

Original Article

Peripheral Immune Challenge Drives Enteric α -Synuclein and Tau Pathology in LRRK2 G2019S Mice

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ABSTRACT: Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by α -synuclein aggregation and dopaminergic neuron loss. Non-motor symptoms, including gastrointestinal dysfunction, often precede motor onset, suggesting a gut origin of pathology. LRRK2 variants increase susceptibility to both PD and Crohn's disease, pointing to a potential link between LRRK2 hyperactivity and early intestinal abnormalities; however, it remains unclear how LRRK2 contributes to enteric α -synuclein pathology and co-occurring proteinopathies. In this study, we investigated the interaction between chronic peripheral inflammation and the LRRK2 G2019S variant using a longitudinal mouse model combining ageing and repeated low-dose LPS exposure. We quantified peripheral cytokine levels, colonic immune cell infiltration (CD4⁺ T cells and macrophages), and the temporal progression of enteric α -synuclein and tau pathology across different ages. Our results showed that chronic LPS exposure induced an age-dependent increase in circulating cytokines and colonic immune infiltration in LRRK2 G2019S mice, accompanied by enhanced enteric α -synuclein accumulation and phosphorylation, alongside elevated tau phosphorylation, compared with wild-type controls. Notably, total neuronal counts remained stable, indicating a distinct pre-degenerative stage of pathology. Overall, our findings demonstrate that chronic peripheral inflammation synergizes with LRRK2 G2019S to trigger early intestinal inflammation and α -synuclein/tau co-pathology in the absence of overt neurodegeneration. These results suggest that LRRK2 acts as a critical link between systemic immunity and neuronal protein homeostasis, increasing the vulnerability of the gut to inflammatory stress. Characterizing this early pathological window provides key insights into PD initiation and highlights the gut as a promising target for early therapeutic intervention.

Keywords: LRRK2, aging, Parkinson's disease, gut-brain axis, peripheral inflammation

INTRODUCTION

Parkinson's disease (PD) is an age-related neurodegenerative disorder with a steadily increasing prevalence. The average age at diagnosis is around 60 years, by which time 50–60% of dopaminergic (DA) neurons have already been lost. This indicates that the

pathological process begins long before the onset of clinical symptoms, underscoring the urgent need to identify early disease mechanisms to enable timely detection and intervention.

Emerging evidence suggests that PD may originate in the gut before manifesting in the brain, implicating an age-dependent dysfunction of the gut-brain axis in disease

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onset [1-3]. In addition to its characteristic motor symptoms, PD is associated with a wide spectrum of non-motor manifestations that substantially contribute to disease burden, including sleep disturbances, hyposmia, and gastrointestinal dysfunction such as constipation and dysphagia [4]. Gastrointestinal (GI) symptoms often precede the clinical diagnosis of PD by years, and Lewy body (LB) pathology within the enteric nervous system (ENS) can be detected up to two decades before motor symptoms emerge [2, 5]. The gut harbors a complex network of neurons, immune cells, and microbial communities that communicate continuously with the central nervous system (CNS) via immune, metabolic, and neuronal pathways [6]. Disruptions in this bidirectional communication, driven by genetic susceptibility and environmental exposures, may contribute to PD pathogenesis.

Among the key genetic risk factors for PD is leucine-rich repeat kinase 2 (LRRK2), a multi-domain protein with kinase and GTPase functions. LRRK2 is highly expressed in both innate and adaptive immune cells and is tightly regulated by inflammatory stimuli [7]. Variants in LRRK2, particularly the G2019S variant, represent the most prevalent known genetic cause of autosomal dominant, late-onset PD and are found in 1-2% of sporadic PD cases worldwide [8]. While both idiopathic and LRRK2-associated PD share age-dependent progression of motor and non-motor symptoms [9], the incomplete penetrance of LRRK2 variants [9] suggests that additional risk factors, such as aging, chronic inflammation, and environmental insults, are required to initiate disease. Notably, several single nucleotide polymorphisms (SNPs) in LRRK2 that enhance kinase activity have been identified as susceptibility loci for both PD and Crohn's disease (CD) [10].

Consistent with its role in intestinal inflammation, transgenic mice overexpressing LRRK2 show increased vulnerability to dextran sodium sulfate (DSS)-induced colitis [10-12], indicating that LRRK2 modulates mucosal immune responses in the gut. There is also growing evidence that LRRK2 plays a critical role in host defense mechanisms, particularly in response to enteric bacterial infections. LRRK2 expression is strongly induced by pro-inflammatory cytokines, including interferon-gamma (IFN- γ) and type I interferons, both of which are elevated during infections [13-15].

Furthermore, LRRK2 modulates inflammation in response to pathogens such as *Salmonella enterica*, *Listeria monocytogenes*, and *Mycobacterium tuberculosis* [16-18]. These findings underscore LRRK2's function beyond neurons, as a key player in innate immunity, especially in the gut where microbial exposure is constant.

Mechanistically, LRRK2 regulates key signaling pathways downstream of pattern recognition receptors

(PRRs), including Toll-like receptors (TLRs) and NOD2, which recognize microbial components such as lipopolysaccharide (LPS) and muramyl dipeptide [17]. Upon activation, LRRK2 modulates downstream effectors including NF- κ B and interferon regulatory factors (IRFs) [19], thereby coordinating the transcription of cytokines, chemokines, and antimicrobial peptides that shape the inflammatory response. Furthermore, LRRK2 has been identified as a critical regulator of the transcription factor NFAT1; this interaction modulates the severity of intestinal inflammation and has been shown to influence susceptibility to inflammatory bowel disease by controlling the production of pro-inflammatory cytokines [14, 20]. One of the most direct roles of LRRK2 in immunity involves the regulation of intracellular bacterial processing. Following phagocytosis, pathogens are directed to phagosomes that mature and fuse with lysosomes to form bactericidal compartments [21]. In the absence of LRRK2, or in the presence of PD-associated variants such as G2019S, phagosome maturation is impaired, resulting in defective bacterial clearance and dysregulated cytokine production, indicative of broader immune dysfunction [21].

Consistent with this, recent work has shown that hyperactive LRRK2 variants linked to both PD and CD disrupt autophagy-dependent Paneth cell homeostasis and promote intestinal inflammation, further highlighting the role of LRRK2 in regulating immune and epithelial integrity within the gut [22].

Although LRRK2-PD clinically resembles sporadic PD, it displays heterogeneous CNS pathologies: roughly half of cases show cortical tau and/or LBs pathology, and α -synuclein oligomers can be present even in the absence of classic LBs [23-25]. Reflecting this variability in α -synuclein and tau co-pathology, pathogenic LRRK2 variants have been reported to exacerbate both α -synuclein and tau aggregation in some animal models, although results differ between models [26-29]. Mechanistically, LRRK2 is recruited to damaged or stressed lysosomes, where it phosphorylates specific Rab GTPases to regulate vesicular trafficking and lysosomal homeostasis. Both α -synuclein and tau aggregates disrupt endolysosomal function, and their clearance relies heavily on lysosome-mediated degradation pathways [30]. By modulating Rab-dependent lysosomal dynamics, LRRK2 may influence the accumulation and propagation of these pathogenic proteins, positioning it as a critical mediator of early α -synuclein and tau pathology in neurodegenerative diseases.

Importantly, both α -synuclein and tau are highly expressed in the ENS and in enteroendocrine cells, where they contribute to neuronal integrity and gut function [31, 32]. A growing body of evidence supports a bidirectional relationship between inflammation and α -synuclein

aggregation, in which immune activation not only responds to misfolded α -synuclein but actively promotes its accumulation and pathogenic conversion. Recent studies have demonstrated elevated colonic α -synuclein levels in response to acute experimental colitis [12]. Notably, increased levels and aggregation of α -synuclein have been reported in patients with inflammatory bowel diseases and during a variety of enteric infections, suggesting that sustained mucosal inflammation creates a permissive environment for synucleinopathy within the gut [33–35]. Although less extensively studied, a similar link has begun to emerge for tau, with inflammatory stimuli shown to enhance tau phosphorylation, impair its clearance, and promote the formation of pathogenic tau species in peripheral and central tissues [36, 37].

Together, these observations underscore inflammation as a shared upstream driver of both α -synuclein and tau pathology, providing a mechanistic framework through which chronic immune dysregulation may initiate or amplify protein aggregation in the ENS. Despite these insights, it remains unclear whether systemic low-grade inflammation directly cooperates with LRRK2 to drive gut inflammation and protein co-pathologies. To address this gap, we hypothesized that chronic low-grade inflammation synergizes with the LRRK2 G2019S variant to promote intestinal immune activation and protein aggregation. To test this, we developed a mouse model integrating three major PD risk factors: LRRK2 genetic susceptibility, chronic inflammation, and aging.

MATERIALS AND METHODS

Animals

Male C57BL/6J wild-type (WT) and C57BL/6J-Tg(Lrrk2-G2019S)2AMjff/J (LRRK2 G2019S) mice (Stock No. 018785) were purchased from The Jackson Laboratory (Bar Harbor, ME, USA). Animals were housed at the C.A.Pi.R. animal facility (University of Catania, Italy) and maintained on a 12-h light/dark cycle with *ad libitum* access to food and water. To minimize potential cage effects, mice were housed in groups of 3–4 per cage and randomly assigned to experimental groups, with a three-week acclimation period prior to the start of experiments. The research project (Authorization n. 345/2019-PR, issued by the Italian Ministry of Health on May 5, 2019) and all experimental procedures were approved by the University Institutional Animal Experimentation and Ethical Committee (OPBA, University of Catania). All procedures were performed in accordance with the European Union Directive (2010/63/EU) and the NIH Guide for the Care and Use of Laboratory Animals.

Lipopolysaccharide treatment

Three-month-old WT and LRRK2 G2019S mice were exposed to intraperitoneal (i.p.) injections of a low dose of lipopolysaccharide LPS (0.1 mg/kg *Escherichia coli* serotype O111:B4, Sigma-Aldrich), administered twice a week for 12 weeks.

To ensure a consistent inflammatory stimulus, injections were performed every Tuesday and Friday morning (between 09:00 and 12:00 h), totaling 24 injections per animal. WT and LRRK2 G2019S mice exposed to i.p. injections of 0.9% sterile NaCl (saline) were used as controls. Clinical evaluations (body weight, coat condition, lethargy, locomotor activity, and grooming behavior) were performed weekly throughout the study until experimental endpoint.

Tissue handling

Mice were deeply anesthetized and transcardially perfused with 0.9% NaCl, followed by 4% paraformaldehyde (PFA) in phosphate buffer (pH 7.2, 4°C). Tissues were carefully harvested, post-fixed for 2–4 hours in 4% PFA, and then cryoprotected in 15% sucrose in phosphate buffer overnight at 4°C. Blood was collected into EDTA-coated tubes, and plasma was separated by centrifugation and stored at –20°C. Tail biopsies from each mouse were genotyped via PCR followed by electrophoretic analysis according to the manufacturer's protocols (The Jackson Laboratory).

