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The topography of cortical microbleeds in frontotemporal lobar degeneration: a post-mortem 7.0-tesla magnetic resonance study

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Abstract

Introduction: *Cerebrovascular lesions are rare in frontotemporal lobar degeneration (FTLD), in contrast to other neurodegenerative diseases. Cortical microbleeds (CoMBs) are frequent in Alzheimer's disease, in particular in cases associated with cerebral amyloid angiopathy. The present study investigates the gyral topographic distribution of CoMBs in post-mortem FTLD brains with 7.0-tesla magnetic resonance imaging.*

Material and methods: *The distribution of CoMBs in 11 post-mortem FTLD brains and in 12 control brains was compared on T2*-GRE MRI of six coronal sections of a cerebral hemisphere. The mean values of CoMBs were determined in twenty-two different gyri. The findings were correlated to those separately observed on neuropathological examination.*

Results: *As a whole there was a trend of more CoMBs in the prefrontal section of FTLD as well as of the control brains. CoMBs were significantly increased in the superior frontal gyrus and the insular cortex ($p \leq 0.001$) and also in the inferior frontal gyrus and the superior temporal gyrus ($p \leq 0.01$).*

Conclusions: *CoMBs in FTLD are only increased in the regions mainly affected by the neurodegenerative lesions. They probably do not reflect additional cerebrovascular disease.*

Key words: *7.0-tesla magnetic resonance imaging, topography of post-mortem cortical microbleeds, frontotemporal lobar degeneration, neurodegenerative diseases.*

Introduction

Frontotemporal lobar degeneration (FTLD) is a heterogeneous disorder with various clinical and histological subtypes [22]. FTLD is the second most common cause of presenile dementia with different genetic subtypes [20]. Despite the fact that most cases have a presenile onset, FTLD is not rare amongst elderly patients [3]. A recent neuropathological study showed that age together with vascular and Alzheimer-related co-pathology contributes

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to the morphological appearance of FTLD-tau [23]. In our recent neuropathological assessment of small cerebrovascular lesions, only a prevalence of white matter changes was observed in the FTLD brains, compared to age-matched controls [10]. These findings are in contrast to the high incidence of macro- and micro-infarcts, and hematomas and small cerebral bleeds observed in brains of patients with Alzheimer's disease (AD), in particular in those with associated cerebral amyloid angiopathy (CAA) [6,7].

Cerebral microbleeds (CMBs) are frequently detected on T2*-weighted gradient-echo magnetic resonance imaging (MRI) of patients with small-vessel diseases [26]. They are also found in asymptomatic patients as well as in those with various degrees of cognitive impairment [5]. The number of CMBs detected during life depends on the MRI characteristics, such as pulse sequence, sequence parameters, spatial resolution, magnetic field strength and image post-processing [17]. 7.0-tesla T2*-weighted gradient-echo MRI is able to detect reliably even the smallest bleeds in the cerebral cortex (CoMBs) on post-mortem brain sections [8]. This technique allows to determine the topographic distribution and to quantify the number of CoMBs on a large number of brain sections [25]. In our previous study we have demonstrated a significant prevalence of CoMBs in the deep cortical layers of the frontal cortex in FTLD [9]. However this frontal predominance of CoMBs is also observed in AD brains with and without CAA and in controls [14].

The aim of the present post-mortem MRI study is to compare the prevalence of CoMBs in twenty-two different cortical gyri of pure FTLD versus controls in order to determine their eventual clinical impact.

Material and methods

Twenty-three patients, followed-up at the Lille University Hospital underwent an autopsy. The cohorts consisted of 11 patients with FTLD and 12 controls who had no clinical history of dementia or stroke. The vascular risk factors were registered from the clinical files. The median age at disease onset of the FTLD patients was 54 (interquartile range of 46-64) years. The clinical phenotype of the FTLD patients was the behavioral variant in 9 and semantic dementia in 2. Two patients had in addition amyotrophic lateral sclerosis. None of them had on neuropathological examination Alzheimer features or some degree of amyloid angiopathy. The post-mortem diagnosis of FTLD was made according to the neuropathological diagnostic and the nosological criteria of the Consortium for FTLD [4]. The main histological subtypes were FTLD-Tau in 3, FTLD-TDP-A in 1, FTLD-TDP-B in 3, FTLD-TDP-C in 3 brains and FTLD-FUS in 1 brain.

