The Consumption of Artificial Sweeteners Aspartame and Sucralose Possibly Induces Progression of Parkinson's Disease Through Mutations in SNCA and LRRK2 Genes

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Abstract

The present study correlates the onset and progression of Parkinson's Disease with the use of artificial sweeteners aspartame and sucralose through neuroinflammation, oxidative stress, and the loss of dopaminergic neurons. In this study, the existing literature was extensively reviewed to identify the genetic mutations causing this neurodegenerative disorder. The mutations which were significantly contributing to the induction of Parkinson's were found in *SNCA* and *LRRK2* genes. To further evaluate the functional aspects of the genetic mutations, the wild type and mutated protein sequences and structures were studied through computational analysis by using tools like multiple sequence alignment and homology modeling. The prolonged consumption of selected artificial sweeteners leads to the generation of free radicals and inflammatory responses in influential neurological regions in Parkinson's Disease, which leads to the loss of dopaminergic neurons. This study concludes that use of selected artificial sweeteners could interact with the pathways involving mutated genes accelerating the development of Parkinson's disease.

Introduction

Artificial sweeteners are at least 200 times sweeter than natural sugars, making them an appealing alternative (Program, 2025). These substitutes like aspartame and sucralose are found in a variety of foods like non-caloric sweeteners, chewing gum, and diet drinks. Aspartame, a methyl ester of dipeptide, breaks down into phenylalanine and aspartic acid, amino acids known to play a role in brain function. Sucralose, similar in structure to the natural sugar sucrose, has a different chemical structure with the hydroxyl group having been replaced with chlorine, leading to increased inflammation in the body. Sucralose and aspartame have been found to be genotoxic, meaning it can cause DNA damage and lead to mutations (Czarnecka, et al., 2021; Schiffman, et al., 2023). Artificial sweeteners can cause adverse effects on the body, especially in the central nervous system, through "cognitive impairments, headache,... and impairment of learning and memory" (Choudhary & Lee, 2018, p. 306).

Parkinson's Disease (PD) is a nervous system disorder that affects movement. Genes can play an influential role in the progression of PD. Recently, Trevisan and colleagues found that up to 15% of PD cases can be attributed to genes (Trevisan, et al., 2024). Dopamine is a neurotransmitter that affects emotions, behavior, and movement. The most well-known marker of PD is the neurodegeneration of dopaminergic neurons in the substantia nigra (SN) and ventral tegmental area (VTA), influential brain regions in PD. The impairment of dopaminergic neurotransmission causes PD. The changes in dopamine levels play a role in the loss of cognitive function and change in behavior that is a symptom of PD (Meder, et al., 2019). Moreover, oxidative stress is an imbalance of free radicals, molecules with unpaired electrons, and antioxidants. Oxidative damage in the brain regions involved in PD can lead to dopamine neurodegeneration. When dopamine oxidizes, it produces dopamine quinones which set off neuroinflammation (Dias, et al., 2013). Neuroinflammation, another cause of PD, can also be caused by cytokine release which regulates the impact of neurotransmitters and neural circuits.

Cytokines play a role in motor activity and cognitive dysfunction. Glial cells are chemical messengers in the brain's immune system. Thus, the chronic secretion of pro-inflammatory cytokines by glial cells can lead to PD (Shaw, et al., 2023).

This study plans to explore the effects of artificial sweeteners on gene functions and brain regions leading to the onset of PD. A deductive method is when general principles are analyzed to make specific conclusions. Using this method, the existing literature will be analyzed to make connections between artificial sweeteners and causes of PD. Furthermore, this study will find which genes and their mutations lead to the causes of PD that have been found. The genetic aspect of PD was chosen because mutations can be susceptible to environmental factors, like the consumption of sweeteners. With this information, protein structures will be retrieved from the Protein Data Bank as well as their amino acid sequences from the UniprotKb database to predict the 3D protein structures of the mutant genes. The mutations, a change in the DNA sequence which affects protein structure and function, of the structure will also be created through homology modeling. After mutations, the gene will be either upregulated or downregulated. Upregulation refers to a gene's increase of receptor sites, whereas downregulation has a decrease in receptor sites (Tanowitz & Von Zastrow, 2010). Gene expression alters depending on the number of receptor sites. Conducting a review and synthesizing current research to connect the effects of sweeteners on PD was done to understand current research that aims to find genes that are being affected by artificial sweeteners. The amino acid sequences of selected genetic mutations were used to find where the mutation occurs through multiple sequence alignment by a comparison of the wild type and mutant protein sequences. The primary sequence of these proteins will be used to create 3D protein structures of genes and their mutations. The aim of this study is to identify genes that come into contact with artificial sweeteners aspartame and sucralose which lead to oxidative stress, inflammation, or neurodegeneration of dopaminergic neurons, ultimately contributing to the onset of Parkinson's Disease.

