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INFLAMMATORY CONTROL OF ANTIDEPRESSANT EFFICACY: AN IMAGING-GENETIC APPROACH

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Abstract

Adverse childhood experiences and recent life stressors are crucial environmental risk factors for the development of Major depression. Furthermore they have been robustly associated with an altered immune/inflammatory state and with changes in brain structure and function; their effect on antidepressant treatment response however is much less clear. Immune/inflammatory alterations have long been recognized as contributors to major depression pathophysiology, and a comprehensive framework is beginning to emerge linking childhood and recent stressors, inflammation, depressive symptomatology and antidepressant response. At the same time major depression is widely regarded to be a complex and heterogeneous disorder, with no single pathophysiological explanation that can be adapted to all cases. A possible source of such heterogeneity is its likely overlap, both phenotypical and pathophisiological, with other affective disorders, such as bipolar disorder. Investigating individual genetic data could provide a tool to disentangle such heterogeneity, providing informations directly linked to the underlying biology of the disease, rather than to its phenotypic presentation.

Therefore in our study, performed on a sample of major depression inpatients, we investigated the effect of childhood and recent stressors on response patterns to antidepressants, immune/inflammatory state and brain structure and function. Further, we tested the association between inflammatory and MRI markers and antidepressant response, also investigating longitudinal changes in brain structure and function after hospital discharge. Finally we tested if and how genetic liabilities affected these associations.

We repeatedly observed significant interactions between childhood and recent stressors in affecting response patterns, inflammation status and brain MRI correlates, giving credence to a diathesis-stress model explaining the effect of childhood and recent traumatic experiences on psychopathology; we observed baseline MRI correlates and longitudinal changes associated with antidepressant resistance in regions strongly associated with neuroplasticity and neurogenesis processes, corroborating the

“neurotrophic theory” of depression and of antidepressant action; finally we found bipolar disorder genetic liability to significantly affect the relation between childhood trauma, inflammation, MRI findings and treatment response, possibly providing a tool to disentangle part of major depression complexity and heterogeneity.

Table of contents

4. Mediation analysis: childhood trauma, inflammation and neuroimaging

5. Early and recent trauma x BD PRS interaction on treatment response, neuroimaging and inflammatory status

6. Relation between neuroimaging and treatment response and resistance

7. Moderated mediation analysis: childhood and recent adversities, neuroimaging and treatment response.

8. Moderated mediation analysis: childhood trauma, BD PRS, neuroimaging and treatment response

9. Effect of inflammatory status on treatment response

10. Effect of CRP on neuroimaging and mediation analysis

11. Longitudinal analysis

Discussion

1. Effect of early and recent adversities on treatment response.

1.1 Childhood adversities

1.2 Recent stressors

2. Effect of early and recent adversities on neuroimaging.

2.1 Grey matter volumes and cortical thickness

2.2 White matter microstructure

2.3 Resting state functional connectivity

3. Effect of early and recent adversities on inflammatory markers.

3.1 Inflammatory ratios

3.2 Serum cytokines and mediation analysis

4. Polygenic risk score moderation

5. Relation between neuroimaging and treatment response and resistance

5.1 Gray matter volumes and cortical thickness

5.2 White matter microstructure

5.3 Resting state functional connectivity

6. Moderated mediation analyses

6.1 Childhood and recent adversities, neuroimaging and treatment response.

6.2 Childhood trauma, BD PRS, neuroimaging and treatment response

7. Effect of inflammatory status on treatment response and mediation analysis

8. Longitudinal analyses

9. Limitations

10. Conclusions

Materials and Methods

1. Participants

2. Clinical and psychometric assessment

3. Treatment response determination

4. Inflammatory markers

5. MRI acquisition and preprocessing

6. Genetic sequencing and polygenic risk scores calculation

7. Statistical analysis

References

Acronyms and abbreviations

ACC: anterior cingulated cortex

ACEs: adverse childhood experiences

AD: Axial diffusivity

ALFF: Amplitude of low frequency fluctuations

BD: Bipolar disorder

BDI: Beck Depression Inventory

BDNF: Brain-derived neurotrophic factor

BMI: body mass index

CNS: Central nervous system

CRP: C-reactive protein

CT: Childhood trauma

CTQ: Childhood Trauma Questionnaire

DA: dopamine

DMN: Default mode network

DSM: Diagnostic and Statistical Manual of Mental Disorders

DTI: diffusion tensor imaging

EA: Emotional abuse

ECT: Electroconvulsive therapy

EN: Emotional neglect

FA: Fractional anisotropy

FC: functional connectivity

fALFF: fractional amplitude of low-frequency fluctuations

FU: follow up

GM: grey matter

HC: healthy control

HDRS: Hamilton Depression Rating Scale

HPA: hypothalamic pituitary adrenal

MAO: Monoamine oxidases

MD: mean diffusivity

MDD: Major depressive disorder

MLR: monocyte to lymphocyte ratio

MRI: Magnetic resonance imaging

NA: noradrenaline

NLR: Neutrophil to lymphocyte ratio

OR: Odds ratio

PA: Physical abuse

PERC: percentage of HDRS decrease

PFC: pre-frontal cortex

PLR: platelet to lymphocyte ratio

PN: Physical neglect

PRS: Polygenic risk score

PSS: Perceived Stress Scale

RCT: randomized controlled trial

RD: Radial diffusivity

RFQ: Risky Families Questionnaire

rs-FC: resting state functional connectivity

SA: Sexual abuse

SII: systemic inflammatory index

SLE: stressful life events

SRE: Schedule of Recent Experience

TBSS: Tract Based Spatial Statistics

TMS: Transcranial magnetic stimulation

TRD: Treatment resistant depression

VBM: Voxel based morphometry

WM: White matter

List of figures and tables

Fig. 11: mediation analysis between childhood physical neglect, GRO-α and Mean diffusivity.

Fig. 12: Effects of childhood trauma x BD PRS interaction on response patterns. Left: remission at discharge. Right: percentage of decrease in HDRS from admission to discharge.

Fig. 13: Effects of childhood trauma x BD PRS interaction on fractional anisotropy.

Fig. 14: brain regions of reduced grey matter volume in treatment resistant patients compared to non-treatment resistant ones.

Fig. 15: brain regions of reduced cortical thickness in treatment resistant patients compared to non-treatment resistant ones.

Fig 16: Top, brain regions of reduced cortical thickness in patients who did not achieve symptom remission at discharge compared to those who did. Bottom, brain regions in which cortical thickness was positively associated with the percentage of HDRS decrease from admission to discharge.

Fig. 17: relation between Hippocampal volumes and response patterns.

Fig. 18: relation between hippocampal volumes and Thase resistance criteria.

Fig. 19: white matter tracts with reduced fractional anisotropy in patients who failed to achieve symptom remission at discharge compared to those who did.

Fig. 20: brain regions of reduced functional connectivity with left Hippocampus (left) and anterior cingulate cortex in treatment resistant patients.

Fig. 21: brain regions of reduced functional connectivity with left Amygdala in patients who achieved symptomatic remission at discharge.

Fig. 22: moderated mediation analysis between SRE-S, childhood emotional abuse, Fractional anisotropy and remission at discharge.

Fig. 23: Moderated mediation analysis between childhood physical neglect, bipolar disorder polygenic risk score, fractional anisotropy and remission at discharge.

