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RESEARCH ARTICLE

A Novel Therapeutic Approach to Parkinson's Disease: Using Transcriptomics to Identify Unique Patterns of Gene Expression including 'Triple Positives,' Toll receptors, and Endotoxin Exposure

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ABSTRACT

Parkinson's disease 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. 20-40% of Parkinson's disease patients also develop dementia within 10 years of diagnosis, and 50-80% develop dementia after 15-20 years.

There is no agreement on objective biomarkers to define the onset of this illness, including commercially available genomic testing, which makes early detection difficult and the identification of prodromal symptoms obscure. Neuropathology of Parkinson's Disease is characterized by the progressive loss of dopaminergic neurons in the substantia nigra, marked by the 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 or delay symptoms, there is no cure. Even if a cure remains elusive, improving quality of life is a reasonable goal, especially if reliable biomarkers are available to diagnose illness and assess the effectiveness of therapy.

This study aims to investigate transcriptomic biomarkers in a subset of Parkinson's Disease (PD) patients with prior exposure to biotoxins, particularly lipopolysaccharides (LPS) from water-damaged buildings. This paper focuses on the role of TLR2, RELA, and associated inflammatory and autophagic pathways as part of a novel model linking environmental exposure and neurodegeneration. The scope includes evaluating these markers for diagnostic and therapeutic utility in CIRS-related PD phenotypes.

We recently reported transcriptomic abnormalities in a deidentified subset of patients with Chronic Inflammatory Response Syndrome characterized by a multisystem, multi-symptom inflammatory and metabolic illness due to prior or ongoing exposure to the interior environment of water-damaged buildings. A transcriptomic diagnostic test, based on mRNA expression, was applied to white blood cells called GENIE (Genomic Expression: Inflammation Explained). The use of transcriptomics provides a deeper understanding of the genomic underpinnings that mediate disease processes and the relationship between gene-environmental interactions in neurodegenerative disorders.

GENIE reliably identifies features that define the specific causation of offending microbial substances in patients who produce systemic inflammation following exposure to the interior of damp buildings, where various resident toxigenic fungi, Actinobacteria, beta-glucans, and/or lipopolysaccharides (LPS, endotoxins) can be found either singly or in combination.

A unique transcriptomic fingerprint was found in symptomatic Parkinson's patients in this population. The genomic grouping was represented by (i) clusterin (CLU), (ii) a panel of coagulation (COAG) genes, and (iii) cytoskeletal tubulin genes (TUB), cumulatively called Triple Positives (TP). We also showed treatment with a published protocol corrected many symptoms while restoring normal gene expression.

In this current paper, we aim to expand the gene set associated with Parkinson's Disease while linking this genomically related pathophysiology to a specific exposure to lipopolysaccharides (LPS), also known as endotoxins. We present four additional genomic biomarkers combined with the prior Triple Positive findings associated with Parkinson's Disease, including patients possibly in the prodromal phase: (i) elevated numbers of cases with genes that reflect specific causation (SC) of illness; (ii) elevated levels of Toll receptor 2 (TLR2); (iii) elevated levels of Akt, focusing on autophagy; (iv) elevated levels of a nuclear transcription gene, RELA, encoding for a component of NFκB.

This is the first paper to relate an expanded set of genomic biomarkers characterizing the inflammatory effects of LPS in humans, which are also observed in animals with experimental Parkinson's Disease. The prior finding of Triple Positives, combined with these four new biomarkers, creates the possibility that clinical Parkinson's Disease could be avoided if biomarkers for dysfunctional genes were identified and treated in the prodromal phase before clinical symptoms and signs of overt disease become apparent.

Acronyms:

Akt Protein Kinase B (a set of three serine/threonine-specific protein kinases)

CD14 cluster of differentiation 14

CIRS chronic inflammatory response syndrome

CLU clusterin

CNS central nervous system

DA dopaminergic neurons

GENIE gene expression, inflammation explained.

LPS lipopolysaccharide

MHM molecular hypometabolism

RELA RELA Proto-Oncogene, NFkB Subunit

SC Specific causation

SYN α synuclein; α-syn; alpha-synuclein

TLR2 Toll receptor 2

TP Triple-positive genes identified.

TUBA4A, TUBB1 tubulins, cytoskeletal genes

VIP vasoactive intestinal polypeptide

WDB water-damaged building (damp building)

wtTIDM wild-type TLR2-interacting domain of MyD88.

