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James Bauer April 14, 2020

The synergistic effects of gastrointestinal inflammation and the G2019S *Lrrk2* mutation on Parkinson's disease associated phenotypes as defined by RNA-sequence analysis

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Abstract

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Parkinson's disease (PD) is the second most common neurodegenerative disease and is further classified as a progressive movement disorder. Specific to its symptoms, PD primarily affects mobility and can lead to constant body tremors, muscle rigidity, and slowness of movement. Certain non-motor symptoms can also become apparent such as cognitive dysfunction, fatigue, and gastrointestinal disturbances. With these symptoms aside, the pathology of PD is primarily due to the degeneration of dopaminergic neurons within the substantia nigra although Lewy body accumulation and inflammation are also involved. Furthermore, certain risk factors such as increasing age and numerous other environmental and genetic factors are all implicated in PD development and therefore affect an individual's susceptibility for developing PD. However, even with a plethora of existing knowledge surrounding PD, the ability of scientists to discern the origin of and reasons for PD development associated with each affected individual is still lacking. As a result, this study sought to better understand both environmental and genetic factors involved in PD development by focusing on the potential synergistic effects of the G2019S *Lrrk2* mutation (one of the most prevalent familial PD mutations) and gut inflammation induced by dextran sodium sulfate (DSS). More specifically, substantia nigra samples from 59 total mice were collected with 41 mice having been previously exposed to a 30-day chronic DSS-induced colitis paradigm and the remaining 18 mice having been treated with water to serve as controls. The statistical program Rstudio was then used to identify differential gene expression by making comparisons between both genotype (B6, WTOE, G2019S) and treatment type (DSS-treated vs water-treated). Differences in gene expression were then assessed by either one-way analysis of variance or a student t-test to verify significance. Overall, these results suggest that genes associated with various cellular processes such as neuroprotection, inflammation, and saliva production may all experience changes in expression as a result of PD associated phenotypes. Therefore, with these findings we hope to contribute to future research surrounding the signaling pathways involved in PD development in order to ultimately aid in the identification and development of potential therapeutics to combat this neurodegenerative disease.

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1. Introduction

1.1. Incidence:

Parkinson's disease (PD) is the second most common neurodegenerative disease behind Alzheimer's, affecting 0.3% of the global population and 1% of people above the age of 60 (De Lau & Breteler, 2006). Furthermore, the number of people across the world affected by PD has doubled from 1990 to 2015, highlighting the idea that PD is one of the fastest growing neurological diseases in terms of prevalence, disability, and deaths (GBD Collaborator Group, 2017). With this in mind, incidence rates of PD development are thought to be around 8-18 per 100,000 individuals, a rate that initially seems small but on a larger scale has a much more substantial effect (De Lau & Breteler, 2006). Importantly, aside from individuals with the disease, PD can also have significant secondary effects on those who are close to and have to care for a PD patient. Generally referred to as "informal" caregivers, the overall role of these individuals is to provide the PD patient with physical, psychological, and social support as they attempt to adapt to living with this disease (Goy et al., 2008). Unfortunately, as a result of this immense workload, numerous studies have found that although providing care for a PD patient can be rewarding, having to sustain care for such a long period of time can also be extremely taxing and can ultimately lead to adverse effects such as depression, fatigue, and increased mortality for caregivers (Mai et al., 2010; Kristjanson et al., 2005). Therefore, for the sake of both PD patients and those suffering from the burden of caring for their loved ones, the need for a clearer understanding of the disease as well as successful treatments is at an all-time high.

1.2. Motor and Non-motor symptoms:

1.2.1. Motor symptoms:

Specific to its symptoms, PD is characterized as a neurodegenerative disorder and can be further classified as a progressive movement disorder in which patients experience abnormal and debilitating motor and non-motor symptoms. More specifically, PD patients may experience motor symptoms such as constant body tremors, muscle rigidity, and overall slowness of movement, which is referred to as bradykinesia (Forno, 1996). Aside from these primary motor symptoms, individuals suffering from PD may also experience numerous secondary motor symptoms ranging from gait disturbances to speech and handwriting problems (Moustafa et al., 2016).

1.2.2. Non-motor symptoms:

It is also important to note that although PD is primarily characterized as a progressive movement disorder, numerous studies have shown that patients with PD may also experience a variety of non-motor symptoms. These symptoms can range from cognitive dysfunction to fatigue and to gastrointestinal (GI) dysfunction. GI dysfunction is particularly compelling as symptoms such as constipation or drooling occur in around 50-80% and 70-80% of patients respectively (Poirier et al., 2016). In addition, other non-motor symptoms such as nausea and dysphagia, a term for having difficulty swallowing, have been observed in patients suffering from PD (Edwards et al., 1991). Furthermore, PD patients have also been found to experience an increase in intestinal permeability due to the dysfunction of important tight junction proteins within the intestine. Coupled with this increase in intestinal permeability, patients also

experience an increase in pro-inflammatory cytokines which in turn can contribute to intestinal inflammation (Devos et al., 2013). Consequently, it has been hypothesized that this increase in both intestinal inflammation and permeability may be important factors in the development of PD-associated neuroinflammatory and neurodegenerative phenotypes. In short, nonmotor gastrointestinal symptoms of PD patients may not only provide another indication of the presence of this disease but more importantly may act as early biomarkers for PD development due to the fact that these nonmotor symptoms, particularly constipation, appear well before the motor ones (Poirier et al., 2016).

1.3. Pathology:

1.3.1. Dopaminergic Neurodegeneration:

With the symptoms and overall characterization of PD in mind, the next logical step would be to outline the underlying pathology of PD, starting with a generalized idea of how this neurodegenerative disease affects the basal ganglia. However, before delving into the connection between the basal ganglia and PD development, it is important to first understand the functional importance of these integral brain areas. More specifically, the basal ganglia are characterized as an interconnected group of subcortical nuclei that control integral biological processes ranging from cognitive planning and emotions to voluntary movement and reward functions. One basal ganglia nucleus in particular, the substantia nigra, has been shown to be especially important for the modulation of these aforementioned processes as it is thought to receive initial inputs into the basal ganglia circuitry. To provide further clarity for the importance of the substantia nigra, scientists have divided this brain area into two distinct regions based on

differences in both morphological and functional features. More specifically, the substantia nigra contains the pars compacta (SNpc) and the pars reticulata (SNpr) which consist of dopaminergic neurons and gamma-aminobutyric acid-ergic (GABAergic) neurons respectively. In terms of their functional differences, the SNpc is known to send dopaminergic projections to the striatum and its substructures which then project further to the globus pallidus, generating the direct and indirect pathways of the basal ganglia. Importantly, these pathways play a significant role in motor control as a proper balance between the direct inhibition of the globus pallidus through the direct pathway and the indirect stimulation of the globus pallidus through the indirect pathway are necessary in order for the correct movement to be initiated. Conversely, due to the GABAergic neurons contained within the SNpr, this brain region takes on an overall inhibitory role and has been shown to be involved in the output projections from the direct and indirect basal ganglia pathways (Sonne & Beato, 2018).

With the clear role that the substantia nigra plays in motor control in mind, it comes as no surprise that alterations and/or loss of function within this brain region play a large part in PD development. More specifically, research has shown that the primary indication of PD development is dopaminergic neuron degeneration within the SNpc which ultimately reduces dopamine levels needed for neurotransmission between the substantia nigra and striatum (Dauer & Przedborski, 2003). Furthermore, past studies have highlighted the idea that motor symptoms generally become apparent after an individual experiences substantial dopaminergic neuron loss within the SNpc, which in some cases may exceed 80% (Zarow et al., 2003; Sulzer & Surmeier, 2013). In any case, as a result of this extensive dopaminergic neuron loss, a change in the overarching basal ganglia circuitry ensues as decreased levels of dopamine have been shown

to decrease the activity along the direct pathway while simultaneously increasing the activity along the indirect pathway (Widnell 2005). As a result of this change in circuitry, individuals would no longer be able to properly control essential biological processes such as voluntary movement and cognitive planning, rendering them helpless to the neurodegenerative nature of PD.

1.3.2. Importance of Dopamine:

With dopaminergic neurodegeneration being a hallmark cause of PD development, it is crucial to understand both the general and specific roles that the neurotransmitter dopamine plays in our body and in PD development respectively. However, before delving into its functional importance, the underlying mechanism of dopamine production is important to consider. More specifically, the mechanism begins with the amino acid L-tyrosine which is converted into the intermediate L-Dihydroxyphenylalanine (L-DOPA) by the enzyme Tyrosine hydroxylase. L-DOPA is then converted into dopamine by the enzyme DOPA decarboxylase although the process does not necessarily end there as dopamine can undergo further enzymatic modifications to produce norepinephrine and epinephrine. Importantly, the initial conversion of L-tyrosine to L-DOPA is regulated by the activity of the tyrosine hydroxylase enzyme, making it the rate-limiting step of this mechanism (Olguin et al., 2015). With the mechanism of dopamine production aside, the anatomical distribution of dopaminergic neurons within our brains is also critical to understand. As aforementioned, a significant population of dopaminergic neurons are located in the substantia nigra, specifically the pars compacta, which contribute to biological processes such as voluntary movement control (Sonne & Beato, 2018). Aside from the substantia nigra, a significant number of dopaminergic neurons have been identified within the ventral tegmental area (VTA) and are known to be responsible for both reward processing and motivation. Importantly, this

neuron population is much less afflicted by neurodegeneration during PD development for as aforementioned the main site of neurodegeneration is within the SNpc (Alberico et al., 2015). Furthermore, dopaminergic neurodegeneration has been shown to have secondary effects on other modulatory systems which dopamine interacts with, such as the serotonergic and cholinergic systems (Braak et al., 2003). As a result, patients suffering from PD experience multiple non-motor symptoms in addition to their already debilitating motor ones (Schapira et al., 2017). In short, dopamine plays a vital role in the maintenance of important biological processes and is clearly linked to PD development, making it a key molecule to focus on when studying this crippling disease.

1.3.3. Role of Lewy Bodies:

Aside from dopaminergic degeneration within the substantia nigra, past research has also highlighted the link between Lewy body accumulation and PD development. However, before exploring this idea further, it is important to first grasp the overall structural features of these Lewy body formations. More specifically, these cellular features are described as protein filled intracellular entities that primarily contain clusters of the protein α-synuclein (Kim et al., 2014). In reference to α-synuclein, past research has shown that this protein may be involved in several possible functions ranging from the maintenance of synaptic vesicles in presynaptic terminals to the regulation of dopamine release in reference to motor control (Kim et al., 2014). Furthermore, research has suggested that the misfolding of α-synuclein is the principal cause behind the formation of these hazardous α-synuclein accumulations i.e. Lewy bodies which as aforementioned can act as neurological markers for PD (Spillantini et al., 1997; Dickson et al., 2009). Additionally, as a result of this misfolding, these neurotoxic α-synuclein aggregates can

cause numerous secondary effects including but not limited to neurodegeneration, neuroinflammation, and ultimately cell death (Wolozin & Behl, 2000). In essence, Lewy body and α-synuclein accumulation ultimately promote neurodegeneration and are therefore important factors to consider when studying the pathology of PD.

1.3.4. Role of Inflammation:

As aforementioned, inflammation both centrally and peripherally has been found to play a key role in PD development (Su & Federoff, 2014). To be more specific, animal models of PD have shown that increased activation of microglia and astrocytes, which are both involved in an individual's inflammatory response, is characteristic of PD development (Deleidi & Gasser, 2013). With this in mind, several studies have also presented the idea that these inflammatory responses coordinated by both microglia and astrocytes may in turn promote dopaminergic neurodegeneration which again is the hallmark pathological characteristic of PD (Deleidi & Gasser, 2013). Aside from this, studies have also shown that increases in specific pro-inflammatory cytokines such as tumor necrosis factor (TNF), interferon gamma (IFN-γ), interleukin-6 (IL-6), and interleukin-1 beta (IL-1β) have all be observed in both the brain and cerebrospinal fluid of patients suffering from PD (Devos et al., 2013). Furthermore, much like with the increased activation of microglia and astrocytes, this increase in pro-inflammatory cytokines has been proposed to also contribute to dopaminergic neurodegeneration (Postuma et al., 2013). Therefore, the link between inflammation and the pathogenesis of PD is quite clear and something that warrants further study in order to understand fully.

1.4. Risk Factors:

1.4.1. Age:

Although the underlying causes of PD development are still relatively unknown, past research has highlighted numerous risk factors that are thought to affect an individual's likelihood of developing this neurodegenerative disease. Surprisingly, aging has actually been shown to be the largest risk factor when it comes PD development as the disease has been observed to have a higher incidence in older populations. More specifically, in addition to the aforementioned statistic that 1% of individuals over 60 develop PD, research has also shown that this prevalence rises to 5% for individuals over 85, highlighting the clear effect that aging can have on PD development (Wood-Kaczmar et al., 2006). With the influence of aging in mind, a study conducted in 2012 measured neuronal loss within the substantia nigra of 750 non-PD affected individuals who had an average age of 88.5 years (Buchman et al., 2012). Strikingly, the researchers found that even in elderly people without PD, almost 250 of the 750 total subjects experienced mild to severe neuronal loss within their substantia nigra with 10% of those affected also showing signs of Lewy body accumulation (Buchman et al., 2012). Further research has shown that compared to other brain regions, the extent of cell loss within the substantia nigra as a result of aging is much greater as some scientists estimate that the percentage of cells lost can range from 4.7% to 9.8% per decade (Rudow G et al., 2008; Fearnley & Lees, 1991; Ma et al., 1999). Specific to dopaminergic neuron populations, considerable cell loss has also been observed as a result of aging with one study estimating that the extent of cell loss may reach almost 50% (Hirsch et al., 1987). In short, aging has been shown to have significant effects on an individual's risk for developing PD as extensive cell death in the substantia nigra and in

dopaminergic populations coupled with other complications that people experience as they age may cause individuals to become more vulnerable to PD development.

