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ORIGINAL COMMUNICATION

Cortical atrophy is relevant in multiple sclerosis at clinical onset

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Abstract *Introduction* Increasing evidence suggests relevant cortical gray matter pathology in patients with Multiple Sclerosis (MS), but how early this pathology begins; its impact on clinical disability and which cortical areas are primarily affected needs to be further elucidated. *Methods* 115 consecutive patients (10 Clinically Isolated Syndrome (CIS), 32 possible MS (p-MS), 42 Relapsing Remitting MS (RR-MS), 31 Secondary Progressive MS (SP-MS)), and 40 age/gender-matched healthy volunteers (HV) underwent a neurological examination and a 1.5 T MRI. Global and regional Cortical Thickness (CTh) measurements, brain parenchyma fraction and T2 lesion load were analyzed. *Results* We found a significant global cortical thinning in p-MS (2.22 ± 0.09 mm), RR-MS (2.16 ± 0.10 mm) and SP-MS (1.98 ± 0.11 mm) compared to CIS (2.51 ± 0.11 mm) and HV (2.48 ± 0.08 mm). The correlations between mean CTh and white matter (WM) lesion load was only moderate in MS ($r = -0.393$, $p = 0.03$) and absent in p-MS ($r = -0.147$, $p = 0.422$). Analysis of regional CTh revealed that the majority of cortical areas were involved not only in MS, but also in p-MS. The type of clinical picture at onset (in particular, pyramidal signs/symptoms and optic neuritis) correlated with atrophy in the corresponding cortical areas. *Discussion* Cortical thinning is a diffuse and early phenomenon in MS already detectable at clinical onset. It correlates with clinical disability and is partially independent from WM inflammatory pathology.

Key words multiple sclerosis · cortical thickness · cortical atrophy · neuronal degeneration

Introduction

Multiple Sclerosis is a common chronic, inflammatory, autoimmune disease of the central nervous system (CNS) and one of the most important neurological causes of invalidity [1–3]. CNS inflammation, a disease hallmark, is thought to be orchestrated by auto-reactive T-lymphocytes primarily directed against myelin antigens [4]. However, the assumption that MS is purely a White Matter (WM) disease is currently questioned on the basis of diverse evidence. First, a number of hystopathological [5–9] and Magnetic Resonance (MR) studies [10–12] have demonstrated that cortical lesions are more common than supposed. Second, N-acetyl-aspartate, a biomarker of neuronal integrity, was found decreased in the cortical gray matter (cGM) of patients with early MS [11, 13]. Third, a substantial neocortical volume loss seems to occur early not only in Primary Progressive [14] but also in Relapsing Remitting MS (RRMS) [15–18], and significantly contributes to disability [17, 19, 20]. Finally, while a comprehensive look at the cGM thickness showed a significant focal thinning in several cortical areas [21], regional analyses of CTh have produced conflicting results [21, 22].

Despite these observations, the question as to whether cortical atrophy is secondary to WM pathology via retrograde degeneration or is a primary process remains unsolved. Our study addressed whether cortical thinning: (i) appears early on in patients with clinically isolated syndromes suggestive of MS or with p-MS; (ii) occurs in subjects with very low WM lesion load as well; (iii) preferentially affects some cortical regions; (iv) correlates with clinical disability.

Methods

Patient population

A total of 115 patients consecutively presenting at the Multiple Sclerosis Centre in Padova, and 40 age- and gender-matched healthy volunteers (HV) were recruited between May 2005 and September 2005 (Table 1). McDonald's criteria [23, 24] were used to differentiate patients into four groups: Clinical Isolated Syndrome (CIS), Possible Multiple Sclerosis (p-MS), Relapsing Remitting Multiple Sclerosis (RR-MS) and Secondary Progressive Multiple Sclerosis (SP-MS). CIS were defined as patients having a first clinical episode suggestive of CNS inflammation but lacking evidence of dissemination in space of lesions. p-MS were defined as patients having a first clinical episode suggestive of CNS inflammation, presenting with a dissemination in space of lesions (i.e., MR according with Barkhof et al. criteria [25] and Tintoré et al. criteria [26], or two or more MR lesions plus demonstration of intrathecal IgG syntesis).

In order to better analyze the correlation between T2 lesion volume in the brain, cortical thinning and disability (EDSS), we did not include in the study the following patients: (1) p-MS with evidence of dissemination in time, but not in space, of lesions (i.e., patients with very low T2 lesion load), (2) CIS of the spinal cord with normal brain MRI.