Introduction

Lipopolysaccharides (LPS), a type of endotoxin, are structural components of the outer cell membranes of Gram-negative bacteria and potent activators of the innate immune system. In animal models, intraperitoneal or intranigral administration of LPS results in microglial activation and subsequent loss of dopaminergic neurons (DA), mimicking features of PD pathology. The relevance of LPS to environmental exposure has been underscored in CIRS, where elevated LPS levels found in house dust and ambient air contribute to systemic inflammation. Repeated LPS exposure may predispose individuals to neuroinflammatory cascades, particularly through Toll-like receptor (TLR) activation, and potentiate alpha-synuclein misfolding and aggregation, which can be caused by defective autophagy.

The pathogenesis of PD is not explained by a single mechanism, as a variety of biological factors, including genetics, mitochondrial dysfunction, oxidative stress, and neuroinflammation, may induce the loss of dopaminergic (DA) neurons, leading to the onset of PD when an unidentified threshold is reached. Previous studies have shown that the intranasal instillation of LPS results in progressive hypokinesia, selective loss of dopamine (DA) neurons, reduction in striatal DA content, and α-synuclein aggregation in aged mice. In a second study, intranasal instillation of LPS also induced a PD-like syndrome in young and aged mice. Other studies have shown that LPS exposure leads to a loss of dopaminergic neurons, accompanied by the activation of microglia and the NFκB pathway.[2,8,9] LPS induced the formation of α-synuclein fibrils[1,3]. TLR 2 and NFκB are upregulated by LPS, with NFκB signaling frequently involved in inflammation following the activation of TLR [2,3]. The finding of increased RELA may be linked to NFκB.[10]

A neuroinflammatory hypothesis of neurodegeneration suggests that endotoxin exposure may cause or contribute to PD. Blood levels of endotoxins are normally low but increase during infections, gut inflammation, gum disease, bacterial infections, and neurodegenerative diseases. Introducing endotoxins into healthy human blood triggers systemic inflammation and activates microglia in the brain. Adding elevated levels of endotoxin to the blood or body of rodents induces microglial activation, priming, and/or tolerance, as well as memory deficits and loss of brain synapses and neurons.

Lipopolysaccharide binding protein (LBP) is a soluble plasma protein that facilitates the transfer of LPS to membrane-bound CD14, which in turn is required for the transfer of LPS[2]. Intravenous injection of 1 ng LPS/kg caused robust microglial activation in most brain areas, as measured by a PET scan. Note that repeated doses of LPS downregulate body responses to LPS, but brain responses to LPS are less downregulated[2].

A single intraperitoneal injection of 5 mg LPS/kg in laboratory rats causes microglial activation in the brains that persists for 12 months and results in loss of dopaminergic neurons in the substantia nigra 10

months later. Multiple intraperitoneal doses of 1 mg LPS/kg, or chronic endotoxin exposure, are used as models for Parkinson's and Alzheimer's Disease[3].

Given that peripheral endotoxemia drives brain pathology, we hypothesize that endotoxin-activated genes are associated with neurodegeneration. Even though it is not ethical to assess this directly in humans, we can use an "experiment in Nature" to confirm that exposure to endotoxins is associated with brain injury. Exposure to water saturation with an activity of water (Aw) greater than 0.9, supporting bacterial growth indoors, creates a risk for patients associated with levels of endotoxins in settled dust greater than 100 Endotoxin units per milligram of dust (EU/mg). Additional sources of LPS, such as sewage, could be associated with massive exposures due to drainage backups, recurrent exposure to air from homes with pets, leakage around toilets with defective wax seals or other plumbing issues.

Toll-like receptors play a crucial role in sensing lipopolysaccharide (LPS) and other microbial components. TLR2 is expressed in microglia, macrophages, and other innate immune cells[14]. In PD, elevated TLR2 expression has been demonstrated in both postmortem brain tissue and peripheral immune cells. (https://pmc.ncbi.nlm.nih.gov/articles/PMC5442366/) Activation of TLR2 in the substantia nigra leads to upregulation of pro-inflammatory cytokines (e.g., IL-1β, TNF-α) and contributes to the loss of dopaminergic neurons[11,12,13,14,15,16,17,18,19,20,21,22]. Data reveal the persistent upregulation of TLR2, even in early or prodromal disease, indicating its potential value as a biomarker and therapeutic target.