1.4.2. Environment:

Aside from age, numerous studies have attempted to identify environmental factors that may increase or reduce an individual's risk of developing PD. More specifically, past research has highlighted numerous potential factors ranging from one's diet to their work environment. For example, one study found that PD patients consumed less food overall, especially protein, when compared back to control patients, ultimately suggesting that a lower protein intake may increase one's risk of PD development (Fall et al., 1999). However, as with most prospective environmental risk factors, there may be multiple explanations for an observation as a lower-protein intake could also mean that individuals are consuming more fats and carbohydrates which have also been shown to increase one's risk of PD development (Logroscino et al., 1996). In terms of a person's work environment, numerous studies have shown that agricultural work, specifically with pesticides, may increase a person's risk of developing PD although importantly other studies have also found agricultural exposure to instead not have an effect (Ho et al., 1989; Koller et al., 1990). Multiple studies have also focused on the effects of smoking and alcohol consumption which surprisingly seem to reduce one's risk of PD development. More specifically, in one such study PD patients were observed to consume alcoholic drinks less often compared to healthy controls with a greater preventative effect observed in females compared to males (Kim et al., 2019). Additionally, a similar preventative effect was seen in individuals who smoked when compared back to non-smoking controls with a greater effect seen in males compared to females (Kim et al., 2019). Therefore, environmental factors such as smoking, alcohol consumption,

pesticide exposure, and even diet all seem to influence PD development although unlike with age the specific roles that each of these factors play are still largely unknown.

1.4.3. Genetics:

Interestingly, up until 20 years ago genetics was not thought to play a significant role in PD development. More specifically, numerous studies looking at monozygotic twins provided evidence to support the claim that genetic factors do not influence PD development citing findings such as the fact that the concordance rate of PD in twins was significantly lower compared to other known genetic diseases (Duvoisin et al., 1981; Eldridge & Ince, 1984). However, with the development and use of molecular genetics techniques, scientists were soon able to identify certain genetic risk factors that may cause an individual to become more susceptible to PD development. In terms of overall influence, over 20 genes have been identified to be responsible for monogenic forms of PD although this does not even come close to explaining all monogenic forms of PD that develop (Billingsley et al., 2018). Specific to known PD associated genes, mutations in the gene *SNCA*, which encodes for the protein α-synuclein, were soon found by researchers to be associated with late-onset PD development, ultimately providing one of the first pieces of evidence to suggest that PD had a heritable genetic component (Polymeropoulos et al., 1997). Furthermore, additional genes such as *PRKN*, which encodes for the protein parkin, and *PARK7*, which encodes for the protein DJ-1, were later found to be key genetic components in recessive forms of early-onset PD (Kitada et al., 1998; Bonifati et al., 2003). As research continued, investigators soon identified the significance of mutations on the *Lrrk2* gene which were found to be responsible for the development of the most frequent monogenic PD type i.e. autosomal dominant PD (Zimprich et al., 2004; Paisan-Ruiz et al., 2004).

Furthermore, it is important to note that although genetics accounts for only 10% of PD cases, both genetic and sporadic PD cases are often pathologically and clinically similar, meaning that insight into genetic PD cases may concurrently add to our understanding surrounding the pathology and clinical features of sporadic PD cases (Klein & Westenberger, 2012; Chai & Lim, 2013). In short, the influence that genetics plays in PD development is unquestionable as mutations in numerous genes have been identified and ultimately associated with PD onset.

1.4.3.1. Importance of the gene Lrrk2

As mentioned previously, the gene Leucine Rich Repeat Kinase 2 (*Lrrk2*) plays a particularly important role in PD development. Specific to its structure, LRRK2 consists of 2527 amino acids and is described as a multi-domain G-protein (Guaitoli et al., 2016). In terms of its functional capabilities, LRRK2 has been proposed to be involved in a variety of roles ranging from cytoskeletal maintenance to autophagic protein degradation and vesicle trafficking (Rideout & Stefanis, 2014). Furthermore, the LRRK2 protein is also expressed in a wide range of tissue types, ultimately making it difficult for scientists to pinpoint both the exact functional properties and distributional properties of this protein (Wallings et al., 2015). With its structure and function aside, it's also important to consider the percent of PD cases that develop as a result of a LRRK2 mutation. Specific to these statistics, LRRK2 is known to be responsible for 1-2% of sporadic PD cases, 5-6% of familial PD cases, and surprisingly 29.7% of PD cases for individuals of Ashkenazi Jewish descent (Chen et al., 2015). Furthermore, it's important to note that these LRRK2 induced PD cases are not solely due to the same singular LRRK2 mutation as there are numerous possible mutations that could occur and ultimately lead to PD development. With this in mind, the G2019S mutation in particular has drawn enormous attention as researchers try to elucidate the role that

LRRK2 mutations play in PD development. Located within the kinase domain of the LRRK2 protein, this mutation involves a glycine to serine substitution at the 2019th amino acid position of LRRK2, resulting in hyperactive kinase activity as well as increased phosphorylation (Luzon-Toro et al., 2007). As a result of this hyperactive kinase activity, both an increase in cell toxicity and cell death have been observed, ultimately showcasing the detrimental effects that this mutation can have (Chen & Wu, 2018). Furthermore, a strong connection between inflammation and the G2019S mutation has been observed as past studies have shown that inflammatory dysregulation may occur as a consequence of this mutation (Dzamko et al, 2016). Therefore, the link between the G2019S mutation within the LRRK2 protein and PD development is quite clear although few studies have attempted to focus on the possible synergistic effects that may occur as a result of a LRRK2 mutation and an induced environmental symptom i.e. gut inflammation.

1.5. Project Goal and Hypothesis:

Therefore, with both environmental influences (i.e. inflammation) and genetic influences (i.e. LRRK2) in mind, this project aims to understand how both factors may work together in order to promote a PD phenotype. As a result, the overarching hypothesis for this work is the idea that an increase in LRRK2 kinase activity due to the G2019S mutation will synergize with intestinal inflammation (DSS-induced colitis) to ultimately promote neuroinflammation and neurodegeneration associated with a PD phenotype. To test this, the effects of intestinal inflammation on the immunohistological, biochemical, and transcriptional profile in midbrain substantia nigra samples were investigated with the long-term goal of identifying additional gene targets for therapeutic intervention to delay or mitigate the progression of the disease.

2. Materials and Methods

2.1. Mouse Models:

Bacterial artificial chromosome (BAC) transgenic mouse strains were used in order to create mouse models that differed in levels of both LRRK2 expression and LRRK2 kinase activity (Table 1). More specifically, the transgenic mice used were either overexpressing the wildtype mouse *Lrrk2* gene (WTOE) or the G2019S mutated *Lrrk2* gene (G2019S). Homozygous male *Lrrk2-G2019S* (B6.Cg-Tg(Lrrk2*G2019S)2Li/J; stock number 012467) and *Lrrk2-WT* (B6.Cg-Tg(Lrrk2)6Li/J; stock number 012466) mice were purchased from the Jackson Laboratory and bred to hemizygosity at Emory University. In order to develop these mice, a BAC was engineered to contain the entirety of the mouse *Lrrk2* gene for the WTOE mice whereas the G2019S mutant mice received a modified BAC strain that included the G2019S mutation (Li et al., 2010). Importantly, the LRRK2 promoter was utilized when formulating the BAC in order to ensure that all cells which endogenously express LRRK2 would successfully express the gene in the transgenic mouse models. Specific to LRRK2 levels, a 6-8-fold increase was observed in both the WTOE and G2019S transgenic mice when compared to normal LRRK2 levels. For a control, littermates were used and characterized as B6 as they expressed normal endogenous levels of LRRK2. Notably, all mouse models did not show any signs of PD development such as α-synuclein accumulation, neurodegeneration, or motor impairment (Li et al., 2010). This in turn ensured that the only differences between the three strains were their LRRK2 expression and LRRK2 kinase activity, which was enhanced to toxic levels in the G2019S mutant mice.

2.2. Chronic DSS-Induced Colitis Paradigm:

The transgenic mice were then subjected to a chronic Dextran Sodium Sulfate (DSS) paradigm in order to induce gut inflammation i.e. colitis. The DSS colitis model is often the experimental model of choice due to the fact that gut inflammation in a DSS-treated mouse closely resembles epithelial damage a human would experience if suffering from colitis (Randhawa et al., 2014). In terms of the chemical properties of DSS, it is described as a negatively charged sulfated polysaccharide that is water soluble, meaning that it can be delivered to animal subjects through their drinking water (Eichele & Kharbanda, 2017). Specific to its mechanism of action, DSS is thought to not directly induce colitis but rather to act as a chemical toxin to epithelium cells within the colon, ultimately causing epithelial damage. Researchers have further proposed that this epithelial damage is due to the ability of DSS to damage the monolayer lining of the epithelial cells, ultimately leading to the dispersal of proinflammatory contents out of the epithelial cells and into underlying tissue (Eichele & Kharbanda, 2017). With this in mind, our DSS-treated transgenic mice initially received 1.5% DSS for a 5-day period *ad libitum*. Following this 5-day period, the mice then received autoclaved tap water that was not treated with 1.5% DSS for an additional 5-days (Figure 1). This experimental timeline was repeated two more times for a total duration of 30 days after which the mice were sacrificed and their brains, specifically the substantia nigra, were harvested for further investigation through procedures such as RNA-sequence analysis.

2.3. RNA Sequence Analysis:

RNA-sequencing (RNA-seq) is an analysis technique that is utilized by researchers to identify both the presence and amount of RNA within a given sample. As a result of these RNA measurements, changes in gene expression i.e. differential gene expression can be assessed by comparing RNA data over time or between distinct samples. In terms of the actual experimental workflow shown in Figure 2, gene reads/counts are first associated and mapped to a reference genome and then gathered into transcripts to continue the analysis (Kukurba & Montogmery, 2016). Once these initial steps are complete, gene expression can ultimately be detected by counting the amount of gene reads that align to the assembled transcripts (Kukurba & Montogmery, 2016). Importantly, gene counts must also be normalized before differential expression analysis in order to correct for multiple sources of variation such as sample differences in sequence composition and the size of library fragments used for analysis (Oshlack & Wakefield, 2009).

Aside from its underlying methodology, RNA-seq analysis offers a handful of important advantages when compared to other sequencing methods such as Tiling microarrays and Expressed Sequence tag (EST) sequencing. For starters, RNA-seq has been shown to not be limited to RNA data for which a corresponding genome exists, allowing for researchers to successfully use this technique even in non-model organisms that still have undetermined genomic sequences (Wang et al., 2009). Additionally, RNA-Seq is known to have very little background signal, allowing for sequences to be accurately mapped back to a specific genomic location (Wang et al., 2009). Moreover, scientists have also praised the accuracy and reproducibility of RNA-Seq as well as the fact that compared to other sequencing techniques

RNA-Seq requires a smaller amount of RNA (Nagalakshmi et al., 2008; Wang et al., 2009). However, as with any experimental technique there are also several limitations that are important to consider. More specifically, generating genomic libraries for RNA-seq analysis are both difficult and error prone as a multitude of steps are involved which may unfortunately lead to the loss of samples. Aside from this, the overall analysis process can be both time-consuming and costly to complete. Nevertheless, even with these potential limitations, RNA-Seq analysis is still considered to be a cutting-edge technique as it allows researchers to effectively and accurately identify differential gene expression.

Therefore, with the importance and overall methodology of RNA-Seq in mind, this technique was utilized in order to identify differential expression when comparing mice of different genotypes and different treatments. Specific to the treatments, as aforementioned certain mice received only autoclaved tap water over the 30-day experimental period in order to serve as control mice that lacked DSS-induced intestinal inflammation. With this in mind, once the substantia nigra samples were harvested at sacrifice, RNA was isolated from samples using TRIzol according to the manufacturer's protocol. Importantly, RNA was then quantified and ultimately sent to Novogene for RNA-seq analysis. Consequently, once the Novogene sequencing was complete, RNA counts for 59 total subjects (Table 2) were obtained and converted into respective comma-separated value (CSV) files in order to allow for further analysis.

The statistical program Rstudio was then used to identify differentially expressed genes through specific R packages such as edgeR, Glimma, and statmod. For the first four rounds of analysis, the threshold of significance was set to a fold change of 2 in order to identify significant genes. However, for the last three rounds of analysis, differential gene expression was evident

on a much smaller scale, resulting in the threshold of significance being lowered to a fold change of 0.1. For more information on this methodology, I recommend the following two sources as they provided clear and effective instructions on how to complete RNA-sequence analysis.

1. Phipson B, Trigos A, Ritchie M, Doyle M, Dashnow H, Law C (2016) RNA-seq analysis in R – Differential expression analysis
2. Chen Y, McCarthy D, Ritchie M, Robinson M, Smyth G (2019) edgeR: differential expression analysis of digital gene expression data User's Guide

2.3.1 Differential Expression Analyses

A total of 7 rounds of analysis were run focusing on various samples and experimental questions.

1. **First sample – All DSS-treated mice**
    a. Experimental Question – How does increased LRRK2 protein and kinase activity levels affect gene expression within the substantia nigra after DSS-induced gastrointestinal inflammation?
2. **Second sample – All DSS-treated males**
    a. Experimental Question – How does increased LRRK2 protein and kinase activity levels affect gene expression within the substantia nigra of male mice after DSS-induced gastrointestinal inflammation?
3. **Third sample – All DSS-treated females**
    a. Experimental Question – How do LRRK2 protein and kinase activity levels affect gene expression within the substantia nigra of female mice after DSS-induced gastrointestinal inflammation?

4. **Fourth sample – All Water-treated mice**
    a. Experimental Question – How does increased LRRK2 protein and kinase activity levels affect gene expression within the substantia nigra of mice without DSS-induced gastrointestinal inflammation?
5. **Fifth sample – B6 water-treated and B6 DSS-treated**
    a. Experimental Question – How does DSS-induced gastrointestinal inflammation relative to water controls affect gene expression in the substantia nigra of B6 mice with endogenous levels of LRRK2?
6. **Sixth sample – WTOE water-treated and WTOE DSS-treated**
    a. Experimental Question – How does DSS-induced gastrointestinal inflammation relative to water controls affect gene expression in the substantia nigra of WTOE mice with increased levels of LRRK2?
7. **Seventh sample – G2019S water-treated and G2019S DSS-treated**
    a. Experimental Question – How does DSS-induced gastrointestinal inflammation relative to water controls affect gene expression in the substantia nigra of G2019S mice with both increased levels of LRRK2 and toxic levels of LRRK2 kinase activity?