Literature Review

Through extensive research, three main causes of Parkinson's Disease that are connected to the consumption of artificial sweeteners have been found. The loss of dopaminergic neurons, oxidative stress, and neuroinflammation have been connected to PD through genetic mutations. This study builds on existing literature by introducing a new aspect into the relationship of increasing and prolonged consumption of sweeteners correlated with PD.

Neuroinflammation

Neuroinflammation is defined as an inflammatory response in the central nervous system, disturbing homeostasis (DiSabato, et al., 2016). This response is typically involved in neurodegenerative diseases, including Parkinson's. Waseem Dar, a researcher at the Translational Neurobiology and Disease Modeling Laboratory in the Department of Life Sciences at the School of Natural Sciences in Shiv Nadar Institution of Eminence, studied aspartame inducing inflammation by microglia to find that aspartic acid causes oxidative stress in immune cells to cause an inflammatory response in the brain (2024). Similarly, Mohammed and colleagues, researchers in the Department of Nutrition and Food Sciences at the National Research Center in Egypt, identified that both aspartame and sucralose induced the increase of

cytokines which lead to neuroinflammation (2024). Prolonged consumption of these sweeteners generates a risk of the identified effects. The metabolites that aspartame and sucralose break down into have been proved to come into contact with the brain's immune system. Reish & Standaert concluded that innate and adaptive immune systems activated in PD, which has a role in neurodegeneration, are primarily triggered by *SNCA* (2016). Another gene *LRRK2* involved in PD is prevalent in inflammation and microglia activity and has been known to mediate neuroinflammation and other inflammatory processes (Lee & Cannon, 2015). G2019S, a mutation of this gene overexpresses *LRRK2* kinase and causes cytotoxicity, defining *LRRK2* as a neurotoxin (Lee & Cannon, 2015). Additionally, mutations in *PINK1, PRKN, and DJ-1* are also associated with neuroinflammation in PD (Liu, et al., 2022). Through Dar's research, aspartame has been linked as a cause of neuroinflammation by activating microglia (2024). This can happen possibly through aspartame interacting with *LRRK2*. *LRRK2* and *SNCA* are two possible genes that come into contact with aspartame and sucralose.

Oxidative Stress

Choudhary & Lee, researchers in the School of Medical Sciences at Universiti Sains Malaysia, conducted a review of the current literature, finding "negative impact[s] on neurobehavioral health" (2018, p. 313). The link between aspartame and increased free radicals along with elevated levels of metabolites aligns with this study's findings related to oxygen level imbalances (Choudhary & Lee, 2018). These metabolites have been found to be methanol and aspartate (Czarnecka, et al., 2021). Griebsch and colleagues, doctoral students in the Experimental Neurology program at Saarland University, have found 3 upregulated genes *PINK1, SOD1/2, and F1S1* that correlate with increased levels of oxidative stress caused by aspartame (2023). *PINK1* is another gene closely related to *SNCA* through its involvement in PD. The literature review by Dias and colleagues confirms that oxidative stress causes dopamine loss in the SN, establishing its connection to PD (2013). Furthermore, a study by Amin, an Assistant Professor of Medical Physiology at Cairo University in Egypt, validates that the artificial sweetener aspartame causes oxidative stress (2018). Kundu researched the effects of sucralose with oxidative stress, finding that sucralose in the body caused oxidative stress and led to a response of upregulated antioxidant genes (2020). Both sucralose and aspartame have been shown to cause oxidative stress which can lead to Parksinon's Disease.

Loss of Dopaminergic Neurons

The most prominent marker of PD is the loss of dopaminergic neurons in the SN, a brain region heavily linked to PD. Carmichael and colleagues, researchers in the Transgenic Section of the Laboratory of Neurogenetics at the National Institutes of Health, concluded that nigrostriatal dopaminergic neurons, neurons found in the SN, have selective regulation for critical dopamine genes, which may be involved in inflammation and oxidative stress (2021). Moreover, Mosharov and colleagues, researchers in the Department of Neurology at Columbia University Medical Center, expand on the death of dopamine neurons by finding that they undergo selective death from the "activity of the L-type Ca2+ channels that create high cytoplasmic Ca2+ levels, an upregulation of DAcyt synthesis by Ca2+, and the presence of α-synuclein [SNCA]" (2009, p. 223). This leads to a high calcium environment and the production of more dopaminergic neurons and ultimately, their death. Oxidative stress has been found to cause dopamine loss

through Dias' research (2013). This increase of free radicals can be attributed to aspartame's metabolites.

The Gap

The impact of artificial sweeteners, oxidative stress, α-synuclein, and inflammation has all been researched in connection to PD. However, these causes have not been directly correlated with each other in a singular study. Additionally, protein structures will be predicted through gene homology modeling using tertiary structures from the PDB database along with their amino acid sequence from the Uniprot database to depict mutant protein structures after direct or indirect contact with the aforementioned sweeteners. Artificial sweeteners aspartame and sucralose have negative effects in the body. By directly or indirectly coming into contact with genes, the artificial sweeteners cause genetic mutations, leading to the onset of PD. This study hypothesizes that the artificial sweeteners interact with specific genes to cause oxidative stress, neuroinflammation, or loss of dopaminergic neurons to ultimately lead to the onset of PD. The primary structure of the proteins will be evaluated before and after contact with artificial sweeteners to determine the change in the amino acid sequence that causes the mutation. This leads to the question: how do the artificial sweeteners aspartame and sucralose contribute to changes in the functional characteristics of genes involved in Parkinson's Disease?