TLR2 receptors are evolutionarily conserved, functioning as pattern recognition and damage-associated molecular pattern (DAMP) elements of innate immunity. Recognition of antigens by Toll receptors is a necessary step in linking innate immune action to antibody formation[16,34]. TLR2 is found in the central nervous system (CNS), producing inflammatory compounds and activating glial cells.[16] It is also linked to apoptosis.[18,38]

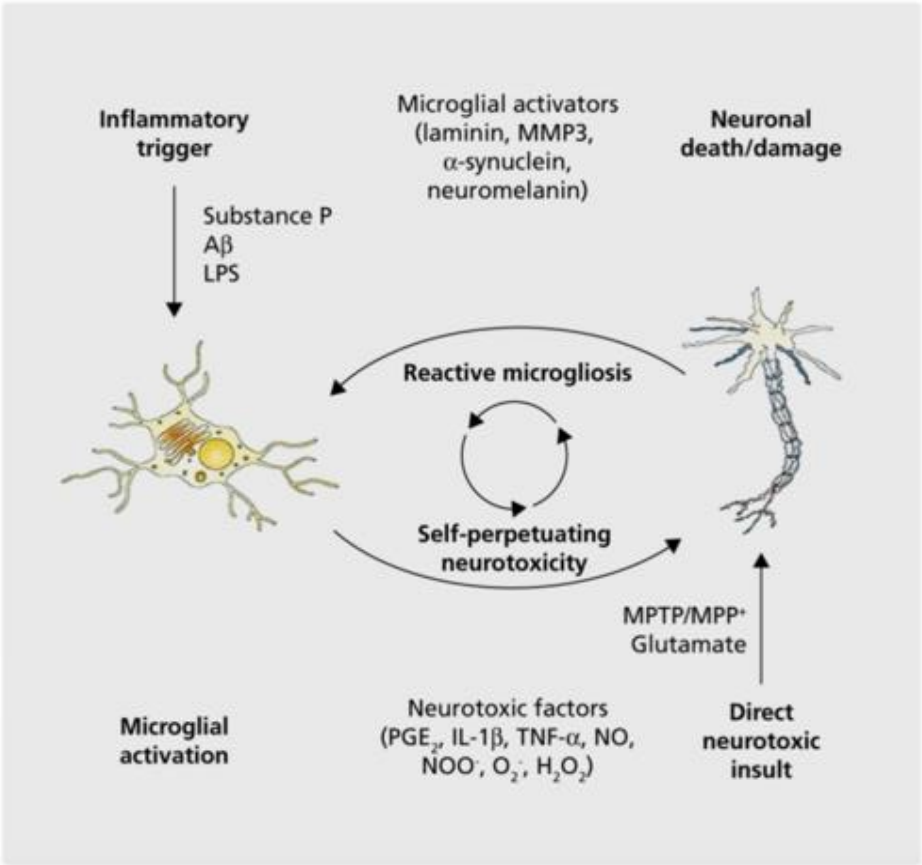
Microglia are the resident immune cells of the central nervous system (CNS), maintaining homeostasis and responding to pathogenic insults. In PD, microglia become chronically activated, especially in the substantia nigra, secreting reactive oxygen species (ROS), nitric oxide (NO), and inflammatory cytokines that exacerbate neuronal injury. Transcriptomic profiles in CIRS-PD reveal elevated expression of microglial markers and pro-inflammatory genes, which correlate with disease severity. This supports a model where environmentally triggered innate immune activation leads to sustained microglial reactivity and progressive neurodegeneration[25,26,27,35,36].

The pathological hallmark of PD is the accumulation of misfolded alpha-synuclein within Lewy bodies and Lewy neurites. Inflammatory signals from microglia enhance alpha-synuclein phosphorylation, misfolding, and aggregation[28]. TLR2 activation contributes directly to the propagation of alpha-synuclein pathology through receptor-mediated endocytosis and intracellular signaling pathways that increase oxidative stress and lysosomal dysfunction[26,29,39]. Thus, the presence of environmental endotoxins may function as upstream initiators of a cascade, leading to alpha-synuclein pathology.