For each round of RNA-Seq analysis, volcano plots were created in order to showcase differential gene expression and provide an idea of which genes were up or down regulated. Furthermore, for significantly differentially expressed genes, strip plots were created in order to showcase their expression across either the three genotypes (B6, WTOE, and G2019S) or across treatments within each genotype (water and DSS). Differences between experimental groups were then assessed by either one-way analysis of variance between genotypes or a student t-

test between treatments. Importantly, shared letters above groups indicate that the groups are not significantly different.

3. Results

3.1. Confirmation of *Lrrk2* expression using RNA Sequence Analysis Technique

The effectiveness of this analysis technique was first confirmed by looking at *Lrrk2* gene expression levels. As shown in Figure 3, in all four initial rounds of analysis, *Lrrk2* gene expression was significantly upregulated in both the WTOE and G2019S mice compared to the B6 control mice. Additionally, a significant increase in *Lrrk2* expression levels was observed in G2019S mice compared to WTOE mice. Importantly, this significant increase in *Lrrk2* expression levels was to be expected for as aforementioned both the WTOE and G2019S mice had a 6-8-fold increase in *Lrrk2* gene expression levels as a consequence of their genotype (Li et al., 2010). Therefore, the above result ultimately provides strong evidence for the accuracy and efficacy of my analysis technique.

3.2. Differentially Expressed Genes – Analyses 1-4

3.2.1. Submandibular gland protein C (Smgc) Gene Expression

As shown in Figure 4, in all four initial rounds of analysis, *Smgc* gene expression was significantly upregulated in G2019S mice compared to both the WTOE and B6 mice. It is also important to note that although *Smgc* gene expression was only slightly increased in WTOE mice compared to B6 mice, it was still found to be a significant difference.

3.2.2. Mucin 19 (Muc19) Gene Expression

As shown in Figure 5, in all four initial rounds of analysis, *Muc19* gene expression was significantly upregulated in G2019S and WTOE mice compared to B6 mice. It is also important to note that there was significant upregulation of *Muc19* gene expression in the G2019S mice compared to the WTOE mice.

3.2.3. Synaptonemal Complex 1 (Sycp1) Gene Expression

As shown in Figure 6, in all four initial rounds of analysis, *Sycp1* gene expression was significantly upregulated in WTOE mice compared to both G2019S and B6 mice. Importantly, there was no significance difference in *Sycp1* gene expression when comparing the B6 and G2019S mice.

3.2.4. Predicted gene 15382 (Gm15382) Gene Expression

As shown in Figure 7, in all four initial rounds of analysis, *Gm15382* gene expression was significantly upregulated in both G2019S and WTOE mice compared to B6 mice. It is also important to note that there was no significant difference in gene expression when comparing the WTOE and G2019S expression levels.

3.3. Differentially Expressed Genes – Analyses 5-7

After looking extensively for evidence of differential gene expression across the three mouse genotypes (B6, WTOE, G2019S), I next turned my attention to differential gene expression between water-treated and DSS-treated mice of the same genotype to answer the question of how DSS-induced intestinal inflammation affects the substantia nigra. Importantly, by shifting my

focus I hoped to be able to pinpoint genes related to inflammation for as aforementioned the key difference between the water-treated and DSS-treated mice is that the DSS-treated mice experienced induced gut inflammation. With this in mind, this section will solely focus and present data on differentially expressed genes that are either related to inflammation or have a clear connection to PD development in order to provide the clearest picture in regard to the link between genetic factors and PD.

3.3.1. B6 water-treated vs B6 DSS-treated mice

There was no significant differential gene expression observed between the water-treated B6 mice and the DSS-treated B6 mice.

3.3.2 WTOE water treated vs WTOE DSS treated mice

As shown in Figure 8, the genes *Dusp5*, *Tmem106b*, *Nrxn3*, *Il10ra*, and *Ppp1r1b* all were significantly upregulated in WTOE mice that received the DSS-treatment compared to WTOE mice that received the water-treatment. Surprisingly, the gene *Rtkn2* showed a different trend as WTOE mice that received the water-treatment experienced a significant upregulation in this gene compared to WTOE mice that received the DSS-treatment.

3.3.3. G2019S water treated vs G2019S DSS treated mice

As shown in Figure 9, the genes *Nfkbia*, *Gadd45g*, and *Bag6* were all significantly upregulated in the G2019S mice that received the water-treatment compared to the G2019S mice that received the DSS-treatment. With these trends in mind, it is also important to note the spreads of the distributions although in all cases a significant upregulation is still observed.

4. Discussion

PD as stated previously is the second most common neurodegenerative disease and can further be classified as a progressive movement disorder. With this in mind, the primary symptoms of PD affect an individual's motor capabilities and can ultimately result in constant body tremors, muscle rigidity, and overall slowness of movement. However, as aforementioned, certain non-motor symptoms may also become apparent in individuals with PD such as cognitive dysfunction, fatigue, and gastrointestinal disturbances. In terms of its pathology, numerous studies have cited the significant degeneration of dopaminergic neurons within the substantia nigra as being the primary cause of PD although other factors such as Lewy body accumulation and inflammation have also been shown to play important roles. Aside from these underlying pathological trademarks, studies have shown that certain risk factors such as increasing age as well as numerous environmental and genetic factors may also affect an individual's susceptibility for developing this neurodegenerative disease. However, even with all of this preexisting knowledge, the ability of scientists to both identify and understand the origin of and reasons for PD development underlying each individual is still somewhat deficient. Therefore, in order to try and further elucidate the factors involved in PD development, both a transgenic mouse model with LRRK2, one of the most prevalent familial PD mutations, and a chronic DSS-induced colitis paradigm to model intestinal inflammation were utilized simultaneously in order to study the synergistic effects of the specific gene *Lrrk2* and gut inflammation respectively. Furthermore, RNA-seq analysis was run on substantia nigra samples collected from the transgenic mice in order to study differential gene expression and ultimately showcase which genes were up or downregulated as a result of both genetic and environmental interventions.

From the four initial rounds of analysis, several intriguing genes were found to be significantly differentially expressed. Firstly, as aforementioned the expression levels of *Lrrk2* were significantly increased in both WTOE and G2019S mice compared to the B6 control mice in all four rounds of analysis. This observation, as mentioned previously, was to be expected though as the differences in *Lrrk2* levels can be attributed to the transgenic mice utilized in this study (Table 1). Interestingly, a significant difference in *Lrrk2* expression was also observed in all four rounds of analysis upon comparing the WTOE mice to the G2019S mice which at first glance may seem rather surprising given the fact that both transgenic strains were reported to have approximately the same fold increase in *Lrrk2* levels (Table 1). With that being said, the G2019S mutation has been shown to not just increase both LRRK2 expression levels and kinase activity but also pro-inflammatory cytokine levels and ultimately peripheral inflammation (Wallings & Tansey, 2019; Dzamko et al., 2016). Furthermore, LRRK2 expression has been shown to increase in response to pro-inflammatory signals (Wallings & Tansey, 2019). Therefore, in the case of this experiment, G2019S mice would not only be exposed to DSS-induced inflammation but also would be more susceptible to peripheral inflammation as a result of the G2019S mutation, meaning that these mice could experience a more significant inflammatory response that in turn may explain the significant differences in LRRK2 expression levels observed between WTOE and G2019S mice.

Aside from *Lrrk2*, several genes associated with salivation were found to be differentially expressed. More specifically, the gene Submandibular gland protein C (*Smgc*) was found to be significantly upregulated in both WTOE and G2019S mice compared to the B6 controls. Moreover, a significant difference was found upon comparing the WTOE mice to the G2019S

mice, ultimately highlighting the fact that G2019S mice experienced the highest expression levels of *Smgc*. Specific to the functionality of this gene, it is known to be associated with the submandibular gland, one of the three main salivary glands in humans, and therefore is necessary for saliva production. Importantly, saliva production is necessary for a variety of integral processes such as swallowing, the ability to initiate digestion, and dental hygiene, which therefore highlights the importance of both *Smgc* and the submandibular gland (Grewal & Ryan, 2019). Aside from this gene's functional significance, past research has also emphasized the connections between PD and both the submandibular gland and saliva production. Specific to the submandibular gland, research has shown that the accuracy of PD diagnosis can be improved by analyzing the accumulation of both Lewy bodies and α-synuclein within the submandibular gland, which as aforementioned are pathological trademarks of PD development (Beach et al., 2014). Aside from the connection between PD and the submandibular gland, excessive drooling has been cited as a non-motor symptom of PD which in turn supports the link between saliva production and PD development (Srivanitchapoom et al., 2014). Therefore, in the context of past research, the upregulation of *Smgc* in G2019S mice should come as no surprise. More specifically, although saliva production was not directly measured, the increase in *Smgc* may be telling of an increased susceptibility to PD development as a result of both the G2019S mutation and the experimental paradigm utilized in order to induce gut inflammation. In short, although the significant upregulation in the *Smgc* gene can be accounted for by the clear connection between the submandibular gland and PD development, further analysis comparing the expression levels of *Smgc* in DSS-treated vs water-treated mice is needed in order to confirm the synergistic effects of the G2019S mutation and gut inflammation and ultimately support our hypothesis.

In addition to *Smgc*, the gene *Muc19* was also observed to be significantly upregulated in both G2019S and WTOE mice when compared back to the expression levels of the B6 control mice. Furthermore, much like with *Smgc* expression, *Muc19* expression levels were significantly upregulated in G2019S mice when compared to WTOE mice. Specific to its functionality, *Muc19* is known to encode the main salivary gel-forming mucin protein in mice and as a result provides lubrication for the tissues within the mouth (Culp et al., 2014). Aside from its functionality, past research has highlighted the connection between *Muc19* and *Smgc* as both are products of a shared gene referred to as *Muc19/Smgc*. More specifically, this gene is known to contain 60 exons split between the two genes as *Smgc* is encoded by exons 1-18 and *Muc19* is encoded by exon 1 as well as exons 19-60 (Das et al., 2010). Furthermore, previous work has shown that an increase in salivary mucins was observed in PD patients, supporting the potential link between *Muc19* and PD development (Masters et al., 2015). Therefore, in the context of previous findings, the upregulation of *Muc19* within G2019S mice is to be expected. Firstly, given that *Smgc* shares the same parent gene as *Muc19*, the differential expression patterns should be similar which as shown in figures 4 and 5 is in fact true. Secondly, the connection between PD and increased salivary mucin production is clearly supported, which much like with *Smgc* could be telling of an increased susceptibility to PD development as a result of both the G2019S mutation and the chronic DSS paradigm. Consequently, although the significant upregulation of the *Muc19* gene can be accounted for by both its co-localization with the *Smgc* gene as well as its connection to PD, further analysis comparing the expression of *Muc19* in DSS-treated vs water-treated mice is needed in order to confirm the synergistic effects of both the G2019S mutation and gut inflammation and ultimately support our hypothesis.

Aside from *Muc19* and *Smgc*, significant upregulation of the gene *Sycp1* was observed in WTOE mice. In reference to this gene's functional importance, *Sycp1* is known to encode a central protein component of the synaptonemal complex which in turn is responsible for the fusion of homologous chromosomes during meiotic prophase. However, unlike with *Muc19* and *Smgc*, no clear connection was apparent between *Sycp1* and PD development based off of previous research. In addition, no significant evidence or support was found by taking a more general focus and looking at the possible connection between PD development and the synaptonemal complex. Therefore, although significant upregulation of *Sycp1* was observed, this result does little to either support or refute our original hypothesis. The same is true for the gene *Gm15382* which was observed to be significantly upregulated in both WTOE and G2019S mice. In terms of its functionality, this gene is classified as a mouse pseudogene and as a result little research has been done on both its functional importance as well as its potential connection to PD development. In short, although significant differential expression was observed in both the genes *Sycp1* and *Gm15382*, the lack of research concerning the possible roles these genes may play in PD development make it difficult to formulate accurate conclusions in regard to why an upregulation is seen. However, with that being said, this lack of research may in turn suggest a new avenue of research in order to either confirm or refute the involvement of these genes and their protein counterparts in PD development.

From the last three rounds of analyses looking specifically at differential gene expression between water-treated and DSS-treated mice, a number of genes were observed to be differentially expressed. However, before getting into more specifics, it is first important to note the lack of differential expression observed between B6 water-treated and B6 DSS-treated mice.

As shown in Table 1, the B6 control mice had normal endogenous levels of both the LRRK2 protein as well as LRRK2 kinase activity. As a result, unlike in both the WTOE and G2019S mice, the proposed synergistic effects of both a *Lrrk2* mutation and induced gut inflammation would not be plausible as the genetic component would be absent. Therefore, with only the environmental influence as a factor, the resulting effects on PD development would be expected to be less, ultimately supporting the finding that significant differential gene expression was not observed upon comparing B6 water-treated vs B6 DSS-treated mice.

With the lack of differential expression in B6 mice aside, it is also important to note the differences in distribution patterns between the first four rounds of analysis I ran and the last three rounds of analysis I ran. More specifically, the data reported from the first four rounds of analysis was much more compact with a smaller distribution compared to the data reported from the last three rounds of analysis which was much more variable and therefore had a much larger distribution. With this in mind, this difference in distribution may be due to the thresholds of significance that were used in order to identify whether or not the change in gene expression that was observed was in fact significant. As aforementioned, the threshold of significance was set at a fold change of 2 for the first four rounds of analysis and after was reduced to a fold change of 0.1 for the final three rounds of analysis as differential gene expression was observed on a much smaller scale which in turn may have affected the distribution of the data that was found. Furthermore, as mentioned previously the first four rounds of analysis and the final three rounds of analysis focused on different comparisons. More specifically, the first four rounds of analysis compared gene expression between genotypes and the final three rounds of analysis compared gene expression between water-treated and DSS-treated mice of the same genotype

in order to understand how different genotypes and gastrointestinal inflammation relative to water controls affect gene expression respectively. Therefore, this difference in comparisons may have also contributed to a difference in distribution patterns that was observed between the different analyses.

