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# No increased cerebrovascular involvement in adult beta-thalassemia by advanced MRI analyses

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ABSTRACT

Beta-thalassemia-related anemia and chronic hypercoagulative state are supposed to cause cumulative cerebrovascular damage with consequent parenchymal/vascular changes and functional impairment. However, recent conventional MRI/MR-angiography investigations failed to show an increased cerebrovascular involvement in beta-thalassemia patients managed according to current treatment guidelines, in spite of significantly decreased full-scale IQ scores. We therefore investigated those patients and controls by means of advanced quantitative MRI analyses (based on magnetization transfer and diffusion tensor imaging) searching for signs of possible cerebrovascular injuries undetected by conventional MRI/MR-angiography. The 3 T-MRI study protocol included diffusion tensor imaging and 3D-multi-echo FLASH sequences for magnetization transfer analysis.

Whole-brain voxel-based analyses showed that magnetization transfer, fractional anisotropy, and mean, radial and axial diffusivity do not differ between healthy controls and beta-thalassemia patients (considered as a whole group or as distinct transfusion dependent and non-transfusion dependent subgroups). No correlation emerged between all the considered MRI metrics and cognitive findings (full-scale IQ) or the main clinical and laboratory data. According to our findings, adult neurologically-asymptomatic beta-thalassemia patients (regardless of clinical severity) do not seem to present an increased disease-related cerebrovascular vulnerability compared to healthy controls downsizing the need of regular brain MRI monitoring, at least when the current treatment guidelines are followed.

## 1. Introduction

With the dramatic improvement in the management of the several systemic beta-thalassemia-related complications, brain involvement has become one of the major concerns for patients and their physicians. Chronic microcytic anemia and hypoxia, cardiovascular involvement due to iron overload [1] and chronic hypercoagulable state [2] supposedly expose patients' brain to cumulative cerebrovascular injury with subsequent possible progressive functional (cognitive) impairment. Indeed, in the last two decades, routine conventional MRI studies have shown a high rate of vascular-like signal changes in the supratentorial white matter even in neurologically asymptomatic patients, [3–5] raising the issue of a regular brain MRI follow up to monitor the cumulative cerebrovascular brain injury. However, most reported MRI abnormalities referred to non-specific, often isolated small vascular-like white matter signal changes that might be encountered, as innocent

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bystanders, even in healthy subjects, especially when the MRI is performed with a high magnetic field scanner (1.5-3T) [6]. In this regard, almost all available brain MRI studies on beta-thalassemia lacked a control group, preventing to identify the true disease-related lesion burden. Recently, a case-control multimodal MRI and MR-angiography study investigated a large sample of transfusion-dependent and non-transfusion-dependent neurologically-asymptomatic adult beta-thalassemia patients with a 3 T MR scanner [7]. This study confirmed the presence of vascular-like changes among patients but yielded to no differences between patients and controls. White matter lesions' incidence rate as well as their number, size and distribution, the presence of silent territorial or lacunar strokes and the rate of intracranial vascular changes (stenosis, aneurysms, sinus venous thrombosis) did not differ among patient subgroups and healthy controls; ergo, the specter of an increased cerebrovascular vulnerability in beta-thalassemia appeared strikingly fading, at least when the current treatment guidelines are followed. However, in spite of these reassuring findings, the same patients' sample showed a significant cognitive impairment, [7,8] suggesting a plausible underlying brain involvement, even in absence of neurological or conventional MRI changes.

