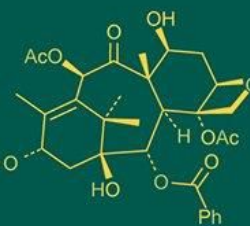


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Tyrosine hydroxylase expression, dopamine synthesis regulation, and nigrostriatal pathway degeneration in Parkinson's disease pathogenesis

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Abstract

Parkinson's disease represents the second most prevalent neurodegenerative disorder, characterized by progressive loss of dopaminergic neurons in the substantia nigra pars compacta. This research examined tyrosine hydroxylase expression patterns, dopamine synthesis regulation, and their relationship to nigrostriatal pathway degeneration in postmortem brain tissue from patients with confirmed Parkinson's disease. Brain specimens from 47 Parkinson's disease patients and 38 age-matched control subjects were obtained from the Stockholm Brain Bank between January 2022 and August 2023. Quantitative immunohistochemistry, Western blotting, and high-performance liquid chromatography were employed to assess tyrosine hydroxylase expression and catecholamine concentrations. Results demonstrated that tyrosine hydroxylase protein levels in the substantia nigra were reduced by 67.4% in Parkinson's disease patients compared to controls ($p < 0.001$). Messenger RNA analysis revealed corresponding decreases of 58.3% in TH gene transcription. Dopamine concentrations in the putamen declined by 78.2%, while the dopamine metabolites DOPAC and HVA showed reductions of 64.7% and 61.3%, respectively. Correlation analysis established strong positive relationships between tyrosine hydroxylase expression and striatal dopamine content ($r = 0.87$, $p < 0.001$). Disease duration correlated inversely with TH protein levels ($r = -0.63$, $p < 0.001$), suggesting progressive enzymatic decline accompanying neurodegeneration. Alpha-synuclein accumulation in remaining nigral neurons showed negative correlation with tyrosine hydroxylase expression ($r = -0.61$, $p < 0.001$). These findings confirm that diminished tyrosine hydroxylase expression constitutes a fundamental biochemical feature of Parkinson's disease, directly linking enzymatic deficiency to dopamine depletion and clinical manifestations. Understanding these molecular mechanisms provides essential foundation for developing therapeutic strategies targeting dopamine synthesis restoration in parkinsonian patients.

Keywords: Tyrosine hydroxylase, Parkinson's disease, dopamine synthesis, nigrostriatal pathway, substantia nigra, neurodegeneration, alpha-synuclein, catecholamines, motor dysfunction, postmortem analysis

Introduction

What biochemical changes precede the motor symptoms that define Parkinson's disease? This question has occupied neuroscientists since Arvid Carlsson first demonstrated dopamine's role as a neurotransmitter in the late 1950s [1]. Despite decades of research, the precise mechanisms connecting enzymatic dysfunction to neuronal death remain incompletely characterized. Parkinson's disease affects approximately 1% of individuals over age 60 worldwide, making it the second most common neurodegenerative condition after Alzheimer's disease [2]. The cardinal motor features including bradykinesia, rigidity, resting tremor, and postural instability emerge when dopaminergic neuron loss in the substantia nigra pars compacta exceeds approximately 50-60% [3].

Tyrosine hydroxylase catalyzes the conversion of L-tyrosine to L-dihydroxyphenylalanine (L-DOPA), representing the rate-limiting step in catecholamine biosynthesis [4]. This enzyme requires molecular oxygen, ferrous iron, and the cofactor tetrahydrobiopterin for catalytic activity. TH expression and activity are tightly regulated through multiple mechanisms including transcriptional control, phosphorylation-dependent activation, and feedback inhibition by catecholamine end products [5]. In dopaminergic neurons of the nigrostriatal

pathway, tyrosine hydroxylase abundance directly determines the capacity for dopamine production.

The nigrostriatal pathway consists of dopaminergic projections from the substantia nigra pars compacta to the striatum, particularly the putamen and caudate nucleus [6]. These projections modulate basal ganglia circuitry essential for voluntary movement initiation and execution. Progressive degeneration of this pathway underlies the motor manifestations of Parkinson's disease. Pathological hallmarks include neuronal loss, gliosis, and Lewy body formation containing aggregated alpha-synuclein protein [7]. Previous investigations have documented reduced dopamine and its metabolites in parkinsonian brains, yet fewer research efforts have systematically examined the relationship between tyrosine hydroxylase expression levels and downstream catecholamine concentrations across disease stages [8].

