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RESEARCH ARTICLE

# A Transcriptomic Fingerprint for Parkinson's Disease Found in Patients with Chronic Inflammatory Response Syndrome: Implications for Diagnosis, Treatment and Prevention

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## ABSTRACT

Parkinson's Disease (PD) is the second most common neurodegenerative disease worldwide, characterized by a movement disorder that includes tremors, micrographia, difficulty initiating and stopping movement, stiffness, raspy voice, constipation, fatigue, anosmia, musculoskeletal pain and loss of balance resulting in significant disability, risk for infection and substantial need for care of activities of daily living. Many people with PD also develop dementia. The onset of illness usually begins during the 6th decade of life. Neuropathology of PD is characterized by progressive loss of dopaminergic neurons of the substantia nigra marked by intracellular accumulation of α-synuclein in the form of Lewy bodies and Lewy neurites. While dopamine-promoting medications are the mainstay of treatment to reduce symptoms, there is no cure. The leading cause of death is pneumonia, and an average life expectancy is 14.5 years.

We recently reported abnormalities in cytoskeletal tubulin genes TUBA4A and TUBB1 associated with die-back of degenerative central nervous system disorders, including Alzheimer's disease, PD and amyotrophic lateral sclerosis (ALS), using a transcriptomic diagnostic test on white blood cells, based on mRNA expression, called GENIE (Genomic expression: inflammation explained). The use of transcriptomics provides a better understanding of the genomic underpinnings mediating disease expression and the relationship between gene-environment interactions in neurodegenerative disorders.

This paper reports our findings of a unique transcriptomic fingerprint, including tubulin genes, densely found in symptomatic PD patients and much younger patients with fewer symptoms. The grouping is represented by clusterin (CLU) and a panel of coagulation (COAG) genes, called "Triple Positives," consistently found in a subset of Chronic Inflammatory Response Syndrome (CIRS) patients, independent of age but invariantly linked to patients with at least three upregulated COAG genes. Treatment with a published CIRS protocol in a small study corrects many symptoms and Triple Positives, regulating normal gene expression.

This fingerprint supports reports of similar transcriptomics in the PD literature but is the first to demonstrate successful resolution of differentially expressed genes in PD using commercially available medications. Finding Triple Positives in CIRS patients younger than 50, in the absence of toxic compounds like MPTP and instead initiated by exposure to WDB, raises the possibility of neuroprotection from PD if intervention were initiated before neurodegenerative changes are underway. While many putative causes of PD have been explored, a genomically mediated model of disease initiation and expression has emerged supporting a potential paradigm shift regarding PD, . Our work offers a unified framework of environment-gene interaction in the PD population connected to contaminated indoor living spaces, which we term CIRS-PD.

## Acronyms

| | |
|---|---|
| **CIRS** | Chronic Inflammatory Response Syndrome |
| **CIRS-PD** | Chronic Inflammatory Illness due to Parkinson's Disease |
| **COAG** | Upregulated coagulation genes |
| **CLU** | Clusterin |
| **GENIE** | Genomic expression: inflammation explained. |
| **MHM** | Molecular hypometabolism |
| **PD** | Parkinson's Disease |
| **SYN** | Nuclear synuclein |
| **Triple Positive(s)** | upregulated TUBA4A or TUBB1; with CLU and 3 or more COAG TUBA4A Tubulin A4A |
| **TUBB1** | Tubulin B1 |
| **VIP** | Vasoactive intestinal polypeptide |
| **WDB** | Water-damaged buildings |

## Introduction

Parkinson's Disease (PD) is a prevalent neurodegenerative disorder characterized by the progressive loss of dopaminergic neurons in the substantia nigra, leading to motor symptoms such as tremors, rigidity, bradykinesia, and postural instability. Despite being the second most common neurodegenerative disease globally, the successful treatment of PD remains elusive. Recent research has increasingly highlighted the role of neuroinflammation as a pivotal factor in the pathogenesis of PD. Neuroinflammation activates the brain's immune response, leading to events that exacerbate neuronal damage and contribute to disease progression[1,30,31,32,33].

Mechanisms underlying PD are not fully understood, but environmental factors like pesticides, toxins, metals, head injury and certain drugs have received significant attention[2]. Recent studies revealed that genetic susceptibility has a crucial role in PD pathogenesis; complex genetic and environmental factors and interactions are necessary to develop PD [2, 24,34,43,45] Monogenic forms of PD constitute < 10% of all cases, but identification of these genes provides insight into molecular mechanisms underlying the disease[2]. Several studies established that oxidative stress, mitochondrial dysfunction and impairment of protein homeostasis contribute to disease mechanisms[2,35,36,38,39]. Neuroinflammation also seems to play a role in PD, but whether it induces harmful effects due to the release of proinflammatory cytokines or has a protective role in the clearance of extracellular debris and production of trophic factors has not yet been established.[2]

Patients with chronic inflammatory response syndrome (CIRS), most often caused by exposure to the interior environment of water-damaged buildings (WDB), show a wide overlap of symptoms with PD[3]. We have published research demonstrating the association between chronic inflammation and specific brain-related changes in the forebrain parenchyma, cortical grey matter, superior lateral ventricle, caudate, and putamen in this population that may explain the similarities in symptoms among common neurodegenerative disorders, their phenotypic expression and causation by exposure to amplified microbial growth[4].

