PCN Psychiatry and Clinical Neurosciences

REGULAR ARTICLE

Abnormal choroid plexus, hippocampus, and lateral ventricles volumes as markers of treatment-resistant major depressive disorder

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Aim: One-third of patients with major depressive disorder (MDD) do not achieve full remission and have high relapse rates even after treatment, leading to increased medical costs and reduced quality of life and health status. The possible specificity of treatment-resistant depression (TRD) neurobiology is still under investigation, with risk factors such as higher inflammatory markers being identified. Given recent findings on the role of choroid plexus (ChP) in neuroinflammation and hippocampus in treatment response, the aim of the present study was to evaluate inflammatory- and trophic-related differences in these regions along with ventricular volumes among patients with treatment-sensitive depression (TSD), TRD, and healthy controls (HCs).

Methods: ChP, hippocampal, and ventricular volumes were assessed in 197 patients with MDD and 58 age- and sex-matched HCs. Volumes were estimated using FreeSurfer 7.2. Treatment resistance status was defined as failure to respond to at least two separate antidepressant treatments. Region of interest volumes were then compared among groups.

Results: We found higher ChP volumes in patients with TRD compared with patients with TSD and HCs. Our results also showed lower hippocampal volumes and higher lateral ventricular volumes in TRD compared with both patients without TRD and HCs.

Conclusions: These findings corroborate the link between TRD and neuroinflammation, as ChP volume could be considered a putative marker of central immune activity. The lack of significant differences in all of the region of interest volumes between patients with TSD and HCs may highlight the specificity of these features to TRD, possibly providing new insights into the specific neurobiological underpinnings of this condition.

Keywords: *biomarkers, choroid plexus, depressive disorder, neurobiology, treatment-resistant.*

http://onlinelibrary.wiley.com/doi/10.1111/pcn.13764/full

Approximately one-third of patients with major depressive disorder (MDD) do not achieve full symptomatic remission and functional recovery after several lines of treatment, experiencing high relapse rates even after episode resolution.[1]

Treatment-resistant depression (TRD) is linked to increased medical costs and reduced quality of life and health status.[2] The clinical course of TRD is often characterized by an early onset and a longitudinal pattern of recurrences with increasing frequency and severity.[3] Its consequences include premature death, suicide, greater impairment in work productivity and daily activities, and heightened utilization of healthcare resources.[4]

In addition, the likelihood of achieving remission decreases with each subsequent treatment failure,[5] while the burden of disease progressively increases over time. Hence, there is a need for reliable predictors of TRD to identify potential new treatment targets and facilitate the rapid diagnosis and selection of the most appropriate treatment combinations.

The neurobiology of MDD is highly complex, with multifactorial causes that include genetic, environmental, biological, and psychosocial factors.[6] Immunopsychiatry and neuroimaging studies continue to provide novel biomarkers of the disease.[7] To what extent TRD might represent a distinct clinical entity, or rather share MDD neurobiology, remains an open research question. A recent systematic review identified numerous risk factors associated with TRD, including worse baseline severity, frequency of recurrences, and duration of the current episode, but also higher levels of circulating inflammatory markers, such as interleukin (IL) 6 and C-reactive protein.[8] Indeed, several markers of an altered immune/inflammatory status, including decreased adaptive and increased innate immunity activity, elevated proinflammatory setpoints, altered cytokine profiles, changes in gene expression patterns in circulating white blood cells, and activation of brain microglia, have been associated with TRD.[9,10]

Concerning neuroimaging, patients with MDD show a multitude of brain abnormalities when compared with healthy controls (HCs),

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Psychiatry and Clinical Neurosciences published by John Wiley & Sons Australia, Ltd on behalf of Japanese Society of Psychiatry and Neurology.

including lower amygdala and hippocampal volumes (HVs); gray matter reductions in the prefrontal, cingulate, orbitofrontal, and parahippocampal cortices; enlarged ventricular volumes; widespread disruption of white matter integrity; and marked abnormalities in resting state functional connectivity.[11–15] However, magnetic resonance imaging (MRI) differences between patients with MDD who have and those who do not have TRD are less consistent. While studies investigating baseline brain volumes to predict response to antidepressants (ADs) report strong associations of larger HVs with positive response to treatment,[16] a recent systematic review focusing on the comparison between TRD and treatment-sensitive depression (TSD) found limited evidence of structural brain abnormalities linked to TRD, again identifying HVs among the few robust correlates.[17] We previously observed a positive association between HV and response to monoaminergic ADs, an effect influenced in females by immune/inflammatory setpoints.[18,19]

Located in the brain ventricles, the choroid plexus (ChP) consists of a layer of ependymal cells surrounding capillaries and loose connective tissue. Classically regarded to be responsible for the production of cerebrospinal fluid (CSF),[20] in recent years ChP's role in physiology and disease has gained renewed interest.[21] Indeed, the ChP is now regarded as the main interface between the brain and the immune system, playing a major role in immune cell trafficking which ensures brain homeostasis in physiological conditions.[22] Furthermore, given its repeatedly observed enlargement in several inflammatory conditions affecting the brain,[23] its use as a possible direct marker of neuroinflammation has been extensively investigated.[24–26]