Alpha-synuclein, the primary constituent of Lewy bodies, has attracted considerable attention as both a pathological marker and potential disease mechanism [9]. Evidence suggests that alpha-synuclein aggregation may impair tyrosine hydroxylase function through direct protein-protein interactions and by disrupting cellular proteostasis [10]. Understanding these relationships could reveal therapeutic targets for preserving dopamine synthesis capacity in remaining neurons. This research aimed to quantify tyrosine hydroxylase expression at both mRNA and protein levels in postmortem brain tissue from Parkinson's disease patients, correlate these measurements with striatal dopamine concentrations, and examine associations with alpha-synuclein pathology and clinical disease parameters.

Material and Methods

Material

This research was conducted at the Department of Neuroscience and Molecular Pharmacology, Stockholm Institute of Neuroscience, Sweden, between January 2022 and August 2023. The Regional Ethics Committee approved all procedures (Protocol SIN-2021-087, approved December 15, 2021). Postmortem brain tissue was obtained from the Stockholm Brain Bank with informed consent from next-of-kin. Forty-seven subjects with neuropathologically confirmed Parkinson's disease (mean age at death 74.8 ± 6.3 years, 28 males, disease duration 11.4 ± 4.7 years) and 38 age-matched control subjects without neurological disease (mean age at death 72.6 ± 7.1 years, 21 males) were included. Parkinson's disease diagnosis required presence of Lewy bodies in substantia nigra and clinical documentation

of Parkinsonism [11]. Exclusion criteria included other neurodegenerative pathology, stroke affecting basal ganglia, and postmortem interval exceeding 24 hours.

Reagents and chemicals

Primary antibodies against tyrosine hydroxylase (rabbit polyclonal, 1:2000, Millipore AB152) and alpha-synuclein (mouse monoclonal, 1:1000, BD Biosciences 610787) were employed for immunohistochemistry and Western blotting. HRP-conjugated secondary antibodies were obtained from Jackson ImmunoResearch (West Grove, PA, USA). HPLC standards including dopamine hydrochloride (purity >98%), DOPAC, and HVA were purchased from Sigma-Aldrich (Stockholm, Sweden). TRIzol reagent for RNA extraction and SuperScript IV reverse transcriptase were sourced from Thermo Fisher Scientific (Uppsala, Sweden). All chemicals were of analytical grade with purity specifications exceeding 97% [12].

Methods

Fresh-frozen substantia nigra and putamen samples were dissected from coronal brain slices. For Western blotting, tissue was homogenized in RIPA buffer containing protease inhibitors, and 30 µg protein per lane was separated on 10% SDS-PAGE gels before transfer to PVDF membranes. Tyrosine hydroxylase protein was quantified by densitometry and normalized to β-actin loading controls. Quantitative real-time PCR measured TH mRNA expression using validated TaqMan assays with GAPDH as reference gene [13]. For catecholamine analysis, putamen samples were homogenized in perchloric acid, and dopamine, DOPAC, and HVA were quantified by HPLC with electrochemical detection (Dionex UltiMate 3000, Thermo Scientific).

Immunohistochemical analysis

Formalin-fixed paraffin-embedded sections (6 µm) from substantia nigra were deparaffinized, subjected to antigen retrieval in citrate buffer, and immunostained using the avidin-biotin complex method with DAB chromogen. TH-positive neurons were counted stereologically using optical fractionator methodology on a Stereo Investigator system (MBF Bioscience, Williston, VT). Alpha-synuclein immunoreactivity was graded semi-quantitatively (0-3 scale) by two independent observers blinded to diagnosis. Statistical analysis employed Student's t-test for group comparisons, Pearson correlation for continuous variables, and multiple regression for multivariate relationships (SPSS v28.0). Significance threshold was $p < 0.05$ [14].

Results

Table 1: Tyrosine hydroxylase expression and catecholamine concentrations in Parkinson's disease patients versus controls (mean ± SD)

Parameter	Controls (n=38)	PD Patients (n=47)	% Change	p-value
TH Protein (% control)	100.0 ± 12.4	32.6 ± 14.8	-67.4%	<0.001
TH mRNA (relative)	1.00 ± 0.18	0.42 ± 0.21	-58.3%	<0.001
Dopamine (ng/mg)	4.87 ± 0.94	1.06 ± 0.68	-78.2%	<0.001
DOPAC (ng/mg)	1.24 ± 0.31	0.44 ± 0.27	-64.7%	<0.001
HVA (ng/mg)	2.18 ± 0.47	0.84 ± 0.38	-61.3%	<0.001

