TITLE PAGE

Title: Associations between structural brain changes and blood neurofilament light chain protein in treatment-resistant schizophrenia

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Word count (excluding title page, abstract, references, figures and tables): 3356 words

Conflict of interest

The author(s) declared the following potential conflicts of interest with respect to the research, authorship and/or publication of this article: OH has acquired research support (for the institution) from AVID Radiopharmaceuticals, Biogen, C2N Diagnostics, Eli Lilly, Eisai, Fujirebio, GE Healthcare, and Roche. In the past 2 years, he has received consultancy/speaker fees from AC Immune, Alzpath, BioArctic, Biogen, Bristol Meyer Squibb, Cerveau, Eisai, Eli Lilly, Fujirebio, Merck, Novartis, Novo Nordisk, Roche, Sanofi and Siemens. The remaining authors have nothing to disclose.

Author contributions

DE, OH, IE, CB, AFS, DV, CP developed conception and design of the work. DE, CW and OH carried out data collection, B-JC, DE, CW and CM performed data analysis. B-JC, DE, CW, CM, AFS, DV and CP made the interpretation of the results and B-JC wrote the first manuscript draft with assistance from DE, CW, CM, AFS and DV. SJ, OH, IE, CB, NT and CP provided critical revision of the manuscript. All authors approved the final version of the manuscript.

Abstract

Background and Hypothesis

Around 30% of people with schizophrenia are refractory to antipsychotic treatment (treatment-resistant schizophrenia; TRS). While abnormal structural neuroimaging findings, in particular volume and thickness reductions, are often observed in schizophrenia, it is anticipated that biomarkers of neuronal injury like neurofilament light chain protein (NfL) can improve our understanding of the pathological basis underlying schizophrenia. The current study aimed to determine whether people with TRS demonstrate different associations between plasma NfL levels and regional cortical thickness reductions compared with controls.

Study Design

Measurements of plasma NfL and cortical thickness were obtained from 39 individuals with TRS, and 43 healthy controls. T1-weighted magnetic resonance imaging sequences were obtained and processed via FreeSurfer. General linear mixed models adjusting for age and weight were estimated to determine whether the interaction between diagnostic group and plasma NfL level predicted lower cortical thickness across frontotemporal structures and the insula.

Study Results.

Significant (false discovery rate corrected) cortical thinning of the left ($p = 0.001$, $\eta^2_p = 0.104$) and right ($p < 0.001$, $\eta^2_p = 0.167$) insula was associated with higher levels of plasma NfL in TRS, but not in healthy controls.

Conclusions.

The association between regional thickness reduction of the insula bilaterally and plasma NfL

may reflect a neurodegenerative process during the course of TRS. The findings of the

present study suggest that some level of cortical degeneration localised to the bilateral insula

may exist in people with TRS, which is not observed in the normal population.

Key Words:
Schizophrenia, treatment-resistant, neurofilament, biomarker, neuroimaging

Introduction

Schizophrenia is a complex and lifelong neuropsychiatric illness affecting nearly 1% of people worldwide. The most enduring model posits that schizophrenia is a neurodevelopmental disorder and postulates that susceptibility genes in combination with environmental risk factors occurring in early life produce a neurodevelopmental lesion that results in schizophrenia two to three decades later.(1–4) However, recent longitudinal neuroimaging studies showing progressive brain changes over the course of the illness, have revived the concept of schizophrenia as a neuroprogressive, perhaps even neurodegenerative, brain disorder in at least a subset of patients with severe disorder.(5,6)

Up to one in three people with schizophrenia inadequately respond to conventional antipsychotic treatment and are described as having treatment-resistant schizophrenia (TRS).(7) TRS is broadly defined as the absence of symptomatic improvement despite adherence to ≥2 different antipsychotic trials of appropriate dosage and duration.(8) People with TRS also tend to have significant residual symptoms, a worse prognosis, and a greater level of social and occupational dysfunction compared to those who respond better to antipsychotic treatment.(9)

