

TGF- β 1 is associated with deficits in cognition and cerebral cortical thickness in first-episode schizophrenia

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Background: Evidence indicates that cytokines are associated with cognitive deficits in schizophrenia; however, the underlying brain-behaviour mechanisms remain unclear. We hypothesized that aberrations in brain structural connectivity mediate the cytokine effect in schizophrenia. **Methods:** In this study, we recruited patients with first-episode schizophrenia ($n = 75$, average illness duration 12.3 months, average medication period 0.6 days) and healthy controls ($n = 44$) of both sexes. We first conducted whole-blood RNA sequencing to detect differentially expressed genes. We also explored transcriptomic data on the dorsal lateral prefrontal cortices (dlPFC) retrieved from the CommonMind Consortium for gene functional clustering; we measured plasma transforming growth factor β 1 (TGF- β 1) levels by enzyme-linked immunosorbent assay; we acquired high-resolution T_1 -weighted MRI data on cortical thickness MRI; and we assessed cognitive function using the validated Chinese version of the MATRICS Consensus Cognitive Battery. We compared these parameters in patients with schizophrenia and controls, and analyzed their associations. **Results:** Patients with schizophrenia had higher TGF- β 1 at both the mRNA level (\log_2 fold change = 0.24; adjusted $p = 0.026$) and the protein level ($12.85 \pm 6.01 \mu\text{g/mL}$ v. $8.46 \pm 5.15 \mu\text{g/mL}$, adjusted $p < 0.001$) compared to controls. Genes coexpressed with *TGFB1* in the dlPFC were less abundant in patients with schizophrenia compared to healthy controls. In patients with schizophrenia, TGF- β 1 protein levels were inversely correlated with cortical thickness, especially of the lateral occipital cortex ($r = -0.47$, adjusted $p = 0.001$), and with the MATRICS Consensus Cognitive Battery visual learning and memory domain ($r = -0.50$, adjusted $p < 0.001$). We found a complete mediation effect of the thickness of the lateral occipital cortex on the negative relationship between TGF- β 1 and visual cognition ($p < 0.05$). **Limitations:** We did not explore the effect of other blood cytokines on neurocognitive performance and cortical thickness. Participants from the CommonMind Consortium did not all have first-episode schizophrenia and they were not all antipsychotic-naïve, so we could not exclude an effect of antipsychotics on TGF- β 1 signalling in the dlPFC. The sample size and cross-sectional design of our study were additional limitations. **Conclusion:** These findings highlighted an association between upregulated blood levels of TGF- β 1 and impairments in brain structure and function in schizophrenia.

Introduction

Schizophrenia is a complex neurodevelopmental disorder. Accumulating evidence suggests that dysregulation of the immune system may be involved in the pathogenesis of schizophrenia, with changes occurring in both the peripheral blood and brain tissue.¹⁻³ Multiple recent studies have observed dysregulation of inflammatory markers in the prefrontal cortex⁴⁻⁶ and blood of people with schizophrenia,⁷⁻⁹ which may converge to (and propagate through) microglia-dependent neuro-

inflammation in association with other neuropathological hallmarks in the brains of people with schizophrenia.¹⁰ Most cytokines are synthesized and secreted by astrocytes and microglia in the brain. Transforming growth factor β 1 (TGF- β 1) is one such brain cytokine.^{11,12} The expression of TGF- β 1 is regulated by neurons in an activity-dependent manner,¹³ and its receptors are expressed on multiple brain cell types.¹⁴ TGF- β 1 signalling is critical for a range of biological processes during brain development and regeneration via the regulation of neurotransmission, synaptic remodelling

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and brain connectivity.^{11,15–18} Accumulated evidence suggests that TGF- β 1 may also be involved in disease onset and cognitive deficits in schizophrenia.^{11,19} Notably, in a previous genetic study by the Psychiatric Genomics Consortium, TGF- β signalling was included among the enriched biological pathways for schizophrenia.²⁰

Although immune genes including *TGFB1* have been implicated in schizophrenia, how they cause cerebral abnormalities and behavioural deficits in schizophrenia remains elusive.^{21,22} We have recently observed that immune-related factors negatively affect cognition in association with brain structural deficits in patients with first-episode^{23–25} and treatment-resistant schizophrenia.²⁶ In the present study, we hypothesized that changes in blood immune genes may be linked to alterations in cerebral cortical structures (such as cortical thinning) and their connectivity, which in turn may lead to cognitive decline in schizophrenia. We used RNA sequencing to examine the blood transcriptomes of patients with first-episode schizophrenia and healthy controls, and to identify differentially expressed genes from which TGF- β 1 family members were surfaced during pathway analysis (Appendix 1, Tables S1 and S2, available at www.jpnp.ca/lookup/doi/10.1503/jpn.210121/tab-related-content). We further explored cortical transcriptomic data from the CommonMind Consortium to identify biological pathways associated with TGF- β 1 the brains of people with schizophrenia. Moreover, we measured participants' plasma TGF- β 1 and cerebral cortical thickness and evaluated the relationships between TGF- β 1, cognition and cerebral cortical thickness.

Methods

Participants

We recruited patients with first-episode schizophrenia ($n = 105$) from the Beijing Huilongguan Hospital. Patients met the diagnostic criteria for schizophrenia according to the Structured Clinical Interview for DSM-IV, and confirmed by 2 psychiatrists. Inclusion criteria were as follows: 18–45 years old and Han Chinese; an illness duration of 3 years or less (less than 1 year on average); and taking antipsychotic medication for less than 2 weeks at the time of the blood draw and cognitive testing.

We recruited age- and sex-matched healthy controls ($n = 52$) from the local community by advertisement.

Among the participants, 75 patients with schizophrenia (average illness duration 12.3 months, average medication period 0.6 days) and 44 healthy controls had mRNA samples that passed quality control for RNA sequencing and were selected for further clinical evaluation. Among the patients with schizophrenia, 29 had been hospitalized for first-episode psychosis and were not taking antipsychotic medication at the time of admission and blood sample collection; 41 had received a first- or second-generation antipsychotic for less than 3 days; and 5 had been medicated for longer (8 d on average). Antipsychotic medication doses were calculated as chlorpromazine equivalents.

We collected complete medical histories of the healthy controls and conducted physical examinations for all participants to identify those with chronic medical or psychiatric conditions. Exclusion criteria for potential healthy controls were as follows: previous diagnosis of an Axis I psychiatric disorder; substance abuse or dependence in the previous 6 months; a history of autoimmune disorders or other significant medical conditions; or receiving anti-inflammatory medications.

All participants provided written informed consent. The study was approved by the institutional ethics committee of Beijing Huilongguan Hospital.

Psychological assessment

We assessed psychopathology in the patients with schizophrenia using the Positive and Negative Syndrome Scale,²⁷ administered independently by 2 psychiatrists on the day blood samples were collected. The intraclass correlation coefficient for the 2 raters was greater than 0.80.