With both the lack of differential expression in B6 mice and differences in distribution patterns aside, 6 genes were found to have significant differential expression when comparing water-treated WTOE to DSS-treated WTOE mice. To be more specific, one such gene was *Dusp5* which showed significant upregulation in the DSS-treated WTOE mice. In reference to its functionality, *Dusp5* is known to encode a protein in the dual specificity protein phosphatase subfamily which is ultimately responsible for negatively regulating kinases within the mitogen-activated protein (MAP) kinase superfamily through dephosphorylation of phosphotyrosine and phosphoserine/threonine residues. Importantly, protein members of the MAP kinase superfamily are associated with integral cellular processes such as cellular differentiation and proliferation. Therefore, with the general functional importance of dual specificity protein phosphatases in mind, *Dusp5* is specifically known to inactivate MAP3/ERK1 which is an essential protein component of the MAP kinase signal transduction pathway. Aside from being a central component of this kinase signal transduction pathway, previous research has also highlighted the potential link between ERK1 signaling and PD development. More specifically, past studies have outlined the idea that inhibition of ERK1 along with ERK2 may lead to the activation of necrotic and apoptotic pathways associated with induced cell-death (Monick et al., 2008). Furthermore, past research has highlighted the potential role that ERK1 activity could play in neuronal survival as when both ERK5 and ERK1/2 pathways were inhibited, a significant decline in dopaminergic

neuron survival was observed after the neurons were exposed to a toxic agent (Bobush et al., 2018). Therefore, in the context of past research, the observed upregulation of *Dusp5* in DSS-treated WTOE mice is a reasonable result. More specifically, an upregulation of *Dusp5* would in turn lead to a more substantial inactivation of ERK1 which ultimately may lead to increased activation of necrotic and apoptotic pathways as well as reduced dopaminergic neuron survival. Consequently, an increase in cellular pathways associated with induced cell-death as well as reduced survival of dopaminergic neurons would increase one's susceptibility for developing PD and ultimately provides support for our hypothesis regarding the synergistic effects that increased LRRK2 kinase activity and expression as well as induced gut inflammation may have in regards to PD development.

Aside from *Dusp5*, the gene *Tmem106b* was also found to be significantly upregulated in DSS-treated WTOE mice compared to water-treated WTOE mice. Specific to its functionality, this gene is categorized as a lysosomal membrane protein and therefore is integral for proper lysosomal function (Arrant et al, 2018). With this in mind, numerous studies have investigated the effects that *Tmem106b* overexpression may have in regard to lysosomal function. More specifically, *Tmem106b* overexpression has been shown to induce detrimental effects on lysosomes such as acidification impairment, reduced mobility and clustering within neurons which together contribute to lysosomal dysfunction (Chen-Plotkin et al., 2012; Stagi et al., 2014). Furthermore, past work on lysosomal dysfunction has shown that the impairment of lysosomal degradation pathways is often thought to be a significant pathogenic characteristic of neurological diseases including PD (Debay et al., 2013). Therefore, in the context of past research, an upregulation of *Tmem106b* would be expected to contribute to a higher susceptibility for

lysosomal dysfunction and therefore a higher likelihood for PD development. In short, in reference to our hypothesis, an upregulation of *Tmem106b* in DSS-treated WTOE mice provides support for the potential synergistic effects of LRRK2 and gut inflammation as this change in gene expression may represent an increased susceptibility to PD development compared to water-treated WTOE mice.

The next gene that showed significant differential expression was *Nrxn3* which was upregulated in DSS-treated WTOE mice. This gene is known to be encode a neural protein referred to as Neurexin 3 which is one of three main neurexin genes (Sudhof, 2008). Additionally, it is important to note that each neurexin gene encodes an α-protein as well as a β-protein from different promoters, resulting in two protein versions that have distinct structural features such as the fact that α-proteins are generally larger than β-proteins (Sudhof, 2008). With the structural features of neurexins aside, these proteins have a very important function within the brain. More specifically, these proteins can be further classified as synaptic cell-adhesion molecules that are essential for both synaptic function and ultimately normal brain function (Sudhof, 2008; Chen et al., 2017). Unfortunately, limited research has been conducted on the potential connections that neurexins have with PD development which in turn makes it rather challenging to draw concrete conclusions regarding why an upregulation was observed in DSS-treated WTOE mice. However, with that being said, past research has stressed the link between synaptic dysfunction and neurodegenerative diseases. More specifically, one study described synaptic dysfunction as a central pathological feature of neurological diseases which ultimately highlights the importance of proper synaptic function (Taoufik et al., 2018). Therefore, much like with the genes *Sycp1* and

Gm15382, this scarcity in research concerning neurexins and PD development may in turn suggest a new research focus for future work.

Differential expression was also observed in reference to the gene *Il10ra* as DSS-treated WTOE mice were seen to have significant upregulation of this gene when compared back to water-treated WTOE mice. Specific to its functionality, *Il10ra* is known to encode the receptor protein associated with the anti-inflammatory cytokine Interleukin 10 (IL-10). With this in mind, it is interesting to note that as an anti-inflammatory cytokine, IL-10 is responsible for dampening an individual's overall inflammatory response by limiting the production of pro-inflammatory molecules in order to ultimately limit tissue damage (Moore et al., 2001). Therefore, with the clear connection between IL-10 and the reduction of inflammation, the upregulation of *Il10ra* makes sense as in response to DSS-induced inflammation the mice would have needed an increase in IL-10 production to combat an increase in inflammation. However, in reference to our hypothesis and the development of a PD phenotype, this finding refutes our proposed concept as if increased LRRK2 levels and kinase activity coupled with induced gut inflammation did in fact increase an individual's susceptibility for PD development, one would expect a reduction in Il10ra. This is because as aforementioned, inflammation is a pathological characteristic of PD development and so with a significant reduction in *Il10ra* expression, an individual would be less equipped to reduce inflammation and as a consequence become even more prone to continued PD development.

Another gene to show significant differential expression was *Ppp1r1b* which experienced an upregulation in expression for DSS-treated WTOE mice when compared to water-treated WTOE mice. In reference to its function, this gene is known to encode for the protein

phosphatase 1 regulatory subunit 1B which is also often times referred as dopamine- and cAMP-regulated neuronal phosphoprotein (DARPP-32). More specifically, this protein product can be activated by either glutamatergic or dopaminergic stimulation which in turn regulates its ability to perform as a phosphatase or kinase inhibitor. Consequently, this gene is linked extensively to dopamine which as aforementioned is the main neurotransmitter that is reduced as a result of PD development. Interestingly, one such study looked at how dopamine depletion as a result of PD affected PPP1R1B/DARPP-32 signaling and found that the loss of dopamine lead to multiple changes in the signaling of these important proteins in both striatonigral and striatopallidal neurons (Meurers et al., 2009). Therefore, with this past research in mind, differential expression of the gene *Ppp1r1b* is to be expected. More specifically, based on our proposed hypothesis regarding the synergistic effects of LRRK2 and gut inflammation, if mice were to become more susceptible to PD development as a result of these factors, certain pathological characteristics such as dopaminergic neurodegeneration would most likely develop which in turn would lead to changes in PPP1R1B/DARPP-32 signaling. In short, this change in expression of *Ppp1r1b* may serve as an indication of changing dopamine levels which in turn would reflect the idea that mice treated with DSS and with increased LRRK2 levels have a greater chance of developing a PD phenotype.

The final gene I included that showed differential expression in WTOE mice was *Rtkn2* which was observed to be significantly upregulated in water-treated WTOE mice when compared to DSS-treated WTOE mice. Specific to its functionality, *Rtkn2* is known to encode the protein product Rhotekin-2 which is part of a group of proteins that are targets for Rho-GTPases. Specific to Rho-GTPases, this protein family is involved in the regulation of significant cellular processes

such as gene transcription (Hill et al., 1995) and cellular survival and death (Heasman & Ridley, 2008; Linseman & Loucks, 2008). Moreover, Rho-GTPases have been implicated in the maintenance of neuronal morphology (Stankiewicz & Linseman, 2014). Finally, these Rho-GTPase molecules have been shown to be involved in the initiation of neuronal apoptosis and therefore play a very important role in induced cell death (Stankiewicz & Linseman, 2014). Therefore, in the context of past research, this upregulation in *Rtkn2* expression within water-treated WTOE mice may be accounted for based off of the functional importance of Rho-GTPases. More specifically, given that *Rtkn2* encodes for a target protein of Rho-GTPases, reduced expression of *Rtkn2* would therefore correlate with reduced activity of Rho-GTPases which in turn could dysregulate the important cellular processes discussed previously. Therefore, although clear connections between *Rtkn2* and PD development cannot be drawn based off of previous work, reduced expression of this gene may in fact have significant effects that with more research may increase an individual's susceptibility for developing neurological conditions such as PD.

With differential expression among WTOE mice aside, my attention next shifted to differentially expressed genes when comparing DSS-treated G2019S mice to water-treated G2019S mice. More specifically, one such gene was *Nfkbia* which was found to be significantly upregulated in water-treated G2019S mice compared to DSS-treated G2019S mice. Specific to this gene's functional importance, *Nfkbia* is known to encode for the protein product IκBα which in turn acts as a negative regulator of the nuclear-factor NF-κB pathway (Ali et al., 2013). Importantly, the nuclear-factor NF-κB pathway is categorized as a pro-inflammatory signaling pathway as the expression of pro-inflammatory genes which encode for cytokines, chemokines, and adhesion molecules are all increased as a result of this pathway (Lawrence, 2009). Therefore,

with this in mind, the upregulation of *Nfkbia* in water-treated G2019S mice is rather reasonable. More specifically, given that *Nfkbia* is known to encode for a protein that ultimately inhibits the pro-inflammatory nuclear-factor NF-κB pathway, a significant reduction in *Nfkbia* expression as seen in the DSS-treated G2019S mice would in turn reduce the inhibition of this pathway and ultimately lead to an increased inflammatory response. Consequently, this differential gene expression provides support for the synergistic effect of increased LRRK2 levels and kinase activity as well as induced gut inflammation. As a result of these interventions, the DSS-treated mice became more susceptible to inflammation and exhibited PD-associated inflammatory phenotypes.

Aside from *Nfkbia*, the gene *Gadd45g* was observed to be significantly upregulated in water-treated G2019S mice compared to DSS-treated G2019S mice. In terms of its functionality, this gene is known to encode a protein within the Gadd45 protein family which overall is responsible for cellular responses to a wide variety of stressful stimuli (Liebermann & Hoffman, 1998; Fornace et al., 2002). Therefore, as a consequence of either physiological or environmental stress-inducing stimuli, research has shown that Gadd45 expression levels increase in response to stress (Moskalev et al., 2012). With the function of this protein family in mind, previous research has also highlighted the connection between Gadd45 and neurodegeneration. More specifically, research in Drosophila showcase the idea that Gadd45 proteins can act as protective measures against neurodegeneration and would ultimately be beneficial against neurodegenerative disorder such as PD (Bgatova et al., 2014). Therefore, in the context of previous research, an upregulation of *Gadd45g* in water-treated G2019S mice is a reasonable outcome. To be more specific, given that the Gadd45 protein family has been shown to mitigate

signs of neurodegeneration, based off of our hypothesis it would therefore make sense that an up-regulation in *Gadd45g* was not seen in the DSS-treated G2019S mice. Additionally, this result may make sense because of the fact that lower levels of *Gadd45g* should correlate with a reduced protective effect against neurodegeneration, meaning that this data ultimately supports our hypothesis regarding the synergistic effect of LRRK2 and gut inflammation as the G2019S mice with both the genetic and environmental risk factors were observed to have significantly lower levels of *Gadd45g* and therefore less of a protective effect against neurodegeneration.

The final gene that I focused on when comparing DSS-treated G2019S mice to water-treated G2019S mice was *Bag6*. Specific to the differential expression that was observed, water-treated G2019S mice were shown to have a significant upregulation in *Bag6* gene expression compared back to DSS-treated G2019S mice. In reference to this gene's functional importance, it is known to encode for the protein Bag6 which is a member of the Bcl-2-associated athanogene (BAG) protein family which importantly is involved in a wide variety of integral cellular processes such as neuron differentiation and stress signaling (Kabbage & Dickman, 2008). Furthermore, past research has highlighted the potential neuroprotective effects that BAG proteins may have as not only is this protein family closely associated with neurodegenerative disease, but it may also aid in the prevention of apoptosis in response to neurotoxicity (Guo et al., 2015). Therefore, much like with *Gadd45g*, an upregulation of *Bag6* within water-treated G2019S mice is supported by previous research. More specifically, given the link between BAG proteins and neuroprotection, the DSS-treated G2019S mice would be expected to have lower expression levels of *Bag6* if in fact LRRK2 and gut inflammation synergistically lead to an increased likelihood of developing a PD phenotype. Therefore, due to the fact that the expression levels of *Bag6* were

shown to be significantly lower in DSS-treated G2019S mice, this data provides support for our overall hypothesis and the idea that as a result of both genetic and environmental risk factors, the G2019S mice experienced an increase in susceptibility to PD-associated phenotypes.

5. Conclusion

The present study showcases a wide variety of differentially expressed genes as a result of mutations in *Lrrk2* and DSS-induced gut inflammation. Although not all genes were found to be directly linked to PD or PD development, many genes did in fact have connections to PD whether through neuroprotection, inflammation, saliva production, or other important cellular processes. Therefore, the findings of this study provide a greater understanding of signaling pathways implicated in PD phenotypes and will hopefully help contribute to the continued exploration of important signaling cascades that may in turn allow for the development of therapeutics to combat the detrimental effects of PD.

