**Regional Cortical Atrophy in patients with RRMS and cognitive impairment: a multi-center study**

J.M. Tillema, H. Hulst, M.A. Rocca, H. Vrenken, M. Steenwijk, A. Rovira, Enzinger, Ciccarelli, de Stefano, ……., M.Filippi, F. Barkhof for the MAGNIMS group***

**Current Institutional Affiliations:**

[xx] Department of Radiology and Nuclear Medicine, VU University Medical Center, Amsterdam, The Netherlands.
[xx] Department of Neurology, Mayo Clinic, 200 First St. SW, Rochester, Minnesota 55905
[xx]
[xx] Neuroscience Campus Amsterdam, Amsterdam, The Netherlands.

*** the steering committee members of MAGNIMS (www.magnims.eu) are…..

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**Introduction:**

Cognitive impairment in patients with Multiple Sclerosis (MS) is a frequent phenomenon, reported in up to 65% of patients. [1, 2] Studies of MRI correlates to cognitive dysfunction have shown that atrophy measures are better predictors of cognitive impairment than white matter lesion volume. [3-6] Furthermore, structural MRI studies have shown relations between cognitive impairment and number of cortical lesions [7], diffusion tensor imaging changes [8, 9], gray matter magnetization transfer ratio [10] and deep gray matter volume loss. [4]

Cortical involvement in MS has been increasingly recognized in recent years and the presence of cortical lesions is well described both on MRI and histopathology. [11-13] Cortical atrophy is associated with cognitive impairment in many neurodegenerative diseases, but also in MS. Cortical atrophy can be assessed with intensity based segmentation methods or via surface based methods. The latter approach provides sub-millimeter measurements of cortical thickness. Such methods have been validated to assess cortical thickness in a variety of neurological conditions. Regional areas of cortical thinning relate to clinical disability scores and lesion volume.[14] Bilateral thinning of the frontal and temporal cortex has been reported in MS using Freesurfer (http://surfer.nmr.mgh.harvard.edu/) [14-16] and other volumetric MRI analysis methods [17, 18]. This cortical thinning might be present already in the earliest stages of MS,[19] even in the absence of cognitive impairment.[15] However, there has been no well-defined large cohort of patient with RRMS and cognitive impairment, where cortical thickness has been studied and related to cognitive function. Involvement of the parietal lobe seems to potentially occur later in the disease and parietal thinning have been reported to be prominent in patients with RRMS and cognitive impairment (CI), where these areas were preserved in patients with RRMS without CI. [15] Another study found that significant cortical thinning was present solely in very small areas of the parietal and frontal lobe in MS patients compared to healthy controls[16], although cognitive status was not included.

Focus on cognitive impairment in MS is an important feature of current research as it affects quality of life [20] and imaging correlates could facilitate the development and treatment of cognitive impairment in MS. To explore the MRI correlates of cerebral cortical atrophy in relation to CI in MS we carried out a multi-center study. In the MAGNIMS network 7 centers participated to evaluate the extent of regional cortical atrophy in patients with RRMS with

cognitive impairment (CI) and preservation of cognition (CP) compared to healthy controls. We used Freesurfer to study cortical thickness in all subjects.

**Methods:**

***Participants:*** This cross-sectional study was conducted in seven MAGNIMS (www.magnims.eu) centers. Institutional ethics review boards in each participating center approved the study and all subjects provided written consent for participation. Inclusion criteria were a diagnosis of clinically definite RRMS and age > 18 years. Each center recruited patients with MS with known cognitive impairment and cognitively preserved patients, as well as healthy controls. Clinical information on patients were collected including Expanded Disability Status Scale (EDSS) at time of MRI, disease duration, use of disease modifying treatments and years of education.

