Multivoxel MRS: right frontal parafalcine cortex – area of neurobiochemical gender differentiation?

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Abstract

OBJECTIVE: To determine the presence of gender neurometabolic differences in healthy men and women by multivoxel magnetic resonance spectroscopy (MRS).
MATERIALS AND METHODS: We performed multivoxel magnetic resonance imaging and spectroscopy in 50 healthy volunteers (27 women and 23 men) using 1.5T scanner. Spectra from 12 different voxels were obtained, covering frontal, paracentral, and parietal white and gray matter. Three dominant signals were analyzed: NAA, tCr and Cho, and expressed as ratios of Cho/tCr, NAA/tCr, NAA/Cho.
RESULTS: There was statistically significant gender difference between Cho/Cr and NAA/Cr metabolites ratio in only one location – the right frontal parafalcine cortex. There was no statistically significant difference in NAA/Cho ratio between men and women.
CONCLUSION: Our study suggests that right frontal parafalcine cortex is a sexually dysmorphic area and supports the value of multivoxel MRS as a method able to define spatial biochemical heterogeneity of the cerebral tissue.

ORIGINAL ARTICLE

INTRODUCTION

The development of sophisticated diagnostic modalities in neuroscience in the last two decades, such as volumetic measurements, proton emission tomography, functional magnetic resonance imaging (f-MRI) and brain topographic electroencephalography, markedly contributed in detection of morphologic differences between male and female brain. Nevertheless, sex differences were absent in majority of available MR spectroscopic studies of brain metabolism (Charles *et al.* 1994; Bernard *et al.* 1996; Pouwels & Frahm 1998; Komoroski *et al.* 1999). However, it has been suggested that alterations of brain metabolites concentration is different between sexes during the process of ageing (Kadota *et al.* 2001; Sijens *et al.* 2003). Also, higher level of NAA was observed in women in the areas of sensorymotor and orbitofrontal cortex (Grachev & Apkarian 2000), while significantly lower NAA/

Cho and higher Cho/Cr ratio were shown in the parietooccipital white matter in men (Wilkinson *et al.* 1997).

The aim of this study was to determine the presence of gender neurometabolic differences in healthy men and women by multivoxel MR spectroscopy (MRS).

MATERIALS AND METHODS

Participants

We conducted an ethical-board-approved study on 50 healthy volunteers aged 30 to 58 years: 26 women (mean age 49.14±3.11) and 24 men (mean age 47.67±3.48). All the subjects signed a fully-informed written consent. Volunteers were screened for histories of diabetes, stroke, coronary artery disease, renal failure, liver failure, alcohol abuse, and psychiatric illness. All participants received a Mini-Mental State Examination. None of the participants were excluded neither for dementia nor history of neurologic or psychiatric diseases. None had undergone or was undergoing any therapeutic treatment.

Magnetic resonance imaging and spectroscopy

Magnetic resonance imaging and spectroscopy were performed on 1.5T scanner (Siemens Avanto Tim, Erlangen, Germany) using matrix head coil (receiver coil) in CP (circularly polarized) mode. Sagital T1 weighted spin-echo sequences with TR/TE of 511/8.7 milliseconds, axial T2 weighted turbo spin-echo (TSE) sequences with TR/TE of 8590/98 ms, coronal T2 TSE TR/TE 5170/105 3 mm slice thickness, were obtained in orthogonal orientation for image guided localization of the spectroscopic imaging slab. Axial FLAIR with TR/TE 8840/109, 5.0 mm slice thickness was obtained to exclude any pathological process. Proton 2D MR Spectroscopic Imaging data sets were acquired with point-resolved spectroscopy TR/TE 1500/135. The CSI slab size: Field of view (FOV) 160×160×160 mm; VOI 80×80×80 mm, thickness 10 mm, was positioned parallel to the axial images, immediately above the corpus callosum along the anterior-posterior commisure to encompass the semioval white matter and the cortical gray matter. Number of phase encoding steps (scan resolution) was 16 in all directions (R-L, A-P and F-H). Interpolation resolution was 16 in all directions resulting in VOI of 10×10×10 mm. Number of acquisitions were 4, scan time 7 min 12 s. The Weighted phase-encoding scheme was applied. Interfering signal contributions from areas outside the VOI were suppressed by 6 saturation regions, manually positioned along the margin of the VOI. The homogeneity of the magnetic field is optimized over the VOI using an automatic, volume-selective shimming method. We took care to position the region of interest in the same way in every subject in order to achieve the highest possible level of reproducibility, taking into account anatomical variations.