Neurocognition assessment

We assessed cognitive function in both patients and controls using the validated Chinese version of the MATRICS Consensus Cognitive Battery (MCCB).²⁸ The MCCB contains assessments of 7 cognitive domains: speed of processing, attention and vigilance, working memory, verbal learning, visual learning, reasoning and problem solving, and social cognition. Initial scores were transformed to Chinese-normalized t scores. We calculated domain scores and total scores for the MCCB.

Whole blood collection and RNA sequencing

We collected whole blood samples for RNA sequencing (5 mL) between 7 and 9 am after overnight fasting using PAXgene blood RNA tubes (Applied Biosystems). Tubes were shaken vigorously for at least 10 seconds after sampling and immediately stored at -80°C . We extracted total RNA using the MagMAX for Stabilized Blood Tubes RNA Isolation Kit (Applied Biosystems), following the manufacturer's instructions.

We quantified RNA and assessed it for purity using NanoDrop spectrophotometry and optical density ratios of 260/280 nm and 260/230 nm. The RNA samples (1 μg per sample) were immediately sent to the Beijing Genomics Institution for mRNA sequencing (after globin mRNA removal) on the BGISEQ-500 platform. The Beijing Genomics Institution confirmed quality controls on RNA samples (RNA integrity number/RNA quality number ≥ 7.0 ; 28S/18S ≥ 1.0). They collected clean data of at least 4 Gb (20 M clean reads) per sample.

RNA sequencing data analysis

We first conducted quality control checks of the fastq files and used bam files to construct an mRNA sequencing count table. We conducted gene expression analysis using DESeq2 on the NetworkAnalyst platform.²⁹ Data with a variance percentile

rank of less than 15% and counts of less than 4 were filtered out. Counts per million reads were transformed and normalized, and fold changes (\log_2FC) were calculated for differentially expressed genes. Those with adjusted p values of less than 0.05 were further analyzed in Panther and DAVID for their biological pathways and molecular function using Gene Ontology (GO) terms (Appendix 1, Tables S1 to S4).

CommonMind Consortium brain RNA sequencing data

We obtained brain tissues for the CommonMind Consortium study from the following brain bank collections: the Mount Sinai National Institutes of Health Brain and Tissue Repository; the University of Pennsylvania Alzheimer's Disease Research Center; the University of Pittsburgh NeuroBioBank and Brain and Tissue Repositories; and the National Institute of Mental Health Human Brain Collection Core.³⁰ We downloaded gene coexpression data from the CommonMind Consortium portal (<https://synapse.org>). To compare mRNA expression levels of TGF- β 1 in the dorsolateral prefrontal cortex (dlPFC) between patients with schizophrenia ($n = 258$) and controls ($n = 279$), we also explored the SZDB database (www.szdb.org),^{31,32} which uses RNA sequencing data published by the CommonMind Consortium.³⁰

Functional clustering analyses of the TGF- β 1 coexpression gene network in the dlPFC

We constructed coexpression networks using weighted gene coexpression network analysis. Details of the statistical analysis are provided by the CommonMind Consortium.³⁰ We further analyzed *TGF β 1*-coexpressed genes in the schizophrenia and control samples for functional clustering and protein-protein interactions using String version 11 (<https://string-db.org>). We set the clustering stringency threshold at 0.4 and the k -means value at 3. We used GO biological pathways for functional annotation analysis, and we corrected enrichment scores using false discovery rate (Appendix 1, Tables S5 to S8).

Measurement of plasma TGF- β 1

We collected blood samples for measurement of plasma TGF- β 1 (5 mL) between 7 and 9 am after overnight fasting and centrifuged them at 5000 rpm for 10 minutes. Plasma was immediately separated and stored at -80°C until assayed.

We determined plasma TGF- β 1 concentrations using an enzyme-linked immunosorbent assay kit (Biosensis) and following the manufacturer's instructions. Intra- and inter-assay variation coefficients were approximately 5%. TGF- β 1 concentrations were expressed in micrograms per millilitre.

MRI acquisition and processing

We acquired brain structural MRI data using a Siemens Prisma 3.0 T MRI scanner with a 64-channel head coil, located at the Beijing Huilongguan Hospital Magnetic Resonance Scanner Centre. We used foam pads to minimize

head motion. We used a sagittal, 3-dimensional, magnetization prepared rapid acquisition gradient echo (MPRAGE) sequence to collect anatomic data. Scan parameters were as follows: echo time 2.98 ms, inversion time 1100 ms, repetition time 2530 ms, flip angle 7° , field of view 256×224 mm, matrix size 256×224 , thickness/gap = 1/0 mm. After the scan, 2 radiologists evaluated image quality, and if there were any artifacts, images were collected again.

Following the ENIGMA protocol (<http://enigma.ini.usc.edu/>), we used FreeSurfer^{33,34} software for data processing (<http://surfer.nmr.mgh.harvard.edu>). Based on the Desikan-Killiany atlas, FreeSurfer software automatically divides the occipital cortex of both hemispheres into 4 parts: the cuneiform leaf, the lateral occipital leaf, the lingual gyrus and the peridistal sulcus. After imputing the corresponding internal instructions, we obtained anatomic parameters for cortical thickness. We measured the thickness of 34 cerebral cortical features from each hemisphere and calculated averages.

Statistical analysis

We then examined data distributions using the Shapiro-Wilk test. We compared demographic and clinical variables between groups using analysis of variance for continuous variables and χ^2 tests for categorical variables. For TGF- β 1 levels and MCCB total score and subscores, we used analysis of covariance, using age, sex, education and body mass index as covariates. We evaluated the relationships among TGF- β 1, MCCB scores and cortical structures using Pearson partial correlation and linear regression, controlled by age, sex, education and body mass index. We performed statistical analyses in SPSS version 23.0 (IBM). For the MCCB and MRI data, we corrected p values for multiple comparisons using the Bonferroni method (e.g., adjusted p values = p values \times 7 for MCCB data and p values \times 34 for MRI data).

We conducted principal component analysis for MRI features to separate the patient and control groups and identify clustering trends with 95% confidence intervals; we conducted heatmap analysis on correlational data using online tools (www.bioinformatics.com.cn). Data are presented as mean \pm standard deviation. All statistical tests were 2-tailed, and an adjusted $p < 0.05$ was considered statistically significant.

Results

Demographic and clinical characteristics

Participants' demographic and clinical characteristics are listed in Table 1. Patients and controls were not statistically different in terms of age, sex or years of education, but patients had a lower mean body mass index than controls (20.90 ± 2.91 kg/m² v. 23.35 ± 3.67 kg/m²; $p = 0.001$). We used these variables as covariates in subsequent analyses. Whole blood cell counts did not differ between patients and controls (data not shown).

The MCCB total and domain index scores are summarized in Table 2. Compared with controls, patients with schizophrenia had worse cognitive performance on all MCCB indices (all adjusted $p < 0.001$).