***Cognitive testing:*** All patients underwent the Brief Repeatable Battery of neuropsychological tests (BRB-N) by trained local personnel in their native language. The BRB-N includes the selective reminding test (SRT), spatial recall test (SPART), controlled oral word association test (COWAT), paced auditory serial addition test (PASAT), symbol digit modalities test (SDMT) and word list generation (WLG). Results of each test were scored according to published rules [REF - ] and the BRB-N has been used for determination of cognitive impairment in Multiple Sclerosis.[1, 2] Individual test scores were combined and z-scores were calculated for four cognitive domains (verbal memory, visual memory, attention and fluency), based on published data from healthy controls. [21] Subjects that failed more than two tests were defined as cognitively impaired (CI).

***MRI data acquisition:*** All subjects at each individual site underwent an MRI using a standardized protocol. The information on scanner manufacturer, 3D-T1 acquisition parameters and enrollment numbers per site are listed in table 1. Additional axial T2/PD images were obtained at all sites for lesion scoring purposes.

***Lesion measurements:*** WM lesions were marked and outlined on the PD/T2 and T1-weighted

images to determine T2 lesion (including T2 lesion volume (T2LL) and T1 lesions (including T1 "black hole" lesion volume (T1BH-LL) using … [software, scoring person?]

***MRI data analysis: cortical thickness and surface based cortical volume***

Surface-based cortical analysis was performed using Freesurfer 5.1.0 (http://surfer.nmr.mgz.harvard.edu), technical aspects of the involved procedures have been described in detail elsewhere. [22-26] Briefly, the processing pipeline includes motion correction, removal of non-brain tissue using a hybrid watershed/surface deformation procedure, intensity normalization, transformation to standard space, segmentation of subcortical white and gray matter, tessellation of gray/white-matter boundary, automated correction of topological defects, and surface deformation to form the gray and white matter boundaries. [24, 27-30] Cortical thickness measures, calculated as the closest distance from the gray/white boundary to the gray/CSF boundary at each vertex on the tessellated surface, capable of detecting sub-millimeter differences between groups.[23] Cerebral cortex was parcellated into six lobar structures based on gyral and sulcal structure, [31, 32] with extraction of average hemispheric and lobar regional cortical thickness and surface based cortical volumetric measures. Surface based volumes were obtained per lobar region and normalized by multiplying the native cortical grey matter volumes with the scaling factor for registration to the MNI template. Cortical thickness measures have been validated against histology [33] and manual measurements [34, 35] and these morphometric procedures have been demonstrated to show good test-retest reliability across scanner manufacturers and across field strengths. [36] (Example in figure 1)

Visual inspection was performed on each scan for accuracy; dural segments were removed manually and white matter control points were placed when initial segmentation steps had suboptimal performance. Subsequent continuation of the processing pipeline resulted in good results in the majority of the scans. Though accurate cortical thickness measures can be affected by juxta-cortical and mixed cortical lesions (extending into the subcortical white matter), we opted to perform the analysis on unfilled T1 weighted images. The potential overfilling of lesions would artificially decrease the cortical thickness and create bias towards exaggerated atrophy in patients with significant lesion load in juxta-cortical distribution. All segmentations in control subjects were of good quality and in the MS group there were some patients that needed manual adjustments of the white matter segmentation maps. To inspect for the lesion burden and the effect on cortical thickness measures we created a color overlay of the lesion maps on the initial segmentation maps, where lesions that were

accurately recognized as white matter were colored green and areas identified as lesions by manual tracing that were classified as non-white matter by the segmentation process were colored red. These maps were used to manually fill the segmentation maps of the lesions accurately and this was fed back into the processing pipeline to calculate white and grey matter surfaces. An example of this is provided in figure 1. When this process did not result in accurate correction of the surface maps, the subject was excluded from further analysis, which led to exclusion of 4 MS cases with a significant burden of subcortical lesions (figure 1C).

***Statistical analysis:*** Statistical analysis of demographic, clinical data and regional thickness and surface based volumes was performed in SPSS 21.0 (Chicago, IL). In normally distributed variables, multivariate GLM was used, using center, age and gender as covariates. Variables not normally distributed were log-transformed and included in the analysis. A p-value $< 0.05$ was considered statistically significant.