The article shows preformed α-synfibrils (PFF) enhance the association between TLR2 and MyD88, leading to microglial activation[3,30]. TLR2-interaction domain of MyD88 peptide-mediated selective inhibition of TLR2 reduces PFF-induced microglial inflammation in vitro. MyD88 stands for myeloid differentiation primary response protein 88, and it is a protein encoded by the MyD88 gene. This protein plays a crucial role in the body's innate immune response, particularly in the signaling pathways triggered by TLRs and IL-1Rs. MyD88 acts as an adapter, connecting the external signals received by these receptors to

the internal signaling pathways that activate immune responses.
(https://medlineplus.gov/genetics/gene/myd88)

Figure 2. Inflammatory triggers, such as lipopolysaccharide, generate a vicious cycle of neuroinflammation, which leads to degeneration of neurons. Aβ, β-amyloid; H2O2, hydrogen peroxide; IL-1β, interleukin 1β; LPS, lipopolysaccharide; MMP3, matrix metalloproteinase 3; MPP+, 1-methyl-4-phenylpyridinium; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NO, nitric oxide: ONOO, peroxynitrite; O2.-; superoxide anion; PGE2, prostaglandin; TNF-α, tumor necrosis factor α Adapted from reference 26: Block ML, Zecca L, Hong JS. Microgliamediated neurotoxicity: uncovering the molecular mechanisms. *Nat Rev Neurosci.* 2007;8(1):57-69. Copyright © 2007, Nature Publishing Group.



In PFF-seeded mice, the nasal administration of the wtTIDM (wild-type TLR2-interacting domain of MyD88) peptide reduces glial inflammation, decreases α-syn spreading, and protects dopaminergic neurons by inhibiting NFκB3. In summary, α-syn spreading depends on the TLR2/MyD88/NFκB pathway, which can be reduced by nasal delivery of wtTIDM[3,12].

Activation of TLR2 requires its association with the downstream adapter protein MyD88 [12,13]. Peptides of the TLR2-interacting domain of MyD88 (TIDM) specifically inhibit the induction of TLR2 activation without inhibiting basal TLR2 activity or the activation of TLRs[3].

The wtTIDM peptide inhibited α-syn spreading in the brain, prevented activation of glial cells and attenuated parkinsonian pathologies in the PFF-seeded sporadic model of PD. Genetic deletion of TLR2 also halted the spreading of α-syn, indicating an indispensable role of TLR2 in α-syn spreading[3].

A summary of the seminal paper by Dutta et al. is instructive. "A peptide corresponding to the TLR2-interacting domain of MyD88 (TIDM) inhibits explicitly the induction of TLR2 or MyD88. The TIDM peptide does not modulate the activation and function of other TLRs; however, it plays a significant role in inducing TLR2-MyD88 signaling in microglial

activation and α-syn pathology. First, α-syn PFF increased the association between TLR2 and MyD88 in microglia. WtTIDM inhibited this physical association in PFF-stimulated microglia. Second, PFF-induced microglial activation is evident from the activation of NF-κB and the expression of various proinflammatory molecules. Selective inhibition of TLR2 by wtTIDM resulted in the attenuation of PFF-induced microglial activation. Third, widespread microglial activation was observed in the central nervous system (CNS) of PFF-seeded mice. Intranasal administration of wtTIDM peptide inhibited microglial activation. Fourth, wtTIDM peptide treatment reduced the α-syn spreading. Fifth, the specificity of TLR2 inhibition by wtTIDM in vivo was confirmed in both α-synucleinopathies and glial inflammation, indicating the requirement of functional TLR2 protein for the functioning of wtTIDM[3].

Autophagy is a cellular degradation pathway critical for clearing aggregated proteins such as alpha-synuclein. In PD, autophagic dysfunction leads to the accumulation of misfolded proteins and contributes to neuronal loss[17]. Experimental models show that LPS and TLR2 activation impairs autophagic flux, reducing the clearance capacity of neuronal and glial cells[17,32]. This impairment increases the alpha-synuclein burden, further stimulating neuroinflammation and cell death. Restoration of autophagic processes, therefore, offers a promising therapeutic avenue.

Autophagy is a defensive mechanism that can be activated in response to pathological changes, such as an increase in reactive oxygen species (ROS) levels. Eventually, it can maintain normal cell proliferation and growth by degrading damaged cells through the lysosomal pathway. Autophagy is a complex, multistep cellular process characterized by the formation of intracellular protein aggregates. If autophagy is blocked, alpha-synuclein accumulates. The activation of autophagy participates in the clearance of these aggregates and thus could be beneficial in decreasing the symptoms of PD.