Methodology

This study focuses on analyzing the genetic sequences of genes that have been found to induce a cause of artificial sweeteners. The three identified causes are oxidative stress, neuroinflammation, and loss of dopaminergic neurons. This study utilized a non-experimental design. Through a concept analysis, a negative correlation will be aimed to establish between artificial sweeteners and PD. This was done by analyzing current research on different aspects of this topic to conduct a deductive analysis. A study by Sharma and colleagues was utilized to understand the methods needed to obtain protein sequence data from databases and utilize applications to predict and view protein structures (2016). This study was only conducted after approval from a review board in the school district.

For this study, a concept analysis was done by analyzing current literature, and it was concluded that this was the most efficient way to collect information by comparing conclusions from previous research to be able to generate a new conclusion. This method allowed for an identification of relationships and patterns to make connections. Conducting a wet lab study would require materials and participants that were not accessible to me. Additionally, there was a time constraint and this research was unfeasible due to the lack of resources. The goal of this study was to understand the relationship between selected sweeteners and the onset of PD using gene and protein sequence databases to develop wild type and mutated protein structures. This study's method worked to answer the question: How do the artificial sweeteners aspartame and sucralose contribute to changes in the functional characteristics of genes in brain regions involved in Parkinson's Disease? This study hypothesized that the aforementioned sweeteners come into direct or indirect contact with PD affiliated genes, causing mutations that contribute to the onset of this progressive movement disorder.

Gene Screening

To understand the relationship between sweeteners and identified genes, further research was conducted into each sweetener and gene individually. With the help of literature and Genecards (https://www.genecards.org/), 50 genes were primarily screened (score > 50) which play a role in PD. These genes are enlisted in Table 1 in the results section. Genecards is a human gene database providing all information on annotated and predicted human genes. This database was utilized because it was the easiest and most efficient to access with the resources available to me. The threshold was set to a score of 50, and the five genes which scored greater than 50 were selected. By reviewing literature about the selected genes enlisted in Table 2 in the results section, I looked for the gene interactions with the selected sweeteners (aspartame and sucralose). Further narrowing down the list, two genes were selected, *SNCA* and *LRRK2*.

Retrieval of Target Protein Sequence

The target protein sequences of selected genes were retrieved from the Uniprotkb database (https://www.uniprot.org/). Uniprot is a database offering protein sequence and annotation data. This is the database listed in Sharma and colleagues' paper, making it easy for me to learn how to retrieve the protein sequences (2016). These sequences specific to homo sapiens were downloaded in FASTA format. This text-based file represents the amino acid sequence for each of the selected genes. The wild type and mutated protein sequences of *SNCA* and *LRRK2* were downloaded from UniprotKb. All of the mutant sequences were not found in the UniprotKb database, and had to be manually altered.

Table 1

Wt/Mutant Genes and UniprotKb ID

Gene	Wild Type/Mutant	UniprotKb ID
SNCA	Wild Type	P37840
LRRK2	Wild Type	Q5S007

Multiple Sequence Alignment

The multiple sequence alignment was done in order to compare the protein sequences of wild type and the mutated genes. This was done using the Align tool of UniProt (https://www.uniprot.org/align). Multiple sequence alignment is the process of three or more amino acid sequences to evaluate their relationships and indicate similarities in sequence patterns. The mismatched part of the protein sequences was highlighted to identify the mutations causing PD and its correlation with the selected sweeteners.

Retrieval of Protein Structure Files

The tertiary structure of proteins (wild type and mutants) were retrieved from the Protein Data Bank (https://www.rcsb.org/). PDB is a global database storing all of the 3D protein structures determined by X-Ray Diffraction, Electron Microscopy, Solid-State and Solution Nuclear

Magnetic Resonance Spectroscopy, and Electron Paramagnetic Resonance. Sharma and colleagues had also used this database in their methods (2016). The PDB files retrieved for wild type are enlisted in Table 2.