Vertex-based General Linear Model (GLM) approach using the QDEC-tool (http://surfer.nmr.mgh.harvard.edu) was applied to analyze the correlation between vertex-based cortical thickness and cognitive testing (z-scores as variable), correcting for center, age and gender. We used a Gaussian smoothing kernel with full width at half maximum of 10 mm. Significance was set at $p<0.01$ with correction for multiple testing.

**RESULTS:**

Demographic and clinical information are summarized in table 2. Sixty patients with RRMS and sixty-five control subjects (58% female) were enrolled. In the patient group, four patients were excluded based on extensive white matter lesion load affecting the Freesurfer process (2 CP, 2 CI). Of the fifty-six patients (63% female), twenty (36%) were defined as cognitively impaired and thirty-six (64%) as cognitive preserved. Of the cognitively impaired patients, 7 subjects had failed 2 tests and 13 subjects had failed 3 or more. Among the cognitively preserved patients, 7 subjects failed 1 test. Control subjects were slightly younger ($p=0.048$) than patients and had similar gender distribution ($p=0.40$). Between cognitively preserved and impaired MS patients, there were differences in age ($p=0.012$), EDSS ($p=0.03$) and disease duration ($p=0.03$), but not gender. In CI patients, the most commonly affected domain was attention ($z=-1.4$), followed by visual memory ($z=-1.1$) and verbal memory ($z=-0.85$). Fluency was not significantly different between CI and CP patients ($p=0.10$).

***Global cortical thickness:*** The average cortical thickness was similar for left and right hemispheres in all subgroups. Average cortical thickness was significantly different between controls (2.54 mm) and MS patients (2.44 mm, $p<0.001$). CI and CP patients had no significant difference in global cortical thickness.

***Vertex-wise cortical thickness:*** Differences between control subjects and MS patients were most pronounced in the superior and middle temporal gyrus and inferior parietal regions. (figure 4A) Direct comparison between CP and CI patients with MS showed no statistical differences in vertex based cortical thickness measures. No significant correlations were seen in this analysis within the MS group for EDSS, disease duration and global z-scores of the BRB-N. There was a significant correlation between white matter lesion volume and cortical thickness (figure 4B).

***Lobar region-wise analysis:*** Regional cortical thickness was defined using the Desikan-Killiany atlas into six lobar regions (cingulate, frontal, insula, occipital, parietal and temporal) and mean thickness in these regions were compared between subgroups. Given the correlation between age and cortical thickness (figure 3), as reported by others [35, 37, 38], we reduced the cognitively preserved patient group to match the CI group for sub-analysis. These subgroups

had no statistically significant different distribution of age, EDSS and disease duration. Using multivariate GLM analysis using center and gender as covariates (table 3) significant differences in cortical thickness were only found to affect the temporal lobe (difference of 0.1 mm between groups, p=0.05), using age, gender and center as covariate. There was a trend towards significance in the cingulate (p=0.055) between CI and CP groups.

***Correlation clinical features and cortical thickness:*** In the MS patient group, partial correlations with correction for age, gender and center showed significant correlations between regional cortical thickness and EDSS (-0.34, p=0.01), age (-0.27, p=0.04) and lesion volumes (both T2-LL (-0.51, p<0.001) and T1BH-LL (-0.46, p<0.001)). These correlations for EDSS and lesion volumes were only statistically significantly different in the frontal, temporal and parietal lobes.

***Correlation cognitive subtest and cortical thickness:***

The obtained z-scores for each of the tested cognitive domains (verbal memory, visual memory, attention and fluency) were correlated to regional cortical thickness in the total MS group using partial correlations with correction for age, gender and center (n=56). This showed significant correlations between verbal memory and cortical thickness in the insula (p=0.035) and between visual memory and parietal cortex (p=0.044), with a trend for occipital cortex (p=0.055).