Immunohistochemistry and image analysis

For intestinal analysis, the distal colon was dissected and fixed in 1% paraformaldehyde for 2 h, washed with 50 mmol/L NH₄Cl, and cryoprotected in 30% sucrose (w/v) at 4°C overnight. Tissue was then embedded in OCT medium, snap-frozen, and stored at –80°C. Cryosections were rehydrated with PBS and blocked with 0.3% Triton X-100 in PBS containing 10% normal goat serum (NGS) for 1 h at room temperature. Samples were incubated overnight at 4 °C with primary antibodies diluted in PBS containing 0.3% Triton X-100 and 1% normal goat serum (NGS). The following antibodies were used: rat anti-CD4 (1:100, Cat# MCA4635T, Bio-Rad), rat anti-CD68 (1:100, Cat# MCA1957T, Bio-Rad), rabbit anti-HuC/D (1:200, Cat# ab184267, Abcam), mouse anti- β III tubulin (Tubb3, clone Tuj1, 1:1000, Cat# 801202, BioLegend), rabbit anti- α -synuclein aggregate (clone MJFR-14-6-4-2, 1:500, Cat# ab209538, Abcam). Following PBS washes, sections were incubated for 1 h with phalloidin-iFluor-594 (1:500, Cat# ab176757, Abcam) and appropriate secondary antibodies: goat anti-rat 488 (Cat# A48262TR), goat anti-rabbit 488 (Cat# A32731TR), goat

anti-mouse 488 (Cat# A32723TR), or goat anti-rabbit 647 (Cat# A32733TR), all from Invitrogen. After washing with PBS, samples were incubated for 5 min at room temperature with DAPI (1:10,000 in PBS) to stain nuclei. Images were acquired using a Leica TCS SP8 confocal microscope equipped with a 63×/1.4 NA oil-immersion objective or a ZEISS Axio Imager with ApoTome 2 (Carl Zeiss) equipped with a 10×/0.3 NA objective and analyzed with ImageJ (version 1.53t).

To normalize immune cell infiltration (CD4+, CD68+), an investigator blinded to the experimental groups examined areas of approximately 0.1 mm² of epithelial-covered villus mucosa from 6-8 fields per mouse. Pan-neuronal HuC/D+ cells were manually quantified in at least 8 random fields across the distal colon from 3 distinct slides per animal (n=6 mice/condition). The mean fluorescence intensity (MFI) of the aggregated α -synuclein specific signal was calculated from 8–10 images taken with a 10× objective covering the entire gut section; a constant threshold was applied to all images to isolate aggregate-specific signals, which were normalized to the total surface area detected by DAPI staining. For the analysis of total and phosphorylated α -synuclein (pS129) and tau (pThr181), gut tissues were paraffin-embedded and sectioned at 5 μ m (n = 6 mice per group). Sections were deparaffinized in xylene and rehydrated through a graded ethanol series. Heat-induced antigen retrieval was performed in 1X Tris-EDTA buffer at 95–98°C for 20 min. Sections were cooled for 35 min, rinsed with 1X Tris-Buffered Saline (TBS), and blocked in 0.2% Triton X-100 and 10% NGS in 1X TBS for 1 h. The following primary antibodies were diluted in 1X TBS with 0.2% Triton X-100 and 5% NGS and incubated overnight at 4°C: rabbit anti-phosphorylated S129 α -synuclein (clone MJF-R13, 1:2000, Cat# ab168381, Abcam), rabbit anti-phosphorylated Tau (Thr181) (1:2000, Cat# 28866-1-AP, Proteintech), rabbit anti-total α -synuclein (clone Syn211, 1:500, Cat# 36-008, Invitrogen), and rabbit anti-total MAPT/Tau (1:500, Cat# 10274-1-AP, Proteintech).

Secondary antibodies and phalloidin were applied in 5% NGS and 0.2% Triton X-100 in 1X TBS for 2 h at room temperature. After washing with PBS, samples were incubated for 10 min with DAPI (1:5000 in PBS). Images were acquired with an Olympus APEXVIEW APX100 fluorescence microscope using a 60× oil-immersion objective (1.42 NA). MFI was analyzed using ImageJ (version 1.54p) within a fixed 0.22 mm² area for each image using the same thresholding criteria previously described.

To validate antibody specificity and distinguish genuine target staining from background noise, negative controls were performed by omitting primary antibodies

and utilizing isotype-matched controls where appropriate. All quantitative data were normalized to the 3-month WT baseline to allow for longitudinal comparison across experimental groups.

Proteinase K treatment of gut sections

Gut tissue sections were mounted onto slides and treated for 15 minutes at room temperature using PBS containing 10 μ g/mL Proteinase K (Promega, V302A). The slides were then rinsed three times in PBS for ten minutes. Following the washes, the slides were blocked for 1 hour at room temperature in PBS with 0.3% Triton X-100 and 10% Normal Goat Serum and subsequently stained according to the immunofluorescence procedure described in the previous section.

Luminex-Based multiplex cytokine analysis

Plasma cytokine levels, including IFN- γ , IL-1 β , IL-2, IL-4, IL-6, IL-10, IL-17A, and TNF- α were measured simultaneously using the multiplexed kit "Mouse High Sensitivity T Cell Magnetic Bead Panel" (MHSTCMAG-70K, MILLIPLEX, EMD Millipore Corp., Billerica, MA, USA). The assay was performed according to the manufacturer's instructions with an overnight incubation step (17 hours) at 4 °C. Samples were analyzed as single values and two controls with corresponding acceptance ranges, provided with the kit, were used for quality control purposes. The readout was performed on a FLEXMAP 3D® instrument (Luminex Corp., Austin, TX, USA). Data were acquired using Luminex xPONENT® software (version 4.3) and mean fluorescence intensity (MFI) was determined. Bio-Plex Manager™ software (version 6.2; Bio-Rad, Hercules, CA, USA) was used for back-calculation of unknown cytokine concentrations.

Statistical analysis

Statistical analyses were performed using GraphPad Prism 10 (GraphPad Software, Boston, MA, USA). Sample sizes (n=6 mice per group) were determined to ensure sufficient power to detect significant pathological and immune differences in accordance with established standards in the field [12]. Data are presented as mean $\pm$ standard error of the mean (SEM). Comparisons between two groups were analyzed using an unpaired, two-tailed Student's t-test. Multiple comparisons were analyzed using two-way analysis of variance (ANOVA), followed by Bonferroni's multiple comparisons test. A p-value of < 0.05 was considered statistically significant.

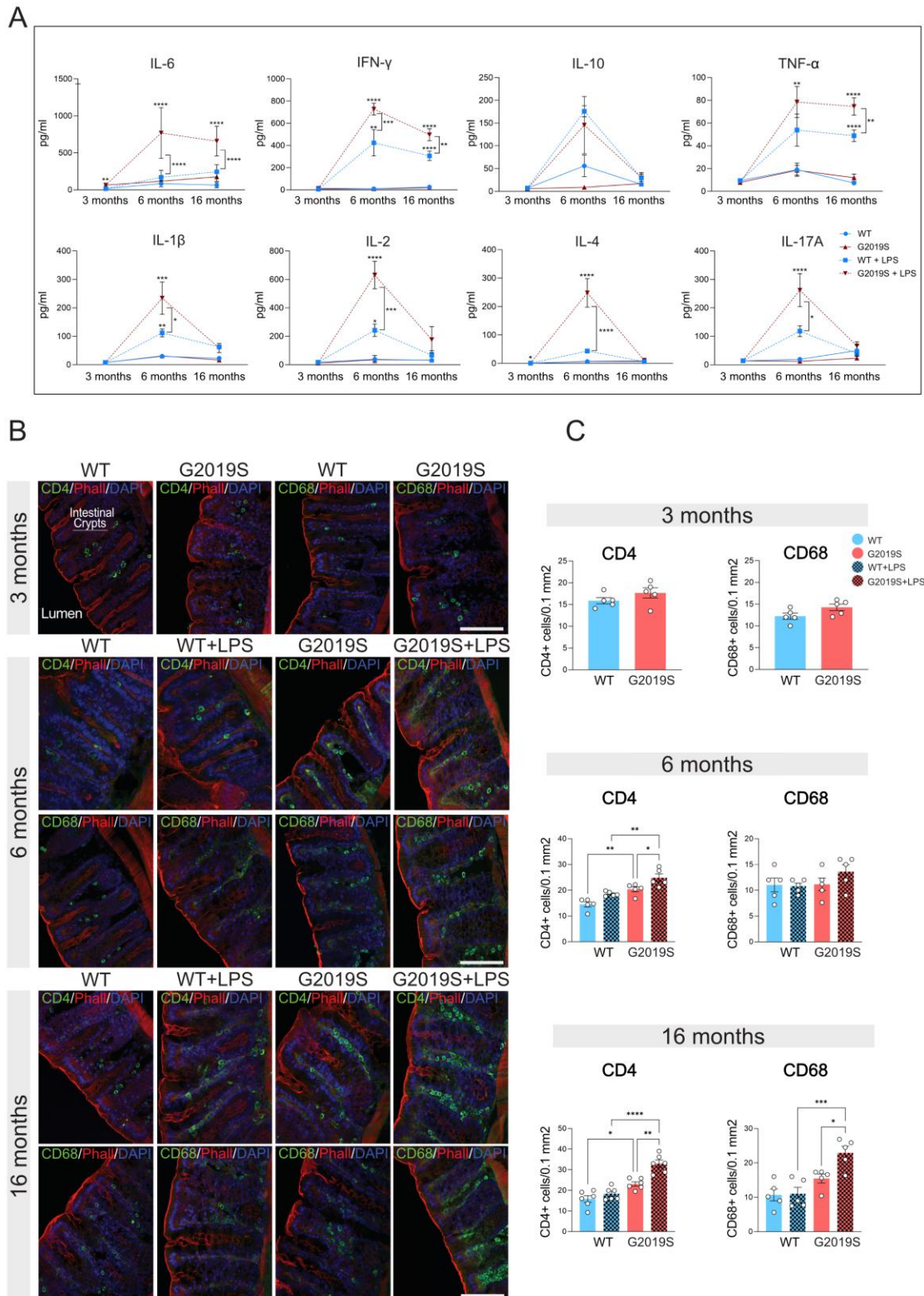


Figure 1. LRRK2 G2019S enhances age-dependent systemic inflammation and promotes colonic immune cell infiltration under chronic LPS exposure. (A) Cytokine profile in the plasma of WT and LRRK2 G2019S mice at the baseline (3 months), 6 and 16 months. Mean $\pm$ SEM, $n = 6$ mice/group. The significances shown are between LRRK2 G2019S and LRRK2 WT under untreated conditions or LPS-treated groups if indicated in brackets: **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, two-way ANOVA with Bonferroni post hoc test; data are expressed as pg/mL. **(B)** Intestinal cryosections

stained against CD4 or CD68 (green), phalloidin (red), and DAPI (blue) from WT and LRRK2 G2019S mice treated with LPS or saline at the indicated time points. Scale bar, 100 μ m. (C) Analysis of CD4⁺ and CD68⁺ cells within colonic sections. Mean $\pm$ SEM; **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, as indicated in the graphs; two-way ANOVA with Bonferroni post hoc test; $n=6$ mice/group. Cells counted from at least 10 high-power fields (100 $\times$)/mouse.

