Assessing the Relationship Between Neuroimaging Analysis of the Substantia Nigra and Dopaminergic System with CSF & Serum Biomarkers in Parkinson's Disease

A thesis submitted in partial fulfilment of the

HONOURS DEGREE of BACHELOR OF

HEALTH AND MEDICAL SCIENCES In

The Discipline of Anatomy

Adelaide Medical School

The University of Adelaide

By Angus McNamara

November 01

ABSTRACT

A current need in Parkinson's disease (PD) is advancing understanding on predicting risk and rate of PD development. A proposed method in doing is through assessing a combination of biomarkers and neuroimaging techniques to evaluate key pathologphysiologies within PD. However, neuroimaging is currently underutilised and underdeveloped in clinical PD and verified biomarkers are yet to be confirmed. This study aims to assess whether differences exist between PD and healthy controls for the structural integrity of the SN measured by MRI, along with measures of CSF alpha synuclein, CSF tau and serum IGF-1. In addition, whether biomarker levels are predictive of SN volume loss will be investigated. SN integrity was determined by comparing SN signal loss detected via manual masking of Axial T2-weighted MRI scans to two control regions: red nucleus (RN) and midbrain. Significantly higher volume loss was found in patients compared to controls in both the SN:RN ($t(69.61) = -5.49$, $P = < 0.01$) and SN:midbrain ($t(57.55) = -6.047$; $P = < 0.001$). Contrastingly however, baseline differences in biomarker data within subjects who underwent MRI analysis yielded no difference between groups ($P > 0.05$). This was reflected in linear regression models, highlighting variation accounted for by biomarkers and covariates was comparable to models of biomarkers in isolation and in conjunction. This suggests that little additive effect exists between biomarkers to improve the predictive power of the model. Overall, the results are insufficient to draw conclusions on the predictive relationship between tested biomarkers and SN integrity.

INTRODUCTION

Parkinson's Disease (PD) is a hypokinetic disorder and is the second most common neurodegenerative disease in the world [1-2]. Currently 80,000 people live with PD in Australia alone, approximately 1/350 of the population[2]. However, this rate is expected to increase as prevalence has steadily risen overtime due to an aging population, making PD a global concern[1].

The characteristic hypokinetic symptoms of PD are bradykinesia and at least one other deficit[3-4]. The presence of these cardinal motor disorders is the current standard for diagnosing patients with PD[8-9]. However, once motor symptoms present, patients have already lost 60-80% of their DA neurons[1,3-4]. This emphasises a need to develop methods of predicting PD, with one proposed method being targeting known pathologies of disease progression and development.

PD pathology greatly alters the circuitry of the basal ganglia, sub-cortical nuclei highly inter-connected with the cortex which is attributed to a variety of functions[5-8]. Within the context of PD, the most prominent function disrupted is the initiation of voluntary movement[5-6]. This is attributed to wholesale loss of dopaminergic (DA) neurons, predominantly in the Substantia Nigra pars compacta (SN), as shown in figure 1:[5]

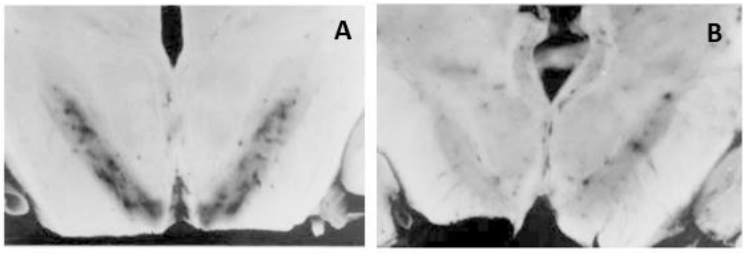


Figure 1*: Ex-vivo midbrain slices comparing prominent SN in healthy (A) against depigmented SN in PD (B)*[3]

Under normal conditions, the SN produces sufficient dopamine to modulate pathways vital for regulating voluntary movement[7-9]. These include a monosynaptic direct pathway (D1) responsible for promoting movement and a polysynaptic indirect pathway (D2) which inhibits it[7-8]. However, PD, dopaminergic input is heavily reduced, resulting in increased activation of the indirect pathway, thereby inhibiting voluntary movement[7].

However, in PD, DA input is heavily reduced thereby resulting in decreased activation of the direct pathway[7-8]. Simultaneously, the inhibitory function dopamine has on the indirect pathway is reduced, resulting in increased activation, with the imbalance in pathways resulting in voluntary movement, emphasised in figure 2:[7-9]

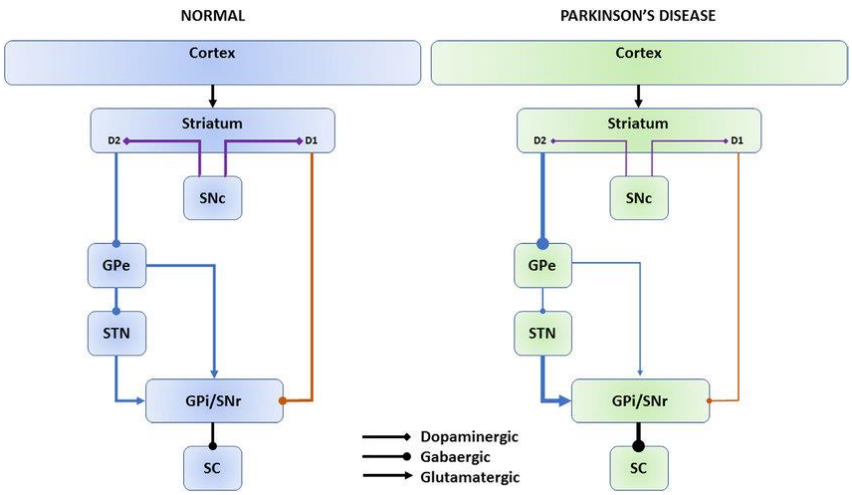


Figure 2*: Changes to the contribution of the Direct Pathway (D1) and Indirect Pathway (D2) in Normal vs Parkinson's disease*[5].

Currently it is unknown what initiates the cascade of events that leads to eventual DA cell death and disease development[10]. However, many biomarkers have been identified as noticeably different in Parkinsonian brains[10]. Understanding the relationship between biomarkers and PD is a major focus in PD research, as their verification will allow for advances in the developing predictive methods.

One prominent biomarker is the protein alpha synuclein (α-syn), acting as the pathological hallmark for PD[1,3,11-12]. While the normal function of α-syn is not well understood, it is highly expressed in synapses, hypothesised to interact with vesicles to provide subtle breaks in neurotransmitter release[13]. For a yet unknown reason, α-syn misfolds in PD, leading to aggregations known as Lewy bodies[1, 11-13]. These induce a neurotoxic environment through many proposed mechanisms such as synaptic deficits and increased oxidative stress, which can perpetuate DA death[11-13].

Additionally, tau proteins, involved in microtubule stabilisation within neurons, have been attributed to PD development[-14]. Hyperphosphorylation of tau leads to abnormal deposition and aggregations in neuronal cell bodies, leading to the development of insoluble neurofibrillary tangles (NFTs)[14-16]. While PD is not considered a typical tauopathy, evidence suggests that NFTs and the interaction between tau and α-syn may contribute PD development[14 -16]. It is hypothesised that accumulation of α-syn in synapses has a positive relationship with tau recruitment which induces damage and reduces axonal transport functioning.[16] This initiates a cycle of increased accumulation of said proteins which would eventually result in DA death.[16]

Finally, interleukin growth factor (IGF-1) is a hormone with endocrine, paracrine and autocrine capabilities and is associated with several functions within the brain[10,17-18]. While little is known about the mechanisms that regulate IGF-1 expression in-vivo, it has many neurophysiological functions that may implicate in PD pathophysiology[10,17-18]. For example, one of the primary risk factors for developing PD is age and evidence shows IGF-1, having known neuroprotective mechanisms, decreases with age[10]. Additionally, males are at higher risk of PD as oestrogen provides women protection via IGF-1[10].