Methods

This retrospective study was conducted using patient data collected between March 2019 and May 2025 through the ProgeneDx CLIA-certified laboratory located in Bedford, Massachusetts, and affiliated clinical practices. All data were de-identified and analyzed by established transcriptomic workflows.

Using de-identified data from an ongoing GENIE roster in a single research center (RCS, Pocomoke, MD) practice with 1,822 confirmed cases of CIRS, confirmed by the presence of elements that met the case definition, retrospectively identified 178 patients with TP. For data analysis without selection bias, we examined the most recent 100 consecutive cases (from 12/1/24 up to 4/15/25) with TP (T3+) prior to CIRS therapy compared to a small group of controls. The study design was a retrospective case/control review of objective biomarkers stratified by the presence of TP. We further stratified normal Dispersion (D-) or elevated Dispersion (D+) of 24 genes. Finally, we sorted our cases by Specific Causation positive for endotoxins (SC+). We created four groups of cases, with sorting creating a concentration of T3 in high dispersion versus low, to assess the effects of known Specific Causation on the incidence of biomarkers. The results of our biomarkers are presented in Tables 1 and 2.

As discussed in our previous study[23], the GENIE test measures the amount of mRNA produced for 188 genes identified as abnormal in CIRS cases compared to controls, matched for age and gender, including ribosomal genes and nuclear-encoded mitochondrial genes. Downregulation of mRNA for these genes has been termed molecular hypometabolism (MHM) since June 2019.

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, fungi and endotoxins, MAPK; and genes CD14, TLR2 and Toll receptor 4 (TLR), 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 harm to the central nervous system from exposure to endotoxins. We excluded all non-endotoxin cases from the current study, finding that SC by Actinobacteria comprised 42% of all cases, endotoxins comprised 28% of all cases, and fungi comprised 7% of all cases. The current study focused on SC for endotoxin exposures.

Standard transcriptomic methods have been employed since March 2019 in all studies. Statistical analysis was performed using the Excel package available at docs.google.com. An alpha score of <.05 was considered significant.

The study population consisted entirely of patients meeting the criteria for chronic inflammatory response syndrome (CIRS), representing a targeted cohort with known environmental exposures. While this design enables a focused investigation into environmentally induced PD subtypes, it limits the generalizability of the findings. Future studies in broader, community-based PD populations are recommended to validate these findings.

RNA Extraction

Venous blood was drawn from the arms into PAXgene RNA blood collection tubes, incubated for 4 hours at room temperature, and 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 > 8 were used for sequencing.

Transcriptomic Analysis

A Nanostring digital analyzer was used to measure gene expression with a custom probe set developed by ProgeneDX, a CLIA-approved laboratory. The GENIE test was designed to assess physiological changes seen in 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 the input material, and the GENIE assay was performed according to the standard protocols for the Nanostring digital analyzer platform.

Data Analysis

The samples for the 1822 GENIE cases 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 calculated using a simple ratio of the metabolic scores (geometric mean of the probe groups) 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 calculate a z score for each subject. Patients with GENIE were de-identified, known to researchers only by numbers, and matched to patient history studies using the same unique identifier number as GENIE.

The results from GENIE were entered into an EXCEL file. GENIE was performed by identifying specific Stages of Therapy: Stage 1 – untreated CIRS; Stage

2- treated according to a published protocol; Stage 3 - post-Vasoactive Intestinal Polypeptide (VIP) treatment; Stage 4 - off all medications with resolved illness; and Stage 5 - confirmed relapse. For this paper, all cases were Stage 1.

A master dataset of the current study, comprising 100 consecutive TP cases, was sorted into two groups: Dispersion > 0.94 (T3 + D+, 48 cases) and Dispersion ≤ 0.94. (T3 + D-, 52 cases). T3 +D+ was further categorized into positive D+ with specific causation for endotoxins (T3+ D+ SC+, 17 cases) and D- with specific causation (T3+ D- SC+, 31 cases). The four subgroups were then analyzed for genes, assigned based on average z scores, with the results presented in Table 1 and Table 2. Data from 10 controls were compared to the four groups.