Figures and Graphs

Genotype	LRRK2 Levels	LRRK2 kinase activity
Control (B6)	Normal endogenous levels	Normal endogenous activity
Wild Type (WTOE)	6-8 fold increase in LRRK2 levels	Intermediate increase in kinase activity
G2019S mutation (G2019S)	6-8 fold increase in G2019S LRRK2 levels	Increase in kinase activity to toxic levels

Table 1: The table above provides a summary of LRRK2 protein expression levels and kinase activity across the transgenic mice used in this study.

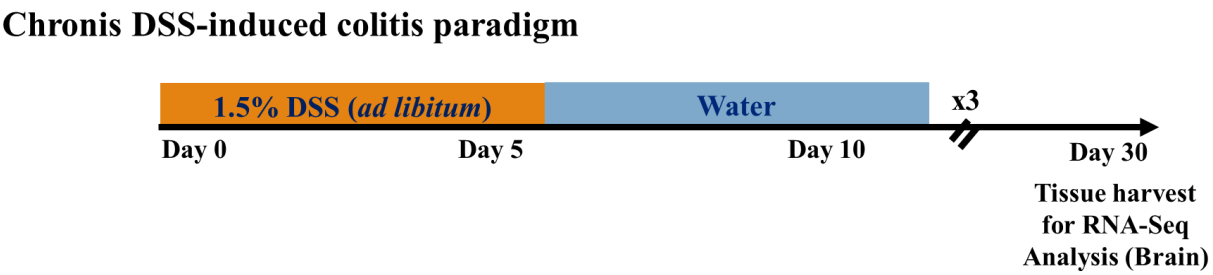
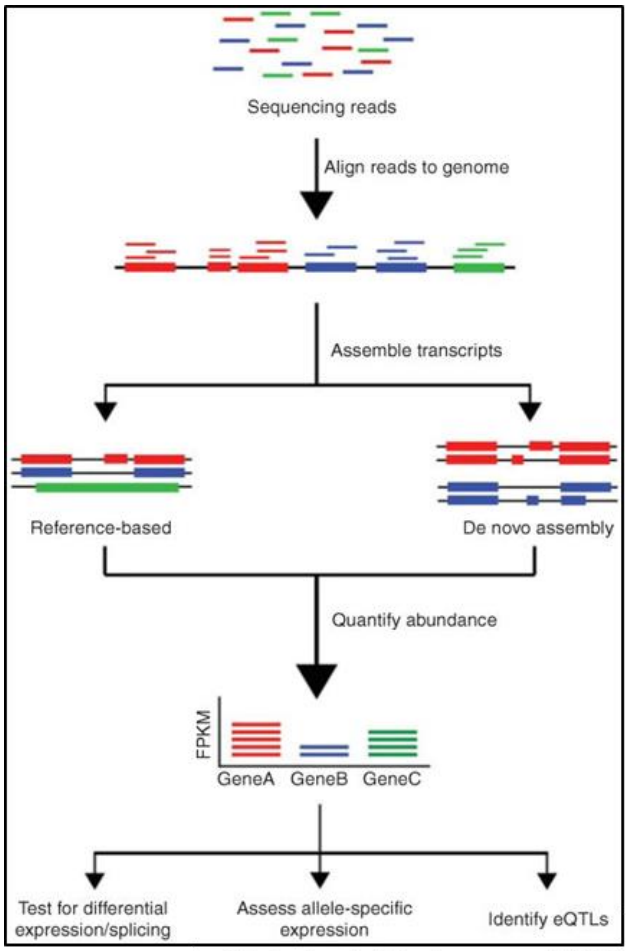


Figure 1: The figure above provides a visualization of the experimental paradigm used to induce intestinal inflammation in LRRK2 mouse models.



(Kukurba & Montgomery, 2016)

Figure 2: The figure above provides a visualization of the RNA sequence experimental workflow (adapted from Kukurba & Montgomery, 2016).

Genotype	B6	WTOE	G2019S	Total
Males treated with Water	3 mice	3 mice	3 mice	9 mice
Females treated with Water	2 mice	2 mice	5 mice	9 mice
Males treated with DSS	9 mice	6 mice	5 mice	20 mice
Females treated with DSS	7 mice	5 mice	9 mice	21 mice
Total	21 mice	16 mice	22 mice	59 mice

Table 2: The table above provides a visualization of the categorical organization of the 59 total mice studied based on their genotype, sex, and treatment.

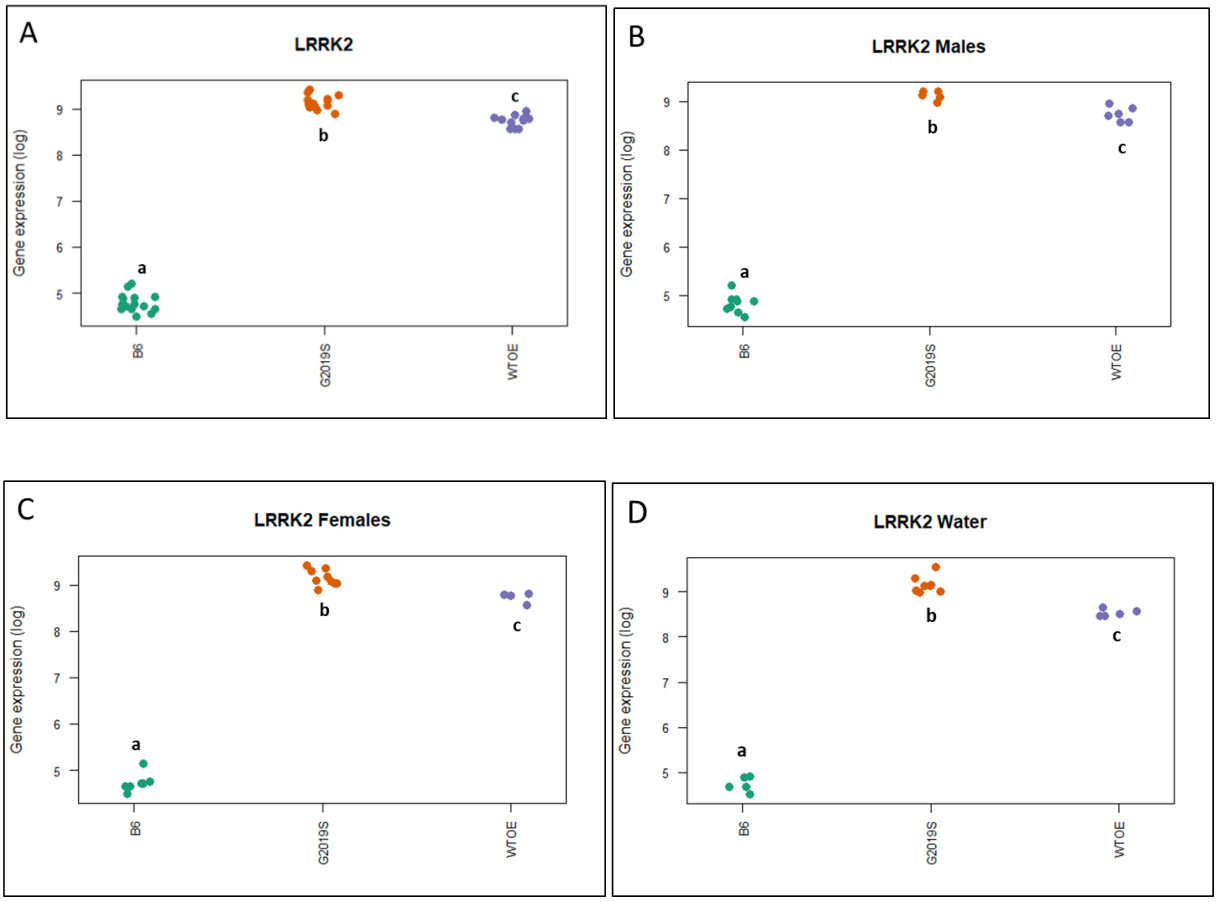


Figure 3: The figure above showcases the *Lrrk2* expression levels across the three mouse genotypes. **(A)** Strip plot of the *Lrrk2* log gene expression levels of all DSS-treated mice. Gene expression is on the y-axis with the corresponding genotype (B6, WTOE, G2019S) on the x-axis. **(B)** Strip plot of the *Lrrk2* log gene expression levels of all male DSS-treated mice. Gene expression is on the y-axis with the corresponding genotype (B6, WTOE, G2019S) on the x-axis. **(C)** Strip plot of the *Lrrk2* log gene expression levels of all female DSS-treated mice. Gene expression is on the y-axis with the corresponding genotype (B6, WTOE, G2019S) on the x-axis. **(D)** Strip plot of the *Lrrk2* log gene expression levels of all water-treated mice. Gene expression is on the y-axis with the corresponding genotype (B6, WTOE, G2019S) on the x-axis.

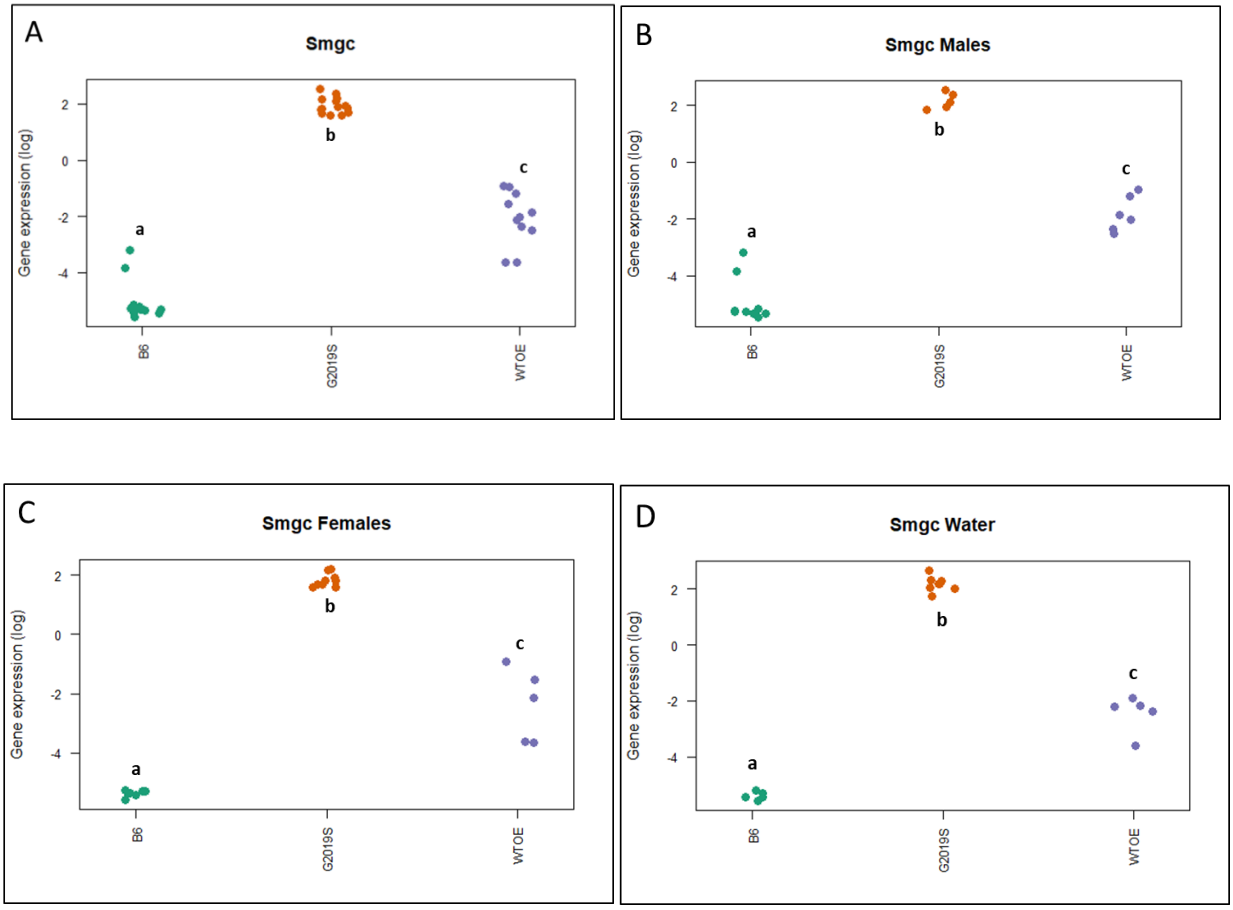


Figure 4: The figure above showcases *Smgc* expression levels across the three mouse genotypes. **(A)** Strip plot of the *Smgc* log gene expression levels of all DSS-treated mice. Gene expression is on the y-axis with the corresponding genotype (B6, WTOE, G2019S) on the x-axis. **(B)** Strip plot of the *Smgc* log gene expression levels of all male DSS-treated mice. Gene expression is on the y-axis with the corresponding genotype (B6, WTOE, G2019S) on the x-axis. **(C)** Strip plot of the *Smgc* log gene expression levels of all female DSS-treated mice. Gene expression is on the y-axis with the corresponding genotype (B6, WTOE, G2019S) on the x-axis. **(D)** Strip plot of the *Smgc* log gene expression levels of all water-treated mice. Gene expression is on the y-axis with the corresponding genotype (B6, WTOE, G2019S) on the x-axis.