Effectively verifying the use of biomarkers requires understanding their relationship with the hallmarks of PD, such as structural integrity of the SN. Currently, the go to standard for evaluating differences in SN integrity is via assessing ex-vivo sections of the midbrain[7]. Therefore, a need exists to utilise in-vivo imaging to evaluate the SN and dopaminergic system. However, current imaging techniques are inadequate and underdeveloped for PD diagnosis.[19]

DaTscans are a form of single photon emission computerized tomography (SPECT) imaging technique that assesses dopamine transporters[20-21]. The radiopharmaceutical ioflupane is injected into the bloodstream where it binds to dopamine transporters within the striatum, whereby SPECT allows visualisation of the amount of transporter present[20-21]. Due to reduced dopamine, lower signal is reported in PD, with abnormal DaTscans and final diagnosis of PD being highly correlated[20-21]. However, it suffers from significant limitations being that it is not entirely sensitive to early PD[20] and is unable to distinguish between other parkinsonian disorders[21]. In addition, from a patient perspective, it is both invasive and very expensive, reducing its benefits in a clinical setting even further[22].

Conversely, Magnetic Resonance Imaging (MRI) allows for structural imaging and therefore will be essential for non-invasive diagnosis of PD[23-24]. MRI in previous research has confirmed increased atrophy of key structures such as the SN[23]. However, it suffers from many drawbacks which reduce its use in clinical PD settings[23-24]. The primary issue is current MRI has difficulty assessing deep brain and subcortical structures[23]. This is associated with low contrast and low differences in spatial resolution between structures, which makes them difficult to distinguish and analyse[23].

Mitigating the drawbacks of current neuroimaging is essential to verifying biomarkers as measures of predicting PD. This is emphasised in prior literature focusing on refining methods of imaging the structures effected throughout PD[23-25]. One primary technique involves

measuring volume loss in the SN using T2-weighted MRI which due to DA death, is higher in PD patients compared to healthy individuals. Proper evaluation of the changes that occur in PD via neuroimaging would allow for its relationship to be evaluated with key biomarkers of PD, hypothesised to be predictive of SN volume loss and dopaminergic system integrity.

Therefore, this study aims to assess whether differences exist between PD and controls for the structural integrity of the SN and dopaminergic system, along with measures of CSF biomarkers alpha synuclein, tau and serum IGF-1. In addition, the relationship between biomarker levels and SN volume loss in PD will be investigated. Overall, this will contribute to improved patient outcomes by advancing the development of a novel technique to predict risk and rate of PD development.

MATERIAL & METHODS

Materials & Cohorts

All information was obtained from the Parkinson's Progression Markers Initiative (PPMI) international database. It is an observational clinical study with the overall goal of verifying biomarkers of PD development and progression and introducing them to therapeutic studies[26]. To properly evaluate said biomarkers, PPMI utilises a comprehensive set of advanced clinical, imaging, and biological sample data and is therefore highly appropriate for the methodology of this project.

The PPMI database has approximately 2000 subjects split into multiple cohorts[26]. In the context of this study, the two following cohorts were extracted: Early PD and healthy controls, with approximately 400 and 200 subjects, respectively. Control subjects were defined as people without PD, 30 years old or older, who do not have a first-degree blood relative with PD[26]. Excluded subjects were patients who did not yield expected corresponding DaTscan data, referred to as "scans without evidence of a dopaminergic deficit" (SWEDD)[26]. Key pieces of data extracted for this study include: Demographic data, MRI, DaTscans and biological sample data.

MRI Masking Protocol

Axial T2-Weighted MRI scans were used to assess the structural integrity of the substantia nigra. These scans were obtained via a Siemens 3T TIM trio scanner model, with a 12 channel

matrix head coil and an acquisition sequence of 5 minutes and 8 seconds, ensuring that phase encoding direction is L/R[26]. Selected scans were subjected to blinded manual masking (n = 32 Control; 64 Patient) by two independent researchers. An imaging programme called FSLeyes was used to create blank overlays of the original image. This allowed for masks of the three regions of interest to be created: the SN, red nucleus (RN) and midbrain, highlighted in figure 4:

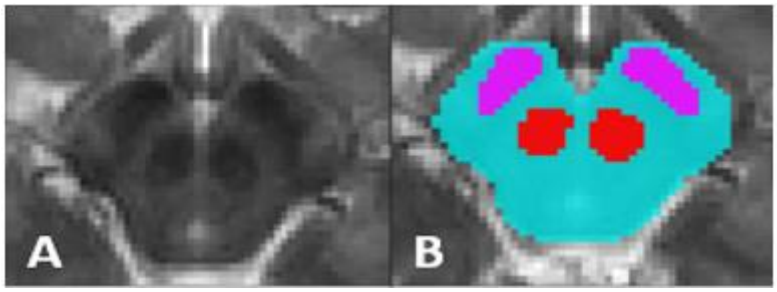


Figure 4*: (A) Cropped Midbrain in Axial T2-Weighted MRI Image which has been manually masked (B) to distinguish the midbrain (blue), RN (red) and SN (pink)*

To ensure adequate masks were created, two variables were taken into consideration: slice number and contrast. A suitable slice number was chosen per image that allowed for all three structures' shapes to be easily distinguished. Slice discrepancies were recorded, in which case the researchers would deliberate what slice number was most appropriate and repeat the masking process.

Properly distinguishing regions, particularly the RN and SN, was done via only including voxels of considerably darker colouration. This is indicative of signal loss, with lighter voxels acting as a border between the two regions. Therefore, an optimal balance between brightness and contrast was essential to properly distinguish borders. This is particularly important in the context of sub-cortical imaging, as two primary issues in segmenting PD structures is low contrast and poor spatial resolution between structures[20].

Region Comparison

The SN is the primary region of interest, as it contains A9 DA neurons, the main neuronal type affected during PD[23-25]. To properly evaluate the expected volume loss in PD, the RN and midbrain acted as control regions, as they are not expected to degenerate in PD. Of interest is that the RN possesses A10 DA neurons, which, despite being practically adjacent to the SN, is spared during PD for a yet unknown reason[27-29]. Region values were average on account of inter-reliability between scorers and converted into SN:midbrain (r = 0.78) and SN:RN (r = 0.44). Ratio calculation allowed for proper evaluation of SN volume loss by accounting for individual differences in size in regions between subjects.

DaTscan

DaTscan data (n = 193 Control, 419 Patient) was utilised as a measure of dopaminergic system integrity. The data used was on the binding potential of dopamine within the mean striatum (globus pallidus left + right & putamen left + right). DaTscan data was used as a method of validating the results collected from the MRI analysis. This was determined via correlation analysis on each subject with MRI data and corresponding DaTscan data (n = 32 Control, 64 Patient).

Biomarkers

For the purposes of this research, the biomarkers selected from the overall data-set included CSF α-syn (n = 190 Control; 414 Patient), phosphorylated tau 181 (p-tau) (n = 176 Control; 379 Patient) and serum IGF-1 (n = 191 Control; 405 Patient). In addition, the dataset was

restricted to assess subjects with MRI data and corresponding biomarker data: α-syn (n = 32 Control; 64 Patient), P-tau (n = 26 control, 57 patient) and IGF-1 (n = 30 control, 63 patient).

Baseline Differences Between Groups

Welch two-sample t-tests were conducted on the data provided to evaluate whether differences exist between patients and controls. This included the SN:RN and SN:midbrain for the MRI analysis, complete mean striatum dataset for the DaTscans, along with separate calculations for the overall and restricted biomarker dataset.

Assessing Relationship Between MRI Analysis and Biomarkers

Determining whether the selected biomarkers are predictive of SN integrity was accomplished via linear regression. Analyses was conducted against α-syn, p-tau or IGF-1 with the SN:midbrain as the dependent variable, chosen due to possessing higher inter-relatability between scorers (r = 0.78) compared to SN:RN (r = 0.44). Scatterplots demonstrating this can be found in appendix 1. Key demographics were accounted for and included as covariates to accurately assess the relationship, including age (years), self-reported gender (male or female), education (years), and diagnosis (PD or control).

To further probe the effect of diagnosis, additional linear regressions looking at the predictive power of each biomarker on SN signal were conducted within both the patient and control groups. Data was only included for subjects who had measurements for both the biomarker of interest and the SN:midbrain ratio.