## DISCUSSION

In this multi-center study we found differences between cortical thickness in control subjects and patients. The magnitude of differences in cortical thickness between the study groups was smaller than previously reported in single-center studies. [15] Similar to a recent multi-center study on cortical thickness in MS,[38] frontal cortical thinning between patients and healthy controls was less strong than previously reported. [14, 15] The average global thickness differences that were found between control and MS groups were in the order of 0.1 mm. This is in contrast with a single center study where these differences in cortical thickness were four to five times larger in respectively mild and severely cognitively impaired patients.[15] The magnitude of our findings in the MS group is comparable to mild cognitive impairment in prior studies. [39] Despite the absence of striking global atrophy between cognitively impaired and preserved patients with MS, we did show (subtle) regional difference between cortical thickness in CP and CI patients. No significant differences were found using vertex based surface comparisons between the two subgroups, likely related to the limited size of the differences between the two subgroups, relatively small sample size and the possible regional heterogeneity of the cortical thinning. Multivariate GLM region-wise analysis revealed significant reductions in cortical thickness in temporal lobe with a trend for reduced cingulate cortical thickness. In addition, worse performance in specific cognitive domains was correlated to regional cortical thickness measures. Attention and visual memory scores were most affected in the CI group, and verbal memory scores correlated to regional cortical thinning in the insula where visual memory scores correlated to parietal thinning.

Total gray matter atrophy and regional analysis using other analysis modalities (e.g. voxel based morphometry (VBM) and other software investigating cortical thickness measures [40, 41]) have previously been reported to show regional variances in gray matter volume in multiple sclerosis, most pronounced in the frontal, temporal and parietal lobes and are present even in the earlier stages of the disease. [17, 40-43] Studies on regional cortical volume loss and cognitive impairment in MS have been limited, but have shown findings of regional variation between cognitively impaired and cognitively preserved patients with relapsing remitting MS. A recent study using VBM found left superior temporal gyrus, left insula and right middle occipital gyrus atrophy in CI compared to CP patients with RRMS. [44] Another study did not replicate these finding and although volumetric differences between CI and CP were found, no significant regional difference between these groups. [8] These were both

relatively small sample studies from single centers and these findings underscore that possibly multifactorial interplay between lesions, white matter integrity and regional deep and cortical gray matter atrophy is important in the development of cognitive impairment. Our multi-center study supports that indeed subtle variations may be present between these patient groups but that there is more pronounced atrophy between MS patients and controls than between subgroups of cognitive functioning. Likely, the heterogeneity in topographic distribution of inflammatory and degenerative aspects of MS limit the use of one single analysis modality to explain all variance in cognitive dysfunction in MS.

Prior studies revealed a correlation between the number of cortical[7] and juxta-cortical lesions[45] in cognitive impairment. We purposefully did not aggressively fill the white matter lesions with normal white matter intensities to avoid artificial underestimation of cortical thickness. This may have resulted in an overestimation of cortical thickness in cases where CI could have been correlated to number of juxta-cortical lesions. Our filling methods gave interpretable results in the majority of the cases; we only had to exclude 4 patients due to the quality of the surface measurements being affected by large number of juxta-cortical lesions. Similar techniques could be further refined in the future with improved acquisitions allowing for determining the effect on cortical atrophy and accurate classified juxta-cortical and mixed lesions.

Limitations of this study include the older age, slightly higher EDSS scores and disease duration of the CI patients compared to CP patients and controls. This was addressed by performing a sub-analysis with younger CP patients to a subgroup analysis where these demographic features were not significantly different. In addition, the study was performed on 1.5T scanners, applying slightly different acquisition protocols in each center. This was corrected for by using an equal number of control MRIs from each center performed on the same scanner and using each center as a covariate for analysis. Future uniform scanning parameters could address such limitations, where on the other hand it reflects the variability in daily practice between different centers that would be the typical setting for everyday practice and within clinical trials.

