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# Cognitive impairment and memory disorders in relapsing–remitting multiple sclerosis: the role of white matter, gray matter and hippocampus

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**Abstract** Cognitive disorders occur in up to 65 % of multiple sclerosis (MS) patients; they have been correlated with different MRI measures of brain tissue damage, whole and regional brain atrophy. The hippocampal involvement has been poorly investigated in cognitively impaired (CI) MS patients. The objective of this study is to analyze and compare brain tissue abnormalities, including hippocampal atrophy, in relapsing–remitting MS (RRMS) patients with and without cognitive deficits, and to investigate their role in determining cognitive impairment in MS. Forty-six RRMS patients [20 CI and 26 cognitively preserved (CP)] and 25 age, sex and education-matched healthy controls (HCs) underwent neuropsychological evaluation and 3-Tesla anatomical MRI. T2 lesion load (T2-LL) was computed with a semiautomatic method, gray matter volume and white matter volume were estimated using SIENAX. Hippocampal volume (HV) was obtained by manual segmentation. Brain tissues volumes were compared among groups and correlated with cognitive performances. Compared to HCs, RRMS patients had significant atrophy of WM, GM, left and right Hippocampus ($p < 0.001$).

Compared to CP, CI RRMS patients showed higher T2-LL ($p = 0.02$) and WM atrophy ($p = 0.01$). In the whole RRMS group, several cognitive tests correlated with brain tissue abnormalities (T2-LL, WM and GM atrophy); only verbal memory performances correlated with left hippocampal atrophy. Our results emphasize the role of T2-LL and WM atrophy in determining clinically evident cognitive impairment in MS patients and provide evidence that GM and hippocampal atrophy occur in MS patients regardless of cognitive status.

**Keywords** Multiple sclerosis · Cognitive disorders · Hippocampal volumes · MRI, lesion load, white matter, gray matter

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## Introduction

Cognitive disorders (CD) are frequently reported in Multiple Sclerosis (MS) occurring in 40–65 % of patients [1]. They start early in the disease, generally worsen over time and are partially independent of physical disability [1]. The most frequent deficits are found in processing speed, working memory, new learning, visual and verbal memory [2]. Cognitive impairment deeply impacts on quality of life and therapy adherence in MS patients; the definition of its pathogenic mechanisms and the identification of markers useful to monitor its evolution might contribute to identify pharmacologic and rehabilitative strategies.

CD have been associated with different magnetic resonance imaging (MRI) measures of brain tissue damage such as T2 lesion load (T2-LL) [3], whole brain [4], ventricular and cortical volumes [5], corpus callosum size [6] and cortical lesions [7]. The clinical relevance of regional cortical volume, including hippocampal volume, has

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recently been explored [8–11]; since the Hippocampus is known to play a critical role in visual and verbal memory [12], frequently compromised in MS [2], it represents an important target to study memory disorders in MS.

Indeed, histo-pathologic, morphological and functional MRI studies have recently reported hippocampal involvement in MS patients showing hippocampal demyelination, atrophy, and functional abnormalities of the cingulated-hippocampus network [13–17]. Moreover, in MS patients, hippocampal atrophy has been correlated with neuropsychological scores [8–11].

Despite the role of hippocampus in cognitive domains compromised in MS patients, the manner in which hippocampal atrophy affects cognitive function in MS is not well established.

To better understand the pathophysiological basis of CD in MS, we performed, an extensive evaluation of cognitive status and MRI measures of brain tissue damage, including hippocampal volume (HV), in MS patients with and without cognitive impairment and in healthy controls (HCs); we also looked at the correlations between brain tissue volumes and cognitive performances.

## Methods

### Study population

We studied 46 patients with relapsing–remitting MS (RRMS) according to revised McDonald criteria [18] and 25 HCs matched for sex, age and education.

MS patients were recruited among those referred to our outpatient MS clinic according to the following inclusion criteria: age 18–60 years (mean $39.6 \pm 7.7$; range 18–57); absence of relapses and steroid therapy for at least 3 months prior to the study; absence of significant anxiety or depression as assessed, respectively, by the State Trait Anxiety Inventory [19] and the Chicago Multiscale Depression Inventory [20].