Immune/inflammatory alterations, including microglia activation and brain infiltration by peripheral leucocytes, are now thought to play a crucial role in the pathophysiology of several psychiatric conditions,[27–29] and the ChP might prove to be a crucial mediator between inflammatory status and psychopathology. Indeed, several studies are beginning to explore the association between ChP volumes and several psychiatric diagnoses.[30,31] In a previous study, we observed enlarged ChP volume in MDD, proportional to the duration of illness, and to circulating levels of inflammatory cytokines, both factors consistently associating to TRD in clinical practice.[32–34]

Although anatomically contiguous, the relationship between hippocampi and ChP is not yet fully understood. Both ChP and hippocampus develop from the mammalian embryonic cortical hem, sharing signaling pathways to shape their progenitor regions and linking the formation of the ChP with embryonic and postnatal hippocampal neurogenesis.[35] In adult life, animal models have shown that the ChP still plays a key role in maintaining and activating the subventricular zone neuroblast pool, thus regulating the regenerative capacity of the adult brain.[36,37] Studies on in vivo human adult neurogenesis are still in its infancy, but postmortem staining supports an adult human neurogenic system originating from the circumventricular organs and encompassing multiple structures, including the hippocampus.[38] Agents transported from blood to lateral ventricles are carried by CSF volume transmission to periventricular structures, including the hippocampus,[39] and possible fluxes of growth factors, neurotrophins, hormones, and leukocytes from ventricular CSF into the hippocampus, and subventricular zone are being studied in humans.[40] The relationship between the two structures could then involve shared mechanisms regulating homeostasis and the development of brain tissue in humans, as it has been well established in animal models.[21]

Therefore, in the present study, given the reported association between inflammatory status and MDD pathophysiology and treatment resistance,[9,10] the role of ChP volume as a putative proxy of neuroinflammation,[22] and the role of HV as a predictor of response,[17] we investigated the association between ChP and HV with TRD in patients with MDD compared with patients with TSD and age- and sex-matched HCs. Furthermore, considering the crucial role of the ChP in CSF secretion and its strong association with ventricular volume,[41,42] as well as the enlargement of ventricular volume as a consequence of general atrophy and cerebral gray matter volume loss,[43–45] we also investigated the association between treatment resistance and brain ventricular volumes.

Methods

Participants and clinical data collection

We studied 197 patients with MDD (*DSM-5* criteria) consecutively admitted to the mood disorder unit of our hospital and 54 age- and sex-matched HCs. Additional inclusion criteria for all groups were age between 18 and 65 years, willingness to participate, absence of other diagnoses on Axis I, intellectual disabilities, drug and alcohol abuse or dependency, and pregnancy. Concerning medical comorbidities, patients with comorbid neurological disorders or conditions known to affect the immune system were excluded from the study, including those with rheumatologic and autoimmune diseases, acute or chronic infections, hematological conditions, or any type of cancer or hematological malignancy. Patients with uncontrolled medical conditions were also excluded from participation. Treatment with pharmacotherapies known to affect the immune system, such as corticosteroids, immunomodulators, and nonsteroidal anti-inflammatory drugs were also an exclusion criterion. Overlapping criteria were adopted for the inclusion of HCs together with the absence of current or history of psychiatric disorders. The sample of the present study partially overlaps with that from our previous study.[34]

After a complete description of the study, written informed consent was obtained. All of the research activities were performed according to the guidelines of the Declaration of Helsinki and approved by the ethics committee of Ospedale San Raffaele (protocol no. 10-06-SO, date of approval: 30 June 2006).

TRD was assessed soon after hospitalization. Using a best-estimation procedure, two pairs of authors independently extracted information about response to AD treatments from all of the available clinical charts, mediating any uncertainty associated with extraction (M.P., M.M., R.Z., and F.B.). Resistance status was investigated through the patients' clinical history up until the moment of MRI scan. TRD was defined as failure to respond to at least two separate AD treatments administered with an adequate dose and duration.[1,46] Established add-on treatment for depression (buproprion, mirtazapine, low-dose aripiprazole, or amisulpride) were considered as a distinct line of treatment. Patients were therefore divided according to their TRD clinical history.

Equivalent doses of imipramine, chlorpromazine, and lorazepam taken at the moment of MRI were calculated for all patients.