In the last years, two highly sensitive advanced MRI quantitative analyses based on relaxometry models, magnetization transfer measurements or water molecule diffusion properties have been developed to investigate in-vivo brain tissue microstructure. The magnetization transfer (MT) saturation technique provides a semiquantitative parameter reflecting the macromolecular brain tissue content that is used as a marker of myelin amount and integrity; current multi-echo three-dimensional fast low-angle-shot (FLASH) MRI acquisition sequences allow high-resolution whole-brain voxel-based quantitative MT analysis [4]. The diffusion tensor imaging (DTI) is a technique that allows the quantification of intra-voxel water molecule movement [9] in terms of mean diffusivity, fractional anisotropy, radial and axial diffusivity. These parameters add remarkable information on white matter maturation and microstructural integrity in the normal and diseased brains providing interesting hints about the nature of brain tissue microstructural changes (e.g. demyelination, axonal loss etc.). MT- and DTI-related analyses are especially sensitive since they can reveal subtle brain parenchymal abnormalities even when conventional MRI appears unremarkable, thus representing, so far, the best available techniques for the “in vivo” detection of cerebrovascular-related changes.

For these reasons, we investigated, by means of MT and DTI techniques, the sample of neurologically asymptomatic beta-thalassemia patients and controls recruited in the above-mentioned studies, [7,8] searching for signs of possible cerebrovascular injuries undetected by conventional MRI.

## 2. Material and methods

### 2.1. Subjects

Beta-thalassemia patients and healthy controls were recruited in four referral centers for beta-Thalassemia. Exclusion criteria were: 1) age < 16 years; 2) contraindications to MRI or 3) history of head trauma, neurosurgery or neurologic disease. All data were acquired between June 2016 and June 2017.

Healthy controls were volunteers recruited mainly among patients' relatives or entourage, to avoid most confounding factors on cerebrovascular involvement (environment, diet, etc.). No control subject was recruited in a hospital setting and the same exclusion criteria were used for the case group. Subjects were interviewed before MRI and those presenting major cerebrovascular risk factors (namely hypertension or diabetes) were excluded.

All recruited subjects participated to previous studies on brain involvement in beta-thalassemia [7,8].

The study protocol was approved by the institutional review board and written informed consent was obtained from each participant.

Study participants underwent brain MRI in a single session on the same 3 T scanner (MAGNETOM Skyra, Siemens, Erlangen Germany) with a 20-channel head coil. The study protocol included:

1) 3D fluid attenuated inversion recovery (FLAIR, TR/TE/TI 5000/387/1800 ms; voxel-size 1x1x1 mm; echo-train length 278; field of view 230; acquisition-time 4 min 32 s). Axial, coronal and sagittal multiplanar reconstructions of the whole brain were obtained from FLAIR (slice thickness 3 mm without interslice gap).
2) 2D echo-planar diffusion weighted imaging (DWI, TR/TE 9500/95 ms; acquisition matrix 128*128; slice thickness 2 mm; interslice gap 0 mm; field of view 256; b-value 1000 s/mm$^2$; number of excitations 1; gradient directions 30; number of slices 70; acquisition-time 6 min 31 s). Trace DWI images and ADC maps were automatically generated.
3) 3D FLASH sequences according to the multi-parametric mapping (MPM) protocol (Weiskopf & Helms, 2008), respectively with predominant T1-, proton density (PD)-, and Magnetization Transfer (MT)-weighting. by appropriate choice of the TR and the flip angle α (TR/α = 18.7 ms/20° for the eT1w scan and 23.7 ms/6° for the PDw and the MTw scans) [10]. The MT-weighting was obtained by applying an off-resonance RF pulse (4 ms duration, 220° nominal flip angle, 2 kHz frequency offset from water resonance) before the RF excitation. Multiple gradient echoes were acquired with alternating readout polarity at six equidistant TEs between 2.4 and 14.7 ms for the MTw, with two additional TEs at 17.2 and 19.6 ms for the PDw and T1w acquisition. All the MPM sequences were acquired at 1 mm isotropic resolution.
4) a 3D anatomical T1-weighted Magnetization Prepared RApid Gradient Echo (MPRAGE) sequence with TR/TE = 2400/2.25 ms, resolution = 1 × 1 × 1 mm$^3$, matrix size = 256 × 256, anterior-posterior phase encoding direction, generalized autocalibrating partially parallel acquisitions (GRAPPA) factor of 2 in phase-encoding direction, bandwidth 200 Hz/Px, non-selective excitation;

### 2.2. MT analysis

MPM datasets were processed in Matlab R2013a (The MathWorks Inc., Natick, MA, USA), using SPM12 (www.fil.ion. ucl.ac.uk/spm) tools and the VBQ batch tool [10].