RESULTS

LRRK2 G2019S enhances systemic inflammatory responses to chronic low-dose LPS exposure

As aging is recognized as the main risk factor influencing both the clinical presentation and progression of sporadic and LRRK2-associated PD, we examined whether the LRRK2 G2019S variant, in combination with chronic moderate systemic inflammation, interacts with the aging process to modulate intestinal and systemic immune responses. To assess the impact of LRRK2 G2019S on gut inflammation and pathology, wild-type (WT) and hemizygous transgenic C57BL/6J-LRRK2 G2019S (LRRK2 G2019S) mice received intraperitoneal injections of low-dose lipopolysaccharide (LPS: 0.1 mg/kg) or saline (NaCl) twice weekly for 12 consecutive weeks. Age- and background-matched C57BL/6 WT non-transgenic mice served as controls. Weekly clinical assessments, including body weight, posture, lethargy, movement reluctance, and grooming behavior revealed that chronic LPS administration did not significantly affect general health parameters or lifespan in either young or aged mice of both genotypes compared to NaCl-injected controls. Mice were sacrificed at 3, 6, and 16 months of age for subsequent analyses. We first measured plasma cytokine levels in WT and LRRK2 G2019S mice in response to LPS at different ages. Unlike previous reports [38, 39], LRRK2 G2019S mice exhibited elevated baseline levels of IL-6 and IL-4 at 3 months of age compared with WT mice (Fig. 1A). Furthermore, 6-month-old LRRK2 G2019S mice treated with LPS showed significantly higher plasma concentrations of IL-6, IL-1 β , IFN- γ , IL-2, TNF- α , IL-17A, and IL-4 than WT counterparts. At 16 months, levels of IL-4, IL-2, and IL-17A returned to baseline values, whereas plasma levels of the pro-inflammatory cytokines IL-6, IFN- γ , and TNF- α remained significantly elevated in LPS-treated LRRK2 G2019S mice compared with WT controls (Fig. 1A).

LRRK2 G2019S promotes colonic infiltration of CD4⁺ T cells and macrophages

To investigate whether the LRRK2 G2019S variant affects local immune cell dynamics in the gut, we analyzed CD4⁺ T-cell and macrophage populations in colonic tissue from WT and LRRK2 G2019S mice at 3, 6, and 16 months of age. These analyses were designed to capture both the baseline immune landscape and

alterations induced by chronic peripheral inflammation. A significant increase in CD4⁺ T-cell infiltration was detected in the colon of untreated LRRK2 G2019S mice starting at 6 months, compared with age-matched WT controls (Fig. 1B, C), indicating an early onset of local immune activation. Chronic LPS administration further enhanced CD4⁺ T-cell infiltration in 6-month-old LRRK2 G2019S mice, whereas no significant changes were observed in WT animals, suggesting a genotype-specific response to peripheral inflammatory stimuli. In parallel, quantification of CD68⁺ macrophages revealed an increase in the colonic tissue of 16-month-old LRRK2 G2019S mice relative to WT controls, which was further exacerbated by chronic LPS exposure (Fig. 1B, C). Together, these findings indicate that LRRK2 G2019S promotes age-dependent immune cell infiltration in the colon and enhances sensitivity to peripheral inflammatory challenges.

Chronic low-grade inflammation triggers α -synuclein aggregation and phosphorylation in the colon of LRRK2 G2019S mice

To investigate whether chronic inflammation and LRRK2 G2019S expression influence α -synuclein pathology in the gut, we performed immunohistochemical analysis of colonic tissues using the aggregate-specific α -syn antibody MJF-14 [40, 41]. Quantitative analysis revealed that LPS treatment significantly increased α -synuclein aggregation in both WT and G2019S mice at 6 and 16 months compared with their respective saline-treated controls (Fig. 2A, B). Importantly, the G2019S mutation further exacerbated this effect, as LPS-treated G2019S mice showed greater α -synuclein accumulation than LPS-treated WT mice at both time points, with the most pronounced difference observed at 16 months (Fig. 2B). Immunofluorescence colocalization analysis at 16 months, combining MJF-14 and tyrosine hydroxylase (TH) staining, revealed that α -synuclein aggregates localize within enteric dopaminergic neurons (Supplementary Fig. 1A, B). Notably, despite the presence of aggregated α -synuclein, we did not detect proteinase K-resistant α -synuclein, suggesting the aggregates may represent non-fibrillar species.

We next assessed α -synuclein pathology by quantifying phosphorylated α -synuclein at serine 129 (pS129 α -syn). At 6 months, LPS-treated G2019S mice exhibited significantly elevated pS129 α -synuclein levels compared with both saline-treated G2019S and LPS-

treated WT mice (Fig. 3A, B). At 16 months, LPS-treated G2019S mice still displayed higher pS129 α -synuclein levels compared with their saline-treated counterparts, while WT mice showed only a modest, non-significant increase. Notably, pS129 α -synuclein levels remained significantly higher in LPS-treated G2019S mice compared with LPS-treated WT animals. In contrast, total

α -synuclein levels did not differ significantly across genotypes or treatment conditions (Supplementary Fig. 2). Collectively, our data demonstrate that chronic inflammatory stress acts in concert with LRRK2 G2019S to drive the accumulation and phosphorylation of α -synuclein in the gut.

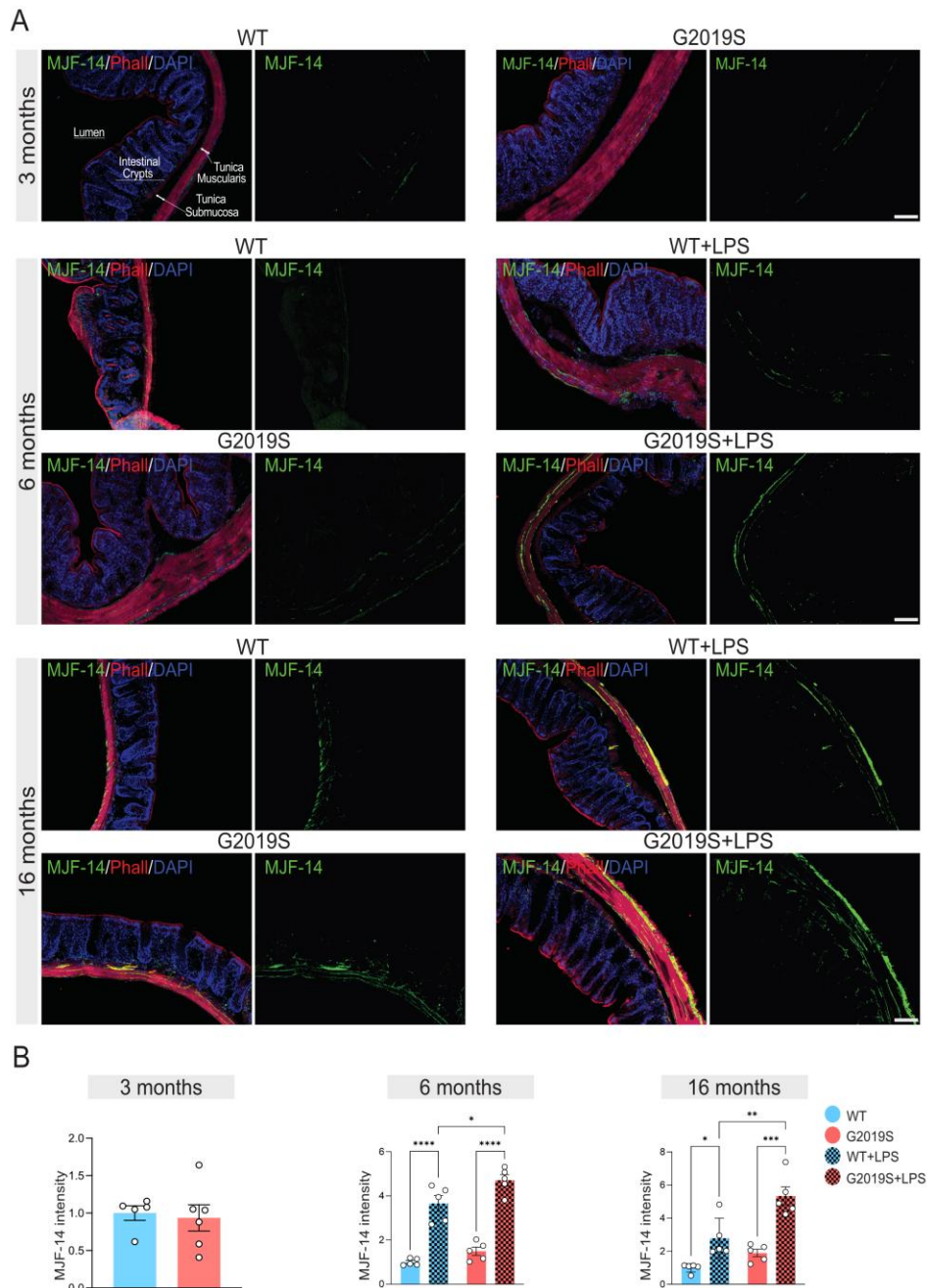


Figure 2. Chronic low-grade inflammation promotes accumulation of aggregated α -synuclein in the gut of LRRK2 G2019S mice. (A) Intestinal cryosections stained for aggregated α -syn (MJF-14, green), phalloidin (magenta), and DAPI (blue) from WT and LRRK2 G2019S mice treated with LPS or saline at the indicated time points. Scale bar, 100 μ m. (B) Quantification of MJF-14 mean fluorescence intensity (MFI) within colonic sections, normalized to the corresponding untreated control. Mean $\pm$ SEM; **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$; two-way ANOVA with Bonferroni post hoc test. Data were acquired from at least 10 high-power fields (100x)/mouse; $n = 6$ mice/group.

