Potential two-step proteomic signature for Parkinson’s disease: Pilot analysis in the Harvard Biomarkers Study

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Abstract

Introduction: We sought to determine if our previously validated proteomic profile for detecting Alzheimer’s disease would detect Parkinson’s disease (PD) and distinguish PD from other neurodegenerative diseases.

Methods: Plasma samples were assayed from 150 patients of the Harvard Biomarkers Study (PD, n = 50; other neurodegenerative diseases, n = 50; healthy controls, n = 50) using electrochemiluminescence and Simoa platforms.

Results: The first step proteomic profile distinguished neurodegenerative diseases from controls with a diagnostic accuracy of 0.94. The second step profile distinguished PD cases from other neurodegenerative diseases with a diagnostic accuracy of 0.98. The proteomic profile differed in step 1 versus step 2, suggesting that a multistep proteomic profile algorithm to detecting and distinguishing between neurodegenerative diseases may be optimal.

Discussion: These data provide evidence of the potential use of a multitiered blood-based proteomic screening method for detecting individuals with neurodegenerative disease and then distinguishing PD from other neurodegenerative diseases.

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Keywords: Parkinson’s disease; Precision medicine; Proteomics; Blood biomarkers; Diagnostic accuracy

1. Background

Parkinson’s disease (PD) is the second most common neurodegenerative disease affecting over 1% of people aged 65 years and older in the United States [1]. The cost of PD to our society was reported to be $23 billion annually in the United States in 2005 [2]. Considering the estimated 15% growth in the elderly US population during the last a consultant to Roche Diagnostics. S.E.O. has received funding from the National Institute on Aging, Michael J. Fox Foundation, Alzheimer’s Association, has multiple patents pending on precision medicine for neurodegenerative diseases; is the founding scientist for Cx Precision Medicine and has served as a consultant to Roche Diagnostics.

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decade, these costs can be expected to increase dramatically as the population ages. Neuropathologically, PD is a progressive disorder of unknown cause affecting multiple neurotransmitter systems. Common nonmotor features of the disease include autonomic failure, urinary incontinence, hallucinations, and dementia [3]. Although a number of treatments have been developed that improve the “dopaminergic deficit,” no treatment has been demonstrated to slow the neuronal degeneration of the substantia nigra neurons. Novel therapeutic approaches are needed with new disease-modifying therapies currently being examined that may ultimately improve patient outcomes.

A major impediment to treatment developments and clinical trials for neurodegenerative diseases is the lack of a sensitive, easily obtained biomarker of disease presence [4–8]. The “cornerstone” to the development of novel disease-modifying therapies in PD is the identification and validation of biomarkers of disease presence and progression [9]. Over the last several decades, the search for biomarkers that have diagnostic and prognostic use in neurodegenerative diseases has grown exponentially [5,10,11] with most work focusing on neuroimaging and cerebrospinal (CSF) methods [5,10–14] and increasingly clinical-genetic algorithms [15,16]. In fact, amyloid-beta (A\(\beta\)) positron emission tomography (PET) scanning tracers and CSF assays have been approved by the Food and Drug Administration for use in the diagnostic process for Alzheimer’s disease (AD), and dopamine transporter single photon emission computed tomography [17] has been established for PD. Recent work suggests CSF markers may also have use in the differential diagnosis of neurodegenerative diseases [18]. Although advance imaging and CSF methods have tremendous potential as biomarkers of PD and other neurodegenerative diseases, invasiveness, accessibility, and cost barriers preclude these from being used as initial detection procedures [6,7,19,20]. Therefore, it has been proposed that blood-based methods require additional investigation [21–23] and may serve as first step in a multitier detection process [19,32] similar to the multistage models used for diagnosing cancer [24].

Here, we test the hypothesis that our previously validated proteomic profile could distinguish AD from PD [31], as well as accurately detect neurodegenerative disease [32]. Therefore, we hypothesize that our blood-based biomarker profile approach may serve to provide a cost- and time-effective means for establishing a rapidly scalable multitiered neurodiagnostic process [19,32] for detecting neurodegenerative disease, including PD. With this initial screening approach, appropriate referrals can be made for subsequent specialty examinations and confirmatory diagnostic biomarkers (imaging, CSF), following the multistage models used for diagnosing cancer [24].