Differently from the commonly used MT ratio (MTR), the semi-quantitative MT saturation parameter obtained from this protocol is implicitly corrected for differences in relaxation times and excitation flip angle [11]. The maps were obtained for each subject using the automated procedure of the VBQ tool which implements the method described in [11]. For the voxel-based statistical comparison the MT maps were classified into GM, WM and CSF using the unified segmentation approach [12]. Subject's maps were non-linearly transformed to the standard MNI space [13] using the diffeomorphic algorithm (DARTEL) implemented in SPM [14] and adapted for quantitative maps [10,14]. During the normalization procedure the maps were spatially smoothed with a Gaussian filter of 3 mm isotropic kernel at full width at half maximum (FWHM). Total intracranial volume (ICV) was calculate for each subject with the standard utility of SPM.

The differences between groups (Healthy controls HC, transfusion-dependent thalassemia patients TDT, and non transfusion-dependent thalassemia patients, NTDT) were assessed voxel-wise using an analysis of variance (ANOVA) test with factor group and considering age and total ICV as covariate. The statistical comparisons were carried out separately across the GM and WM using explicit masks obtained averaging the smoothed, Jacobian-modulated tissue probability maps in MNI space across all subjects. Voxels were assigned to the tissue class for which the probability was maximal and were excluded if neither the GM nor the WM probability exceeded 20% [15]. Differences were searched (in each tissue) for each contrast on the post-hoc *t*-test (HC vs TDT, HC vs NTDT, HC vs (TDT + NTDT) and TDT vs NTDT).

Additionally, the age effect was evaluated on the entire group (HC, NTDT, TDT).

Statistical thresholds were applied at $p < 0.05$ after family-wise error (FWE) correction for multiple comparisons using Gaussian random field theory as implemented in SPM over the whole volume of the GM/WM mask.

### 2.3. DTI analysis

DWI images were preprocessed and analyzed in FSL [16]. First, the DWI images were corrected for eddy current-induced distortions and subject movement using FSL "eddy" tool [17], then multiple diffusion metrics, including fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD) and radial diffusivity (RD) were estimated. The voxel-wise analysis for each of the latter metrics were performed using tract-based spatial statistics (TBSS) [18]. Group differences were evaluated for each contrast (HC vs TDT, HC vs NTDT and HC vs (TDT + NTDT)) including age as covariate. Multiple comparison correction to test significant correlations was performed with FSL randomise tool [18,19].

## 3. Results

Among 152 adult beta-thalassemia patients followed in the participating sites, 81 patients had been enrolled in previous cognitive and conventional MRI/MR-angiography studies.(Tartaglione 1 e 2) Among them, 72/81 (mean-age 34.7 ± 11.0 years, range 15–65; mean-hemoglobin 9.4 ± 1.0 g/dl; 48 TDT and 24 NTDT) had MRI suitable for the present analysis; 57 healthy volunteers also consented to the study (mean-age 33.9 ± 10.8 years, range 17–66). Sample main clinical and laboratory data, including disease history and severity, are reported in the Table 1.

Whole-brain voxel-based analyses showed that magnetization transfer, fractional anisotropy, mean diffusivity, radial and axial diffusivity do not differ between healthy controls and beta-thalassemia patients (considered as a whole group or as distinct transfusion dependent and non-transfusion dependent subgroups). As expected, MT maps showed age-dependent MT decrease in both healthy controls and beta-thalassemia patients, mainly symmetrically along the white matter of the forcipes major and minor ($p < 0.05$ FWE corrected, see Fig. 1). No correlation emerged between all the considered MT- and DTI-derived metrics and cognitive findings (full scale IQ) or the main clinical and laboratory data, including hemoglobin level and number of circulating nucleated red blood cells (nRBC).

