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Metabolite ratios in the posterior cingulate cortex do not track cognitive decline in Parkinson's disease in a clinical setting

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ABSTRACT

Introduction: Parkinson's Disease (PD) is classified as a motor disorder, but most patients develop cognitive impairment, and eventual dementia (PDD). Predictive neurobiomarkers may be useful in the identification of those patients at imminent risk of PDD. Given the compromised cerebral integrity in PDD, we investigated whether brain metabolites track disease progression over time.
Methods: Proton Magnetic Resonance Spectroscopy (MRS) was used to identify brain metabolic changes associated with cognitive impairment and dementia in PD. Forty-nine healthy participants and 130 PD patients underwent serial single voxel proton MRS and neuropsychological testing. At baseline patients were classified as either having normal cognitive status (PDN, n = 77), mild cognitive impairment (PDMCI, n = 33), or dementia (PDD, n = 20). Posterior cingulate cortex (PCC) was examined to quantify N-acetylaspartate (NAA), choline (Cho), creatine (Cr), and myo-inositol (mI). A hierarchical Bayesian model was used to assess whether cognitive ability and other covariates were related to baseline MRS values and changes in MRS over time.
Results: At baseline, relative to controls, PDD had significantly decreased NAA/Cr and increased Cho/Cr. However, these differences did not remain significant after accounting for age, sex, and MDS-UPDRS III. At follow-up, no significant changes in MRS metabolite ratios were detected, with no relationship found between MRS measures and change in cognitive status.
Conclusions: Unlike Alzheimer's disease, single voxel MR spectroscopy of the PCC failed to show any significant association with cognitive status at baseline or over time. This suggests that MRS of PCC is not a clinically useful biomarker for tracking or predicting cognitive impairment in Parkinson's disease.

1. Introduction

In addition to the classic motor symptoms of rigidity, tremor, and bradykinesia, many Parkinson's disease (PD) patients experience cognitive impairments. These cognitive problems worsen as the disease progresses, ultimately leading to dementia in the majority [1,2]. However, there is considerable variation (range 2–20 years) between onset of PD and the emergence of dementia [3]. This delay provides a window for potential therapeutic intervention [2,4]. Neuroimaging is an attractive option for identifying neurobiomarkers of cognitive status, especially mild cognitive impairment (PDMCI), and the progression to dementia. Suitable biomarkers may facilitate timely interventions to slow cognitive decline.

One candidate is proton Magnetic Resonance Spectroscopy (MRS). This technique allows in vivo measurement of the metabolites N-acetylaspartate (NAA, a neuronal marker), choline (Cho, a cell membrane turnover marker), creatine (Cr, an energy metabolism marker; typically used as an internal control metabolite in MRS analysis), and myo-Inositol (mI, a glial cell marker) [5,6].

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Abnormal metabolic ratios (NAA/Cr, Cho/Cr and mI/Cr) in the posterior cingulate cortex (PCC) have been identified in Alzheimer's disease (AD), as well as other neurodegenerative diseases such as fronto-temporal dementia [7]. A recent meta-analysis of single voxel MR spectroscopy in the PCC of patients with dementia and mild cognitive impairment (MCI) of the Alzheimer's type reported accelerated metabolic changes over time in the PCC of MCI patients relative to controls [8]. Metabolic changes measured by MRS in such degenerative disorders have been linked to neuronal loss, axonal injury and compromised neuronal energy metabolism [9], but the value of MRS as a similar biomarker in PD remains uncertain.

In this study, we used single-voxel proton MRS to investigate the integrity of the PCC in PD over time. The PCC exhibits high resting state metabolism, is a key hub in the fMRI-identified default mode network, and is highly involved in multiple cognitive processes [10–12]. Additionally, it is one of the first areas to be compromised in early Alzheimer's disease [9]. It is also affected in PD, showing compromised metabolism (via positron emission tomography), reduced perfusion (via arterial spin labeling MRI), and cortical thinning (via structural MRI) [13–15]. Furthermore, cross sectional MRS in the PCC has identified reduced NAA/Cr in both PDD and non-demented PD [16–18], therefore suggesting it as a potential biomarker to track Parkinson's progression. However, abnormal MRS in PD cross-sectional studies is not a universal finding and provides no information on whether such a measure reflects progression [19]. We therefore investigated the relationship between single-voxel proton MRS metabolite ratios and PD-related cognitive decline, cross-sectionally and over time, in order to evaluate its utility as an imaging biomarker. In order to make any possible findings immediately applicable in the clinic, we used automated, scanner-quantified MRS ratios [20,21].

