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MRS in Early and Presymptomatic Carriers of a Novel Octapeptide Repeat Insertion in the Prion Protein Gene

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Abstract

To evaluate the proton MR spectroscopy (¹H MRS) changes in carriers of a novel octapeptide repeat insertion in the Prion Protein Gene (*PRNP*) and family history of frontotemporal dementia with ataxia. Four at-risk mutation carriers and 13 controls were compared using single voxel, short TE, ¹H MRS from the posterior cingulate gyrus. The mutation carriers had an increased choline/creatine, $p=0.003$ and increased myoinositol/creatine ratio, $p=0.003$. ¹H MRS identified differences in markers of glial activity and choline metabolism in pre- and early symptomatic carriers of a novel *PRNP* gene octapeptide insertion. These findings expand the possible diagnostic utility of ¹H MRS in familial prion disorders.

Search terms

MRS; MRI; familial prion disorders; frontotemporal dementia

INTRODUCTION

MR spectroscopy (¹H MRS) has demonstrated the ability to detect unique patterns of brain metabolite levels in asymptomatic carriers of genetic forms of neurodegenerative dementias^{1–3}. In the prion encephalopathies MRS has identified changes in markers of neuronal integrity and glial cells^{3, 4}. Most of these studies have been in symptomatic patients and focused on abnormal areas revealed by other MRI sequences or in areas most commonly affected by pathology.

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Kejal Kantarci MD, MS from the Department of Radiology, Mayo Clinic, Rochester, Minnesota conducted the statistical analysis

COMPETING INTERESTS

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In this study of pre and early-symptomatic offspring carriers of a novel 288 base pair octapeptide repeat insertion in the prion protein gene (*PRNP*), we assessed the ability of short TE single voxel MRS to detect neurometabolic changes in an area of the brain, the posterior cingulate cortex (PCC), devoid of MRI changes in these cases. Because of the extensive connectivity of the PCC we hypothesized that the carriers would have early neurometabolite changes compared to age matched controls.

METHODS

Subjects

Carriers were recruited through the Alzheimer Disease Research Center of the Mayo Clinic because of known family history of a genetic PrP disorder. Each case underwent formal neuropsychometric testing and neurologic exam and informants were interviewed by trained staff to assess functional status. Parents of the carriers developed symptoms of frontotemporal dementia starting with cognitive and behavioral changes and later developed gait ataxia and seizures in their 5th decade that progressed over 7–10 years.

Table 1 provides details regarding demographic cognitive and clinical data for the cases, one of which was identified by informants to have demonstrated cognitive and functional decline in the previous 12–18 months.

Controls (n = 13) were free of any significant health, neurological or psychiatric disorders and matched by age to cases. This study was approved by the Institutional Review Board, and informed consent for participation was obtained from every subject or an appropriate surrogate.

¹H MRS and MRI

All subjects underwent ¹H MRS and MRI studies within a week of the clinical evaluation. Single voxel ¹H MRS and MRI studies were performed on a 3 Tesla scanner using an 8-channel phased array head coil (General Electric Medical Systems, Milwaukee, WI). After sagittal T1-weighted and Fluid attenuated inversion recovery (FLAIR) scans, We used a fully automated analysis package PROBE/QUANT (GE Healthcare) for the analysis of the MR spectra ⁵. Point resolved spectroscopy (PRESS) sequence with TR = 2000 ms, TE = 30 ms, 2048 data points and 128 excitations were used for the examinations.

An 8 cm³ (2x2x2cm) voxel, prescribed on a mid-sagittal T1 weighted image, included right and left posterior cingulate gyri and inferior precuneate gyri⁶. Metabolite intensity ratios calculated at the end of each PROBE /SV acquisition were analyzed. Quantifying metabolite intensities by referencing to a physiologically stable internal standard in neurodegenerative dementias, creatine (Cr) is preferred in clinical ¹H MRS, because internal referencing does not require correction for coil loading, atrophy and relaxation times and can readily be used in clinical practice with standard equipment and vendor-provided processing software. According to our quality control criteria, the spectra were excluded if the Cr signal to noise ratio is <5, however none of the MR spectra in the current study failed the qc procedure.