Neuroimaging studies implicate widespread brain structural abnormalities in schizophrenia throughout the course of the disease, including in chronic schizophrenia, at first-episode psychosis, and even among cohorts at high risk for psychosis.(10–15) Among the most widely replicated neuroimaging findings in schizophrenia is cortical volume loss, in particular, of frontal and temporal areas.(16,17) Cortical volume, which is by definition the product of cortical thickness and cortical surface area, may erroneously confound these two separate and partly unrelated neuroanatomical features. Emerging evidence suggests that although cortical thickness and surface area are both heritable features, they are likely under distinct and independent sets of genetic and phenotypic influences.(18,19) Surface area is

understood to be predominantly under neurodevelopmental and genetic influence whereas

cortical thickness tends to be more affected by neurodegenerative and environmental

factors.(15,20) Regional and global cortical thickness reductions have also been

demonstrated in schizophrenia, implicating cortical thinning in the neuropathology and

symptomatology of the illness.(12,15,21–23) It has therefore been postulated that findings of

abnormal brain volumes in schizophrenia may be predominantly driven by cortical thickness

changes rather than surface area loss.(15) Taken together, changes to cortical thickness may

better describe the aetiological and pathological (perhaps neurodegenerative) processes

driving disease progression in schizophrenia.

As TRS is often associated with structural neuroimaging abnormalities,(13,34–36) it

is plausible that cortical volume and thickness reductions may be associated with elevations

of markers of neuroaxonal injury. A novel and minimally invasive approach to assessing

neuronal integrity involves measurement of fluid biomarkers. Neurofilaments are a family of

neuron-specific cytoplasmic proteins that confer stability to neuronal axons.(24,25)

Neurofilament light chain protein (NfL) is the lowest molecular weight, most soluble, and

most abundant of the neurofilament protein family.(26) Large-calibre myelinated axons are

particularly enriched in NfL, and as a marker of neuronal injury/degeneration, numerous

studies have demonstrated elevated cerebrospinal fluid (CSF) and blood levels of NfL in a

broad range of neurological and neurodegenerative disorders.(24,26–29) Several studies to

date have investigated NfL in schizophrenia.(30–33) Despite largely negative findings at the

group level, disease-driven axonal injury may instead be a feature of a subset of people with

psychoses, such as those with more severe phenotypes.

The primary aim of this study was to investigate the association between plasma NfL

levels and cortical thickness in people with TRS compared to matched controls. Brain regions

of interest included frontal, temporal, and the insula. The primary hypothesis was that raised

plasma NfL levels will be associated with cortical thinning of these brain structures in TRS
patients, but not among healthy controls.

Methods and materials

Participants

Samples and data were made available to the current study on behalf of the Cooperative
Research Centre (CRC) for Mental Health. Data collection for the psychosis cohort including
clinical assessment information, blood sampling and neuroimaging was conducted by the
Melbourne Neuropsychiatry Centre (Department of Psychiatry, University of Melbourne).
We have reported NfL values for this cohort previously.(33) The CRC Psychosis Study
protocol and this project were approved by the Melbourne Health Human Research Ethics
Committee (ethics IDs 2012.069 and 2020.142, respectively). All participants provided
informed, written consent before participating.

Individuals with TRS were recruited from a range of inpatient and outpatient
psychiatric services across Melbourne, Australia from 2012 to 2017 as part of the CRC
Psychosis Study. Treatment-resistance was defined by current clozapine use and inclusion
criteria for the TRS group included a schizophrenia diagnosis and being aged 18 to 65 years.
A comparison group of unrelated, age- and sex-matched controls who did not have
schizophrenia was also drawn from the same catchment site as the patient group.

All recruited patients underwent a process of comprehensive psychopathology and
neuropsychology assessments as well as a medical history. The MINI International
Neuropsychiatric Interview(37) was administered to all participants in order to confirm a
diagnosis of schizophrenia in the patient group and to rule out psychiatric disorders in healthy

controls. Additional clinical measures were recorded for both groups including other medical comorbidities, a family history of mental illness, and substance abuse history.

