DYNAMIC VOLUMETRIC CHANGES OF HIPPOCAMPAL SUBFIELDS IN CIS PATIENTS: A 2-YEAR MRI STUDY

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INTRODUCTION AND AIMS

Lesional and atrophy data[1,2] support the evidence of gray matter (GM) involvement in patients with clinically isolated syndrome (CIS) suggestive of multiple sclerosis (MS).

The hippocampus is affected early in MS, with characteristic subregional patterns of involvement at different stages of the disease and in different disease clinical phenotypes.

The subgranular layer of the dentate gyrus (DG), in the CA4 region of the Cornus Ammonis, experiences neurogenesis and there is *in-vivo* evidence of its expansion in relapsing-remitting MS patients.[3]

Aims

To assess if regional hippocampal variations occur in CIS patients and if they show any correlation with focal white matter lesions and short-term evolution to clinically defined MS.

METHODS

Exclusion and inclusion criteria, MRI acquisition and hippocampal analysis are explained in **Table 1**.

Table 1. Summary of exclusion and inclusion criteria of the study population, clinical assessment, MRI acquisition, hippocampal segmentation and statistical analysis.

Study population	Clinical evaluation	MRI acquisition	Statistical analysis
Exclusion criteria (CIS and HC) No significant medical illnesses/substance abuse, history of psychiatric or other neurological diseases, steroid admininstration within the previous month. **Inclusion criteria (CIS only)** First neurological event within the previous 3 months. Exclusion of other, subclinical, neurological relapses.	**Neurological assessment** Expanded Disability Status Scale (**EDSS**) at baseline and after 3 (M3), 12 (M12) and 24 (M24) months.	**Structural MRI (1.5 T scanner)** **1) Axial DE TSE** **2) Sagittal 3D T1-weighted MPRAGE**: TR=2000 ms, TE=3.93 ms, inversion time=1100 ms, number of sections=208, section thickness=0.9 mm, matrix size=256 x 224, FOV=236 x 270 mm2) **3) Post-contrast sagittal 3D T1-weighted MPRAGE**: 0.1 mmol/kg of gadolinium [Gd]-DTPA; acquisition delay: 5 minutes, same parameters as the pre-contrast sequence **MRI analysis** Quantification of T2, T1 and Gd- lesion volume (LV) (**Jim 6.0 software**) at each timepoint for each hemisphere; number of new T2, T1 and Gd lesions at each timepoint and assessement of DIS and DIT following 2011 Polman criteria. Refilling of T1-hypointense lesions[4], quantification of normalized brain volume (NBV) and longitudinal percent brain volume changes (PBVC) using the **SIENAx and SIENA software.** **Hippocampal segmentation** Manual hippocampal segmentation from the 3D T1-weighted images (**MultiTracer software**) was performed according to a standardized procedure[5,6] and global volumes derived. Radial atrophy distribution was assessed using 3D parametric surface mesh models (**LONI Shape Tools**). Location of hippocampal subfields was defined by superimposing on the average hippocampus obtained from a HC group.	**Between-group comparisons**: non-parametric **Mann-Whitney and Pearson $\chi 2$** (demographic, clinical and conventional MRI data) between HC and CIS, adjusted for age or disease duration, as appropriate. **Longitudinal analysis**: **Wilcoxon test** for paired data. **Analysis of variance adjusted for age**: local differences in radial distance between HC and CIS patients at two time points, at equivalent locations. **Pearson correlation, controlling for age**: correlation between radial size in CIS patients and ipsilateral T2-, T1- and gadolinium lesion volume (LV). The previous analysis were repeated comparing non-converting patients (nc-CIS) and patients converted to clinically defined MS (c-CIS) at the end of the follow-up.

RESULTS

The main clinical and conventional MRI data are shown in Table 2.

At baseline, compared to non-converters (nc), c-CIS had higher male/female ratio (6/15 *vs* 1/14, p <0.01), whereas they did not differ for age, EDSS, disease duration, CSF oligoclonal bands, T2 LV, T1 LV, Gd LV and NBV (data not shown).

Table 2. Main demographic, clinical and conventional MRI data of healthy controls (HC) and clinically isolated syndrome (CIS) patients at baseline and of CIS patients during the follow-up.