Patients and HCs taking psychoactive drugs or substances that might interfere with neuropsychological examination (NPE) were excluded. Other exclusion criteria were claustrophobia and all contraindications for undergoing MRI investigation.

The study was approved by local Ethic Committee and a signed informed consent was obtained by all participants according to the Declaration of Helsinki.

### Clinical and neuropsychological assessment

All participating subjects underwent neurological evaluation (NE) and NPE; in MS patients, disability was assessed by the expanded disability status scale (EDSS)

[21]. Both NE and NPE took place the same day of MRI scan; NPE was performed using the version A of the Rao's brief repeatable battery (BRB), which incorporates subtests of verbal memory acquisition and delayed recall [selective reminding test (SRT) and SRT-delayed recall (SRT-D)], visuo-spatial memory acquisition and delayed recall [10/36 spatial recall test (10/36-SPART) and 10/36-SPART-delayed recall (10/36-SPART-D)], concentration, sustained attention and information processing speed [paced auditory serial addition test at 3 (PASAT3″) and 2 (PASAT2″) seconds, symbol digit modalities test (SDMT)], and verbal fluency on semantic stimulus [word list generation (WLG)] [22]. Moreover, we administered the stroop color–word interference test (SCWIT) to better investigate executive functions. Neuropsychological scores were converted into $Z$ scores using the available normative data of the Italian population [23]. Test failure was defined as a $Z$ score $\leq 2$. Patients were categorized as cognitively impaired (CI) when they failed at least two tests of the NPE, with at least one exploring hippocampal functions (verbal and/or visuo-spatial memory) [23]. Contrariwise RRMS patients who did not fail any test or just one, excluding verbal or visuo-spatial memory tests, were classified as cognitively preserved (CP).

### MRI scanning protocol

MRI datasets were acquired on a 3-T GE Medical System (Milwaukee, WI, USA) scanner equipped with an 8-channel parallel head coil. The following images were acquired: (1) fast spin echo (FSE) dual-echo (DP-T2) [repetition time (TR) = 3,080, echo time (TE) 1/TE2 = 24/120 ms, axial slices = 88, matrix = 256 × 384, field of view (FOV) = 240 mm, thickness = 3 mm, interslice gap = 0 mm]; (2) T2-fluid-attenuated inversion recovery (T2-FLAIR) [TR = 9,000, TE = 1200, inversion time (TI) = 2500, axial slices = 44, matrix = 224 × 448, FOV = 240 mm, thickness = 3 mm, interslice gap = 0 mm]; (3) high-resolution 3D T1 (GE sequence IR-FSPGR, TR = 6988 ms, TI = 1100 ms, TE = 3.9 ms, flip angle = 10, voxel size = 1 × 1 × 1.2 mm$^3$).

### T2-LL measurement

Hyperintense T2 lesions were first identified on FSE dual-echo images by a single operator blinded to the patients' cognitive status using the T2-FLAIR images as further reference. T2 lesions' contours were transferred on electronic MRI data by means of a semiautomatic method implemented in Medical Image Processing, Analysis and Visualization application (MIPAV version 4.2.2, http://mipav.cit.nih.gov/). The same software was used to compute the global T2-LL for each subject.

## Brain atrophy measurement

On high-resolution 3D T1-weighted images, normalized volumes of the whole brain were obtained by the SIENA (Structural Image Evaluation using Normalization of Atrophy) software, a fully automated and accurate method for measuring longitudinal and cross-sectional changes in brain volume [24] which is freely available as part of the FMRIB Software Library (FSL) (http://www.fmrib.ox.ac.uk/fsl). In the current study the cross-sectional version (SIENAX) was used. As a first step, this software performs brain segmentation from non-brain tissue. As a second step, the software creates a probabilistic brain mask making sure that certain structures such as eyes and optic nerves have not been included in the brain segmentation. Finally, MR imaging random field model-based segmentation [25] is used to segment the brain image into different tissue types giving a normalized volume of total brain, GM and WM.

To reduce the influence of WM lesions in the measurement of brain volumes, the lesion filling tool of the FSL (http://www.fmrib.ox.ac.uk/fsl) was used on 3D T1 datasets [26].