Brain imaging

All patients underwent MRI. T1-weighted images were acquired on two 3.0 Tesla scanners: (Ingenia CX, Philips, the Netherlands), using a 32-channel sensitivity encoding SENSE head coil (T1-weighted MPRAGE sequence: TR 8.00 ms, TE 3.7 ms, field of view FOV = 256 mm, matrix = 256 × 256, in-plane resolution 1 × 1 mm), yielding 182 transversal slices with a thickness of 1 mm; (Gyroscan Intera, Philips, the Netherlands), employing an 8-channel SENSE head coil (T1-weighted MPRAGE sequences: TR 25.00 ms, TE 4.6 ms, field of view FOV = 230 mm, matrix = 256 × 256, in-plane resolution 0.9 × 0.9 mm, yielding 220 transversal slices with a thickness of 0.8 mm). Volumetric brain segmentation was performed using the fully automated and validated FreeSurfer image analysis suite v 7.2 (http://surfer.nmr.mgh.harvard.edu/). Standard reconstruction procedures, which delineate brain anatomy into cortical and subcortical labels, were used. The processing includes motion correction,[47] automated Talairach transformation, segmentation of the subcortical deep gray matter volumetric structures,[48,49] intensity normalization,[50] tessellation of the gray matter–white matter boundary, and automated topology correction.[51,52] Segmentation of ChP, lateral ventricles, hippocampus, and total ICV were obtained with the FreeSurfer "recon-all" function[49] (Fig. 1). Volumes of structures were automatically acquired in voxel. All segmented structures were visually inspected for accuracy by a neuroimaging expert who examined each image

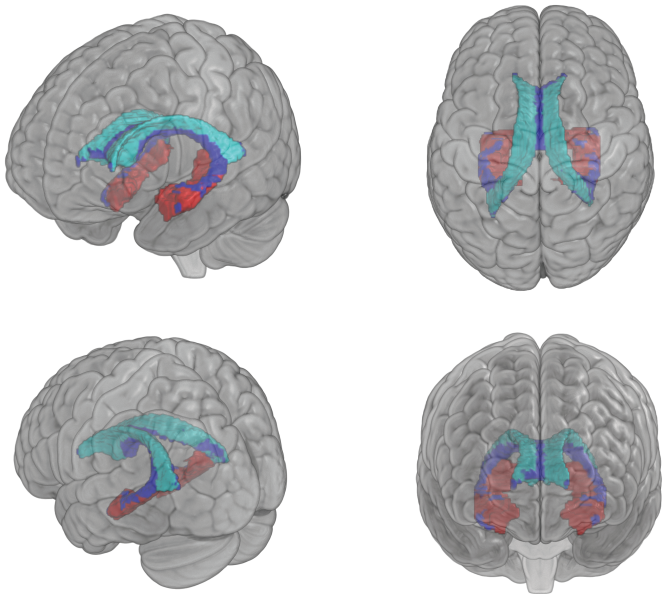

Fig. 1 FreeSurfer standard templates from Desikan-Killiany Atlas for lateral ventricle (sky blue), choroid plexus (blue), and hippocampus (red) overlaid on the 152 MNI brain template.

segmentation by overlaying the segmentation label of each structure on the T1-weighted brain scan.

Volume of ChPs, hippocampi, and ventricles were all per mille (‰) normalized for ICV before being entered into the analyses with the formula: region of interest [ROI] × 1000/ICV, as previously suggested, and proved effective for detecting group differences in mood disorders.[34,53]

Statistical analyses

Statistica StatSoft 12 (TIBCO Software Inc) was used for all statistical analyses. We tested the normality of the distributions of outcome variables with the Shapiro–Wilk W test, and the homogeneity of variances for group effects with the Levene test.

The relationships between ROI volume and imipramine, lorazepam, and chlorpromazine equivalents were tested with rank-based Spearman correlations.

To account for the expected nonnormal distribution of brain volumes, the multiple covarying variables, and the *a priori* expected significant interaction with several independent factors (age, sex), we tested the effect of predictors on outcomes by combining, in subsequent steps, Kruskal-Wallis ANOVA by ranks, machine learning (ML) multivariate regression techniques to perform feature reduction and prediction of effects handling multicollinearity among predictors, and finally confirmed the independent factors effects on TRD with a nonparametric generalized linear model (GLZM). This robust approach has been shown to successfully reveal the complex relationship between immunological variables and clinical phenotypes and outcomes in the field of mood disorders.[7,54–57]

Significant differences across groups in ROI volumes identified through Kruskal-Wallis ANOVA were subsequently confirmed in the context of GLZM, entering ROI volume as the dependent variable and group as a three-level factor, accounting for the effect of age, sex, and scan.

To perform a feature reduction by selecting the factors of interest in predicting TRD, we used partial least-squares (PLS) regression, an ML technique that models the relationships between sets of observed variables with latent variables, to define a linear regression model by projecting the predicted variables and the observable variables to a new space. Diagnosis and TRD were entered as dependent variables, and clinical (age and sex) and biological (volumes of the brain structures of interest, significantly differing in TRD groups) variables were entered in the model as predictors, together with MRI scan. Accuracy and significance of the predictive value of the model was assessed by using the nonlinear iterative PLS algorithm[58] and optimizing by cross-validation the number of PLS components to extract (A), then calculating R^2X (an A-dimensional vector, to record the explained variance of the data matrix of predictors by each PLS component), R^2Y (an A-dimensional vector, to record the explained variance of response variables by each PLS component), Q^2 (predicted variation, to measure R^2Y applied to a test set with cross-validation procedure, in order to assess the predictive relevance of the endogenous constructs); and for each variable predictive weights (w), and the variable importance in projection (VIP) values, to estimate the contribute of each variable to the explanation of Y variance and the direction of effect. K-fold cross-validation was performed by randomly splitting the data into k folds of roughly equal size, in order to estimate the error rate of the predictive algorithm by resampling the analysis data on k-1 folds to then assess the performance on the final test fold. Significance of the model and of variable contributions were defined by $Q^2 > 0$ and VIP >1.[59–62] This approach was proven effective in selecting predictors in a randomized controlled trial with multiple biological and clinical predictors and outcomes.[57] Given the preliminary nature of the study, however, a cutoff value at VIP >0.8 for mining variables potentially contributing to prediction was considered.[63] Again, this approach proved valid for testing treatment effects in randomized controlled trials.[57,64,65]