2. Participants and methods

This study was approved by the Upper South Ethics Committee of the New Zealand Ministry of Health. All participants, or significant others where appropriate, provided written informed consent. A convenience sample of 130 PD patients, comprising those with normal cognitive status (PDN, n = 77); mild cognitive impairment (PDMCI, n = 33); or dementia (PDD, n = 20), was recruited from the Movement Disorders Clinic at the New Zealand Brain Research Institute, Christchurch, New Zealand, between May 2007 and August 2013. All satisfied the UK Parkinson's Society criteria for idiopathic PD [22]. Forty-nine healthy adults were recruited to match the PD patients for mean age, years of education and sex ratio. Exclusion criteria included atypical parkinsonian disorder; prior learning disability; history of other neurologic conditions including moderate–severe head injury, stroke, vascular dementia; and major psychiatric or medical illness in the previous six months. Patients diagnosed with dementia (PDD, n = 20) at baseline were not followed further as dementia was considered an endpoint, but data from this group were included for baseline comparisons.

Of the 110 non-dementia PD cases, 64 were re-imaged on at least one other occasion over the subsequent four years for a total of 106 follow-up scans. Of the 49 healthy controls at baseline, 40 individuals were similarly re-imaged, with a total of 59 follow-up scans. These follow-up assessments occurred at approximately two and four years after baseline. Demographic details are presented in Table 1. Fig. 1B depicts the timings of when scanning was performed in each individual.

2.1. Clinical and cognitive assessment

Motor severity was assessed with Hoehn and Yahr (H&Y) [23] staging and part III of the Movement Disorder Society Unified Parkinson's Disease Rating Scale (MDS-UPDRS III) [24]. Montreal Cognitive Assessment (MoCA) was used as a screening tool for cognitive performance [25]. A comprehensive neuropsychological battery assessed cognitive status consistent with the Movement Disorder Society (MDS) recommended domains [2] (executive function – letter, action and category fluency, category switching, Trails B and Stroop interference; attention, working memory, and processing speed – digits forward and backward, digit ordering, map search, Stroop color and word reading, and Trails A; learning and memory – California Verbal Learning Test-short form short and long delay recall, and the Rey–Osterrieth complex figure short and long delay; visuospatial/visuoperceptual function – judgment of line orientation, fragmented letters and Rey–Osterrieth complex figure copy; and language – Boston naming, DRS-2 similarities subtest, and ADAS-Cog) Standardized scores from the constituent neuropsychological tests were averaged to provide individual cognitive domain scores. Global cognitive ability for each participant was then expressed as an aggregate z score obtained by averaging four domain scores (non-normality of the language scores precluded their inclusion) [26]. Consistent with the MDS task force level II diagnostic criteria, PDMCI patients did not have significantly impaired functional activities of daily living, verified by interview with a significant other, and scored $\geq$1.5 standard deviations (or equivalent) below normative data on at least two measures within at least one of the five cognitive domains [26]. MDS criteria were also used to diagnose dementia (PDD) [1]. Accordingly, patients were classified at baseline as either having normal cognitive status (PDN, n = 77), mild cognitive impairment (PDMCI, n = 33), or dementia (PDD, n = 20) [27]. Table 1 summarizes the neuropsychological assessment results.

2.2. MRS acquisition

Magnetic Resonance spectroscopy (MRS) data were acquired on a 3.0 T General Electric Signa HDxt scanner (GE Medical Systems, Wauwatosa, WI) using an eight channel head coil. The imaging protocol included: (1) a T1-weighted 3D spoiled gradient recalled echo sequence (echo time = 2.8 ms, repetition time = 6.6 ms, inversion time = 400 ms, flip angle = 15°, acquisition matrix = 256 × 256, 170 slices, field of view = 250 mm, slice thickness = 1 mm, voxel size = 0.98 × 0.98 × 1.0 mm^3), and (2) a single voxel Point Resolved Spectroscopy (PRESS) acquisition, echo time = 35 ms, repetition time = 1500 ms, voxel size = 20 × 20 × 30 mm^3, number of averages = 128. At each time point, one of three experienced MR radiographers placed the spectroscopy voxel of interest (VOI) in the midline posterior cingulate cortex (PCC) of the brain (Figure S.1). Pre-scan shimming was performed to achieve full-width half maximum (line width) of $\leq$13 Hz [28]. Table S.1 demonstrates the mean linewidth and water suppression values for the study groups.