Table 1

| Characteristic | PD | Neurodegenerative controls | Healthy controls |
|---------------|----|----------------------------|-----------------|
| Total, N      | 50 | 50                         | 50              |
| Male/female, N| 25/25 | 25/25                  | 25/25           |
| UPDRS         | 49.6 ± 23.9 | -                      | -               |
| Age           | 72.4 ± 9.4 | 72.64 ± 10.3            | 69.08 ± 9.7     |
| MMSE          | 26.5 ± 3.7 | 20.4 ± 6.7              | 29.2 ± 1.6      |
| PD medications| 36 (72%) | 0 (0%)                   | 0 (0%)          |

Abbreviations: MMSE, Mini-Mental State Examination; PD, Parkinson’s disease; UPDRS, unified Parkinson’s disease rating scale.

2. Methods

2.1. Subjects

The study sample included 150 patients from the HBS (PD n = 50; other neurodegenerative diseases n = 50, controls n = 50). The other neurodegenerative diseases category included AD (n = 12), frontotemporal dementia (n = 25), progressive supranuclear palsy (n = 7), and corticobasal degeneration (n = 6) (Table 1). HBS is a longitudinal, case-control study that tracks clinical phenotypes and linked biospecimens of individuals with neurodegenerative diseases and controls without neurologic disease. High-quality biosamples and high-resolution clinical phenotypes are longitudinally tracked over time. HBS was designed for the primary goal of developing biomarkers that track disease progression and allow go/no go decisions in phase II clinical trials. The HBS specifically fosters research across neurodegenerative diseases, such as the proof-of-concept study described in this study. HBS has been published extensively [15,33–40].

2.2. Proteomics

Plasma samples were assayed using two technological platforms. The proteomic assays were conducted using
two automated systems. The electrochemiluminescence (ECL) assays from our previously validated AD blood screen were captured via the multiplex platform QuickPlex from Meso Scale Discovery per our previously published methods [31,32], with assay preparation performed via automation using the Hamilton Robotics StarPlus system. We recently reported the analytic performance of each of these markers for >1300 samples across multiple cohorts and diagnoses (normal cognition, mild cognitive impairment, AD) [32]. The assays are reliable, and our experience with these assays show excellent spiked recovery, dilution linearity, coefficient of variation (CV), and detection limits. Interassay and intraassay variability has been excellent. A total of 250 μl of plasma was used to assay the following markers: fatty acid–binding protein (FABP), β2-microglobulin, pancreatic polypeptide, C-reactive protein, intercellular adhesion molecule 1, thrombopoietin, α2-macroglobulin, eotaxin 3, tumor necrosis factor α, tenasin C, interleukin (IL)-5, IL6, IL7, IL10, IL18, IL309, factor VII, vascular cell adhesion molecule 1, thymus and activation regulated chemokine, and serum amyloid A. With automation, the average CV for these assays on >1000 samples in our laboratory has been excellent with nearly all having CVs < 10% and 62% having CVs < 5%. Given the recent surge in the literature examining ultrasensitive blood-based markers of neuropathological markers in neurodegenerative diseases, here the Simoa assays for Aβ40, Aβ42, tau, α-synuclein, and neurofilament light polypeptide were conducted using the automated HD-1 analyzer from Quanterix. The performance of the assays in our laboratory from >1000 samples has been excellent with all CVs ≤ 5%.

2.3. Proteomic profile

In our prior work, we have generated and cross-validated an AD proteomic profile across platforms [28,31], cohorts [28,30,32,41,42], species (human, mouse) [31], tissue (brain, serum, plasma) [31], and ethnicities (non-Hispanic white, Mexican American) [28,43]. In our preliminary work, we found that this same proteomic profile could discriminate PD from AD [31]. In that work, we found that the relative importance of the proteins varied between PD and AD, but the overall algorithm was still highly accurate in detecting both diseases. In a subsequent study, we found that our proteomic profile was highly accurate in detecting neurodegenerative diseases [32]. Therefore, here we sought to cross-validate and expand on that work by demonstrating the accuracy of our proteomic profile approach for detecting neurodegenerative diseases and discriminating PD from other neurodegenerative diseases.