Table 2
Wt/Mutant Proteins and PDB ID

Protein	Wild Type/Mutant	PDB ID
SNCA	Wild Type	8CE7
LRRK2	Wild Type	9C61

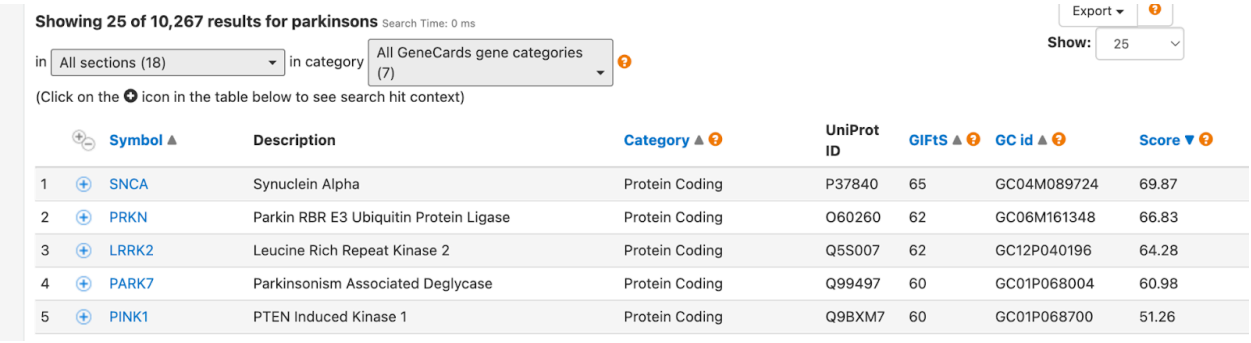
The protein structures were viewed using BIOVIA Discovery Studio Visualizer 2024. This application was recommended by my research mentor. It is a molecular modeling application which enables users to view, share and analyze protein structures.

Tertiary Structure Prediction of Proteins
To predict the tertiary structure of protein sequences, the FASTA files of selected Wt and mutant genes retrieved from UniProtKb and were submitted to Swiss-Model Server (https://swissmodel.expasy.org/interactive#structure). The homology modeling approach was used by Swiss-Model Server to determine the 3D protein structures because it is the easiest webtool for me to use to create the PDB file. The homology modelling approach uses the template to predict the structure of submitted FASTA files. It finds the best possible match against the input sequences. The predicted structures were viewed in Discovery studio and the mutations were also topologically highlighted.

Results & Discussion
Gene Screening
The Genecards database was utilized to conduct an initial search of influential genes involved in PD. This database revealed that 10,267 genes were correlated with Parkinson's Disease. Genecards gave each gene a score based on a search platform Elasticsearch 7.11. Figure 1 displays the genes as a result of the initial screenings from the Genecards database. *SNCA* was first on the list with a score of 69.87, and *LRRK2* was third on the list with a score of 64.28.

Figure 1

Initial Results of Gene Screening of Influential Genes in Parkinson's Disease from the Genecards Database



Showing 25 of 10,267 results for parkinsons Search Time: 0 ms

in All sections (18) in category All GeneCards gene categories (7)

(Click on the ⊕ icon in the table below to see search hit context)

Export Show: 25

	Symbol	Description	Category	UniProt ID	GIFtS	GC id	Score
1	SNCA	Synuclein Alpha	Protein Coding	P37840	65	GC04M089724	69.87
2	PRKN	Parkin RBR E3 Ubiquitin Protein Ligase	Protein Coding	O60260	62	GC06M161348	66.83
3	LRRK2	Leucine Rich Repeat Kinase 2	Protein Coding	Q5S007	62	GC12P040196	64.28
4	PARK7	Parkinsonism Associated Deglycase	Protein Coding	Q99497	60	GC01P068004	60.98
5	PINK1	PTEN Induced Kinase 1	Protein Coding	Q9BXM7	60	GC01P068700	51.26

Note. The results of the initial gene screening for influential genes in PD from the Genecards database. The selected genes all had scores above 50.

Sequence Alignment

Wild type sequences of *SNCA* and *LRRK2* were selected from a list of influential genes (Figure 1) involved in PD from Genecards and retrieved from the UniprotKb database. In order to locate the mutated amino acid, the protein sequences of *SNCA* and *LRRK2* were compared to its wild type. The wild type and mutated protein sequences were aligned using the multiple sequence alignment tool in UniprotKb. The alignment results for *SNCA* and *LRRK2* are depicted in Figure 2. Three mutant proteins of *SNCA* and one of *LRRK2* were found, each mutation contributing to one of the three causes of PD: neuroinflammation, oxidative stress, or loss of dopaminergic neurons. The E46K mutation causes oxidative stress (Greenbaum, et al., 2005); The A30P mutation causes the loss of dopaminergic neurons (Wise-Scira, et al., 2013). The A53T mutation causes neuroinflammation and the loss of dopaminergic neurons (Podoly, et al., 2010); The G2019S mutation of *LRRK2* causes neuroinflammation (Ren, et al., 2019). Thus, E46K, A53T, A30P, and G2019S were chosen for this study. All of the protein sequences for both wild type and mutant are listed in Table 3.