This paper explores the emerging concept of a transcriptomic fingerprint in PD patients, particularly those with CIRS, and its implications for early diagnosis, treatment, and potential prevention. By examining the specific gene expression patterns associated with PD and their intersection with chronic neuroinflammation, this study aims to understand the molecular mechanisms underpinning PD and propose novel therapeutic intervention avenues. The potential for early intervention based on these molecular markers offers a promising strategy to alter the disease trajectory and improve patient outcomes.

Attempts to find genomic biomarkers are ongoing[5, 6,41,44,] but no consistent input from identified transcriptomic biomarkers has shown benefit from therapy brought into clinical use. Treatment with levodopa/carbidopa combinations improves symptoms, but inexorable progressive neurologic deterioration is the expected outcome. The search for specific gene biomarkers that show the preservation of dopaminergic neurons has only recently demonstrated potential clinical relevance[1,2,7,37,40,42,44].

In a recent paper, Shen et al.[6] analyzed data from a combination of publicly available gene expression data at GEO (Gene Expression Omnibus, NCBI, NIH), using two separate studies conducted on PD, as well as freshly collected blood samples from 103 PD outpatients at their hospital. After building a machine learning model with the first set of GEO data and validating it with their second set of GEO data, the group identified 39 significant, differentially expressed genes that were then used to build a protein-protein interaction (PPI) network. Of those 39 significant genes, 11 are also known to play a substantial role in CIRS (TLR4, CD14, PTGS1, GP6, ITGB3, GP9, ITGA2B, ILIB, PF4, TUBA4A and TUBB1) and are included in the transcriptomic test called GENIE, offered commercially by Progene DX since 2020. The PPI build identified 10 "hub" genes, or genes with significant degrees of interaction (CD36, P2RY12, PF4, ITGB3, ITGA2B, ITGB5, GP1BA, VCL, GP6, and GP9). Of these ten hub genes, GP6, GP9, ITGA2B, ITGB3 and PF4 are found on GENIE under the category of coagulation.

Part of our concern in establishing a gene-based classification for PD is the absence of typical symptoms in younger patients. This problem has been addressed in previously published work, where, in 1967, Hoehn and Yahr defined five stages of PD based on a level of clinical disability[8]. These stages are used to describe how motor symptoms progress in PD. Stages one and two are early stages; stages two and three are mid-stage, and stages four and five are advanced. Another stage classification was published by The International Parkinson Movement Disorder Task Force, recognizing three stages in early Parkinson's disease[9]. These include a preclinical phase where the degeneration of dopamine-producing neurons has already begun, but clinical symptoms are not yet evident. In the prodromal phase, symptoms start to emerge, but they are insufficient by themselves to make a definitive diagnosis of PD. In the clinical phase, symptoms are present and are

recognizable. Reading scales have also been used, as espoused by the Movement Disorder Society (www.movedisorder.org accessed 7/31/24), to stage Parkinson's.

Our work aims to identify a predictive transcriptomic fingerprint that characterizes the "pre-prodromal" phase before any phenotypical manifestations and neurodegenerative changes occur. If an identifiable predictive transcriptomic fingerprint is modifiable through specific treatment protocols, we may be able to arrest the expression and progression of PD.

Studies have shown the vital role of neuroinflammation in PD pathology as summarized in Lancet Neurology[10], in which a systematic process of signaling, genetics, genomics and the immune system analyzes the global burden of PD. We seek the main transcriptome features of hereditary PD. Cytokines contribute to neuronal death and influence neurodegenerative pathways such as tau phosphorylation and amyloid protein processing[24-29].

Karaaslan et al. identified inflammation and regulation of T-cell genes showing differentially expressed genes (DEG) in peripheral blood cells of PD patients by comparing 30 PD patients and 30 controls[2]. Flow cytometry determined the frequency of regulatory T cells (Tregs) in PBMC. A total of 361 DEGs (143 upregulated and 218 downregulated) were identified after Gene Spring analysis. DEGs participated in 28 biological processes, 12 cellular components and 26 molecular functions. Pathway analyses demonstrated that upregulated genes enriched in p53 (CASP3, TSC2, ATR, MDM4, CCNG1) and PI3K/Akt (IL2RA, IL4R, TSC2, VEGFA, PKN2, PIK3CA, ITGA4, BCL2L11) signaling pathways. The results pinpointed expression alterations, particularly in inflammation and survival genes. Peripheral blood ratios of a subset of regulatory T cells (Tregs) were associated with altered disability scores of PD[2].

Hypercoagulation represents another neuropathologic feature of PD as an independent risk factor. The role of upregulated genes, GP9, GP6 and GP1BA, are enriched in chemokine signaling pathways, which are crucial for platelet death and activation for monocyte migration[6]. The three upregulated platelet proteins GP9, GP6 and GP1BA are physiologic collagen receptors that bind collagen.[46]. GP6 is a major platelet receptor, as is CLU, and plays crucial roles in promoting platelet activation and atherosclerosis by interacting with exposed collagen on injured vessel walls. These findings highlight the roles of macrophage migration and platelet activation in the pathology of PD, with the date of onset not specified.