2.4. Statistical analysis

Statistical analyses were conducted using R (V 3.3.3) statistical software [44] and SPSS 24 (IBM). Diagnostic accuracy was calculated via receiver operating characteristic (ROC) curves. First, support vector machine (SVM) analyses were used to discriminate controls from neurodegenerative disease (i.e., PD/Other), with resulting diagnostic accuracy statistics generated (step 1). Then, SVM analysis was restricted only to PD versus other neurodegenerative diseases (step 2). SVM analyses were conducted with internal 5-fold cross-validation. In our prior work, we have found that the overall proteomic profile varies between different neurodegenerative diseases [31]. Therefore, our two-step approach was used to capitalize on these differences to increase accuracy and also to allow for the overall algorithm to be more robust and avoid multilevel analyses simultaneously. The latter reduces risk for error and sample overidentification.

3. Results

Descriptive statistics of the sample are provided in Table 1. The average age of the sample was 71.37 years (standard deviation [SD] = 9.9). There were even numbers of males and females across all three groups. An analysis of variance showed there were no significant age differences among the PD group, the healthy control group, and the other neurodegenerative disorders group (F(2, 147) = 2.04, P = .13). There were significant group differences in Mini-Mental State Examination score among the three groups (F(2, 118) = 39.9, P = .<.001). Tukey’s honestly significant difference post hoc analysis revealed that participants with PD (M = 26.5, SD = 3.7) scored significantly lower than healthy controls (M = 29.2, SD = 1.6) but higher than those with other neurodegenerative diseases (M = 20.4, SD = 6.7).

In step 1, our SVM-based proteomic profile was highly accurate in detecting neurodegenerative disease (PD and other) as compared to normal controls. The overall area under the receiver operating characteristic curve (AUC) was 0.94 with an observed sensitivity (SN) of 0.92 and specificity (SP) of 0.65. Table 2 shows all the correct and incorrect predictions while the variable importance plot and ROC curve are presented in Fig. 1. Inclusion of demographic factors did not significantly change the AUC.

In the step 2, the overall SVM-proteomic profile also showed excellent accuracy at distinguishing PD from other...
neurodegenerative diseases. In this model, the AUC = 0.98, SN = 0.94, and SP = .89. Table 3 shows all classifications (correct and incorrect) while the variable importance plot and ROC curve are presented in Fig. 2. Inclusion of demographic factors did not significantly change the AUC.

When reviewing the variable importance plots (Figs. 1 and 2), the overall profiles for discriminating PD/other neurodegenerative diseases from controls were different than the profile for discriminating PD from other neurodegenerative diseases, as was the case in our prior work. The top 10 markers for discriminating neurodegenerative diseases from controls were as follows: neurofilament light polypeptide, pancreatic polypeptide, FABP3, IL18, IL7, thymus and activation regulated chemokine, thrombopoietin, α-synuclein, Eotaxin 3, and IL5. However, the top 10 variables for discriminating PD from other neurodegenerative diseases were intercellular adhesion molecule 1, vascular cell adhesion molecule 1, Aβ42, B2M, Tenascin C, Aβ40, tumor necrosis factor alpha, pancreatic polypeptide, thymus and activation regulated chemokine, and IL6. Fig. 3 provides box plots by protein across the three diagnostic groups for all proteins.

Table 3
Classification accuracy for proteomic profile for distinguishing PD from other neurodegenerative diseases

| Predicted         | SVM model | PD | AD/FTD/others |
|-------------------|-----------|----|---------------|
| PD                |           | 44 | 7             |
| AD/FTD/others     |           | 3  | 55            |
| Sensitivity       |           | 93.6%|             |
| Specificity       |           | 88.7%|             |
| AUC               |           | 0.98|               |

Abbreviations: AD, Alzheimer’s disease; AUC, area under the receiver operating characteristic curve; FTD, frontotemporal dementia; PD, Parkinson’s disease; SVM, support vector machine.

Given our prior work looking at our proteomic profile in AD, we conducted preliminary analyses with (1) only our AD proteomic algorithm, (2) only the Simoa assays, and (3) all markers combined for discriminating PD from AD as well as PD from controls in this sample. For PD versus AD, the Simoa assays alone yielded an excellent SN of 1.0 but only an SP of 0.25. However, our standard ECL proteomic profile (described earlier) yielded a superior balance of SN (also 1.0) and SP (0.75). When the Simoa assays were combined with our standard ECL proteomic panel, there was a modest increase in SP to 0.80. When distinguishing PD from controls, the Simoa assays yielded an SN = 0.74 and SP = .83. Our standard ECL profile yielded an improved SN = 0.92 and SP = .90. The combined algorithm with our ECL and Simoa assays resulted in an increase of SP to 0.94. These results are very preliminary given the size of the sample.