Metabolite ratios, with creatine (Cr) as the reference metabolite, were produced using scanner software (PROBE-Q, GE Medical Systems); this included NAA/Cr, Cho/Cr, and mI/Cr. The fully automated PROBE-Q package involves (1) Setting a global frequency fit parameter; (2) performing line-width and line-shape enhancement by appropriate apodisation of the time-domain signal; (3) Fourier transformation of the signal to the appropriate frequency resolution and number of points; (4) calculation of a baseline correction from the frequency-domain signal; (5) and curve fitting the desired regions of the frequency-domain signal. There were two software upgrades over the duration of the study (starting from scanner software v14, through v15 and lastly v16), but acquisition parameters were unchanged.

Table 1
Subject demographics and baseline neuropsychological assessment results.

	HC mean ± SD	PDN mean ± SD	PDMCI mean ± SD	PDD mean ± SD	F value	p
n	49	77	33	20		
Sex (M/F)	33/16	51/26	21/12	17/3	(3175) = 1.8	0.39
Age (yrs)	68 ± 8	65 ± 8	69 ± 7	73 ± 7	(3175) = 7.8	<0.001
Education (yrs)	13 ± 3	13 ± 3	13 ± 3	12 ± 2	(3175) = 1.4	0.64
Disease duration (yrs)	NA	3.7 ± 5.3	5.4 ± 6.4	9.7 ± 8.9	(2127) = 24.4	<0.001
LED (mg/day)	NA	290 ± 389	404 ± 463	723 ± 379	(2127) = 8.5	<0.001
Anti-Cholinergics (yes:no)	NA	5:72	6:27	4:16	(2127) = 2.0	0.10
MoCA	27 ± 2	26 ± 2.2	23 ± 2.4	17 ± 3.7	(3171) = 93.5	<0.001
H&Y	NA	1.9 ± 0.7	2.2 ± 0.7	3.2 ± 0.8	(2127) = 24.8	<0.001
UPDRS-III	NA	31 ± 15	36 ± 15	58 ± 21	(2127) = 22.5	<0.001

Disease duration was calculated as time from diagnosis.
F values calculated by simple ANOVA across groups $p < 0.05$.
Abbreviations: HC = Healthy Controls, PDN = Parkinson's Disease with Normal cognitive ability, PDMCI = Parkinson's Disease with Mild cognitive Impairment, PDD = Parkinson's Disease with Dementia, LED = Levodopa Equivalent Dose, MoCA = Montreal Cognitive Assessment, H&Y = Hoehn and Yahr scale, UPDRS-III = Unified Parkinson's Disease Rating Scale-part three, SD = Standard Deviation, and NA = not applicable.