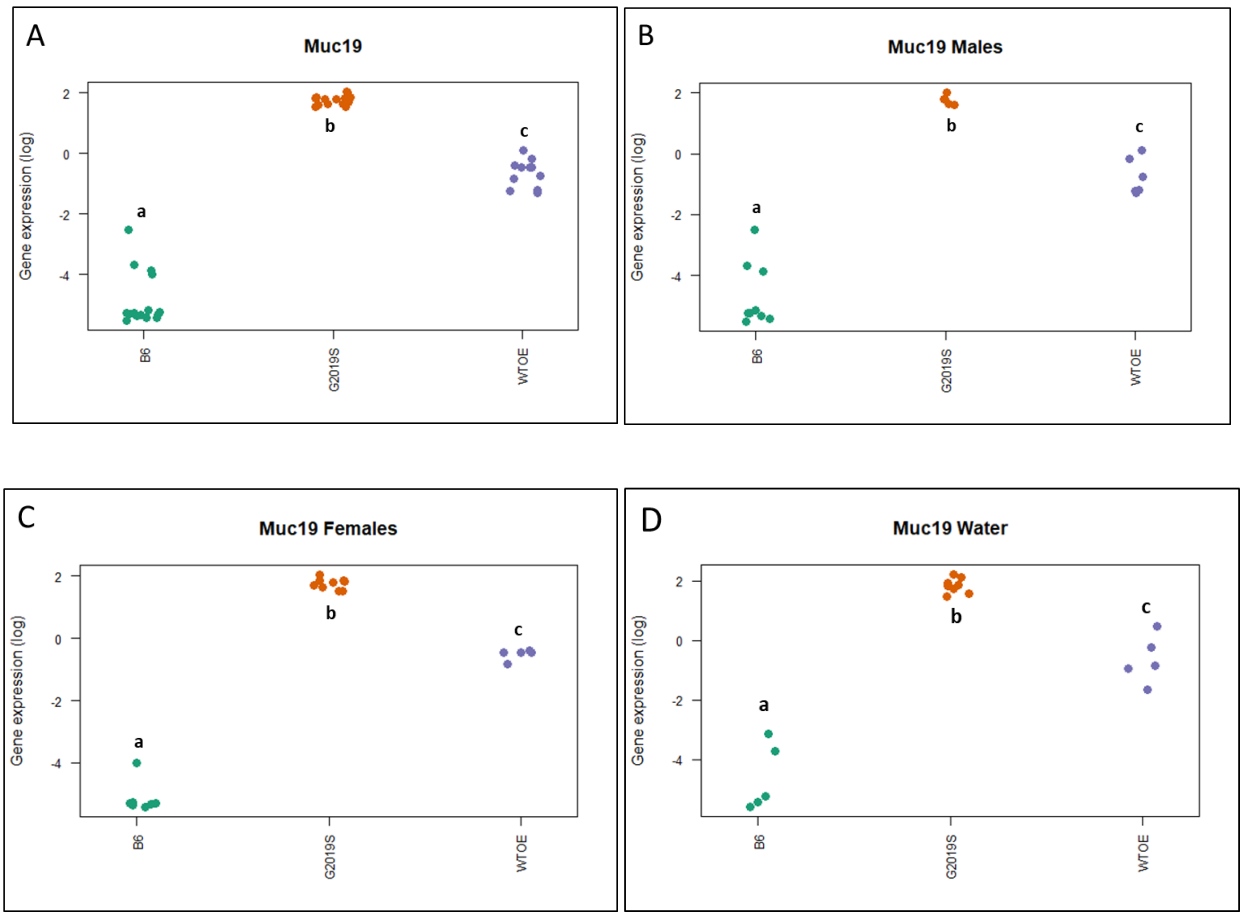


Figure 5: The figure above showcases *Muc19* expression levels across the three mouse genotypes. **(A)** Strip plot of the *Muc19* log gene expression levels of all DSS-treated mice. Gene expression is on the y-axis with the corresponding genotype (B6, WTOE, G2019S) on the x-axis. **(B)** Strip plot of the *Muc19* log gene expression levels of all male DSS-treated mice. Gene expression is on the y-axis with the corresponding genotype (B6, WTOE, G2019S) on the x-axis. **(C)** Strip plot of the *Muc19* log gene expression levels of all female DSS-treated mice. Gene expression is on the y-axis with the corresponding genotype (B6, WTOE, G2019S) on the x-axis. **(D)** Strip plot of the *Muc19* log gene expression levels of all water-treated mice. Gene expression is on the y-axis with the corresponding genotype (B6, WTOE, G2019S) on the x-axis.

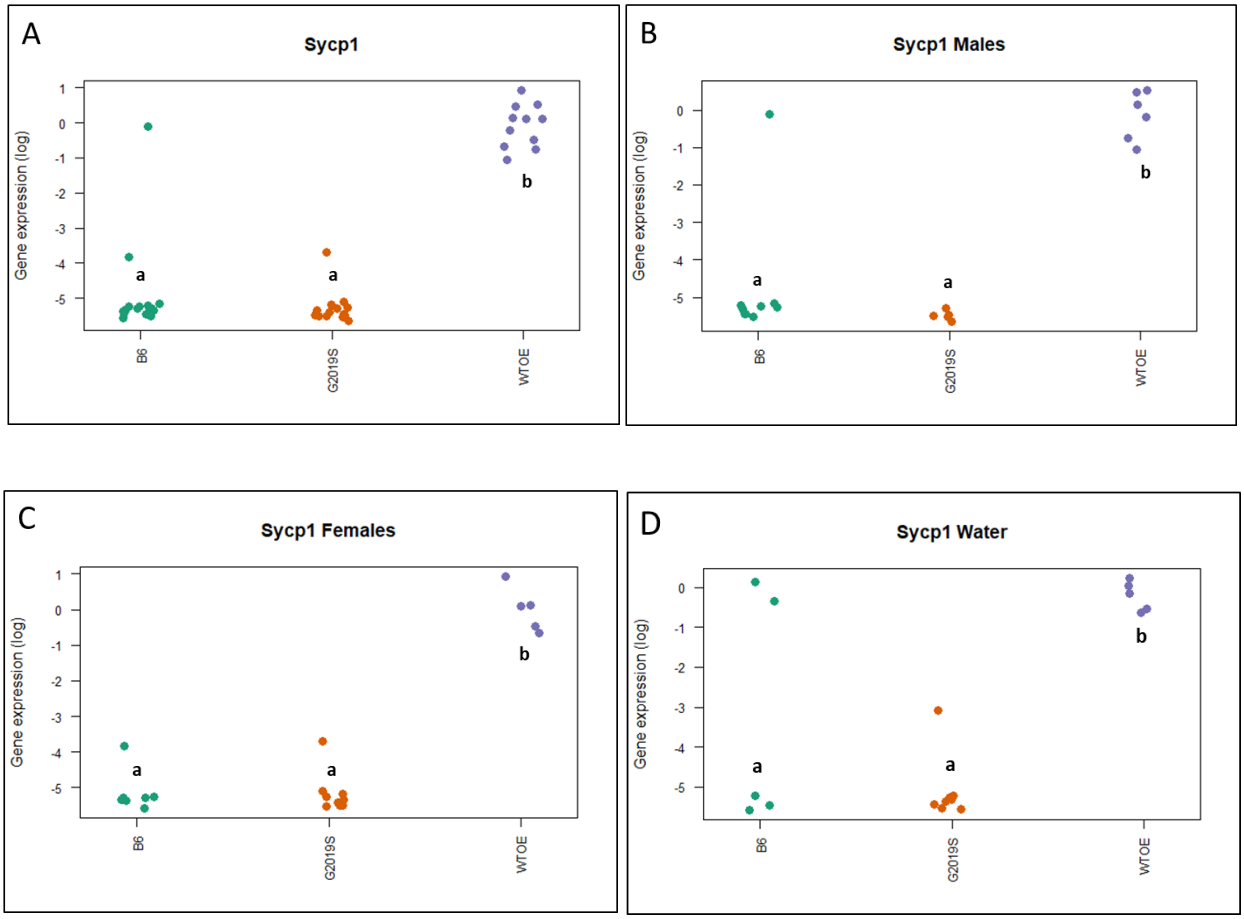


Figure 6: The figure above showcases *Sycp1* expression levels across the three mouse genotypes. **(A)** Strip plot of the *Sycp1* log gene expression levels of all DSS-treated mice. Gene expression is on the y-axis with the corresponding genotype (B6, WTOE, G2019S) on the x-axis. **(B)** Strip plot of the *Sycp1* log gene expression levels of all male DSS-treated mice. Gene expression is on the y-axis with the corresponding genotype (B6, WTOE, G2019S) on the x-axis. **(C)** Strip plot of the *Sycp1* log gene expression levels of all female DSS-treated mice. Gene expression is on the y-axis with the corresponding genotype (B6, WTOE, G2019S) on the x-axis. **(D)** Strip plot of the *Sycp1* log gene expression levels of all water-treated mice. Gene expression is on the y-axis with the corresponding genotype (B6, WTOE, G2019S) on the x-axis.

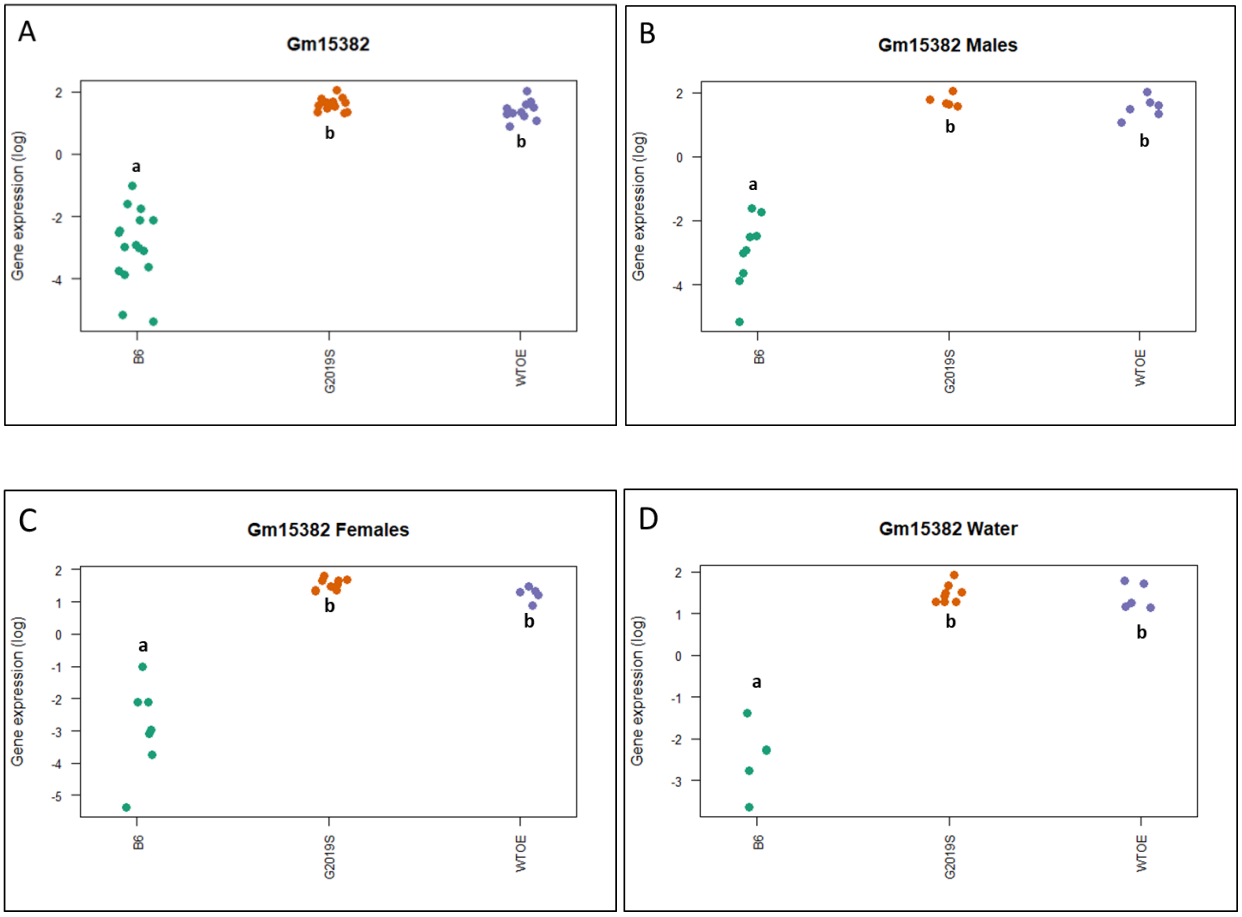


Figure 7: The figure above showcases *Gm15382* expression levels across the three mouse genotypes. (A) Strip plot of the *Gm15382* log gene expression levels of all DSS-treated mice. Gene expression is on the y-axis with the corresponding genotype (B6, WTOE, G2019S) on the x-axis. **(B)** Strip plot of the *Gm15382* log gene expression levels of all male DSS-treated mice. Gene expression is on the y-axis with the corresponding genotype (B6, WTOE, G2019S) on the x-axis. **(C)** Strip plot of the *Gm15382* log gene expression levels of all female DSS-treated mice. Gene expression is on the y-axis with the corresponding genotype (B6, WTOE, G2019S) on the x-axis. **(D)** Strip plot of the *Gm15382* log gene expression levels of all water-treated mice. Gene expression is on the y-axis with the corresponding genotype (B6, WTOE, G2019S) on the x-axis.