To confirm the effects of the variables selected in the PLS, they were entered as factors into a GLZM regression with a logit link function, on TRD (yes/no) in patients with MDD, considered as a binomial measure.[66] Parameter estimates were obtained with iterative reweighted least squares maximum likelihood procedures. The significance of the effects was calculated with the likelihood ratio (LR) statistic, thus providing a test of the increment in the log-likelihood attributable to each current estimated effect.[67,68]

Results

Clinical and demographic features are summarized in Table 1. The distribution of brain volumes was not normal for both ChP volumes (left: Shapiro–Wilk $W = 0.985$, $P = 0.006$; right: $W = 0.971$, $P < 0.001$), HVs (left: $W = 0.977$, $P < 0.001$; right: $W = 0.973$, $P < 0.001$), and ventricles volumes (left: $W = 0.908$, $P < 0.001$; right = 0.888, $P < 0.001$). Brain volumes of the three brain structures were partially intercorrelated: ChP directly correlated with ventricular volume (left: Spearman rank order $\rho = 0.508$, $P < 0.001$; right: $\rho = 0.390$, $P < 0.001$) and the hippocampus negatively correlated with ventricular volume (left: $\rho = -0.355$, $P < 0.001$; right: $\rho = -0.370$, $P < 0.001$), but ChP and hippocampus were unrelated.

Brain volumes of three brain structures significantly differed according to diagnosis and to TRD (Fig. 2).

Left ChP ‰ volumes significantly differed among the three groups (Kruskal-Wallis test: $H = 10.829$ $p = 0.0045$). Post hoc analyses showed significantly higher volumes in patients with TRD compared with both HCs ($z' = 2.880$, $P = 0.0119$) and patients without TRD ($z' = 2.617$, $P = 0.0266$), with no significant differences between the last two groups. The effect remained significant when correcting for age, sex, and scan (GLZM LR $\chi^2 = 6.839$, $P = 0.033$). No significant effect was observed for right ChP volume.

Similar results were identified for bilateral hippocampal ‰ volumes (left: $H = 9.467$, $P = 0.0088$; right: $H = 7.660$, $P = 0.0217$). Post hoc analyses showed lower HVs in patients with TRD compared with HCs (left: $z' = 2.880$, $P = 0.0119$; right: $z' = 2.693$, $P = 0.0212$), but not with patients without TRD (left: $z' = 2.075$, $P = 0.1139$; right: $z' = 1.678$, $P = 0.2803$), with no significant differences between the last two groups. The effect remained significant for the left hippocampus when correcting for age, sex, and scan (GLZM LR $\chi^2 = 7.381$, $P = 0.025$), with only marginal effects for the right hippocampus (GLZM LR $\chi^2 = 5.961$, $P = 0.051$).

Table 1. Clinical and demographic characteristics of the studied sample

	HCs	Patients with TSD	Patients with TRD	$t/\chi^2/F$	*P* value
Age (years)	50.166 ± 10.464	47.656 ± 10.878	53.317 ± 9.348	7.644	<0.001
Female/male sex	36/18	68/25	62/42	4.006	0.135
Illness duration (years)	-	12.612 ± 11.272	18.366 ± 12.189	−3.290	0.001
Number of episodes	-	2.480 ± 2.473	4.861 ± 7.187	−2.719	0.007
Baseline HDRS	-	22.341 ± 6.531	21.758 ± 6.480	0.601	0.549
Imipramine equivelent (mg)	-	164.475 ± 99.777	187.340 ± 102.703	−1.577	0.116
Chlorpromazine equivelent (mg)	-	17.212 ± 38.337	25.136 ± 72.972	−0.936	0.350
Lorazepam equivelent (mg)		1.630 ± 1.428	1.652 ± 1.997	−0.087	0.931
Left ChP (mm^3)	418.524 ± 135.294	426.179 ± 155.320	497.248 ± 145.576	7.720	<0.0011
Right ChP (mm^3)	518.742 ± 157.410	509.869 ± 190.714	566.494 ± 164.603	2.932	0.055
Left hippocampus (mm^3)	3842.278 ± 380.722	3728.879 ± 395.861	3686.787 ± 425.504	2.624	0.074
Right hippocampus (mm^3)	3957.850 ± 373.793	3822.696 ± 382.842	3813.329 ± 484.495	2.296	0.103
Left lateral ventricle (mm^3)	8317.996 ± 4510.045	9103.684 ± 4510.634	11,545.525 ± 5114.269	10.440	<0.001
Right lateral ventricle (mm^3)	7474.072 ± 4315.689	8003.978 ± 3580.773	10,427.044 ± 4841.978	11.556	<0.001
ICV (mm^3)	1,440,818.47 ± 166,450.02	1,421,346.70 ± 182,754.18	1,465,857.42 ± 188,047.87	1.484	0.229

Continuous variables are expressed as means ± SDs.
ChP, choroid plexus; HC, healthy control; HDRS, Hamilton Depressive Rating Scale; ICV, intracranial volume; TRD, treatment-resistant depression; TSD, treatment-sensitive depression.