Each patient was clinically assessed using the Kurtzke Expanded Disability Status Scale (EDSS) [27]. For all patients with CIS or p-MS the MRI scan was obtained within 6 months of symptom onset. Steroid therapy in the month prior to MR acquisition was also an exclusion criterion. Informed consent was obtained from all patients and HV. The study was approved by the local Ethics Committee.

Table 1 Demographics characteristics of study population

	HV	CIS	p-MS	RR-MS	SP-MS	All patients
N°	40	10	32	42	31	115
M:F	15:25	2:8	11:21	15:27	13:18	34:66
Age *	36.2 ± 10.2 (18–65)	37.1 ± 6.4 (28–50)	32.1 ± 10.4 (13–51)	36.1 ± 10.9 (18–58)	44.1 ± 12.2 (28–63)	36.2 ± 11.3 (13–63)
Positve oligoclonal bands	Nd	4	28	35	29	72
Disease duration (years) *	Nd	0.66 ± 0.34 (0.2–1)	0.7 ± 0.3 (0.1–1.1)	6.8 ± 3.9 (1–16)	10.5 ± 6.1 (1–25)	4.8 ± 5.2 (0.1–25)
Therapy						
None	0	10	29	7	9	52
Immunomodulatory	0	0	3	33	6	38
Immunosuppressive	0	0	0	2	16	9
EDSS *	Nd	1.1 ± 0.8 (0–2.0)	1.4 ± 1.0 (0–3.5)	2.0 ± 1.1 (1.0–5.5)	5.8 ± 1.1 (3.0–7.0)	2.3 ± 1.8 (0–7.0)
T2LV (cm^3) *	Nd	0.2 ± 0.3 (0–0.7)	3.0 ± 2.6 (0.2–8.8)	8.0 ± 8.8 (0.4–40.2)	8.7 ± 10.4 (1.0–36.7)	5.7 ± 7.7 (0–40.2)
BPF (%) *	85.4 ± 1.9 (88.7–81.1)	84.5 ± 3.4 (78.4–88.6)	83.7 ± 3.2 (75.4–89)	81.4 ± 4.5 (69.1–88.2)	79.2 ± 4.2 (73.7–85.6)	82.0 ± 4.3 (73.7–89)
WMF (%)	45.0 ± 1.8	44.5 ± 0.3	44.3 ± 2.7	44.5 ± 3.0	44.2(2.7	44.3 ± 2.4
GMF (%)	40.4 ± 1.5	39.9 ± 1.4	39.4 ± 2.7	37.2 ± 4.9	35.0 (3.3	37.7 ± 2.9

Image acquisition

All images were acquired using a 1.5 T Philips Achieva (Philips Medical Systems, Best, Netherlands) with 33 mT power gradient, slew rate 160 T/m/s. No major hardware upgrades were carried out on the scanner during the study and weekly quality assurance sessions took place to monitor measurement stability. Five different sets of images were achieved: three *3D Fast Field Echo (FFE)* axial plane sequences with the off-center positioned on zero (120 contiguous axial slices, echo time (TE) 4.6 millisecond, repetition time (TR) 25 millisecond, flip angle 30, slice thickness 1.2 mm, matrix 256 ± 256); one *Fluid Attenuated Inversion Recovery (FLAIR)* sequence with 50 contiguous axial slices (TE 120 millisecond, TR 10000 millisecond, inversion time 2500 millisecond, slice thickness 3.0 mm, matrix 256 ± 256). Finally, conventional *Turbo-Spin Echo Dp/T2 (TSE)* and *Spin Echo (SE) T1* post contrast sequences were also obtained.

Lesion identification and measurements

MR scans were reviewed by an experienced neuroradiologist (AM) and a neurologist (MC) who were blinded to the patients' clinical status. On a T1-post contrast image, the number of T1 Gadolinium-enhancing lesions were identified. Using a semiautomatic thresholding technique [28], included in a software developed at NIH, Medical Images Processing, Analysis and Visualization (MIPAV) (http://mipav.cit.nih.gov) lesions were segmented on the FLAIR images providing a T2 hyperintense (T2LV) lesion volume. Total T2LV was calculated by multiplying lesion area by slice thickness and was reproducible to less than 5% as coefficient of variation, in serial measurements.

Brain parenchyma fraction evaluation

Brain Parenchyma Fraction (BPF), Grey Matter fraction (GMF) and White Matter Fraction (WMF) were computed as previously described [29, 30] on the 3D-FFE image by using MIPAV. The BPF, GMF and WMF calculations were performed using two main algorithms including: (1) the brain extraction tool (BET) [31] to extract brain from non-brain tissue on the 3D-FFE sequence; (2) a tissue segmentation program (Fuzzy C-mean) to segment the extracted brain image into GM, WM, CSF, and background [28]. All tissue fraction measures have been corrected for lesion misclassification.