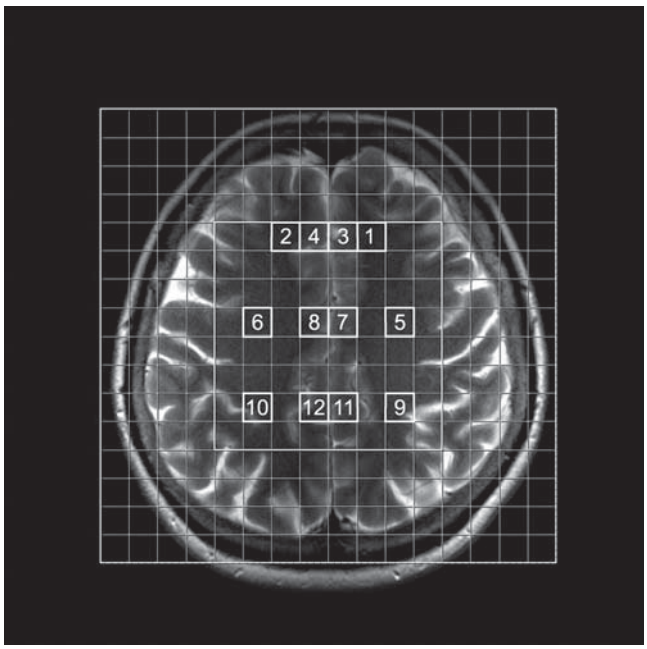

Fig. 1. Axial MR image of a healthy 22-year old man shows the outlines of the spectroscopic VOI and typical position of 12 voxels in the centrum semiovale. Six voxels were in bilateral anterior, middle and posterior regions containing predominantly white matter (voxel numbers 1, 2, 5, 6, 9, 10) and six voxels were in bilateral mesial cortex with mostly gray matter of the anterior, middle and posterior regions (voxel numbers 3, 4, 7, 8, 11, 12).

Data Analysis

The raw data were evaluated automatically using a commercially available spectral analysis software package (Syngo Multi Modality Workplace version VE23A). The post processing protocol included: water reference processing by averaging 20 adjacent points, removing the residual water signal from the spectrum by subtracting it from the time signal and frequency shift correction of the water signal, Hanning filter 512 ms width, Zero-filling from 512 to 1024 data points and Fourier transformation. After baseline correction by polynomial fitting and phase correction, the spectra were quantified using Gaussian curve fittings to measure the areas under the peaks. In order to test gender differences, 12 individual voxels were selected: six areas were located in bilateral parasagittal anterior, middle, and posterior cortices, characterized primarily by frontal, paracentral, and parietal mesial gray matter, and six were in lateral anterior, middle, and posterior regions containing predominantly frontal, precentral, and parietal white matter. (Figure 1). A total of 600 spectra were analyzed in this study. The raw data were evaluated automatically by using a comercially available spectral analysis software package (Syngo MultiModality Workplace version VE23A). The post processing protocol included: water reference processing by averaging 20 adjacent points, removing the residual water signal from the spectrum by subtracting it from the time signal and frequency shift correction of the water signal, Hanning filter 512 ms width, Zero-filling from 512 to 1024 data points

and Fourier transformation. After baseline correction by polynomial fitting and phase correction, the spectra were quantified using Gaussian curve fittings to measure the areas under the peaks. Three dominant signals were analyzed: Choline (Cho) at 3.21, Creatine plus phosphocreatine (tCr) at 3.04 and N-Acetylaspartate (NAA) at 2.02 ppm and expressed as ratios of Cho/tCr, NAA/tCr, NAA/Cho (Figure 2). Descriptive statistics included: normality of distribution tests (measure of the asymmetry skewness and peakedness of the probability distribution, Kurtosis and Kolmogorov-Smirnov test), Mean, Standard Deviation (SD), Minimum and Maximum, Coefficient of Variance (CV) and Confidence Interval. Using both Multivariate analysis of variance (MANOVA) and Discriminant Analysis (DA) we tested overall gender differences. When MANOVA was significant we applied Analysis of Variance (ANOVA) to evaluate the gender differences in twelve locations. DA was used to determine which location account the most for the differences between genders.

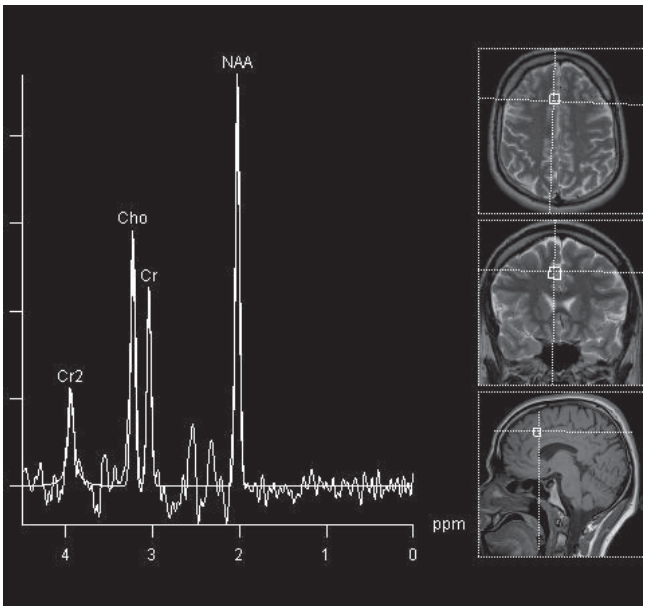

Fig. 2. Spectra obtained in right frontal parafalcine cortex.

RESULTS

According to the descriptive and dispersion parameters, the sampling distribution was approximately normal for all ratios in all locations observed.