## 4. Discussion

According to our findings, beta-thalassemia patients (regardless of clinical severity) do not seem to present an increased disease-related structural brain damage pointing to an increased cerebrovascular vulnerability compared to healthy controls, at least when the current treatment guidelines are followed. These findings, obtained with updated, microstructural-sensitive, advanced MRI analyses applied on high-quality 3T scanner imaging, strengthen previous observations regarding the lack of increased brain parenchymal and intracranial vascular involvement on conventional MRI/MR-angiography.

As a whole, these findings might appear unexpected. In beta-thalassemia a hypercoagulable state has been widely recognized above all among NTDT patients. Indeed, several serum and blood cell abnormalities in beta-thalassemia can potentially contribute to an increased risk of systemic thrombotic events [2]. However, the evidence of an increased vulnerability to cerebrovascular events is less well defined especially in patients treated according to the current guideline for beta-thalassemia. In fact, most studies on the brain referred to countries with lower hemoglobin level management and drug availability or lacked of a control group that could take into account also the general

**Table 1**
Baseline characteristics.

| | | Beta-thalassemia patients | | | Controls |
|---|---|---|---|---|---|
| | | Whole sample | TDT | NTDT | |
| # | | 72 | 48 | 24 | 57 |
| Age, years | | 34.7 ± 11.0 | 37 ± 9.9 | 29.2 ± 11.7* | 33.9 ± 10.8 |
| range | | (15–65) | (15–65) | (16–52) | (17–66) |
| Female sex, # (%) | | 49 (61.5) | 35 (63.6) | 14 (60.9) | 38(66.7) |
| Splenectomized, # (%) | | 38 (48.7) | 30 (54.5) | 8 (34.8) | 0 |
| Age of first transfusion, (mo) | | n.a. | 29.1 ± 42.5 | n.a. | n.a. |
| median - IQR | | | 24–30 | | |
| Transfusion Index (ml/kg/year) | | n.a. | 152.3 ± 57.6 | n.a. | n.a. |
| median - IQR | | | 143.4–68.2 | | |
| Mean Hemoglobin, g/dl | | 9.4 ± 1.0 | 9.2 ± 0.8 | 9.7 ± 1.1 | n.a. |
| On Aspirin, # (%) | | 33 (45.8) | 24 (50.0) | 9 (37.5) | 0 |
| Ferritin, ng/mL | | 646.9 ± 515.4 | 788.3 ± 546.4 | 364.0 ± 290.8 | n.a. |
| median- IQR | | 500–628 | 714.5–637 | 290–303,5 | |
| LIC, mg/gdw | | 4.7 ± 3.7 | 4.1 ± 2.9 | 6.7 ± 5.3 | n.a. |
| median - IQR | | 3.7–5.1 | 3.5–3.7 | 6.2–7.6 | |
| History of thrombotic events | | 2 (2.8) | 2 (4.2) | 0 | 0 |
| CT, # (%) | DFO | 13 (18) | 11 (22.9) | 2 (8.3) | |
| | DFP | 3 (4.2) | 3 (6.2) | 0 | n.a |
| | DFX | 37 (51.4) | 33 (68.7) | 4 (16.7) | |
| | DFX + DFO | 1 (1.4) | 1 (2.1) | 0 | n.a. |
| PLT (x103/uL) | | 474.7 ± 284.2 | 517.0 ± 256.9 | 390.1 ± 321.5 | |
| median - IQR | | 406–460 | 486.5–530.2 | 221.5–365.5 | |
| WBC (x103/uL) | | 13.3 ± 17.6 | 11.6 ± 6.9 | 16.5 ± 28.9 | n.a. |
| median - IQR | | 8.7–9.3 | 9.8–11.0 | 7.8–5.5 | |
| nRBC (x103/uL) | | 14.9 ± 23.4 | 13.1 ± 21.5 | 17.8 ± 26.6 | n.a. |
| median - IQR | | 8.5–15.4 | 5.6–13.9 | 9.6–15.1 | |