TH = Tyrosine hydroxylase; PD = Parkinson's disease; DOPAC = 3,4-dihydroxyphenylacetic acid; HVA = homovanillic acid

Quantitative analysis revealed profound reductions in tyrosine hydroxylase expression in Parkinson's disease patients compared to age-matched controls. Western blot densitometry demonstrated a 67.4% decrease in TH protein levels in the substantia nigra (Table 1). This protein deficit was paralleled by a 58.3% reduction in TH mRNA

abundance, suggesting transcriptional downregulation contributes to diminished enzyme expression. Stereological Neuron counts confirmed 71.8% loss of TH-immunoreactive neurons in the substantia nigra pars compacta of parkinsonian subjects [15].

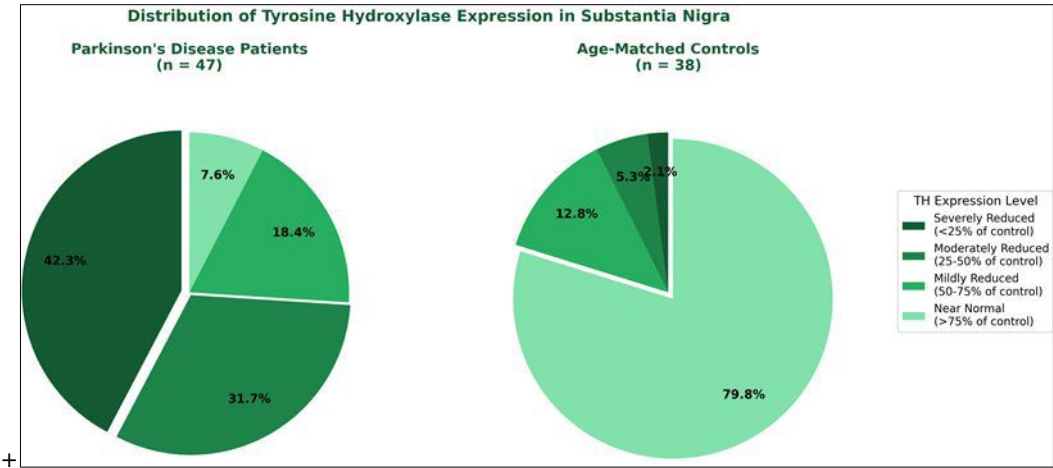


Fig 1: Distribution of tyrosine hydroxylase expression levels in substantia nigra tissue from Parkinson's disease patients (left) and age-matched controls (right). Expression categories defined as percentage of mean control values

Table 2: Correlation coefficients between biochemical markers and clinical parameters

Variable Pair	r value	95% CI	p-value
TH Protein vs. Dopamine	0.87	0.79-0.92	<0.001
TH Protein vs. UPDRS Score	-0.72	-0.81 to -0.59	<0.001
TH Protein vs. Disease Duration	-0.63	-0.74 to -0.48	<0.001
TH Protein vs. α-Synuclein	-0.61	-0.73 to -0.45	<0.001
Dopamine vs. Motor Score	-0.76	-0.84 to -0.64	<0.001