**SPECIFIC GENES FOR COAGULATION**
(FROM www.genecards.org. ACCESSED 8/17/24)

**F13A1 Coagulation factor XIII chain.** Encodes Factor XIII A subunit. Coagulation factor XIII is the last compound to be activated in the blood coagulation cascade.

**F5 Coagulation factor V.** Encodes a crucial factor in the blood coagulation cascade. It circulates in plasma and is converted by releasing the activation peptide by thrombin in coagulation.

**GP6 Glycoprotein VI platelet** Encodes a platelet glycoprotein. The protein is a receptor for collagen that initiates the platelet activation signaling cascade upon collagen binding. Plays a key role in platelet procoagulant activity and subsequent fibrin and thrombin formation.

**GP9 Glycoprotein 9 platelet** Encodes a small membrane glycoprotein found on the surface of platelets. It serves as a protein receptor for von Willebrand's factor.

**ITGA2B Integrin subunit alpha 2B** Encodes a member of the integrin alpha chain family of proteins. It is processed to form alpha IIb/beta-3 integrin cell adhesion receptors. This receptor plays a crucial role in blood coagulation by mediating platelet aggregation. It is a receptor for fibronectin, fibrinogen, plasminogen, thrombospondin and vitronectin. It leads to rapid platelet aggregation to plug ruptured endothelial surfaces physically.

**ITGB3 Integrin subunit alpha 3** Encodes the integrin beta chain beta 3. It is known to participate in cell adhesion and surface signaling.

**PF4 platelet factor 4** Encodes a member of the CXC chemokine family. The protein has a high affinity for heparin and participates in platelet aggregation. It is an inhibitor of hematopoiesis, angiogenesis and T-cell function.

**SELP selectin P** Encodes a protein redistributing to the cell membrane during platelet activation and mediates the interactions of activated endothelial cells or platelets with leukocytes.

**THBS1 thrombospondin 1** Encodes a protein that is an adhesive glycoprotein that mediates cell-to-cell and cell-to-matrix interactions. It binds to fibrinogen, fibronectin, laminin, Type V collagen and integrins. It plays a role in platelet aggregation, angiogenesis and tumorigenesis.

**TREM1 triggering receptor expressed on myeloid cells 1** Encodes a protein that amplifies neutrophil and monocyte-mediated inflammatory responses triggered by bacterial and fungal infections by stimulating the release of pro-inflammatory chemokines and cytokines and increasing surface expression of cell activation markers.

**CLU clusterin** is involved in fundamental biological processes, including cell death, tumorigenesis and neurodegeneration.

**TUBB1 tubulin beta 1 class VI.** Encodes a member of the beta-tubulin family. It is specifically expressed on platelets and megakaryocytes.

## Methods

**Transcriptomic Test: GENIE**
The GENIE test measures the amount of mRNA produced for 188 genes found to be abnormal in CIRS cases compared to controls matched by age and gender, including ribosomal genes and nuclear-encoded

mitochondrial genes. Down-regulation of mRNA for these genes has been termed molecular hypometabolism (MHM) since 2016. Additional genes of interest in GENIE that have been reported in the peer-reviewed literature on CIRS include cytokines; TGF beta-1 receptors 1, 2, and 3; coagulation elements; genes involved with apoptosis and cytoskeletal- tubulins TUBB1 and TUBA4A; and ribosomal stress response genes, including specific indicators of exposure to Actinobacteria and endotoxins, MAPK; and genes CD14 and Toll receptor 4, respectively. Although the treatment of CIRS cases with a published protocol results in the resolution of these gene abnormalities, it is unknown how gene expression may contribute to harm to the central nervous system.

Standard transcriptomic methods have been used since 3/19 in all study subjects. Statistical analysis was performed according to the Excel package at docs.google.com. An alpha score of <.05 was considered significant.

**RNA Extraction**

Venous blood was drawn from the arms into PAXgene RNA blood collection tubes (http://www.preanalytix.com/product-catalog/blood/rna/products/paxgene-blood-rna-tube/), incubated for four hours at room temperature, then frozen at -80 ° C until RNA extractions were performed. Total RNA was extracted with the Qiagen PAXgene Blood miRNA system kit according to the manufacturer's protocol. Total RNA was analyzed using an Agilent 2100 bioanalyzer (Agilent Technologies, USA) for RNA integrity and then quantified using a NanoDrop NS-2000 (Wilmington, DE). Only samples with Agilent RIN scores $\geq$ 8 were used for sequencing.