Cortical thickness measurements

Measurement of global and regional cortical thickness was performed using a semiautomatic technique, the FreeSurfer, extensively described elsewhere [32, 33].

A data set of three anatomical 3D-FFE was employed. Images were registered using a rigid body registration tool with 6 degrees of freedom and Sinc as interpolation tool. From this, an averaged data set was obtained and intensity variations, due to magnetic field unhomogeneities, were corrected. A normalized-intensity image was subsequently created and used to build models of each individual cortical surface. An automatic procedure involving segmentation of the cortical WM, tessellation of the GM/WM junction, inflation of the folded surface tessellation patterns, and automatic correction of topological defects in the resulting manifold was applied [34]. Thereafter, a manual editing procedure followed in order to correct surface inaccuracies (which may result from incorrect topology correction). GM/WM and pial surfaces were then identified and thickness was defined as the shortest distance between the GM/WM and phial surface models [33]. Each subject's reconstructed brain was morphed to an average spherical surface representation to align sulcal and gyral features across subjects. This transformation was used to map thickness measurements into a common spherical coordinate system.

In order to evaluate the methodological limitations of CTh analysis, the same observer (M.C.) repeated CTh measurements in 10 patients and in 10 healthy volunteers who underwent two different MRI in the same week. A intraobserver Coefficient Variation (COV) was <0.5% for both global and regional CTh measurements.

Statistical analysis

Global CTh has been quantified as an average of mean thickness of all cortical areas (right and left) across patients. Tukey test [35], a statistical test specifically designed for mean pair-wise comparison analyses was applied on global CTh for mean's comparison of all patient groups. The same test was applied in order to compare the means of primary motor, visual and sensory CTh in patients with difference type of onset.

Since the analysis on global CTh gives no statistical information about the weight that each single regional CTh variables has on the difference among groups, a t-test with Bonferroni's correction was applied on regional CTh variables (mean of left and right areas) to test differences between patient groups. CTh variables have been sorted according to their percentage of change (PC). Percent reductions in regional CTh were calculated using the following formula: $PC = [(a-b)/b] * 100$, where "*a*" and "*b*" are, respectively, the CTh of patients and HVs, respectively. For percent reduction calculation, values coming from left and right hemispheres were averaged and considered as a single measurement.

Possible correlations between pairs of variables (clinical and demographic) were quantified using Pearson's correlation coefficient. Since there is no difference among mean CTh in CIS and HV group, the correlations were performed considering p-MS and MS (RRMS + SPMS) groups only. Even in this case, the mean left and right CTh and the Bonferroni's correction have been used. All statistical analyses were performed using SPSS v.12 and R, an open source statistical package available at http://www.r-project.org.

Results

Global cortical thickness measurements

Two distinct clusters could be identified on the base of global CTh. The first including CIS and HV, with a mean CTh of 2.51 ± 0.11 mm and 2.48 ± 0.08 mm, respectively, and the second including p-MS, RRMS and SPMS, respectively, with 2.22 ± 0.09 mm, 2.16 ± 0.10 mm and 1.98 ± 0.11 mm. The global CTh was significantly different ($p < 0.005$, Tukey's statistical test with Bonferroni's correction) between the two clusters, while only a trend ($0.005 < p < 0.05$) was observed when RRMS were compared to SPMS (99.5% CI, Fig. 1).

Moreover, as reported in Table 1, two distinct clusters could be identified on the base of BPF and GMF. While RRMS and SPMS showed a significantly higher ($p < 0.001$) cerebral and cortical atrophy compared to CIS and HV, p-MS showed a trend that did not reach the statistical significance.

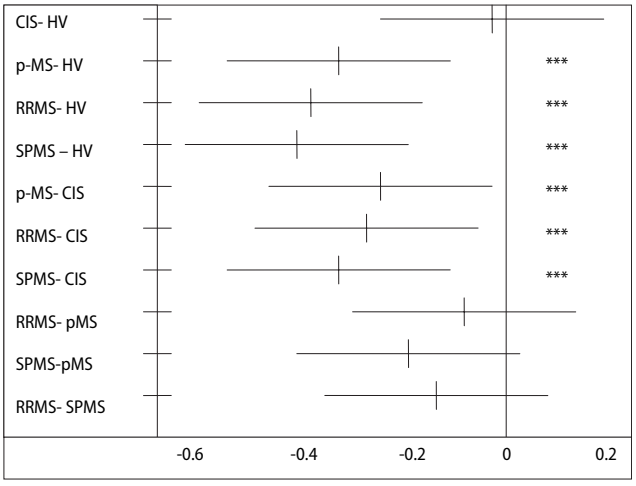

Fig. 1 Tukey test of comparison of global CTh between the five groups analyzed. Confidence interval (99.5%) of groups' mean CTh differences
*** $p < 0.005$ shows confidence interval (99.5%) for each comparison. The same statistical results were obtained considering mean right and left CTh separately