Neurofilament light chain protein assay protocol

Fasting blood samples were obtained from all participants and plasma was stored at $-80°C$. Plasma concentration of NfL was measured using a single molecule array NF-Light Advantage Kit (SR-X) according to the manufacturer's instructions (Quanterix Corporation, Lexington, MA USA). SR-X digital immunoassays have a mean limit of detection of 0.0552 pg mL^{-1}. All samples were diluted 1:4. Four internal control samples of pooled plasma were included in every plate. The mean intra-plate coefficient of variability (CV) was 4.97%, and the average inter-plate CV was 6.59%.

Two extreme outliers were noted, one in the control group (45-year-old male, plasma NfL 46.2 pg mL^{-1}, z-score = 6.28) and another in the TRS group (48-year-old female, plasma NfL 21.4 pg mL^{-1}, z-score = 3.98). A generally accepted cut-off value for extreme values is a z-score of 3 or more. As both outliers were more than three standard deviations above the means of their respective groups, they were excluded from all analyses. Outlier samples were measured three times, and levels did not differ by more than 10% each time.

Image data acquisition

Magnetic resonance imaging (MRI) sequences were acquired in a Siemens Avanto 3T Magnetom TIM Trio scanner. For each participant, a T1-weighted Magnetisation-Prepared Rapid Acquisition Gradient Echo sequence was obtained with the following sagittal imaging parameters: 176 sagittal slices of 1 mm thickness without gap, field of view of 250 x 250 mm^2, repetition time of 1980 ms, echo time of 4.3 ms, flip angle of 15°, and an acquisition

matrix of 256 x 256. A final reconstructed voxel resolution of 0.98 x 0.98 x 1.0 mm^3 was

achieved.

Image processing and estimation of cortical thickness

MRI data was processed with the FreeSurfer software package, version 6.0

(https://surfer.nmr.mgh.harvard.edu/) to estimate cortical thickness. FreeSurfer automatically

labels the surface of the brain to construct a cortical map based on probabilistic

information.(38,39) In brief, the automated processing pipeline consisted of intensity

normalisation, skull stripping, automated Talairach transformation, and corrections for

motion, signal intensity and topology.(39–44) Next, cortical surfaces were reconstructed.

This was achieved by overlaying grey-white matter boundaries to form the white matter

surface layer, and grey-CSF boundaries to create the pial surface layer—the cortex being the

region defined by the white matter and pial boundaries.(40) All reconstructed surfaces were

visually checked for defects in skull stripping, intensity normalisation, white-grey matter

segmentation and motion artefact by a qualified technician. Errors in boundary demarcation

were manually corrected via FreeSurfer's native editing tools, following a standardised

editing protocol. Edited images were reprocessed, and the output visually inspected for

inaccuracies again. These steps were repeated until all surface errors were corrected. The

cortical surface was then parcellated into 68 regions according to the Desikan-Killiany

atlas.(45) An estimate of the average cortical thickness for each region was obtained by

averaging across all vertices of the given region for each subject.

Statistical analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) versions 28 & 29.

Analysis of demographic measures

Group differences of demographic (such as age at sample, sex, and years of schooling) and clinical (such as illness duration, and degree of functional impairment) measures were compared using chi-square tests for categorical variables, and independent samples t-tests for continuous variables. Statistical significance was defined as $p < 0.05$. Missing data was dealt with using pairwise deletion.

Analysis of between-group differences in plasma neurofilament light chain protein level and cortical thickness

Independent samples t-tests were used to compare levels of plasma NfL between the healthy controls and people with TRS.

Group differences in cortical thickness were examined using ANCOVAs, with age and weight used as covariates. Results were then corrected for multiple comparisons in each hemisphere using a false discovery rate (FDR) of $p < 0.05$.[47]

Analysis of interactions between plasma neurofilament light chain protein level and group

To determine whether differential relationships exist between plasma NfL levels and cortical thickness by group, a series of general linear mixed models (GLMMs) with random effect of clinical site were estimated. GLMMs in the present study were all adjusted for age given its relationship with plasma NfL levels.[24] Weight was also included as a covariate due to emerging evidence of a negative association between plasma NfL levels and weight which

has been attributed to the dilutional effect of increased blood volume.(46) Each model
included the main effects of group membership and plasma NfL level, in addition to age and
weight as covariates. The interaction term (NfL-by-Group) was included in the model to
investigate whether NfL- and group-related differences of the thickness measures were
significant. In brief, NfL-by-Group determines whether membership in either diagnostic
group (i.e., healthy control or TRS) significantly modifies the association between plasma
NfL and thickness of each brain structure.