**Transcriptomic Analysis using Nanostring.**

A Nanostring digital analyzer was used to measure gene expression with a custom probe set developed by ProgeneDX as a research-use-only assay called GENIE. The GENIE test was designed to assess for CIRS. Specific metabolic gene names were anonymized due to confidentiality restrictions. GENIE contains 174 genes of research interest and 14 housekeeping genes for normalization. Approximately half of the research genes (80) in the assay comprise a metabolic panel with multiple probes averaged using a geometric mean to describe gene expression for the following elements: sizeable ribosomal subunit (17 probes), small ribosomal subunit (14 probes), large mitoribosome subunit (8 probes), small mitoribosome subunit (7 probes), ATP synthase (8 probes), Cytochrome C oxidase (8 probes), mitochondrial inner and outer translocases (8 probes) and NADH dehydrogenase; ubiquinone (10 probes). Two hundred nanograms of total RNA were used as input material, and the GENIE assay was performed according to standard protocols for the Nanostring digital analyzer platform.

## Data Analysis

The samples for GENIE were compared with a control database of 70 healthy, normal adult GENIE results. All samples were normalized using the geometric mean of the 14 housekeeping genes. The metabolic panel results were achieved using a simple ratio of the metabolic scores (geometric mean of the probe groups of probes above) divided by the standard, healthy, and control averages. For the remaining 94 genes assayed in GENIE, the mean and standard deviation of the control group were used to generate a *z* score for subjects.

Patients with GENIE were deidentified, known to researchers only by numbers, and matched to patient history studies using the same unique identifier number used for GENIE.

The results were derived by entering the data into an EXCEL file from the results of GENIE. GENIE was performed by identifying a specific Stage, i.e., 1 was untreated; 2 was treated according to a published protocol; 3 was after vasoactive intestinal polypeptide (VIP) treatment; 4 was off all medications with resolved illness; and 5 was confirmed relapse.

## Results

We noted an increased incidence of the combination of (i) upregulated tubulin genes TUBA4A and TUBB1 with (ii) elevated clusterin (CLU) and (iii) coagulation genes (COAG). The COAG gene panel in GENIE comprises ten genes; the link from upregulated COAG genes to CLU and tubulins was densely associated with three or more COAG genes. The deidentified history delineation roster of prior illnesses for these patient samples yielded 21 cases of PD but not Alzheimer's or ALS. We polled users of GENIE who submitted a total of 77 samples to report this finding (see acknowledgements).

The observed linkage of three sets of genes held consistently true for the non-random series of 77 patients. We then evaluated 102 consecutive GENIE tests in patients who had CIRS in July 2024, identifying 36 cases with this unique linkage of genes, now called "Triple Positives." Non-triple positives in 66 patients served as our control for Triple Positives. These patients also had CIRS but not Triple Positives.

We then used a retrospective study design to examine de-identified cases of gene activity results compared to controls from a library of 210 genes in 1822 patients. 171 Triple Positives were identified, as shown by *z* scores > 1.29 in cases compared to controls. We used the same protocol for reporting gene activation as reported previously. We further defined the data to compare instances of Triple Positives by four stages, representing: 1) before treatment; 2) after the first eleven steps of a published treatment protocol[5]; 3) during therapy with VIP; and 4) after all treatment was completed, respectively[11].

Finally, we queried our roster of 10 untreated PD cases, all demonstrating Triple Positive DEG. Additionally, 11 treated PD cases showed no expression of Triple Positives.

Therefore, we report a unique finding among patients with PD based on a small literature of pathway analysis. A simple observation evolved into a case/control, treated/untreated study. We found more women than men in our four groups (Table 1). The age range of subjects was 4 to 83. Molecular hypometabolism (MHM), normally found in 90% of untreated CIRS cases, was found in 73% of Triple Positives study cases, with only 30% found to have IRS2 elevation. Non-triple positive CIRS cases typically had 60% positive IRS2.

**TABLE 1:** Four studies by age and gender

| | Male | Female | Mean Male Age | Mean Female Age | Existing Controls |
|---|---|---|---|---|---|
| 77 NON-RANDOM CASES | 34 | 43 | 43.4 | 50.8 | |
| 102 CONSECUTIVE PATIENTS | 36 | 66 | 47 | 44 | 66 |
| RETROSPECTIVE CASES | 65 | 106 | 48.7 | 51.3 | AGE 48 MALE AGE 52 FEMALE |
| TREATED/NOT | 10 | 11 | 64 | 56 | |

The four study groups demonstrate the desegregation of gene linkages with the Triple Positive. Given the absence of significant classification error at 1.3% (three unlinked TUBB1 and two unlinked CLU out of 371 cases), the finding of specificity and sensitivity suggests but does not confirm, that this fingerprint is unique to PD.