Previously obtained informed consent of the patients or from the nearest family allowed an autopsy for diagnostic and scientific purposes. The brain tissue samples were acquired from the Lille Neuro-Bank of the Lille University, federated to the Centre de Ressources Biologiques that acted as an institutional review board.

One fresh cerebral hemisphere was deeply frozen for biochemical examination. The remaining hemisphere, the brainstem and most of the cerebellum were fixed in formalin for 3 weeks.

The patients with FTLD were compared concerning the incidence of CoMBs to the control group.

Neuropathological examination

The diagnosis of FTLD was made according to a standard procedure examining samples from the primary motor cortex, the associated frontal, temporal and parietal cortex, the primary and secondary visual cortex, the cingulate gyrus, the basal nucleus of Meynert, the amygdaloid body, the hippocampus, basal ganglia, mesencephalon, pons, medulla and cerebellum. Slides from paraffin-embedded sections were immune-stained for protein tau, β-amyloid, α-synuclein, prion protein and TDP-43. FUS histochemistry was performed in Tau and TDP negative cases.

A quantitative evaluation of the cerebrovascular lesions was performed on a standard coronal section of a cerebral hemisphere, at the level of the mammillary body according to a previously described method [6].

MRI examination

Six coronal sections of a cerebral hemisphere from each brain were submitted to MRI: one at the prefrontal level in front of the frontal horn, one of the frontal lobe at the level of the head of the caudate nucleus, a central one near the mammillary body, a post-central one, a parietal one at the level of the splenium corporis callosi and one at the level of the occipital lobe.

Table I. Brain regions and gyri of interest on magnetic resonance imaging

Frontal lobe	Temporal lobe	Parietal lobe	Occipital lobe
Frontalis inferior	Temporalis inferior	Postcentralis	Lingualis
Frontalis medius	Temporalis medius	Insula	Precuneus
Frontalis superior	Temporalis superior	Parietalis inferior	Cuneus
Precentralis	Hippocampus	Parietalis medius	Occipitotemporalls
Rectus	Dentatus	Parietalis superior	
Orbitalis	Parahippocampalis	Cinguli	

We used a 7.0-tesla MRI Bruker BioSpin SA with an issuer-receiver cylinder coil of 72 mm inner diameter (Ettlingen, Germany), according to a previously described method [8]. The brain sections, previously cleaned from formalin, were placed in a plastic box filled with salt-free water, the size of which did not allow significant tissue movements. Three MRI sequences were used: a positioning sequence, a T2 sequence and a T2* sequence. The positioning sequence allowed determination of the three-directional position of the brain section inside the magnet. The thickness of the T2 images was 1 mm. The field of view was a 9 cm-square slide that was coded by a 256 matrix giving a voxel size of 0.352 × 0.352 × 1 mm. T2 weighted images were obtained by using RARE sequence (Rapid Acquisition with Relaxation Enhancement) with repetition time (TR), echo time (TE) and RARE factor of 2,500 ms, 33 ms and 8, respectively. The acquisition time of this sequence was 80 s The thickness of the T2* images was 0.20 mm.

Table II. Comparison of the patients' characteristics, vascular risk factors and ranking values of the different neuropathological lesions in the control brains and those with frontotemporal lobar degeneration (FTLD)

Items	Control (n = 12)	FTLD (n = 11)	p value
Median age in years (IQR) at decease	67 (58-78)	67 (62-68)	0.52
Gender (% of males)	42	27	0.67
Vascular risk factors (%)			
Arterial hypertension	17	18	1.0
Diabetes	17	18	1.0
Hypercholesterolemia	17	18	1.0
Smoking	0	0	1.0
Antithrombotic drug use	25	18	1.0
Neuropathological lesions: ranking scores (standard deviations)			
White matter changes	0.3 (0.8)	1.5 (1.1)	**0.01**
Lacunar infarcts	0.0 (0.0)	0.2 (0.7)	0.70
Territorial infarcts	0.0 (0.0)	0.0 (0.0)	1.0
Haematomas	0.1 (0.3)	0.0 (0.0)	0.70
Cortical microinfarcts	0.1 (0.3)	0.1 (0.3)	0.60
Cortical microbleeds	0.1 (0.3)	0.9 (0.8)	0.04