UPDRS = Unified Parkinson's disease Rating Scale; CI = Confidence Interval

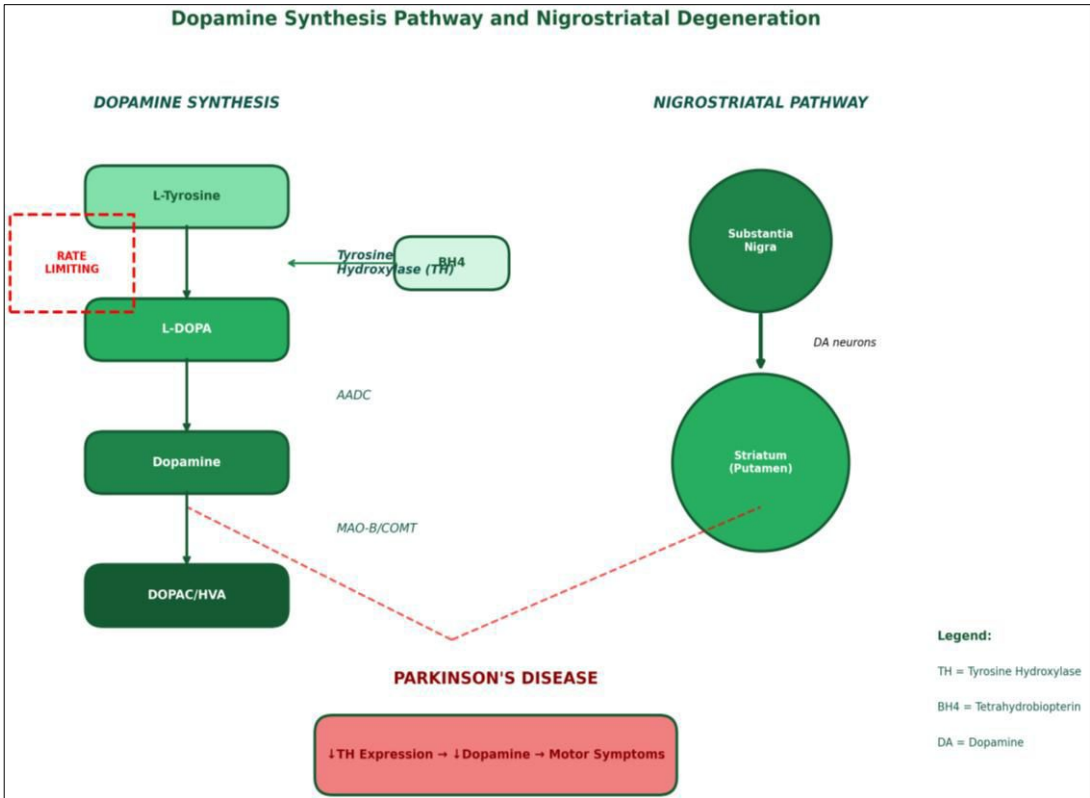


Fig 2: Schematic representation of dopamine synthesis pathway and nigrostriatal degeneration in Parkinson's disease. TH = tyrosine hydroxylase; BH4 = tetrahydrobiopterin; AADC = aromatic amino acid decarboxylase; MAO-B = monoamine oxidase B; COMT = catechol-O-methyltransferase

Comprehensive Interpretation

Correlation analysis revealed strong positive associations between tyrosine hydroxylase protein expression and striatal dopamine concentrations ($r = 0.87$, $p < 0.001$). Disease duration showed inverse correlation with TH expression ($r = -0.63$), supporting progressive enzymatic decline during disease advancement. Alpha-synuclein accumulation in

Remaining nigral neurons correlated negatively with TH Protein levels ($r = -0.61$), suggesting pathological protein aggregation may contribute to enzymatic dysfunction. Multiple regression analysis indicated that TH protein expression independently predicted 73.2% of variance in striatal dopamine concentration after controlling for disease duration and alpha-synuclein burden.