**Table 2:** COAG genes by stage from retrospective library

| COAG | ST 1 | CLU 94 | | | ST 2 | CLU 52 | | | ST. 3 | CLU 28 | | | ST. 4 | CLU 16 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | N= | 3 | PV. | | N= | 3 | PV. | | N= | 3 | PV. | | N= | 3 | PV |
| F13A1 | | 31 | 25 | 81 | | 5 | 5 | 100 | | 15 | 15 | 100 | | 6 | 6 | 100 |
| | | | | | | | | | | | | | | | | |
| F5 | | 33 | 17 | 52 | | 4 | 4 | 100 | | 27 | 12 | 44 | | 5 | 2 | 40 |
| GP6 | | 38 | 30 | 79 | | 11 | 6 | 54 | | 26 | 17 | 65 | | 10 | 9 | 90 |
| GP9 | | 24 | 21 | 88 | | 10 | 5 | 50 | | 15 | 11 | 73 | | 12 | 10 | 83 |
| ITGA2B | | 33 | 29 | 88 | | 5 | 5 | 100 | | 22 | 19 | 87 | | 12 | 11 | 92 |
| ITGB3 | | 33 | 30 | 91 | | 9 | 7 | 78 | | 26 | 22 | 85 | | 16 | 12 | 75 |
| PF4 | | 8 | 8 | 100 | | 5 | 3 | 60 | | 17 | 14 | 82 | | 7 | 7 | 100 |
| SELP | | 21 | 19 | 90 | | 5 | 4 | 80 | | 17 | 15 | 88 | | 6 | 6 | 100 |
| THBS1 | | 39 | 25 | 64 | | 13 | 5 | 38 | | 24 | 18 | 75 | | 16 | 13 | 81 |
| TREM1 | | 11 | 11 | 100 | | 3 | 2 | 67 | | 24 | 19 | 79 | | 8 | 7 | 87 |

ST= STAGE. N= TOTAL NUMBER OF SPECIFIC COAG GENES. 3= NUMBER OF TIMES SPECIFIC COAG GENES APPEAR WITH AT LEAST 2 OTHER COAG GENES. PV= PREDICTIVE VALUE: A GIVEN SPECIFIC GENE WILL APPEAR IN A TRIPLE POSITIVE

We calculated a prediction value for each of the 10 COAG genes for each treatment stage. The highest values were for F13A1, ITGA2B, SELP, and ITGB3. The most-found genes were GP6, GP9, ITGB3, ITGA2B and F13A1. An advantage of the GENIE panel was the inclusion of 10 genes. We have no data looking at other COAGs, but the published work of Strickland et al. on the role of COAG, fibrinogen, in Alzheimer 's[15-23] ensures that further advances in the study of COAG in PD are forthcoming. The finding of extremely high PV suggests that coagulation is more critical than reported in PD.

## Discussion

The findings presented in this study offer a compelling narrative for the early diagnosis, treatment, and potential prevention of Parkinson's Disease (PD) by identifying a unique transcriptomic fingerprint, particularly in patients with Chronic Inflammatory Response Syndrome (CIRS). The combination of upregulated tubulin genes (TUBA4A and TUBB1), Clusterin (CLU), and a panel of coagulation (COAG) genes—termed the "Triple Positive" fingerprint--which represents a significant biomarker for PD, has been shown in one of our four study groups to correct with treatment for CIRS. We cannot answer the question of causation of Parkinson's but remain convinced that the treatment can be expanded in patients who still mobility issues and tremors after the Triple Positive genes have been corrected. Triple Positives patients will need to be followed into the indeterminate future to assess the significance and efficacy of transcriptomic-induced molecular injury.

The genes mentioned in the dataset—MHM, IRS2, CLU, # coag, and TUBB1—are related to various biological processes. Here is how they might relate to Parkinson's disease, based on what is known about these genes and their roles:

**1. CLU (Clusterin)**

- **Role in Neurodegeneration:** CLU, also known as clusterin, is a chaperone protein that plays a role in protein folding, cell death, and neuroprotection. It has been implicated in several neurodegenerative diseases, including Alzheimer's disease. In Parkinson's, its role could be related to its involvement in cellular stress responses and the regulation of apoptosis (cell death), both of which are relevant to the neuronal loss seen in Parkinson's.
- **Potential as a Biomarker:** Given its consistent expression and its involvement in neurodegenerative processes, CLU could be a potential biomarker for Parkinson's disease progression or response to therapy.

**2. IRS2 (Insulin Receptor Substrate 2)**

- **Role in Insulin Signaling:** IRS2 participates in insulin signaling pathways. Increasing evidence links insulin resistance to Parkinson's disease, as insulin signaling is crucial for neuronal survival and function. Disruptions in insulin signaling have been observed in Parkinson's to be diminished, suggesting that IRS2 could play a role in the disease, potentially through

mechanisms related to energy metabolism and neuronal health.

- **Neuroprotective Pathways:** Insulin signaling pathways, where IRS2 plays a crucial role, have been shown to have neuroprotective effects, which could be relevant in slowing Parkinson's disease.

3. **MHM Molecular Hypometabolism**

A fundamental finding in GENIE of decreased production of mRNA due to reduced activity of ATP synthases, reduced electron transport activity and reduced production of translocases needed to transport pyruvate across the outer mitochondrial membrane resulting in (i) reduced production of protein by ribosomes, and (ii) reduced mitochondrial production of ATP.

**4. Coagulation Genes**

- **Blood-Brain Barrier Integrity and Inflammation:** Coagulation-related genes are not typically linked to Parkinson's disease. However, the integrity of the blood-brain barrier and systemic inflammation[47,48] is increasingly recognized as important in neurodegenerative diseases. Coagulation factors could influence Parkinson's indirectly through these mechanisms.
- **Vascular Contributions to Neurodegeneration:** There is some evidence suggesting that vascular factors, including those related to coagulation, might contribute to the progression of neurodegenerative diseases, including Parkinson's. This could make coagulation genes relevant, particularly in understanding the vascular component of Parkinson's.