Introduction

1. Major depression

Major depressive disorder (MDD) is a common and highly debilitating condition that affects millions of individuals worldwide: its 12 moth prevalence is roughly 6%, with a lifetime risk of about 15% and a 2:1 female to male ratio (Malhi & Mann, 2018, Marx, Penninx et al., 2023). It ranks first as a cause of disability among psychiatric conditions worldwide, and is associated to substantial mortality, mainly due to comorbid conditions such as obesity and substance abuse or to suicidalty (Lam, McIntosh et al., 2016).

MDD core symptoms include depressed mood and feelings of worthlessness, reduced interest or pleasure in most activities (Anhedonia), suicidal ideation or thoughts of death, but also loss of energy, psychomotor agitation or retardation, insomnia or hypersomnia, marked decrease or increase in appetite, and reduced ability to think or concentrate (Malhi & Mann, 2018, Marx et al., 2023). According to DSM-5 criteria, to diagnose MDD such symptoms must be present for at least 2 consecutive weeks, cause a clinically meaningful distress or impairment, and not be otherwise explained by an underlying medical condition, substance abuse, or another psychiatric condition such as Schizophrenia or bipolar disorder (Vahia, 2013).

1.2 Etiology and pathophysiology

MDD etiology is complex, multifactorial and still somewhat poorly understood (Malhi & Mann, 2018, Marx et al., 2023). Offspring of individuals with MDD have twice the risk of developing the disease compared to the general population (Kendall, Van Assche et al., 2021); despite this, the contribution of genetic liability to the development of MDD appears to be lower than most other psychiatric conditions, with hereditability estimates of around 40% (Pettersson, Lichtenstein et al., 2019).

The relatively low genetic contribution to the development of the disease might underlie the importance of environmental risk factors in MDD pathophysiology; indeed numerous environmental risk and protective factors have been shown to influence MDD

onset and course. Low socioeconomic status, lack of social support, but also an unhealthy lifestyle (physical inactivity, smoking, unhealthy diet), obesity and other somatic illnesses all increase the risk of developing MDD (Marx et al., 2023, Østergaard, Waltoft et al., 2013).

Among environmental determinants of the disease, early life and recent adverse experiences appear to play a pivotal role. Childhood trauma has been robustly associated to the development of depression both in adolescence and in adult life (LeMoult, Humphreys et al., 2020, Xiang & Wang, 2021); several types of trauma have been implicated, ranging from physical and sexual abuse, physical neglect, but also emotional abuse and neglect and an adverse family environment (Infurna, Reichl et al., 2016). Exposure to various types of trauma particularly during middle childhood (6-13 years) appears to confer the highest risk (Li, Gao et al., 2022). Crucial in the development of depression are also severe stressful life events (Kessler, McLaughlin et al.), such as divorce, death of a close family member, and job loss (Monroe, Slavich et al., 2007): some authors suggest that more than 50% of first depression episodes are preceded by SLE, with lower rates in subsequent recurrent episodes (Monroe & Harkness, 2005).

Several processes have been implicated to explain the development of MDD; at the same time no single model or mechanism appears to be able to fully explain all aspects of the disease, and a comprehensive pathophysiological model of MDD is still elusive (Malhi & Mann, 2018, Marx et al., 2023).

The serendipitously discovered first antidepressant agents – Iproniazid and Imipramine – were soon shown to be able to increase intersynaptic monoamines concentration, through MAO or reuptake inhibition. This originated the classical monoamine hypothesis of depression – if antidepressants act enhancing monoaminergic neurotransmission, depression might stem from monoamines deficiency (Hirschfeld, 2000); although this theory might today appear simplistic and even naïve, it is worth noting that still today most antidepressants act modulating monoaminergic neurotransmission. However several observations highlight the limitations of this hypothesis: studies attempting to associate serotonin plasma levels, tryptophan depletion, or genetic variation of the SERT gene to depressive symptomatology appear

to provide weak and inconsistent results (Moncrieff, Cooper et al., 2023); in the last decades, new drugs have appeared – such as ketamine, lamotrigine, brexanolone – that exert antidepressant activities without directly affecting monoaminergic neurotransmission (Gonda, Dome et al., 2023); furthermore, while monoaminergic antidepressant produce a rapid increase of monoamines availability in the synaptic cleft, their clinical efficacy is seen only after weeks of treatment (Commons & Linnros, 2019).

Several lines of evidence link alteration of the immune-inflammatory status to depression. Depressed individuals have been robustly shown to have elevated inflammatory indicators compared to healthy controls both in serum and in CSF, although this elevation might affect only a subset of MDD patients (Beurel, Toups et al., 2020, Harsanyi, Kupcova et al., 2022, Kiecolt-Glaser, Derry et al., 2015). Administration of pro-inflammatory compounds (such as interferon or LPS) induces depressive phenotypes in rodents and human alike, and several anti-inflammatory drugs have been shown to have antidepressant proprieties (Kohler, Krogh et al., 2016). Furthermore patients with chronic inflammatory and autoimmune diseases, but also milder form of immune alterations such as allergies, present increased rates of depressive symptomatology (Pryce & Fontana, 2017).

The impact of inflammatory status on depression can also be seen in an evolutionary framework. For most of human evolution infections were the predominant cause of death; this provided a strong selective pressure on the human immune system, resulting in an inflammatory bias that for millennia represented an evolutionary advantage. In close relation to immune ones, a set of behavioral responses evolved to face infections, promote energy conservation and wound healing: such responses, collectively called "sickness behavior", included social avoidance and hypervigilance, and are speculated to be at least partially reflected in the depressive syndrome (Miller & Raison, 2016).

Several mechanistic explanations to link inflammation and depression have been put forward: pro-inflammatory cytokines are able to induce the expression of monoamines reuptake transporters, thus reducing their availability in the synaptic cleft; NA and DA synthesis is affected as well, through a reduction of needed enzymatic co-factors (such as tetrahydrobiopterin) that are sensitive to inflammation-induced oxidative stress;

finally pro-inflammatory cytokines activate the enzyme indoleamine 2,3-dioxygenase (Martinuzzi, Barbosa et al.), which breaks down tryptophan, serotonin precursor, into kynurenine, thus reducing its availability for the neurotransmitter synthesis. Furthermore kynurenine can be converted by activated microglia into quinolinic acid, an NMDA agonist: this, coupled with the inflammation induced reduced astrocyte glutamate uptake and increased release, can lead to enhanced glutamatergic neurotransmission and possibly excitotoxicity (Miller, Maletic et al., 2009, Miller & Raison, 2016). The combined effect of excessive glutamate neurotransmission and inflammation-induced oxidative stress can then lead to reduced neurogenesis and neuroplasticity, partially through their effect on BDNF and other brain growth factors (Miller & Raison, 2016, Zhang, Yao et al., 2016).

Indeed a more comprehensive – albeit still incomplete – explanatory framework of depression emerged in the last decades, stresses the importance of neurotrophic factors in originating depressive symptomatology and in determining antidepressant efficacy (Caviedes, Lafourcade et al., 2017, Duman & Li, 2012). BDNF is a crucial and abundant neurotrophic factor that promotes neuronal survival and neurogenesis; accordingly it is highly expressed in the hippocampus and PFC, where it also regulates neurotransmitter release and synaptic plasticity (Yu & Chen, 2011). BDNF has been found to be reduced in serum and CSF, and markers of reduced neurogenesis have been identified in post-mortem studies of untreated depressed patients (Rana, Behl et al., 2021). Interestingly various types of antidepressant interventions – "classical" monoaminergic and glutamatergic antidepressants, ECT and even psychotherapy – have been shown to restore BDNF levels, and indeed modulation of BDNF signaling has been proposed as a common route of action of antidepressants (Björkholm & Monteggia, 2016, Castrén & Monteggia, 2021). From the observation that both monoaminergic antidepressants and ketamine are able to directly bind BDNF receptor, TrkB, stemmed the provocative hypothesis that direct action on NTRK2 contributes to the efficacy of these drugs (Casarotto, Girych et al., 2021).