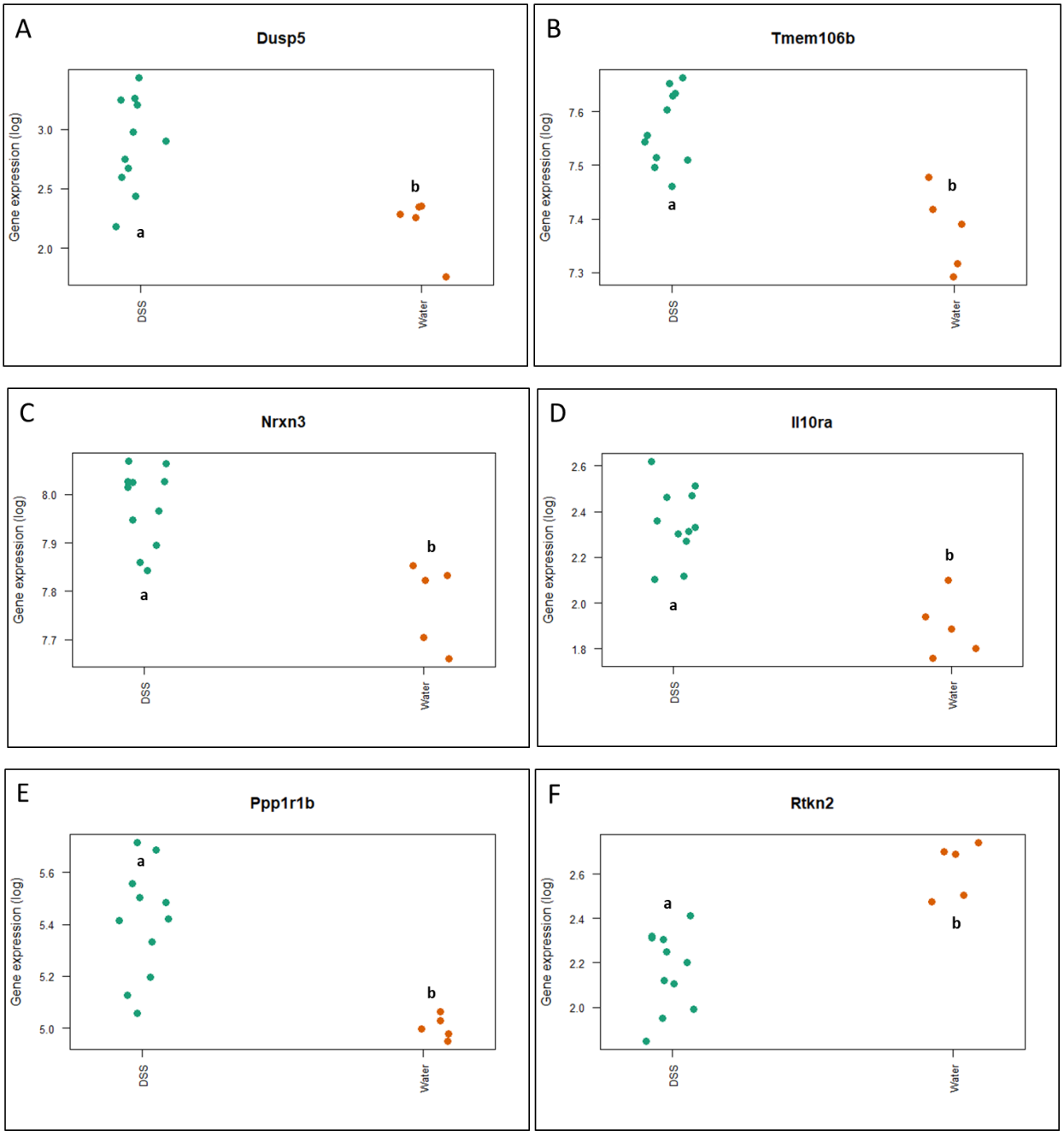


Figure 8: This figure shows differential Gene expression between water-treated and DSS-treated WTOE mice (A) Strip plot of the *Dusp5* log gene expression levels. Gene expression is on the y-axis with the corresponding treatment (DSS, Water) on the x-axis. **(B)** Strip plot of the *Tmem106b* log gene expression levels. Gene expression is on the y-axis with the corresponding treatment (DSS, Water) on the x-axis. **(C)** Strip plot of the *Nrxn3* log gene expression levels. Gene expression is on the y-axis with the corresponding treatment (DSS, Water) on the x-axis **(D)** Strip plot of the *Il10ra* log gene expression levels. Gene expression is on the y-axis with the corresponding treatment (DSS, Water) on the x-axis. **(E)** Strip plot of the *Ppp1r1b* log gene expression levels. Gene expression is on the y-axis with the corresponding treatment (DSS, Water) on the x-axis. **(F)** Strip plot of the *Rtkn2* log gene expression levels. Gene expression is on the y-axis with the corresponding treatment (DSS, Water) on the x-axis.

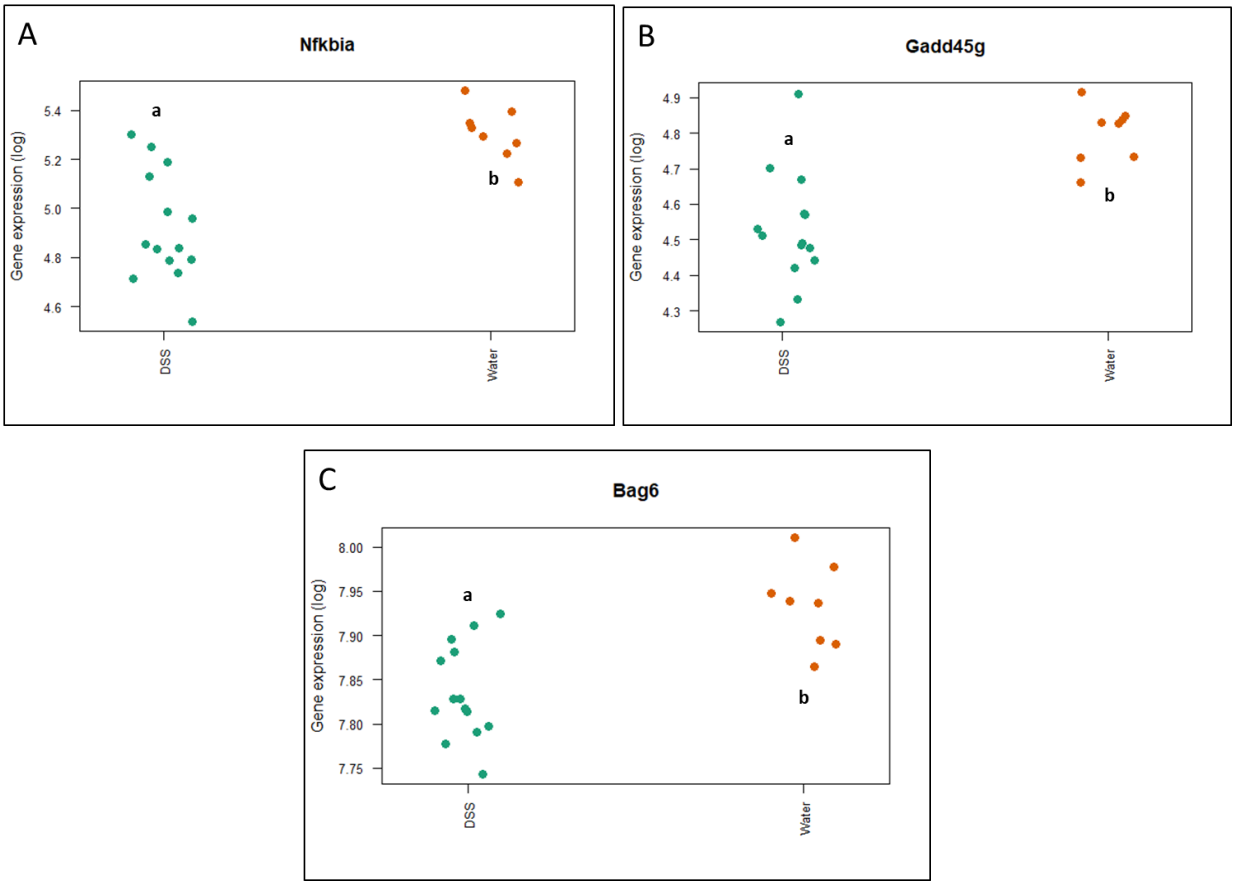


Figure 9: The figure above showcases differential Gene expression between water-treated and DSS-treated G2019S mice (A) Strip plot of the *Nfkbia* log gene expression levels. Gene expression is on the y-axis with the corresponding treatment (DSS, Water) on the x-axis. **(B)** Strip plot of the *Gadd45g* log gene expression levels. Gene expression is on the y-axis with the corresponding treatment (DSS, Water) on the x-axis. **(C)** Strip plot of the *Bag6* log gene expression levels. Gene expression is on the y-axis with the corresponding treatment (DSS, Water) on the x-axis

Works Cited

Alberico SL, Cassell MD, Narayanan NS (2015) The vulnerable ventral tegmental area in Parkinson's disease. Basal Ganglia. 5:51-55

Ali S, Hirschfeld AF, Mayer ML, Fortuno ES, Corbett N, Kaplan M, Wang S, Schneiderman J, Fjell CD, Yan J, Akhabir L, Aminuddin F, Marr N, Lacaze-Masmonteil T, Hegele RG,

Arrant AE, Nicholson AM, Zhou X, Rademakers R, Roberson ED (2018) Partial Tmem106b reduction does not correct abnormalities due to progranulin haploinsufficiency. Mol Neurodegener. 13: 32

Beach TG, Adler CH, Dugger BN, Serrano G, Hidalgo J, Henry-Watson J, Shill HA, Sue LI, Sabbagh MN, Akiyama H (2014) Submandibular Gland Biopsy for the Diagnosis of Parkinson's Disease. J Neuropathol Exp Neurol 72:130-136

Becker A, Chan-Yeung M, Hancock REW, Kollmann TR, Daley D, Sandford AJ, Lavoie PM, Turvey SE (2013) Functional Genetic Variation in *NFKBIA* and Susceptibility to Childhood Asthma, Bronchiolitis, and Bronchopulmonary Dysplasia. The Journal of Immunology. 190: 3949-3958

Bgatova N, Dubatolova T, Omelyanchuk L, Plyusnina E, Shaposhnikov M, Moskalev A (2014) Gadd45 expression correlates with age dependent neurodegeneration in Drosophila melanogaster. Biogerontology. 16: 53-61

Billingsley KJ, Bandres-Ciga S, Saez-Atienzar S, Singleton AB (2018) Genetic risk factors in Parkinson's disease. Cell and Tissue Research. 373: 9-20

Bobush A, Niewiadomska G, Filipek A (2018) Role of Mitogen Activated Protein Kinase Signaling in Parkinson's Disease. Int J Mol Sci. 19: 2973

Bonifati V, Rizzu P, Squitieri F, Krieger E, Vanacore N, van Swieten JC, … Heutink P (2003) DJ-1(PARK7), a novel gene for autosomal recessive, early onset parkinsonism. Neurological Sciences: Official Journal of the Italian Neurological Society and of the Italian Society of Clinical Neurophysiology. 24: 159–160

Braak H, Tredici KD, Rub U, De Vos RAI, Jansen Steur ENH, Braak E (2003) Staging of brain pathology related to sporadic Parkinson's disease. Neurobiol. Aging. 24:197-211

Buchman A.S., Shulman J.M., Nag S., Leurgans S.E., Arnold S.E., Morris M.C., Schneider J.A., Bennett D.A. (2012) Nigral pathology and Parkinsonian signs in elders without Parkinson disease. Ann. Neurol. 71:258–266.

Chai C, Lim KL (2013) Genetic Insights into Sporadic Parkinson's Disease Pathogenesis. Curr Genomics. 14: 486-501

Chen H, Zhao EJ, Zhang W, Lu Y, Liu R, Haung X, Cieskielski-Jones AJ, Justice MA, Cousins DS, Peddada S (2015) Meta-analyses on prevalence of selected Parkinson's nonmotor symptoms before and after diagnosis. Transl. Neurodegener. 4:1-8

Chen LY, Jiang M, Zhang B, Gokce O, Sudhof TC (2017) Conditional Deletion of All Neurexins Defines Diversity of Essential Synaptic Organizer Functions for Neurexins. Neuron. 94: 611-625

Chen ML, Wu RM (2018) LRRK2 gene mutations in pathophysiology of the ROCO domain and therapeutic targets for Parkinson's disease: a review. J Biomed Sci. 25

Chen-Plotkin AS, Unger TL, Gallagher MD, Bill E, Kwong LK, Volpicelli-Daley L, Busch JI, Akle S, Grossman M, Van Deerlin V, Trojanowski JQ, Lee VMY (2012) *TMEM106B*, the risk gene for frontotemporal dementia, is regulated by the microRNA-132/212 cluster and affects progranulin pathways. J Neurosci. 32:11213–11227

Culp DJ, Robinson B, Cash MN, Bhattacharyya I, Stewart C, Cuadra-Saenz G (2014) Salivary Mucin 19 Glycoproteins: Innate Immune Functions in Streptococcus mutans-Induced Caries in Mice and Evidence for Expression in Human Saliva. Journal of Biological Chemistry 1-23

Das B, Cash MN, Hand AR, Shivazad A, Grieshaber SS, Robinson B, Culp DJ (2010) Tissue Distribution of Murine Muc19/Smgc Gene Products. Journal of Histochemistry & Cytochemistry. 58: 141-156

Dauer W, Przedborski S. (2003) Parkinson's disease: mechanisms and models. Neuron. 39:889–909.

Debay B, Martinez-Vicente M, Caldwell GA, Caldwell KA, Yue Z, Cookson MR, Klein C, Vila M, Bezard E (2013) Lysosomal Impairment in Parkinson's Disease. Mov Disord. 28: 725-732

De Lau LML & Breteler MMB (2006) Epidemiology of Parkinson's disease. Lancet Neurol. 5: 525-535

Deleidi M, Gasser T (2013) The role of inflammation in sporadic and familial Parkinson's disease. Cell. Mol. Life Sci. 70:4259-4273

Devos D, Lebouvier T, Lardeux B, Biraud M, Rouaud T, Pouclet H, Coron E, Bruley des Varannes S, Naveilhan P, Nguyen JM, Neunlist M, Derkinderen P (2013) Colonic Inflammation in Parkinson's disease. Neurobiology of Disease 50:42-48

Dickson DW, Braak H, Duda JE, Duyckaerts C, Gasser T, Halliday GM, Hardy J, Leverenz JB, Del Tredici K, Wszolek ZK, Litvan I. (2009) Neuropathological assessment of Parkinson's disease: refining the diagnostic criteria. Lancet Neurol. 8:1150–1157.