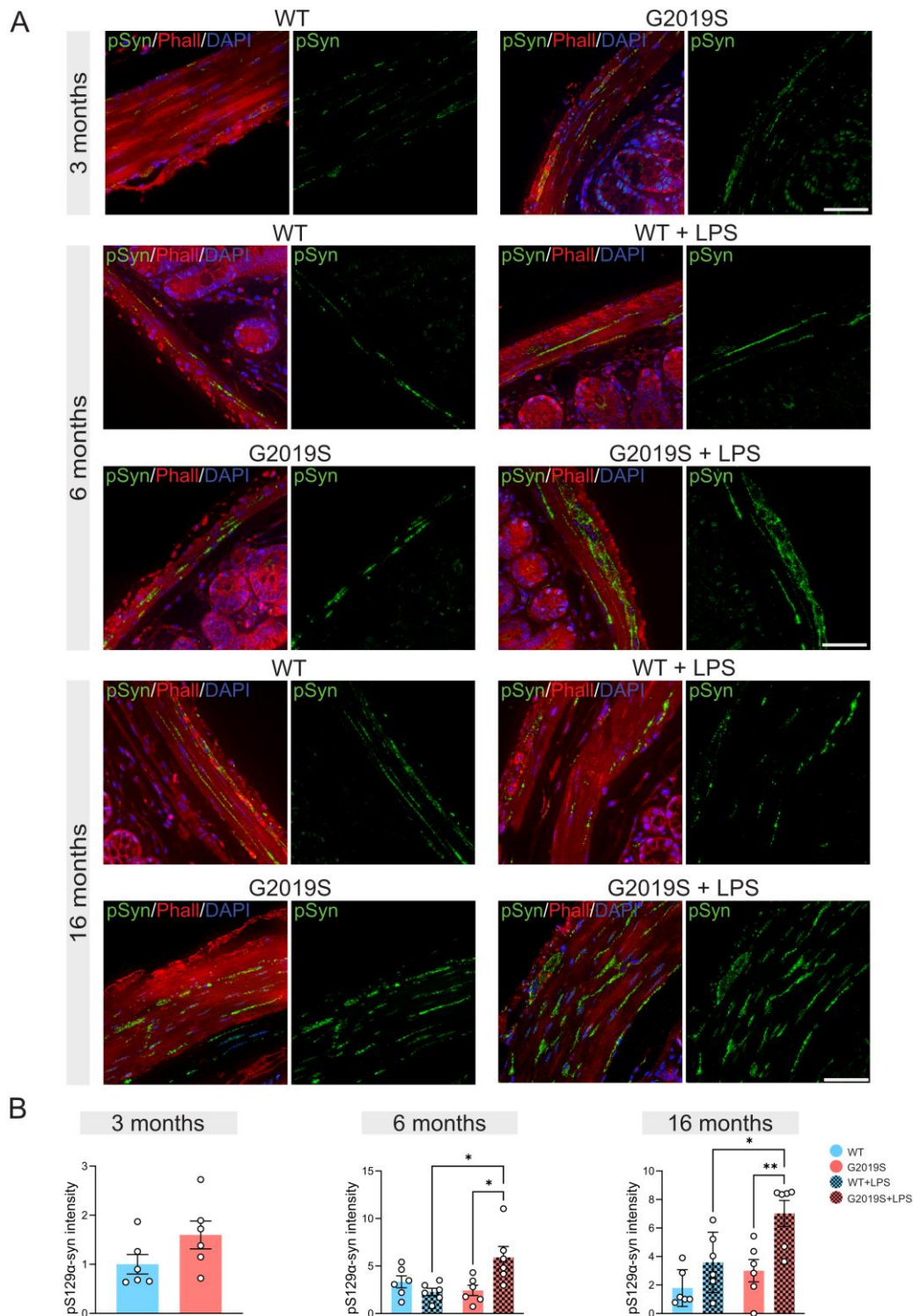


Figure 3. Chronic low-grade inflammation promotes early pS129 α -syn accumulation in the gut of LRRK2 G2019S mice. (A) Intestinal cryosections stained against phalloidin (red), pS129 α -syn (green) and DAPI (blue) from WT and G2019S mice treated with LPS or saline at the indicated time points. Scale bar, 50 μ m. (B) Quantification of pS129 α -syn mean fluorescence intensity (MFI), normalized to the corresponding untreated control. Mean $\pm$ SEM; ** $p < 0.01$; * $p < 0.05$; two-way ANOVA with Bonferroni post hoc test. Data were acquired from at least 4 different fields/section, with 60x oil-immersion objective; $n=6$ mice/group.

Chronic low-grade inflammation promotes tau pathology in the colon of LRRK2 G2019S mice

Accumulating evidence indicates that LRRK2 may influence tau pathology, another hallmark of neurodegeneration. LRRK2 can modulate tau phosphorylation, aggregation, and spreading in the CNS, particularly under conditions of cellular stress or

inflammation, suggesting a shared mechanism with α -synuclein pathology [23, 42]. This positions LRRK2 as a potential mediator linking peripheral immune activation to early tau and α -synuclein accumulation in the gut. To dissect the possible link between LRRK2 and intestinal tau pathology, we assessed the phosphorylation of tau at threonine 181 (pTau-Thr181), a recognized biomarker of early-stage tauopathy [43, 44].

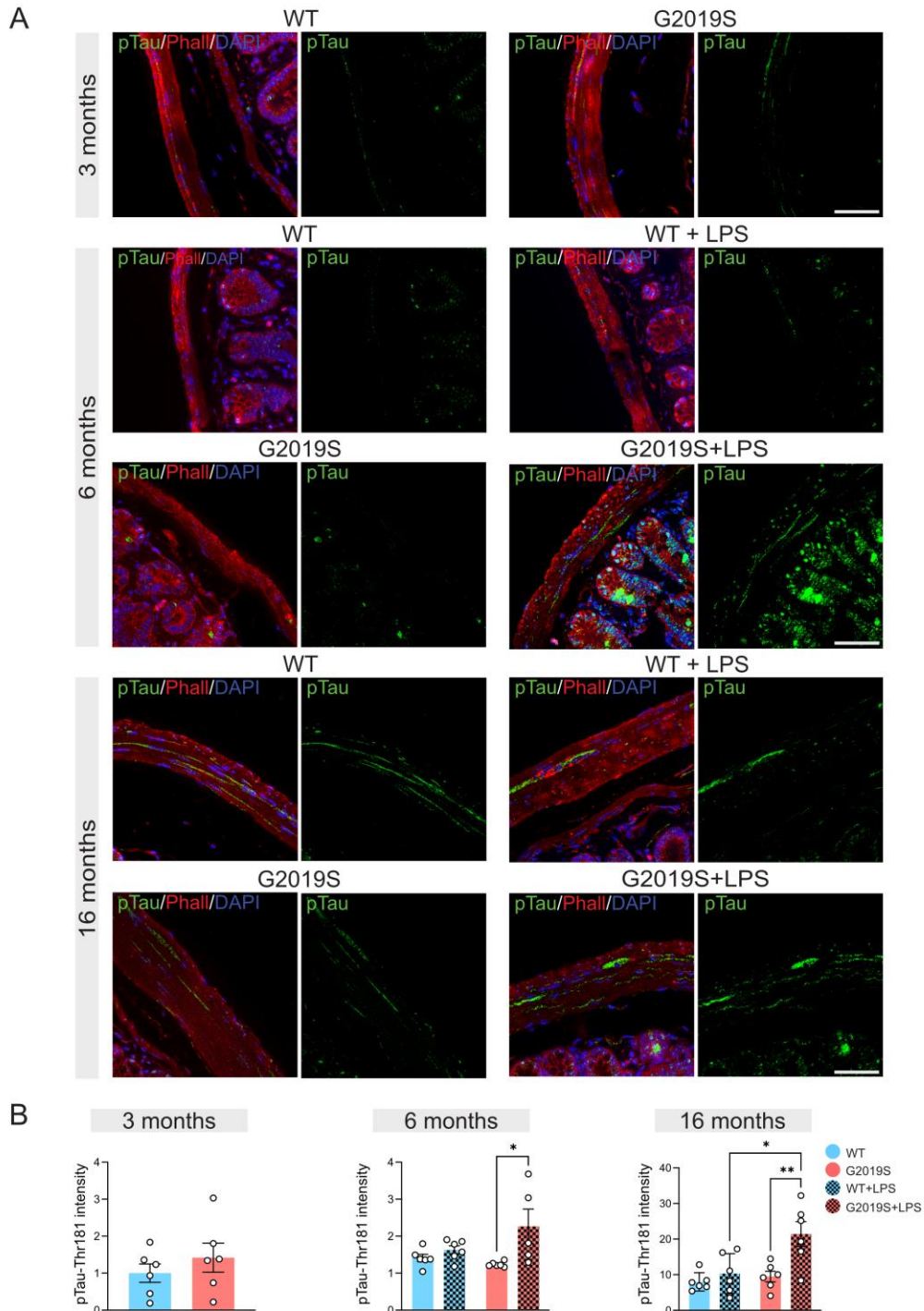


Figure 4. Chronic low-grade inflammation promotes tau pathology in the gut of LRRK2 G2019S mice. (A) Intestinal cryosections stained against phalloidin (red), pTau-Thr181 (green) and DAPI (blue) from WT and G2019S mice treated with LPS or saline at the indicated time points. Scale bar, 50 μ m. (B) Quantification of pTau-Thr181 mean fluorescence intensity (MFI), normalized to the corresponding untreated control. Mean $\pm$ SEM, ** $p < 0.01$, * $p < 0.05$; two-way ANOVA with Bonferroni post hoc test. Data were acquired from at least 4 different fields/section, with 60x oil-immersion objective; $n = 6$ mice/group.

At 3 months, pTau-Thr181 levels showed a non-significant increase in LRRK2 G2019S mice compared with WT controls (Fig. 4A, B). At 6 months, LPS-treated LRRK2 G2019S mice exhibited significantly higher pTau-Thr181 levels compared with their saline-treated counterparts (Fig. 4A, B). At 16 months, this effect persisted, with LPS-treated LRRK2 G2019S mice displaying significantly elevated pTau-Thr181 levels compared with both saline-treated G2019S and LPS-

treated WT mice (Fig. 4A, B). In contrast, total tau levels did not differ significantly across genotypes or treatment conditions (Supplementary Fig. 3). Together, these findings demonstrate that LRRK2 G2019S enhances susceptibility to inflammation-induced tau phosphorylation in the gut, promoting an early and sustained accumulation of pathological pTau-Thr181 that persists with aging.