Table 1: Demographic characteristics of patients with early-stage schizophrenia and healthy controls

Characteristic	Patients with schizophrenia (n = 75)*	Healthy controls (n = 44)*	t or χ^2	p value
Sex, M/F	34/41	24/20	1.20	0.27
Age, yr	28.61 ± 6.90	30.07 ± 7.49	-1.07	0.29
Education, yr	13.01 ± 3.77	14.28 ± 2.18	-2.02	0.05
BMI, kg/m ²	20.90 ± 2.91	23.35 ± 3.67	-3.92	0.001
Illness duration, mo	12.25 ± 17.85	NA	NA	NA
Positive and Negative Syndrome Scale				
Positive symptom score	21.79 ± 4.91	NA	NA	NA
Negative symptom score	17.56 ± 6.00	NA	NA	NA
General psychopathological symptom score	36.92 ± 6.98	NA	NA	NA
Total score	76.26 ± 12.81	NA	NA	NA

NA = not applicable.
*Data are mean ± standard deviation unless otherwise indicated.

Table 2: MCCB scores and TGF-β1 levels

Characteristic	Patients with schizophrenia*	Healthy controls*	t or χ^2	p value
MCCB domain score				
Speed of processing	42.86 ± 13.06	59.00 ± 6.93	-8.36	< 0.001
Attention/vigilance	39.19 ± 13.33	58.05 ± 11.58	-7.33	< 0.001
Working memory	46.65 ± 10.94	58.62 ± 5.48	-7.50	< 0.001
Verbal learning and memory	46.74 ± 14.06	55.76 ± 7.36	-4.36	< 0.001
Visual learning and memory	44.20 ± 12.47	56.21 ± 6.17	-6.66	< 0.001
Reasoning and problem solving	46.14 ± 12.92	57.84 ± 5.04	-6.66	< 0.001
Social cognition	44.55 ± 13.44	54.47 ± 9.73	-4.01	< 0.001
Composite score	44.83 ± 9.86	59.83 ± 4.40	-10.52	< 0.001
TGF-β1, µg/mL	12.85 ± 6.01	8.46 ± 5.15	3.94	< 0.001

MCCB = MATRICS Consensus Cognitive Battery; TGF-β1 = transforming growth factor-β1.
*Data are mean ± standard deviation.

TGFB1 mRNA higher in the whole blood of patients than controls

A total of 1332 differentially expressed genes were detected by the RNA sequencing of blood samples from patients with schizophrenia and controls; of those, 567 were down-regulated and 755 were upregulated (adjusted $p < 0.05$; Figure 1A and Appendix 1, Table S1). Panther pathway analysis of molecular function showed that these differentially expressed genes mainly regulated molecular binding (32.50% hit) and catalytic activity (22.50% hit; Figure 1B). The top 3 rankings for Panther biological process were cellular process (GO:0009987, 53.8% hit), metabolic process (GO:0008152, 34.3% hit) and biological regulation (GO:0065007, 33.8% hit). Gene clusters enriched in the GO and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses done in DAVID are listed in Appendix 1, Table S2. Notably, functional clustering analysis of these differentially expressed genes by DAVID molecular function highlighted the top-ranking genes involved in TGF-β receptor binding (Appendix 1, Table S2). Of these, *TGFB1* was significantly higher in patients with schizophrenia than in con-

trols ($\log_2FC = 0.24$, adjusted $p = 0.026$), and its receptors *TGFB1R1* ($\log_2FC = -0.24$, adjusted $p = 0.019$) and *TGFB1R3* ($\log_2FC = -0.45$, adjusted $p = 0.004$) were both lower in patients with schizophrenia than in controls (Figure 1C and Appendix 1, Table S3 and S4).

TGFB1-coexpressed genes in the dlPFC involved in different biological pathways in patients than controls

To better understand the function of TGF-β1 in the human brain, we further explored RNA sequencing data from the dlPFC of deceased people with schizophrenia ($n = 258$) and control participants ($n = 279$) provided by the CommonMind Consortium.³⁰ We retrieved a weighted gene coexpression network analysis list containing *TGFB1*-coexpressed genes and conducted gene functional clustering and protein-protein interaction analyses to compare the characteristics of TGF-β1-associated gene networks between patients with schizophrenia and controls. The analyses showed that *TGFB1* was coexpressed with a different and smaller number of genes in patients with schizophrenia than in controls (Appendix 2, Table S5), constituting a less prominent interaction network