We found a correlation between structural and clinical parameters and cortical thinning, but none of these were independently predictive of cognitive dysfunction. We found no statistical significances in lesion volumes between the CP and CI group, although a trend towards larger lesion volumes in CI

was seen, but the influence of white matter lesions was not assessed topographically. Such correlations to lesion location and micro-structural properties of the involved white matter tracts could be useful. White matter integrity, assessed by diffusion tensor imaging, was recently described to be related to cognitive impairment in MS.[8, 46] Other modalities have been explored for their predictive properties, including GM magnetization transfer ratios which was found to be a long term predictor of the development of cognitive impairment, and other studies have described a predictive role for thalamic atrophy in the development of CI in MS. Imaging correlation of cognitive impairment in RRMS thus far is lacking a specific substrate and has equal dissociative properties as the paradox between classical WM lesion detection and the extent of physical disability. The likelihood of a single structural etiology being the determinant of the development of cognitive impairment in MS is low and the application of multimodal structural and functional imaging techniques is probably where future study directions should go. Combining such imaging modalities in future longitudinal with uniform imaging protocols will further elucidate the role of specific structural involvement in their development of difficulties in specific cognitive domains. The use of regional cortical thickness measures could become a useful additional tool in such future multi-center studies.

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**Table 1: MRI protocols used per center for acquisition of 3D T1 images (all performed on 1.5 T)**

| | Amsterdam | Barcelona | Graz | London | Milan | Napoli | Siena |
|---|---|---|---|---|---|---|---|
| Manufacturer | GE | SIEM | SIEM | SIEM | PHIL | GE | PHIL |
| TR | 7.8 | 2.3 | 1.9 | 2.2 | 8.3 | 5.5 | 10 |
| TE | 3.0 | 3.0 | 2.2 | 2.9 | 3.7 | 1.8 | 4.0 |
| *n* | 15 | 15 | 18 | 15 | 19 | 19 | 20 |

| ***Table 1: Demographics*** | | | | |
|---|---|---|---|---|
| | MS (n=56) | | Controls (n=65) | p-value |
| Age | 39.2 | | 35.9 | 0.048 [a] |
| Gender (F/M) | 35/21 | | 38/27 | 0.65 [b] |
| Cognition | *Preserved* | *Impaired* | | |
| *n* | 36 | 20 | - | |
| *Age* | 36.9 (8.1) | 43.3 (8.9) | - | 0.012 [a] |
| *EDSS* | 1.9 (1.0) | 2.5 (1.3) | - | 0.030 [c] |
| *Disease duration* | 6.8 (4.4) | 10.8 (8.4) | - | 0.031 [c] |
| *Education* | 13.7 (3.1) | 13.5 (3.4) | | 0.87 |