In this framework, antidepressant-induced increase in BDNF-TrkB signaling could reverse the detrimental consequences of chronic stress, thus leading to antidepressant effects (Harmer, Duman et al., 2017). Furthermore a novel hypothesis posits that

antidepressants, increasing brain plasticity, enhance brain reactivity to the surrounding environment (Branchi, 2011), underlying the importance of environmental stimuli in affecting response to treatment.

1.3 Nosological and genetic considerations

Finally, when addressing MDD pathophysiology, some nosological considerations are warranted. The modern definition of MDD mainly stems from the DSM diagnostic categorization, which is often referred as "neo-kraepelinial", indicating a direct inspiration to the work of Emil Kraepelin (Compton & Guze, 1995). However, our current conceptualization of MDD has little resemblance to the original Kraepelinian conceptualization, originating from the Karl Leonhard distinction between bipolar and unipolar recurrent psychoses and then adapted in the original DSM-III conceptualization to include also non-psychotic mood presentations and the psychoanalytic concept of "neurotic depression", excluded from DSM-III (Ghaemi, 2013). Neurotic depression had striking differences from the modern definition of MDD, being typically mild rather than severe, chronic and constant rather than episodic and recurrent, and with a strong presence of anxious symptoms alongside mood ones. Kraepelin on the other hand conceptualized mood disorders mainly with the concept of manic depressive illness, a condition characterized by episodic mood swings of both polarity followed by a complete *restitutio ad integrum* (this allowing its distinction from *Dementia praecox*), whose essential features were recurrency and episodic nature rather than episode polarity (Kraepelin, 1913). Merging these two very distinct concepts into our modern definition of MDD originated a somewhat hybrid diagnostic entity (Ghaemi & Dalley, 2014). Indeed some authors consider our current definition of MDD too wide and of BD too narrow, and propose a classification of mood disorders into MDI (which would include BD and a portion of MDD patients) and neurotic depression, possibly closer to the real world clinical picture (Geddes & Andreasen, 2020).

Possibly also in light of these nosological considerations, MDD is today widely considered to be a clinically and phenotypically heterogeneous disorder (Lynall & McIntosh, 2023). The dramatic advances made by psychiatric genetic research in the

last decade could prove to be crucial in disentangling MDD heterogeneity (Buch & Liston, 2021): polygenic risk scores (PRS) can now be calculated for most psychiatric conditions, allowing for a (yet incomplete) individual estimation of the genetic liability for a given disorder (Murray, Lin et al., 2021).

Since the relatively recent introduction of PRS calculation, modern genetic research of mood disorders seems to confirm the existence of a partially shared genetic background in patients ranging from MDD to schizoaffective disorders (Coleman, Gaspar et al., 2020): polygenic risk scores of sub-threshold, single episode and recurrent MDD, BD type 2 and type 1 and schizoaffective disorder exhibit high levels of correlation, with stronger associations between disorder more clinically alike (i.e. between BD type 2 and recurrent MDD or BD type 1 and schizoaffective disorder) (Coleman et al., 2020). This is because many of the genetic variants associated with psychopathology are shared among disorders – that is, they exhibit pleiotropy (Giangrande, Weber et al., 2022, Richards, Cardno et al., 2022). At the same time some genetic variants appear to be disorder specific, or shared selectively with only some psychiatric conditions, contributing to the complexity of psychiatric disorders genetic architecture (Richards et al., 2022).

Indeed Wiste and colleagues investigated the effect of BD PRS on the clinical features of the STAR*D major depression sample (Wiste, Robinson et al., 2014); interestingly, they found that higher BD PRS associated with earlier age of onset, history of suicide attempt and presence of sub-clinical manic symptoms, all features classically regarded to reflect bipolar vulnerability (Frankland, Roberts et al., 2018, Patella, Jansen et al., 2019).

1.4 Treatment

Antidepressants (AD) are the mainstay of MDD pharmacological treatment. Despite the abundance of available AD molecules, almost all act by increasing monoamines (particularly 5-HT) in the intersynaptic cleft, either inhibiting their intracellular reuptake blocking monoamines high affinity transporters (SERT, NET and DAT) or inhibiting their degradation via inhibition of MAOs (Harmer et al., 2017). In the last decades

however newer antidepressant drugs with novel mechanisms of actions – such as ketamine or brexanolone - are beginning to emerge (Jarończyk & Walory, 2022, Wang & Dwivedi, 2021). Antidepressant efficacy is somewhat modest in real world settings, especially in determining a full resolution of depressive symptoms: in the STAR*D study, only about a third of patients achieved symptom remission with first line treatments, and two third after 4 lines of treatment (Sinyor, Schaffer et al., 2010).

Despite its relative frequency, no universal accepted definition of TRD exist, and its place in psychiatric nosography is still a matter of debate (Gaynes, Lux et al., 2020); according to a relatively accepted definition, treatment resistance can be defined by the failure to respond to at least two antidepressant treatments with different mechanism of action, administered for sufficient time and at adeguate dose (Sforzini, Worrell et al., 2022).

For AD resistant patients today available options include augmentation strategies with various compounds (lithium, SGAs, T3 among others) and physical therapies such as TMS and ECT, but the potential of newer AD molecules in TRD patients is beginning to show promising results (Ruberto, Jha et al., 2020).

1.5 Neuroimaging studies

MDD has been widely investigated through various neuroimaging techniques.

Concerning brain structure, MDD patients compared to HCs appear to show lower GM brain volumes in several clusters, including bilateral insulae and temporal regions, middle and anterior cingulate and orbitofrontal cortex and bilateral hippocampi and parahippocampal gyri, and possibly increased GM volumes in occipital regions (Wise, Radua et al., 2017). Some of these alterations appear to be also observed in BD, anxiety disorders and chronic pain (Brandl, Weise et al., 2022), and are paralleled by analogous reductions in cortical thickness (Schmaal, Hibar et al., 2017).

Of particular interest, reduced hippocampal volumes appear to be a stable and robust finding of MDD neuroimaging studies (Zhao, Du et al., 2014), already apparent in first episode drug-naïve patients (Cole, Costafreda et al., 2011) and with a tendency to

worsen and to associate with cognitive deficits over subsequent episodes (aan het Rot, Mathew et al., 2009). Hippocampal volumes are speculated to reflect impaired neuroplasticity and neurogenesis, and could be therefore directly reflective of MDD pathophysiology (Boku, Nakagawa et al., 2018).

A less clear picture emerges concerning white matter microstructure, with some DTI studies reporting fractional anisotropy reductions in MDD compared to healthy controls (Jiang, Zhao et al., 2017), while others falling to find such an association (Koshiyama, Fukunaga et al., 2020).

Various alterations have also been identified in rs-fMRI studies when comparing MDD and HCs: interestingly MDD patients exhibit increased ALFF (a possible measure of intrinsic brain activity) in ACC and orbitofrontal cortexes and in bilateral insulae, and reduced in occipital and cerebellar regions, with topological patterns somewhat reminiscent of GM alterations (Gong, Wang et al., 2020). Concerning restng state functional connectivity, alterations in connectivity patterns between PFC, ACC, insula and amygdala are often reported, albeit at times inconsistently (Li, Chen et al., 2022).