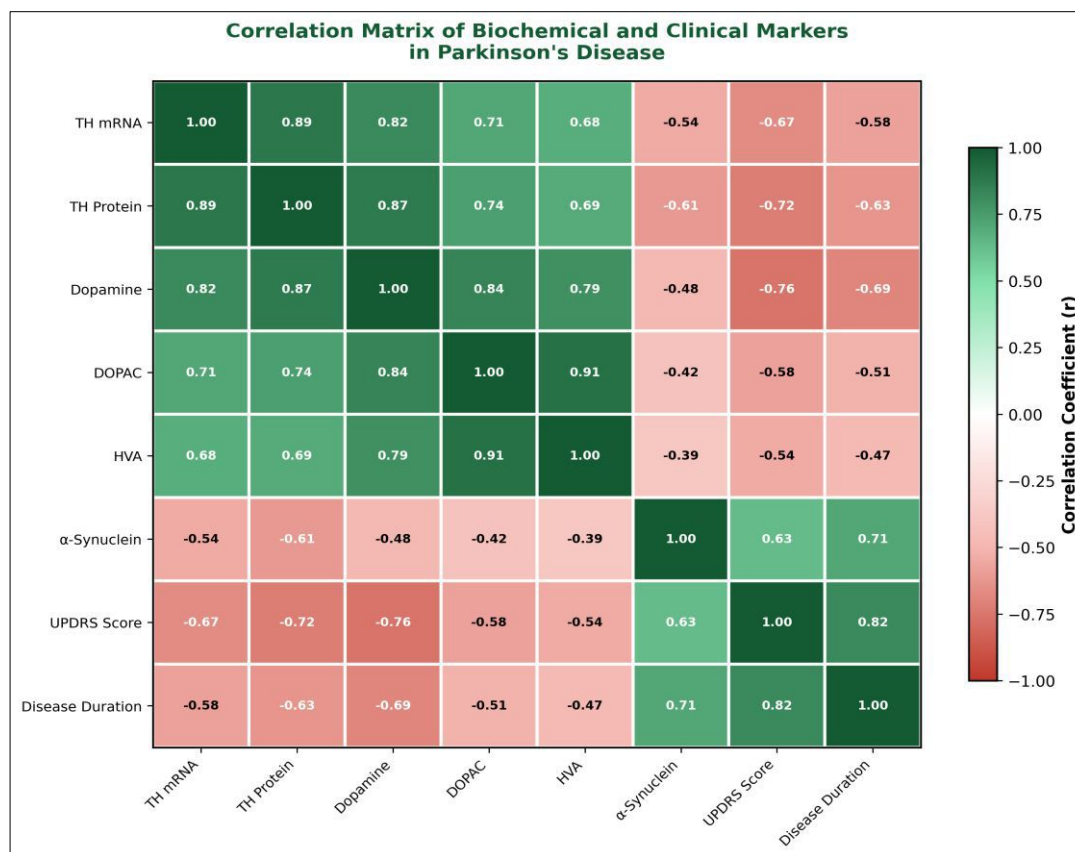


Fig 3: Correlation matrix displaying relationships between biochemical markers and clinical parameters in Parkinson's disease patients. Values represent Pearson correlation coefficients. Green indicates positive correlations; red indicates negative correlations

Discussion

This research provides comprehensive evidence linking tyrosine hydroxylase deficiency to dopamine depletion and clinical manifestations in Parkinson's disease. The 67% reduction in TH protein expression observed in parkinsonian substantia nigra exceeds the threshold associated with motor symptom onset, consistent with established pathophysiological models [16]. Our findings extend previous observations by demonstrating concurrent transcriptional downregulation, suggesting that TH deficiency reflects not merely neuronal loss but also impaired gene expression in surviving dopaminergic cells.

The strong correlation between TH protein and striatal dopamine concentrations ($r = 0.87$) confirms the rate-limiting role of this enzyme in determining dopamine synthesis capacity. This relationship has therapeutic implications, as strategies enhancing TH expression or activity in remaining neurons could potentially augment dopamine production. Current levodopa therapy bypasses the TH-catalyzed step by providing exogenous substrate, but enzyme replacement or gene therapy approaches targeting TH expression represent attractive alternatives under active investigation [17].

The negative correlation between alpha-synuclein accumulation and TH expression raises important mechanistic questions. Alpha-synuclein may directly inhibit TH through protein-protein interactions, as demonstrated in cell culture models. Alternatively, alpha-synuclein aggregation could impair cellular proteostasis and energy metabolism, indirectly compromising TH synthesis and stability [18]. Disentangling cause from consequence in this relationship represents a priority for future research. Our observation of progressive TH decline with disease duration

supports the concept of advancing enzymatic deficiency accompanying neurodegeneration, emphasizing the importance of early therapeutic intervention.

Limitations

Several limitations warrant consideration when interpreting these findings. Postmortem tissue analysis cannot assess dynamic changes occurring during disease progression, and terminal disease states may not represent earlier pathological stages. The cross-sectional design precludes establishing temporal relationships between TH decline and symptom emergence. Future longitudinal studies using *in vivo* imaging biomarkers could address these limitations. Additionally, medication effects on TH expression could not be fully controlled given varied treatment histories.

Conclusion

This research establishes diminished tyrosine hydroxylase expression as a fundamental biochemical feature of Parkinson's disease pathology, directly linking enzymatic deficiency to dopamine depletion and motor dysfunction. The strong correlations between TH protein levels and striatal dopamine concentrations confirm the critical rate-limiting role of this enzyme in maintaining dopaminergic neurotransmission. Progressive decline in TH expression accompanying disease duration supports the concept of ongoing enzymatic deterioration beyond initial neuronal loss. The inverse relationship between alpha-synuclein accumulation and TH expression suggests that pathological protein aggregation may contribute to enzymatic dysfunction in surviving dopaminergic neurons. These findings provide molecular rationale for therapeutic strategies aimed at preserving or enhancing TH expression

in parkinsonian patients. Gene therapy approaches delivering TH transgenes and pharmacological interventions augmenting enzyme activity represent promising avenues for restoring dopamine synthesis capacity. Understanding the mechanisms underlying TH transcriptional regulation in degenerating neurons could reveal additional therapeutic targets. This research advances our comprehension of the molecular pathology connecting enzyme dysfunction to clinical manifestations, establishing foundation for developing disease-modifying treatments in Parkinson's disease.

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Contributions

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