Bilateral lateral ventricle ‰ volumes were also significantly different among groups (left: $H = 27.362$, $P < 0.0001$; right: $H = 25.316$, $P < 0.0001$). Post hoc analysis revealed higher volumes in TRD compared with both HCs (left: $z' = 4.809$, $P < 0.0001$; right: $H = 4.700$, $P < 0.0001$) and patients without TRD (left: $z' = 3.821$, $P = 0.0004$; right: $H = 3.537$, $P = 0.0012$), with no significant differences between the last two groups. The effects remained significant when correcting for age, sex, and scan (left: GLZM LR $\chi^2 = 16.196$, $P < 0.001$; right: GLZM LR $\chi^2 = 16.569$, $P < 0.001$).

Using a multivariate PLS predictive ML modeling, including bilateral ChP, bilateral hippocampus, bilateral ventricles ‰ volumes, age, sex, and MRI as factors, TRD was significantly predicted by a

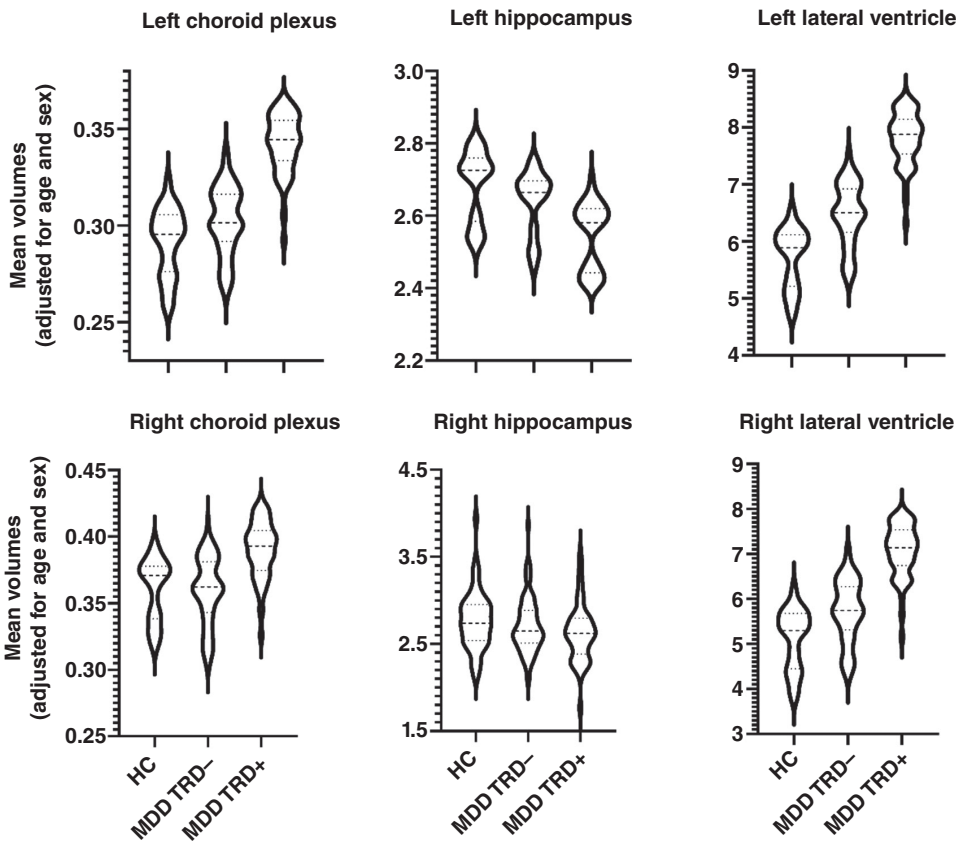

Fig. 2 Mean volumes of choroid plexus, hippocampus, and lateral ventricles in healthy controls (HCs) and in patients with major depressive disorder (MDD) divided according to positive or negative history of treatment-resistant depression (TRD). Values have been ‰ scaled for total intracranial volume and adjusted for age and sex. Violin plots show median (solid line) and quartiles (dotted lines).

model with one significant component explaining 4.8% of variance (coefficient for the whole model = 0.272, Y loading for predicting TRD = 0.663; $R^2X = 0.307$; $R^2Y = 0.048$; $Q^2 = 0.036$). The variables significantly contributing to predict outcomes were higher left ChP volume ($w = 0.364$, VIP = 1.207), lower bilateral HVs (left: $w = 0.336$, VIP = 1.115; right: $w = 0.336$, VIP = 1.016), and higher bilateral ventricular volume (left: $w = 0.487$, VIP = 1.615; right: $w = 0.495$, VIP = 1.643). Older age showed a marginal effect ($w = 0.306$, VIP = 0.905), and right ChP, sex, and scanner type were nonsignificant (Fig. 3).

