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# High serum levels of transforming growth factor $\beta$ 1 are associated with increased cortical thickness in cingulate and right frontal areas in healthy subjects

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## Abstract

**Background:** Transforming growth factor  $\beta$  (TGF- $\beta$ ) is a cytokine having multiple functions in the central nervous system such as promoting repair mechanisms in degenerative diseases and stroke. To date, however, its neuroprotective effects in non-disease conditions have not been studied

**Methods:** With the aim of exploring the relationship between peripheral TGF- $\beta$ 1 expression and brain structural integrity, 70 healthy participants underwent high-resolution structural T1-weighted magnetic resonance imaging scans and blood sampling. Data were processed to obtain brain cortical thickness and serum concentrations of TGF- $\beta$ 1. We investigated the correlation between TGF- $\beta$ 1 and cortical thickness using both region-of-interest- and vertex-based approaches.

**Findings:** Region-of-interest-based analysis of the cortical mantle showed a correlation between TGF- $\beta$ 1 serum concentrations and cortical thickness bilaterally in cingulate and right frontal and temporal areas. Similar results emerged in the vertex-based analysis, where significant correlations were found bilaterally in cingulate and right frontal cortices.

**Conclusions:** These results suggest that TGF- $\beta$ 1, through its role in down-regulating inflammatory processes, might have a beneficial effect on the structural integrity of the brain in physiological states.

**Keywords:** TGF- $\beta$ , Cortical thickness, Inflammation, Neuroprotection

## Background

In mammals, transforming growth factor  $\beta$  (TGF- $\beta$ ) is a multifunctional cytokine which exists as three closely related isoforms (TGF- $\beta$ 1, TGF- $\beta$ 2 and TGF- $\beta$ 3) that bind to the same receptors and exert similar functions but with different spatiotemporal control of their expression patterns [1].

Interestingly, TGF- $\beta$  effects are prominent in brain development [2] and its signaling might control the size of a specific brain area by modulating self-renewal of neural stem cells [3]. In addition, an ongoing and potent trophic role for this anti-inflammatory cytokine has been

identified in TGF- $\beta$ 1-deficient unlesioned as well as injured adult brain [4].

TGF- $\beta$  is also involved in down-regulating inflammatory reactions to injury and in promoting repair mechanisms [5], and has been implicated in the pathophysiology of chronic neurodegenerative disorders and stroke [6]. Indeed, it has been shown that a reduction of TGF- $\beta$  signaling increases amyloid deposition and degeneration in transgenic Alzheimer's Disease (AD) mice [7], although negative effects of TGF- $\beta$  in neurodegeneration have also been described [8,9]. In the vasculature, TGF- $\beta$  modulates atherosclerosis and restenosis [10]. There is also a great deal of evidence, primarily from animal studies, that TGF- $\beta$  plays a crucial protective role in reducing infarct size following cerebral ischemia [11,12].

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Although TGF- $\beta$  has been recognized as a neuroprotective factor, the mechanisms underlying the protective effects have yet to be clarified. In vivo evidence for a beneficial role of TGF- $\beta$  in human brain is scarce or limited to pathological conditions such as ischemia and neurodegenerative diseases, and the pattern of such protection in physiological conditions has not yet been thoroughly investigated. It could be that individual TGF- $\beta$  phenotypes exert different levels of protection from brain pathologies such as neurodegenerative diseases, vascular deficits and aging.

Following this line of reasoning, the hypothesis of a relationship between TGF- $\beta$  expression and brain structural integrity would not be unjustified. Thus, the aim of the present pilot study was to determine whether there is an association between peripheral TGF- $\beta$ 1 expression (i.e. concentrations in serum samples) and structural integrity of the brain (i.e. cortical thickness) in healthy human subjects.

## Methods

### Subjects

Seventy healthy subjects (27 males, 43 females; mean age  $\pm$  sd =  $31 \pm 8.5$  years, range 18-48; mean education  $\pm$  sd =  $15.6 \pm 2.8$  years, range 8-18) were recruited from universities, community recreational centres and hospital personnel by local advertisement. The inclusion criteria were age between 18 and 50 years and suitability for MRI scanning. Exclusion criteria included: i) suspicion of cognitive impairment or dementia based on a Mini Mental State Examination (MMSE) [13] score  $\leq 24$ , and confirmed by clinical neuropsychological evaluation using the Mental Deterioration Battery [14] and NINCDS-ADRDA diagnostic criteria for dementia [15], ii) subjective complaints of memory difficulties or of any other cognitive deficits, iii) major medical illnesses, iv) current or reported psychiatric or neurological disorders, v) known or suspected history of alcoholism or drug dependence, vi) MRI evidence of focal parenchymal abnormalities or cerebrovascular diseases, and vii) presence of systemic inflammatory diseases and/or treatment with anti-inflammatory drugs at the time of the assessment.