Duvoisin RC, Eldridge R, Williams A, Nutt J, Calne D (1981) Twin study of Parkinson disease. Neurology 31: 77–80

Dzamko N, Rowe DB, Halliday GM (2016) Increased Peripheral Inflammation in Asymptomatic Leucine-Rich Repeat Kinase 2 Mutation Carriers. Movement Disorders. 31: 889-897

Edwards LL, Pfeiffer RF, Quigley EMM, Hofman R, Balluff M (1991) Gastrointestinal Symptoms in Parkinson's Disease. Movement Disorders 6:151-156

Eichele DD, Kharbanda KK (2017) Dextran sodium sulfate colitis murine model: An indispensable tool for advancing our understanding of inflammatory bowel diseases pathogenesis. World J Gastroenterol. 23:6016-6029

Eldridge R, Ince SE (1984) The low concordance rate for Parkinson's disease in twins: A possible explanation. Neurology. 34: 1354-1356

Fall PA, Fredrikson M, Axelson O, Grane'rus AK (1999) Nutritional and Occupational Factors Influencing the Risk of Parkinson's Disease: A Case-Control Study in Southeastern Sweden. Movement Disorders. 14: 28-37

Fearnley J.M., Lees A.J. (1991) Ageing and Parkinson's disease: substantia nigra regional selectivity. Brain. 114: 2283–2301.

Fornace AJ Jr, Amundson SA, Do KT, Meltzer P, Trent J, Bittner M (2002) Stress-gene induction by low-dose gamma irradiation. Mil Med. 167: 13-5

Forno LS (1996) Neuropathology of Parkinson's Disease. Journal of Neuropathology and Experimental Neurology 55:259-272

GBD 2015 Neurological Disorders Collaborator Group (2017) Global, regional, and national burden of neurological disorders during 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet Neurol. 16: 877-897

Goy ER, Carter J, Needs L (2008) Needs and experiences of caregivers for family members dying with Parkinson Disease. J Palliat Care. 24: 69-75

Grewal JS, Ryan J (2019) Anatomy, Head and Neck, Submandibular Gland. StatPearls.

Guaitoli G, Raimondi F, Gilsbach BK, Gomez-Llorente Y, Deyaert E, Renzi F, Li X, Schaffner A, Jagtap PKA, Boldt K,…Gloeckner CJ (2016) Structural model of the dimeric Parkinson's protein LRRK2 reveals a compact architecture involving distant interdomain contacts. PNAS. 113: 4357-4366

Guo JF, He S, Kang JF, Xu Q, Hu YC, Zhang HN, Wang CY, Yan XX, Tang BS (2015) Involvement of Bcl-2-associated athanogene (BAG)-family proteins in the neuroprotection by rasagiline. Int J Clin Exp Med. 8: 18158-18164

Heasman SJ, Ridley AJ (2008) Mammalian Rho GTPases: new insights into their functions from *in vivo* studies. Nat. Rev. Mol. Cell Biol. 9: 690–701

Hill CS, Wynne J, Treisman R (1995) The Rho family GTPases RhoA, Rac1, and CDC42Hs regulate transcriptional activation by SRF. Cell. 81:1159–1170

Hirsch E.C., Graybiel A.M., Duyckaerts C., Javoy-Agid F. (1987) Neuronal loss in the pedunculopontine tegmental nucleus in Parkinson disease and in progressive supranuclear palsy. Proc. Natl. Acad. Sci. U.S.A. 84: 5976–5980.

Ho S, Woo J, Lee G (1989) Epidemiologic study of Parkinson's disease in Hong Kong. Neurology. 39:1314-1318

Kabbage M, Dickman MB (2008) The BAG proteins: a ubiquitous family of chaperone regulators. Cell Mol Life Sci. 65: 1390-1402

Kim R, Yoo D Jung YJ, Han K, Lee JY (2019) Sex differences in smoking, alcohol consumption, and risk of Parkinson's disease: A nationwide cohort study. Parkinsonism and Related Disorders 1-6

Kim WS, Kagedal K, Halliday GM (2014) Alpha-synuclein biology in Lewy body diseases. Alzheimers Res Ther. 5

Kitada T, Asakawa S, Hattori N, Matsumine H, Yamamura Y, Minoshima S, … Shimizu N (1998) Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. Nature. 392: 605–608

Klein C, Westenberger A (2012) Genetics of Parkinson's Disease. Cold Spring Harb. Perspect Med. 2

Koller W, Veter-Overfield B, Gray C (1990) Environmental risk factors in Parkinson's disease. Neurology. 40: 1218-1221

Kristjanson LJ, Anoun S, Oldham L (2005) Palliative care and support for people with neurodegenerative conditions and their carers. Int J Palliat Nurs. 12: 368-377

Kukurba KR, Montgomery SB (2016) RNA Sequencing and Analysis. Cold Spring Harb. Protoc. 11: 951-969

Lawrence T (2009) The Nuclear Factor NF-κB Pathway in Inflammation. Cold Spring Harb. Perspect. Biol. 1

Liebermann DA, Hoffman B (1998) MyD genes in negative growth control. Oncogene 17: 3319-3329

Li JQ, Tan L, Yu JT (2014) The role of the LRRK2 gene in Parkinsonism. Mol Neurodegener. 9: 1-17

Linseman DA, Loucks FA (2008) Diverse roles of Rho family GTPases in neuronal development, survival, and death. Front. Biosci. 13:657–676

Li X, Patel JC, Wang J, Avshalumov MV, Nicholson C, Buxbaum JD, Elder GA, Rice ME, Yue Z (2010) Enhanced Striatal Dopamine Transmission and Motor Performance with LRRK2 Overexpression in Mice Is Eliminated by Familial Parkinson's Disease Mutation G2019S. J Neurosci. 30: 11788-1797

Logroscino G, Marder K, Coˆte´ L, Tang MX, Shea S, Mayeux R (1996) Dietary lipids and antioxidants in Parkinson's disease: a population-based, case-control study. Ann Neurol. 39:89-94

Luzon-Toro B, Rubio de la Torre E, Delgado A, Perez-Tur J, Hilfiker S (2007) Mechanistic insight into the dominant mode of Parkinson's disease-associated G2019S LRRK2 mutation. Hum Mol Genet. 16:2031-2039

Ma S.Y., Roytt M., Collan Y., Rinne J.O. (1999) Unbiased morphometrical measurements show loss of pigmented nigral neurones with ageing. Neuropathol. Appl. Neurobiol. 25: 394–399.

Mai T, Schnepp W, Hobmann U (2010) The situation of people suffering from Parkinson's disease and their relatives in the mirror of literature – an overview. Pflege. 23: 81-98

Masters JM, Noyce JN, Warner TT, Giovannoni G, Proctor GB (2015) Elevated salivary protein in Parkinson's disease and salivary DJ-1 as a potential marker of disease severity. Parkinsonism & Related Disorders 21:1251-1255

Meder D, Herz DM, Rowe JB, Lehericy S, Siebner HR (2019) The role of dopamine in the brain – lessons learned from Parkinson's disease. NeuroImage. 190:79-93

Meurers BH, Dziewczapolski G, Shi T, Bittner A, Kamme F, Shults CW (2009) Dopamine Depletion Induces Distinct Compensatory Gene Expression Changes in DARPP-32 Signal Transduction Cascades of Striatonigral and Striatopallidal Neurons. J Neurosci. 29: 6828-6839

Monick MM, Powers LS, Barrett CW, Hinde S, Ashare A, Groskreutz DJ, Nyunoya T, Coleman M, Spitz DR, Hunninghake GW (2008) Constitutive ERK MAPK activity regulates macrophage ATP production and mitochondrial integrity. J. Immunol. 180:7485–7496.

Moore KW, de Waal Malefyt R, Coffman RL, O'Garra A (2001) Interleukin-10 and the interleukin-10 receptor. Annu Rev Immunol. 19: 683-765

Moskalev AA, Smit-McBride Z, Shaposhnikov MV, Plyusnina EN, Zhavoronkov A, Budovsky A, Tacutu R, Fraifeld VE (2012) Gadd45 proteins: Relevance to aging, longevity, and age-related pathologies. Ageing Res Rev. 11: 51-66

Moustafa AA, Chakrayarthy S, Phillips JR, Gupta A, Keri S, Polner R, Frank MJ, Jahanshahi M (2016) Motor symptoms in Parkinson's disease: A unified framework. Neuroscience & Biobehavioral Reviews. 68: 727-740

Nagalakshmi U, Wang Z, Waern K, Shou C, Raha D, Gerstein M, Snyder M (2008) The transcriptional landscape of the yeast genome defined by RNA sequencing. Science. 320: 1344-1349

Olguin HJ, Guzman DC, Garcia EH, Mejia GB (2015) The Role of Dopamine and Its Dysfunction as a Consequence of Oxidative Stress. Oxidative Medicine and Cellular Longevity. 2016

Oshlack A, Wakefield MJ (2009) Transcript length bias in RNA-seq data confounds systems biology. Biol Direct. 4

Paisán-Ruíz C, Jain S, Evans EW, Gilks WP, Simón J, Van der Brug M et al (2004) Cloning of the gene containing mutations that cause PARK8-linked Parkinson's disease. Neuron. 44: 595–600

Parkinson's Disease Society (2008) Life with Parkinson's today – room for improvement. The UK's largest every survey of people with Parkinson's and carers. Northern Ireland. London: Parkinson's Disease Society

Poirier AA, Aube B, Cote M, Morin N, Di Paolo T, Soulet D (2016) Gastrointestinal Dysfunctions in Parkinson's Disease: Symptoms and Treatments. Parkinson's Disease 2016

Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, … Nussbaum RL (1997) Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. Science. 276: 2045–2047

Postuma RB, Gagnon JF, Pelletier A, Montplaisir J. Prodromal autonomic symptoms and signs in Parkinson's disease and dementia with Lewy bodies. Movement Disorders 28:597-604

Randhawa PK, Singh K, Singh N Jaggi AS (2014) A review on chemical-induced inflammatory bowel disease models in rodents. Korean J Physiol Pharmacol. 18:279-288

Rideout HJ, Stefanis L (2014) The Neurobiology of LRRK2 and its Role in the pathogenesis of Parkinson's disease. Neurochem Res. 39: 576-592

Rudow G., O'Brien R., Savonenko A.V., Resnick S.M., Zonderman A.B., Pletnikova O., Marsh L., Dawson T.M., Crain B.J., West M.J., Troncoso J.C. (2008) Morphometry of the human substantia nigra in ageing and Parkinson's disease. Acta Neuropathol. 115: 461–470.

Schapira AHV, Chaudhuri KR, Jenner P (2017) Non-motor features of Parkinson's disease. Nat. Rev. Neurosci. 18:435-450

Sonne J, Beato MR (2018) Neuroanatomy, Substantia Nigra. StatPearls.

Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M (1997) Alpha-synuclein in Lewy bodies. Nature. 388:839-840

Srivanitchapoom P, Pandey S, Hallett M (2014) Drooling in Parkinson's Disease: a review. Parkinsonism Relat Disord 20:1109-1118

Stagi M, Klein ZA, Gould TJ, Bewersdorf J, Strittmatter SM (2014) Lysosome size, motility and stress response regulated by fronto-temporal dementia modifier TMEM106B. Mol Cell Neurosci. 61:226–240.

Stankiewicz TR, Linseman DA (2014) Rho family GTPases: key players in neuronal development, neuronal survival, and neurodegeneration. Front. Cell. Neurosci.

Subbannayya Y, Pinto SM, Bosl K, Prasad TSK, Kandasamy RK (2019). Dynamics of dual specificity phosphatases and their interplay with protein kinases in immune signaling. Int. J. Mol. Sci. 20: 1-23

Sudhof TC (2008) Neuroligins and neurexins link synaptic function to cognitive disease. Nature. 455: 903-911

Sulzer D, Surmeier DJ (2013) Neuronal vulnerability, pathogenesis, and Parkinson's disease. Mov Disord. 28:41–50.

Su X, Federoff HJ (2014) Immune Responses in Parkinson's Disease: Interplay between Central and Peripheral Immune Systems. BioMed Research International. 2014:1-9

Takagawa T, Kitani A, Fuss I, Levine B, Brant SR, Peter I, Tajima M, Nakamura S, Strober W (2018) An increase in LRRK2 suppresses autophagy and enhances Dectin-1-induced immunity in a mouse model of colitis. Sci Transl Med 10

Taoufik E, Kouroupi G, Zygogianni O, Matsas R (2018) Synaptic dysfunction in neurodegenerative and neurodevelopmental diseases: an overview of induced pluripotent stem-cell-based disease models. 8: 1-19

Wallings RL, Tansey MG (2019) LRRK2 regulation of immune-pathways and inflammatory disease. Biochemical Society Transactions. 47: 1581-1595

Wallings R, Manzoni C, Bandopadhyay R (2015) Cellular processes associated with LRRK2 function and dysfunction. FEBS Journal 282: 2806-2826

Wang Z, Gerstein M, Snyder M (2009) RNA-Seq: a revolutionary tool for transcriptomics. Nat Rev Genet. 10: 57-63

Whetten-Goldstein K, Cutson T, Zhu C, Schenkman M (2000) Financial burden of chronic neurological disorders to patients and their families: what providers need to know. Neurol Rep. 24: 140-144

Widnell K (2005) Pathophysiology of motor fluctuations in Parkinson's disease. Mov Disord 20:17-22

Wolozin B, Behl C (2000) Mechanisms of neurodegenerative disorders: Part 1: protein aggregates. Arch Neurol. 57:793-796

Wood-Kaczmar A, Gandhi S, Wood NW (2006) Understanding the molecular causes of Parkinson's disease. Trends Mol. Med. 12: 521-528

Yan X, Liu L, Li H, Huang L, Yin M, Pan C, Qin H, Jin Z (2016) Dual specificity phosphatase 5 is a novel prognostic indicator for patients with advanced colorectal cancer. American Journal of Cancer Research. 6: 2323-2333

Zarow C, Lyness SA, Mortimer JA, Chui HC (2003) Neuronal loss is greater in the locus Coeruleus than nucleus basalis and substantia Nigra in Alzheimer and Parkinson diseases. Arch Neurol. 60:337–41

Zimprich A, Biskup S, Leitner P, Lichtner P, Farrer M, Lincoln S, … Gasser T (2004). Mutations in LRRK2 cause autosomal-dominant parkinsonism with pleomorphic pathology. Neuron. 44: 601–607