The brain structures that were investigated in the present study accounted for all
frontotemporal regions as well as the insula as these cortical structures are most consistently
implicated in schizophrenia neuroimaging studies.(15,22,34) The ENIGMA Schizophrenia
Working Group identified a loco-regional pattern of cortical thinning across 4474 patients
with schizophrenia compared to 5098 healthy controls, with the largest effect sizes in frontal
and temporal areas.(15) Zhao et al. (2022) similarly demonstrated cortical thickness
reductions across the frontotemporal areas, as well as the right insula in a cohort of people
with chronic schizophrenia.(22) A complete list of cortical regions included in the present
study is available in Table S1 in the supplement.

Correction for multiple comparisons was made using a FDR of $p < 0.05$.(47) Hence,
significance for the NfL-by-Group interaction would indicate that the trendlines between
cortical thickness and plasma NfL level are significantly different between the two groups,
when adjusted for age and weight. Bias-corrected and accelerated (BCa) confidence intervals
(CI) were generated for all GLMMs via bootstrapping using 2000 replicates. Partial eta
squared (η^2_p) was also reported to give a measure of effect size for the NfL-by-Group
interaction term and the main effects of plasma NfL.

Results

Sample characteristics

A total of 82 participants from the initial pool of volunteers recruited to the CRC Psychosis Study Treatment Resistant Schizophrenia Biobank had both plasma samples as well as MRI scans available. Full demographic and clinical variables are presented in Table 1. Thirty-nine participants had a diagnosis of TRS and a group of 43 control participants were also included in the study. The TRS group had a mean illness duration of 16.3 years with an average illness onset at 21.6 years. The TRS and control groups did not differ in age ($p = 0.433$) nor in sex ($p = 0.097$).

Individuals with TRS completed fewer years of schooling ($p < 0.001$) and had lower current IQ scores ($p < 0.001$) compared to matched controls. People with TRS were also more likely to have comorbidities like epilepsy ($p = 0.004$) and dyslipidaemia ($p = 0.015$) versus control subjects. Tobacco and other substance use disorders were more common among participants with TRS, although alcohol use was not different between the two groups ($p = 0.286$). People with TRS were also more likely to have had a previous head injury than controls ($p = 0.003$).

Twenty participants with TRS (51.3%) reported having a family history of mental health disorders, with psychotic illnesses being the largest contributor. The opposite trend was observed in healthy controls where non-psychotic disorders (23.3%) predominated among those with a family history of mental illness. 24.3% of TRS participants (9/37) had received electroconvulsive therapy.

Group differences in neurofilament light chain protein

At the group level, there was no significant mean difference between levels of plasma NfL in people with TRS compared to controls (Table 2). Controls had a mean NfL of 6.1 pg/mL compared to 5.5 pg/mL recorded for people with TRS.

Group differences in cortical thickness

In comparison with controls, TRS participants were found to have reduced cortical thickness of the insula bilaterally as well as all frontotemporal structures, except for the transverse temporal bilaterally, right pars triangularis, left pars orbitalis, and left rostral middle frontal regions (Table 3).

Interactions between neurofilament light chain protein and cortical thickness

Region of interest (ROI) analysis of frontotemporal and insula cortical thicknesses showed a significant NfL-by-Group interaction in the left and right insula ($p = 0.001$, and $p < 0.001$, FDR-corrected, respectively). This was interpreted as a statistically significant difference in the relationship between plasma NfL and cortical thickness of the bilateral insula across the two groups. Specifically, a higher plasma NfL level significantly were associated with lower cortical thickness of these structures among participants with TRS but not in controls. Interaction plots of the left and right insula with regression lines and 95% CIs are presented in Figure 1. Trendlines and CIs are separated by diagnostic group (i.e., control or TRS).