	HC	CIS Baseline	P	M3	p(3-0)	M12	p(12-3)	M24	p(24-12)
Number	14	36	-	-	-	-	-	-	-
Female/Male	10/4	29/7	n.s.*	-	-	-	-	-	-
Mean age (range) [years]	33.6 (23-49)	30.5 (18–47)	n.s.**	-	-	-	-	-	-
Median disease duration (range) [days]	-	22.6 (2–60)	-	-	-	-	-	-	-
Median EDSS (range)	-	2.0 (0.0-6.0)	-	1.0 (0.0-3.0)	**<0.001*** **	1.5 (0.0-4.0)	n.s.***	1.5 (0.0-4.0)	1***
Mean T2 LV (SD) [ml]	-	3.8 (4.9)	-	3.7 (4.8)	n.s.***	4.4 (7.6)	n.s.***	4.1 (4.9)	n.s.***
Mean T1 LV (SD) [ml]	-	1.2 (1.9)	-	1.4 (2.2)	n.s.***	1.4 (3.2)	n.s.***	1.4 (2.3)	n.s.***
Mean Gd LV (SD) [ml]	-	0.0 (0.0)	-	0.0 (0.1)	n.s.***	0.0 (0.1)	n.s.***	0.0 (0.1)	n.s.***
Mean (SD) NBV [ml] and PBVC (%)	1561 (85)	1552 (96)	n.s.**	+ 0.3 (0.57)	n.s.***	- 0.37 (0.61)	n.s.***	-0.86 (1.26)	**<0.05*** **
Mean (SD) R normalised hippocampal volume [ml]	3.71 (0.4)	3.64 (0.4)	n.s.**	3.66 (0.4)	n.s.***	3.65 (0.3)	n.s.***	3.51 (0.3)	**<0.001*** **
Mean (SD) L normalised hippocampal volume [ml]	3.62 (0.4)	3.55 (0.4)	n.s.**	3.65 (0.3)	**0.01*** **	3.52 (0.3)	n.s.***	3.33 (0.3)	**<0.001*** **

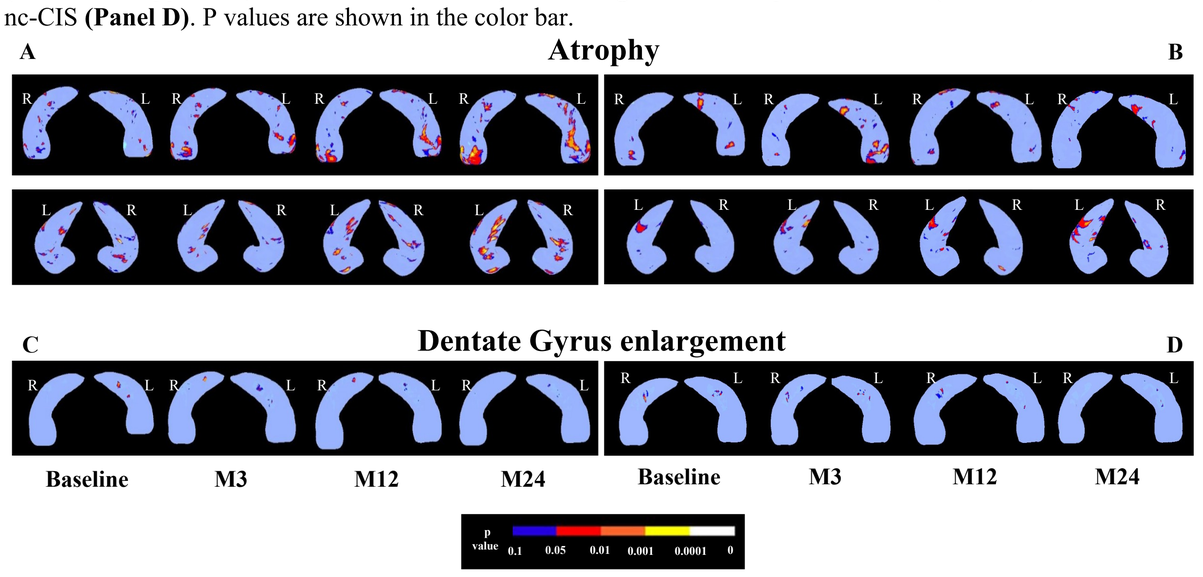
p= *Pearson $\chi 2$, **Mann-Whitney, ***Wilcoxon test for paired data.