Results

Table 1. Gene Expression Z-Scores for Coagulation and Cytoskeletal Genes (TP Panel) Stratified by Dispersion and Specific Causation (SC)

	N=	F5	GP6	GP9	IGTA2B	ITGB3	PF4	SELP	THBS1	TREML	CLU	TUBA4A	TUBB1
T3+D+	48	0.91	3.18	2.03	2.96	2.62	3.51	5.66	3.06	1.84	3.91	3.9	1.92
T3+D-	52	0.16	2.3	1.67	1.9	2.53	1.51	3.72	1.21	1.83	2.93	2.3	2.51
T3+D+ SC+	17	1.34	3.06	2.03	2.85	2.48	3.63	5.66	2.72	1.88	4.52	4.26	1.94
T3+D- SC+	31	1.8	2.16	2.16	1.69	2.32	1.53	3.39	1.58	1.48	3.08	2.59	2.29
Control	10	1.1	-0.2	-0.1	-0.7	-0.5	-0.2	-0.8	0.2	-0.5	-0.7	-0.6	-0.7

Values represent mean Z-scores for genes implicated in Triple Positive (TP) profiles across subgroups. T3+ indicates Triple Positive; D+ = Elevated Dispersion; SC+ = Specific Causation by endotoxins; Control = unaffected comparison group. Genes include coagulation (e.g., F5, PF4), cytoskeletal tubulins (TUBA4A, TUBB1), and clusterin (CLU).

Table 2. Neuroinflammatory and Autophagy

	N=	Akt	IRS2	CYTO	RELA	NFκB	MAPK3	TLR2	ENDO SC	BIOM	PFP	DISP	ACTINO SC
T3+D+	48	1.76	1.95	4.17	1.98	-1.33	1.99	1.3	65%	11.19	6.06/8	1.78	63%
T3+D-	52	-0.24	-0.02	1.13	0.55	-0.97	0.88	0.36	35%	7.75	5.52/8	0.77	35%
T3+D+ SC+	17	2.19	2.42	4.47	2	-1.45	2.69	1.88	100%	11.4	6.13/8	1.91	79%
T3+D- SC+	31	-0.09	0.63	1.47	0.68	-0.79	1.39	1.32	100%	8.94	5.59/8	0.78	59%
Control	10	-2	-1.3	1	-0.9	-0.8	-0.2	-0.8	0	0	0	0.4	0

Values represent Z-scores for selected genes related to autophagy (e.g., Akt, IRS2), inflammation (RELA, MAPK3, TLR2), and specific causation indicators. "ENDO SC BIOM" = proportion of cases with endotoxin-specific biomarkers; PFP = positive fingerprint pattern; DISP = dispersion score for 24-gene cluster; ACTINO SC = proportion positive for Actinobacteria-specific markers.

All subgroups were different from the controls. The T3+D+ group exhibited more dysregulation than the T3+D-group; additionally, T3+D+ had higher z scores than T3+D-. Following stratification by specific causation for endotoxins, the differences in subgroups were demonstrably enhanced in T3+D+SC+.

The COAG genes showed slightly higher z-scores in T3+D+ than in T3+D+SC+ (Table 1), but T3+D+SC+ had significantly higher z-scores than T3+D-SC+ for each gene in Table 2. The role of dispersion was shown by differences in T3+D+SC+ compared to T3+D-SC+.

In Table 2, Akt, IRS2, the number of different cytokines, RELA, MAPK3, and TLR2 were highest in the T3+D+SC+ group. For Table 1, the five genes with the highest values were observed, with four genes higher in T3+D+ and two (SELP, GP9) showing equal values, accompanied by extraordinary z scores for PF4, SELP, CLU, and TUBA4A. Given that CLU and TUBA4A were components of TP, as presented in our earlier publication, finding elevated z scores for CLU and TUBA4A is not surprising.

The genes Akt and RELA have not been recognized as biomarkers; elevated TLR2 has been identified as a risk factor for specific endotoxin causation (IN TEXT, introduction, above). The magnitude of the elevation of the z-score for TLR2 has not been previously demonstrated. Another striking finding is the number of upregulated housekeeping genes seen in T3+D+ and T3+D+SC+ compared to our controls, T3+D- and T3+D-. Overall, T3+D+ cases show dramatic gene activation compared to CIRS cases without elevated dispersion.