RESULTS

Demographics

Demographic data for the entire cohort is reported in Table 1. No significant differences (P > 0.05) were found between patients and controls in age, education, or gender, as shown in table 1:

Table 1: *Demographic characteristics of PD patients versus healthy controls*

Demographics	**PD Patients**		**Controls**		**t-Test**	***p***
	N	**Mean ± SD**	**N**	**Mean ± SD**	**t(df)**	
Age (years)	423	(61.66 ± 10.17)	196	(60.82 ± 10.19)	-0.91(335)	.364
Education (years)	423	(15.60 ± 3.05)	196	(16.04 ± 3.05)	1.92(389)	.055
Gender	423	**(%)**	196	**(%)**	0.29(377)	.772
Female	277	44.75	126	20.35		
Male	146	23.60	70	11.30		

MRI Analysis of SN Structural Integrity

The SN was compared to two brain regions: the midbrain and RN. Ratios were created between the SN and the comparator control regions, where higher ratios correspond to increased signal loss detected within the SN, interpreted as higher levels of volume loss. High correlations existed for independent SN:midbrain and SN:RN values (r = 0.774) before being averaged with another independent evaluator to be analysed. Figure 5A represents the SN:midbrain ratio,

showing that patients (0.142 ± 0.021) had significantly higher volume loss (t (57.55) = -6.05; P = < 0.001) compared to controls (0.116 ± 0.024).

In addition, the SN:RN ratio yielded similar results, as shown in figure 5B, highlighting significantly higher volume loss (t (69.61) = -5.49; P = <0.001) in Patients (1.41 ± 0.23) compared to Controls (1.181 ± 0.23)

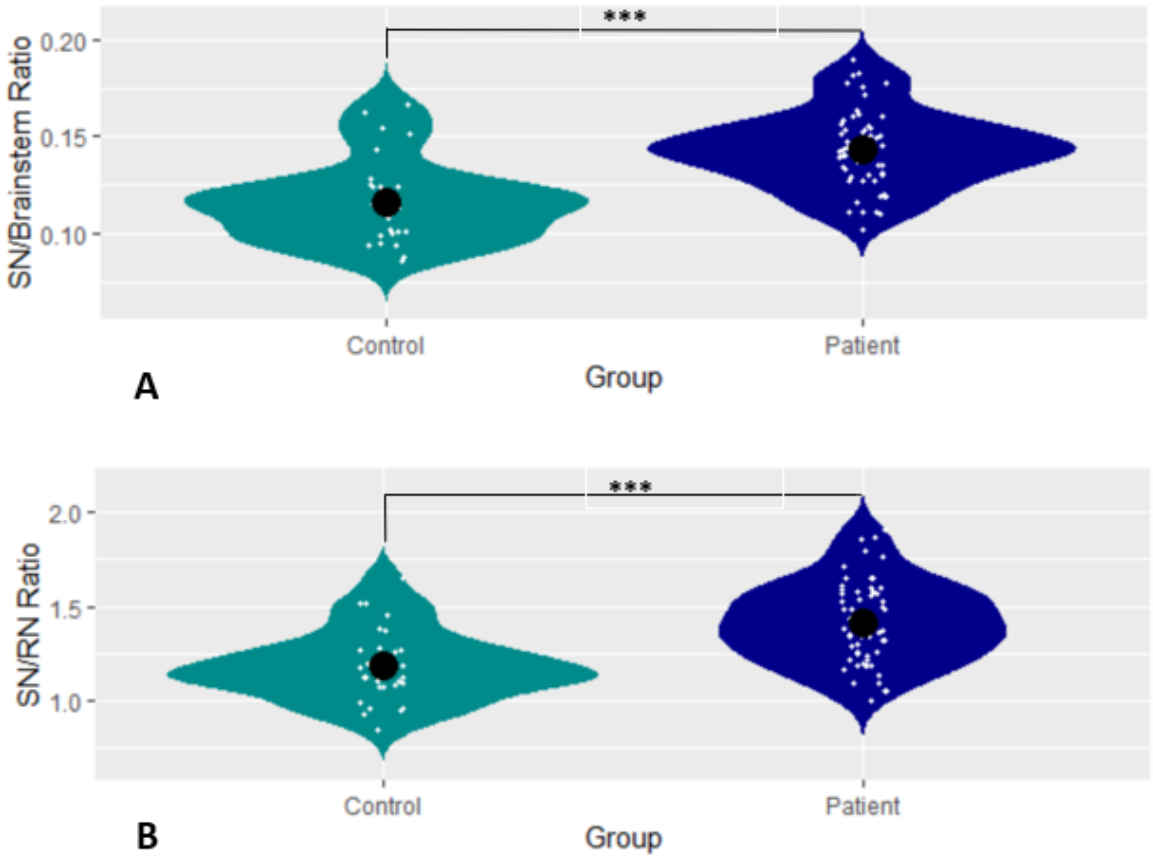


Figure 5: *Volume loss in the SN, as measured by ratios analysis with the midbrain (A) and RN (B), was higher in individuals with PD compared to Controls* ***** = P < 0.001 Between Patient and Control Groups*

DaTscan Analysis of Dopaminergic Binding Potential in Striatum

Dopamine binding in the mean striatum was evaluated to assess the overall integrity of the dopaminergic system. Figure 6 demonstrates that DaTscan results were consistent with the MRI structural integrity analysis. Overall, patients (1.411 ± 0.72) showed significantly lower (t (292.80) = 24.40; P = <0.001) binding in the striatum compared to controls (2.563 ± 0.72).

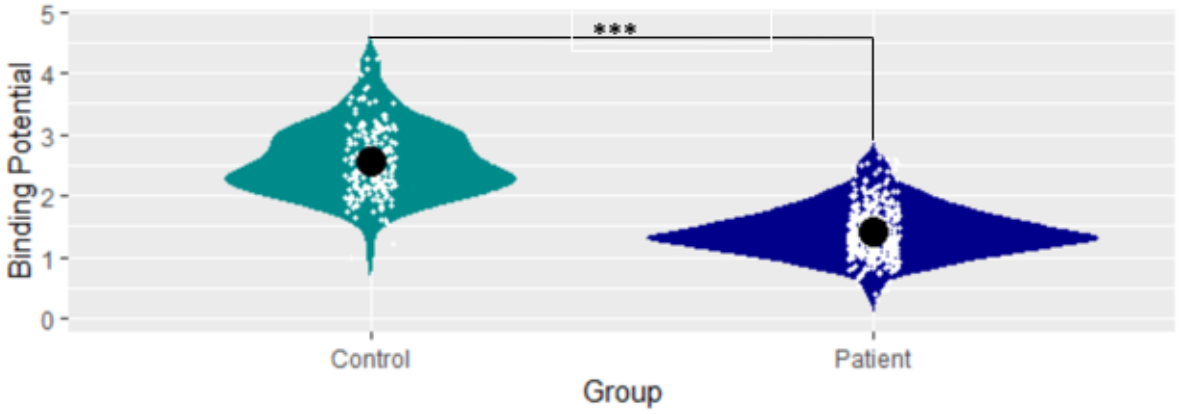


Figure 6: *Dopaminergic System Integrity, as measured by dopamine binding potential in the striatum via DaTscan analysis, was lower in Patients compared to Controls* *** = *P < 0.001 Between Patient and Control Groups*

Correlation Between SN Structural Integrity and Dopaminergic Binding in Striatum

In addition, as a measure of validating the results found by MRI analysis, a correlation between DaTscan and both ratio analyses was conducted (n = 96). Both correlation analyses yielded similar results as DaTscan data possessed a weak to moderate negative relationship with the SN:midbrain (r = -0.34) and a weak negative relationship with the SN:RN (r = -0.29), detailed in figure 7:

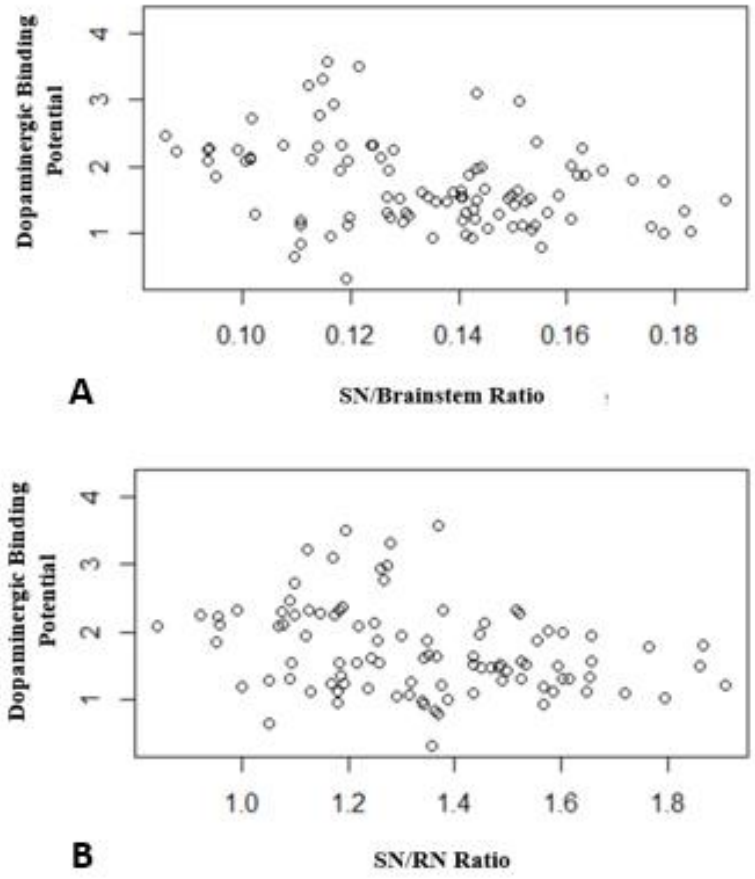


Figure 7*: Scatterplots highlighting the relationship between DaTscans and (A) SN:Brainstem and (B) SN:RN*

Differences in Biomarker Levels

Baseline differences between patients and controls were analysed in three biomarkers: CSF α-syn, CSF p-tau and serum IGF. Figure 8A demonstrates CSF levels of α-syn (pg/mL) being significantly lower (2.975 (331.93), P = 0.003) in patients (1506.7 ± 32.76) compared to controls (1695.19 ± 54.22). Similarly, p-tau (pg/mL) results, as shown in figure 8B, demonstrate patients (14.87 ± 0.27) had significantly lower levels (4.550 (41.38); P = < 0.001) than controls (17.52 ± 0.63). Contrastingly, serum IGF-1 (ng/mL) values showed no significant

difference (t (362.12) = -0.56, P = 0.65) between Controls (134.5 ± 4.06) and Patients (136.70 ± 2.70), as depicted in Figure 8C.

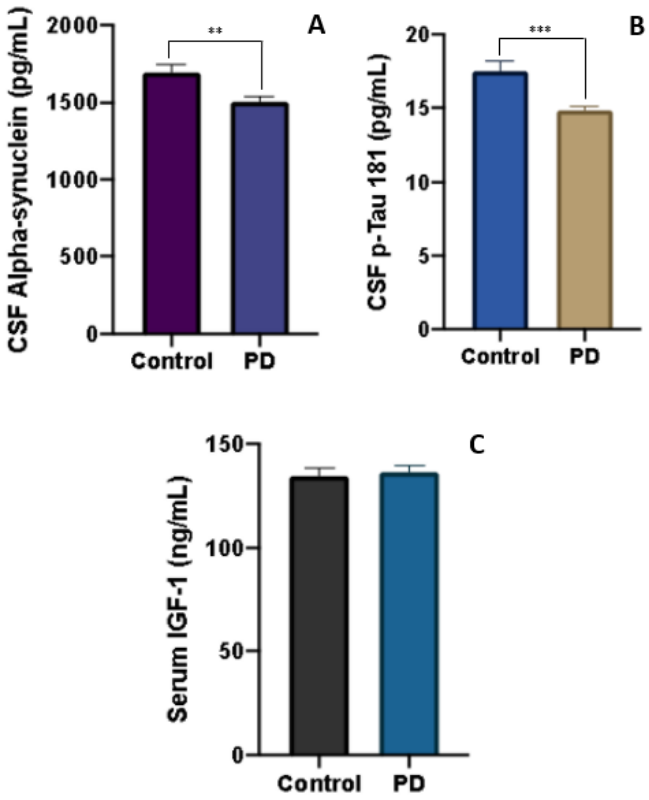


Figure 8: *Mean ± SEM of (A)* α-syn *(B) P-tau and (C) IGF-1 in both Patients and Controls in Complete Dataset.*

*** = P < 0.005; *** = P < 0.001 Between Patient and Control Groups*

Baseline differences were also calculated on a restricted dataset which only included subjects who had an MRI and had a corresponding biomarker of interest. Figure 9C demonstrates that like baseline data, serum IGF-1 (n = 30 Control, 63 Patient) showed no significant difference (t (54.85) =0.33, P = 0.73) between patients (5.97 ± 0.65) and controls (5.97 ± 0.65). Unlike the baseline data, however, Figure 9A emphasises that CSF α-syn (n = 32 Control, 64 Patient) in patients (1554.14 ± 158.75) was not significantly different (t (78.5) = -.49, P = 0.66) to

controls (1565.55 ± 96.91). Likewise, Figure 9B shows that p-tau (n = 26 Control, 57 Patients) in patients (5.97 ± 0.65) was not significantly different (t (39.41) = 1.14, P =0.20) to controls (5.97 ± 0.65).

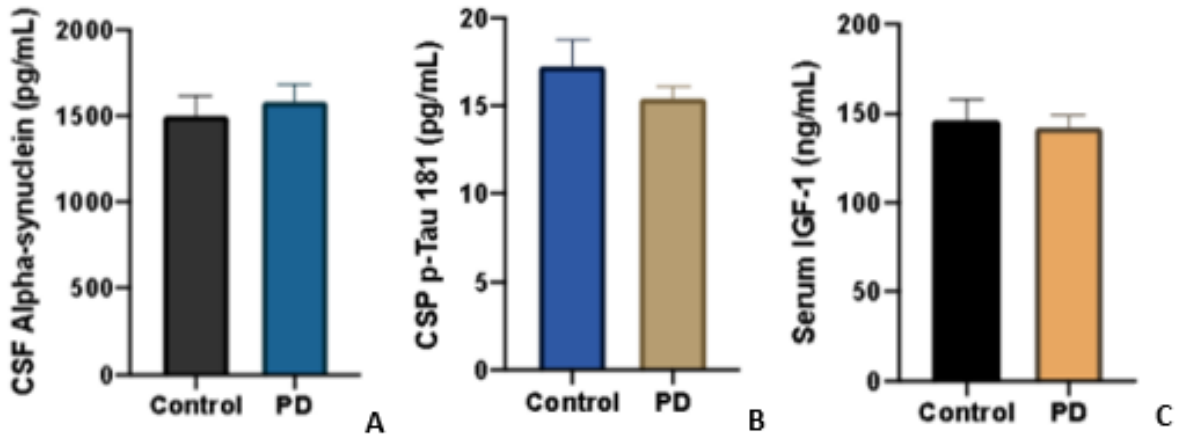


Figure 9: *Mean ± SEM of (A)* α-syn *(B) p-tau and (C) IGF-1 in both Patients and Controls, restricted to subjects with both MRI analysis and corresponding biomarkers*

Linear Regression Models

Linear regression was conducted with SN:midbrain as the dependent variable and the biomarkers of interest as independent variables, with additional covariates such as age, gender, education, and diagnosis added into the model. Therefore, this assessed how well levels of α-syn, p-tau or IGF-1, in conjunction with the other key variables, could predict volume loss in the SN.

Firstly, the SN:midbrain relationship with all biomarkers included was assessed (n = 96). A moderate positive linear relationship exists, confirmed by Pearson's correlation coefficient (R) of 0.51. No significant relationships were found between SN:midbrain and any of the biomarkers of interest (P > 0.05). However, a significant relationship was found with diagnosis (P < 0.001). The slope coefficients formed the following regression equation:

SN:midbrain = .115 ± .000 (Age) ± .007 (Gender) ± .000 (Education) ± .029 (Diagnosis) ± 3.87^{-6} (α-syn) ± .000 (p-tau) ± 4.81^{-5} (IGF-1)

In addition, the R^2 value was 0.44, so 44% of the variation in the SN:midbrain can be explained by the independent variables.