Abbreviations: EDSS=expanded disability status scale; LV=lesion volume; SD=standard deviation; NBV=normalised brain volume; PBVC=percentage brain volume change; R=right; L=left; M3=3-month follow-up; M12=12-month follow-up; M24=24-month follow-up.

Radial mapping analysis: CIS patients showed clusters of reduced radial distance (RD) in the Cornu Ammonis 1 (CA1) at baseline (p<0.01 right, p<0.05 left), progressively extending to the subiculum. RD negatively correlated with ipsilateral T2 and T1 lesion volume. Increased RD of the DG appeared in the right hippocampus after three (p<0.01) and 12 months (p<0.05), and in the left hippocampus at baseline (p<0.05) and after 3 months (p 0.05).

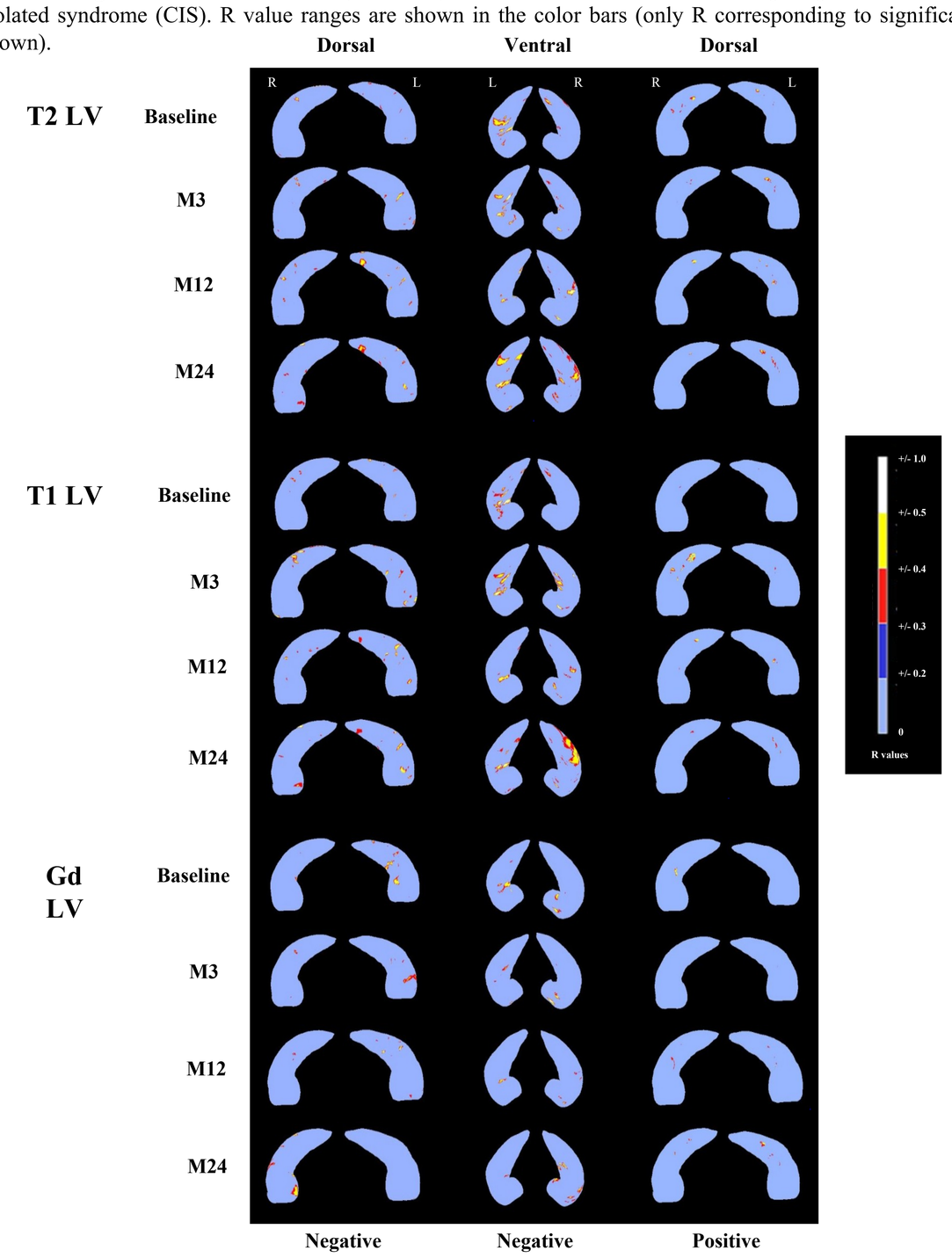
Similarly, compared to nc-CIS, c-CIS showed a significantly reduced RD in the CA1 subfield of the head (p range 0.05-0.001 bilaterally) and of the left tail (p range 0.05-0.001) at baseline, extending in the head, especially in the left hippocampus at M3 (p range right head 0.05-0.001, left head and tail 0.05-0.001) and M12 (p range right tail 0.05-0.001, left tail 0.05-0.001) and M24 (p range 0.05-0.001 for both left and right tail). At baseline, M3 and M12 follow-up, c-CIS showed a bilaterally increased RD of the DG compared to nc-CIS, that disappeared at M24 (Figure 1).