Other biologically plausible regions such as the left middle and inferior temporal gyri, while not significant regions following FDR-correction, however these showed similar effect sizes. Plasma NfL did not predict differences in cortical thickness between people with TRS

and controls in other brain structures (Supplementary Figures S1 & S2). Complete results are

presented in Table 4.

Detailed results of GLMMs with main effects of plasma NfL separated by diagnostic

group are available in Supplementary Tables S1 & S2.

Discussion

This study investigated the utility of plasma NfL as a marker of neuroimaging abnormalities

in a cohort of clozapine-treated TRS patients, compared to controls. The main finding is that

plasma NfL levels predicted lower cortical thickness of the insula bilaterally among people

with TRS, compared to healthy controls. Thus, the primary hypothesis regarding associations

between plasma NfL and decreased thickness of discrete cortical areas was partially

supported. These findings suggest that regional neurodegenerative changes may be occurring

in a schizophrenia cohort that represents severe and chronic disease. Although the exact

mechanism of how schizophrenia pathology may contribute to neuroimaging abnormalities is

unclear, the association between NfL and neuroimaging abnormalities in the TRS group

raises the possibility of a neurodegenerative course that specifically affects neuronal axons.

To the best of our knowledge, this is the first study to explore plasma NfL in the context of

neuroimaging in a large, well-characterised group of people with TRS (defined by current

treatment with clozapine). We propose that plasma NfL, an established marker of neuronal

injury and neurodegeneration, is associated with cortical thinning of key brain structures

often implicated in schizophrenia.

The association of cortical thickness reductions of the right and left insula with

plasma NfL in people with TRS versus controls was a key and novel finding. The insula is

understood to have extensive connections to brain areas that process external sensory

information, which if disrupted may contribute to a diminished ability to discriminate between internally- and externally-generated sensory information.(48) Misrepresentation of self-sources of speech as distinct and external has therefore been posited as a potential substrate for auditory hallucinations in schizophrenia.(49) Our findings are comparable with those of Van Erp et al.,(15) who demonstrated a discrete pattern of cortical thinning across the brain in schizophrenia compared to healthy controls, including the insula bilaterally. They further suggest that clozapine dose is associated with a higher degree of cortical reduction, while a reduced insular thickness corresponds to an earlier age of onset and greater duration of illness in people with schizophrenia. Volumetric reductions of the insula have even been observed in first-episode psychosis(50,51) as well as in individuals at ultra-high-risk of psychosis.(52) A functional MRI study also reported altered and poorly differentiated functional connectivity profiles between the anterior and posterior insulae and their targets in schizophrenia.(53) Diminished insula functional connectivity is also associated with longer illness duration which may suggest a progressive loss of connectivity of this network along the clinical course of the illness. Our finding of an association of plasma NfL levels and thinning of the insular cortex bilaterally may at least to some degree reflect aberrant connectivity of this region in schizophrenia.

Our results of a relationship between thinning of the insula bilaterally, and plasma NfL levels supports the notion of a neurodegenerative process occurring in severe forms of schizophrenia. Cortical thickness represents the density and arrangement of neurons, glial cells, dendritic processes, and synaptic spines.(54) Disruption to one or more of these neural components in localised areas of the brain may account for findings of cortical thinning associated with NfL among people with TRS compared to controls. Taken together, we propose the possibility of an active neurodegenerative process leading to axonal degeneration and injury, of which NfL is a marker, in patients who fail to respond adequately to

antipsychotics. In addition to the conceptualisation of schizophrenia as a psychiatric illness, the current study adds weight to previous bodies of work speculating about the possibility of a neurodegenerative component.(6,55,56) These findings do not rule out the involvement of other neuropathological processes in severe schizophrenia, only that axonal injury may represent one mechanism driving these structural neuroimaging changes.