The study was approved and undertaken in accordance with the guidance of our local Ethics Committee and written consent was obtained from all participants.

### TGF- $\beta$ measurement

Peripheral blood samples were obtained in the early morning from all subjects by venipuncture of an upper limb. Serum TGF- $\beta$ 1 levels were measured by a quantitative enzyme immunoassay (ELISA) technique using a specific TGF- $\beta$ 1 kit (Human TGF- $\beta$  CytoSet, Biosource, Camarillo, CA, USA) according to the manufacturer's instructions. The calibrator consisted of recombinant human TGF- $\beta$ 1.

All samples were measured in duplicate and respective mean values were calculated. The limit detection of the assay was 30 pg/ml and the intra- and inter-assay coefficients of variability were 2.8% and 12.5%, respectively.

### MRI acquisition and cortical thickness analysis

All 70 participants underwent the same imaging protocol, which included standard clinical sequences (FLAIR, DP-T2-weighted) and a whole-brain high resolution T1-weighted sequence obtained using a modified driven equilibrium Fourier transform (MDEFT) sequence (TE/TR = 2.4/7.92 ms, flip angle: 15°, voxel-size:  $1 \times 1 \times 1$  mm<sup>3</sup>) with a 3T Allegra MR imager (Siemens, Erlangen, Germany).

MRI-based quantification of cortical thickness was performed using the Freesurfer (v. 4.05) software package <http://surfer.nmr.mgh.harvard.edu>. This method has already been described in detail [16,17]. Images were first corrected for intensity of non-uniformity and registered via affine transformation (12 parameters) to Montreal Neurological Institute (MNI) space [18]. Then, images underwent a further intensity normalization using a different automated algorithm and were automatically skull stripped [16]. Next, the entire cortex was visually inspected prior to analysis. The data from 70 subjects were deemed to require manual correction, which included: a) setting intensity normalization control points where brain matter was erroneously skull stripped, b) adjusting watershed parameters of the skull strip, and c) visual inspecting and correcting of the automatic subcortical segmentation. All processes (i.e. skull stripping and segmentation) were inspected by an expert neuroradiologist who was blinded to the aim of the study.

For each subject, thickness measurements across the cortex were computed by finding the point on the gray-white matter boundary surface that was closest to a given point on the estimated pial surface (and vice versa) and obtaining the average of these two values [19]. The accuracy of the thickness measures derived from this technique was validated by direct comparisons with manual measurements on postmortem brains and direct comparisons with manual measurements on MRI data [20,21]. The surface representing the gray-white matter border was "inflated" [22]. Differences among individuals in the depth of gyri-sulci were normalized, and each subject's reconstructed brain was then morphed and registered to an average spherical surface representation that optimally aligned sulcal and gyral features across subjects [21]. Finally, cortical maps were smoothed with a 10-mm full-width at half maximum Gaussian kernel.

For each subject mean thickness values were then calculated for 33 regions of interest (ROIs) in each hemisphere, using the Destrieux atlas [23], implemented in the Freesurfer software.

### Statistical analyses

Association between TGF- $\beta$ 1 and cortical thickness was investigated using both a ROI-based and a vertex-based approach. The former was performed by calculating Pearson's *r* correlation coefficients between TGF- $\beta$ 1 serum levels and each ROI mean thickness. As this is a pilot study, we accepted the false-positive risk with an uncorrected level of significance set at  $p < .05$ . The vertex-based analysis was performed using the Qdec module, implemented in Freesurfer. For each hemisphere, estimation of statistical effects was generated by computing a general linear model (GLM) of the effects of TGF- $\beta$ 1 on cortical thickness at each vertex. We modeled cortical thickness data using a linear regression analysis with TGF- $\beta$ 1 as the variable of interest and age as the nuisance variable. In this case, the threshold of statistical significance was increased at  $p < .001$ , which has been reported as a reasonable threshold for reporting results of neuroimaging studies when no corrections for multiple comparisons are made [e.g. [24]].

### Results and discussion

As shown in Table 1 ROI-based analyses revealed several brain areas (primarily in frontal and cingulate cortices of both hemispheres) in which there was a significant correlation between TGF- $\beta$ 1 serum values and cortical thickness. Specifically, correlations were found bilaterally in caudal anterior cingulate ( $r = .363$ ,  $p = .0018$  for left hemisphere;  $r = .330$ ,  $p = .005$  for right hemisphere) and bilaterally in the rostral anterior cingulate cortices ( $r = .247$ ,  $p = .039$  for left hemisphere;  $r = .439$ ,  $p < .001$  for right hemisphere); in the pars opercularis of the inferior frontal cortex ( $r = .273$ ,  $p = .021$ ) and in the rostral portion of the middle frontal area ( $r = .243$ ,  $p = .043$ ) and in the superior temporal cortex ( $r = .278$ ,  $p = .019$ ) of the right hemisphere only.