Discussion

TLR2-MEDIATED NEUROINFLAMMATION AND SPECIFIC CAUSATION

Elevation of Toll-like receptor 2 (TLR2) genetic expression, as observed in transcriptomic profiling and previous immunohistochemical analyses, underscores its centrality in environmentally triggered neuroinflammatory processes. Unlike TLR4, which primarily recognizes lipopolysaccharide (LPS) from Gram-negative bacteria, TLR2 has broader reactivity, binding lipopeptides and peptidoglycans from Gram-positive bacteria, as well as fungal wall components. This dual recognition is particularly significant in chronic inflammatory response syndrome (CIRS), where mixed microbial exposures (including Gram-negative, Gram-positive, fungal, and actinobacterial) from water-damaged buildings converge.

The present study demonstrates TLR2 upregulation in PD patients with specific causation linked to endotoxin exposure (SC+) and correlated with increased RELA and MAPK3, indicating convergence on the NF-κB signaling pathway. This pathway drives the transcription of pro-inflammatory cytokines and contributes to the sustained activation of microglia. Importantly, RELA, a subunit of NFκB, shows substantial elevation in SC+ subgroups, while total NFκB scores are paradoxically low. This disparity may reflect post-transcriptional modulation or compensatory feedback mechanisms—a topic meriting further study.

This study has several limitations, including its retrospective design, the absence of longitudinal follow-up, and the selection of patients from a specialized patient pool. The results are hypothesis-generating and require validation in larger, prospective studies that include broader demographics and community controls. Despite these constraints, transcriptomic data offers novel insights into the environmental etiology of PD and lays the groundwork for biomarker-driven intervention strategies.

MyD88, MAPK, AND THE INFLAMMATORY CASCADE

Upon TLR2 stimulation, the MyD88 adaptor protein is recruited to the receptor's intracellular domain, activating IL-1 receptor-associated kinases (IRAKs), which subsequently engage downstream mediators such as MAPK3 and NFκB (via RELA). This cascade leads to Th1 cytokine dominance and a transcriptional

profile consistent with neuroinflammatory pathology. The current data support a TLR2 → MyD88 → MAPK3/NFκB → cytokines axis, with dispersion analysis suggesting this cascade is especially exaggerated in the T3+D+SC+ subset.

Moreover, clusterin (CLU) and tubulin isoforms (TUBA4A, TUBB1) are elevated in the same group, linking cytoskeletal destabilization to the inflammatory burden. Clusterin's role in proteostasis and immune modulation may represent an adaptive—but overwhelmed—response to persistent inflammation.

MICROGLIAL PRIMING AND NEUROTOXICITY

Transcriptomic indicators of microglial activation, such as the upregulation of RELA and TLR2, align with a well-documented pathological phenomenon in PD: microglial priming. Repeated low-dose LPS exposures and beta-glucans, akin to those found in damp building environments, may not provoke an acute response but instead prime microglia, making them hypersensitive to subsequent insults. This "trained immunity" leads to excessive production of ROS, NO, and TNF-α, which in turn propagate dopaminergic neuronal loss.

Overlap may also occur with the Dectin-1/Akt/mTOR/HIF-1α signaling pathway. Notably, the induction of the mTOR/HIF-1α axis acts at two levels: i) driving the metabolic shift towards a glycolytic phenotype with consequences on the epigenetic status of the cell, ii) upregulating the expression of glycolysis-related genes.

Persistent activation of microglia, particularly in the substantia nigra, correlates with the clinical motor symptoms of PD. Our data and animal literature support the hypothesis that low-grade, chronic exposure to endotoxin drives microglial reactivity, thereby facilitating neurodegeneration over time.

RELA AS A NOVEL BIOMARKER

The identification of RELA as a distinct and elevated marker—despite modest NFκB values—warrants emphasis. As a transcription factor integral to NFκB signaling, RELA's upregulation may reflect a chronic inflammatory transcriptional state decoupled from its established regulation. This phenomenon is particularly apparent in the T3+D+SC+ subgroup and may explain why traditional markers of inflammation fail to detect this deeper, transcriptomically embedded pathology.

Given that RELA activity contributes to the persistence of inflammation and impaired autophagy, its prominence in this cohort strengthens the case for its use as an early detection and stratification tool in CIRS-PD (PD resulting from CIRS).