2. Early life and recent adversities and treatment response

While childhood trauma is an established risk factor for the development of adult psychopathology and use of psychotropic medications *tout court* (Anda, Brown et al., 2007), its relation with treatment efficacy and treatment resistance in depression is much less clear.

A French outpatients study, performed solely on 256 TRD patients, found a strong association between (self-reported) childhood trauma (CT) and depression severity; when investigating the relation between CT and symptom remission at 1y FU, a weak association was found, which however vas no longer significant when taking into account baseline depression severity (Yrondi, Aouizerate et al., 2020).

A large multicenter study investigated the relation between CT and response and remission rates at 8w FU in a sample of 1008 MDD outpatients randomized to receive Escitalopram, Sertraline of Venlafaxine. While no effect of overall trauma was

identified, the presence of childhood abuse (physical, emotional or sexual) significantly reduced the possibility of achieving response or remission at 8 weeks, specifically when occurring between the ages of 4 and 7 (Williams, Debattista et al., 2016).

Another study performed on 454 patents recruited in a TRD-specialized center investigated the effects of childhood traumatic events on depression severity and treatment outcomes: while the authors identified an association between ACEs, depression severity, suicide attempts and inpatient admissions, no significant association was identified between childhood trauma of any type and treatment resistance (Giampetruzzi, Tan et al., 2023).

Indeed, a recent systematic review on determinants and predictors of treatment resistance in MDD did not identify CT as a risk factor for TRD, while baseline depression severity, repeatedly associated to ACEs, significantly affected the risk of treatment resistance (O'Connor, Hewitt et al., 2023).

Finally a recent meta-analysis of 20 RCTs and nine open label trials (n=6830) investigated the effect of CT on change in depression severity from baseline to the end of the acute treatment phase: while individuals reporting CT had significantly higher depression severity both at baseline and after treatment, no difference was identified in changes of depressive symptomatology; CT+ patients had significant and similar depression improvements than CT-, both when treated with psychotherapy or pharmacotherapy, with no difference related to the trauma type. Furthermore, a significant regional difference emerged, with larger reductions of depressive symptomatology in CT+ patients reported in studies performed in North America (Kuzminskaite, Gathier et al., 2022).

Concerning the effect of recent major life events on response patterns, a recent systematic review pooled data from 6 RCTs of adults treated for depression in primary care settings (n=2858). Individuals reporting major life events in the preceding six months (including disputes, loss, debt, divorce, serious illness, being victim of violence, legal issues, job loss) had significantly worse prognoses at 3-4 months FU, with significantly higher residual depressive symptoms and lower remission rates (Buckman, Saunders et al., 2022).

A large study performed on more than 700 outpatients found no CT x recent adversities interaction in affecting response patterns to non-pharmacological treatments for mild-moderate depression in primary care (Yacaman-Mendez, Hallgren et al., 2019), while their possible interaction in affecting response and resistance to pharmacological treatments in more severe cases od MDD has not been assessed.

3. Early life and recent adversities and neuroimaging

The effects of ACEs on brain structure and function have been extensively investigated, both in healthy subjects and in psychiatric patients. A very large study performed on non-psychiatric samples associated childhood trauma to reduced brain volume and cortical surface particularly in frontal and parietal lobes and in subcortical structures such as the hippocampus, thalamus and nucleus accumbens (Madden, Atkinson et al., 2023); similar results were also reported by a meta-analysis of voxel-wise structural neuroimaging studies (Paquola, Bennett et al., 2016). Fronto-parietal structural abnormalities associated to ACEs seem also to be paralleled by alterations in functional connectivity patterns (Heany, Groenewold et al., 2018).

Concerning psychiatric patients, a recent meta-analysis investigated structural and functional brain differences between MDD patients with or without a positive history of ACEs (Antoniou, Lambourg et al., 2023): ACEs were associated to reduced GM volumes in right hippocampus, insula, and bilateral superior temporal and frontal gyri; concerning rs-FC, one well powered study found emotional abuse and neglect to be associated with increased rs-FC between dorsal attention and sensorimotor network, while physical abuse and neglect to increased connectivity between dorsal an ventral attention networks, and between cingulo-opercular and visual network (Yu, Linn et al., 2019).

While the effects of ACEs on grey matter volume and functional connectivity have been extensively explored, fewer studies investigated the effect of CT on WM integrity (Cassiers, Sabbe et al., 2018), particularly in psychiatric samples. A recent meta-analysis (Lim, Howells et al., 2020) performed on 14 Tract based spatial statistic (TBSS) studies identified six clusters of reduced fractional anisotropy (FA) in

individuals exposed to childhood maltreatment, relative to non-maltreated controls. However studies included in the analysis were performed both on healthy individuals and on psychiatric patients (suffering from PTSD, anxiety, eating disorders, depression, bipolar disorder or schizophrenia, among others); interestingly in the subgroup analysis performed on the three studies including exclusively healthy individuals, FA differed between traumatized and non-traumatized individuals only in the corpus callosum, while the only study performed entirely on subjects suffering from major depression (Tatham, Ramasubbu et al., 2016) found high depression severity and experiences of childhood physical or emotional neglect to predict higher FA. This was partially confirmed by a subsequent study (Graziano, Bruce et al., 2019) investigating the effect of childhood bullying on 186 individuals suffering from major depression, which identified higher FA in participants with a self-reported history of bullying.

Interestingly the one study found to strongly reduce the statistical significance of the above-mentioned meta-analysis when excluded in the jackknife analysis was the only one entirely performed on bipolar subjects (Lim et al., 2020, Stevelink, Abramovic et al., 2018). There were only two studies included in the meta-analysis with samples exceeding 100 participants: one was performed on 240 veterans with or without a history of childhood sexual abuse, physical abuse or family violence, and found no difference in FA values between the two groups (Corbo, Amick et al., 2016); the other investigated 251 patients with bipolar disorder and 163 healthy controls, reporting partially different results: indeed, while patients with bipolar disorder with childhood abuse had widespread reductions of FA relative to patients without, the authors found no differences between healthy individuals with and without abuse (Stevelink et al., 2018). A subsequent study by our group performed on 100 MDD and 100 bipolar patients also identified a significant diagnosis interaction in the effect of CT on white matter integrity, with widespread reductions of FA associated with trauma apparent only in bipolar subjects and not in patients with major depression (Corbo et al., 2016).

Taken together these findings seem to point to a diagnosis-specific effect of ACEs on white matter integrity, with strong and widespread reductions on FA found only in bipolar disorder (Poletti, Paolini et al., 2022, Stevelink et al., 2018) and possibly an opposite effect in MDD patients [68, 69]. Interestingly the negative association between

CT and FA seems to extend beyond bipolar disorder also to patients with psychotic or schizophrenia-spectrum disorders (Asmal, Kilian et al., 2019, Poletti, Mazza et al., 2015).

Concerning recent adversities, stressful life events (SLE) have been associated to reduced GM volumes in left medial orbitofrontal and prefrontal cortex, insulae and ACC by various large studies performed on healthy samples (Ansell, Rando et al., 2012, Ringwald, Meller et al., 2021); furthermore SLE were associated with longitudinal volumetric reductions in left medial prefrontal cortex over a two year period, more evident in subjects reporting also ACEs (Ringwald, Pfarr et al., 2022b). Concerning depressed patients, a large study identified a negative association between recent stressful life events and grey matter volume again in left medial orbitofrontal cortex; of note, a significant interaction with childhood abuse was identified, with the negative association being present only in subjects reporting CA (Ringwald, Pfarr et al., 2022a).