**Table 2:** Summary of demographic and clinical features between study groups.

[a] student t-test, [b] Chi-square, [c] Mann-Whitney

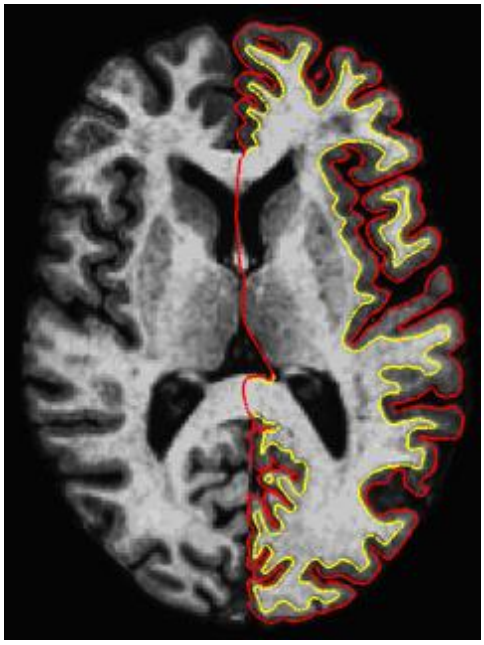

**Figure 1: example MS patient. (supplement, out or combine with figure 2?)**

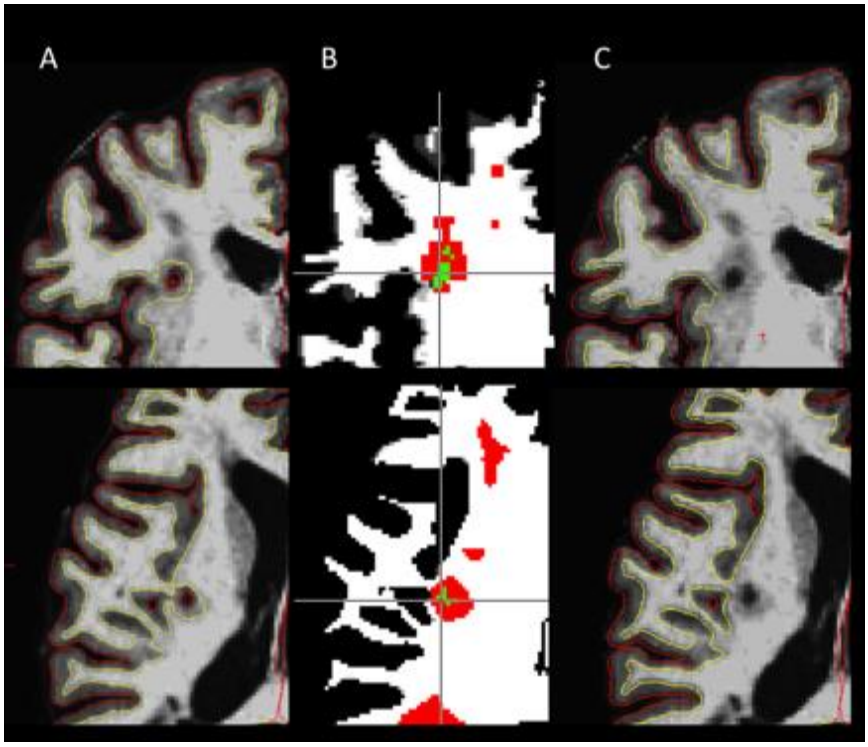


**Figure 2:**

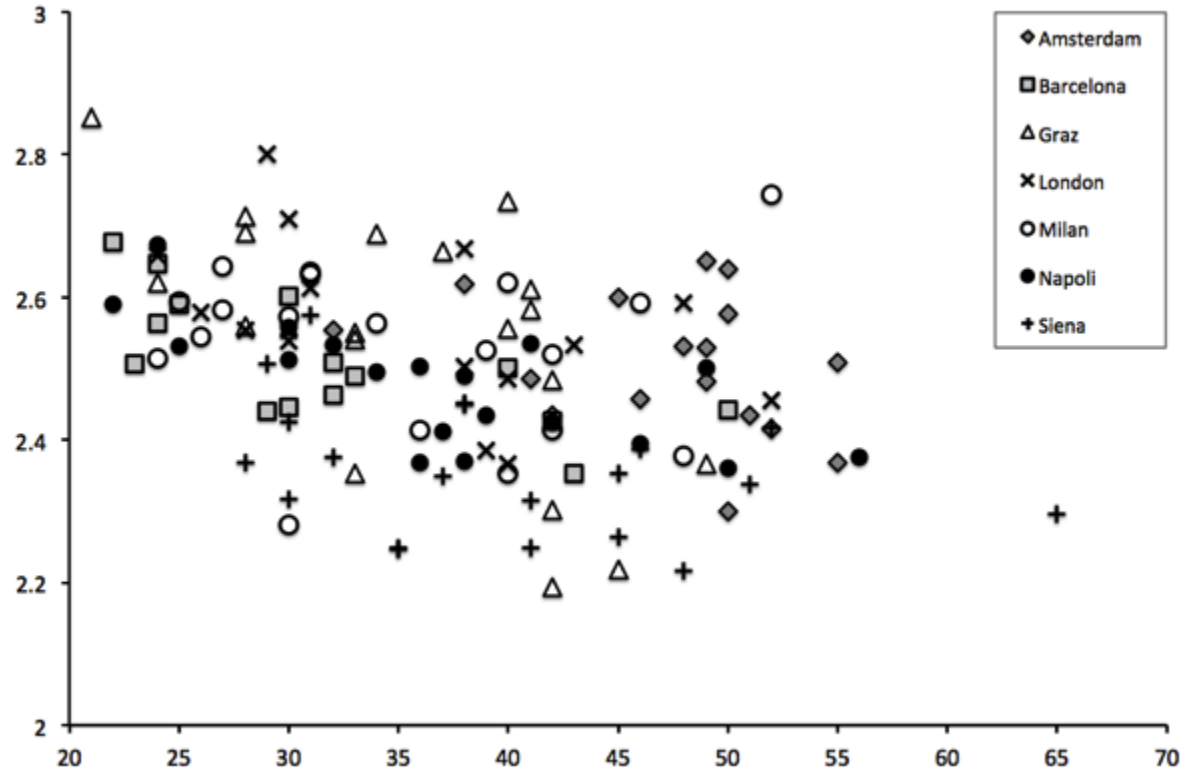


**Figure 3:** Mean Cortical Thickness from all subjects per age by center

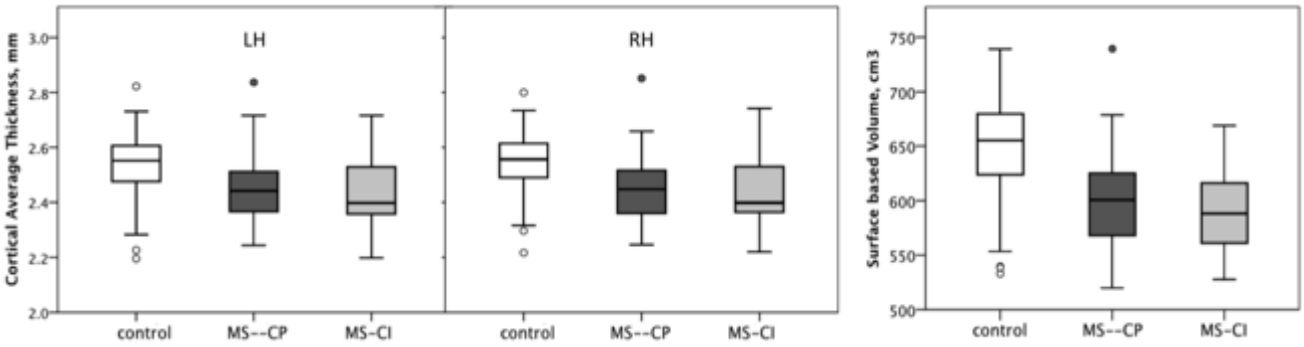


**Figure 3:** Box-plots with cortical thickness per hemisphere (LH; left hemisphere and RH; right hemisphere) and total normalized cortical grey matter (right-hand panel) for control (white, n=65), MS-CP (MS, cognitive preserved, dark gray, n=36) and MS-CI (MS, cognitive impaired, light gray, n=20) groups. Boxplot colors are white for controls, dark grey for CP subgroup, light grey for CI subgroup.