Regional cortical thickness measurements

As expected, in agreement with the statistical equivalence in global CTh between CIS and HV, the regional analysis did not show significant thinning in any cortical areas in CIS (Table 2 and Fig. 2a). Contrariwise, most of the cortical areas analyzed were found significantly reduced in p-MS, RRMS and SPMS groups even after Bonferroni's correction ($p < 0.0005$) (Table 2 and Fig. 2b–d). Precentral (primary motor area), frontal and some occipital areas (including a primary visual area) were the most affected, with about a 25% change in comparison to the HV group. The postcentral gyrus (primary sensory area) was also particularly affected. Table 2 shows the cortical thickness values of the 23 principal cortical areas. Complete list of cortical areas analyzed are available as supplemental material.

CTh and disability

No correlation was found between mean CTh and EDSS in either p-MS ($r = -0.09$, $p = 0.635$) or MS groups ($r = -0.212$, $p = 0.113$). However, since CTh is a global measure, whereas EDSS sums up various regional impairments (e.g., motor, sensory and visual), we performed a second subset of correlations considering single Functional System Scores (FSS) and the corresponding cortical areas. The correlation between motor FSS score and the precentral gyrus thinning was highly significant in both p-MS ($r = -0.487$, $p = 0.006$) and MS ($r = -0.626$, $p < 0.001$). The correlation was also significant between the visual FSS score and the primary visual cortex thinning in p-MS ($r = -0.489$, $p = 0.006$) and MS ($r = -0.389$, $p = 0.02$), while the sensory FSS score and primary sensory cortex did not significantly correlate in either group (p-MS: $r = -0.19$, $p = 0.304$; MS: $r = -0.25$, $p = 0.058$).

A low correlation was observed between precental gyrus and sensory FSS score ($r = -0.129$, $p = 0.065$ for p-MS group; $r = -0.214$, $p = 0.037$ for MS group) while no correlation between precentral gyrus and visual FSS score ($r = -0.101$, $p = 0.078$ for p-MS group; $r = -0.155$, $p = 0.062$ for MS group) was found.

No correlation was found between primary sensory cortex and visual or motor FSS, nor between primary visual cortex and motor and sensory FSS.

Regional CTh is predicted by the type of clinical onset

We evaluated whether cortical thinning in primary motor, sensory and visual cortex was predicted by the type of onset in CIS and p-MS. Primary motor cortex was significantly thinner in patients with piramidal onset (1.70 ± 0.12 mm, $p = 0.023$ for Precentral gyrus, and 2.01 ± 0.15, $p = 0.018$ for Superior Frontal gyrus), than in patients with other clinical presentation (Fig. 3a). The association of primary sensory cortex thinning with sensory onset shows a trend, but did not reach statistical significance. (1.75 ± 0.21 mm, $p = 0.067$) (Fig. 3b).

Interestingly, optic neuritis as clinical onset was predictive of a significant thinning of the primary visual cortex (1.67 ± 0.21, $p = 0.035$ for Occipital Pole and 1.32 ± 0.10, $p = 0.021$ for Calcarine solcus). (Fig. 3c). Patients with RRMS and SPMS have also been examinated, but, as expected, no correlation between clinical onset and regional CTh was found in these subgroups.

Correlation between CTh and other MRI metrics

No correlation was demonstrated between mean CTh and T2LV in p-MS ($r = -0.147$, $p = 0.422$), while in MS the correlation was only modest ($r = -0.393$, $p = 0.03$). The correlations between mean CTh and BPF, GMF, WMF were, respectively, $r = 0.448$, $p = 0.011$; $r = 0.401$, $p = 0.026$; $r = 0.051$, $p = 0.787$ in the p-MS, and $r = 0.312$, $p = 0.018$; $r = 0.310$, $p = 0.019$; $r = -0.121$, $p = 0.369$ in the MS group.