TDT: transfusion dependent thalassemia; NTDT: non transfusion dependent thalassemia; LIC: liver iron concentration; ICT: iron chelation therapy; DFO: deferoxamine; DFP: deferiprone; DFX: deferasirox; PLT: platelet; WBC: white blood cell; nRBC nucleated red blood cell; laboratory data collected as mean of last year's values; n.a.: not available/applicable.

* TDT significantly older than NTDT ($p < 0.01$).

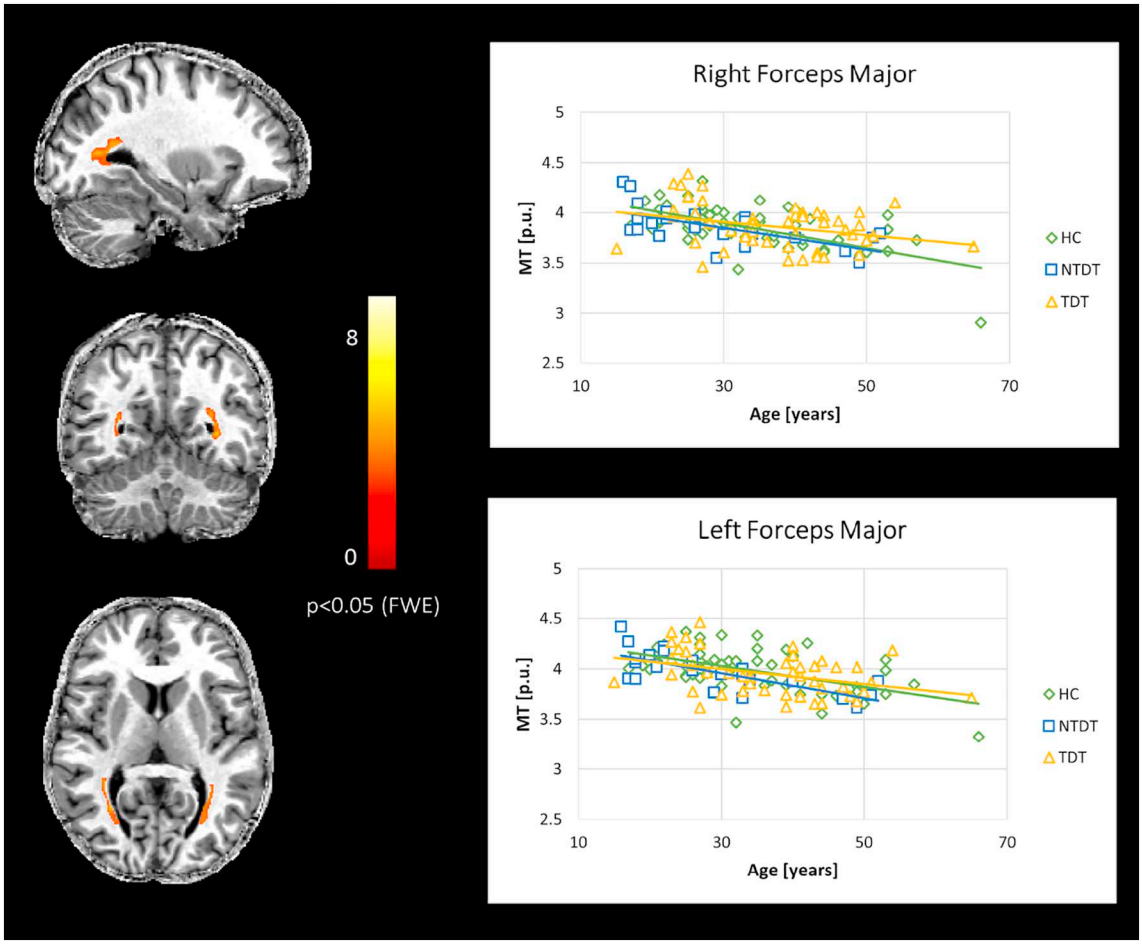


**Fig. 1.** Left: Statistical t-map (red-yellow color-scale), superimposed on a single subject MT maps, showing the relation between age and MT values. Right: Scatter plots and linear trends of the mean values of MT vs age, in the bilateral significant clusters of the forceps major, for the three groups. HT = healthy controls; TDT = transfusion-dependent thalassemia patients; NTDT = non transfusion-dependent thalassemia patients; MT = Magnetization Transfer; FWE = family-wise error correction for multiple comparisons. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

population burden of artery, sinus venous or vascular-like parenchymal changes. A recent study showed that both TDT and NTDT well managed patients do not present an increased risk of cerebrovascular changes at conventional MRI compared to healthy controls. The present study, seems to fiercely confirm that the specter of an increased cerebrovascular risk in beta-thalassemia might be, under specific conditions, not supported by scientific evidence. Whether this finding represents the abatement of the hypercoagulable state due to the current treatment or whether it reflects a lower vulnerability of the brain towards a systemic hypercoagulable state remains undetermined.

To note, up to 50–60% of beta-thalassemia patients might show vascular like white matter changes at conventional MRI [4,5,7]. However, most white matter changes (FLAIR hyperintensities) are isolated and/or relatively small thus resembling what is commonly seen in normal subjects when investigated with high field MRI not only for frequency rate but also for global number and size.(Tartaglione 1) Consistently to white matter changes, also quantitative ultrastructural analyses did not reveal any difference in terms of brain integrity between patients and controls. Coherently, the subanalyses of MT- and DTI-derived parameters did not reveal among patients any association with hemoglobin levels, degree of ineffective erythropoiesis and percentage of abnormal circulating red cells.