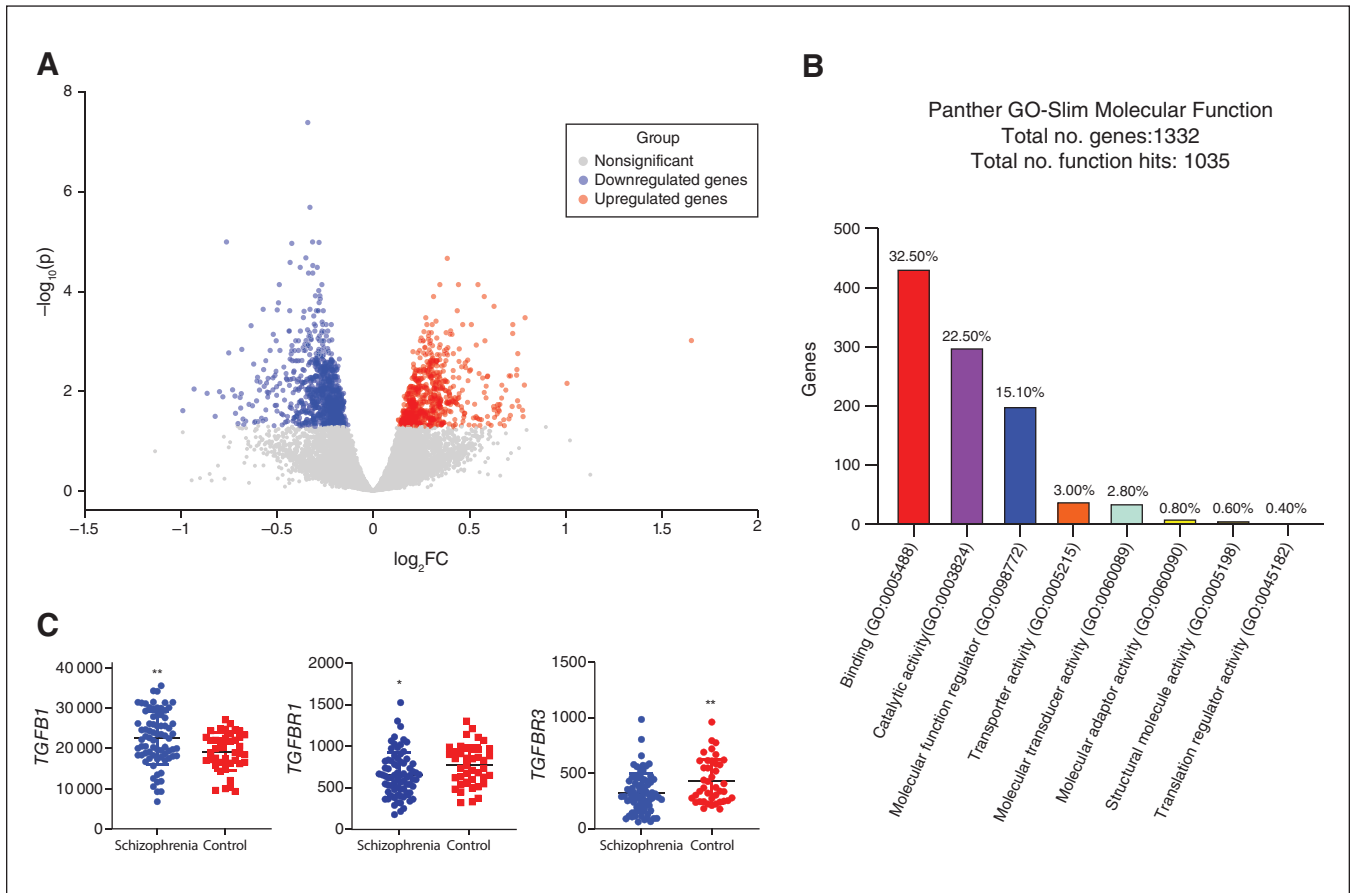


Figure 1: RNA sequencing analysis in the peripheral blood of patients with schizophrenia and healthy controls. (A) Volcano plot representing differentially expressed genes among patients with schizophrenia and healthy controls. Only differentially expressed genes with adjusted $p < 0.05$ are shown in colour. (B) Functional enrichment analysis of the list of differentially expressed genes in the Panther database. Enriched Gene Ontology terms (GO-Slim Molecular Function) are presented. (C) RNA sequencing counts for *TGFB1* and its receptors *TGFB1* and *TGFB3* in patients with schizophrenia and controls. *TGFB1* was upregulated and *TGFB1* and *TGFB3* were downregulated in patients with schizophrenia compared to controls. One-way analysis of covariance. * $p < 0.05$; ** $p < 0.01$; $\log_2FC = \log_2$ fold change.

(Figure 2). These TGF- β 1-associated genes were also enriched in different biological pathways, showing that the top-ranked GOs in the control group were involved in the regulation of receptor signalling and developmental process (Appendix 1, Tables S6 and S8), but the top-ranked GO in patients with schizophrenia was for extracellular matrix organization (Appendix 1, Tables S7 and S8).

TGF- β 1 plasma levels higher in patients versus controls

As shown in Table 2, TGF- β 1 levels were significantly upregulated in the plasma of patients with schizophrenia relative to controls ($12.85 \pm 6.01 \mu\text{g/mL}$ v. $8.46 \pm 5.15 \mu\text{g/mL}$; $p < 0.001$). We found no significant difference in TGF- β 1 levels between women and men with schizophrenia. To evaluate the potential effect of antipsychotic medication on TGF- β 1 levels, we analyzed the regression between chlorpromazine dosages and TGF- β 1 levels and found no significant correlation. We also compared TGF- β 1 levels between drug-naïve and medicated patients, as well as between patients with fewer than 3 days of

antipsychotic medication and those with a longer period of treatment, but we found no group differences in these separate analyses (data not shown).

We also explored dlPFC transcriptomic data from the CommonMind Consortium,³⁰ but brain mRNA levels of TGF- β 1 did not differ between the schizophrenia and control groups this cohort (adjusted $p > 0.05$; Appendix 1, Figure S1).

Reduced thickness of the lateral occipital cortex and positive association with visual cognition in schizophrenia

We next compared cerebral cortical structures in 34 brain regions measured by MRI, including the visual cortex and associated areas (Brodmann areas 17 to 19) of patients with schizophrenia and healthy controls. As summarized in Table 3 and Figure 3A, multiple cortical regions showed significantly reduced thickness in patients with schizophrenia compared with controls. The reduction was most striking in the mean thickness of the lateral occipital cortex (LOC; adjusted $p < 0.001$, Table 3 and Figure 3B). Both the