SLEs appear to also have substantial effects on WM microstructure: a large study identified a negative association between life events and FA in more than 700 MDD patients (Flinkenflügel, Meinert et al., 2023), and SLE induced alteration of WM microstructure has been also connected to depression onset and severity, with significant indirect effects (Wang, Wang et al., 2022b).

4. Early life and recent adversities and inflammatory status

Childhood adversities have profound impact on the immune/inflammatory status in adulthood. Peripheral levels of CRP, IL-6 and TNF-α have been found to be positively associated to ACEs in healthy and psychiatric samples alike (Baumeister, Akhtar et al., 2016). Indeed CT has been identified as a risk factor for the development of rheumatic diseases and, interestingly, multiple sclerosis (Rehan, Khan et al., 2023). When specifically investigating depressed patients, IL-6 and TNF- α levels are robustly reported to be elevated in CT+ individuals, while results concerning CRP are somewhat more inconsistent (Gill, El-Halabi et al., 2020).

While robust evidence links childhood adversities to an altered inflammatory status, the relation between stressful life events and inflammation in adulthood appears to be less clear: recent stressors were not associated to peripheral inflammatory markers both in healthy participants and in depressed patients (Grosse, Ambrée et al., 2016, van Ockenburg, Tak et al., 2015). At the same time a study investigating the effect of stressful events on peripheral CRP, IL-6 and soluble urokinase plasminogen activator receptor (suPAR, a novel inflammatory marker), found no association with the first two, but a significant positive association between life events and suPAR; furthermore the study identified a significant interactions with ACEs, with individuals with a positive history of CT exhibiting higher suPAR levels in response to recent stressful events (Bourassa, Rasmussen et al., 2021).

Given the established impact of CT on inflammatory status and on the development of depression later in life, and the suspected contribution of inflammatory alteration to MDD pathophysiology, a new framework is starting to emerge, linking all these aspects together; it is indeed hypothesized that childhood psychosocial stressors affect immune system development, which in turn has consequences on brain development, long-term functioning and on the development of psychopathology (Danese & J Lewis, 2017). Indeed a large study, performed on more than 4000 children, found childhood adverse events to be associated to increased levels of IL-6, in turn associated to the development of later internalizing symptoms, with significant indirect effects (Flouri, Francesconi et al., 2019).

5. Treatment response and neuroimaging

Numerous studies investigated brain correlates and predictors of treatment response and remission in MDD through various MRI methodologies.

Various studies have detected structural correlates in grey matter (GM) volumes, particularly in parts of the limbic system and frontal cortex (like the hippocampus, amygdala, anterior cingulate cortex (ACC), and prefrontal cortex), which are connected to both the diagnosis of Major Depressive Disorder (MDD) and how individuals

respond to treatment (Enneking, Leehr et al., 2020, Kang & Cho, 2020, Klok, van Eijndhoven et al., 2019).

A consistent finding in MDD imaging is the decreased volume of the hippocampus, a crucial area for memory and emotional processing (Campbell, Marriott et al., 2004, Schmaal, Veltman et al., 2016). Several studies have explored its connection to treatment response. An investigation focusing on pre-treatment hippocampal volumes found that responders tended to have higher volumes compared to non-responders (MacQueen, Yucel et al., 2008). This outcome was verified in a recent study with a larger sample size concentrating explicitly on hippocampal volumes (Nogovitsyn, Muller et al., 2020). Additionally, the hippocampus was identified in a voxel-based morphometry (VBM) study, conducted on a group comprising individuals with unipolar and bipolar depression, as one of the brain regions influencing response to antidepressant treatment (Sämann, Höhn et al., 2013).

Another area frequently studied in major depression is the ACC. A meta-analysis involving 41 studies and 4101 individuals revealed reduced volumes in the ACC among patients with MDD compared to controls (Wise et al., 2017). In terms of its relation to treatment response, greater baseline thickness in the ACC was linked to more significant symptom improvement during follow-up in one study (Phillips, Batten et al., 2015). Similarly, higher GM volumes in the ACC were associated with quicker and more substantial improvement rates in a VBM study conducted with a small group (Chen, Ridler et al., 2007).

Other brain regions in which reduced GM volumes seem to associate with worse treatment response are: dorsolateral prefrontal cortex, middle and inferior frontal gyri, orbitofrontal cortex, insula, fusiform, and lingual gyri (Enneking et al., 2020).

The relation between white matter integrity and antidepressant response has been much less investigated. A relatively large multisite study found lower AD values to predict worse response to SSRIs after 8 weeks (Davis, Hassel et al., 2019). Sparse studies on patients with treatment resistant depression on the other hand seem to report reduced FA when comparing them to non-TRD patients (Runia, Yücel et al., 2022).

Numerous studies investigated rs-fMRI patterns associated with treatment response and resistance in MDD. Results have been often inconsistent, also due to methodological heterogeneity and small sample sizes. As noted by a recent systematic review of the topic however, reduced rs-FC within the DMN and of the DMN with other networks seem to be associated with treatment resistance; this finding seem however to be paralleled by an increased spontaneous neuronal activity in several DMN regions in TRD, as measured via ALFF or fALFF analyses (Long, Du et al., 2020, Runia et al., 2022).

6. Treatment response and inflammatory status

Altered inflammatory markers have been repeatedly linked to MDD treatment response and resistance. Two separate meta-analytic studies identified higher baseline IL-8 levels in patients non-responding to treatment (Gasparini, Callegari et al., 2022, Liu, Wei et al., 2020); one of the meta-analyses identified also higher CRP levels in non-responders (Gasparini et al., 2022), while the other reported only a trend towards significance for CRP (Liu et al., 2020).

A systematic review investigated the effect of baseline inflammatory status on treatment response taking into account the class of prescribed pharmacoterapies: while they found higher baseline inflammatory markers to be predictive of worse treatment response to predominantly serotoninergic drugs, an opposite effect was identified for treatment acting also on noradrenergic, dopaminergic or glutamatergic neurotransmission, with higher inflammation predicting *better* treatment response (Arteaga-Henríquez, Simon et al., 2019).

Expression patterns of several genes linked to the immune function have also been found to be altered according to treatment resistance and response: among the genes found to be differentially expressed FKBP1A, an immunomodulatory gene, FAM19A4, involved in macrophages activity, and NFIB and FKBP5, involved in HPA axis regulation (Amasi-Hartoonian, Pariante et al., 2022).

7. Longitudinal changes in MRI findings and inflammatory status

Several studies investigated longitudinal changes in MDD patients after AD treatment.

Concerning grey matter, hippocampal volumetric increases after AD treatment have been repeatedly reported (Enneking et al., 2020); furthermore, one study reported a differential effect between responders and non-responders, with volumetric increases in the first group and decreases in the second (Phillips et al., 2015). Analogous results have been reported concerning prefrontal regions, with a general tendency towards volumetric increases and a differential effect according to response status (Phillips et al., 2015).

A larger body of literature investigated longitudinal GM changes after ECT: consistent findings indicate volumetric increases in the hippocampus–amygdala complex, and cortical thickness increases in temporal regions and in ACC; numerous studies also reported volumetric increases in the basal ganglia (Enneking et al., 2020).

Reports of longitudinal changes in measures of WM integrity after antidepressant treatment appear to be much less consistent, also due to often small sample sizes employed: one study reported significant decreases in AD, RD and MD and no changes in FA one week after citalopram administration (Seiger, Gryglewski et al., 2021); another well powered study on the other hand found no change in DTI metrics after 8 weeks SSRI treatment [100]. Antidepressants and particularly TCAs use appears to be associated to worsening white matter hyperintensities in older samples (Grool, van der Graaf et al., 2013, Steffens, Chung et al., 2008).