Results of the vertex-based analysis are summarized in Figure 1 and Table 2. In particular, significant results were found in rostral and caudal portions of bilateral anterior cingulate cortices, in the pars opercularis and triangularis of the right inferior frontal cortex and in the rostral part of the right middle frontal area.

Thus, the results in this study, in which we analyzed TGF- $\beta$ 1 serum levels and brain cortical thickness in a

large sample of healthy subjects, showed positive correlations in several brain areas, particularly the bilateral cingulate cortices and right frontal areas. Although these results are observational, they provide the first in-vivo support for the hypothesis that this cytokine has a putative protective effect in physiological conditions.

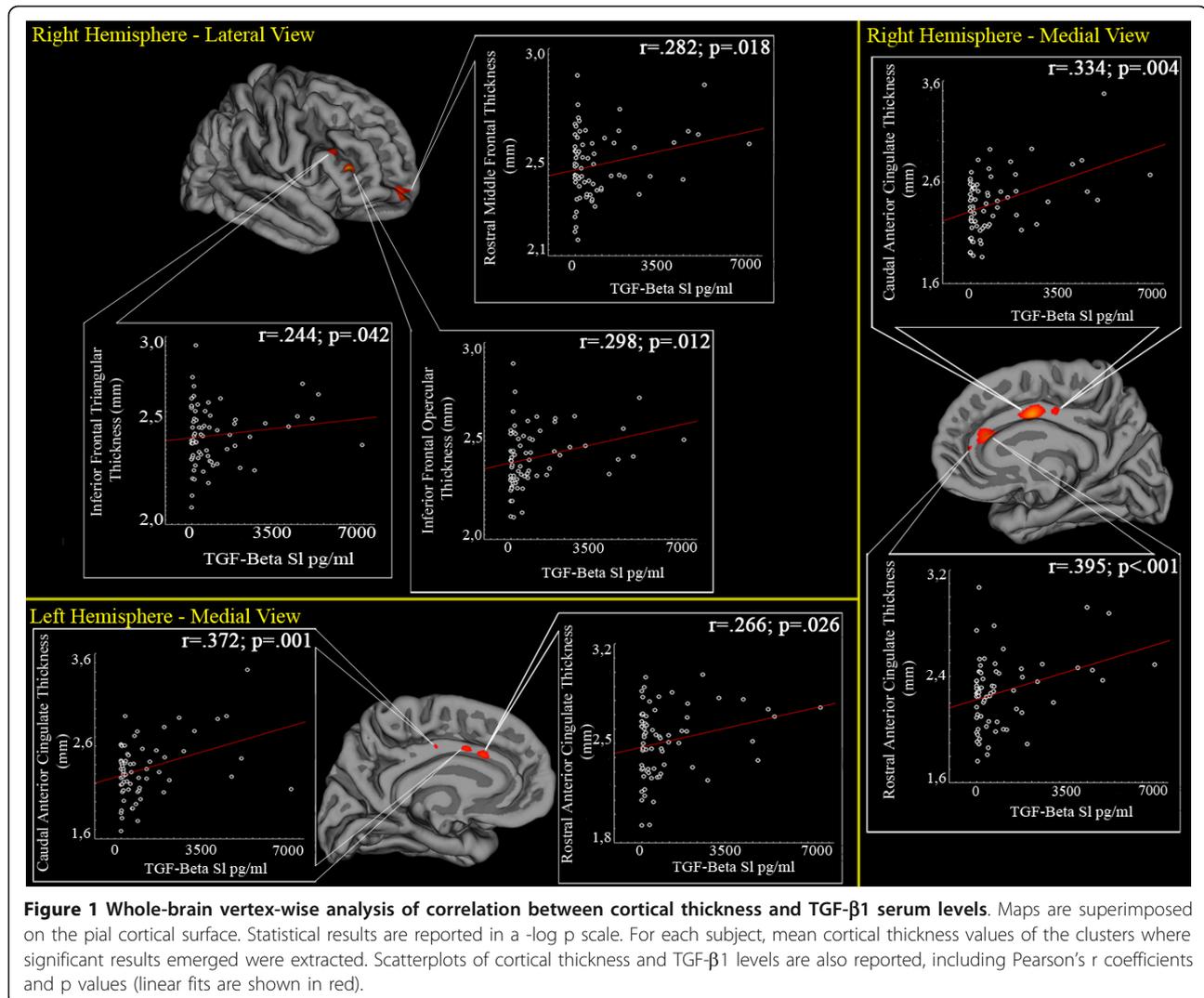
In this view, the role of TGF- $\beta$ 1 could be central in down-regulating inflammatory processes, because converging evidence suggests that peripheral levels of inflammation are associated with activation of central inflammatory mechanisms (through direct penetration of blood-brain barrier or via activation of the afferent vagus nerve) and might adversely affect cognition and brain structure. Indeed, Yaffe and colleagues [25] found that serum markers of inflammation, especially IL-6, likely predict cognitive decline in well-functioning elderly individuals. Along these lines, Marsland and coworkers [26] used a computational structural neuroimaging method (optimized voxel-based morphometry) to evaluate the relationship between plasma IL-6 levels and hippocampal grey matter volume in a sample of 76 relatively healthy community volunteers. They found a strong inverse correlation between IL-6 levels and hippocampal gray matter and argued that low-grade systemic inflammation might presage subclinical cognitive decline in part via structural neural pathways. This result has been confirmed in a more recent study [27] which showed a pattern of cortical thinning associated to levels of systemic inflammation in older persons without dementia.