Discussion

Several cross-sectional studies have described diffuse cerebral and cortical atrophy in MS brain, even in patients with short disease duration [15–18]. A

Table 2 Analysis of regional cortical thickness in p-MS, MS and HV group

	CIS			p-MS			RRMS			SPMS			HV	
	Mean	SD	% Change	Mean	SD	% Change	Mean	SD	% Change	Mean	SD	% Change	Mean	SD
Frontal inferior	2.77	0.22	−0.2%*	2.38	0.24	−14.3%***	2.20	0.35	−20.9%***	2.04	0.36	−26.7%***	2.78	0.24
Frontal middle	2.69	0.19	0.9%*	2.18	0.20	−18.2%***	2.11	0.33	−21.0%***	1.87	0.34	−29.8%***	2.67	0.20
Frontal superior	2.70	0.23	−2.2%*	2.28	0.22	−17.5%***	2.13	0.28	−22.6%***	1.90	0.28	−31.3%***	2.76	0.25
Precentral	2.20	0.17	−2.7%*	1.81	0.15	−20.3%***	1.72	0.15	−24.1%***	1.50	0.16	−33.8%***	2.27	0.18
Central	1.65	0.18	−1.2%*	1.37	0.11	−17.9%***	1.36	0.20	−18.3%***	1.14	0.20	−31.8%***	1.67	0.18
Subcentral	2.73	0.27	3.8%*	2.10	0.24	−20.1%***	2.00	0.35	−23.8%***	1.80	0.36	−31.6%***	2.63	0.28
Paracentral	1.98	0.22	−0.2%*	1.64	0.16	−17.5%***	1.61	0.18	−18.9%***	1.51	0.19	−24.0%***	1.99	0.22
Parietal inferior	2.52	0.20	−2.3%*	2.43	0.20	−5.8%*	2.37	0.34	−7.9%**	2.31	0.35	−10.4%***	2.58	0.21
Parietal superior	2.16	0.14	−0.8%*	1.91	0.18	−12.2%***	1.90	0.28	−12.8%***	1.70	0.28	−21.8%***	2.17	0.16
Postcentral	2.04	0.17	−1.1%*	1.79	0.20	−13.4%***	1.72	0.25	−16.7%***	1.59	0.26	−23.0%***	2.06	0.19
Subcallosal	2.66	0.40	−2.1%*	2.46	0.45	−9.4%*	2.56	0.53	−5.7%*	2.45	0.55	−9.6%**	2.71	0.41
Temporal inferior	2.87	0.24	0.0%*	2.57	0.30	−10.5%***	2.46	0.42	−14.3%***	2.45	0.44	−14.6%***	2.87	0.25
Temporal middle	3.06	0.20	−0.8%*	2.73	0.24	−11.4%***	2.58	0.34	−16.3%***	2.49	0.35	−19.2%***	3.08	0.21
Temporal superior	2.94	0.27	−0.7%*	2.56	0.22	−13.7%***	2.44	0.37	−17.6%***	2.40	0.37	−18.9%***	2.96	0.28
Insular long	3.32	0.30	−1.1%*	2.99	0.39	−10.9%**	2.83	0.53	−15.7%***	2.62	0.55	−21.9%***	3.36	0.32
Insulat short	3.45	0.27	−0.9%*	3.16	0.45	−9.3%**	3.03	0.63	−13.0%***	2.94	0.63	−15.6%***	3.48	0.28
Occipital inferior	2.47	0.24	−1.7%*	2.19	0.26	−13.1%***	2.13	0.29	−15.2%***	1.89	0.30	−24.9%***	2.51	0.26
Occipital lateral	2.69	0.26	−0.7%*	2.09	0.23	−22.8%***	2.09	0.42	−22.9%***	2.08	0.43	−23.3%***	2.71	0.27
Occipital medial	1.97	0.21	−3.0%*	1.78	0.14	−12.6%***	1.82	0.19	−10.6%***	1.79	0.19	−11.9%***	2.03	0.22
Occipital middle	2.61	0.21	−0.4%*	2.45	0.21	−6.7%**	2.31	0.24	−12.0%***	2.22	0.25	−15.3%***	2.62	0.21
Occipital pole	1.98	0.18	−0.4%*	1.84	0.14	−7.4%**	1.73	0.14	−12.8%***	1.68	0.15	−15.5%***	1.99	0.19
Occipital superior	2.09	0.19	−1.1%*	1.87	0.13	−11.5%***	1.82	0.21	−14.1%***	1.77	0.23	−16.5%***	2.12	0.21
Calcarine s	1.68	0.19	−3.1%*	1.58	0.14	−8.9%***	1.60	0.12	−7.7%***	1.53	0.12	−11.6%***	1.74	0.20