Table 3

Wild Type and Mutant Protein Sequences

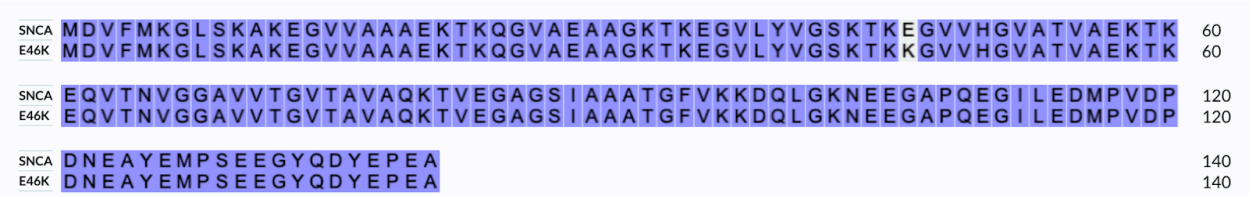
Gene	Protein Sequence
SNCA	MDVFMKGLSKAKEGVVAAAEKTKQGVAEAAGKTKEGVLYVGSKTKEGVVHGVATVAEKTKEQVTNVGGAVVTG VTAVAQKTVEGAGSIAAATGFVKKDQLGKNEEGAPQEGILEDMPVDPDNEAYEMPSEEGYQDYEPEA
A53T	MDVFMKGLSKAKEGVVAAAEKTKQGVAEAAGKTKEGVLYVGSKTKEGVVHGVTTVAEKTKEQVTNVGGAVVTG VTAVAQKTVEGAGSIAAATGFVKKDQLGKNEEGAPQEGILEDMPVDPDNEAYEMPSEEGYQDYEPEA

E46K	MDVFMKGLSKAKEGVVAAAEKTKQGVAEAAGKTKEGVLYVGSKTKKGVVHGVATVAEKTKEQVTNVGGAVVTG VTAVAQKTVEGAGSIAAATGFVKKDQLGKNEEGAPQEGILEDMPVDPDNEAYEMPSEEGYQDYEPEA
A30P	MDVFMKGLSKAKEGVVAAAEKTKQGVAEAPGKTKEGVLYVGSKTKEGVVHGVATVAEKTKEQVTNVGGAVVTG VTAVAQKTVEGAGSIAAATGFVKKDQLGKNEEGAPQEGILEDMPVDPDNEAYEMPSEEGYQDYEPEA
LRRK2	EEVAVKIFNKHTSLRLLRQELVVLCHLHHPSLISLLAAGIRPRMLVMELASKGSLDRLLQQDKASLTRTLQHRIALH VADGLRYLHSAMIIYRDLKPHNVLLFTLYPNAAIIAKIADYGIAQYCCRMGIKTSEGTPGFRA
G2019 S	EEVAVKIFNKHTSLRLLRQELVVLCHLHHPSLISLLAAGIRPRMLVMELASKGSLDRLLQQDKASLTRTLQHRIALH VADGLRYLHSAMIIYRDLKPHNVLLFTLYPNAAIIAKIADYSIAQYCCRMGIKTSEGTPGFRA

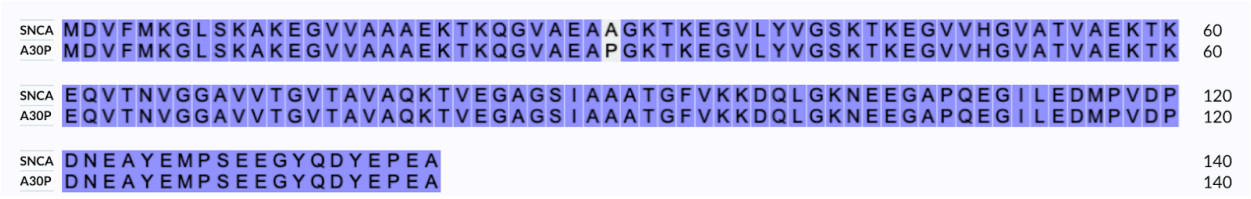
The alignment of the wild type and mutant protein sequences for the *SNCA* and *LRRK2* genes revealed that each mutant sequence differed from its wild type by one amino acid, marking them as single point pathogenic mutations. In Figure 2, the alignment of wild type (*SNCA* and *LRRK2*) and mutated sequences (E46K, A30P, A53T, and G2019S) revealed the substitution of E46K from glutamic acid to lysine at the 46th position, A30P from alanine to proline at the 30th position, A53T from alanine to threonine at the 53rd position, and G2019S from glycine to serine at the 2019th position.

Figure 2

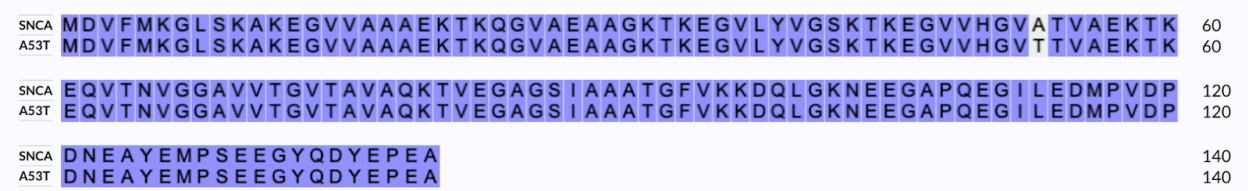
Results of Multiple Sequence Alignment of SNCA and LRRK2 Wild Type and Mutant Protein Sequences