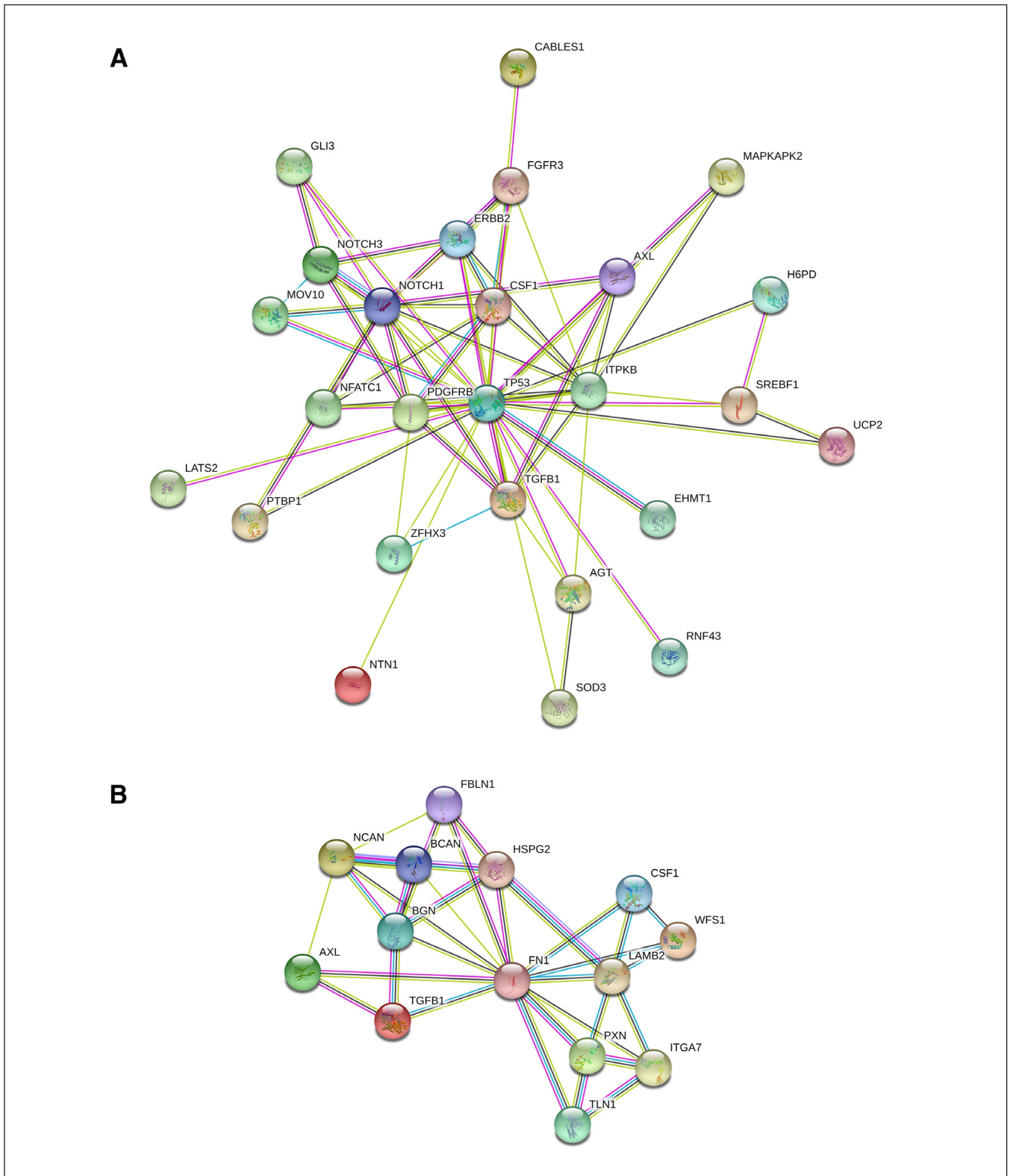


Figure 2: TGF-β1-interacting protein networks in the dorsolateral prefrontal cortex of patients with schizophrenia and healthy controls. Protein-protein interaction network of *TGFB1* coexpressed genes in (A) healthy controls and (B) patients with schizophrenia. We analyzed *TGFB1*-coexpressed genes retrieved from the CommonMind Consortium portal using String (version 11). Clustered molecules are represented as nodes tagged with their gene symbols. The interacting strength between 2 nodes is represented by the thickness of the line.

Table 3: Average thickness of the visual cortex and associated cortical areas

Region	Thickness, mm*			Adjusted <i>p</i> value
	Patients with schizophrenia	Healthy controls	<i>t</i>	
Lateral occipital cortex	2.20 ± 0.13	2.31 ± 0.11	-4.12	< 0.001
Inferior parietal cortex	2.49 ± 0.15	2.62 ± 0.10	-4.64	< 0.001
Supramarginal gyrus	2.57 ± 0.13	2.69 ± 0.11	-4.74	< 0.001

*Data are mean ± standard deviation.

left and right LOC were thinner in patients with schizophrenia compared to controls (Appendix 1, Table S9). Other cortical regions related to visual cognition and working memory, such as the inferior parietal cortex and the supramarginal gyrus, were also decreased in patients versus controls (Table 3 and Appendix 1, Table S9). However, we found no change in cortical thickness of the dlPFC (adjusted $p > 0.05$).

We also studied the relationship between visual cognition and brain cortical structures and found that it was positively correlated with multiple cortical regions in patients with schizophrenia, but not in healthy controls (Figure 3C). Specifically, the mean thickness of the LOC ($r = 0.42$, adjusted $p = 0.001$; Figure 3D), as well as the thickness of the left LOC ($r = -0.39$, unadjusted $p = 0.007$; Figure 3E) and the right LOC ($r = 0.42$, unadjusted $p = 0.003$; Figure 3F) were most significantly related to visual learning and memory scores in patients with schizophrenia. These relationships were non-significant in healthy controls.

Scores on the Positive and Negative Syndrome Scale were not significantly correlated with MCCB scores, TGF- β 1 levels or LOC thickness in patients with schizophrenia.

Negative association between TGF- β 1 and visual cognition in schizophrenia

We further observed associations between plasma TGF- β 1 levels and MCCB total score and subscores. TGF- β 1 plasma levels were negatively associated with MCCB total scores ($r = -0.48$, adjusted $p = 0.001$) in the schizophrenia group alone and in the schizophrenia and healthy control groups combined ($r = -0.40$, adjusted $p < 0.001$). After Bonferroni correction for multiple comparisons, the most impressive correlations were for visual learning and memory ($r = -0.50$, adjusted $p < 0.001$; Figure 4A) and for social cognition ($r = -0.41$, adjusted $p = 0.004$) in patients with schizophrenia (Appendix 1, Figure S2). These correlations remained significant when we evaluated the subgroup of patients with fewer than 3 days of antipsychotic treatment for visual learning and memory ($r = -0.45$, adjusted $p = 0.002$, other data not shown). Other measurements of cognition, including speed of processing, attention and vigilance, working memory, verbal learning, reasoning and problem solving were non-significant in patients with schizophrenia. In the control group, TGF- β 1 levels were not correlated with MCCB total or subscale scores.

Negative association between TGF- β 1 and LOC thickness in schizophrenia

When we explored the associations between plasma TGF- β 1 levels and brain cortical structures, we found multiple negative associations in patients with schizophrenia, but healthy controls showed only trends of positive correlations in most cases (Figure 4B). In particular, TGF- β 1 levels showed the strongest negative correlations with the mean thickness of the LOC ($r = -0.47$, adjusted $p = 0.001$; Figure 4C), as well as the thickness of the left LOC ($r = -0.35$, unadjusted $p < 0.01$; Figure 4D) and the right LOC ($r = -0.49$, adjusted $p < 0.001$; Figure 4E). These findings remained significant when we analyzed a subgroup of patients with fewer than 3 days of antipsychotic treatment ($r = -0.45$, adjusted $p = 0.002$; $r = -0.34$, adjusted $p = 0.02$; $r = -0.46$, adjusted $p = 0.001$).

Effect of TGF- β 1 on visual cognition mediated via LOC thickness

We assessed whether the LOC mediated the association between TGF- β 1 and MCCB visual cognition score. The negative effect of TGF- β 1 on the visual learning and memory score was fully mediated by mean LOC thickness in patients with schizophrenia (path AB: $\beta = -0.29$ [95% confidence interval -0.74 to -0.06], $p < 0.05$; Figure 5). In healthy controls, none of the mediation analyses showed significance.

Discussion

The present study showed the following main findings for patients with first-episode schizophrenia: increases in blood TGF- β 1 mRNA levels and plasma TGF- β 1 protein levels; decreases in the thicknesses of multiple cortical regions, particularly the LOC, and reduced neurocognitive performance; negative association between plasma TGF- β 1 levels and both visual cognition and LOC thickness, as well as a positive association between LOC thickness and visual cognition; a full mediation effect of the LOC on the relationship between TGF- β 1 and visual cognition.