The posterior cingulate gyrus region is our standard MRS protocol for several important reasons relating to spectral quality⁶. The quality and reliability ¹H MR spectra from the posterior cingulate is significantly superior to the spectra from the frontal lobe, owing to greater magnetic susceptibility artifacts at the skull base. This is particularly true for short echo time spectra, and quantification of mI requires a short echo time ¹H MRS acquisition.⁷. Furthermore, the posterior cingulate voxel location can be identified by clear anatomical landmarks by trained technicians, which is critical for longitudinal serial measurements.

Genetic Analysis

The single coding exon of the *PRNP* gene was PCR amplified and sequenced using the following three primer sets:

1F; CAACATAAATATGGGACTCTG and 1R; GGCTTGTTCCACTGACTG,
2F; TGGAGGATGGAACACTGG and 2R; GGTGCTGTACTCATCCATG, and
3F; GCAGTGACTATGAGGAC and 3R; AGAGAAGAAAGAGTGAGACAC.

Following the initial identification of the 288bp insertion in the proband, primers 1F and 2R were used to span the mutation in subsequent reactions⁸.

Statistical Analysis

Standard descriptive statistics were performed, where possible, for the cases as a group in order to conceal subjects' genetic status. Between-group comparisons of ¹H MRS metabolite ratios were performed using Wilcoxon rank-sum tests.

RESULTS

Demographics and Clinical Data

The median age of our cases was 33 years, similar to the controls, 32. One subject had been diagnosed as symptomatic by informant report but the other cases were considered cognitively normal. All subjects demonstrated evidence of a postural tremor and abnormal eye movements, (Table 1). Performance on the Mattis Dementia Rating Scale showed wide variability with the greatest deficits in attention and memory components⁶.

¹H MRS

(Figure 1) demonstrates the range in values for 3 separate metabolite ratios compared to creatine as a reference value and the N-acetylaspartate (NAA) to myoinositol (mI) ratio⁷. Compared with controls, increased choline/creatine; increased myoinositol (mI)/Cr ratio were found. For the NAA/Cr ratio there were no differences between carriers and non-carriers, although NAA/Cr levels were widely varied.

Structural MRI

T1 images demonstrated evidence of mild cerebellar atrophy in one carrier with normal PCC volumes, compared with a normal control, (Figure 2). Diffusion Weighted Imaging (DWI) sequences were evaluated for all subjects and were normal to visual read. For illustrative purposes the spectroscopic profiles are provided.

DISCUSSION

In pre and early symptomatic carriers of a novel *PRNP* gene octapeptide insertion, we identified abnormal metabolite levels of choline and myoinositol in the posterior cingulate cortex (PCC). These changes may indicate the presence of increased glial cell activity and, consequent to or independent of, increased neuronal membrane turnover or altered acetylcholine metabolism in the absence of any significant loss of neuronal integrity.

As an early marker of disease ¹H MRS identified differences in carriers of an octapeptide insertion in the *PRNP* gene at an age that was approximately one decade before symptom onset in the previous generations. This further substantiates the sensitivity of this modality in identifying changes in cases in the earliest stages of hereditary dementias^{2, 3}. Although these findings are non-specific, in known OPRI mutation carriers it might prove helpful as a

recent report of a heterogeneous group of symptomatic subjects with *PRNP* repeat insertions found only 30 % had abnormal structural MRI⁹.

Because these cases were early and pre-symptomatic, we doubt that this neurometabolic pattern might be unique to the *PRNP* octapeptide-gene-associated neurodegenerative process. The few other studies that have assessed the utility of ¹H MRS in prion disorders have looked at sporadic cases or familial disorders with *PRNP* point mutations such as Gerstmann-Sträussler-Scheinker disease (GSS)^{3, 4, 10–12}. Nonetheless similarities to these studies identify some commonalities of aberrant prion protein (PrP). Specifically, the elevation of mI/Cr, in both the presence and absence of significant decrease in the NAA/Cr level, even in areas of brain without atrophy has commonly been identified in prion disorders. As the mI signal is mainly present in glial cells, the increase identified in this and other studies, including asymptomatic gene carriers with point mutations, may represent glial proliferation which pathologically is linked to the astrogliosis seen in prion disorders. However studies of presymptomatic microtubule associated tau protein (*MAPT*) carriers, as well as presymptomatic carriers of the presenilin 1 (*PS1*) and amyloid precursor protein gene (*APP*) mutations MRS showed abnormalities before symptoms appeared^{2, 3}. Thus, the changes we observed by ¹H MRS are unlikely unique to any single neurodegenerative disorder.