AUTOPHAGIC IMPAIRMENT AND ALPHA-SYNUCLEIN AGGREGATION

Transcriptomic elevations in Akt and IRS2 suggest perturbation of the PI3K/Akt/mTOR signaling axis, a key regulator of autophagy. Inhibition of autophagic flux leads to the intracellular accumulation of misfolded proteins, notably alpha-synuclein, which exacerbates neuroinflammatory damage and contributes to the formation of Lewy bodies. Our findings reinforce that defective autophagy is not merely a downstream consequence, but may also be upstream of symptom onset, particularly in individuals who are genetically or environmentally predisposed.

A Model of Endotoxin Effects in Humans with PD

The findings from GENIE transcriptomic analysis, coupled with environmental exposure histories, support a novel model of Parkinson's Disease (PD) in humans, seen in a subset of PD patients, as a neurodegenerative illness initiated or exacerbated by biotoxin exposure, particularly lipopolysaccharides (LPS) from water-damaged buildings. This model posits that individuals with genetic and epigenetic susceptibilities develop CIRS, contributing to chronic activation of innate immune pathways. Persistent activation of Toll-like receptor 2 (TLR2) on microglia and other immune cells leads to sustained inflammation, oxidative stress, and impaired protein clearance. This cascade promotes the misfolding and aggregation of alpha-synuclein, driving the neurodegenerative process characteristic of PD.

Our data supports the assignment of biomarkers in untreated PD. In a cohort of PD patients with TP, we identified both RELA and Akt as novel biomarkers, which explain the basis for the autoinflammatory and autophagy abnormalities observed in PD (www.genecard accessed May 20, 2025). RELA is a subunit of NFkB, with a z score of 2.00, but NFκB has a negative z score. The significance of this finding is unclear.

Therapies for PD

Traditional therapies for PD focus on symptomatic management, primarily targeting dopaminergic signaling. However, recognizing inflammatory and environmental underpinnings opens a new therapeutic dimension. Protocols used for CIRS, such as eliminating biotoxin exposure, using binding agents, VIP therapy, and transcriptomic normalization, show promise in reversing the gene abnormalities that underlie neuroinflammatory damage. When applied in early PD or CIRS-PD cases, these treatments may help arrest or even reverse disease progression. This implies that long-considered irreversible PD could benefit from personalized, root-cause therapeutic approaches.

Conclusions

This study introduces a paradigm-shifting model of Parkinson's Disease (PD) pathogenesis wherein chronic exposure to environmental endotoxins, particularly lipopolysaccharides (LPS) from water-damaged buildings, initiates a sustained neuroinflammatory cascade mediated by Toll-like receptor 2 (TLR2). Transcriptomic analysis via the GENIE platform reveals a constellation of gene expression abnormalities—elevated TLR2, RELA, Akt, MAPK3, and IRS2—correlated with specific causation from endotoxins and robust microglial activation.

In this current paper, we aimed to expand the gene set associated with Parkinson's Disease while linking this genomically related pathophysiology to a specific exposure to lipopolysaccharides (LPS), also known as endotoxins. We present four additional genomic biomarkers combined with the prior Triple Positive findings associated with Parkinson's Disease, including patients possibly in the prodromal phase: (i) elevated numbers of cases with genes that reflect specific causation (SC) of illness; (ii) elevated levels of Toll receptor 2 (TLR2); (iii) elevated levels of Akt, focusing on autophagy; (iv) elevated levels of a nuclear transcription gene, RELA, encoding for a component of NFκB.

In a significant subset of patients, we propose that PD may not be a spontaneous neurodegenerative process but the outcome of a biotoxin-initiated, gene-environment interaction manifesting as Chronic Inflammatory Response Syndrome (CIRS). This environmental neurotoxicity model presents new avenues for early detection, primarily through transcriptomic biomarkers that precede the onset of clinical symptoms.

These insights support a therapeutic shift from symptomatic dopaminergic strategies toward interventions that interrupt TLR2-MyD88-NFκB signaling, restore autophagy, and modulate innate immunity.

Finally, environmental screening, transcriptomic profiling, and early-stage intervention, particularly in genetically susceptible populations, represent a new frontier in PD prevention and personalized treatment. These findings justify the expansion of research into biotoxin-induced neuroinflammatory diseases, placing environmental health at the forefront of neurodegenerative disease management.

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