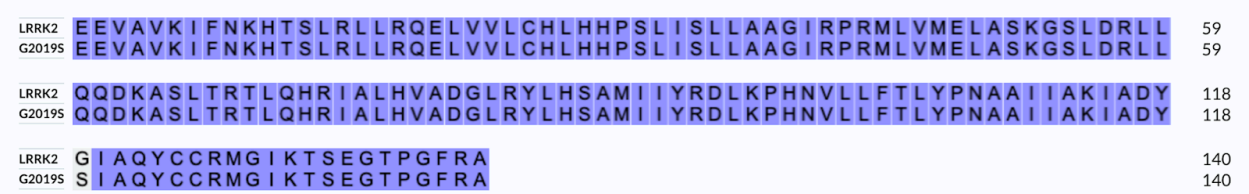
A. SNCA and E46K Sequence Alignment



B. SNCA and A30P Sequence Alignment



C. SNCA and A53T Sequence Alignment



D. LRRK2 and G2019S Sequence Alignment

Note. Multiple Sequence Alignment of *SNCA* and *LRRK2* wild type and mutant protein sequences (A) *SNCA* and E46K alignment had an amino acid change from glutamic acid to lysine at the 46th position (B) *SNCA* AND A30P alignment had an amino acid change from alanine to proline at the 30th position (C) *SNCA* and A53T had an amino acid change from alanine to threonine at the 53rd position (D) *LRRK2* and G2019S had an amino acid change from glycine to serine at the 2019th position.

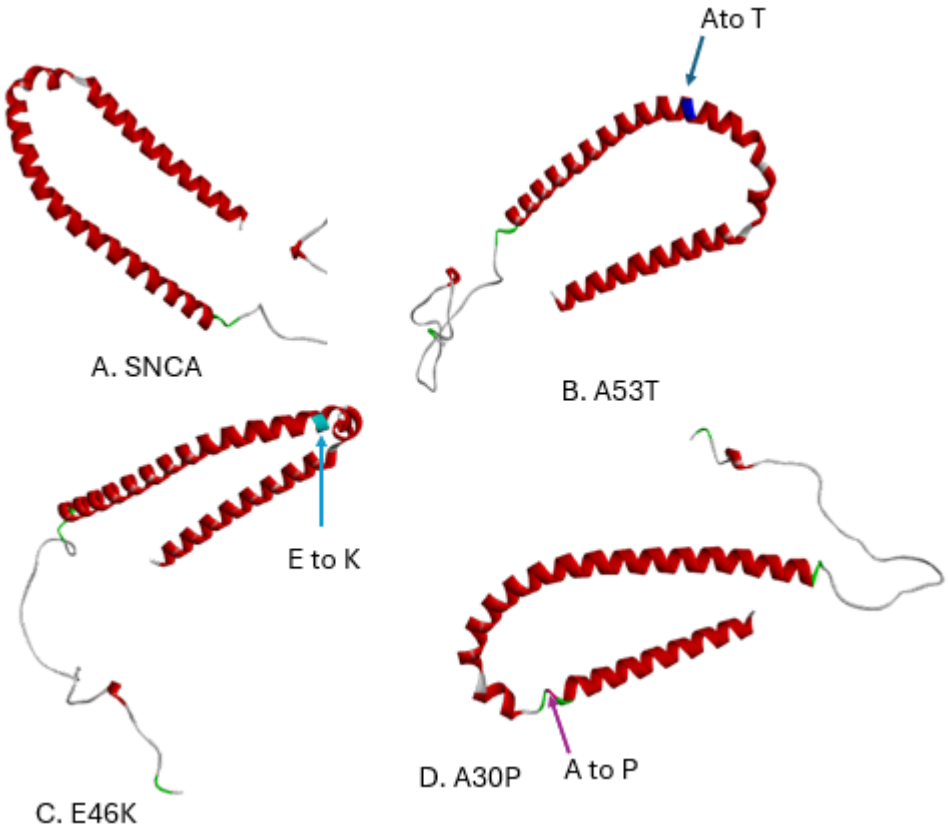
SNCA has 140 amino acids and all can be seen in the figure above. All three of the mutations occurred in the N-terminal domain, a common location for missense mutations. The role of the N-terminal domain is to interact with phospholipid membranes and micelles. Mutations in this region, especially from position 32-58, affect the protein's stability and tendency to aggregate (Siddiqui, et al., 2016). *LRRK2* has 2,527 amino acids. To make it easier to view and perform the multiple sequence alignment, the *LRRK2* sequence was compared using 120 amino acids, starting from glutamic acid at the 1,920th position and ending at alanine at the 2,040th position. The G2019S mutation occurred during this shortened sequence. The alignments displayed the missense mutations which are caused by a substitution in the DNA nucleotide sequence, leading to an alteration in the protein sequence.