The significance of the elevation in choline is uncertain but has been identified in dementia with Lewy bodies, frontotemporal dementia and HIV and has been subsequently proposed to represent increased cell membrane turnover, altered acetylcholine metabolism and inflammation^{2, 6, 13}. Contrary to our results a previous study in spontaneous CJD did not identify differences compared to controls⁴. Neuropathology in the parents of our cases identified neurofibrillary tangles in the basal nucleus of Meynert which could result in altered cortical acetylcholine metabolism. Likewise, we might speculate that the elevated choline signal might be a reflection of inflammation paralleling that of mI given the striking similarities in pattern demonstrated in figure 1.

Alternatively, the MRS changes in our mutation carriers may not represent ongoing direct neurodegeneration in this area, but rather might reflect the down-stream effect of neurodegeneration in an area of the brain polysynaptically associated with the PCC^{14–16}. Recent work in non-human primates as well as functional connectivity MRI in the non-task specific state has identified correlations between the cerebellar cortex, particularly the posterior and lateral hemispheres and multiple, non-motor areas of the cerebral cortex, including the posterior-parietal cortex^{15, 16}. Thus the neurometabolic pattern revealed in this study in the PCC may reflect an early remote glial response to the cerebellar cell loss. This likely supports the lack of NAA decrease in this area of the brain and in relatively asymptomatic cases. We might expect the NAA to decrease as cognitive symptoms progress. Although a longer echo time might have provided a more reliable NAA signal we do not feel that our findings these findings to be unreliable. In fact the finding that the elevation in mI is an earlier feature of neurodegeneration than NAA has been confirmed in independent cohorts^{6, 17, 18} and pathologically validated¹⁹ in mild cognitive impairment and Alzheimer's disease and the inclusion of a long echo time MRS NAA measurement did not change this finding¹⁸. Furthermore, a similar study in of familial PrP point mutations also revealed an isolated elevations of MI in the absence of a decreased NAA³. Additionally, Because we found a significant decrease in NAA/mI in FTLN patients in the posterior cingulate voxel in a previous study⁶, we felt confident in this acquisition for the current analysis. We also referenced the metabolites to water as the reference with similar results (supplemental figure). Further work, following these subjects serially with structural MRI and MRS will be required, along with the possibility of correlating pathology at different stages of the disorder where possible.

Given the small number of cases our results require caution in interpretation. The lack of direct comparison to other neurodegenerative disorders at the early and presymptomatic phases precludes us from testing the specificity of MRS in prion disorders. Furthermore, we hypothesized that the carriers would have neurometabolite changes in the PCC because of the wide range of connectivity of this region with the rest of the brain. However it is possible that these metabolic abnormalities are more generalized across the brain. Nonetheless, given the changes identified in the PCC, a more extensive examination of the cortex, particularly those areas most affected at post-mortem study, might prove MRS to be a valuable pre-symptomatic biomarker in genetic prion disorders.

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Figure 1. ^1H MRS Metabolite Ratios for Cases and Controls

*- p value < 0.01, Wilcoxon rank-sum test; Cho- choline; Cr- creatine; ml- myoinositol; NAA-N-acetylaspartate

Figure 2. Sagittal MRI & ^1H MRS profile for Single Control and Case

A- Control

B- Case

Table 1

Clinical and Demographic Data

		Cases (n= 4)
Age;Median (range)		33 (28–38)
CDR;Median (range)		0.5 (0–0.5)
dysprosodia		3/4
Abnormal affect		4/4
hypokinesia		2/4
Tremors		4/4
Parkinsonism		0/4
ataxia		1/4
KSTM [*] ;Median (range)		31 (28–35)
DRS [†] ; Z score Median (range)	Total	−0.55 (0.54- -2.36))
	Attention	−1.56 (0.92- -3.44)
	Initiation/ Perseveration	0 (0.50- -0.50)
	Construction	0.33 (0.33)
	Conceptualization	0.31 (0.69- -2.77)
	Memory	−1.10 (0.33- -.2.05)

* Kokmen Short Test of Mental State- Maximum 38;

† Mattis Dementia Rating Scale- Maximum 144