Ongoing drug treatments did not significantly differ according to TRD. Equivalent doses of imipramine and lorazepam did not significantly correlate with any ROI volume. Equivalent doses of chlorpromazine positively correlated with left ChP (Spearman $R = 0.142$, $P = 0.038$) and bilateral ventricles volumes (left: $R = 0.203$, $P = 0.003$; right: $R = 0.178$, $P = 0.009$), and negatively correlated with bilateral hippocampus volumes (left: $R = –0.165$, $P = 0.016$; right: $R = –0.183$, $P = 0.007$). When repeating the above ML PLS analysis and introducing chlorpromazine equivalents as an additional factor (defined as zero in HCs), the effects of all of the ROIs remained significant, with the addition of chlorpromazine equivalents ($w = 0.307$, VIP = 1.064), leading to a marginal increase (from 4.8% to 5.4%) in explained variance.

With explanatory GLZM logistic modeling, significant effects of the ML selected ROIs on TRD (yes/no) in patients with MDD were also confirmed for both left ChP volume (LR $\chi^2 = 7.233$, $P = 0.0072$), left HV (LR $\chi^2 = 4.968$, $P = 0.0258$), and bilateral ventricular volumes (left: LR $\chi^2 = 9.629$, $P = 0.0019$; right: LR $\chi^2 = 11.343$, $P = 0.0008$). Right HV showed a marginal effect (LR $\chi^2 = 2.814$, $P = 0.0934$). Age always showed a significant effect, but chlorpromazine equivalents never did, and this variable could always be discarded in the models because of nonsignificantly incrementing the log-likelihood attributable to the ROIs estimated effects.

When controlling for the effect of illness duration, associations were still significant (LChP: LR $\chi^2 = 7.118$, $P = 0.008$; LVent: LR $\chi^2 = 6.106$, $P = 0.013$; RVent: LR $\chi^2 = 6.642$, $P = 0.010$; LHipp: $\chi^2 = 4.476$, $P = 0.034$).

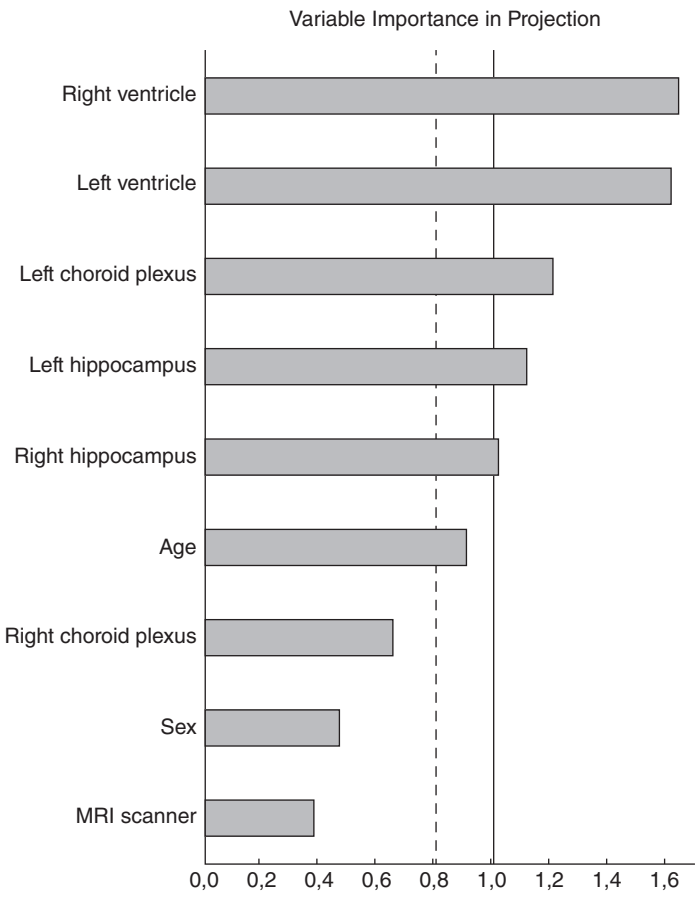

Fig. 3 Variables predicting diagnosis and treatment-resistant depression, listed according to their relative predictive power at linear regression. MRI, magnetic resonance imaging.