**5. TUBB1 (Tubulin Beta-1)**

- **Cytoskeleton and Neuronal Function:** Tubulin proteins are key cytoskeleton components crucial for maintaining neuronal structure and function. Abnormalities in cytoskeletal proteins, including tubulin, have been linked to neurodegenerative diseases, including Parkinson's. TUBB1's role in cytoskeleton dynamics might be relevant in understanding the cellular changes in Parkinson's.
- **Neuronal Transport and Stability:** Proper functioning of the cytoskeleton is essential for axonal transport, a process often disrupted in Parkinson's disease. TUBB1 might be involved in this aspect, making it a potential marker for the disease.

## Summary:

- **CLU**: Likely related to neuroprotective mechanisms and could be a biomarker for Parkinson's.
- **IRS2**: Involved in insulin signaling, which has been linked to Parkinson's pathophysiology.
- **MHM**: This may have an indirect role through metabolic processes.
- **# coag**: Potentially relevant through vascular contributions and systemic inflammation.
- **TUBB1**: Related to cytoskeleton integrity, which is crucial for neuronal function and transport, processes disrupted in Parkinson's.

The role of these genes suggests that they could be part of a broader network of biological processes relevant to Parkinson's disease, including neuroprotection, metabolism, vascular health, and cytoskeletal integrity. Further research could elucidate their specific contributions to Parkinson's pathology and their potential as biomarkers or therapeutic targets.

GENETIC FINGERPRINT VALIDATION

Applying the logical structure of *modus ponens*—if P implies Q, and P is true, then Q must be true—the study's findings align with this reasoning. If we posit that specific gene expression patterns are predictive of PD (P), and these patterns are indeed observed in the CIRS population (P is true), then it follows that these patients are at risk for PD (Q). The repeated identification of the "Triple Positive" in both symptomatic and asymptomatic patients, particularly those with CIRS, supports the hypothesis that these genetic markers are precursors to or early indicators of PD.

The study's use of the GENIE transcriptomic test strengthens this argument. The consistency of these biomarkers across different patient groups—both treated and untreated—provides robust evidence for their role in PD pathology. Furthermore, the observation that CIRS treatment protocols can reverse the abnormal gene expression linked to PD underscores the potential for early intervention to alter the disease's trajectory. If the fingerprint is present (P), and treatment reverses it (R), then one might infer that effective treatment could prevent the progression to full-blown PD (Q).

IMPLICATIONS OF A PREDICTIVE FINGERPRINT

The discovery of this fingerprint in younger, asymptomatic patients introduces the possibility of a preclinical phase of PD where intervention could be most effective. This aligns with the hypothesis that neurodegenerative diseases like PD may have a long latency period where underlying genetic changes occur before clinical symptoms manifest. Identifying these changes through transcriptomic analysis could lead to earlier and more targeted therapeutic strategies, potentially delaying or even preventing the onset of PD.

Moreover, the study's findings suggest that environmental factors, particularly exposure to water-damaged buildings (WDB), may significantly develop PD in genetically susceptible individuals. This environment-gene interaction framework aligns with the growing body of evidence that neuroinflammation and oxidative stress are critical factors in the pathogenesis of PD. Therefore, the "Triple Positive" fingerprint may represent a valuable diagnostic tool for identifying individuals at substantial risk due to environmental exposures.

TREATMENT AND REVERSAL OF GENE EXPRESSION

The study demonstrates that the "Triple Positive" fingerprint can be corrected through CIRS treatment protocols. This finding suggests that addressing the underlying inflammatory processes associated with CIRS can mitigate the gene expression abnormalities linked to PD. Using VIP and other anti-inflammatory therapies offers a novel approach to managing PD risk in CIRS patients. If the fingerprint is reversible through treatment (P), and the treatment is applied (P is true), then it is reasonable to expect a reduction in PD incidence (Q).

This approach could revolutionize how we manage PD, shifting the focus from symptomatic treatment to

prevention and early intervention. However, the study acknowledges that larger-scale research is needed to validate these findings and determine the most effective treatment protocols for different patient populations.

## Conclusions

Identifying a "Triple Positive" transcriptomic fingerprint in CIRS patients at risk for PD significantly advances our understanding of the disease's etiology and potential treatment strategies. This study supports the hypothesis that specific gene expression patterns may serve as early indicators of PD, particularly in individuals exposed to environmental factors like water-damaged buildings.

The potential for early intervention based on these genetic markers offers a promising avenue for altering the course of PD. The study's findings suggest that targeted treatment protocols can reverse the gene expression abnormalities associated with PD, potentially preventing the progression of the disease in at-risk individuals. However, further research with larger cohorts is essential to validate these results and establish robust clinical guidelines.

In summary, discovering the "Triple Positive" fingerprint offers new hope for early diagnosis, treating, and preventing PD, particularly in CIRS patients. By integrating environmental and genetic factors into our understanding of PD, this research opens new avenues for personalized medicine and improved patient outcomes.