Our findings must be interpreted in the context of some limitations. Firstly, the cross-sectional design of the study meant that it was not possible to investigate whether longitudinal plasma NfL increases are associated with progressive brain changes in TRS. While progressive changes have been explored in schizophrenia previously,(5,16,17,57) no studies to date have examined such changes in association with NfL levels. Secondly, all participants with TRS recruited to the study were receiving clozapine and so it was not possible to disentangle pathological disease-driven brain changes from those arising due to, or accelerated by, clozapine use. Thirdly, the lack of specificity of NfL as a marker of neuronal insult and injury inherently limits the conclusions that can be drawn about the disease-driven pathogenic processes in schizophrenia. NfL is elevated in response to various neurodegenerative, inflammatory, vascular, and traumatic insults to neuronal axons.(24) Fourthly, the present study was unable to compare findings of the TRS group to a cohort of non-TRS patients in order to determine whether NfL-related neuroimaging abnormalities are a feature common across all schizophrenia patients, or whether they are unique to TRS. The utility of plasma NfL in predicting neuroimaging changes specific to patients exhibiting treatment-resistance was unexplored but should be a priority of future studies. Finally, participants recruited to the TRS cohort of our study were largely patients with long-standing illness. As a result, the present study was unable to investigate the clinical utility of NfL in detecting structural brain abnormalities in earlier stages of the schizophrenia disease course.

The current study provides evidence for an association between plasma NfL and reduced thickness in discrete brain structures, namely the bilateral insula, in a group of TRS patients compared to controls. These findings suggest that neuronal insult may be occurring in patients who respond inadequately to treatment, adding weight to a neurodegenerative model of schizophrenia and the notion of a subgroup of poor-outcome, severe schizophrenia represented by treatment-resistance. NfL and other markers of neurodegeneration are likely to be important correlates of clozapine-treated TRS patients. Large-scale prospective longitudinal studies are required to confirm findings presented in the current study. Future studies should also aim to appraise the clinical utility of plasma NfL in distinguishing TRS from non-TRS patients, as a marker of treatment response, as well as exploring NfL in other stages of the disorder and in at-risk cohorts.

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Tables and Figures

Table 1. Demographic and clinical characteristics.

	Control	TRS
N	43	39
Age at sample, years	39.6 [36.1, 43.1]	37.9 [35.1, 40.6]
Sex, *n* female (%)	16 (37.2%)	8 (20.5%)
Age at onset, years	–	21.6 [19.6, 23.6] (n=38)
Duration of illness, years	–	16.3 [14.2, 18.4] (n=38)
PANSS total	29.7 [29.0, 30.4]	58.7 [54.3, 63.1]
PANSS positive	7.2 [7.0, 7.4]	15.2 [13.2, 17.2]
PANSS negative	6.7 [6.3, 7.1]	16.3 [14.3, 18.3]
PANSS general	15.7 [15.4, 16.1]	27.2 [25.4, 29.1]
Years of schooling	15.9 [15.0, 16.9]	12.2 [11.2, 13.3]
Current smoker (last 12 months)	7 (16.2%)	15/36 (41.7%)
Diagnosis of alcohol use disorder	6 (14.0%)	9 (23.1%)
Other substance use disorder	4 (9.3%)	11 (28.2%)
Hypertension	1 (2.3%)	4/38 (10.5%)
Diabetes	0%	3/38 (7.9%)
Dyslipidaemia	0%	5 (12.8%)
Weight, kg	73.5 [70.4, 76.6]	95.4 [88.7, 102.2]
Current IQ	111.7 [107.2, 116.2] (n=40)	88.5 [82.3, 94.7] (n=38)
Clozapine level, µg/mL	–	464.9 [379.3, 550.6] (n=38)
History of head injury	7 (16.3%)	18 (46.2%)
Epilepsy	0%	7 (17.9%)
Other neurological disorders	0%	8 (20.5%)
Family history of mental illness	13 (30.2%)	20 (51.3%)
psychotic	3 (7.0%)	14 (35.9%)
non-psychotic	10 (23.3%)	6 (15.4%)
Problems with birth	4/42 (9.5%)	10/38 (26.3%)
Ever had ECT	0%	9/37 (24.3%)

Note: Data reported as mean [95% CI] or *n* (%).