## Hippocampus segmentation

3D-T1 images were re-oriented along the anterior–posterior commissure line, setting the anterior commissure as the origin of the spatial coordinates. Normalization was carried out through a 12-parameter affine transformation on a customized template [27]. A blinded tracer outlined the hippocampi, following a standardized and validated protocol [28] and using an interactive software program developed at the LONI [Laboratory of NeuroImaging, University of California at Los Angeles (http://www.loni.ucla.edu/Software/MultiTracer)] previously used in Alzheimer's disease neuroimaging initiative (ADNI) project [28] and in MS patients [11]. Each hippocampus comprised approximately 30–40 consecutive slices and segmentation process took approximately 30 min per subject.

Since the HV is influenced by the head size, the absolute HV was multiplied by the volume correction factor determined by SIENAX. The normalized HV (NHV) was used for data analysis.

To test intra-rater reproducibility of hippocampal segmentation, our blinded tracer evaluated the scans of 25 randomly selected subjects twice, 3 weeks apart. Intra-class correlation coefficient was 0.95 for intra-rater assessment.

## Data analysis

All data were analysed using the STATA version 12.0 software package (StataCorp, College Station, Texas, http://www.stata.com).

The Shapiro–Wilk test and graphical methods were used to assess the normal distribution of data. In case of normal distribution, the data are presented as mean and standard deviation (SD); in case of deviation from the normal distribution, the data are presented as median and interquartile range (IQR).

A descriptive statistic for the collected data was performed. Between-group differences were tested by unpaired two-tailed Student $t$ test for independent samples or the Mann–Whitney $U$ test. HCs were compared to the whole group of MS patients and then separately to CI and CP patients. Finally CI and CP patients were compared each other. The significance level was set at 0.05.

Correlations between different MRI parameters and clinical/NPE data were analysed by Pearson correlation coefficient ($r$) or Spearman's rank correlation coefficient ($r^2$), controlling for anxiety and depression.

## Results

Our RRMS population included 26 CP and 20 CI patients. Clinical and demographic characteristics of all study participants are summarized in Table 1. Patients and HCs were matched for age, sex and education. All subjects were right-handed. Disease duration ($p = 0.70$), number of relapses in the previous two years ($p = 0.37$) and EDSS score ($p = 0.33$) were not statistically different between CI and CP patients. The proportion of patients failing each neuropsychological test in the whole MS group, CI and CP groups is summarized in Table 2.

As previously shown [29], in both HCs and MS patients, right NHV [HCs (mean = 4.103, SD = 0.364); (MS patients mean = 3.552, SD = 0.512)] was significantly higher than the left one [HCs (mean = 3.993, SD = 0.335); MS (mean = 3.421, SD = 0.488)] (HCs: $p = 0.02$; RRMS: $p < 0.001$). Therefore, statistical analyses were performed separately for right and left NHV. Right and left NHV were significantly lower in the whole group ($p < 0.001$), CI ($p < 0.001$) and CP ($p \leq 0.001$) MS patients when compared to HCs. Right ($p = 0.24$) and left NHV ($p = 0.17$) were not significantly different between CP and CI patients (Table 3). GM volume (GMV) ($p \leq 0.001$) and WM volume (WMV) ($p \leq 0.001$) were significantly smaller in the whole group of MS patients compared to HCs (Table 3). These differences were still significant when comparing HCs to CI ($p \leq 0.001$) or CP (GM: $p = 0.002$; WM: $p = 0.006$) separately. GMV was not statistically different between CI and CP patients ($p = 0.25$), while WMV was significantly smaller in CI as compared to CP patients ($p = 0.01$) (Table 3). LL was significantly higher in CI patients as compared to CP ones ($p = 0.02$) (Table 3).