Cortical thinning or grey matter loss in schizophrenia may be caused by multiple mechanisms, including neuronal maldevelopment and neurodegeneration. To the best of our knowledge, this study is the first to assess the correlations between the thickness of the LOC and other associated cortical regions and TGF- β 1 levels in patients with schizophrenia and

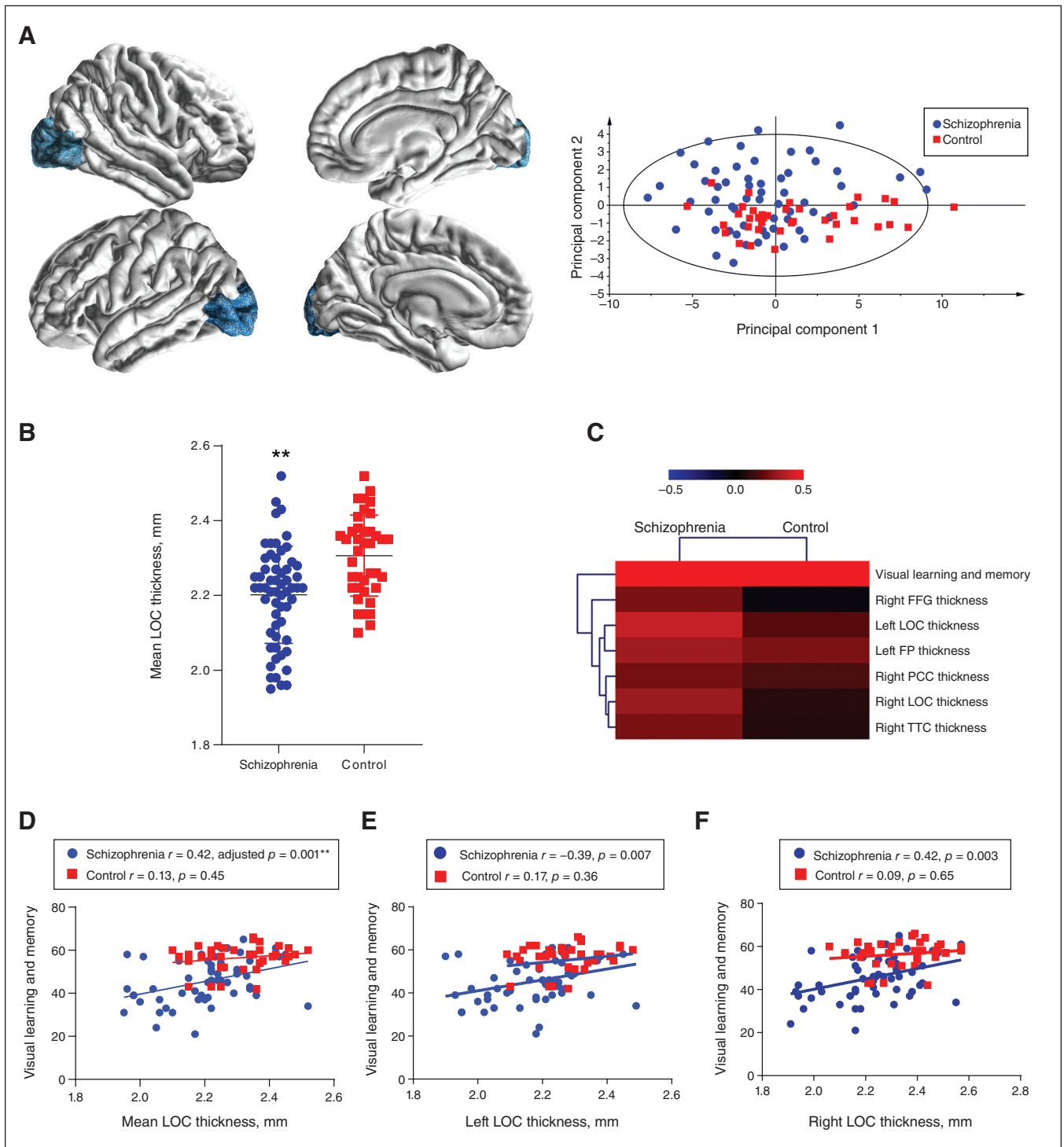


Figure 3: Positive associations between visual brain cortical structures and cognitive performance in patients with schizophrenia. (A) An exemplar graph showing the location and shape of the LOC and a principal component analysis scatter plot showing the separation of the schizophrenia and healthy control groups by all 34 cerebral cortical MRI measures. In the imaging graphs, blue represents a decrease in cortical thickness. (B) The mean LOC thickness was significantly lower in patients with schizophrenia than in controls (2.20 ± 0.13 mm v. 2.31 ± 0.11 mm, adjusted $p < 0.001$). Data are expressed as mean \pm standard deviation. (C) A heatmap of MRI features, which bears significant correlations with MATRICS Consensus Cognitive Battery visual scores in the schizophrenia group, shows hierarchical clustering of the features. (D–F) Visual learning and memory scores were positively associated with mean LOC thickness (adjusted $p = 0.001$), left LOC thickness (unadjusted $p = 0.007$) and right LOC thickness (unadjusted $p = 0.003$) in patients with schizophrenia but not controls. **Adjusted $p < 0.01$. FFG = fusiform gyrus; FP = frontal pole; LOC = lateral occipital cortex; PCC = pericalcarine cortex; TTC = transverse temporal cortex.