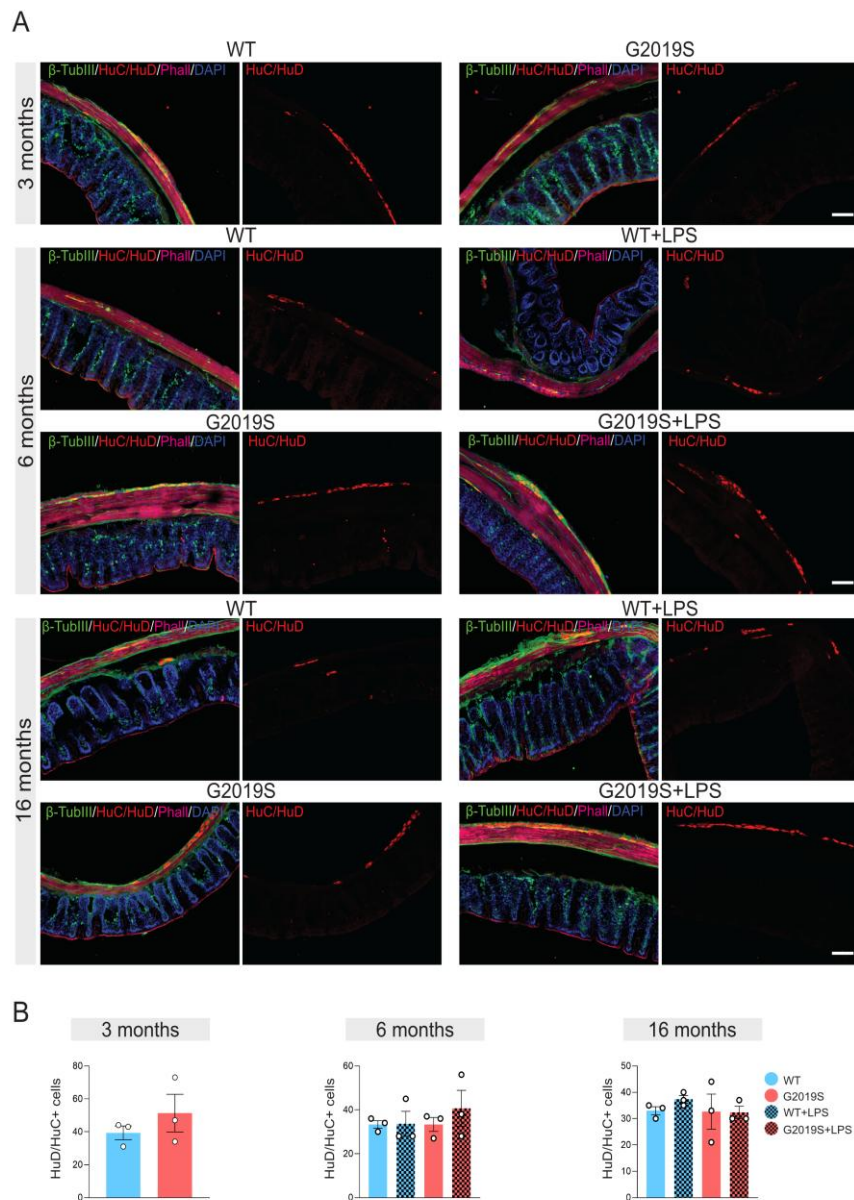


Figure 5. LRRK2 G2019S and chronic inflammation do not affect enteric neuron numbers. (A) Intestinal cryosections stained against HuC/D (red), β -III-Tubulin (green), phalloidin (magenta), and DAPI (blue) from WT and G2019S mice treated with LPS or saline at the indicated time-points. Scale bar, 100 μ m. (B) Quantification of HuC/D-positive cells within colonic sections. Mean $\pm$ SEM; two-way ANOVA followed by Bonferroni's multiple comparisons test revealed no significant differences between groups ($p > 0.05$). Data were acquired from at least 10 high-power fields (100x)/mouse, $n = 6$ mice/group.

LRRK2 G2019S and chronic inflammation do not affect enteric neuron numbers

Given that PD-associated pathology in the gut can involve protein aggregation without necessarily causing overt neuronal loss [45, 46], we next assessed the potential impact of inflammation and LRRK2 genotype on enteric neuron integrity. Quantification of HuC/D-positive neurons revealed no significant differences in neuronal counts across treatment groups or genotypes at any of the time points analyzed (Fig. 5A, B). Collectively, these findings indicate that LRRK2 G2019S predisposes to increased pathological pS129 α -synuclein and pTau-Thr181 accumulation in the colon in response to chronic low-grade inflammation, likely mediated by age-dependent immune cell infiltration.

DISCUSSION

In this study, we examined how chronic low-grade systemic inflammation interacts with the LRRK2 G2019S variant and aging to influence colonic inflammation and the development of α -synuclein and tau pathology in the gut. We found that low-grade peripheral immune activation induced an age-dependent rise in circulating cytokines, along with increased infiltration of CD4⁺ T cells and macrophages into the colonic tissue of LRRK2 G2019S mice. This heightened inflammatory response was accompanied by greater α -synuclein aggregation and phosphorylation, as well as increased tau phosphorylation, relative to WT controls. These findings suggest that LRRK2 serves as a critical link between peripheral immune responses and early proteinopathy changes in the gut. Importantly, these alterations occurred in the absence of overt neuronal loss, indicating a pre-degenerative stage of gut pathology. Together, our data support the concept that the gut may represent an early site of protein aggregation, where LRRK2-mediated dysregulation of immune signaling contributes to the initial stages of PD-like pathology. Moreover, we observed that systemic immune alterations, including elevated cytokine levels and increased CD4⁺ T-cell infiltration, emerge as early as 6 months of age in LRRK2 G2019S mice, suggesting that peripheral immune activation potentially drives local protein aggregation. These findings align with the growing evidence that peripheral immune mechanisms modulate the onset and progression of PD, providing a mechanistic link between

genetic susceptibility, environmental triggers, and early proteinopathy in the gut.

PD is a complex disorder affecting both the central and peripheral nervous systems, producing a broad spectrum of motor and non-motor symptoms [47, 48]. Its etiology likely reflects a convergence of genetic susceptibility and environmental factors, including aging, inflammation, and microbial exposures. Among genetic contributors, LRRK2 stands out for its dual role in neuronal and immune regulation, including modulation of α -synuclein and tau pathology, mitochondrial function, and glial reactivity. LRRK2 kinase activity is increased in both midbrain dopaminergic neurons and microglia in postmortem brains of patients with idiopathic PD, implicating it as a contributor to sporadic, age-dependent PD [49]. Rodent models of LRRK2 have provided important insights, although many transgenic and knock-in models lack robust neurodegeneration [50–53]. Overexpression of pathogenic LRRK2 variants, including G2019S, can induce pathological α -synuclein and tau accumulation, altered dopamine homeostasis, and autophagic or mitochondrial impairments [54–56]. However, overexpression systems can introduce artifacts, and viral vector approaches, while informative, may not replicate the peripheral expression patterns of LRRK2 observed in human PD [55, 57]. Knock-in models, in which the variant is introduced into the endogenous locus, offer a more physiological strategy but also have inherent limitations. Differences in regulatory regions between mouse and human LRRK2 may lead to altered tissue-specific expression or dysregulated responses to environmental or exogenous stimuli [58, 59]. Although LRRK2 G2019S knock-in mice reproduce aspects of PD susceptibility, they generally develop only mild phenotypes, suggesting that additional environmental or inflammatory challenges may be required to unmask early pathological features. Consistent with this, studies using LRRK2 G2019S knock-in mice have shown that an exaggerated inflammatory response in the gut emerges only after exposure to an environmental trigger, such as DSS-induced colitis [10]. This response was markedly more severe than in wild-type mice and was sufficient to induce neuroinflammation. However, pronounced motor deficits and exacerbated neurodegeneration occurred only when gut inflammation coincided with a second pathogenic insult represented by α -synuclein injection in the brain [10]. In line with these findings, a recent study on SARS-CoV-2 infection demonstrated that neither viral

exposure nor genetic predisposition alone was sufficient to trigger PD-like pathology [60]. Infection with SARS-CoV-2 did not trigger parkinsonian features in wild-type mice. In contrast, genetically susceptible LRRK2 G2019S mice developed marked neuroinflammation and substantia nigra degeneration only when SARS-CoV-2 infection was followed by exposure to a subtoxic dose of MPTP [60]. Together, these findings reinforce the concept that LRRK2-linked vulnerability requires an additional environmental ‘hit’ to precipitate the pathological cascade, better reflecting the multifactorial and age-dependent disease progression observed in patients with PD.

This convergence of LRRK2’s roles in intestinal immunity and neuroinflammation highlights its central position within the gut-brain axis. Early-life infections, microbial dysbiosis, and chronic gut inflammation may engage and potentiate LRRK2-dependent immune pathways, thereby initiating or accelerating neurodegenerative processes. Beyond these triggers, aging emerges as the most dominant and pervasive driver of PD, fundamentally reshaping homeostasis across the gut-brain axis. In the enteric compartment, aging is associated with impaired epithelial barrier integrity, compositional and functional alterations of the microbiota, and a chronic state of systemic low-grade inflammation, all of which may synergize with and amplify the consequences of LRRK2 hyperactivation [61, 62]. At the cellular level, this increased vulnerability reflects a progressive erosion of proteostatic and metabolic homeostasis, prominently involving dysfunction of the autophagy-lysosomal system and depletion of essential metabolic cofactors [63, 64]. This age-dependent decline is driven by dysregulation of nutrient- and stress-sensing pathways, including sustained mTOR signaling and impaired lysosomal biogenesis, which together reduce autophagic flux by compromising autophagosome formation and degradative capacity. As a result, cellular quality-control systems progressively fail, leading to inefficient clearance of damaged proteins and dysfunctional organelles. In parallel, age-associated NAD⁺ depletion further weakens sirtuin-dependent mitochondrial quality-control pathways, including mitophagy, thereby promoting mitochondrial dysfunction, chronic cellular stress, and the establishment of a senescence-associated pro-inflammatory state. Collectively, these converging failures in metabolic, lysosomal, and mitochondrial maintenance lower the threshold for proteotoxic stress and facilitate the accumulation and potential propagation of misfolded protein species.

In this context, aging acts as a critical permissive and integrative factor that links systemic inflammation and impaired cellular clearance mechanisms, thereby creating

a biological environment that is highly permissive for the emergence and progression of neurodegenerative pathology in PD.

Our study integrates three PD risk factors, LRRK2 genetic susceptibility, chronic low-grade systemic inflammation, and aging, in a mouse model. We demonstrate that even sub-threshold doses of LPS trigger significant recruitment of CD4⁺ T cells to the colon and potentiate α -synuclein and tau co-pathology in LRRK2 G2019S mice, without causing overt neuronal loss in the gut. These findings mirror observations in human PD, where enteric α -synuclein and tau pathology can precede motor symptoms by decades [37, 65, 66]. Furthermore, our data indicate that peripheral immune dysregulation, through elevated cytokines such as IL-6, IFN- γ , and TNF- α , may amplify local protein aggregation and potentially modulate CNS pathology via the gut-brain axis. The persistent elevation of IL-6, TNF- α , and IFN- γ in aged LRRK2 G2019S mice suggest a shift toward chronic innate inflammation (“inflammaging”), while the decline in IL-4, IL-2, and IL-17A reflects loss of adaptive immune competence (“immunosenescence”). Together, these findings suggest that LRRK2-driven immune dysregulation interacts with chronic low-level inflammation and aging to promote a sustained pro-inflammatory milieu, which may contribute to increased PD susceptibility or progression [38, 67, 68].