IQR – interquartile range

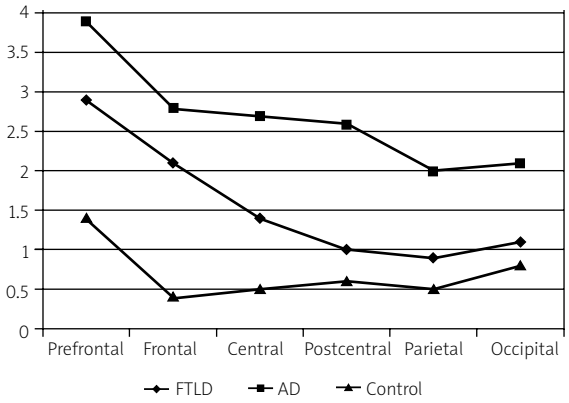

Fig. 1. Mean values of cortical microbleeds in the six coronal sections on T2*-weighted gradient-echo (GRE) magnetic resonance imaging of brains with frontotemporal lobar degeneration compared to those with Alzheimer's disease and to controls. Although their numbers are different between the three groups, they all display a similar anterior-posterior decreasing gradient of cortical microbleeds.

The field of view was also a 9 cm^2. It was coded by a 512 matrix, giving a voxel size of 0.176 × 0.176 × 0.2 mm. The slice thickness corresponded to the upper part of the brain section. This sequence was a GRE sequence with a short TR of 60 ms and TE of 22 ms, a flip angle of 30° and number of excitation of 20. The acquisition time of the sequence was 10 minutes.

The total number and the location of CoMBs was determined in 22 gyri of each brain (Table I) by consensus evaluation by three observers (JDR, FA, ND) blinded to the neuropathological diagnosis and based on comparison of brain sections of an anatomical atlas [19]. The inter-rater reliability resulted in an interclass correlation coefficient of 0.79. The mean values of CoMBs in FTLD brains were compared to the controls.

Statistical analyses

Univariate comparisons of unpaired groups were performed with the Fisher's exact test for categorical data. The non-parametric Mann-Whitney *U*-test was used to compare continuous variables. The significance level, two-tailed, was set at ≤ 0.01 for significant and ≤ 0.001 for highly significant. Values set at ≤ 0.05 and more than > 0.01 were considered as marginal significant and not included as relevant due to the relative small sample sizes.

Results

The FTLD and control groups did not show any statistical differences according to age, gender distribution and vascular risk factors. However, on the