Discussion

This study provides the first evidence, to our knowledge, of higher volumes of ChP in patients with a history of TRD compared with both patients without TRD and HCs. The possible specificity of this novel TRD-related feature is highlighted by the lack of significant differences in ChP volume between patients without TRD and HCs. These findings could provide further evidence of the link between TRD and neuroinflammation,[69,70] as ChP volume is considered a proxy of central immune system activity.[31,34]

TRD posits a significant clinical challenge because of its resistance to conventional therapies and worse clinical features, including more severe and persistent depressive symptoms, increased risk of suicidal behavior, higher rates of psychiatric comorbidity, and greater functional impairment.[46,71,72] Moreover, TRD is associated with poorer long-term treatment outcomes, increased healthcare costs, and elevated societal burden.[73]

A widely accepted model of immune-mediated depression hypothesizes an effect of peripheral immune/inflammatory alterations on brain physiology and depressive symptoms through their action on specific gating structures such as the blood–brain barrier and the ChP.[74,75] Indeed, previous studies identified larger ChP volume in MDD, finding associations with both central glia activation[31] and peripheral cytokines levels.[34] Similar findings were observed in different inflammatory pathologies such as multiple sclerosis,[76] systemic lupus erythematosus,[77] and Alzheimer disease,[24] as well as in other psychiatric conditions such as schizophrenia and bipolar disorder.[30,34,42]

A strong relationship between peripheral and central inflammatory states have already been established,[78] and indeed ChP enlargement has been associated with higher permeability, immune cell infiltration,[79,80] and epithelial cell expression of adhesion molecules, chemokines, and molecules involved in leukocyte recruitment.[81,82] We previously observed higher ChP volumes in patients with mood disorders, proportional to levels of circulating proinflammatory cytokines.[34] Taking into account the stronger inflammatory signature of patients with TRD compared with patients without TRD,[33,83] ChP volume enlargement in TRD could indeed be considered novel evidence of the involvement of immune/inflammatory processes in affecting AD response patterns. As previous studies in patients with MDD have shown increased ChP compared with HCs, we can hypothesize that these results may have been influenced by the proportion of patients with TRD MDD, which is known to range between one-third and one-half of the MDD population.[1] The results reported in this paper might better characterize patients with MDD and disentangle possible confounding factors affecting ChP volume in depression.

Inflammatory status in depression is emerging as a putative treatment target,[84] and significant improvements of both depressive symptoms and inflammatory markers have been observed in patients with TRD who have higher baseline levels of CRP and TNF-α treated with the immunomodulatory infliximab,[85] minocycline,[86] and low-dose IL-2.[57] Concerning conventional AD treatments, a recent study found ChP volume to be associated with response patterns to sertraline,[41] with responders having smaller ChP volumes than nonresponders. Our result appears to support and expand this finding, also linking larger ChP volumes to TRD status.

Hippocampal volumes were also associated with treatment resistance in our sample; specifically, for left hippocampus, lower volumes

were identified in patients with TRD compared with both patients without TRD and HCs. Lower HVs are a robust MRI correlate of major depression[14] and among the few robust predictors of poor AD response, as observed with prospective observation of treatment outcomes.[19,87] However, how HVs in MDD compared with those of HCs according to treatment resistance status has been rarely investigated. In this regard, our data, in addition to confirming the presence of lower HVs in patients with TRD compared with treatment-sensitive patients, seem to suggest that HVs differentiate HCs only from patients with treatment-resistant MDD.

The hippocampus is a primary site of adult neurogenesis.[19,88] While the exact nature of its volumetric reduction in MDD is still debated, preclinical studies seem to exclude widespread cell apoptosis and neuronal death, suggesting structural changes to be related to reduced neurogenesis, dendritic and axonal changes, and glial alterations.[89,90] At the same time, the hippocampus appears to be crucial in AD response: various types of AD treatments have been shown to enhance hippocampal neurogenesis,[91] and neurogenesis inhibition through x-ray irradiation hampers AD responses in mouse models.[92]

AD activity has been demonstrated for numerous drugs with several mechanisms of action, including the enhancement of serotoninergic,[93] noradrenergic,[94] and dopaminergic[95] neurotransmission, GABA[96] and glutamate[97] modulation, and effects on second messenger systems.[98] In recent years, a common explanatory framework of AD action has emerged, proposing BDNF–TrkB signaling enhancement as a common final mechanism of action of AD drugs.[99] BDNF is the most abundant neurotrophic factor in the adult brain, crucial in regulating synaptic plasticity and hippocampal neurogenesis,[100] and is implicated in responses to both "classical" monoaminergic and novel ADs,[101] with some authors even suggesting a direct effect of several AD drugs on its high-affinity receptor TrkB.[102]

The association between hippocampus structure and physiology and AD response might also be strongly affected by inflammatory status. Indeed, several studies report an inverse association between inflammatory markers and HVs,[103,104] possibly through their effect on BDNF signaling.[105] In a previous study by our group, we identified a significant effect of peripheral inflammatory status on AD response through its effect on HVs.[18] However, in the present study, we found no association between ChP and hippocampal volumes, possibly suggesting that immune/inflammatory alterations reflected by ChP enlargement do not directly affect the hippocampal trophic state. This might also stress the likely complex interaction between various immunological alterations, partially reflected by peripheral and central immune markers and brain physiology in psychiatric conditions, for which a complete understanding is still lacking.

In agreement with our hippocampus findings, AD resistance status was associated with larger lateral ventricular volumes, with progressively increasing volumes from HCs to patients with and without TRDs. As for HVs, no significant differences were observed between HCs and treatment-sensitive patients.