**Table 1** Clinical and demographic characteristics of healthy controls (HCs) and MS patients (Pts) cognitively impaired (CI) and preserved (CP)

|  | HCs | MS Pts | CP | CI | HCs vs MS Pts | HCs vs CP | HCs vs CI | CP vs CI |
|---|---|---|---|---|---|---|---|---|
| N | 25 | 46 | 26 | 20 | $p$ | $p$ | $p$ | $p$ |
| Mean (SD) age, years | 36.3 (9.2) | 39.6 (7.7) | 40.0 (5.8) | 39.1 (9.8) | 0.11 | 0.09 | 0.33 | 0.68 |
| N M/F | 8/17 | 17/29 | 9/17 | 8/12 | 0.68 | 0.84 | 0.58 | 0.71 |
| Mean (SD) education, years | 14.2 (3.3) | 12.6 (3.5) | 12.5 (3.2) | 12.7 (4.0) | 0.06 | 0.07 | 0.16 | 0.89 |
| Mean (SD; range) disease duration, years | – | 11.7 (6.6; 1–25) | 12.0 (7; 1–25) | 11.3 (6.1; 2–24) | – | – | – | 0.70 |
| Median (range) EDSS score | – | 2.5 (1–6.0) | 2.5 (1.5–6) | 3.0 (1–6.5) | – | – | – | 0.33 |
| Relapse 2 years (range) |  | 1 (0–2) | 1 (0–2) | 1 (0–2) |  |  |  | 0.37 |

**Table 2** Proportion of patients failing neuropsychological tests

| Neuropsychological test | N of MS Pts (%) | N of CI Pts (%) | N of CP Pts (%) |
|---|---|---|---|
| SRT/LTS | 10/46 (22 %) | 10/20 (50 %) | 0/26 (0 %) |
| SRT/CLTR | 10/46 (22 %) | 10/20 (50 %) | 0/26 (0 %) |
| 10/36 SPART | 10/46 (22 %) | 10/20 (50 %) | 0/26 (0 %) |
| SDMT | 18/46 (39 %) | 18/20 (90 %) | 0/26 (0 %) |
| PASAT 3″ | 10/46 (22 %) | 9/20 (45 %) | 1/26 (4 %) |
| PASAT 2″ | 5/46 (11 %) | 5/20 (25 %) | 0/26 (0 %) |
| SRT/D | 11/46 (23 %) | 11/20 (55 %) | 0/26 (0 %) |
| 10/36 SPART/D | 7/46 (15 %) | 7/20 (35 %) | 0/26 (0 %) |
| WLG | 14/46 (30 %) | 11/20 (55 %) | 3/26 (12 %) |
| SCWIT | 17/46 (37 %) | 14/20 (70 %) | 3/26 (12 %) |

*RRMS* relapsing–remitting multiple sclerosis, *CI* cognitively impaired, *CP* cognitively preserved, *SRT, SRT/D* selective reminding test and selective reminding test-delayed recall, *LTS* long-term storage, *CLTR* consistent long-term retrieval, *10/36, 10/36D* 10/36 spatial recall test and 10/36 spatial recall test-delayed recall, *SDMT* symbol digit modalities test, *PASAT 3″* paced auditory serial addition test at 3 s, *PASAT 2″* paced auditory serial addition test at 2 s, *WLG* word list generation, *SCWIT* stroop color word interference test

In the whole RRMS group, clinical data (disease duration, EDSS) did not show any correlation with hippocampal atrophy.

In the whole group of RRMS patients, the correlation analysis showed correlations between some cognitive tests' performances and MRI measures of brain damage (T2-LL volume, hippocampal, WM and GM atrophy). Particularly GM atrophy was associated with visuo-spatial memory performances while atrophy of WM and left Hippocampus were correlated with verbal memory performances (Table 4). Finally, T2-LL volume was correlated with executive functions, visuo-spatial memory, processing speed and sustained attention performances (Table 4).

In the CI subgroup, significant correlations were found between verbal memory tests and atrophy of WM [LTS ($r = 0.46$; $p = 0.05$), CLTR ($r = 0.55$; $p = 0.01$), D-SRT ($r = 0.45$; $p = 0.05$)] and left Hippocampus [LTS ($r = 0.46$; $p = 0.04$), CLTR ($r = 0.55$; $p = 0.01$)].

## Discussion

In the present study, we assessed MRI structural brain abnormalities, including hippocampal atrophy, in RRMS patients (with and without cognitive impairment) compared to HCs. We focused the attention on the hippocampus as it plays a crucial role in cognitive performances commonly impaired in MS patients (i.e., visual and verbal memory) [2, 22]. Therefore, it represents an interesting target of study to understand mechanisms underlying CD in MS.