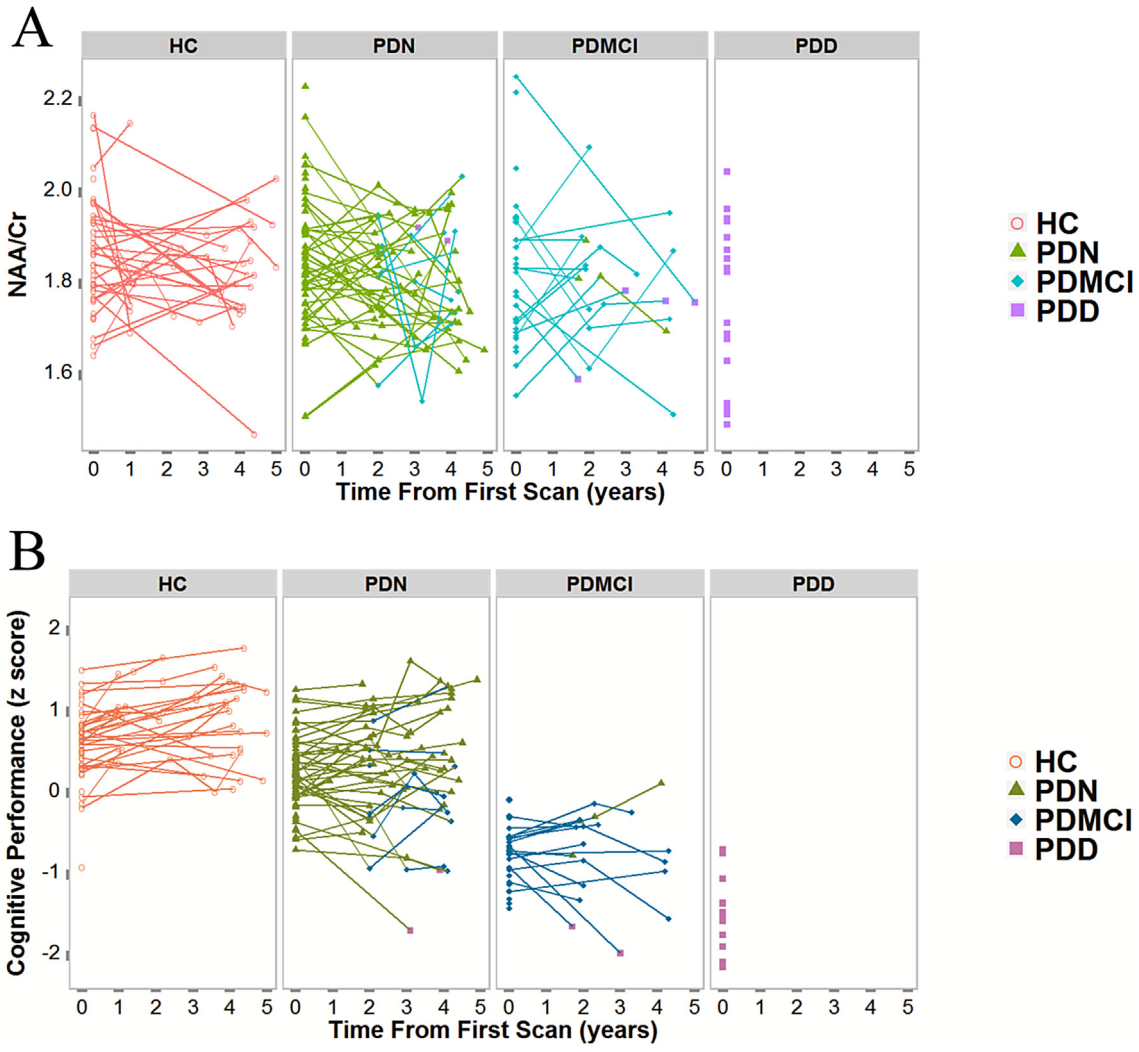

Fig. 1. (A) Indicates NAA/Cr over time (in years) in each cognitive group. Each point represents an assessment and multiple assessments within a single individual are connected with lines. Colors indicate cognitive status at each assessment: Red indicates control, green PDN, blue PDMCI, and purple PDD. (B) Trajectory of global cognitive z score over time by cognitive group. Additional plots for other metabolites are available in the supplementary material. HC = Healthy Control, PDN = Parkinson's disease with normal cognition, PDMCI = Parkinson's disease with mild cognitive impairment, PDD = Parkinson's disease with dementia. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2.3. Statistical analysis

We used R (R Core Team, 2013) and Rstan (RStan: Stan Development Team, Version 2.5.0, 2014) to fit separate Bayesian hierarchical models for global cognitive ability (global z) and the MRS ratios (NAA/Cr, Cho/Cr, mI/Cr). Varying intercepts and slopes

were included per subject, modeling their baseline value and change over time. Subject-level predictors included were baseline cognitive status (control, PDN, PDMCI or PDD), age at baseline, sex, and years of education and, in the case of the MRS ratios, measurement-level predictors of scanner version, line width, and UPDRS III were added. Variances differed by cognitive groups (and also the model allowed for heteroscedasticity). Multiple comparison corrections effectively increase the size of uncertainty around an estimate, thus requiring effects to be further from 0 and distorting the sizes of effects. A Cauchy prior centered around zero fixes the underlying issue of randomness increasing the size of effects by shrinking the estimates instead. Therefore, Cauchy priors (mean 0, scale 0.5) were used for subject-level and measurement-level predictors and half-Cauchy priors (mean 0, scale 1) were used for variance parameters [29,30]. The hierarchical model and Cauchy priors work around a potential multiple comparisons issue by pooling and shrinking estimates [31]. The intercept value of the model corresponds to the cognitive or MRS ratio of a healthy female control subject, of mean age and education. Mean parameter estimates (which can be interpreted as absolute effect sizes) are given along with a 95% probability interval (given the model and the data, the parameter will be within the interval with 95% probability). Results are considered significant in a classical sense if the probability interval does not contain 0. The intercept estimates for each subject from the hierarchical model were used to assess the correlations between MRS values and global cognitive ability. Similarly, the slope estimates were used to assess the correlation between change in MRS values and change in global cognitive ability. Separate linear models were used to assess only baseline scans of individuals with follow-up to determine the influence of conversion to dementia on MRS values.