Mean cortical thickness (mm), Std. Dev, and % change vs. HV, for the most important cortical areas
* p value not significant. ** $p < 0.001$. *** $p < 0.0005$ Significant after Bonferroni's correction
% Change = $(a-b)/b*100$ where a is a is CTh of p-MS or MS group and b is CTh of HV

longitudinal study carried out in patients with "CIS suggestive of MS" (CIS and p-MS included) showed a progressive cortical atrophy (i.e., decrease in GMF) in those subjects who developed a confirmed diagnosis of MS within 3 years from clinical onset [16]. Our study extends these observations showing, across different phases of the disease, an increase in cerebral atrophy (i.e., decrease in BPF), that is already significant when the diagnosis of p-MS (first clinical episode with evidence of dissemination in space of lesions) is achieved. This finding is even more interesting if we consider that cerebral atrophy was found to correlate better with GMF than with WMF.

The sample size of our study enabled us to investigate the regional CTh in patients having different degrees of disability and disease duration. Compared to previous studies on smaller sample sizes, which described a reduction in the overall mean CTh, but gave conflicting results on regional CTh [21, 22], one of the most original results of our study concerns the identification of the cortical areas earlier and primarily involved in the process. Indeed, although a diffuse cortical thinning was found to affect almost all cortical areas, primary motor and primary visual cortex were clearly the most affected, even in patients suffering from p-MS.

The most original finding of our study is the significant correlation between the type of clinical picture at onset and atrophy in the corresponding cortical areas. This correlation was striking in patients with pyramidal signs/symptoms at onset, whose primary motor cortex was found particularly thin. Interestingly, the primary visual cortex was also markedly thin in patients who presented with optic neuritis. This phenomenon did not reach the significance in patients with a sensory onset, may be because of the "multi-step" pathway of the sensory system. We believe that these findings confirm a relevant contribute of retrograde axonal degeneration in determining cortical atrophy.

Moreover, the comparison of CIS and p-MS groups gave us the opportunity to evaluate cortical atrophy in a very early disease phase. These groups include patients with low WM lesion load and low degree of disability. While CIS had a mean CTh similar to HV, p-MS (though at their first clinical episode) showed a significantly lower CTh, similar to that of RRMS and SPMS. This suggests that cGM degeneration is already present in the early phase of brain inflammation, when white matter damage appears to be only modest.

The lack of cortical thinning in CIS patients can be explain by both the low number of CIS patients included in the study as well as their clinical heterogeneity. Indeed, not all CIS will develop MS.

CIS here described, are currently enrolled in a longitudinal clinical and MR study, based on a larger