The co-occurrence of α -synuclein and tau pathologies in the gut mirrors observations in LRRK2-PD brains, where cortical tau and LB pathology frequently overlap [69, 70]. For instance, a recent work has shown that α -synuclein positivity is associated with higher levels of phosphorylated tau-181 in the cerebrospinal fluid and increased tau deposition, correlating with a more aggressive disease course [71]. Consistent with these findings, studies using co-pathology mouse models have shown that the presence of both proteins triggers pronounced infiltration of peripheral CD4⁺ and CD8⁺ T cells into the brain [72, 73].

This interaction appears to establish a detrimental feedback loop in which protein co-aggregation amplifies inflammation, thereby accelerating neurodegeneration.

However, the temporal sequence of these pathogenic events in the brain remains debated [74], with growing evidence suggesting that tau pathology may precede and even trigger α -synuclein aggregation. Several studies report substantial neuronal loss and dysfunction in the substantia nigra before the appearance of LB pathology [75–78], while others show that tau accumulation alone can drive severe neurodegeneration and parkinsonism in the absence of synucleinopathy [69, 79, 80]. These observations support the hypothesis that tau pathology may act as an upstream event contributing to α -synuclein aggregation in PD. In this context, our observation of a

similar α -synuclein-tau co-pathology in the gut suggests that this pathogenic synergy is not restricted to the brain but may originate in the ENS, potentially contributing to the initiation and propagation of pathology along the gut-brain axis [42, 72, 74].

Our findings suggest that early-life exposure to chronic, low-dose systemic inflammation interacts with the LRRK2 G2019S variant to shape the trajectory of α -synuclein pathology in an age-dependent manner. LPS treatment was administered at 3 months of age for 12 weeks, a developmental window in which the immune system remains highly responsive and environmental modifiers may exert long-lasting effects.

Consistent with this, LRRK2 G2019S mice exhibited a robust increase in pS129 α -synuclein at 6 months, indicating that inflammatory stimulation enhances α -synuclein phosphorylation.

LPS administration triggered a marked increase in pathogenic tau phosphorylation at 6 months, which persisted in aged mice. A direct mechanistic link between LRRK2 and the observed co-pathologies is supported by evidence that both α -synuclein and tau aggregation impair lysosomal and endolysosomal function via pathways directly modulated by LRRK2 through Rab GTPases [30, 81-83]. Specifically, the LRRK2 G2019S variant induces lysosomal abnormalities, including altered morphology, reduced acidification, and impaired autophagic flux [84].

These defects are associated with disrupted α -synuclein degradation, leading to the accumulation of detergent-insoluble α -synuclein and its enhanced neuronal release.

Moreover, lysosomal overload induced by lysosomotropic drugs recruits LRRK2 and its substrate Rab10 to the lysosomal surface, promoting Rab10 phosphorylation and exosome-mediated release of insoluble α -synuclein from microglia and macrophages [85]. In line with these findings, recent studies reported that the LRRK2-mediated phosphorylation of Rab proteins (specifically pRab10 and pRab12) is elevated not only in PD but also in other neurodegenerative disorders characterized by altered α -synuclein or tau pathology, such as Alzheimer's disease and progressive supranuclear palsy [81, 86]. Importantly, the phosphorylated Rabs were not randomly distributed but were highly concentrated on the membranes of granulovacuolar degeneration (GVD) bodies, which are small vesicles considered hallmarks of cellular stress and closely associated with endolysosomal and autophagic dysfunction. The number of pRab-positive GVD bodies increased with advancing Braak stages of tau pathology, and at later stages, pRab markers were also observed within mature protein aggregates, including neurofibrillary tangles (tau) and Lewy bodies (α -synuclein) [81].

Our findings support a model in which LRRK2 hyperactivity sensitizes the gut to inflammatory triggers, promoting early co-pathologies in the gut that may act as seeds for CNS propagation.

Notably, the preservation of enteric neuron numbers suggests that protein aggregation precedes neuronal loss, marking a prodromal phase that may be amenable to early therapeutic intervention. Although damage to the enteric nervous system is a common feature across PD models, the extent of neuronal loss varies considerably among them [87]. For instance, pronounced loss of enteric neurons has been reported in A53T transgenic and rotenone-treated mice, whereas MPTP-treated models exhibit neuronal stress without overt degeneration [87]. Similarly, studies on human post-mortem tissues report inconsistent results: some describe a reduction in enteric neuron numbers in PD patients, while others find no significant difference compared to healthy controls [45, 88, 89].

However, neuron counts alone may not fully capture gut pathology, as enteric neurons can exhibit functional impairments, including altered signaling or synaptic activity, even without cell death [45].

Moreover, quantifying total enteric neurons is notoriously challenging, contributing to conflicting results.

Methodological variability begins with sample preparation (whole mounts versus tissue sections), marker selection (e.g., Giemsa, Cuproline blue, NADH diaphorase, PGP 9.5, HuC/D), and normalization strategies (serosal area, gut length, ganglion, or ganglionic area) [90-92]. Marker expression may be downregulated in metabolically stressed or diseased neurons, potentially leading to apparent, but not actual, cell loss. Myenteric neuron density also varies along the intestinal tract, being generally lower in the small intestine than the large intestine, and within segments, highlighting the importance of regional specificity.

Limitations and Future Perspectives

While our findings provide novel insights into the initiation of enteric proteinopathy, several limitations should be acknowledged. First, our systemic LPS model differs from primary lumen-initiated mucosal injury models. However, it captures key features of the chronic endotoxemia observed in PD patients with increased intestinal permeability [93]. This "systemic-to-enteric" axis is further supported by the clinical association between the Systemic Immune-Inflammation Index and PD risk, highlighting peripheral inflammation as a potential driver of disease initiation in genetically susceptible individuals [94]. Second, the impact of biological sex on LRRK2-associated PD pathology

remains complex. Clinical studies in LRRK2 mutation carriers have yielded inconsistent findings, with some reporting higher disease penetrance in females and others observing no significant sex-related differences [95-98]. Our decision to focus on males was guided by recent evidence showing that prodromal intestinal inflammation exacerbates LRRK2 G2019S endophenotypes in a sex-dependent manner, with females displaying relative protection against α -synuclein aggregation [12]. By prioritizing the male sex, we increased the sensitivity of our model to detect subtle, pre-degenerative pathological changes. Future studies incorporating female cohorts will be essential to complete our knowledge of the LRRK2-gut axis and to unravel the specific biological mechanisms underlying the sex-specific resilience observed in this model. Addressing these factors will be crucial for the development of early, sex-stratified therapeutic interventions targeting the gut-immune interface.

Conclusions

Overall, this work supports the concept that LRRK2 mediates a convergence of genetic and environmental risk factors, linking peripheral immune activation, intestinal inflammation, and early α -synuclein/tau co-pathology. Our findings underscore the importance of examining gut-brain interactions and peripheral immunity in PD and suggest that targeting early inflammatory and protein aggregation events in the gut may provide opportunities for disease-modifying interventions.

Data availability

The authors declare that all data supporting the findings of this study are available in this article and its supplementary information file.

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Author Contributions

C.G., C.T., and F.L. collected the samples and performed the mouse injections. C.G., F.B., C. Giu., and M.M. performed the experiments. M.D., C.G., C. Giu., F.B., and M.M. analyzed the results. C.G. and M.D. wrote the manuscript. All authors contributed to reviewing and approved the final version of the manuscript.

Supplementary Materials

The Supplementary data can be found online at: www.aginganddisease.org/EN/10.14336/AD.2026.0342.

References

- [1] Fasano A, Visanji NP, Liu LW, Lang AE, Pfeiffer RF (2015). Gastrointestinal dysfunction in Parkinson's disease. *Lancet Neurol*, 14:625-639.
- [2] Stokholm MG, Danielsen EH, Hamilton-Dutoit SJ, Borghammer P (2016). Pathological alpha-synuclein in gastrointestinal tissues from prodromal Parkinson disease patients. *Ann Neurol*, 79:940-949.
- [3] Braak H, Del Tredici K, Rub U, de Vos RA, Jansen Steur EN, Braak E (2003). Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiology of aging*, 24:197-211.
- [4] Schapira AHV, Chaudhuri KR, Jenner P (2017). Non-motor features of Parkinson disease. *Nat Rev Neurosci*, 18:435-450.
- [5] Schrag A, Horsfall L, Walters K, Noyce A, Petersen I (2015). Prediagnostic presentations of Parkinson's disease in primary care: a case-control study. *Lancet Neurol*, 14:57-64.
- [6] Fung TC, Olson CA, Hsiao EY (2017). Interactions between the microbiota, immune and nervous systems in health and disease. *Nat Neurosci*, 20:145-155.
- [7] Watanabe R, Buschauer R, Bohning J, Audagnotto M, Lasker K, Lu TW, et al. (2020). The In Situ Structure of Parkinson's Disease-Linked LRRK2. *Cell*, 182:1508-1518 e1516.
- [8] Zimprich A, Biskup S, Leitner P, Lichtner P, Farrer M, Lincoln S, et al. (2004). Mutations in LRRK2 cause autosomal-dominant parkinsonism with pleomorphic pathology. *Neuron*, 44:601-607.
- [9] Marder K, Wang Y, Alcalay RN, Mejia-Santana H, Tang MX, Lee A, et al. (2015). Age-specific penetrance of LRRK2 G2019S in the Michael J. Fox Ashkenazi Jewish LRRK2 Consortium. *Neurology*, 85:89-95.
- [10] Cabezedo D, Tsafaras G, Van Acker E, Van den Haute C, Baekelandt V (2023). Mutant LRRK2 exacerbates immune response and neurodegeneration in a chronic model of experimental colitis. *Acta Neuropathol*, 146:245-261.