The power to detect a difference in the PD-D group in this study, given the sample size, analysis methods, and similar effect sizes as seen in AD [8], is 90% for NAA/Cr and 30% for Cho/Cr and mI/Cr.

The anonymized data, along with the code for the hierarchical Bayesian statistical models, are available as supplementary material.

3. Results

3.1. Participant demographics

Age, H&Y and MDS-UPDRS III increased significantly across the PD cognitive sub-groups. Unsurprisingly, the PDN group had the shortest disease duration, PDMCI was intermediate, and PDD the longest. Likewise, levodopa equivalent dose (LED) was highest in PDD, intermediate in PDMCI, and lowest in PDN. Groups did not differ significantly in years of education (Table 1).

At baseline, 60% of the PD patients were receiving levodopa replacement and 11% were on anticholinergic therapy. While antiparkinsonian medications may influence the MRS measures [32], we did not identify any significant effect of medication on MRS ratios (largest $T < 1.88$). Of the 351 individual assessments that included the full suite of MRS spectra, clinical, and neuropsychological testing, 43 (11 controls, 21 PDN, nine PDMCI, and two PDD) were excluded due to partial or complete failure to quantify MR spectroscopy ratios. This generally occurred due to widening of the spectral peaks and poor water suppression, which was most likely due to head motion. The remaining 308 individual assessments were included in further analyses.

No significant differences were identified when we investigated whether the excluded subjects differed from the remaining study. Figure S.2 shows examples of acceptable and degraded quality spectra.

3.2. Neuropsychological assessment and MR spectroscopy markers

At baseline, global cognitive z score decreased across cognitive groups, in a stepwise fashion from healthy controls to PDN, PDMCI and PDD. In contrast, there were no significant differences in any of the MRS ratios across groups (Table 2). When covariates (age, sex and UPDRS III) were not considered, NAA/Cr and Cho/Cr were significantly different in PDD relative to controls (Table S.2). Scanner software version did not affect the MRS ratios. At follow-up, relative to the controls' rate of global cognitive z score change, cognitive ability in PDN and PD-MCI groups declined at a faster rate. We found no significant change in any of the MRS ratios over time in any of the groups (Table 3 and Figure S.3). Moreover, those who converted ($n = 6$) from PDN or PD-MCI to PDD were not significantly different from those that remained cognitively stable in terms of baseline MRS values or rate of MRS change. Hence, converters were included in our analysis (Table S.3). The relationships between MRS measures and global cognitive ability were very weak ($R2 = 0.02$ for NAA/Cr, $R2 = 0.0003$ for Cho/Cr, $R2 = 0.0002$ for mI/Cr). This was also the case for the relationships between change in MRS measures and change in global cognitive ability ($R2 = 0.01$ for NAA/Cr, $R2 = 0.004$ for Cho/Cr, $R2 = 0.003$ for mI/Cr).

4. Discussion

This is the first study to examine the association between changes in brain metabolites in posterior cingulate cortex (via single voxel proton MRS) and changes in cognitive ability in PD over time. At baseline, when PDN, PDMCI and PDD were compared to controls, no significant group differences in MRS ratios were found, once age and motor impairment was accounted for. More importantly, longitudinal assessment for up to four years found no significant change in MRS metabolite ratios and no significant relationship between participants' cognitive performance tests and MRS measures.

Table 2
Group differences at baseline (with age, sex, UPDRS III, scanner version and spectrum line width as covariates).

	HC (95% PI)	PDN − HC (95% PI)	PDMCI − HC (95% PI)	PDD − HC (95% PI)
Global cognitive ability	0.53 (0.37–0.70)	−0.32[a] (−0.48 to −0.16)	−1.23[a] (−1.42 to −1.03)	−1.53[a] (−2.33 to −0.60)
NAA	369 (337–402)	0.34 (−2.25–5.05)	−3.87 (−22.42–1.75)	0.84 (−11.01–20.22)
Cho	110 (99–122)	0.07 (−2.08–2.45)	−1.85 (−8.36–0.88)	1.07 (−3.33–13.17)
mI	76 (65–87)	−0.19 (−2.66–1.62)	−0.50 (−4.12–1.33)	6.43 (−1.24–44.61)
NAA/Cr	1.64 (1.52–1.76)	0.013 (−0.06–0.08)	0.002 (−0.07–0.07)	−0.248 (−0.53–0.01)
Cho/Cr	0.50 (0.45–0.56)	0.01 (−0.02–0.04)	0.01 (−0.02–0.05)	0.08 (−0.056–0.21)
mI/Cr	0.3478 (0.27–0.42)	0.0003 (−0.04–0.04)	0.0272 (−0.02–0.08)	0.1615 (−0.01–0.32)