Cho/Cr

The overall differences between gender groups in twelve locations were significant for Cho/Cr (MANOVA, $p<0.05$; DA, $p<0.05$). An analysis of variance ANOVA showed that the effect of examinees' gender was significant in location 4 ($p<0.05$) (Table 1). Results of t-test for location 4 are shown in Table 2. According to discriminant analysis (DA) the location 4 contributes most to discrimination among genders for Cho/Cr, Discrimination coefficient DC=0.468.

NAA/Cr

On the basis of MANOVA ($p=0.086$) there was no statistically significant difference between genders for NAA/Cr, but DA ($p<0.05$) showed that gender difference existed in some locations. By applying ANOVA in all 12 locations we found signifficant difference in location 4 ($p\leq0.05$) (Table 3). Results of t-test for location 4 are shown in Table 4. According to discriminant analysis the location 4 contributes most to discrimination among genders for NAA/Cr (DC=0.372).

NAA/Cho

We found no statistically significant difference in NAA/Cho (MANOVA, $p=0.719$; DA, $p=0.093$).

Tab. 1. Results of Analysis of Variance (ANOVA) Comparing Cho/Cr between genders in twelve locations.

Location	F	*p*-value
1	0.820	0.370
2	0.391	0.535
3	0.256	0.616
4	11.492	0.001
5	0.209	0.650
6	1.383	0.246
7	1.348	0.252
8	0.067	0.798
9	0.066	0.798
10	0.391	0.535
11	0.495	0.485
12	3.154	0.082

Tab. 2. T-test for Cho/Cr in location 4.

Location	Mean		t	*p*-value
	Male	Female		
4	1.237	1.083	3.390	0.001

DISCUSSION

Advancements in volumetric measurements of the brain segments revealed that inferior parietal lobule was markedly larger in men than in women (Frederikse *et al.* 1999). The volume of this structure was significantly more prominent on the left-hand side. On the contrary, it has been found that women had 23% and 13% more voluminous area of Brocka and Wernicke, respectively, compared to men (Schlaepfer *et al.* 1995). Kadota *et al.* found significant regional and sex differences during the process of maturation, growth and

Tab. 3. Results of Analysis of Variance (ANOVA) Comparing NAA/Cr between genders in twelve locations.

Location	F	*p*-value
1	2.892	0.096
2	1.908	0.174
3	0.477	0.493
4	4.020	0.050
5	0.054	0.816
6	1.426	0.238
7	0.224	0.638
8	0.266	0.608
9	0.055	0.815
10	2.547	0.117
11	0.017	0.897
12	0.188	0.667

Tab. 4. T-test for NAA/Cr in location 4.

Location	Mean		t	*p*-value
	Male	Female		
4	1.732	1.572	2.005	0.050

ageing. Spectra were obtained from the specific voxels of 2.5cm^3 in the supratentorial gray matter and white matter of the centrum semiovale. But this study was focused exclusively on NAA/Cho ratio (Kadota *et al.* 2001). Sijens *et al.* measured Cho/NAA ratio and found increasing sex differences at the advanced age of 77, while yet not statistically significant at the mean age of 73 (Sijens *et al.* 2003). Our study showed significant gender neurometabolic difference exclusively in the right frontal parafalcine cortex in the group where majority of examinees were mid-aged volunteers, with Cho/Cr ratio being increased in men. No significant gender differences in any of analyzed locations were noted regarding NAA/Cho ratio.

Right frontal parafalcine cortex, including anterior cingulate girus is presumed to play a role in executive processes. Morphologic studies showed a high degree of fisurization variability within cingulate girus (Huster *et al.* 2007; Vogt *et al.* 1995; Vogt *et al.* 2003). It has been found in numerous studies that prefrontal cortex is sensitive to sex and gonadal hormone environment of animals. Young adult males, when compared to young adult females, have greater spine density of both apical and basilar dendritic branches (Kolb & Stewart 1991; Kritzer 1999; 2000). The sex difference in dendritic arborisation of layer V and layer IV neurons of this area in both rats and meadow voles has been reported (Kolb & Stewart 1991; Markham & Juraska 2002).

Our multivoxel MRS study showed that right frontal parafalcine cortex is a sexually dysmorphic area. It strongly supports the value of multivoxel MRS. This method is able to define spatial heterogeneity of the mass and adds new information relevant to both diagnostic purposes and clinical management (Nelson 2003; Kozic *et al.* 2007). Recent publications showed the presence of marked biochemical abnormalities on MRS not only in patients with normal MRI but also in mutation carriers with no clinical manifestation of the disease (Ostojic *et al.* 2009). Recent study of three-dimensional multivoxel MRS of the healthy hippocampus showed marked biochemical differences among the head, body and tail of this structure with excellent resolution of spectra with voxel sizes of only 0.5 cm^3, suggesting that this diagnostic modality may have more potential applications not only in research but in clinical work as well (Ostojic *et al.* 2010). However, our results most likely could not yet be implemented in routine clinical practice since the neurometabolic differences become obvious only when larger population is examined and sophisticated statistical analyses are performed.

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