Concerning rsFC, a relatively large study identified reduced connectivity of the subcallosal cingulate cortex with the left medial motor cortex in remitted MDD patients after 12 week CBT or pharmacotherapy (Dunlop, Cha et al., 2023). Another study identified widespread within and between network decreases in rsFC after 8 weeks of antidepressant therapy (Li, Su et al., 2021).

Longitudinal changes in peripheral inflammatory markers have also been reported after AD treatment. A systematic review and meta-analysis of changes in cytokines and chemokines levels after AD treatment identified significant decreases in IL-6, TNF-α, IL-10 and CCL-2; however no relation with response patterns was identified (Köhler,

Freitas et al., 2018). A newer meta-analysis extended such results, identifying decreases also in IL-2 and serum Cortisol (Fernandes, Scotti-Muzzi et al., 2022).

Aim of the work

The present study has several aims: first to investigate the effect of childhood and recent adversities (and of their interaction) on antidepressant treatment response, inflammation status and MRI measures in patients with major depression; second to identify inflammatory patterns and MRI correlates associated with treatment response, and to investigate possible mediating roles between inflammation, environmental stressors, MRI findings and patterns of treatment response. Third, to assess the role of genetic liabilities in affecting the relation between our variables of interest, thus investigating their possible role in disentangling major depression heterogeneity. Finally, to investigate MRI longitudinal correlates after antidepressant treatment.

Results

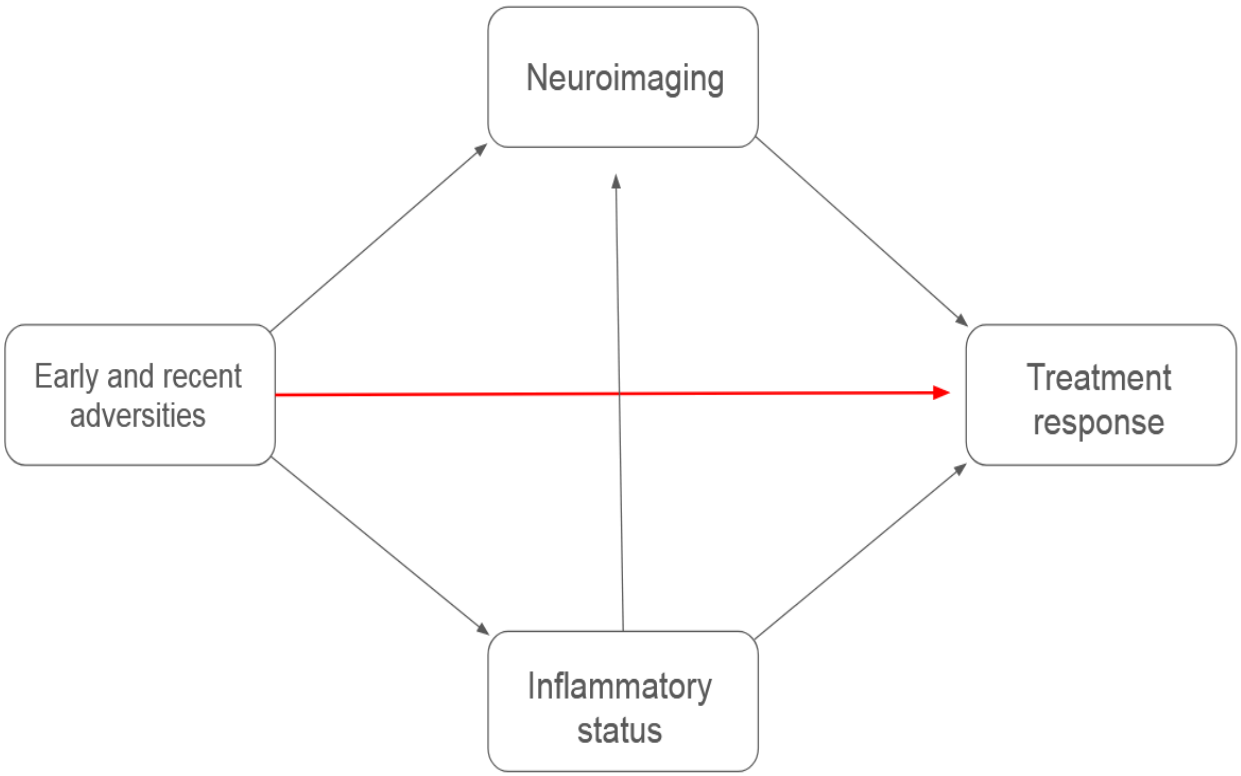
Details on sample clinical and demographic variables can be seen in table 1.

	Whole sample (n=220)	**Non-TRD** (n=113)	**TRD** (n=107)	**χ^2/t-test p**
Age	50.50 ± 10.46	49.27 ± 10.42	51.79 ± 10.40	0.074
Sex (F/M)	142/78	80/33	62/45	*0.046**
Years of education	12.50 ± 4.06	12.48 ± 3.91	12.52 ± 4.23	0.939
Age of onset	34.34 ±12.46	34.83 ± 11.33	33.77 ± 13.66	0.548
BMI	24.74 ± 7.03	25.07 ± 8.88	24.37 ± 4.20	0.482
Imipramine equivalents	214.16 ± 85.82	206.48 ± 86.31	223.42 ± 84.93	0.230
Hospitalization (days)	28.20 ± 11.25	25.04 ± 7.52	32.04 ± 13.66	*<0.001***

HDRS (admission)	22.64 ± 6.86	23.00 ± 6.60	22.25 ± 7.16	0.463
HDRS (discharge)	5.83 ± 5.13	5.02 ± 4.49	6.76 ± 5.67	*0.032**
% HDRS decrease	75.6 ± 20.5	79.2 ± 17.8	71.6 ± 22.6	*0.020**
BDI (admission)	15.72 ± 8.06	14.50 ± 7.50	17.06 ± 8.49	0.065
CTQ total score	39.93 ± 12.83	41.58 ± 14.73	38.27 ± 10.43	0.088
Physical abuse	6.32 ± 2.92	6.84 ± 3.52	5.79 ± 2.05	*0.021**
Emotional abuse	8.37 ± 4.05	9.17 ± 4.73	7.57 ± 3.05	*0.011**
Sexual abuse	5.89 ± 2.75	5.97 ± 2.95	5.80 ± 2.55	0.691
Physical neglect	7.11 ± 3.07	7.54 ± 3.49	6.68 ± 2.51	0.072
Emotional neglect	12.21 ± 4.88	12.34 ± 5.25	12.07 ± 4.49	0.717
Minimization	9.02 ± 3.03	9.09 ± 3.22	8.95 ± 2.84	0.759
RFQ	28.12 ± 10.81	29.72 ± 11.66	26.43 ± 9.60	*0.024**
PSS	28.33 ± 6.14	28.23 ± 6.35	28.44 ± 5.96	0.833
SRE-N	26.93 ± 50.59	28.73 ± 59.26	25.03 ± 39.61	0.598
SRE-S	562.87 ±469.14	573.81 ± 514.76	551.39 ± 418.21	0.732

Table 1: clinical and demographic variables.

1. Effect of early life and recent adversities on treatment response and resistance



Childhood trauma was positively associated with baseline depressive scores: this was true for HDRS (EN: r=0.20, p=0.014; EA: r=0.17, p=0.041; RFQ: r=0.18, p=0.029) and BDI (CTQ: r=0.25, p=0.002; PA: r=0.20, p=0.14; EA: r=0.19, p=0.017; PN: r=0.16, p=0.043; RFQ: r=0.22, p=0.005) baseline scores.