As previously reported, our MS patients showed significant WM and GM atrophy relative to HCs [30, 31]. Furthermore, in line with a previous study using similar hippocampal segmentation software [11], we reported right and left hippocampal atrophy in MS patients with respect to HCs [8–11, 17]. Conversely, our CI RRMS patients showed exclusively WM atrophy and higher T2-LL when compared to CP patients. Therefore, we mainly confirm the results of a previous study showing the presence of hippocampal atrophy in MS patients and the absence of major hippocampal atrophy in CI with respect to CP patients [17]. We also confirm some studies investigating correlations between cognitive performances and MRI measures of brain damage, such as the relationship between T2-LL and processing speed/sustained attention performances and between left hippocampal atrophy and verbal memory [8, 30, 31]. The latter correlation reflects the structural and functional hippocampal organization [32]. However, previously reported correlations between cognitive performances and MRI parameters are not univocal, suggesting that cognitive dysfunctions in MS may depend on different factors such as T2-LL, lesion location (in WM and GM), global or regional GM/WM atrophy and normal appearing WM/GM damage.

Our results are in line with several recent studies that found that WM damage produces the major contribution to CD in MS, whereas GM damage adds only a small increment to the variance due to WM impairment [33–36]. Specifically, the slowed processing speed and particularly, the processing inefficiency were primarily associated with WM damage [37, 38].

Although our CI patients had been strictly selected for impairment of hippocampal domains, over 90 % of them showed also processing speed or attention deficits as generally reported in MS population; therefore, we hypothesize that the observed memory disorders may be due to an inadequate acquisition secondary to information processing insufficiency provoked by WM atrophy and higher T2-LL operating a disconnection among different cognitive networks [39, 40]. This hypothesis is also supported by several studies using advanced MRI techniques [i.e. functional MRI and diffusion weighted imaging (DTI)] showing that CD in MS mostly result from cortico-cortical or cortico-subcortical disconnection phenomena associated with abnormal functional connectivity (FC) [33, 40–45]. In CI patients, DTI studies found WM integrity changes, in relevant cognition areas, correlating with cognitive performances [33, 40–42]. FC changes at rest and during cognitive tasks were found in MS patients and to a major extent in CI patients [43–45]. Furthermore, FC changes may predate hippocampal atrophy as demonstrated either with functional MRI studies during memory task performances [17] or with resting-state functional MRI studies [16]. Interestingly, the fore-mentioned studies also found

**Table 3** MRI characteristics of healthy controls (HCs) and RRMS patients [whole MS group, cognitively preserved (CP) and impaired (CI)]

| | HCs | MS Pts | CP | CI | HCs vs MS Pts | HCs vs CP | HCs vs CI | CP vs CI |
|---|---|---|---|---|---|---|---|---|
| N | 25 | 46 | 26 | 20 | p | p | p | p |
| Median of GM volume (IQR), ml | 845.1 (791.6; 891.0) | 783.0 (762.4;810.0) | 790.2 (766.2; 826.1) | 773.4 (755.6; 798.0) | <0.001 | 0.002 | <0.001 | 0.25 |
| Mean of WM volume (SD),ml | 685.0 (40.5) | 644.0 (35.1) | 654.8 (34.2) | 629.3 (31.3) | <0.001 | 0.006 | <0.001 | 0.01 |
| Mean left hippocampal normalized volume (SD), ml | 3.993 (0.335) | 3.421 (0. 488) | 3.508 (0.439) | 3.308 (0.534) | <0.001 | <0.001 | <0.001 | 0.17 |
| Mean Right hippocampal normalized volume (SD), ml | 4.103 (0.364) | 3.552 (0.512) | 3.631 (0.503) | 3.450 (0.518) | <0.001 | 0.001 | <0.001 | 0.24 |
| Median T2-LL volume (IQR), ml | – | 6.624 (2.784; 17.517) | 5.403 (2.455; 9.577) | 14.260 (3.523; 23.812) | – | – | – | 0.02 |

*GM* gray matter, *WM* white matter, *CSF* cerebrospinal fluid, *T2-LL* T2-lesion load