- [11] Takagawa T, Kitani A, Fuss I, Levine B, Brant SR, Peter I, et al. (2018). An increase in LRRK2 suppresses autophagy and enhances Dectin-1-induced immunity in a mouse model of colitis. *Sci Transl Med*, 10.
- [12] Fang P, Yu LW, Espey H, Agirman G, Kazmi SA, Li K, et al. (2024). Sex-dependent interactions between prodromal intestinal inflammation and LRRK2 G2019S in mice promote endophenotypes of Parkinson's disease. *Commun Biol*, 7:570.
- [13] Gardet A, Benita Y, Li C, Sands BE, Ballester I, Stevens C, et al. (2010). LRRK2 is involved in the IFN-gamma response and host response to pathogens. *J Immunol*, 185:5577-5585.
- [14] Panagiotakopoulou V, Ivanyuk D, De Cicco S, Haq W, Arsic A, Yu C, et al. (2020). Interferon-gamma signaling synergizes with LRRK2 in neurons and microglia derived from human induced pluripotent stem cells. *Nat Commun*, 11:5163.
- [15] Hakimi M, Selvanantham T, Swinton E, Padmore RF, Tong Y, Kabbach G, et al. (2011). Parkinson's disease-linked LRRK2 is expressed in circulating and tissue immune cells and upregulated following recognition of microbial structures. *J Neural Transm (Vienna)*, 118:795-808.
- [16] Shutinoski B, Hakimi M, Harmsen IE, Lunn M, Rocha J, Lengacher N, et al. (2019). Lrrk2 alleles modulate inflammation during microbial infection of mice in a sex-dependent manner. *Sci Transl Med*, 11.
- [17] Zhang Q, Pan Y, Yan R, Zeng B, Wang H, Zhang X, et al. (2015). Commensal bacteria direct selective cargo sorting to promote symbiosis. *Nat Immunol*, 16:918-926.
- [18] Hartlova A, Herbst S, Peltier J, Rodgers A, Bilkei-Gorzo O, Fearn A, et al. (2018). LRRK2 is a negative regulator of Mycobacterium tuberculosis phagosome maturation in macrophages. *EMBO J*, 37.
- [19] Russo I, Kaganovich A, Ding J, Landeck N, Mamais A, Varanita T, et al. (2019). Transcriptome analysis of LRRK2 knock-out microglia cells reveals alterations of inflammatory- and oxidative stress-related pathways upon treatment with alpha-synuclein fibrils. *Neurobiol Dis*, 129:67-78.
- [20] Liu Z, Lee J, Krummey S, Lu W, Cai H, Lenardo MJ (2011). The kinase LRRK2 is a regulator of the transcription factor NFAT that modulates the severity of inflammatory bowel disease. *Nature immunology*, 12:1063-1070.
- [21] Lee H, Flynn R, Sharma I, Haberman E, Carling PJ, Nicholls FJ, et al. (2020). LRRK2 Is Recruited to Phagosomes and Co-recruits RAB8 and RAB10 in Human Pluripotent Stem Cell-Derived Macrophages. *Stem Cell Reports*, 14:940-955.
- [22] Sun S, Hodel M, Wang X, De Vicente J, Haritunians T, Debebe A, et al. (2024). Macrophage LRRK2 hyperactivity impairs autophagy and induces Paneth cell dysfunction. *Sci Immunol*, 9:eadi7907.
- [23] Henderson MX, Sengupta M, Trojanowski JQ, Lee VMY (2024). Correction to: Alzheimer's disease tau is a prominent pathology in LRRK2 Parkinson's disease. *Acta Neuropathol Commun*, 12:110.
- [24] Jensen NM, Vitic Z, Antorini MR, Viftrup TB, Parkkinen L, Jensen PH (2025). Abundant non-inclusion alpha-synuclein pathology in Lewy body-negative LRRK2-mutant cases. *Acta Neuropathol*, 149:41.
- [25] Sekiya H, Franke L, Hashimoto Y, Takata M, Nishida K, Futamura N, et al. (2025). Widespread distribution of alpha-synuclein oligomers in LRRK2-related Parkinson's disease. *Acta Neuropathol*, 149:42.
- [26] (2022). Erratum: Volpicelli-Daley et al., "G2019S-LRRK2 Expression Augments alpha-Synuclein Sequestration into Inclusions in Neurons". *J Neurosci*, 42:718.
- [27] Dues DJ, Ma Y, Nguyen APT, Offerman AV, Beddows I, Moore DJ (2023). Formation of templated inclusions in a forebrain alpha-synuclein mouse model is independent of LRRK2. *Neurobiol Dis*, 188.
- [28] Bailey RM, Covy JP, Melrose HL, Rousseau L, Watkinson R, Knight J, et al. (2013). LRRK2 phosphorylates novel tau epitopes and promotes tauopathy. *Acta Neuropathol*, 126:809-827.
- [29] Mikhail F, Calingasan N, Parolari L, Subramanian A, Yang L, Flint Beal M (2015). Lack of exacerbation of neurodegeneration in a double transgenic mouse model of mutant LRRK2 and tau. *Hum Mol Genet*, 24:3545-3556.
- [30] Alessi DR, Pfeffer SR (2024). Leucine-Rich Repeat Kinases. *Annu Rev Biochem*, 93:261-287.
- [31] Chapelet G, Beguin N, Castellano B, Grit I, de Coppet P, Oullier T, et al. (2023). Tau expression and phosphorylation in enteroendocrine cells. *Front Neurosci*, 17:1166848.
- [32] Derkinderen P, Rolli-Derkinderen M, Chapelet G, Neunlist M, Noble W (2021). Tau in the gut, does it really matter? *J Neurochem*, 158:94-104.
- [33] Barbut D, Stolzenberg E, Zasloff M (2019). Gastrointestinal Immunity and Alpha-Synuclein. *J Parkinsons Dis*, 9:S313-S322.
- [34] Stolzenberg E, Berry D, Yang D, Lee EY, Kroemer A, Kaufman S, et al. (2017). A Role for Neuronal Alpha-Synuclein in Gastrointestinal Immunity. *J Innate Immun*, 9:456-463.
- [35] Prigent A, Lionnet A, Durieu E, Chapelet G, Bourreille A, Neunlist M, et al. (2019). Enteric alpha-synuclein expression is increased in Crohn's disease. *Acta Neuropathol*, 137:359-361.
- [36] Prigent A, Chapelet G, De Guilhem de Lataillade A, Oullier T, Durieu E, Bourreille A, et al. (2020). Tau accumulates in Crohn's disease gut. *FASEB J*, 34:9285-9296.
- [37] Lionnet A, Wade MA, Corbille AG, Prigent A, Paillusson S, Tasselli M, et al. (2018). Characterisation of tau in the human and rodent enteric nervous system under physiological conditions and in tauopathy. *Acta Neuropathol Commun*, 6:65.
- [38] Kozina E, Sadasivan S, Jiao Y, Dou Y, Ma Z, Tan H, et al. (2018). Mutant LRRK2 mediates peripheral and central immune responses leading to neurodegeneration in vivo. *Brain*, 141:1753-1769.
- [39] Littelljohn D, Rudyk C, Dwyer Z, Farmer K, Fortin T, Hayley S, et al. (2018). The impact of murine LRRK2

- G2019S transgene overexpression on acute responses to inflammatory challenge. *Brain Behav Immun*, 67:246-256.
- [40] Lieknina I, Reimer L, Pantelejevs T, Lends A, Jaudzems K, El-Turabi A, et al. (2024). Structural basis of epitope recognition by anti-alpha-synuclein antibodies MJFR14-6-4-2. *NPJ Parkinsons Dis*, 10:206.
- [41] Lassen LB, Gregersen E, Isager AK, Betzer C, Kofoed RH, Jensen PH (2018). ELISA method to detect alpha-synuclein oligomers in cell and animal models. *PLoS One*, 13:e0196056.
- [42] Xiang J, Tang J, Kang F, Ye J, Cui Y, Zhang Z, et al. (2025). Gut-induced alpha-Synuclein and Tau propagation initiate Parkinson's and Alzheimer's disease co-pathology and behavior impairments. *Neuron*, 113:3300-3302.
- [43] Ghahremani M, Wang M, Chen HY, Zetterberg H, Smith E, Ismail Z, et al. (2023). Plasma Phosphorylated Tau at Threonine 181 and Neuropsychiatric Symptoms in Preclinical and Prodromal Alzheimer Disease. *Neurology*, 100:e683-e693.
- [44] Cano A, Capdevila M, Puerta R, Arranz J, Montreal L, de Rojas I, et al. (2024). Clinical value of plasma pTau181 to predict Alzheimer's disease pathology in a large real-world cohort of a memory clinic. *EBioMedicine*, 108:105345.
- [45] O'Day C, Finkelstein DI, Diwakarla S, McQuade RM (2022). A Critical Analysis of Intestinal Enteric Neuron Loss and Constipation in Parkinson's Disease. *J Parkinsons Dis*, 12:1841-1861.
- [46] Bu LL, Huang KX, Zheng DZ, Lin DY, Chen Y, Jing XN, et al. (2020). Alpha-Synuclein Accumulation and Its Phosphorylation in the Enteric Nervous System of Patients Without Neurodegeneration: An Explorative Study. *Front Aging Neurosci*, 12:575481.
- [47] Braak H, Ghebremedhin E, Rub U, Bratzke H, Del Tredici K (2004). Stages in the development of Parkinson's disease-related pathology. *Cell Tissue Res*, 318:121-134.
- [48] Obeso JA, Stamelou M, Goetz CG, Poewe W, Lang AE, Weintraub D, et al. (2017). Past, present, and future of Parkinson's disease: A special essay on the 200th Anniversary of the Shaking Palsy. *Mov Disord*, 32:1264-1310.
- [49] Di Maio R, Hoffman EK, Rocha EM, Keeney MT, Sanders LH, De Miranda BR, et al. (2018). LRRK2 activation in idiopathic Parkinson's disease. *Sci Transl Med*, 10.
- [50] Garcia-Miralles M, Coomaraswamy J, Habig K, Herzig MC, Funk N, Gillardon F, et al. (2015). No dopamine cell loss or changes in cytoskeleton function in transgenic mice expressing physiological levels of wild type or G2019S mutant LRRK2 and in human fibroblasts. *PLoS One*, 10:e0118947.
- [51] Li Y, Liu W, Oo TF, Wang L, Tang Y, Jackson-Lewis V, et al. (2009). Mutant LRRK2(R1441G) BAC transgenic mice recapitulate cardinal features of Parkinson's disease. *Nat Neurosci*, 12:826-828.
- [52] Yue M, Hinkle KM, Davies P, Trushina E, Fiesel FC, Christenson TA, et al. (2015). Progressive dopaminergic alterations and mitochondrial abnormalities in LRRK2 G2019S knock-in mice. *Neurobiol Dis*, 78:172-195.
- [53] Tsika E, Kannan M, Foo CS, Dikeman D, Glauser L, Gellhaar S, et al. (2014). Conditional expression of Parkinson's disease-related R1441C LRRK2 in midbrain dopaminergic neurons of mice causes nuclear abnormalities without neurodegeneration. *Neurobiol Dis*, 71:345-358.
- [54] Liu G, Sgobio C, Gu X, Sun L, Lin X, Yu J, et al. (2015). Selective expression of Parkinson's disease-related Leucine-rich repeat kinase 2 G2019S missense mutation in midbrain dopaminergic neurons impairs dopamine release and dopaminergic gene expression. *Hum Mol Genet*, 24:5299-5312.
- [55] Ramonet D, Daher JP, Lin BM, Stafa K, Kim J, Banerjee R, et al. (2011). Dopaminergic neuronal loss, reduced neurite complexity and autophagic abnormalities in transgenic mice expressing G2019S mutant LRRK2. *PLoS One*, 6:e18568.
- [56] Outeiro TF, Harvey K, Dominguez-Meijide A, Gerhardt E (2019). LRRK2, alpha-synuclein, and tau: partners in crime or unfortunate bystanders? *Biochem Soc Trans*, 47:827-838.
- [57] Dusanich J, Kochubey O, Stafa K, Young SM, Jr., Zufferey R, Moore DJ, et al. (2011). A rat model of progressive nigral neurodegeneration induced by the Parkinson's disease-associated G2019S mutation in LRRK2. *J Neurosci*, 31:907-912.
- [58] West AB, Cowell RM, Daher JP, Moehle MS, Hinkle KM, Melrose HL, et al. (2014). Differential LRRK2 expression in the cortex, striatum, and substantia nigra in transgenic and nontransgenic rodents. *J Comp Neurol*, 522:2465-2480.
- [59] Langston RG, Rudenko IN, Cookson MR (2016). The function of orthologues of the human Parkinson's disease gene LRRK2 across species: implications for disease modelling in preclinical research. *Biochem J*, 473:221-232.
- [60] Chatterjee D, Kurup D, Smeyne RJ (2025). Environmental exposures and familial background alter the induction of neuropathology and inflammation after SARS-CoV-2 infection. *NPJ Parkinsons Dis*, 11:86.
- [61] Conway J, De Jong EN, White AJ, Dugan B, Rees NP, Parnell SM, et al. (2025). Age-related loss of intestinal barrier integrity plays an integral role in thymic involution and T cell ageing. *Aging Cell*, 24:e14401.
- [62] Palmer JE, Wilson N, Son SM, Obrocki P, Wrobel L, Rob M, et al. (2025). Autophagy, aging, and age-related neurodegeneration. *Neuron*, 113:29-48.
- [63] Zhang J, Wang HL, Lautrup S, Nilsen HL, Treebak JT, Watne LO, et al. (2025). Emerging strategies, applications and challenges of targeting NAD(+) in the clinic. *Nat Aging*, 5:1704-1731.
- [64] Lautrup S, Sinclair DA, Mattson MP, Fang EF (2019). NAD(+) in Brain Aging and Neurodegenerative Disorders. *Cell Metab*, 30:630-655.
- [65] Shannon KM, Keshavarzian A, Mutlu E, Dodiya HB, Daian D, Jaglin JA, et al. (2012). Alpha-synuclein in colonic submucosa in early untreated Parkinson's disease. *Mov Disord*, 27:709-715.