Larger lateral ventricular volumes have been associated with earlier age of onset[13] and nonresponse to sertraline[41] in MDD; furthermore, volumetric reductions have been observed after selective serotonin reuptake inhibitors treatment, possibly corroborating a neurotrophic model of AD action.[106] Our study suggests lateral ventricle volumes to be strongly associated with TRD.

Ventricle volumes physiologically increase with age and are found to be abnormally elevated in several brain pathological states[107–109]; as such, they may be regarded as an overall indicator of the brain trophic state. At the same time, the ChP is the main brain structure responsible for CSF production,[110] and, in the inflammatory condition, ChP could increase the secretion of CSF as a homeostatic response. Indeed, the ChP has been shown to increase CSF production after LPS-induced inflammation,[111] possibly promoting transmission of immune signals into the brain,[112] and fostering the clearance of harmful compounds such as amyloid β peptide.[113] The correlation between ventricles and both hippocampi volumes and ChPs, but not between the last two, could point to the presence of two ongoing distinct processes in TRD (one related to the trophic state and HVs and the other to neuroinflammation and ChP volumes) both independently reflected in ventricular volumes.

It is interesting to note that for ChP, hippocampi, and ventricle volumes, no significant distinction emerged between HCs and patients with TSD, with significant differences only with participants with TRD. Our results support the search for specific neurobiological features associated with TRD.[17]

Furthermore, a significant effect on treatment-resistant status was only found for the left hippocampus and ChP volumes and not those on the right. Concerning the hippocampus, several studies have shown that left HVs are smaller than right HVs.[114] This asymmetry may be attributable to differences in glutamate-induced synaptic plasticity, hypothalamic-pituitary-adrenal axis activation, and adult neurogenesis,[115] all processes thought to be involved in TRD. Similarly, also for ChP volumes, results in the literature reported larger volumes in the left hemisphere, possibly due to lateralized differences in cell adhesion and inflammatory marker expression.[116]

We observed a correlational association between levels of antipsychotics (chlorpromazine equivalents) at the time of study and ROI volumes, with the same direction of effect associated with TRD (larger ventricles and ChP, smaller hippocampus). Our results are supported by a previous study on patients with first-episode schizophrenia who were within a 2-week antipsychotics initial stabilization period where an association was found between allostatic load and higher ChP volume.[117] The interpretation of this finding is hampered by the cross-sectional nature of our study. In agreement with our observation, antipsychotic medications have been associated with smaller gray and white matter volumes,[118] and the largest meta-analysis of cross-sectional MRI studies in schizophrenia to date showed that reductions in whole-brain gray matter volume are associated with the dose of antipsychotics taken at the time of scanning.[119] Medication effects on brain volumes can overlap with the influences of clinical and demographic variables, which closely interact with medication status. Therefore, longitudinal studies of specific medications are needed to distinguish the direct effects of the medication on the brain from other moderating or disease-related effects.[120]

The strengths of the present study include a focused research question, formulated based on new findings and emerging literature on the topic. However, our results must be viewed in light of some limitations. First, the study was retrospective and cross-sectional in nature, and, therefore, inferences cannot be made about causality in the relationship of TRD status and the observed brain differences, as longitudinal data would be needed to define it. Recruitment in a single center and its cross-sectional nature limits the generalizability of our findings. Given its real-world setting, patients were prescribed a wide variety of psychotropic drugs. We took into account prescribed drug equivalents, but we cannot exclude a possible effect of single specific drugs. Furthermore, as patients were scanned during their hospital stay, the majority was undergoing drug treatment switch, augmentation or dosage increase, which we could not account for in our analyses. The entirety of our sample consisted of inpatients admitted to our clinical ward. This might imply that the clinical severity of our TSD sample is greater than what is usually observed in outpatient settings, hampering the general applicability of our findings. Although the presence of a major medical or neurological condition was an explicit exclusion criteria, the relatively older age of our sample meant that we could not completely rule out the presence of brain pathological states in their prodromal phases, which could affect both neuroimaging data and resistance patterns. Numerosity between groups varied, with significantly fewer HCs than patients with MDD. MRI acquisitions were performed on two separate scans. We applied no harmonization technique, but we did enter a "scan" covariate in all of our analyses.

Conclusion

The current study provides novel MRI correlates of AD resistance, contributing to a better neurobiological characterization of TRD. The identified biomarkers hold promise, though their potential usefulness in clinical practice will require further investigation.

Acknowledgments
Fondazione CARIPLO grant 2019-3682 (FB); PNRR-MAD-2022-12375859 (FB).

Disclosure statement
The authors declare no conflicts of interest.

Author contributions
B.B.: acquisition and analysis of data and drafting of the manuscript or figures. M.P.: acquisition and analysis of data and drafting of the manuscript or figures. M.M., C.M., L.R., and E.M.T.M.: data acquisition. R.Z.: patient recruitment. C.C.: patient recruitment. F.B.: conception and design of the study, drafting of the manuscript or figures, and funding acquisition.

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