the overall burden in PD. Peripheral autonomic neuropathy has been reported to occur more frequently in PD patients [13] and is likely to contribute to decreased autonomic innervation of the lacrimal glands and thus reduced TF production.

In proteomic analysis, 571 tear proteins were identified in both sample pools, which is in the range of previously published trials on TF with respect to differences in terms of MS analysis technique and study design [14]. Among those, we identified four groups of proteins with different abundancies: proteins that were detected in both groups but were increased or decreased in PD compared to the CTR group and proteins that were exclusively found either in the PD or the CTR group, respectively.

Like other MS studies, highly abundant proteins can mask the presence of less abundant ones. Additionally, the phenomenon of so-called impression cytology (IC) has to be considered, whereby the placement of the Schirmer test strip can remove layers of epithelial cells at the ocular surface resulting in an input of cellular proteins to the TF proteome [15]. The analysis of GO terms suggests that only about one in ten of the identified proteins in our study is known to occur exclusively intracellularly. Nearly the same percentage was identified as of exclusively extracellular origin, whereas almost 80% of the identified proteins are known to occur in both compartments. Recently, an increased expression of S100, annexin and glutathione S-transferase superfamily was documented in IC samples of DES patients [16]. Interestingly, our analysis revealed proteins from S100 superfamily (i.e. [S100A7], [S100A8] and [S100A11]), Peroxiredoxin-6 [PRDX6], Annexin-A5 [ANXA5] and Glutathione S-transferase-A1 [GSTA1] to be upregulated in the PD group. These proteins thus could represent the proteomic correlate of DES pathology in PD patients and may be derived from IC. Strikingly, none of these proteins belonged to the group, which was exclusively identified in PD, suggesting that PD-specific changes are different from DES-specific alterations of the proteome.

When we annotated both, increased proteins in PD and proteins that were exclusively found in PD, almost 40% of all corresponding biological process terms were related to *lipid metabolism*, while more than 10% were annotated to *oxidative stress*. For example, several proteins from the apolipoprotein superfamily (i.e. [ApoD], [ApoA4] and [ApoA1]) were increased in tears of PD patients. All of them are known to be involved in lipid metabolism in neurodegeneration: Apolipoprotein D (ApoD) has an important role in binding and transporting cholesterol, as well as maintaining cholesterol homeostasis during reinnervation and regeneration [17]. In PD patients, elevated levels of ApoD were reported in glial cells of substantia nigra [17].

Serum paraoxonase/arylesterase 1 [PON1], an enzyme that is capable of metabolizing organophosphates, was identified as one of the exclusive proteins in the PD group. Polymorphisms in PON1 were linked to an increased risk of PD [18].

PPI network analysis revealed a contribution of upregulated proteins in PD in *vesicle-mediated transport*, *secretion*, *response to stress* and *wound healing*. A subset of proteins, e.g. Alpha-2-macroglobulin [A2M], Alpha-1-antiproteinase [SERPINA1] and Profilin 1 [PFN1], were attributed to all of the above-mentioned processes: Alpha-2-macroglobulin (A2M), a well-known component of Lewy bodies, inhibits proteases and can bind to several cytokines [19]. Several polymorphisms in A2M gene were found to be a susceptibility variant for sporadic PD [20] and a trend to elevated plasma levels of A2M was found in PD patients [21]. Alpha-1-antiproteinase (A1A), a product of the human gene SERPINA1, is involved in coagulation and inhibiting the activation of proinflammatory cytokines. Levels of A1A were upregulated in CSF samples of PD patients [22]. Mutations in the PFN1 gene, encoding for the actin-binding protein Profilin 1, were recently linked to several neurodegenerative disorders [23].