Figure 1. Surface distribution of regions of significant local atrophy (dorsal view upper line, ventral view lower line) in CIS patients *vs* HC (**Panel A**) and in c-CIS *vs* nc-CIS (**Panel B**) at baseline and after 3, 12 and 24 months (dorsal view upper line, ventral view lower line). DG enlargement in CIS patients *vs* HC (**Panel C**) and in c-CIS *vs* nc-CIS **(Panel D)**. P values are shown in the color bar.



Analysis of correlation: In CIS patients, T2 LV was correlated with hippocampal RD reduction, bilaterally, in the subiculum at baseline (p range 0.05-0.01 right, 0.05-0.001 left) and at M3 (p range 0.05-0.01 right, 0.05-0.001 left), and in the CA1 subfield of the tail at M12 and M24 (p range 0.05-0.01 right, 0.05-0.01 left) (r values, corresponding to significant p, are shown in Figure 2). Similar results, with different p and r values, were found for T1 LV (Figure 2), whereas Gd LV showed a weaker correlation with RD reduction during the whole study (Figure 2). T2 LV was positively correlated with DG enlargement of left hippocampus at baseline (p range 0.01-0.001) and M3 (p range 0.01-0.001) and right hippocampus at M12 (p range 0.01-0.001 right). T1 LV was positively correlated with right DG expansion at M3 and M12 ($p < 0.001$) (Figure 2).

Figure 2. Probability maps of the correlations between radial distance (RD) and ipsilateral T2 lesion volume (LV), T1 LV and gadolinium LV at baseline, 3-month, 12-month and 24-month follow-up in patients with clinically isolated syndrome (CIS). R value ranges are shown in the color bars (only R corresponding to significant p are shown).



CONCLUSIONS

- Regional hippocampal changes occur in the early phase of the disease and are characterized by atrophy of the CA1 and subiculum and early expansion of the DG.
- Similarly, patients converting to MS showed a relative atrophy of the CA1 subfield (especially in the left hippocampus) and a relative enlargement of the DG if compared with non-converters.
- Hippocampal volume abnormalities are dynamic and are modulated by inflammation, as suggested by the correlation between DG expansion and white matter lesional measures.

DISCLOSURES

L. Cacciaguerra, E. Pagani, S. Mesaros, J. Dackovic, I. Dujmovic-Basuroski, J. Drulovic and P. Valsasina have nothing to disclose.

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REFERENCES: 1) Calabrese et al., Neurology 2011; 2) Kolber et al., Journal of Neurology 2015; 3) Rocca et al., Human Brain Mapping 2015; 4) Chard et al., Journal of Magnetic Resonance Imaging 2010; 5) Pruessner et al., Cerebral Cortex 2000; 6) Thompson et al., Neuroimage 2007.