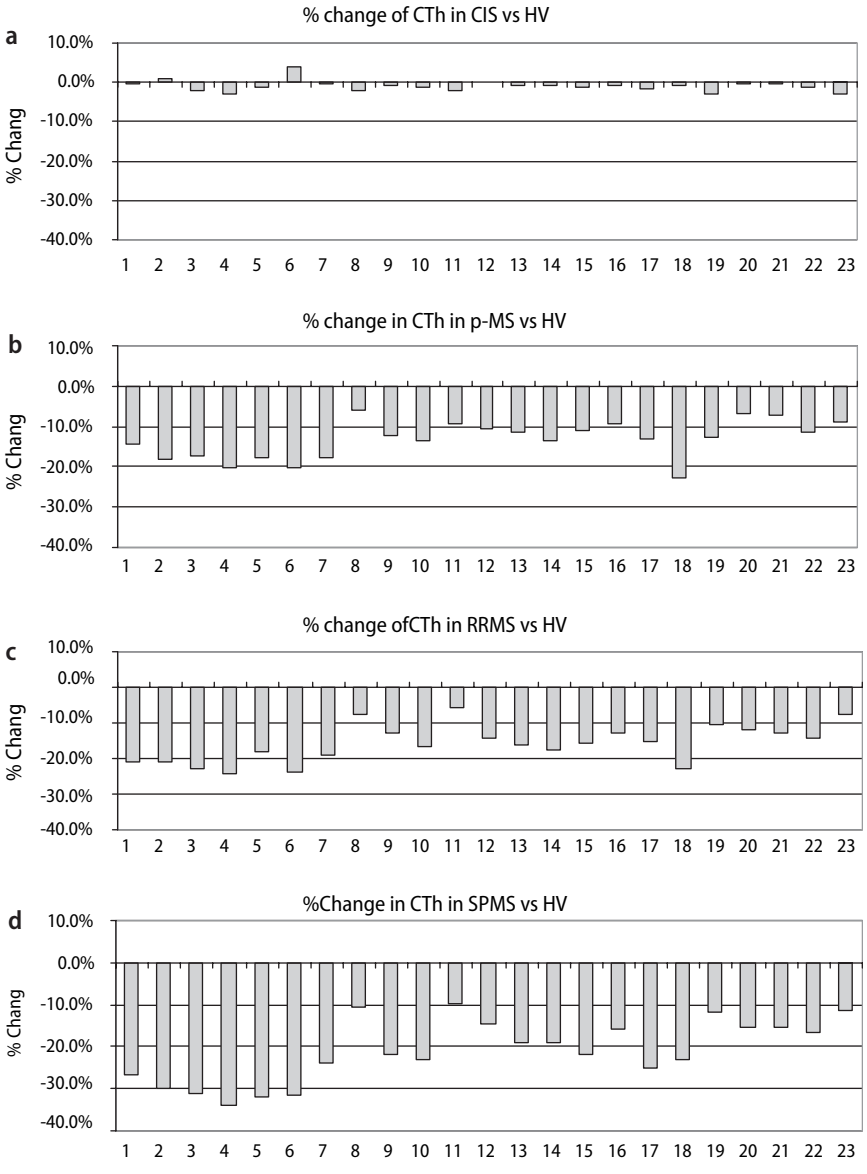

Fig. 2 Percent change of CTh of the most important cortical areas in CIS (**a**), p-MS (**b**), RRMS (**c**) and SPMS (**d**) toward HV. 1 = Frontal Inferior gyrus, 2 = Frontal Middle gyrus, 3 = Frontal Superior gyrus, 4 = Precentral gyrus, 5 = Central solcus, 6 = Subcentral gyrus, 7 = Paracentral gyrus, 8 = Parietal Inferior gyrus, 9 = Parietal Superior gyrus, 10 Postcentral gyrus, 11 Subcallosal gyrus, 12 Temporal Inferior gyrus, 13 = Temporal Middle gyrus, 14 = Temporal Superior gyrus, 15 = insular Long gyrus, 16 Insular Short gyrus, 17 = Occipital Inferior gyrus, 18 = Occipital Lateral gyrus, 19 = Occipital Medial gyrus, 20 = Occipital Middle gyrus, 21 = Occipital Pole, 22 = Occipital Superior gyrus, 23 = Calcarine solcus

number of patients, which will likely allow us to analyze CTh in those patients who will develop MS.

Of course, the observation that the value of global CTh is not statistically different between RRMS and SPMS patients does not indicate that neuronal loss in the cortex starts early and then stops during the course of the disease, but rather reflects the impact of inflammation on neuronal loss in the early phases of the disease [36]. From a statistical point of view, it has to be considered the "overcare" of Bonferroni's correction effect. This observation further stresses the importance of an early immunomodulatory treatment in MS.

Important information about the *origin* of cortical atrophy may arise from the correlation between global CTh and T2LV. In our study, the correlation is only *moderate* in MS and *not significant* in p-MS, thus suggesting that WM inflammation, as depicted by T2LV, and cortical thinning are partly independent processes at clinical onset. Although inflammatory lesions in the WM may wax and wane in volume and sometimes do not leave residual iperintense signal in T2 weighted sequences, the possibility that cortical thinning in p-MS group may be related to axonal damage in the so-called "normal appearing WM" seems to be improbable given the low T2LV observed in this group of patients and their very short disease duration. In addition, global CTh correlates significantly with GMF in both groups, whereas no correlation between mean CTh and WMF could be