Treatment resistance and childhood trauma appeared to be inversely associated: increasing CTQ total (p=0.043, OR=0.92), physical abuse (p=0.022, OR=0.85), emotional abuse (p=0.003, OR=0.86), physical neglect (p=0.045, OR=0.88) and RFQ (p=0.033, OR=0.97) scores reduced the possibility of treatment resistance. Similar effects were observed for the possibility of achieving symptom remission at discharge, which increased with higher levels of CTQ total (p=0.020, OR=1.04) and physical neglect (p=0.008, OR=1.24) (Fig. 1).

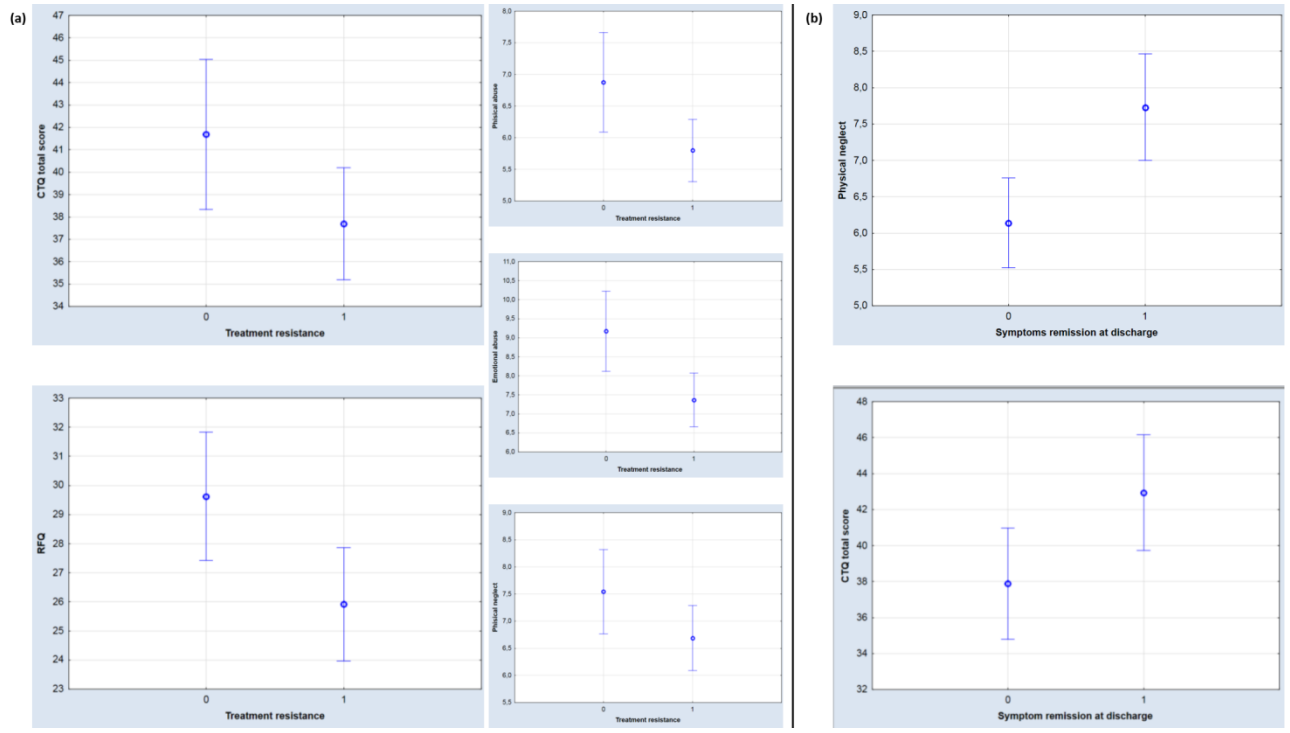


Fig. 1: relation between childhood adversities and response patterns. (a): treatment resistance. (b): remission at discharge.

PSS or SRE scores had no effect on treatment resistance or remission at discharge. However a significant interaction between recent and childhood adverse events was identified: patients with a higher number ($p<0.001$) or score ($p=0.001$) of recent adverse events had higher chance of achieving symptom remission upon discharge for low CTQ scores, while with high scores of childhood trauma recent adversities rendered symptom remission less likely (Fig. 2). This pattern was repeated for the emotional neglect ($p=0.021$ and $p=0.004$), emotional abuse ($p<0.001$ and $p=0.004$), physical neglect ($p=0.004$ and $p<0.001$) CTQ subscales and for RFQ ($p=0.045$).

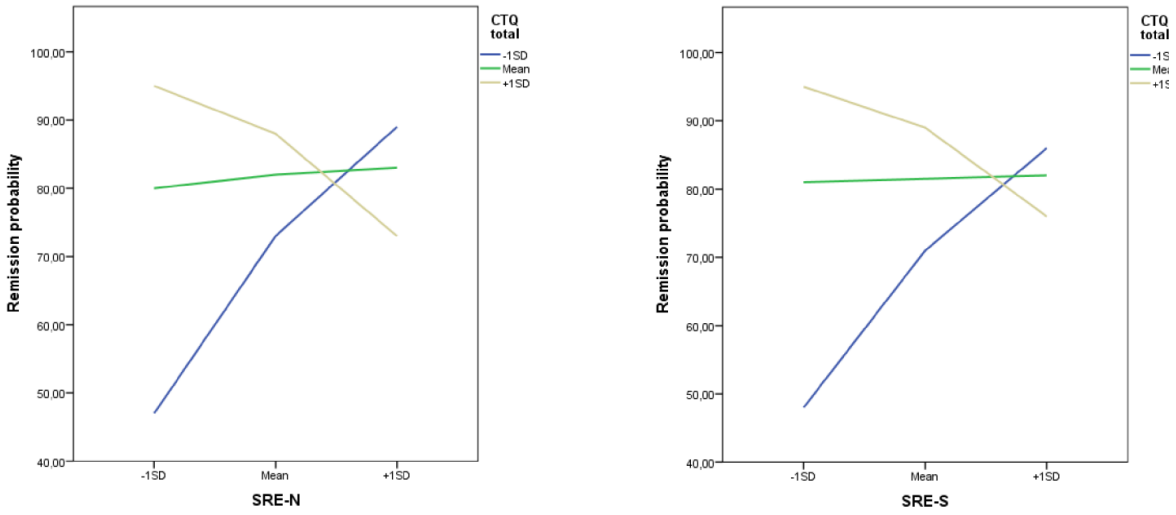
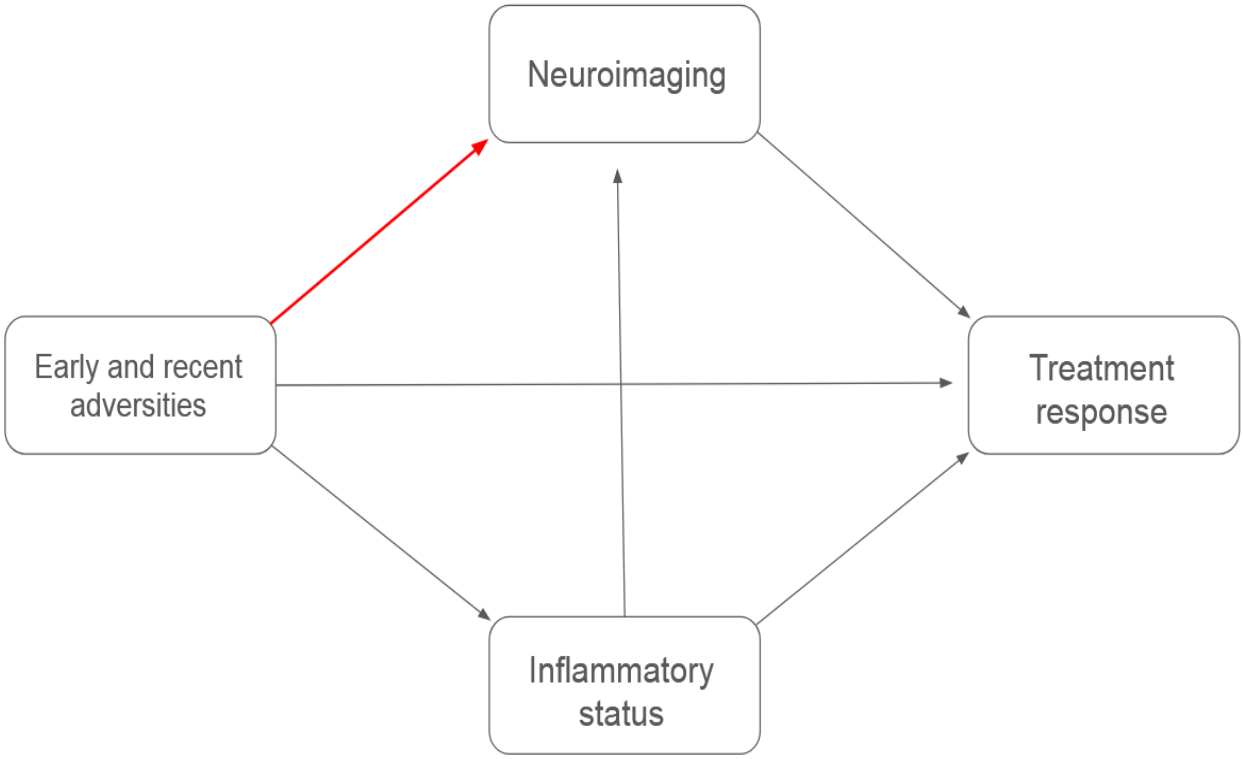