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## References


1. Elbaz A, Carcaillon L, Kab S, et al. Epidemiology of Parkinson's disease. *Rev Neurol 2016; 172: 14-26.*
2. Karaaslan Z, Ozlem T. Sanli E, et al. Inflammation and regulatory T cell genes are differentially expressed in peripheral blood mononuclear cells of Parkinson's disease patients. *Scientific Reports 2021; 11: 2316.*
3. Shoemaker R, Heyman A, Lark D. TUBB1, TUBA4A and MAPK as indicators of die-back degenerative central nervous system disease in patients sickened by specific exposure to the interior environment of water-damaged buildings. *Medical Research Archives 2023; 11: 1-11*
4. Shoemaker R, McMahon S, Ryan J, et al. Intranasal VIP restores volume in multiple grey matter nuclei in patients with CIRS. *Int Med Rev 2017; 3: 1-14.*
5. Kurvits L, Lattekivi F, Reimann E, et al. Transcriptomic profiles in Parkinson's disease. *Experimental Biology and Medicine 2021; 246: 284-295.*
6. Shen J, Chang X, Wang-Lun C, et al. Identification of Parkinson's disease-related pathways and potential risk factors. *Journal of International Medical Research 2020; 48: 1-13*
7. Tan E, Chao Y, West A, et al. Parkinson's disease and the immune system association, mechanisms and therapeutics. *Nature Rev Neurology 2020; 16: 303-318.*
8. Hoehn M and Yahr M. Parkinsonism: onset, progression and mortality. *Neurology 1967; 17:427-42*
9. www.movedisorder.com accessed 7/31/24.
10. GBD 2016 Parkinson's Disease Collaborators. Global, regional and national burden of Parkinson's Disease, 1990-2016. *Lancet Neurology 2018; 17; 939-953.*
11. Shoemaker R, Johnson K, Jim L, Berry Y, Dooley M, Ryan J, McMahon S. Diagnostic process for CIRS: A consensus statement report of Surviving Mold. *Int Med Rev,2018; 4:1-46.*
12. Cortes-Cantell M, Zamolodchikov D. Ahn H, et al. Fibrinogen and altered hemostasis in Alzheimer's disease. *J Alzheimer's Dis 2012; 32: 599-608.*
13. Zamolodchikov D, Strickland S. A possible new role for AB in vascular and inflammatory dysfunction in Alzheimer's disease. *Thromb Res 2016; Suppl 2: S59-61.*
14. Ahn H, Chen Z, Zamolodchikov D, et al. Interactions of B-amyloid peptide with fibrinogen and coagulation factor XII may contribute to Alzheimer's disease. *Curr Opin Hematol 2017; 25: 427-431*
15. Melchor J, Strickland S. Tissue plasminogen activator in central nervous system physiology and pathology. *Thromb Haemost 2005; 93: 655-660.*
16. Singh P, Simoes-Pires E, Chen Z, et al. Lecanemab blocks the effects of the AB/fibrinogen complex on blood clots and synapse toxicity in organotypic culture. *PNAS 2024; 121: 17: e2314450121.*
17. Norris E, Strickland S. Fibrinogen in the nervous system: *Glia Beware. Neuron 2017; 96: 951-953.*
18. Singh P, Badimon A, Chen Z, et.al. The contact activation system and vascular factors as alternative targets for Alzheimer's disease therapy. *Res Pract Thromb Haemost 2021; 5: e12504.*
19. Tataryn N, Singh V, Dyke J, et al. Vascular endothelial growth factor associated dissimilar cerebrovascular phenotypes in two different mouse models of Alzheimer's disease. *Neurobiol Aging 2021; 107: 96-108.*
20. Ahn H, Baker S, Norris E, Strickland S. Inflaming the Brain. *Neuron 2019; 101: 991-993.*
21. Chen Z, Singh P, Calvano M, et.al. A mechanism for the enhanced toxicity of beta-amyloid protofibrils in Alzheimer's disease. *PNAS 2023; 36: e2309389120.*
22. Baker S, Strickland S. A critical role for plasminogen in inflammation. *J. Exp. Med. 2020; 217: 4: e20191865.*
23. Strickland S. Blood will out: vascular contributions to Alzheimer's disease. *Clin Invest 2018; 128: 556-563.*
24. Shihabuddin L, Brundin P, Greenamyre J, et.al. New frontiers in Parkinson's disease: from genetics to clinic. *J Neurosci 2018; 9375-9382.*
25. Do C, Tung J, Elizabeth D, et al. Web-based genome-wide association study identifies two novel loci and a substantial genetic component for Parkinson's disease. *PLoS Genet 2011; 7: e1002141.*
26. Hernandez D, Nalls M, Ylikotila P, et al. Genome wide assessment of young onset Parkinson's disease from Finland. *PLoS One 2012; 7: e41859.*
27. Ritchie M, Phipson B, Wu D, et al. LIMMA powers differential expression analyses for RNA-sequencing and microarray studies. *Nuclei Acids Res 2015; 43: e47.*
28. Alam Q, Alam M, Mushtaq G, et al. Inflammatory process in Alzheimer and Parkinson's disease: significant role of cytokines. *Curr Pharm Des 2015; 22: 541-548.*
29. Mutez E, Nkiliza A, Belarbi K, et al. Involvement of immune system, endocytosis and EIF2 signaling in both genetically determined and sporadic forms of Parkinson's disease. *Neurobiol Dis 2014; 63: 165-170.*
30. Obeso, J.A., Stamelou, M., Goetz, C.G., Poewe, W. & Land, AE Past, present and future of Parkinson's disease: a special essay on the 200th anniversary of the shaking palsy. *Mov. Disord.201 7; 32, 1264-131).*
31. Kalia, LV & Lang, A.E. Parkinson's disease. *Lancet 2015; 386, 896-912.*
32. Johnson M, Stecher B, Labrie V, et al. Triggers, facilitators and aggravators: redefining Parkinson's disease pathogenesis. *Trends in Neurosci,2019; 42: 4-13.*
33. Chao, Y., Wong, S. C. & Tan E. K. Evidence of inflammatory system involvement in Parkinson's disease. *Biomed. Res. Int. 2014; 308654.*
34. Holmans, P., Moskvina, V., Jones, L., Sharma, M. & Vedernikov, A. A pathway-based analysis provides additional support for an immune-related genetic susceptibility to Parkinson's disease. *Hum. Mol. Genet. 2013; 22: 1039-1079.*
35. Gruden, M.A., Yanamandra, K., Kucheryanu, V.G, Bocharova, O. R. & Sherstnev, V. V. Correlation between protective immunity to a-synuclein aggregates, oxidative stress and inflammation. *Neuroimmunomodulation 2012; 19: 334-342.*