Thus, we can speculate that if systemic inflammatory markers have a detrimental effect on the structural integrity of the brain (i.e. reduced cortical thickness), TGF- $\beta$ 1 might have a neuroprotective effect through its role in down-regulating inflammation.

Interestingly, the positive correlations between TGF- $\beta$ 1 levels and cortical thickness found in the present study were mainly located in brain areas involved in high-level cognitive processes (i.e. executive functions) such as the frontal areas and the cingulate cortex. Moreover, in animal studies the latter area was associated to a 37% increase of TGF- $\beta$ 1 mRNA 12 h after occlusion of the middle cerebral artery [28] and an increased expression of brain-derived neurotrophic factor (BDNF) 2 h after

**Table 1 Relationships between region of interest (ROI) mean cortical thicknesses and TGF- $\beta$ 1 serum levels for the 70 healthy participants**

Left hemisphere ROIs			Right hemisphere ROIs		
	Pearson's r	p-value		Pearson's r	p-value
Caudal anterior cingulate	.363	.0018	Rostral anterior cingulate	.439	.0001
Rostral anterior cingulate	.247	.0393	Caudal anterior cingulate	.330	.0051
			Superior temporal	.278	.0194
			Inferior frontal opercular	.273	.0217
			Rostral middle frontal	.243	.0428



transient focal ischemia [29]. Therefore, the cingulate cortex might be a key area in which putative neuroprotective effects of TGF-β1 occur, thus preventing negative agents such as aging, degeneration or cerebrovascular

diseases. Nevertheless, the data presented here cannot completely address this issue because the study was cross-sectional and the participants were free from brain pathology.

**Table 2 Vertex-based relationships between TGF-β1 and cortical thickness.**

Left hemisphere				
Anatomical region	Extent (mm <sup>2</sup> )	p	t	x,y,z {mm}
Caudal anterior cingulate	40	.0005	3.29	-6, 22, 23
Rostral anterior cingulate	11	.0009	3.04	-1, 32, 7
Right hemisphere				
Anatomical region	Extent (mm <sup>2</sup> )	p	t	x,y,z {mm}
Inferior frontal lobe, pars triangularis	83	.00003	4.48	44, 33, 8
Rostral anterior cingulate	153	.0001	3.97	5, 29, -3
Caudal anterior cingulate	218	.00014	3.84	3, 25, 16
Rostral middle frontal	145	.0003	3.54	21, 58, -11
Inferior frontal lobe, pars opercularis	53	.0004	3.33	50, 25, 19

Coordinates are reported in MNI space

A limitation of the present study is that we measured serum level of TGF- $\beta$ 1, which might not accurately reflect levels in the cerebrospinal fluid (CSF) or in brain regions. However, cytokines readily cross the blood-brain barrier, suggesting that serum levels should correlate well with levels in the CSF [30]. Further, there is evidence of a correlation between TGF- $\beta$  levels in serum and CSF of patients with advanced AD [31].

## Conclusion

Overall, the results of the present study show that TGF- $\beta$ 1 serum concentrations are associated with greater cortical thickness in bilateral cingulate and right frontal areas in subjects without neuropsychiatric diseases. This suggests that TGF- $\beta$  might also have a beneficial effect on the structural integrity of the brain even in physiological states.

Future studies should take on the challenge of longitudinally studying the role of TGF- $\beta$  in protecting the brain from degeneration and injury, possibly by collecting data to analyze brain microstructural integrity (i.e. diffusion tensor imaging).

## Abbreviations

CSF: cerebrospinal fluid; IL-6: interleukin-6; MRI: magnetic resonance imaging; ROI: region of interest; TGF- $\beta$ : transforming growth factor  $\beta$ .

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## Authors' contributions

GS, CC and PB directed the work, contributed to designing the study and reviewed the data; FS performed ELISA assay and TGF- $\beta$  measurements; FP performed MRI analyses and wrote the manuscript. All authors read and approved the final manuscript.

## Competing interests

The authors declare that they have no competing interests.

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