4. Discussion

In the present study, we cross-validated in an independent cohort the findings that our previously validated proteomic profile for AD can also (1) detect neurodegenerative diseases and (2) discriminate PD from other neurodegenerative diseases. In detecting neurodegenerative disease versus controls, the current AUC was 0.94 with an observed SN of 0.92 and SP of 0.65. When distinguishing PD from other neurodegenerative diseases, the overall accuracy improved to an AUC = 0.98, SN = 0.94, and SP = .89.

While our proteomic profile has previously been validated for detecting AD and neurodegenerative diseases combined, there is also significant literature to implicate many of these markers in PD. For example, multiple inflammatory markers such as tumor necrosis factor alpha, C-reactive protein, and IL6 have previously been linked with PD [45,46], and
inflammation has been shown to improve after exercise interventions in persons with PD [47,48]. Mollenhauer et al. [49] found FABP to be differentially expressed in PD and dementia with Lewy bodies compared with controls, and FABP was among the top 10 markers in discriminating PD from AD in our prior work [31]. A meta-analysis of 877 PD cases and 1296 controls found polymorphisms associated with alpha-2-macroglobulin (rs669 in particular) are associated with the risk for PD [50]. Recent work demonstrated that the VCAM1 gene was one of 7 novel genes that displayed significant changes in PD [51] while Tan et al. [52] also recently showed that intercellular adhesion molecule 1 was a hub that participated in the pathogenesis of PD. Scherzer et al [40] found differential expression of PD gene α-synuclein (SNCA) in PD, and low SNCA transcript abundance predicted cognitive decline longitudinally in PD [40]. Therefore, there is substantial extant of literature to support the underlying rationale for these markers being altered PD.

It is important to put these SN and SP estimates into perspective relative to the specific context of use. All first-line screening tools are designed to rule out disease, not rule in disease given the population base rates of disease presence. Therefore, assuming a 20% neurodegenerative disease base rate in the population of those aged 65 years and older, the SN = 0.92 and SP = 0.64 would yield a negative predictive power of 0.97 with a positive predictive power of 0.39 using Bayesian statistics for appropriate calculations. This means that a trial would be accurate in saying that a specific patient should not undergo a lumbar puncture, PET scan, or additional clinical evaluations 97% of the time, thereby allowing large-scale screening at substantially reduced cost. Our group has previously provided the same sorts of calculations for AD clinical trials [32].

This work also provides novel data when putting the newly designed ultrasensitive assays of amyloid, tau, α-synuclein, and neurofilament light polypeptide in context with other proteomic markers. In our prior work, our refined algorithm has been highly accurate in detecting both AD and PD. Here, we cross-validate the accuracy of the approach for detecting PD in an independent cohort (HBS). However, we also demonstrate that adding these new markers may increase the accuracy. On the other hand, these new markers were not very accurate at detecting PD or distinguishing PD from AD alone. The SN of 1.0 obtained by both approaches is likely an artifact of sample size and will not hold in larger samples. The current team is assaying additional PD samples to (1) cross-validate the current findings in independent samples/cohorts and (2) working to build a larger database for combined analyses across cohorts for a clinically relevant estimate of the overall accuracy of these algorithms and markers. If cross-validated, this approach should be applied prospectively within the specific population reflective of the intended context of use as the current group is actively doing with our AD blood screen.

There are limitations to the present study. First, the sample size is relatively small, and the results are proof of concept and must be validated in independent cohorts and larger sample sizes. Instead of splitting the sample into training and test samples, internal 5-fold cross-validation was conducted. However, the results strongly support the justification for such validation studies, which are being carried out by the current team. Second, addition analyses are needed to determine the impact of preanalytic conditions on the assay performance as we have previously pointed out in the AD space [53]. Interestingly, our group recently assayed an independent cohort of PD and dementia with Lewy bodies with preanalytic protocols different from
HBS and found comparable diagnostic accuracy [54]. Additional variables such as fasting duration, storage time, medication status, and so forth should be examined in future studies. Third, the reliability of the findings over time should also be tested. The usability of any blood test is reliant on the accuracy of the test over time. Therefore, longitudinal application of the blood test to the same samples over time is warranted. Finally, the current analyses do not compare the blood proteomics to CSF-defined or PET scan–defined pathology. It is certainly possible that the blood proteomic profiles are detecting underlying amyloid-, tau-, or α-synuclein-related pathologies, but that has not yet been tested. It is also possible that the algorithms, specifically of the underlying targeted pathology (e.g., amyloid vs. tau), would be different. This should also be tested. If validated, the next step will be to determine the scalability of the methods to meet the population needs at the primary care office level.