 

36. Hirsch, E. C. & Hunot, S. Neuroinflammation in Parkinson's disease: a target for neuroprotection? *Lancet Neurol. 2009; 8: 382-397*.
37. Duffy M, Collier T, Patterson J. Lewy body-like alpha synuclein inclusions trigger reactive microgliosis prior to nigral degeneration. *J. Neuroinflammation 2018 15; 129*.
38. Lin, C. H., Chen, C.C., Chiang, H. L., Liou, J. M. & Chang, C. M. Altered gut microbiota and inflammatory cytokine responses in patients with Parkinson's disease. *J. Neuroinflammation 2019; 16, 129)*.
39. Mogi, M., Harada, M., Narabayashi, H., Inagaki, H. & Minami, M. Interleukin (IL)-1B, IL-2, IF-4, IL-6 and transforming growth factor a levels are elevated in ventricular cerebrospinal fluid in juvenile parkinsonism and Parkinson's disease. *Neurosci. Lett. 1996; 211: 13-16*.
40. Wang, W. et al. Caspase-1 causes truncation and aggregation of the Parkinson's disease-associated protein a-synuclein. *Proc. Natl Acad. Sci USA 2016; 113: 9587-9592*.
41. Kempuraj, D., Selvakumar, G.P., Zaheer, S., Thangavel, R. & Ahmed, M.E. Cross-talk between glia, neurons and mast cells in neuroinflammation associated with Parkinson's disease. *J. Neuroimmune Pharmacol. 2018; 13: 100-112*.
42. Stefanis, L. et al. How is alpha-synuclein cleared from the cell? J. *Neurochem. 2019; 150: 577-590*.
43. Ahmed, I., Tamouza, R., Delord, M., Krishnamoorthy, R. & Tzourio, C. Association between Parkinson's disease and the HLA-DRB1 locus. *Mov. Disord. 2012; 27: 1104-1110*.
44. Kustrimovic, N. et al. Parkinson's disease patients have a complex phenotypic and functional Th1 bias: cross-sectional studies of CD4+Th1/Th2/T17 and Treg in drug-naïve and drug-treated patients. *J. Neuroinflammation 2018; 15: 205*.
45. McGeer, P. L., Itagaki, S. & McGeer, E. G. Expression of the histocompatibility glycoprotein HLA-DR in neurological disease. *Acta Neuropathol. 1988; 76:: 550-557*.
46. Ham, J. H., Yi, H., Sunwoo, M. K., Hong, J. Y. & Sohn, Y. H. Cerebral microbleeds in patients with Parkinson's disease. *J. Neurol. 2014; 261: 1628-1635*.
47. Liu, Z., Huang, Y., Cao, B. B., Qiu, Y. H. & Peng, Y. P. Th17 cells induce dopaminergic neuronal death via LFA-1/ICAM-1 interaction in a mouse model of Parkinson's disease. *Mol. Neurobiol.2017; 54: 7762-7776*
48. Mogi, M., Harada, M., Narabayashi, H., H., Inagaki, H. & Minami, M. Interleukin (IL)-1 beta, IL-2, IL-4, IL-6 and transforming growth factor-alpha levels are elevated in ventricular cerebrospinal fluid in juvenile parkinsonism and Parkinson's disease. *Neurosci. Lett. 1996; 221: 13-16*.

 