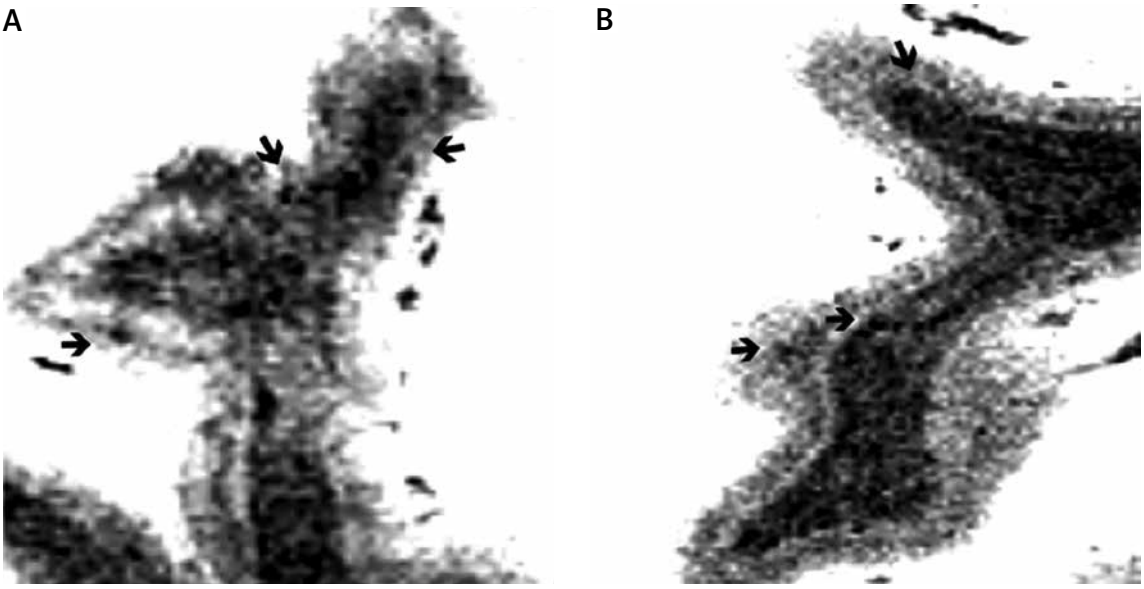

Fig. 2. Cortical microbleeds (arrows) on T2*-weighted gradient-echo (GRE) magnetic resonance imaging in the gyrus frontalis superior (**A**) and in the gyrus frontalis inferior (**B**).

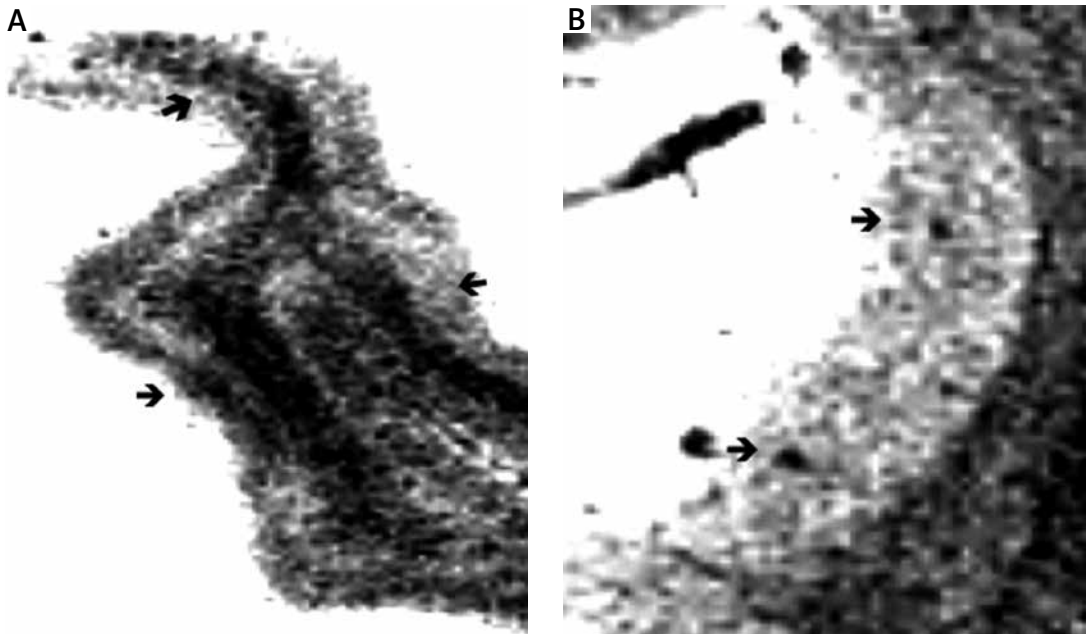

Fig. 3. Cortical microbleeds (arrows) on T2*-weighted gradient-echo (GRE) magnetic resonance imaging of the gyrus temporalis superior (**A**) and in the insular cortex (**B**).

neuropathological examination, white matter changes were significantly increased in the FTLD group compared to the control group ($p = 0.01$), while only marginally increased for the CoMBs. The other cerebrovascular lesions were rare and not statistical different between both groups (Table II).

When comparing on MRI the mean values of CoMBs in the six coronal sections on T2* GRE MRI an anterior-posterior decreasing gradient was observed in the FTLD as well as in the AD and the control brains (Fig. 1).