- [66] Forsyth CB, Shannon KM, Kordower JH, Voigt RM, Shaikh M, Jaglin JA, et al. (2011). Increased intestinal permeability correlates with sigmoid mucosa alpha-synuclein staining and endotoxin exposure markers in early Parkinson's disease. *PLoS One*, 6:e28032.
- [67] Brockmann K, Apel A, Schulte C, Schneiderhan-Marra N, Pont-Sunyer C, Vilas D, et al. (2016). Inflammatory profile in LRRK2-associated prodromal and clinical PD. *J Neuroinflammation*, 13:122.
- [68] Wallings RL, Tansey MG (2019). LRRK2 regulation of immune-pathways and inflammatory disease. *Biochem Soc Trans*, 47:1581-1595.
- [69] Henderson MX, Sengupta M, Trojanowski JQ, Lee VMY (2019). Alzheimer's disease tau is a prominent pathology in LRRK2 Parkinson's disease. *Acta Neuropathol Commun*, 7:183.
- [70] Compta Y, Parkkinen L, O'Sullivan SS, Vandrovcova J, Holton JL, Collins C, et al. (2011). Lewy- and Alzheimer-type pathologies in Parkinson's disease dementia: which is more important? *Brain*, 134:1493-1505.
- [71] Franzmeier N, Roemer-Cassiano SN, Bernhardt AM, Dehsarvi A, Dewenter A, Steward A, et al. (2025). Alpha synuclein co-pathology is associated with accelerated amyloid-driven tau accumulation in Alzheimer's disease. *Mol Neurodegener*, 20:31.
- [72] Webster JM, Yang YT, Miller AT, Zane A, Scholz K, Stone WJ, et al. (2025). Tau, amyloid-beta and alpha-synuclein co-pathologies synergistically enhance neuroinflammation and neuropathology. *bioRxiv*.
- [73] Vacchi E, Kaelin-Lang A, Melli G (2020). Tau and Alpha Synuclein Synergistic Effect in Neurodegenerative Diseases: When the Periphery Is the Core. *Int J Mol Sci*, 21.
- [74] Tsafaras G, Cabezudo D, Wetzels L, Tsakogias I, Abey A, Bentea E, et al. (2025). The G2019S LRRK2 mutation exacerbates alpha-synuclein and tau neuropathology through divergent pathways in Parkinson's disease models. *Acta Neuropathol*, 150:51.
- [75] Kalia LV, Lang AE, Hazrati LN, Fujioka S, Wszolek ZK, Dickson DW, et al. (2015). Clinical correlations with Lewy body pathology in LRRK2-related Parkinson disease. *JAMA Neurol*, 72:100-105.
- [76] Buchman AS, Yu L, Wilson RS, Leurgans SE, Nag S, Shulman JM, et al. (2019). Progressive parkinsonism in older adults is related to the burden of mixed brain pathologies. *Neurology*, 92:e1821-e1830.
- [77] Hirsch EC, Graybiel AM, Agid Y (1989). Selective vulnerability of pigmented dopaminergic neurons in Parkinson's disease. *Acta Neurol Scand Suppl*, 126:19-22.
- [78] Chu Y, Hirst WD, Federoff HJ, Harms AS, Stoessl AJ, Kordower JH (2024). Nigrostriatal tau pathology in parkinsonism and Parkinson's disease. *Brain*, 147:444-457.
- [79] Zhukareva V, Trojanowski JQ, Lee VM (2004). Assessment of pathological tau proteins in frontotemporal dementias: qualitative and quantitative approaches. *Am J Geriatr Psychiatry*, 12:136-145.
- [80] Jellinger KA (2009). Absence of alpha-synuclein pathology in postencephalitic parkinsonism. *Acta Neuropathol*, 118:371-379.
- [81] Buck SA, Malankhanova T, Strader S, Ma EB, Yim S, Pratt HW, et al. (2025). LRRK2 kinase-mediated accumulation of lysosome-associated phospho-Rabs in tauopathies and synucleinopathies. *Acta Neuropathol*, 150:44.
- [82] Obergasteiger J, Frapporti G, Lamonaca G, Pizzi S, Picard A, Lavdas AA, et al. (2020). Kinase inhibition of G2019S-LRRK2 enhances autolysosome formation and function to reduce endogenous alpha-synuclein intracellular inclusions. *Cell Death Discov*, 6:45.
- [83] Taymans JM, Mutez E, Sibrán W, Vandewynckel L, Deldycke C, Bleuse S, et al. (2023). Alterations in the LRRK2-Rab pathway in urinary extracellular vesicles as Parkinson's disease and pharmacodynamic biomarkers. *NPJ Parkinsons Dis*, 9:21.
- [84] Schapansky J, Khasnavis S, DeAndrade MP, Nardozi JD, Falkson SR, Boyd JD, et al. (2018). Familial knockin mutation of LRRK2 causes lysosomal dysfunction and accumulation of endogenous insoluble alpha-synuclein in neurons. *Neurobiol Dis*, 111:26-35.
- [85] Abe T, Kuwahara T, Suenaga S, Sakurai M, Takatori S, Iwatsubo T (2024). Lysosomal stress drives the release of pathogenic alpha-synuclein from macrophage lineage cells via the LRRK2-Rab10 pathway. *iScience*, 27:108893.
- [86] Nielsen LK, Frost JLI, Vaughan DP, Real R, Fumi R, Jensen MT, et al. (2026). Biomarkers of Leucine-Rich Repeat Kinase 2 (LRRK2) and Lysosomal Dysfunction in Progressive Supranuclear Palsy. *Mov Disord*.
- [87] McQuade RM, Singleton LM, Wu H, Lee S, Constable R, Di Natale M, et al. (2021). The association of enteric neuropathy with gut phenotypes in acute and progressive models of Parkinson's disease. *Sci Rep*, 11:7934.
- [88] Annerino DM, Arshad S, Taylor GM, Adler CH, Beach TG, Greene JG (2012). Parkinson's disease is not associated with gastrointestinal myenteric ganglion neuron loss. *Acta Neuropathol*, 124:665-680.
- [89] Baumratov AS, Antony PM, Ostaszewski M, He F, Salamanca L, Antunes L, et al. (2016). Enteric neurons from Parkinson's disease patients display ex vivo aberrations in mitochondrial structure. *Sci Rep*, 6:33117.
- [90] Kapur RP (2013). Counting neurons is not as easy as 'one-two, three'. *Neurogastroenterol Motil*, 25:549-553.
- [91] Michel K, Kuch B, Dengler S, Demir IE, Zeller F, Schemann M (2022). How big is the little brain in the gut? Neuronal numbers in the enteric nervous system of mice, Guinea pig, and human. *Neurogastroenterol Motil*, 34:e14440.
- [92] Sorensen L, Humenick A, Poon SSB, Han MN, Mahdavian NS, Rowe MC, et al. (2024). Gut Analysis Toolbox - automating quantitative analysis of enteric neurons. *J Cell Sci*, 137.
- [93] Liao PH, Tung HY, Lim WS, Jang JR, Li H, Shun CT, et al. (2024). Impaired gut barrier integrity and reduced colonic expression of free fatty acid receptors in patients with Parkinson's disease. *Neurol Sci*, 45:5297-5307.

-
- [94] Liu F, Ran Q, Zhang H, Chen J (2025). The Systemic Immune-Inflammation Index and the Risk of Parkinson's Disease in the U.S.: A Cross-Sectional Study. *J Clin Med*, 14.
- [95] Chen W, Yan X, Lv H, Liu Y, He Z, Luo X (2020). Gender differences in prevalence of LRRK2-associated Parkinson disease: A meta-analysis of observational studies. *Neurosci Lett*, 715:134609.
- [96] Cilia R, Siri C, Rusconi D, Allegra R, Ghiglietti A, Sacilotto G, et al. (2014). LRRK2 mutations in Parkinson's disease: confirmation of a gender effect in the Italian population. *Parkinsonism Relat Disord*, 20:911-914.
- [97] San Luciano M, Wang C, Ortega RA, Giladi N, Marder K, Bressman S, et al. (2017). Sex differences in LRRK2 G2019S and idiopathic Parkinson's Disease. *Ann Clin Transl Neurol*, 4:801-810.
- [98] Gan-Or Z, Leblond CS, Mallett V, Orr-Urtreger A, Dion PA, Rouleau GA (2015). LRRK2 mutations in Parkinson disease; a sex effect or lack thereof? A meta-analysis. *Parkinsonism Relat Disord*, 21:778-782.