Overall, the current findings are strongly supportive of follow-up application of the current proteomic profiles to larger biorepository samples, such as the full HBS cohort. The current team is working toward that goal. Ultimately, the goal is to provide clinicians and companies with a rapidly scalable tool (or tools) that can streamline and increase access (while cost containing) to novel clinical trials to improve patient outcomes.

Fig. 3. Box plots for the top 10 variables. Abbreviations: AD, Alzheimer’s disease; PD, Parkinson’s disease; DLB, dementia with Lewy bodies; IL, interleukin.
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REFERENCES

1. Systematic Review: Literature was identified and reviewed using PubMed. Several articles described the importance of rapid and cost-effective biomarkers for neurodegenerative diseases, including Parkinson’s disease (PD). However, no such blood-based biomarkers currently exist as a first step in a multi-tiered neurodiagnostic process.

2. Interpretation: Our findings show that a blood-based biomarker profile can detect neurodegenerative disease (PD/other) and distinguish PD from other neurodegenerative diseases.

3. Future Directions: This article provides support for the notion that a blood-based biomarker profile can accurately detect PD and even distinguish PD from other neurodegenerative diseases. Future work will be conducted to expand the sample size, particularly among other neurodegenerative diseases. A blood-based biomarker profile for PD would be of tremendous use for screening into novel therapeutic trials.
Nancy Reagan Research Institute of the Alzheimer’s Association and the National Institute on Aging Working Group [see comment] (erratum appears in Neurobiol Aging 1998 May-Jun;19(3):285). Neurobiol Aging 1998;19:109–16.

[11] O’Bryant SE, Hobson V, Hall JR, Warring SC, Chan W, Massman P, et al. Brain-derived neurotrophic factor levels in Alzheimer’s disease. J Alzheimers Dis 2009;17:337–41.

[12] Graff-Radford J, Boeve BF, Pedraza O, Verman TJ, Przybelski S, Lesnick TC, et al. Imaging and acetycholinesterase inhibitor response in dementia with Lewy bodies. Brain 2012;135:2470–7.

[13] Colloby SJ, Firbank MJ, Pakrasi S, Lloyd JJ, Driver I, McKeith IG, et al. A comparison of 99mTc-exetamizetide and 123I-FP-CIT SPECT imaging in the differential diagnosis of Alzheimer’s disease and dementia with Lewy bodies. Int Psychogeriatr 2008;20:1124–40.

[14] McKeith I, O’Brien J, Walker Z, Tatsch K, Boocock J. Dworkin. Sensitivity and specificity of dopamine transporter imaging with 123I-FP-CIT SPECT in dementia with Lewy bodies: a phase III, multicentre study. Lancet Neurolog 2007;6:305–13.

[15] Liu G, Boot B, Locascio JJ, Jansen IE, Winder-Rhodes S, Eberly S, et al. Specifically neuropathic Gaucher’s mutations accelerate cognitive decline in Parkinson’s. Ann Neurol 2016;80:674–85.

[16] Liu G, Locascio JJ, Corvol JC, Boot B, Liao Z, Page K, et al. Prediction of cognition in Parkinson’s disease with a clinical-genetic score: a longitudinal analysis of nine cohorts. Lancet Neurol 2017;16:620–9.

[17] Park E, Hwang YM, Lee CN, Kim S, Oh SY, Kim YC, et al. Differential Diagnosis of Patients with Inconclusive Parkinsonian Features Using [18F]FP-CIT PET/CT. Nucl Med Mol Imaging 2014;48:106–13.

[18] Kaelst L, Kuhlmann A, Wedekind D, Stoeck K, Lange P, Zerr I. Using cerebrospinal fluid marker profiles in clinical diagnosis of dementia with Lewy bodies, Parkinson’s disease, and Alzheimer’s disease. J Alzheimers Dis 2014;38:63–73.

[19] O’Bryant SE, Mielke MM, Rissman RA, Lista S, Vanderstichele H, Zetterberg H, et al. Blood-based biomarkers in Alzheimer disease: Current state of the science and a novel collaborative paradigm for advancing from discovery to clinic. Alzheimers Dement 2017;13:45–58.