Mean difference estimates for PDN, PDMCI, and PDD are relative to controls. 95% PI = 95% Probability Interval.
Abbreviations: HC = Healthy Controls, PDN = Parkinson's Disease with Normal cognitive ability, PDMCI = Parkinson's Disease with Mild cognitive Impairment, PDD = Parkinson's Disease with Dementia, NAA = N-acetylaspartate, Cho = Choline, mI = Myo-inositol, Cr = Creatine.
[a] Significantly different relative to healthy controls.

Table 3
Annual rate of change of global cognitive ability and MRS measures (with age, sex, UPDRS III, scanner version and spectrum line width as covariates).

	HC unit/year (95% PI)	PDN unit/year − HC (95% PI)	PDMCI unit/year − HC (95% PI)
Global cognition	0.007 (−0.0368–0.0510)	−0.068[a] –(−0.1278 to −0.0058)	−0.141[a] –(−0.2296 to −0.0410)
NAA	3.625 (−0.1612–8.4370)	0.112 (−1.9765–2.6536)	−0.391 (−5.1579–1.6808)
Cho	2.036 (0.3164–3.6604)	0.297 (−0.8298–1.7050)	−0.206 (−1.8846–0.9263)
mI	2.002 (0.1816–3.7114)	0.473 (−0.5728–2.0672)	−0.335 (−2.0346–0.8710)
NAA/Cr	−0.013 (−0.0333–0.0064)	−0.008 (−0.0265–0.0108)	−0.003 (−0.0292–0.0216)
Cho/Cr	0.002 (−0.0059–0.0101)	0.004 (−0.0045–0.0122)	−0.004 (−0.0157–0.0072)
mI/Cr	0.007 (−0.0065–0.0203)	0.005 (−0.0086–0.0179)	−0.001 (−0.0281–0.0096)

Values with negative signs represent the decrease in either the subjects' global cognitive score or MRS measures over time. 95% PI = 95% Probability Interval.
Abbreviations: HC = Healthy Controls, PDN = Parkinson's Disease with Normal cognitive ability, PDMCI = Parkinson's Disease with Mild cognitive Impairment, PDD = Parkinson's Disease with Dementia, NAA = N-acetylaspartate, Cho = Choline, mI = Myo-inositol, Cr = Creatine.
[a] Significantly different relative to healthy controls.

4.1. MRS metabolites at baseline

Several smaller cross-sectional studies have examined the PCC in Parkinson's disease using MR spectroscopy, and with inconsistent findings. Griffith and colleagues reported that NAA/Cr was reduced in PDD (n = 12) relative to controls in one study [16] and relative to both controls and non-demented patients in another [17]. Similarly, Camicioli et al. reported reduced NAA/Cr in non-demented PD (n = 12) relative to controls [18]. However, three other studies (n = 44, 12 and 20, respectively), consistent with our findings here, reported no significant difference in NAA/Cr between controls and cognitively unimpaired PD patients [19,28,33]. A more recent study revealed that relative to both controls and non-demented patients, a significant increase in Cho/Cr in PDMCI was found, but not in PDD, perhaps due to the small number of PDD patients (n = 6) [34].

NAA is generally regarded as a marker reflecting neuronal integrity [5,6]. Dautry and colleagues reported that reduction in NAA reversed after ceasing neurotoxic administration to rats and primates, suggesting that neuronal dysfunction precedes cell degeneration [35]. Similarly, NAA can exhibit reversible changes. In humans for example, MR spectroscopy observations of initially reduced NAA followed by later recovery have been reported in multiple sclerosis, global brain ischemia, and acute brain injury [36–38]. These observations suggest that the decrease in NAA may not be specific to neuronal loss, but may reflect interruption of neuronal metabolism that, in some cases, is reversible [39].