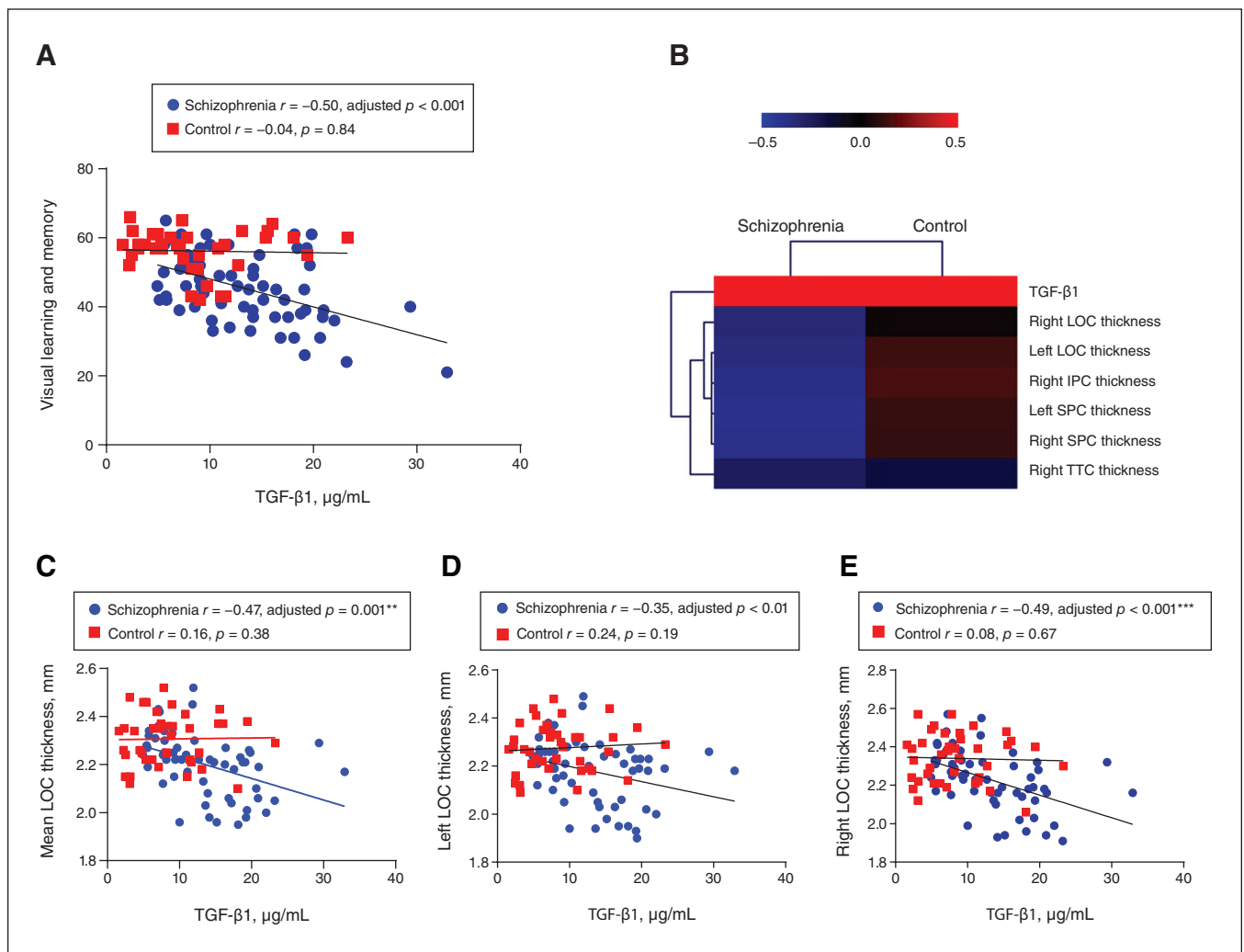


Figure 4: Negative correlations of TGF- β 1 with visual cognitive performance and LOC thickness in schizophrenia. (A) TGF- β 1 levels were negatively associated with visual learning and memory scores in patients with schizophrenia, but not in healthy controls. (B) A heatmap of TGF- β 1, which bears significant correlations with MRI features, shows hierarchical clustering of the features. (C–E) TGF- β 1 levels were negatively correlated with the mean thickness of the LOC and with the thickness of the left LOC (unadjusted $p = 0.01$) and right LOC in patients with schizophrenia, but not healthy controls. **Adjusted $p < 0.01$; ***adjusted $p < 0.001$. IPC = inferior parietal cortex; LOC = lateral occipital cortex; SPC = superior parietal cortex; TGF- β 1 = transforming growth factor β 1; TTC = transverse temporal cortex.

healthy controls. We showed that increased TGF- β 1 levels in patients with schizophrenia were associated with significant LOC thinning, leading to worsened performance on a visual learning and memory scale.

Our finding of higher blood cell mRNA and plasma protein levels of TGF- β 1 in patients with schizophrenia was in line with most previous studies.^{19,35–37} A meta-analysis by Miller and colleagues has also confirmed a significant increase in TGF- β 1 in the blood during acute exacerbations of schizophrenia, which were normalized with antipsychotic treatment.³⁸ Upregulation of TGF- β 1 in peripheral blood mononuclear cells was also correlated with positive symptoms in drug-free patients.³⁹ However, some other studies have found no change or a reduction in TGF- β 1 in the cerebrospinal fluid²¹ or blood of patients with schizophrenia.^{22,40,41} The inconsistency in these results is likely influenced by sample

size, illness process, antipsychotic medications or a combination of these.

The upregulation of blood TGF- β 1 may imply a biased immune function in patients with schizophrenia. Interestingly, we observed opposite changes in *TGFB1* (upregulation) and its receptors *TGFB1R1* and *TGFB1R3* (downregulation) in our blood RNA sequencing data. The cause of such an opposite relationship is intriguing but may reflect a compensational feedback mechanism to balance pro- and anti-inflammatory cytokine production via TGF- β 1-receptor-mediated signalling in target cells. However, we did not observe a broad change in pro- or anti-inflammatory cytokines in blood RNA sequencing, except for a minor population (2.3% hit) representing the immune system process (GO_BP:0002376) that involved some chemokine and TNF superfamily members (Appendix 1, Table S1 and S2), the significance of which warrants further investigation.

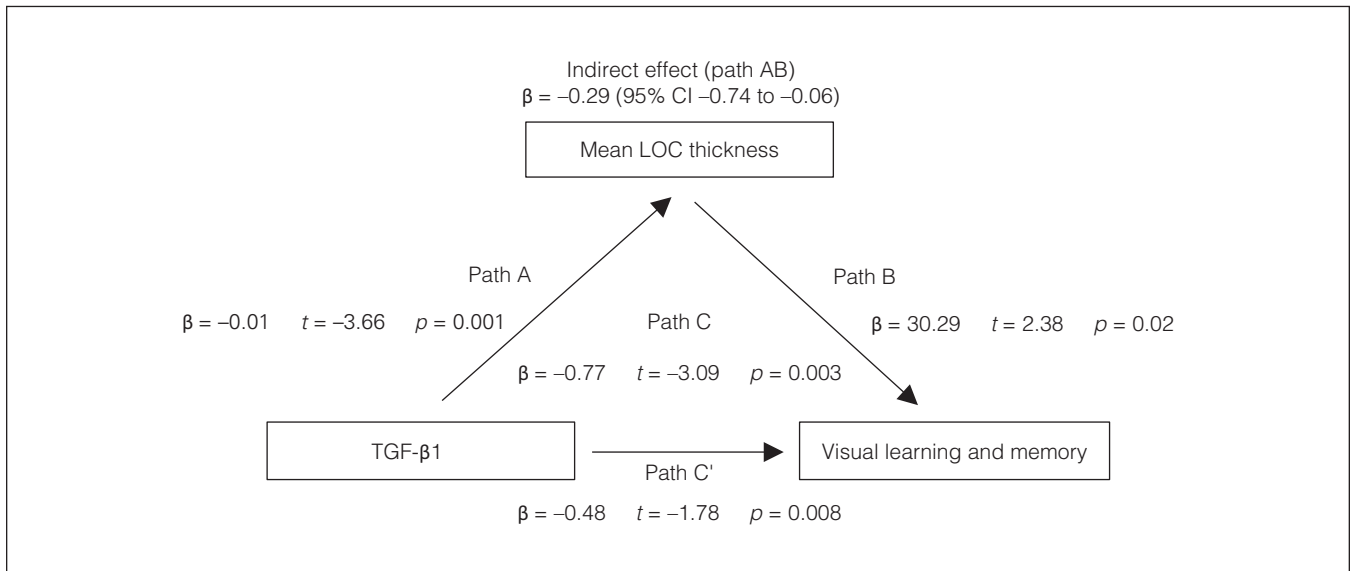


Figure 5: The LOC mediates the negative effect of TGF-β1 on visual cognitive performance in schizophrenia. We performed mediation analyses to examine the effect of the LOC on the correlation between TGF-β1 and visual learning and memory in patients with schizophrenia. Path A represents a direct relationship between TGF-β1 and mean LOC thickness; path C' represents a direct relationship between TGF-β1 and visual learning and memory; path B represents a direct relationship between mean LOC thickness and visual learning and memory; and path C represents a relationship between TGF-β1 and visual learning and memory, considering mean LOC thickness. Path AB reflects an indirect mediating effect of LOC thickness between TGF-β1 and visual learning and memory; $p < 0.05$. CI = confidence interval; LOC = lateral occipital cortex; TGF-β1 = transforming growth factor β1