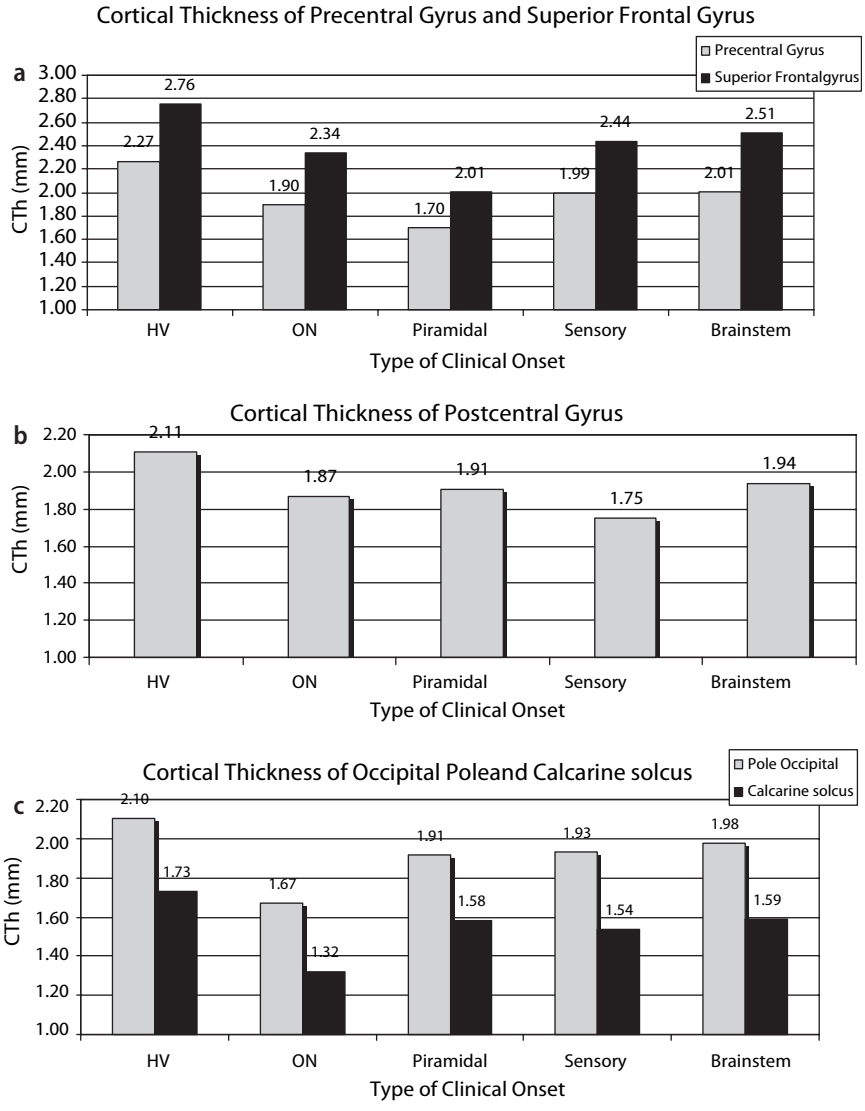

Fig. 3 (**a**) Cortical thickness of precentral and superior frontal gyrus (primary motor cortex) in healthy volunteers (HV), in patients with optic neurits (ON) or piramidal, sensory or brainstem dysfunction at onset. (**b**) Cortical thickness of postcentral gyrus (primary sensory cortex) in healthy volunteers (HV), in patients with optic neurits (ON) or piramidal, sensory or brainstem dysfunction at onset. (**c**) Cortical Thickness of occipital pole and calcarine solcus (primary visual cortex) in healthy volunteers (Hv), in patients with optic neuritis (ON) or piramidal, sensory or brainstem dysfunction at onset

demonstrated. Taken all together, these results widen previous findings [15, 17] indicating that cortical atrophy in early MS is only partly related to WM damage, but rather mainly expression of a primary pathological process taking place in cGM.

Finally, a very poor correlation between mean CTh and EDSS was observed. This is in line with previous studies in which other MR parameters of brain damage (i.e., T2 lesion load, T1 lesion load, BPF, WMF and GMF) showed only moderate or no correlation with clinical disability [37, 38] Since patients with spinal cord syndrome were not included in the study, and clinically silent lesions in the spinal cord are exceptional, these results may be explained with the intrinsic limitations of EDSS as measure of disability. Thus, we exploited the high sensitivity of FreeSurfer to correlate regional CTh measurements with the corresponding Neurostatus's functional system (FS) scores. The finding that pyramidal and visual FS scores correlated with the primary motor area and the primary visual cortex pathology, respectively, while the correlation between the sensory FS score and the pathology of primary sensory area was very poor, further points out that EDSS is inadequate to depict brain damage in MS patients.

We are aware that our study has the limits of the cross-sectional studies and does not describe the dynamics of cortical thinning development. However, the high number of patients enrolled in the study and their clinical stratification, well represent the clinical phases of MS. A 2-years longitudinal study in this cohort of patients is in progress.

Conclusions

Our study, based on a large cohort of patients, shows that cortical thinning is a diffuse and very early phenomenon in MS, and can be demonstrated as soon as at the first clinical episode, when the criterion for a diagnosis of possible MS is achieved. Such phenomenon is also observed in patients with very low T2LV and is not exclusively dependent on the WM inflammatory damage. Indeed, our findings suggest two degenerative components in MS cortical pathology, one expression of neuronal damage in inflammatory lesions, the other taking place in the cortex, probably as a primary local process. Finally, cortical atrophy in primary motor and visual areas significantly correlates with disability in the corresponding functional systems. Our findings further suggest that research on drugs with neuroprotective effects needs to be strengthened and that the application of CTh measurements should be considered as surrogate marker of disease in clinical trials.

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