In agreement with three earlier studies, we found no significant change in Cho/Cr or mI/Cr [16,17,34]. Choline containing compounds are considered to be cell membrane markers. Gliosis involves high membrane turnover [40–42] and choline concentration is at least three times higher in glial cells than neurons. [43], On the other hand, mI is regarded as a glial cell marker [6,44] and so many have linked the increased mI peak in MRS to gliosis. A recent pathological study revealed that glial cells have a deleterious role in the initiation and progression of PD [45]. Our results suggest that any measurable change of Cho/Cr and mI/Cr in PDD is relatively small, at least in the PCC. However, when covariates were removed from the model, NAA/Cr and Cho/Cr were both significantly different in PDD at baseline. As the PDD group was older, had more males, and with greater motor severity, this finding emphasises that covariates must be considered to obtain an accurate estimate of the independent effect of cognitive impairment.

4.2. Longitudinal observations

The present study included a large number of PD patients followed for up to four years after initial assessment (a total of 351 MRI scans), with comprehensive clinical and neuropsychological evaluation, allowing cognitive classification based on the MDS level II criteria [1,26]. The control group exhibited stable cognitive status over time, measured by global cognitive z score. The PDN group demonstrated decreasing cognitive performance over time relative to controls, while PDMCI had the highest rate of decline over time (PDD was an endpoint and therefore were not followed up). That we did not find useful patterns of MRS change over time indicates that MRS may not be a feasible longitudinal biomarker of cognitive ability in PD. Fig. 1 demonstrates the inability of NAA/Cr to track cognitive progression; (A) shows a relatively random trajectory of MRS change across the four cognitive groups, while (B) displays cognitive z score over time for comparison.

This failure of spectroscopic measures to track cognitive decline in PD is perhaps surprising given the positive results in Alzheimer's disease (AD). A recent meta-analysis reported that seven MR spectroscopy studies in AD have identified abnormal metabolic changes over time in PCC of MCI patients relative to controls [8]. Our findings suggest that the posterior cingulate cortex in PD is not as useful a metabolic indicator as it is for AD. We did not include a group of Alzheimer's disease patients. Future studies directly comparing AD and PD will be better placed to further investigate this issue.

In this study, we assessed only the PCC. However, many other brain regions are involved in PD [15,46]. It is therefore possible that brain regions other than the PCC may show abnormal MRS ratios. There is evidence to suggest that abnormal MRS ratios exist in pre-supplementary motor areas, anterior cingulate cortex, and occipital lobe [19,28,34]. It is possible that these other brain areas may hold more promise of capturing disease-related MRS change.

This study was of a clinical nature. For that reason, we used the MRS ratios as quantified by the scanner software (PROBE-Q). This automated approach would have made any positive findings immediately available clinically [20,21]. However, more sophisticated curve-fitting and processing (e.g. LCModel [47]) might have yielded more accurate concentration estimates. More advanced processing techniques may be superior in handling residual water and macromolecule removal, allowing more accurate quantification of metabolites and providing adequate power to measure additional metabolites [48]. However, previous work suggests that the PROBE-Q software provides acceptable inter- and intra-site variability [49] and does not necessarily provide significantly different results to more advanced methods, such as LCModel [48]. Furthermore, the scanner-derived clinical methods have successfully shown association with cognitive abilities over time in Alzheimer's disease [50–52]. However, we emphasize that the results presented were obtained from automated clinical software; it is therefore possible that offline processing may provide more accurate estimates of metabolite concentrations. But given the clinical nature of the study, we

believe that the use of the fully automated, verified, and clinically applicable (i.e. time efficient) PROBE-Q did not negatively affect our results.

Here, we report MRS ratios, with Cr as the reference metabolite. Ratios help correct for signal variations, regional susceptibility changes and partial volume effects, at a cost of reduced sensitivity and specificity [53]. It is generally assumed that Cr (the denominator of each ratio) remains stable, but this may not always be the case [5,39]. Some studies have shown that Cr is unstable in healthy individuals [21] and others report change with normal aging [54]. In our sample, we observed no significant change in Cr concentrations over time. Therefore, as in clinical practice, we used Cr as an accepted internal reference so that our findings could be compared with other reports in the literature [55,56].

Metabolite concentrations and relaxation times may vary with brain compartment, age or disease severity [53]. While tissue segmentation is ideal to account for such differences we did not perform the step due to its impracticality in a clinical setting.