Separate linear regressions were then conducted on individual biomarkers, with covariates still included, producing the following outputs in table 2:

***Table 2**: Key outcomes extracted from linear regression analyses conducted on individual biomarker relationships with SN:midbrain *** = P < 0.001, / = P > 0.05*

	N	R	R2	Slope Coefficient	P-value
α-syn	96	.63	.40		
Constant				.131	
Age				.000	/
Gender				.006	/
Education				.000	/
Diagnosis				.028	***
α-syn				1.48^{-6}	/
P-Tau	83	.64	.41		
Constant				.132	
Age				.000	/
Gender				.007	/
Education				.000	/
Diagnosis				.029	***
p-tau				.000	/
IGF-1	93	.64	.41		
Constant				.118	
Age				.000	/
Gender				.007	/
Education				.000	/
Diagnosis				.029	***
IGF-1				4.12^{-5}	/

Consistent with the results from SN:midbrain overall analysis, diagnosis was the only independent variable shown to have a consistent significant relationship (P < 0.001) with

volume loss. Graphs highlighting the correlation between diagnosis and each biomarker can be found in Appendices 2 – 5. In addition, further detail on the regression analyses outputs found in Appendices 6 – 8.

Additionally, to probe the effect of diagnosis on the predictive power of the biomarkers of interest on SN volume, separate linear regressions were conducted within both the patient and control groups on the overall SN:midbrain, shown in table 3:

***Table 3**: Key outcomes extracted from linear regressions on all biomarkers and their relationship with SN:midbrain within both the PD and healthy control groups. * = P < 0.05, / = P > 0.05*

	CONTROL					**PATIENT**				
	N	**R**	**R2**	**Slope Coefficient**	**P Value**	**N**	**R**	**R2**	**Slope Coefficient**	**P Value**
	32	.52	.27			64	43	.18		
Constant				.19					.90	
Age				-.001	*				-5.04^{-5}	/
Gender				.003	/				.012	*****
Education				.001	/				.001	/
α-syn				-9.20^{-6}	/				2.34^{-6}	/
p-tau				.00	/				3.01^{-5}	/
IGF-1				.00	/				.00	*****

In addition, linear regressions were conducted on the influence of individual biomarkers on volume loss, highlighted in Table 4:

***Table 4**: Key outcomes extracted from linear regressions on individual biomarkers relationship with SN:midbrain within both the PD and healthy control groups. * = P < 0.05, / = P > 0.05*

	CONTROL					PATIENT				
	N	**R**	**R2**	**Slope Coefficient**	**P Value**	**N**	**R**	**R2**	**Slope Coefficient**	**P Value**
α-syn	32	.42	.18			64	.32	.10		
Constant				.142					.128	
Age				-.001	/				.002	/
Gender				.006	/				.059	/
Education				.001	/				.018	/
α-syn				-4.82^{-6}	/				3.16^{-6}	/
P-Tau	26	.44	.19			57	.31	.09		
Constant				.144					.130	
Age				-.001	/				.000	/
Gender				.006	/				.010	/
Education				.001	/				.001	/
p-tau				.000	/				.000	/
IGF-1	30	.43	.18			63	.38	.14		
Constant				.151					.096	
Age				-.001	*				-1.20^{-5}	/
Gender				.006	/				.012	*
Education				.001	/				.001	/
IGF-1				-5.85^{-5}	/				9.50^{-5}	*

For further information on the regression outputs conducted within both the Patients and Control groups, refer to Appendices 9 – 11 and Appendices 12 – 14, respectively.

DISCUSSION

The development of non-invasive methods of predicting risk and rate of developing PD is a highly developing field, as presently none exist. Currently, diagnostic criteria are dependent on of cardinal motor symptoms, at which point patients have undergone high levels of deterioration of their SN and overall dopaminergic system[3-4]. Therefore, studies have been conducted to target specific pathologies which are known to contribute to DA death and could therefore act as biomarkers of disease progression and development[10-17]. Effectively, validating the use of such biomarkers requires adequate neuroimaging as a comparative measure, which is underdeveloped and underutilised in clinical PD settings[19-22]. Therefore, this study investigated such gaps via validating neuroimaging structural integrity of the SN and determining whether it has a relationship with potentially predictive biomarkers. Overall, the results of this study to not provide sufficient data to fully support the relationship of α-syn , p-tau or IGF-1 with SN structural integrity.

Previous studies highlight the difficulty of verifying biomarkers to assess their efficacy as predictive methods[30-31]. Therefore, this study aimed to address this via assessing its relationship with T2-weighted MRI analysis. Previous findings show T2-weighted MRI can successfully evaluate atrophy of structures susceptible to dopaminergic loss in PD, primarily the SN[23-24]. Baseline MRI data supported this, as this study showed significantly lower ratios for patients compared to controls in both SN:midbrain and SN:RN, indicative of higher volume loss patients (Figure 5). In addition, linear regressions with diagnosis included as a covariate consistently showed that diagnosis possessed a significant relationship with SN:midbrain (Table 2). This demonstrates that the methodology employed throughout this study was an effective measure of evaluating SN volume loss. However, previous studies support the idea of employing a multi-modal imaging approach to validate and improve the translatability of imaging data[32-33]. To address this, DaTscan data was used to evaluate the dopaminergic system

integrity to act as a comparative measure against MRI . Results showed significantly lower levels of dopaminergic binding in the striatum in PD compared to controls (Figure 6). DaTscans on their own do not provide sufficient evidence to diagnose PD as they cannot distinguish between other disorders with prominent dopaminergic loss[20-21]. However, it has reported success in confirming diagnosis, particularly in patients who exhibit atypical clinical symptoms, making it a potentially valid measure to confirm MR[-20]. This was partially supported by the results as both the SN:RN and SN:Brainstem possessed a negative relationship with DaTscan analysis; however, the correlations were only weak to moderate (figure 7).

Evaluating baseline differences in selected biomarkers using the entire PPMI database yielded significantly lower levels of CSF α-syn and p-tau in PD patients compared to controls (figure 7A & 7B). This is consistent with literature, attributing lowered levels in CSF due to aggregations, forming Lewy bodies and NFT's for α-syn and p-tau, respectively[11-16]. Lewy body pathology has been heavily researched due to being the pathological hallmark of PD, highly contributing to dopaminergic death in the SN by inducing neurotoxicity[1,12-13]. Previous studies have shown that CSF levels of α-syn are lower in patients compared to healthy controls, which is reflected on the baseline differences of alpha synuclein on the overall dataset[33-36]. However, many disorders share this pathology contributing to disease progression, including dementia with Lewy bodies (DLB) and Alzheimer's disease (AD)[33-36]. Therefore, research into the efficacy of using CSF biomarkers to provide differential diagnosis between disorders is essential. Currently, previous literature shows that α-syn can provide differentiation between PD and AD[33-36]. However, it struggles to distinguish between other Parkinsonian disorders and Dementia with Lewy Bodies[33-36]. This highlights the disadvantages of using single biomarkers in distinguishing PD and the need to use other validated biomarkers in conjunction [11, 37]. Therefore, the potential of using p-tau as a complimentary biomarker is highly promising.

While PD is not a traditional tauopathy, research has shown increasing evidence to support the role of p-tau and α-syn interactions in disease progression[14-15]. It is suggested that α-syn and phosphorylated tau have synergistic effects that perpetuate neurotoxicity. In fact, p-tau has been found that NFTs can be found surrounding Lewy bodies, supporting the interactive mechanisms of α-syn and p-tau[14-15]. Because of this, p-tau is a heavily researched PD biomarker, with previous literature highlighting CSF levels of p-tau being lower in PD compared to controls, consistent with the baseline data within this study[38-39]. However, similarly to α-syn, it cannot make a distinction between other disorders, such as multiple system atrophy or progressive supranuclear palsy[38-39].Therefore expanding the usefulness of assessing biomarkers in conjunction should include biomarkers representative of other pathologies that contribute to PD.

Lewy body pathology could also be attributed to changes in specific hormone expressions throughout the brain. A prime example is IGF-1, a known protective factor against α-syn aggregation, with lower IGF-1 levels at early PD being associated with worse outcomes such as hastened disease progression[39-41]. Therefore, changes in IGF-1 expression could contribute to the PD development. In this study, it was found that serum IGF-1 levels were not significantly different in PD patients compared to healthy controls (Figure 8C). This is consistent with previous literature which highlight IGF-1 being higher in moderate PD but indistinguishable in early PD, while others show no significant differences between PD and controls at all[39-41]. Serum IGF-1 has reportedly limited success as a potential biomarker for various reasons. For example, subjects exhibit high inter- and intraindividual variability[39-41]. Also, unlike CSF biomarkers, serum IGF-1 may not be truly reflective of the changes in central pathophysiolog[41]. However, studies have shown similar expression of CSF and serum IGF-1 within PD patients, therefore, future studies should test the efficacy of utilising CSF IGF-1 to improve translatability[42-43].