[20] Henchcliffe C, Dodel R, Beal MF. Biomarkers of Parkinson’s disease and Dementia with Lewy bodies. Prog Neurobiol 2011;95:601–13.

[21] Shiltabans A, Henchcliffe C. Biomarkers in Parkinson’s disease: An update. Curr Opin Neurol 2012;25:460–5.

[22] Henriksen K, O’Bryant SE, Hampel H, Trojanowski JQ, Montine TJ, Jeromin A, et al. The future of blood-based biomarkers for Alzheimer’s disease. Alzheimer’s Dement 2014;10:115–31.

[23] Hennecke G, Scherzer CR. RNA biomarkers of Parkinson’s disease: developing tools for novel therapies. Biomark Med 2008;2:41–53.

[24] Gold LS, Klein G, Carr L, Kessler L, Sullivan SD. The emergence of diagnostic imaging technologies in breast cancer: Discovery, regulatory approval, reimbursement, and adoption in clinical guidelines. Cancer Imaging 2012;12:13–24.

[25] Hansson O, Janelidze S, Hall S, Magdalino N, Lees AJ, Andreasen N, et al. Blood-based NL1: A biomarker for differential diagnosis of parkinsonian disorder. Neurology 2017;88:930–7.

[26] Green H, Zhang X, Tiktova K, Volakakis N, Brodin L, Berg L, et al. Alterations of p11 in brain tissue and peripheral blood leukocytes in Parkinson’s disease. Proc Natl Acad Sci U S A 2017;114:2735–40.

[27] Chatterjee P, Roy D. Comparative analysis of RNA-Seq data from brain and blood samples of Parkinson’s disease. Biochem Biophys Res Commun 2017.

[28] O’Bryant SE, Xiao G, Barber R, Reisch J, Doody R, Fairchild T, et al. A serum protein-based algorithm for the detection of Alzheimer disease. Arch Neurol 2010;67:1077–81.

[29] O’Bryant SE, Xiao G, Barber R, Reisch J, Hall J, Cullum CM, et al. A blood-based algorithm for the detection of Alzheimer’s disease. Dement Geriatr Cogn Disord 2011;32:55–62.

[30] O’Bryant SE, Xiao G, Barber R, Hrubinger R, Wilhelmsen K, Edwards M, et al. A blood-based screening tool for Alzheimer’s disease that spans serum and plasma: findings from TARC and ADNI. PLoS One 2011;6:e28992.

[31] O’Bryant SE, Xiao G, Zhang F, Edwards M, German DC, Yin X, et al. Validation of a serum screen for Alzheimer’s disease across assay platforms, species, and tissues. J Alzheimers Dis 2014;42:1325–35.

[32] O’Bryant SE, Edwards M, Johnson L, Hall J, Villarreal AE, Britton GB, et al. A blood screening test for Alzheimer’s disease. Alzheimer’s Dement (Amst) 2016;3:83–90.

[33] Cho HH, Cahill CM, Vanderburg CR, Scherzer CR, Wang B, Huang X, et al. Selective translational control of the Alzheimer amyloid precursor protein transcript by iron regulatory protein-1. J Biol Chem 2010;285:31217–32.

[34] Ding H, Sarokhan AK, Roderick SS, Bakshi R, Maher NE, Ashourian P, et al. Association of SNCA with Parkinson: replication in the Harvard NeuroDiscovery Center Biomarker Study. Mov Disord 2011;26:2283–6.

[35] Hakimi M, Selvanantham T, Swinton E, Padmore RF, Tong Y, Kabbagh G, et al. Parkinson’s disease-linked LRRK2 is expressed in circulating and tissue immune cells and upregulated following recognition of microbial structures. J Neural Transm 2011;118:795–808.

[36] Hu Y, Chopra V, Chopra R, Locascio JJ, Liao Z, Ding H, et al. Transcriptional modulator H2A histone family, member Y (H2AFY) marks Huntington disease activity in man and mouse. Proc Natl Acad Sci U S A 2011;108:17141–6.

[37] Scherzer CR, Eklund AC, Morse LJ, Liao Z, Locascio JJ, Fefer D, et al. Molecular markers of early Parkinson’s disease based on gene expression in blood. Proc Natl Acad Sci U S A 2007;104:955–60.