In this study, we used a TE = 35 ms. One of the benefits of using long TE is producing less complicated spectra, where spectra will have flatter baselines and better water suppression due to the reduction of macromolecules effect. However, this may reduce the overall spectra amplitudes. Similarly, the use of very short TE (<20 ms) may also have a detrimental effect on the metabolite signals [57]. Seven studies that investigated the PCC in healthy controls and PD patients with varying TE (3× TE ≈ 30 ms, 2× TE ≈ 140 ms, and 2× TE = 80 ms) reported different findings. Two used TE ≈ 30 ms and reported deceased NAA/Cr [16,17], while Lewis et al. [28] (TE = 35 ms) found no change in the metabolites between PD and controls. The two studies that used TE = 80 ms, reported inconsistent findings [19,33]. Finally, studies implementing longer TE values (TE ≈ 140 ms) found disease-related changes in MRS metabolites [18,34]. While one group [Griffith et al.] has reported reduced NAA/Cr with TE ≈ 30 ms, the current results and those of Lewis et al., who also used a TE = 35 ms, question the clinical utility of single voxel MRS at TE ≈ 35 ms of PCC in reflecting progression in PD.

We attempted to minimize the potential effects of a number of confounding factors. Anti-Parkinsonian medication can influence MRS measures [32,58]. We therefore examined the effect of medication (LED and anticholinergics) and found no significant effect of medication on MRS ratios. Due to the long-term serial nature of the study, two software upgrades occurred during data collection, but acquisition parameters remained unchanged. We found no significant difference in MRS ratios across scanner software version.

5. Conclusion

With a large sample size and comprehensive neuropsychological assessment, we were unable to identify any significant change in MRS parameters relating to cognitive status at baseline or over time, once motor symptom severity and age were accounted for. Our findings suggest that MRS, of the PCC at least, is not a clinically useful biomarker of longitudinal change in cognitive impairment in Parkinson's disease.

Contributors

Almuqbel: Drafting/revising the manuscript, study concept and design, analysis and interpretation of data, acquisition of data, statistical analysis.

Melzer: Revising manuscript, study concept and design, interpretation of data, acquisition of data, study supervision, statistical analysis.

Myall: Revising manuscript, statistical analysis and advice, interpretation of data.

MacAskill: Revising manuscript, study concept and design, interpretation of data, obtaining funding.

Pitcher: Revising manuscript, acquisition of data, study coordination.

Livingston: Revising manuscript, acquisition of data, study coordination.

Wood: Revising manuscript, acquisition of data, study coordination.

Keenan: Revising manuscript and clinical interpretation of data.

Dalrymple-Alford: Revising manuscript, study concept and design, interpretation of data, study supervision, obtaining funding.

Anderson: Revising manuscript, study concept and design, interpretation of data, study supervision, obtaining funding.

Conflict of interest

Mustafa Almuqbel is a student at the University of Otago and received a doctoral scholarship from the University of Otago and a Saudi Arabian government PhD scholarship.

Tracy Melzer is employed by the University of Otago and has received support from the Canterbury Medical Research Foundation, Lotteries Health Research, New Zealand Brain Research Institute, Health Research Council of New Zealand, Brain Research New Zealand Rangahau Roro Aotearoa, and the Neurological Foundation of New Zealand.

Daniel Myall is employed by the New Zealand Brain Research Institute and has received support from the Canterbury Medical Research Foundation.

Michael MacAskill is employed by the University of Otago and has received research support from the Canterbury Medical Research Foundation, Neurological Foundation of New Zealand and the New Zealand Brain Research Institute.

Toni Pitcher is employed by the University of Otago and has received research support from the Neurological Foundation of New Zealand and the New Zealand Brain Research Institute.

Leslie Livingston is employed by the University of Otago and has received support from the Neurology Trust and the New Zealand Brain Research Institute.

Kyla-Louise Wood is a student at the University of Canterbury and received a doctoral scholarship from the New Zealand Brain Research Institute.

Ross Keenan is employed by Christchurch Radiology Group.

John Dalrymple-Alford is employed by the University of Canterbury and has received support from the Canterbury Medical Research Foundation, the Health Research Council of New Zealand, the Supreme Grand Royal Arch Chapter (Freemasons) of New Zealand, the Neurological Foundation of New Zealand, Lotteries Health Research, Brain Research New Zealand Rangahau Roro Aotearoa, and the Government Accident Compensation Corporation of New Zealand.

Tim Anderson is employed by the University of Otago, has received research support from the Canterbury Medical Research Foundation, the Health Research Council of New Zealand, and The Neurological Foundation of New Zealand, the Michael J Fox Foundation, Brain Research New Zealand Rangahau Roro Aotearoa, and has received honoraria from Boehringer Ingelheim and Allergan.

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Appendix A. Supplementary data

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