The mean values of CMBs in FTLD brains were significantly higher in the frontal superior gyrus and in the insular cortex ($p \leq 0.001$) (Fig. 2) and significantly in the frontal inferior and the temporal superior gyri ($p \leq 0.01$) (Fig. 3), compared to the control brains (Table III).

Discussion

The present study confirms that associated cerebrovascular lesions are rare in FTLD due to tau, TAR DNA-binding protein 43 and FUS [24]. Although in brains of elderly patients suffering from neurodegenerative diseases multiple pathologies are usually present [2], our series consist of pure types of FTLD allowing to determine more exactly the topography of CoMBs related to this disease spectrum. Despite a similar global bleeding load in FTLD and in age-matched control brains [11], a similar anterior-posterior decreasing gradient of CoMBs is observed on the sequential hemispheric sections. This is also common to AD brains with or without CAA [14]. This similarity is probably related to the larger development of the frontal lobe compared to the parietal and occipital lobes [21].

However, the main findings in this study are that the CoMBs predominate in the superior and inferior frontal gyri, the superior temporal gyrus and the insular cortex, regions that are most affected by the neurodegenerative process [1].

The findings in FTLD share similarity with those observed in our post-mortem 7.0-tesla MRI study in progressive supranuclear palsy, in which small bleeds are mainly found around the dentate nucleus of the cerebellum and in the tegmentum pontis, where also the major neurodegenerative changes occur [12].

There is no evidence for increased angiogenesis and microglial activation in the neuropathologically most affected regions of different neurodegenerative diseases such as in Alzheimer, Parkinson, progressive supranuclear palsy, and incidental Lewy body disease. Such neo-angiogenic vessels could contribute

Table III. Comparison of the mean values (standard deviations) of cortical microbleeds between different gyri of frontotemporal lobar degeneration (FTLD) and controls

Gyrus	C (n = 12)	FTLD (n = 11)	p value
Frontalis inferior	0.8 (0.9)	2.4 (1.6)	**< 0.01**
Frontalis medius	1.2 (1.4)	2.7 (1.7)	0.04
Frontalis superior	1.2 (1.4)	3.8 (1.6)	**< 0.001**
Precentralis	1.1 (1.5)	1.9 (1.2)	0.10
Rectus	0.4 (0.7)	1.0 (0.8)	0.04
Orbitalis	0.8 (1.1)	1.3 (1.1)	0.24
Temporalis inferior	0.4 (1.0)	1.2 (1.2)	0.03
Temporalis medius	0.5 (0.8)	1.3 (0.9)	0.02
Temporalis superior	0.6 (1.1)	1.7 (0.8)	**< 0.01**
Hippocampus	0.4 (0.8)	0.4 (0.6)	0.45
Dentatus	0.5 (1.1)	0.9 (1.3)	0.09
Parahippocampalis	0.7 (1.2)	1.5 (1.1)	0.04
Postcentralis	0.7 (0.9)	1.4 (1.0)	0.03
Insula	0.0 (0.0)	1.3 (1.3)	**< 0.001**
Parietalis inferior	0.3 (0.4)	0.9 (0.9)	0.13
Parietalis medius	1.2 (1.4)	0.9 (0.9)	0.93
Parietalis superior	0.6 (0.5)	1.2 (1.1)	0.24
Cinguli	0.4 (0.6)	0.7 (0.5)	0.15
Lingualis	0.3 (0.9)	0.7 (1.2)	0.08
Precuneus	0.5 (0.8)	0.6 (0.9)	0.74
Cuneus	0.5 (0.9)	0.8 (1.5)	0.56
Occipitotemporalis	0.6 (10)	1.5 (1.1)	0.03

to neuroinflammation and lead to disruption of the blood-brain barrier [15,16]. The prevalence of CoMBs and the white matter changes in FTLD brains should not be considered as the hallmark of cerebrovascular diseases. Also the increased iron accumulation observed in the basal ganglia of brains with FTLD should not be related to small-vessel ischemic disease [13] as recently proposed [18].

The fact that the CoMBs prevail in the regions with the most prominent neurodegenerative lesions indicates that blood-brain barrier impairments may interact with the severity of the neurodegeneration in FTLD and are secondary phenomena without impact on the clinical features of the disease.

Disclosure

Authors report no conflict of interest.

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