Strikingly, more than 50% of all biological processes annotated to proteins that were down-regulated in PD or exclusively not found in this group, related to *immune response*. A similar annotation resulted from PPI network analysis. Lactotransferrin [LTF], Clusterin [CLU], Galectin 3 [LGALS3], and Beta-2 microglobulin [B2M] belong to this subset of downregulated proteins: Lactotransferrin, a highly abundant protein produced by the lacrimal and Meibomian glands, is known to be decreased in DES [24] and is involved in antimicrobial defense. Its decrease suggests a DES-like dysfunction of the lacrimal function unit in PD. Clusterin (Apolipoprotein J, ApoJ) is a member of the small heat shock protein family. It contributes to lipid transport and, as a molecular chaperone, it is responsible for aiding protein folding of secreted proteins, clearance of cellular debris and apoptosis. It is localized within Lewy bodies and is increased in CSF and serum of PD patients [25]. Finally, Beta-2 microglobulin (B2M) was previously shown to be decreased in DES [26]. Since it is important for cell surface expression, it's reduction may indicate reduced innate immunity and increased susceptibility to infections. Our data suggests that PD patients, in contrast to their age-matched peers, show a significant immune dysfunction in the ocular compartment, which could promote and contribute to visual dysfunction in PD.

5. Conclusion

Proteomic analysis has a high potential to identify biomarker candidates. The relative non-invasiveness of TF collection renders this body fluid a highly promising biomarker source. Being a pilot study, our analysis has, however, clear limitations. Importantly, the small sample size, the low number of control subjects and the use of pooled samples have to be considered when interpreting our results. Although our pilot study was based on the analysis of TF pools and did not allow to conclude on the proteome of individual patients, the overall proteomic analysis revealed previously unknown alterations in the protein composition in PD patients compared to controls. While sample pooling could be a limitation in terms of reduced power, it is valid on the discovery level considering balancing budgetary, effort and time constraints, and proteomic output [27]. Moreover, because pooling minimizes the amount of information lost below the detection threshold, the use of pooled data is preferable [28]. Sample pooling in the discovery stage has been successfully performed to analyze proteomic differences in TF samples between clinical phenotypes of DES [29].

Our results confirm that DES-like pathology is present in PD patients, but at the same time PD-specific proteomic differences are

observed, which are not common to DES patients. Furthermore, a dysregulation of TF proteins involved in lipid metabolism, oxidative stress, vesicle secretion and immune response in PD is observed and reinforces the contribution of these mechanisms in PD pathogenesis. Some of the differentially regulated proteins may thus qualify as diagnostic TF-based biomarkers in the future, which will have to be confirmed in more extensive trials assaying larger patient cohorts and individual samples of this easily accessible biomarker source.

6. Authors' role

1. Research project:
   A. Conception: P Lingor, F Grus
   B. Organization: M Boerger, P Lingor, S Funke, F Grus
   C. Execution: M Boerger, S Funke, F Maass, AK Wuestemann
2. Statistical Analysis:
   A. Design: M Boerger, P Lingor, S Funke, A Leha
   B. Execution: M Boerger, P Lingor, A Leha, AE Roser
   C. Review and Critique: M Boerger, P Lingor, S Funke, A Leha
3. Manuscript Preparation:
   A. Writing of the first draft: M Boerger, P Lingor, S Funke
   B. Review and Critique: M Boerger, P Lingor, S Funke, F Grus, AE Roser, F Maass, M Bähr

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.parkreldis.2019.03.001.

Disclosure

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The work described has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. All participants gave written consent.

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List of abbreviations

AD: Alzheimer's disease
α-syn: Alpha-synuclein
BST: Basic secretion test
BULCMS: Bottom-up liquid chromatography electrospray ionization tandem mass spectrometry
CSF: Cerebrospinal fluid
CTR: Controls
DES: Dry eye syndrome
FDR: False discovery rate
GO: Gene ontology
mHY: Modified Hoehn and Yahr (Scale)
MS: Mass spectrometry
PD: Parkinson's disease
PD-NMS: Parkinson's Disease-Non-Motor Symptoms (Scale)
PPI: Protein-protein interaction
REM: Rapid eye movement
TF: Tear fluid
TPC: Total protein concentration
UPDRS: Unified Parkinson's disease rating scale