[38] Scherzer CR, Grass JA, Liao Z, Pepivani I, Zheng B, Ekland AC, et al. GATA transcription factors directly regulate the Parkinson’s disease-linked gene alpha-synuclein. Proc Natl Acad Sci U S A 2008;105:10907–12.

[39] van Bitterswijk M, Gullati S, Smoot E, Jaffa M, Maher N, Hyman BT, et al. Anti-superoxide dismutase antibodies are associated with survival in patients with sporadic amyotrophic lateral sclerosis. Amyotroph Lateral Scler 2011;12:430–8.

[40] Locascio JJ, Eberly S, Liao Z, Liu G, Hoeising AN, Duong K, et al. Association between alpha-synuclein blood transcripts and early, neuroimaging-supported Parkinson’s disease. Brain 2015;138:2659–71.

[41] Villarreal AE, O’Bryant SE, Edwards M, Grajales S, Britton GB, Panama Aging Research Initiative. Serum-based protein profiles of Alzheimer’s disease and mild cognitive impairment in elderly Hispanics. Neurodegener Dis Manag 2016;6:203–13.

[42] Edwards M, Hall J, Williams B, Johnson LA, O’Bryant SE. Molecular markers of amnestic mild cognitive impairment among Mexican Americans. J Alzheimers Dis 2016;49:221–8.

[43] O’Bryant SE, Xiao G, Edwards M, Devous M, Gupta VB, Martins R, et al. Biomarkers of Alzheimer’s disease among Mexican Americans. J Alzheimer’s Dis 2013;34:841–9.

[44] R_Development_Core_Team. R: A language and environment for statistical computing: 2009. Available from http://www.R-project.org. Accessed October 2018.

[45] Kim SW, Kang HJ, Bae KY, Shin IS, Hong YJ, Ahn YK, et al. Interactions between pro-inflammatory cytokines and statins on depression in patients with acute coronary syndrome. Prog Neuropsychopharmacol Biol Psychiatry 2018;80:250–4.

[46] Hall S, Janelidze S, Surova Y, Widner H, Zetterberg H, Hansson O. Cerebrospinal fluid concentrations of inflammatory markers in Parkinson’s disease and atypical parkinsonian disorders. Sci Rep 2018;8:13276.

[47] Landers MR, Navalta JW, Murtishaw AS, Kinney JW, Pirio Richardson S. A high-intensity exercise boot camp for persons with Parkinson disease: a phase II, pragmatic, randomized clinical trial of
feasibility, safety, signal of efficacy, and disease mechanisms. J Neurol Phys Ther 2019;43:12–25.

[48] Al-Jarrah MD, Erekat NS. Treadmill exercise training could attenuate the upregulation of Interleukin-1beta and tumor necrosis factor alpha in the skeletal muscle of mouse model of chronic/progressive Parkinson disease. NeuroRehabilitation 2018;43:501–7.

[49] Mollenhauer B, Steinacker P, Bahn E, Bibl M, Brechlin P, Schlossmacher MG, et al. Serum heart-type fatty acid-binding protein and cerebrospinal fluid tau: marker candidates for dementia with Lewy bodies. Neurodegener Dis 2007;4:366–75.

[50] Guo X, Tang P, Li X, Chong L, Zhang X, Li R. Association between two alpha-2-macroglobulin gene polymorphisms and Parkinson’s disease: a meta-analysis. Int J Neurosci 2016;126:193–8.

[51] George G, Valiya Parambah S, Lokappa SB, Varkey J. Construction of Parkinson’s disease marker-based weighted protein-protein interaction network for prioritization of co-expressed genes. Gene 2019;697:67–77.

[52] Tan C, Liu X, Chen J. Microarray analysis of the molecular mechanism involved in Parkinson’s disease. Parkinsons Dis 2018;2018:1590465.

[53] O’Bryant SE GV, Henriksen K, Edwards M, Jeromin A, Lista S, Bazenet C, et al. Guidelines for the standardization of preanalytic variables for blood-based biomarker studies in Alzheimer’s disease. Alzheimer’s Dement 2015;11:549–60.

[54] O’Bryant SE FT, Zhang F, Pedraza O, Wszolek ZK, Commo T, Julovitch D, et al. A proteomic signature for dementia with Lewy bodies. Alzheimers Dement (Amst) 2019;11:270–6.