Tertiary Structure Prediction

In order to determine the tertiary structures of wild type and mutated proteins the homology modeling approach of protein structure determination was used. The structures were predicted using Swiss-Model Server to produce PDB files which were viewed in BIOVIA Discovery Studio Visualizer. In Figure 3, the *SNCA* wild type and mutant structures are displayed. Section A depicts the *SNCA* wild type structure; Section B depicts the A53T mutation as dark blue with an amino acid mutation at the 53rd position of alanine to threonine; Section C depicts the E46K mutation as light blue with an amino acid change at the 46th position of glutamic acid to lysine; Section D depicts the A30P mutation as pink with an amino acid change at the 30th position of alanine to proline. All predicted mutant structures still maintain the alpha-helix structure of the wild type.

Figure 3

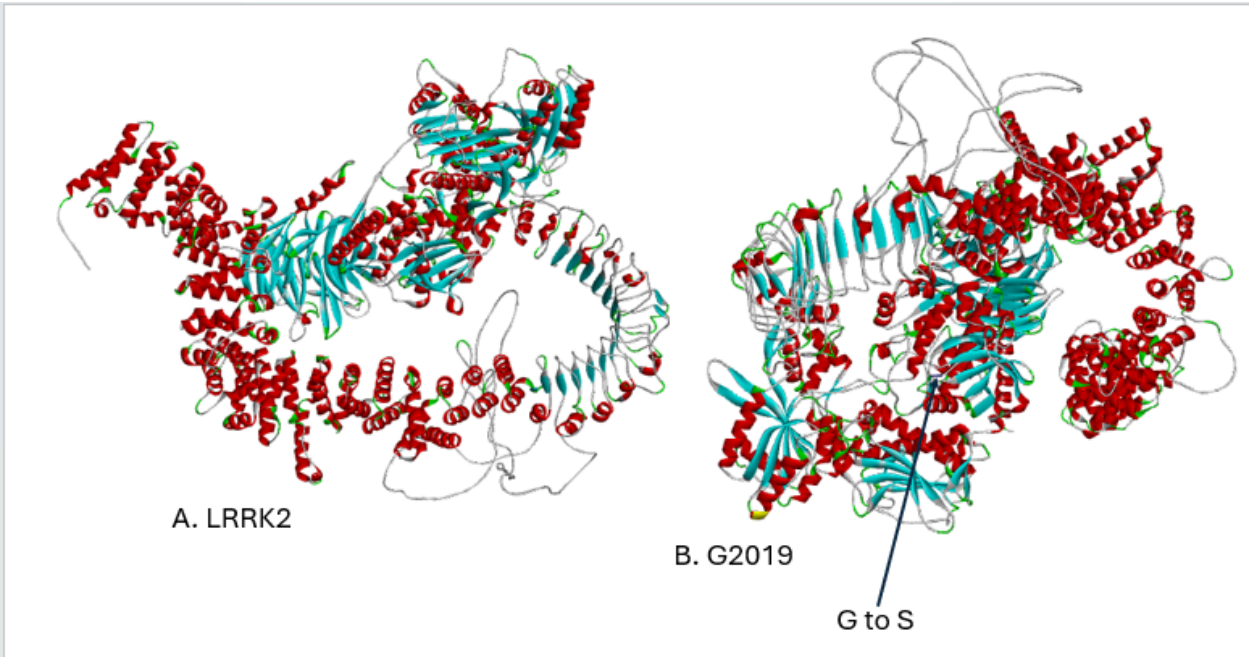
SNCA Wild Type and Mutant Tertiary Structure Prediction



Note. SNCA Wild Type and Mutant Protein Structures (A) *SNCA* wild type structure (B) A53T mutation with amino acid substitution of alanine to threonine at the 53rd position (C) E46K mutation with amino acid substitution of glutamic acid to lysine (D) A30P mutation with amino acid substitution of alanine to proline

In Figure 4, the *LRRK2* wild type and mutant structures are displayed. *LRRK2* is a protein kinase with multiple, independent domains. Section A depicts the *LRRK2* wild type structure, and Section B depicts its G2019S mutation in dark blue with an amino acid change at the 2019th position from glycine to serine.

Figure 4
LRRK2 Wild Type and Mutant Tertiary Structure Prediction



Note. LRRK2 wild type and mutant predicted tertiary structures (A) *LRRK2* wild type structure (B) G2019S mutant structure with an amino acid change at the 53rd position from glycine to serine