**Table 4** Correlation between cognitive tests performances and brain volumes (GM, WM, left NHV, right NHV) and T2-lesion load total volume (T2-LL) in the whole MS patients' group

| | GM N 46 | | WM N 46 | | T2-LL N 46 | | Left NHV 46 | | Right NHV 46 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | r | p | r | p | r | p | r | p | r | p |
| SRT/LTS | 0.13 | 0.38 | **0.29** | **0.05** | −0.27 | 0.07 | **0.33** | **0.03** | 0.27 | 0.07 |
| SRT/CLTR | 0.09 | 0.57 | 0.28 | 0.06 | −0.25 | 0.09 | **0.36** | **0.02** | 0.28 | 0.06 |
| 10/36 SPART | **0.33** | **0.03** | 0.004 | 0.98 | −0.16 | 0.28 | 0.05 | 0.74 | 0.02 | 0.88 |
| SDMT | 0.27 | 0.07 | 0.24 | 0.11 | **−0.38** | **0.01** | 0.22 | 0.14 | 0.19 | 0.20 |
| PASAT 3″ | 0.14 | 0.37 | 0.25 | 0.09 | **−0.41** | **0.01** | 0.17 | 0.25 | 0.16 | 0.30 |
| PASAT 2″ | 0.15 | 0.32 | 0.27 | 0.07 | −0.26 | 0.08 | 0.21 | 0.17 | 0.15 | 0.31 |
| SRT-D | −0.04 | 0.77 | 0.25 | 0.10 | −0.12 | 0.42 | 0.12 | 0.42 | 0.07 | 0.64 |
| 10/36 SPART-D | **0.34** | **0.02** | 0.17 | 0.25 | **−0.46** | **0.001** | 0.15 | 0.32 | 0.13 | 0.40 |
| WLG | 0.11 | 0.48 | 0.09 | 0.57 | −0.26 | 0.09 | −0.04 | 0.79 | −0.04 | 0.80 |
| **SCWIT** | 0.18 | 0.23 | 0.28 | 0.07 | **−0.29** | **0.05** | 0.21 | 0.16 | 0.22 | 0.15 |

Bold values indicate statistically significant correlation

*SRT/LTS* selective reminding test/long-term storage, *SRT/CLTR* selective reminding test/consistent long-term retrieval, *10/36 SPART* 10/36 spatial recall test, *SDMT* symbol digit modalities test, *PASAT* paced auditory serial addition test, *SRT-D* selective reminding test-delayed, *10/36 SPART-D* 10/36 spatial recall test-delayed, *WLG* word list generation, *SCWIT* stroop color–word interference test

that damage of specific WM structures may have a significant impact on resting-state FC, while GM damage do not colocalize with resting-state FC changes.

Although we mainly confirmed previous findings, our study's strength could be summarized in two main points: the sample selection and the detailed manual delineation of hippocampus following a standardized and validated segmentation protocol [11, 27, 28]. Indeed, to have a sample as homogeneous as possible, we recruited exclusively RRMS patients with similar clinical features and disability score. Differently from previous studies [8–11, 17], we divided MS patients in CP and CI, using a specific and validated battery (BRB) exploring all typical MS impaired domains and hippocampal functions (visuo-spatial and verbal memory). Moreover, in contrast to the majority of studies, we also acquired neuropsychological data from a control group [8–11].

With respect to the hippocampal manual segmentation, it is worthwhile to consider that several studies performing direct comparison between manual and automated hippocampal volume measurements showed that automated procedures give highly reproducible measurements but generate larger and less precise volumes than manual techniques [46, 47].

However, some limitations of the present study should be taken into account: (1) the sample size was small; (2) T2-LL and brain atrophy were not regionally assessed; (3) double inversion recovery pulse sequences, useful to detect hippocampal focal lesions, were not acquired; (3) CI patients were identified according to the criterion of "at least one abnormal memory test"; probably the inclusion of patients with only memory deficits, although difficult to select, would have provided more informative results on the role of hippocampal atrophy in this group of patients.

In conclusion, our study confirms and expands previous studies showing a significant GM and hippocampal atrophy in all MS patients, regardless of global cognitive status; it also emphasizes the role of WM atrophy and T2-LL in determining cognitive impairment, including memory disturbances, likely interrupting neuronal pathway involved in information processing.

Future studies, with a longitudinal design and a larger sample size, including patients with only memory deficits and preserved other cognitive domains, are warranted to better elucidate the role of hippocampal atrophy in determining CD in MS.

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