ECT, electroconvulsive therapy; PANSS, Positive and Negative Syndrome Scale; TRS, treatment-resistant schizophrenia.

Table 2. Between-group comparisons of plasma neurofilament light chain protein level.

	Control	TRS
N	43	39
Age at sample, years	39.6 (11.4)	37.9 (8.4)
Sex, *n* female (%)	16 (37.2%)	8 (20.5%)
Plasma NfL, pg/mL	6.1 [5.3, 6.8]	5.5 [4.6, 6.4]

Note: Data reported as mean [95% CI] or *n* (%).
CI, confidence interval; NfL, neurofilament light chain protein.

Table 3. Between-group comparisons of cortical thickness.

Hemisphere	Structure	Control		TRS		Control vs. TRS	
		Mean	SD	Mean	SD	F-statistic	η^2_p
Left	Caudal middle frontal	2.604	0.122	2.481	0.132	22.650*	0.227
	Lateral orbitofrontal	2.657	0.103	2.571	0.121	12.885*	0.143
	Medial orbitofrontal	2.421	0.115	2.377	0.126	7.184*	0.085
	Entorhinal	3.508	0.254	3.263	0.318	10.000*	0.115
	Pars opercularis	2.625	0.115	2.531	0.139	15.406*	0.167
	Pars orbitalis	2.647	0.161	2.573	0.176	3.513	0.044
	Pars triangularis	2.475	0.159	2.421	0.167	7.351	0.087
	Rostral middle frontal	2.351	0.111	2.322	0.122	3.644	0.045
	Superior frontal	2.742	0.124	2.607	0.120	38.999*	0.336
	Precentral gyrus	2.668	0.141	2.576	0.156	9.351*	0.108
	Inferior temporal	2.845	0.129	2.704	0.113	23.676*	0.235
	Middle temporal	2.915	0.115	2.792	0.119	26.293*	0.255
	Superior temporal	2.863	0.139	2.781	0.158	7.539*	0.089
	Transverse temporal	2.523	0.213	2.437	0.232	3.019	0.038
	Insula	3.030	0.163	2.922	0.182	9.656*	0.111
Right	Caudal middle frontal	2.528	0.105	2.389	0.129	25.187*	0.246
	Lateral orbitofrontal	2.605	0.119	2.471	0.136	25.782*	0.251
	Medial orbitofrontal	2.356	0.128	2.56	0.161	9.622*	0.111
	Entorhinal	3.580	0.362	3.343	0.432	6.193*	0.074
	Pars opercularis	2.560	0.143	2.482	0.141	6.467*	0.077
	Pars orbitalis	2.626	0.141	2.487	0.152	18.367*	0.193
	Pars triangularis	2.411	0.140	2.357	0.154	3.121	0.039
	Rostral middle frontal	2.230	0.091	2.192	0.102	5.024*	0.061
	Superior frontal	2.659	0.118	2.512	0.113	31.918*	0.293
	Precentral gyrus	2.595	0.143	2.510	0.162	7.160*	0.085
	Inferior temporal	2.859	0.118	2.756	0.149	11.789*	0.133
	Middle temporal	2.903	0.116	2.810	0.126	15.556*	0.0168
	Superior temporal	2.855	0.142	2.782	0.170	5.169*	0.063
	Transverse temporal	2.462	0.215	2.425	0.241	0.705	0.009
	Insula	3.085	0.132	2.974	0.139	14.103*	0.155

*Note: *p* < 0.05, FDR-corrected.*

SD, standard deviation; TRS, treatment-resistant schizophrenia.

Table 4. General linear mixed models and their corresponding plasma NfL-by-Group interaction term for each brain structure thickness.