Baseline differences of biomarkers were also conducted on a restricted dataset which only included subjects with MRI analysis (figure 8). Contrary to the previous baseline data results, no significant differences were found across any biomarkers. This may be due to the significantly smaller sample size, resulting in an underpowered dataset. This is reported in previous literature, highlighting lack of power as a significant barrier in verifying differences in biomarkers across diseases[44-45].

In fact, underpowered data may drive the relationships between biomarkers and SN volume loss determined via linear regression. Models on overall SN:midbrain highlighted that a large percentage of the variation in SN:midbrain could be attributed to the independent variables. However, no significant relationships were found in any biomarkers. This was supported by models with each biomarker in isolation predicting very similar variance, indicating that little additive effect exists to improve the predictive power of the model. This is reflected in previous studies as while biomarker research has progress in identifying potential biomarkers via baseline differences, no fully validated biomarker is yet available for PD[46-47].

Additionally, the models distinguishing patients from Controls resulted in much lower R2 scores. This could be attributed to both omitting diagnosis as an independent variable, indicating it accounted for majority of the variation, and a further reduced sample size. Like the complete cohort model, neither α-syn or p-tau were significantly different. However, in PD, IGF-1 was a significant predictor of SN:midbrain, whereas this was not present control group, indicating that IGF-1 contributed to volume loss of the SN noted in patients. However, due to the low sample size and contrast results between IGF-1 baseline and linear regression data, it may be that the results are not truly representative. Therefore, definitive conclusions cannot be drawn, and future research will need to assess the predictive power of IGF-1 in a larger dataset.

PROGESSIONAL AND FUNDING ACKNOWLEDGEMENTS

I would like to personally thank the Neurosurgical Research Foundation for providing funding essential for this study to be undertaken. I express my gratitude to the authors of the PPMI database for allowing the University of Adelaide to gain access to the database to extract information necessary for the methodology of this study. Finally, thank you Lyndsey Collins-Praino, Irina Bateu, Mark Jenkinson and Benjamin Ellul for the assistance and guidance throughout the course of this year.

REFERENCES

1. Gómez-Benito M, Granado N, García-Sanz P, Michel A, Dumoulin M & Moratalla R (2020). Modeling Parkinson's Disease with the Alphα-synuclein Protein. *Frontiers in Pharmacology*, **11**.
2. Parkinson's Australia (2020). Statistics on Parkinson's. Available at: <https://www.parkinsons.org.au/statistics>
3. Meder D, Herz D.M, Rowe J.B, LehéricynS & Siebner H.R (2019). The role of dopamine in the brain-lessons learned from Parkinson's disease. *Neuroimage***190**, 79-93.
4. Cheng H, Ulane C & Burke, R (2010). Clinical progression in Parkinson disease and the neurobiology of axons. *Annals of Neurology* **67(6)**, 715-725.
5. Triarhou L.C. (2013). Dopamine and Parkinson's disease. In *Madame Curie Bioscience Database*, Landes Bioscience.
6. DeMaagd G & Philip, A (2015). Parkinson's disease and its management: part 1: disease entity, risk factors, pathophysiology, clinical presentation, and diagnosis. *Pharmacy and therapeutics* **40(8)**, 504.
7. Pretegiani E & Optican L.M (2017). Eye movements in Parkinson's disease and inherited parkinsonian syndromes. *Frontiers in neurology **8***, 592.
8. Freeze B, Kravitz A, Hammack N, Berke J & Kreitzer A (2013). Control of Basal Ganglia Output by Direct and Indirect Pathway Projection Neurons. *Journal of Neuroscience* **33(47)**, 18531-18539.
9. Meder D, Herz D.M, Rowe J.B, LehéricynS & Siebner H.R (2019). The role of dopamine in the brain-lessons learned from Parkinson's disease. *Neuroimage **190***, 79-93.

10. Castilla-Cortázar I, Aguirre G, Femat-Roldán G, Martín-Estal I & Espinosa L (2020). Is insulin-like growth factor-1 involved in Parkinson's disease development? *Journal of Translational Medicine* **18(1)**, 70.
11. Emamzadeh F.N & Surguchov A (2018). Parkinson's disease: biomarkers, treatment, and risk factors. *Frontiers in neuroscience **12***, p.612.
12. Stefanis L (2012). ~~a Syn~~α-synuclein in Parkinson's Disease. *Cold Spring Harbor Perspectives in Medicine* **2(2)**, a009399
13. Henderson M, Trojanowski J & Lee, V (2019). α-Synuclein pathology in Parkinson's disease and related α-synucleinopathies. *Neuroscience Letters* **709**, 134316.
14. Avila, J., Lucas, J.J., Perez, M.A.R. and Hernandez, F., 2004. Role of tau protein in both physiological and pathological conditions. *Physiological reviews **84*(2)**, 361-384.
15. Schraen-Maschke S, Sergeant N, Dhaenens C.M, Bombois S, Deramecourt V, Caillet-Boudin, M.L, Pasquier F, Maurage C.A, Sablonniere B, Vanmechelen E. & Buee L (2008). Tau as a biomarker of neurodegenerative diseases. *Biomarkers Med.* **2**, 363– 384.
16. Zhang X, Gao F, Wang D, Li C, Fu Y, He W. & Zhang J (2018). Tau pathology in Parkinson's Disease. *Frontiers in neurology **9***, 809.
17. Bernhard F.P, Heinzel S, Binder G, Weber, K, Apel A, Roeben B, Deuschle C, Maechtel M, Heger T, Nussbaum S & Gasser T (2016). Insulin-like growth factor 1 (IGF-1) in Parkinson's disease: potential as trait-, progression-and prediction marker and confounding factors. *PLoS One **11*(3)**, e0150552
18. Ghazi Sherbaf F, Mohajer B, Ashraf-Ganjouei A, Mojtahed Zadeh M, Javinani A, Sanjari Moghaddam H, Shirin Shandiz M & Aarabi M.H. (2018). Serum insulin-like growth factor-1 in Parkinson's disease; study of cerebrospinal fluid biomarkers and white matter microstructure. *Frontiers in endocrinology **9***, 608.

19. Stoessl A.J. (2011). Neuroimaging in Parkinson's disease. *Neurotherapeutics* ***8*****(1),** pp.72-81.
20. Seifert K.D. & Wiener, J.I. (2013). The impact of DaTscan on the diagnosis and management of movement disorders: A retrospective study. *American journal of neurodegenerative disease*, ***2*****(1)**, 29.
21. Oravivattanakul S. Benchaya L, Wu G, Ahmed A, Itin I, Cooper S, Gostkowski M, Rudolph J, Appleby K, Sweeney P & Fernandez H (2015). Dopamine Transporter (DaT) Scan Utilization in a Movement Disorder Center. *Movement Disorders Clinical Practice* **3(1)**, 31-35.
22. Ba F. & Martin W.W (2015). Dopamine transporter imaging as a diagnostic tool for parkinsonism and related disorders in clinical practice. *Parkinsonism & related disorders* ***21*****(2),** 87-94.
23. Tuite P.J, Mangia S & Michaeli S (2013). Magnetic resonance imaging (MRI) in Parkinson's disease. *Journal of Alzheimer's disease & Parkinsonism* **03(03)**, p.001.
24. Blair J.C, Barrett M.J, Patrie J, Flanigan J.L, Sperling S.A, Elias W.J. & Druzgal T.J. (2019). Brain MRI Reveals Ascending Atrophy in Parkinson's Disease Across Severity. *Frontiers in neurology* **10**, 1329.
25. Manjón, J., Bertó, A., Romero, J., Lanuza, E., Vivo-Hernando, R., Aparici-Robles, F. and Coupe, P., 2020. pBrain: A novel pipeline for Parkinson related brain structure segmentation. *NeuroImage: Clinical* **25**, p.102184.
26. Parkinson's Progression Markers Initiative (2020) Landmark Study of Parkinson's Disease, Available at: < https://www.ppmi-info.org/>
27. Matsui H. (2017) Dopamine system, cerebellum, and nucleus ruber in fish and mammals. *Development, Growth & Differentiation* **59(4)**, 219-227.