For ethical reasons, we did not obtain the hemoglobin levels of the healthy controls close to the MRI evaluation. However, the lack of association between MT- or DTI-derived parameters and hemoglobin levels among patients and, above all, the lack of differences at both conventional and advanced quantitative MRI analyses between patients and controls seem to rule out any pathogenic role of anemia severity for cerebrovascular changes at least when beta-thalassemia patients are managed according to the current treatment guidelines.

The underlying pathology of FLAIR hyperintense foci is unknown. They most likely are a heterogeneous assemblage of parenchymal changes, both in beta-thalassemia patients and healthy controls. Considering the relatively wide age-range of our study populations, foci of relative hypomyelination leading to higher water content but also pathologic aging-related changes such as demyelination and gliosis might all be possible explanations [6]. The lack of differences between patients (and patient subgroups) and healthy controls at conventional MRI and at advanced MRI analyses suggests that the nature of these changes is similar among patients and controls.

Obviously, this study had also some limits mostly related to the sample size. Only a few dozens of patients (47 TDT and 24 NTDT patients) and controls (57 subjects) were recruited in the present study. Larger samples might help to validate our results even though the detection of minimal cerebrovascular differences requiring larger series would likely have a limited clinical impact.

Overall, therefore, our findings do not support the need of regular brain MRI monitoring in adult neurologically-asymptomatic beta-thalassemia patients, since the putative cumulative multifactorial cerebrovascular brain injury does not seem to occur effectively. Moreover, the absent correlation between cognitive performances and advanced MRI analysis findings seems to minimize the role of cerebrovascular

involvement in causing the observed cognitive impairment, whose pathogenesis remains still elusive and should be likely searched in other disease-related frameworks.

Being this the first study applying these advanced MRI techniques in beta-thalassemia, our conclusions cannot be obviously considered definitive and further studies are needed to validate our findings under different socio-economic and treatment conditions.

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