Hemisphere	GLMM	Plasma NfL-by-Group interaction			
		B*	BCa 95% CI	η^2_p	*p*-value
Left	Caudal middle frontal	0.010	[−0.009, 0.027]	0.011	0.265
	Lateral orbitofrontal	0.014	[−0.003, 0.035]	0.029	0.100
	Medial orbitofrontal	0.001	[−0.023, 0.027]	0.000	0.908
	Entorhinal	0.051	[0.010, 0.088]	0.057	0.030
	Pars opercularis	0.007	[−0.015, 0.030]	0.006	0.488
	Pars orbitalis	0.009	[−0.022, 0.038]	0.006	0.472
	Pars triangularis	0.010	[−0.016, 0.035]	0.007	0.445
	Rostral middle frontal	0.009	[−0.012, 0.027]	0.011	0.315
	Superior frontal	0.014	[−0.003, 0.035]	0.028	0.119
	Precentral gyrus	0.026	[0.003, 0.047]	0.056	0.026
	Inferior temporal	0.026	[0.006, 0.048]	0.088	0.012
	Middle temporal	0.027	[0.008, 0.039]	0.098	0.008
	Superior temporal	0.025	[0.002, 0.043]	0.057	0.027
	Transverse temporal	0.030	[−0.005, 0.058]	0.031	0.060
	Insula	0.039	[0.017, 0.071]	0.104	0.001*
Right	Caudal middle frontal	0.020	[0.002, 0.036]	0.052	0.033
	Lateral orbitofrontal	0.009	[−0.021, 0.031]	0.009	0.326
	Medial orbitofrontal	0.025	[0.005, 0.048]	0.056	0.022
	Entorhinal	0.063	[0.000, 0.126]	0.044	0.026
	Pars opercularis	0.017	[−0.004, 0.038]	0.028	0.103
	Pars orbitalis	0.010	[−0.022, 0.032]	0.008	0.528
	Pars triangularis	0.010	[−0.012, 0.025]	0.009	0.331
	Rostral middle frontal	0.006	[−0.011, 0.020]	0.008	0.418
	Superior frontal	0.012	[−0.008, 0.031]	0.019	0.231
	Precentral gyrus	0.028	[0.005, 0.049]	0.065	0.023
	Inferior temporal	0.026	[0.005, 0.051]	0.072	0.011
	Middle temporal	0.021	[0.000, 0.038]	0.061	0.052
	Superior temporal	0.022	[−0.033, 0.040]	0.040	0.060
	Transverse temporal	0.004	[−0.034, 0.037]	0.001	0.784
	Insula	0.040	[0.021, 0.063]	0.167	<0.001*

*Note: *p* < 0.05, FDR-corrected.*
BCa, bias-corrected and accelerated; CI, confidence interval; GLMM, general linear mixed model; NfL, neurofilament light chain protein; TRS, treatment-resistant schizophrenia.
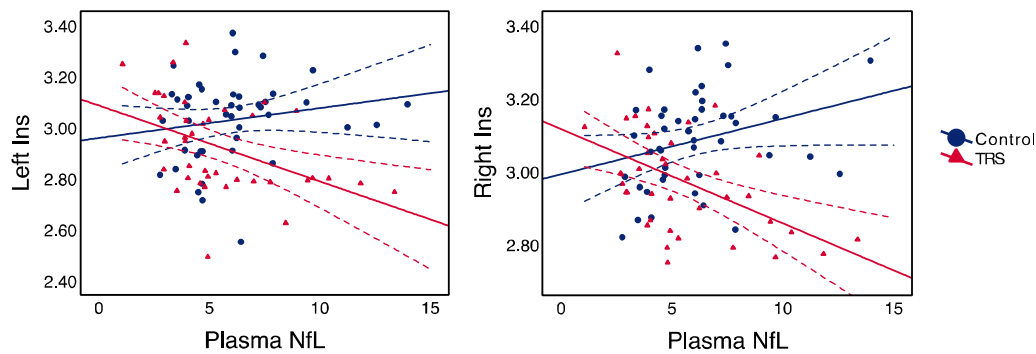
Figure 1. Interaction plots with trendlines and 95% confidence intervals for the left and right insula. Plasma NfL in pg/mL; thickness in mm. *Note:* Ins, insula; NfL, neurofilament light chain protein.