28. Chung C.Y, Seo H, Sonntag K.C, Brooks A, Lin L. & Isacson, O. (2005). Cell type-specific gene expression of midbrain dopaminergic neurons reveals molecules involved in their vulnerability and protection. *Human molecular genetics* ***14*****(13)**, 1709-1725.
29. Grealish S, Jönsson, M.E, Li M, Kirik D, Björklund A. & Thompson L.H. (2010). The A9 dopamine neuron component in grafts of ventral mesencephalon is an important determinant for recovery of motor function in a rat model of Parkinson's disease. *Brain* ***133*****(2)**, 482-495.
30. Delenclos M, Jones D.R, McLean P.J. & Uitti R.J. (2016). Biomarkers in Parkinson's disease: Advances and strategies. *Parkinsonism & related disorders*, *22*, S106-S110.
31. Eusebi P. Hansson O, Paciotti S, Orso M, Chiasserini D, Calabresi P, Blennow K. & Parnetti L. (2017). Cerebrospinal fluid biomarkers for the diagnosis and prognosis of Parkinson's disease: protocol for a systematic review and individual participant data meta-analysis. *BMJ open* ***7*****(11)**, e018177.
32. Bowman F.D, Drake D.F. & Huddleston D.E. (2016). Multimodal imaging signatures of Parkinson's disease. *Frontiers in neuroscience* ***10***, 131.
33. Nemmi F, Pavy-Le Traon A, Phillips O.R, Galitzky M, Meissner W.G, Rascol O. & Péran P. (2019). A totally data-driven whole-brain multimodal pipeline for the discrimination of Parkinson's disease, multiple system atrophy and healthy control. *NeuroImage: Clinical* ***23***, 101858.
34. Tateno F, Sakakibara R, Kawai T, Kishi M. & Murano T. (2012). Alphα-synuclein in the cerebrospinal fluid differentiates synucleinopathies (Parkinson Disease, dementia with Lewy bodies, multiple system atrophy) from Alzheimer disease. *Alzheimer Disease & Associated Disorders* ***26*****(3)**, 213-216.
35. Hall S, Öhrfelt A, Constantinescu R, Andreasson U, Surova Y, Bostrom F, Nilsson C, Håkan W, Decraemer H, Någga K, Minthon L, Londos E, Vanmechelen E, Holmberg

B, Zetterberg H, Blennow K & Hansson O. (2012) Accuracy of a panel of 5 cerebrospinal fluid biomarkers in the differential diagnosis of patients with dementia and/or parkinsonian disorders. Arch Neurol **69(11)**, 1445-1452.

36. Wang Z.Y, Han Z.M, Liu Q.F, Tang W, Ye K & Yao Y.Y. (2015) Use of CSF α-synuclein in the differential diagnosis between Alzheimer's disease and other neurodegenerative disorders. Int Psychogeriatr. **27(9)**, 1429-1438.
37. He R, Yan X, Guo J, Xu Q, Tang B. & Sun Q. (2018). Recent advances in biomarkers for Parkinson's disease. *Frontiers in aging neuroscience **10***, 305.
38. Kandimalla R.J, Prabhakar S, Wani W.Y, Kaushal A, Gupta N, Sharma D.R, Grover V.K, Bhardwaj N, Jain K. & Gill K.D. (2013). CSF p-Tau levels in the prediction of Alzheimer's disease. *Biology open **2*****(11)**, 1119-1124.
39. Murakami H, Tokuda T, El-Agnaf O.M, Ohmichi T, Miki A, Ohashi H, Owan Y, Saito Y, Yano S, Tsukie T. & Ikeuchi T, (2019). Correlated levels of cerebrospinal fluid pathogenic proteins in drug-naïve Parkinson's disease. *BMC neurology **19*****(1)**, 113.
40. Godau J, Knauel K, Weber K, Brockmann K, Maetzler W, Binder G. & Berg D. (2011). Serum insulinlike growth factor 1 as possible marker for risk and early diagnosis of Parkinson disease. *Archives of neurology **68*****(7)**, 925-931.
41. Bernhard F.P, Heinzel S, Binder G, Weber K, Apel A, Roeben B, Deuschle C, Maechtel M, Heger T, Nussbaum S. & Gasser T. (2016). Insulin-like growth factor 1 (IGF-1) in Parkinson's disease: potential as trait-, progression-and prediction marker and confounding factors. *PLoS One **11*****(3),** e0150552
42. Mashayekhi F, Mirzajani E, Naji M. & Azari M. (2010). Expression of insulin-like growth factor-1 and insulin-like growth factor binding proteins in the serum and

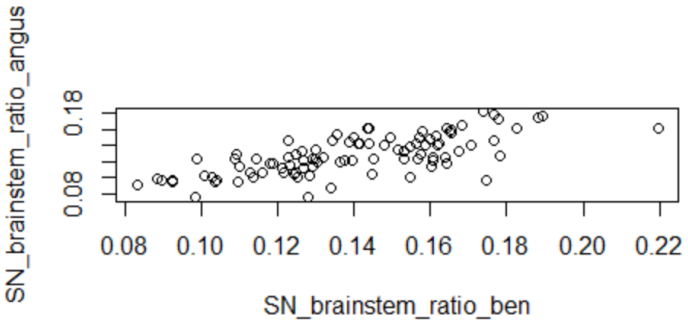
cerebrospinal fluid of patients with Parkinson's disease. *Journal of Clinical Neuroscience* ***17*****(5)**, 623-627.

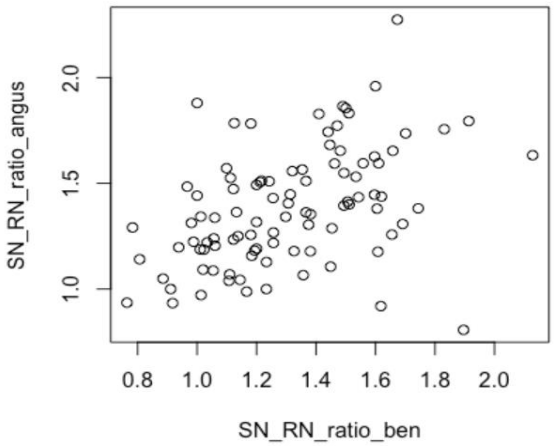
43. Johansson P, Åberg D, Johansson J.O, Mattsson N, Hansson O, Ahrén B, Isgaard J, Åberg N.D, Blennow K, Zetterberg H & Wallin A. (2013). Serum but not cerebrospinal fluid levels of insulin-like growth factor-I (IGF-I) and IGF-binding protein-3 (IGFBP-3) are increased in Alzheimer's disease. *Psychoneuroendocrinology* ***38*****(9)**, 1729-1737.

44. Schnack H.G. & Kahn, R.S. (2016). Detecting neuroimaging biomarkers for psychiatric disorders: sample size matters. *Frontiers in psychiatry* ***7***, 50.
45. Santaella A, Kuiperij H.B, van Rumund A, Esselink R.A, van Gool A.J, Bloem B.R. & Verbeek M.M. (2020). Inflammation biomarker discovery in Parkinson's disease and atypical parkinsonisms. *BMC neurology* ***20*****(1)**, 1-8.
46. Marino S. Ciurleo R. Di Lorenzo G. Barresi M. De Salvo S. Giacoppo S. Bramanti A. Lanzafame P. & Bramanti P. (2012). Magnetic resonance imaging markers for early diagnosis of Parkinson's disease. *Neural regeneration research* ***7*****(8)**, 611.
47. Miller D.B. & O'Callaghan J.P. (2015). Biomarkers of Parkinson's disease: present and future. *Metabolism* ***64*****(3)**, S40-S46.