TGF-β1 levels in the peripheral blood and brain may be aligned, because it has been reported that TGF-β1 can pass the blood–brain barrier via P-glycoprotein efflux transporters expressed on brain capillary endothelial cells,^{42,43} and TGF-β1 concentrations in the blood and the cerebrospinal fluid were parallel with each other.²¹ Furthermore, studies have shown that TGF-β1 plays a very important role in blood–brain barrier function and angiogenesis. For instance, the formation and maturation of the blood–brain barrier relies on interactions between endothelial cells and astrocytes via TGF-β-mediated activation of integrin $\alpha v\beta 3$.⁴⁴ Given that changes in blood–brain barrier structure and permeability have been identified in psychiatric disorders, allowing peripheral inflammatory effects on the brain,⁴⁵ this may also explain the influence of peripheral TGF-β1 in schizophrenia.

In the brain, aberrant TGF-β1 expression affects the formation of cellular and subcellular structures as well as their remodelling. TGF-β is a potent inducer of astrocytes derived from radial glia and is one of the principal factors for microglial development. Astrocytes may sustain a noninflammatory microglial phenotype through TGF-β, and astrocytic TGF-β overdrive pushes microglia to dysfunctional synaptic pruning.¹¹ At the embryonic stage, inactivation of TGF-β signalling impairs neuronal development as well.^{15–17}

To further investigate the underlying mechanisms of TGF-β1 function in the brains of people with schizophrenia, we analyzed TGF-β1 coexpressed genes in postmortem dlPFC samples of people with schizophrenia using the CommonMind Consortium data resource.³⁰ The frontal cortex — particularly the prefrontal cortex — is known to orchestrate the hierarchical

processing of visual and spatial working memory, including visual recognition memory and scene-specific memory for objects, as well as the associated emotional memory.^{46,47} We observed that although TGF-β1 mRNA levels in the dlPFC did not differ between patients with schizophrenia and healthy controls, coexpressed genes that mainly regulate cell surface receptor signalling and developmental processes were compromised in patients with schizophrenia. The schizophrenia-specific biological process was driven instead toward extracellular matrix remodelling, which may be related to changes in neuropil and synaptic remodelling in schizophrenia, as described below. Nevertheless, the overlapping coexpressed genes *AXL* and *CSF1*, conserved in both healthy controls and patients with schizophrenia, are inflammatory response regulators, implying that brain developmental or metabolic processes could intercalate with glia-mediated inflammatory processes, and these may all involve TGF-β1. However, the dlPFC gene expression data from the CommonMind Consortium may have been affected by antipsychotic treatment or other medications.

We have also provided evidence that peripheral TGF-β1 may negatively affect cognitive performance in patients with schizophrenia, particularly visual learning and memory, implying that increased circulating levels of TGF-β1 may affect synaptic connectivity and cognition in the brains of patients with schizophrenia via the aforementioned biological pathways. Indeed, evidence has shown that increased TGF-β1-modulated glutamate receptor expression in the hippocampus¹⁵ and neuronal C1q expression and synaptic pruning in the developing visual system¹⁸ promoted radial glia–astrocyte differentiation in the cerebral cortex in mice.⁴⁸ Mice lacking

TGF- β signalling in dopaminergic neurons were hyperactive and exhibited deficits in reversal learning and memory formation.^{16,17} However, other studies have revealed that over-expressed TGF- β 1 can cause aberrant synaptic function⁴⁹⁻⁵¹ and reduced neurogenesis,⁴⁸ likely detrimental for cognition. A study also showed that people with schizophrenia who displayed poor verbal fluency and decreased volumes in the Broca area had elevated peripheral cytokine levels, including TGF- β 1.⁵²

The above evidence indicates plural potencies of TGF- β 1 in steering the cognitive impairment that is a core component of the symptomatology of schizophrenia and accounts for related poor functional outcomes. Cytokines, either synthesized locally by brain cells or infiltrated from the blood, affect cognition depending on their type, concentration and spatio-temporal expression patterns.⁵³ On one hand, our findings reinforce the hypothesis of inflammatory influence on the pathogenesis of schizophrenia. On the other hand, they reveal the complicated functions of pleiotropic cytokines (in this case anti-inflammatory TGF- β 1) in regulating synaptic function and cognition.¹¹

Our results also suggest that TGF- β 1 levels in the peripheral blood may be a useful biomarker for detecting cortical or cognitive impairment in patients with early-stage schizophrenia. We observed a significant decrease in the thickness of the LOC, along with several other related regions, and this decrease had a significant positive association with visual learning and memory scores, indicating that these regions are probably the substrate of deficits in early visual processing. Consistent with this finding, altered structure and function of the occipital cortex has been implicated in schizophrenia by several other imaging studies.⁵⁴⁻⁵⁶ For instance, an MRI study⁵⁵ suggested that patients with schizophrenia had relatively intact grey matter volume in the primary visual area but reduced bilateral grey matter volume in vision-associated areas. Excessive grey matter loss in the occipital lobe, accompanied by severe perceptual impairments after visual stimulus in some patients with schizophrenia, has also been reported.⁵⁶ Earlier studies of postmortem tissues in the primary visual cortex in schizophrenia found reduced cortical thickness but an abnormally high density of small neurons, implicating reduced neuropils (axonal and dendritic tissues) as a likely cause of cortical thinning.⁵⁷⁻⁵⁹ Such reduction in neuropils could be related to a decrease in synaptic density, long thought to be a contributor to the pathophysiology of schizophrenia.⁶⁰ However, another study has suggested decreased thickness in the occipital cortex was less consistent in people with schizophrenia, and that the temporal and frontal cortices were more consistently different.⁶¹

Limitations

There were 4 major limitations in our study that should be acknowledged. First, although TGF- β 1 levels were associated with LOC thickness and visual cognition, these observations were only correlational, not causative. Second, we did not explore the effect of other cytokines on neurocognitive performance and cortical thickness. The role of cytokines in the pathogenesis of schizophrenia requires better elucidation in

future studies. Third, participants from the CommonMind Consortium did not all have first-episode psychosis, and they were not all antipsychotic-naïve; therefore, we cannot exclude the possible effect of antipsychotics on TGF- β 1 signalling in the dlPFC data. Finally, the heterogeneity of region-specific cortical thinning in patients with schizophrenia may have caused wide confidence intervals as a result of our small sample size and decreased the precision of results.

Conclusion

Overall, our results provide deeper insight into the potential role of TGF- β 1 in influencing cortical structure (especially the occipital lobe) and cognition in patients with schizophrenia. This better understanding may provide new clues for the prevention or treatment of cognitive deficits in patients with schizophrenia.

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