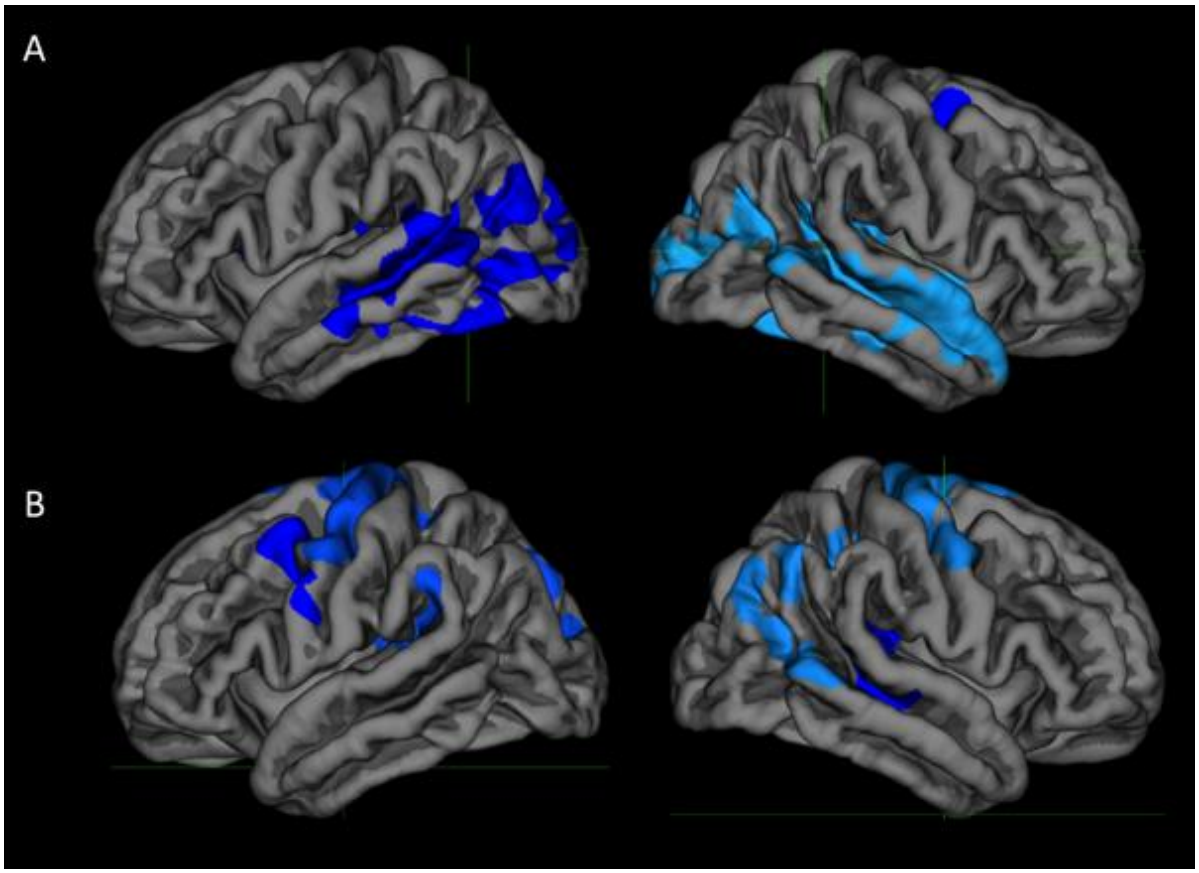


**Figure 4:** GLM model of vertex based surface thickness. Upper panel (A) Controls vs MS patients. Lower panel (B) shows within MS group analysis with T2 lesion volume (log scale) as a covariate. Blue areas are with correction for multiple comparisons ($p<0.01$). (Monte carlo simulation $p < 0.01$). Analyses were corrected for center, age and gender.

**Table 3:** Region-wise Multivariate analysis between age matched groups (corrected for gender and center with post-hoc analysis between sub-groups

| | *CP (n=20)* | *CI (n=20)* | *p-value* |
|---|---|---|---|
| Mean CTh (mm) | 2.47 | 2.42 | 0.24 |
| *Frontal* | 2.50 | 2.49 | 0.95 |
| *Temporal* | 2.76 | 2.67 | 0.050 * |
| *Occipital* | 2.05 | 2.00 | 0.12 |
| *Parietal* | 2.35 | 2.29 | 0.13 |
| *Cingulate* | 2.60 | 2.50 | 0.055 |
| *Insula* | 2.95 | 2.95 | 0.89 |
| Cortical GM Volume | 598 | 583 | 0.23 |
| WM volume | 594 | 572 | 0.27 |
| Log-T2-LL | 3.62 | 3.90 | 0.08 |
| Log-T1BH-LL | 3.38 | 3.66 | 0.09 |