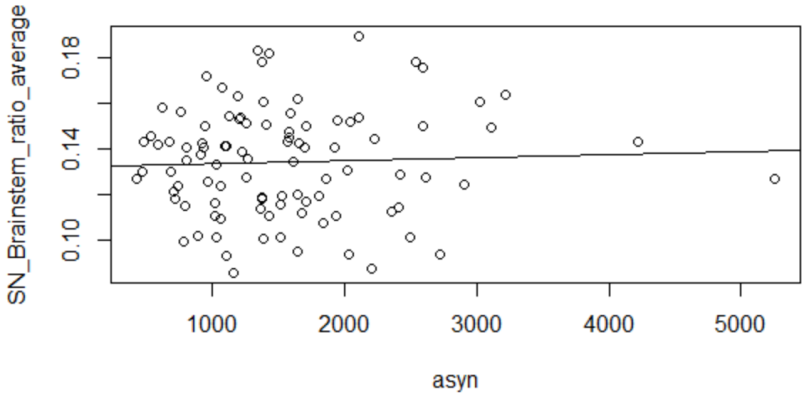
APPENDIX

Appendix 1: Inter-relatability scatterplots

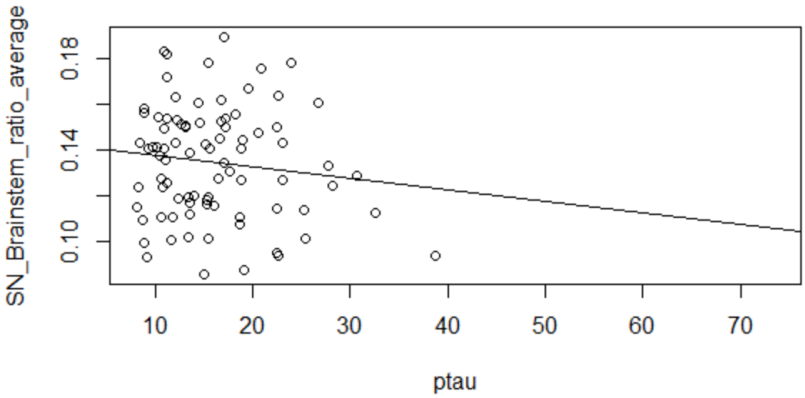




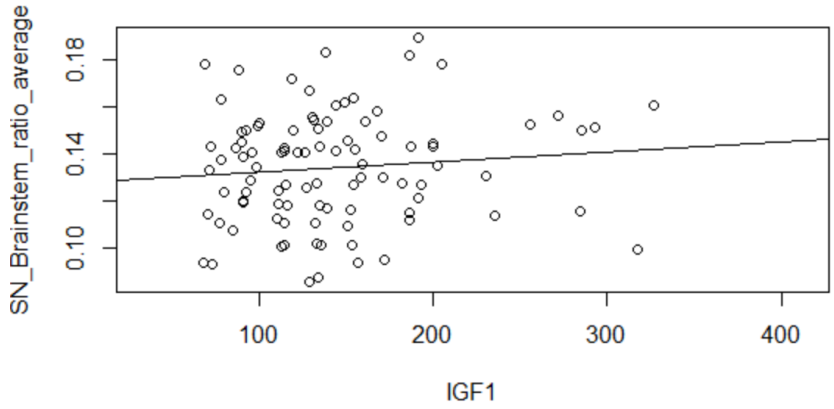
Appendix 2: Relationship between Diagnosis with α-syn



Appendix 3: Relationship between Diagnosis with p-tau



Appendix 4: Relationship between Diagnosis with IGF-1



Appendix 5: SN:Brainstem Overall Linear Regression Coefficient Outputs

Model		Unstandardized Coefficients B	Unstandardized Coefficients Std. Error	Standardized Coefficients Beta	t	Sig.
1	(Constant)	.115	.025		4.677	.000
	Age	.000	.000	-.139	-1.470	.146
	Gender	.007	.005	.149	1.666	.100
	Education	.000	.001	.027	.316	.753
	Diagnosis	.029	.004	.576	6.568	.000
	asyn	3.873E-6	.000	.124	1.069	.288
	ptau	.000	.000	-.112	-.957	.342
	IGF1	4.812E-5	.000	.116	1.216	.228

Appendix 6: SN:Brainstem A-syn Linear Regression Coefficient Outputs

Model		Unstandardized Coefficients B	Unstandardized Coefficients Std. Error	Standardized Coefficients Beta	t	Sig.
1	(Constant)	.131	.018		7.345	.000
	Age	.000	.000	-.204	-2.404	.018
	Gender	.006	.004	.127	1.519	.132
	Education	.000	.001	.014	.165	.869
	Diagnosis	.028	.004	.587	7.116	.000
	asyn	1.482E-6	.000	.051	.602	.548

Appendix 7: SN:Brainstem P-tau Linear Regression Coefficient Outputs

Model		Unstandardized Coefficients B	Unstandardized Coefficients Std. Error	Standardized Coefficients Beta	t	Sig.
1	(Constant)	.132	.021		6.333	.000
	Age	.000	.000	-.188	-2.124	.037
	Gender	.007	.004	.140	1.601	.113
	Education	.000	.001	.026	.297	.767
	Diagnosis	.029	.004	.576	6.611	.000
	ptau	.000	.000	-.041	-.461	.646

Appendix 8 – SN:Brainstem IGF-1 Linear Regression Coefficient Outputs

Model		Unstandardized Coefficients B	Unstandardized Coefficients Std. Error	Standardized Coefficients Beta	t	Sig.
1	(Constant)	.118	.021		5.683	.000
	Age	.000	.000	-.149	-1.676	.097
	Gender	.007	.004	.147	1.756	.083
	Education	.000	.001	.011	.137	.891
	Diagnosis	.028	.004	.590	7.171	.000
	IGF1	4.123E-5	.000	.100	1.130	.262

Appendix 9 – SN:Brainstem A-syn – PD – Linear Regression Coefficient Outputs

Model		Unstandardized Coefficients B	Unstandardized Coefficients Std. Error	Standardized Coefficients Beta	t	Sig.
1	(Constant)	.128	.026		4.961	.000
	asyn	2.402E-6	.000	.108	.845	.402
	Age	.000	.000	-.109	-.872	.387
	Gender	.009	.005	.224	1.744	.086
	Education	.001	.001	.096	.772	.443

Appendix 10 – SN:Brainstem P-Tau – PD – Linear Regression Coefficient Outputs

Model		Unstandardized Coefficients B	Unstandardized Coefficients Std. Error	Standardized Coefficients Beta	t	Sig.
1	(Constant)	.130	.028		4.651	.000
	Age	.000	.000	-.125	-.935	.354
	Gender	.010	.006	.241	1.764	.084
	Education	.001	.001	.101	.765	.448
	ptau	.000	.001	.034	.250	.804

Appendix 11 - SN:Brainstem IGF-1 – PD – Linear Regression Coefficient Outputs

Model		Unstandardized Coefficients B	Unstandardized Coefficients Std. Error	Standardized Coefficients Beta	t	Sig.
1	(Constant)	.096	.029		3.314	.002
	Age	-1.199E-5	.000	-.006	-.045	.964
	Gender	.012	.005	.299	2.385	.020
	Education	.001	.001	.129	1.056	.295
	IGF1	9.503E-5	.000	.267	2.058	.044

Appendix 12 - SN:Brainstem - A-syn – Control – Linear Regression Coefficient Outputs

Model		Unstandardized Coefficients B	Unstandardized Coefficients Std. Error	Standardized Coefficients Beta	t	Sig.
1	(Constant)	.142	.029		4.855	.000
	Age	-.001	.000	-.347	-1.550	.133
	Gender	.006	.008	.140	.785	.440
	Education	.001	.002	.074	.358	.723
	asyn	-4.824E-6	.000	-.152	-.778	.444

Appendix 13 – SN:Brainstem – Ptau – Control – Linear Regression Coefficient Outputs

Model		Unstandardized Coefficients B	Unstandardized Coefficients Std. Error	Standardized Coefficients Beta	t	Sig.
1	(Constant)	.144	.037		3.936	.001
	Age	-.001	.000	-.397	-1.834	.080
	Gender	.006	.008	.131	.685	.500
	Education	.001	.002	.134	.642	.527
	ptau	.000	.001	-.109	-.547	.590

Appendix 14 – SN:Brainstem – IGF-1 – Control – Linear Regression Coefficient Outputs

Model		Unstandardized Coefficients B	Unstandardized Coefficients Std. Error	Standardized Coefficients Beta	t	Sig.
1	(Constant)	.151	.033		4.591	.000
	Age	-.001	.000	-.535	-2.270	.031
	Gender	.006	.008	.146	.825	.416
	Education	.001	.002	.164	.790	.436
	IGF1	-5.851E-5	.000	-.179	-.860	.397