Discussion

Parkinson's Disease is the second most common neurodegenerative disorder. Parkinson's has both motor and non-motor symptoms. This movement disorder is characterized by symptoms like rigidity, rest tremor, and bradykinesia. Some of the most influential genes include both *SNCA* and *LRRK2*. *SNCA* is found in both early and late onset familial PD while *LRRK2* is typically found in late onset. Aspartame and sucralose are two of the most common artificial sweeteners, synthetically designed sugars. The effects of prolonged consumption of artificial sweeteners in neurodegeneration is an emerging topic of study. The pathological hallmark of PD is the aggregation of α-synuclein misfolded proteins. This impairs the normal function of *SNCA* like vesicle transport and lysosome activity, ultimately impairing cellular function. The predicted tertiary structures highlight the exact location of the mutation, leading to overexpression of the gene. According to Jahabardeen, et al., *SNCA* aggregation leads to neuronal dysfunction and neurodegeneration (2024). All of the mutations for *SNCA* identified in this study cause *SNCA* aggregation due to overexpression of the gene after mutation. The E46K mutation leads to oxidative damage that causes PD through *SNCA* aggregation and loss of DN (Reiszadeh Jahromi, et al., 2021). The A53T mutation produces damaged mitochondria through overexpression of the mutant gene (Mullin & Schapira, 2013). Moon & Paek have directly correlated mitochondrial dysfunction to the onset of PD which can occur through oxidative stress to cause a loss of dopaminergic neurons (2015). Lo Bianco and colleagues have confirmed reduced levels of dopaminergic neurons in the SN due to the presence of the A30P mutation (2002). These mutations and its effects contribute to significant causes of *SNCA* aggregation

and of PD. Similarly, prolonged consumption of aspartame and sucralose leads to the same aforementioned effects as the three *SNCA* mutations. Therefore, both pathways of aspartame and sucralose may come into direct or indirect contact with all of the identified mutations of *SNCA* to result in a mutation and overexpression of the gene. *LRRK2* codes for a protein kinase that phosphorylates proteins that engage in a wide variety of cellular functions. The G2019S is a major mutation of *LRRK2*, and its most well known effect is neuroinflammation. Lu and colleagues noticed increased microglial activity in the SN in rats with the G2019S mutation (2022). Neuroinflammation is a prominent hallmark of PD, causing the loss of dopaminergic neurons. Similar to the effects of the G2019S mutation, aspartame induces inflammation by microglia, and both aspartame and sucralose induce cytokine increase as a response to the increased levels of neuroinflammation after consumption (Dar, 2024; Mohammed, et al., 2024). All four of the mutations contribute to at least one major identifier and cause of PD. If both artificial sweeteners and genetic mutations result in the same effect, then artificial sweeteners may also have an effect on the genes, causing a mutation to occur. As the identified sweeteners and mutations lead to the same causes, there may be a relationship between them as increased levels of consumption are correlated with the overexpression of the mutated genes as they both cause one major pathological cause of PD. This study highlighted the genes and mutations to focus on in the relationship between prolonged consumption of artificial sweeteners and the progression of artificial sweeteners.

Limitations & Future Research

A major limitation of this study is the inability to establish proof of direct contact. Only a predicted conclusion was made. Limited access to resources to conduct an in-vitro study prevented a conclusion with experimental evidence from being established. The correlation that this study concluded utilized other studies as a foundation for the relationship. The multiple sequence alignment and homology modeling were only able to confirm the location of the mutation. This study sets the stage for future studies with the conclusion of two influential genes in this relationship. Future research can establish this proof through an experiment on a cellular level in a lab on a cell line like SH-SY5Y like Griebsch and colleagues utilized to examine a direct or indirect point of contact between the pathways of sucralose and aspartame with the occurrence of *SNCA* and *LRRK2* mutations (2023). Furthermore, direct relationships can also be established using mouse models or docking done through a program. Additionally, the String database can be used to view the pathways and relationships the artificial sweeteners aspartame and sucralose take in the body after consumption to identify the contact that sweeteners make with genes to identify if the relationship is direct or indirect.

Conclusion

The results of this study concluded that artificial sweeteners sucralose and aspartame may come into contact with and cause the *SNCA* and *LRRK2* mutations. The significance of this study was the conclusion of the influence that *SNCA* and *LRRK2* have in the onset of PD through the consumption of aspartame and sucralose. The four mutations identified in this study were E46K, A30P, A53T, and G2019S. Each of these mutations were a single point pathogenic mutation, so the wild type structure was maintained after a mutation occurred. The mutations contribute to at least one of the identified causes of PD: neuroinflammation, oxidative stress, or the loss of DN. Neuroinflammation and oxidative stress contribute to the loss of DN. *SNCA*

aggregation is a major pathological hallmark of PD. All three of the causes contribute to *SNCA* aggregation, contributing to the onset of PD. The three identified mutations of *SNCA* in this study were E46K, A53T, and A30P. G2019S, the one mutation of *LRRK2* identified in this study, is a major mutation that directly contributes to neuroinflammation. Aspartame and sucralose have also been implicated in one of the three aforementioned causes of PD. Through this study, PD and its causes have been correlated to the consumption of artificial sweeteners. This answers the gap by bridging this relationship by highlighting the influential causes and genetic mutations of PD. Therefore, this study assumes that the pathways of identified sweeteners are predicted to have direct or indirect contact with the genes to cause a mutation, thereby contributing to the onset of PD. Ultimately, the consumption of artificial sweeteners aspartame and sucralose may increase the risk of the onset of PD by causing genetic mutations of *SNCA* and *LRRK2*.

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