Fig. 2: Effect of recent x childhood adversities interaction on response patterns.

2. Effect of of early life and recent adversities on neuroimaging.



Voxel based morphometry and cortical thickness

We identified no significant effect of ACEs on brain volumes or cortical thickness.

PSS was negatively associated with brain volumes in two bilateral symmetrical clusters (clus. 1: central operculum R, p=0.014; clus. 2: central operculum L, p=0.024); this was paralleled by a negative association between PSS and cortical thickness in the opercular part of the inferior frontal gyrus (p=0.043) and in the middle cingulate gyrus (p=0.039) (Fig. 3).

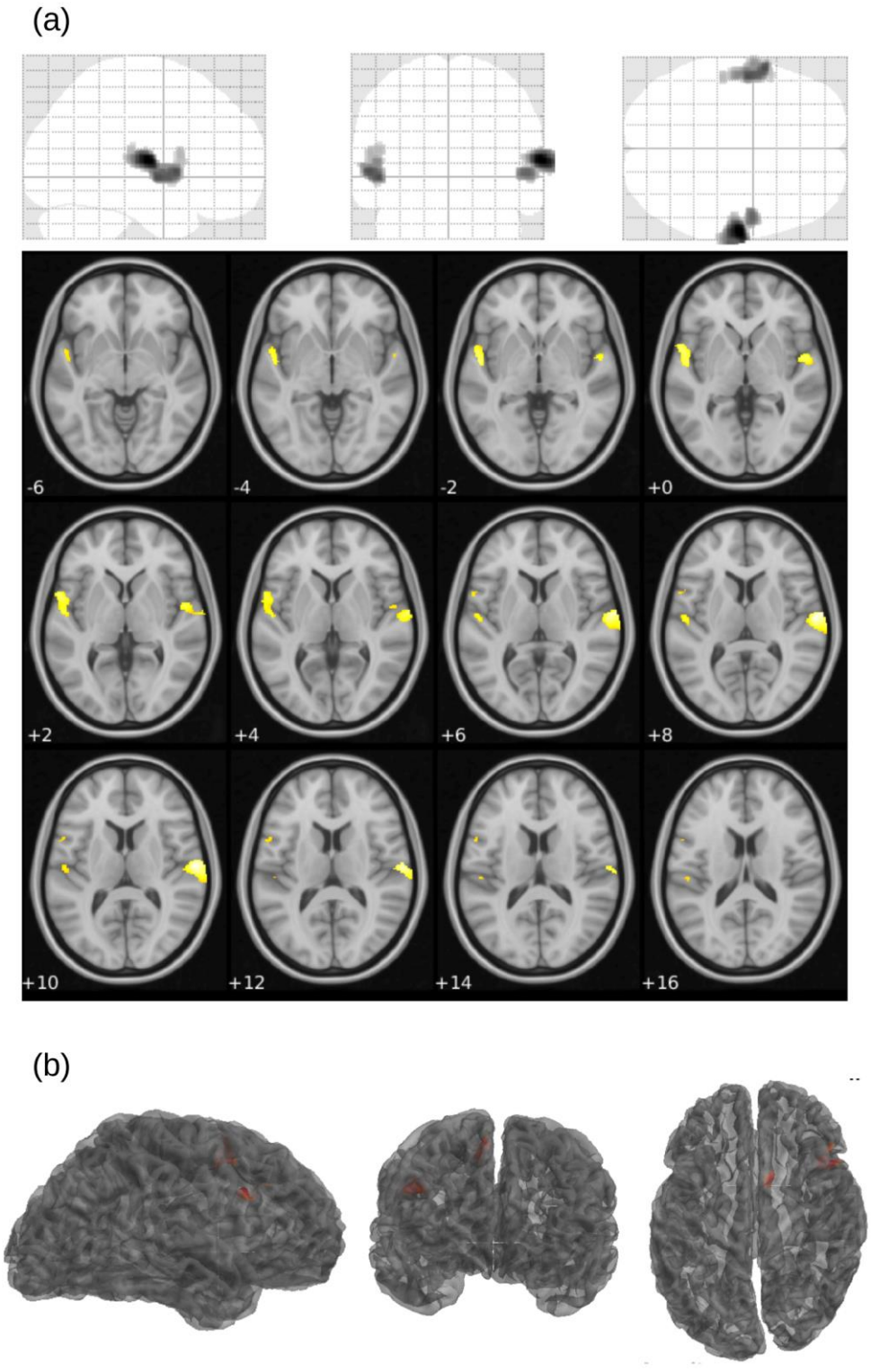


Fig. 3: negative association between perceived stress and grey matter. (a): voxel based morphometry. (b): cortical thickness

No association emerged between SRE-N or SRE-S and either brain volumes or cortical thickness. On the other hand significant interaction between ACEs and PSS, SRE-N and SRE-S were identifided.

Concerning PSS the following interactions emerged.

For VBM, PSS x physical abuse on two clusters (frontal pole R, p=0.021; middle temporal gyrus R, p=0.026), with negative associations between PSS and GM volumes for subjects without a positive history of physical abuse (MSFG and frontal pole R, p<0.001; central operculum R, p=0.024; central operculum L, p=0.032) and a trend towards a positive association in subjects reporting childhood physical abuse (frontal pole R, peak pFWE=0.048, STG R p=0.085) (Fig. 4) (Tab. 2).

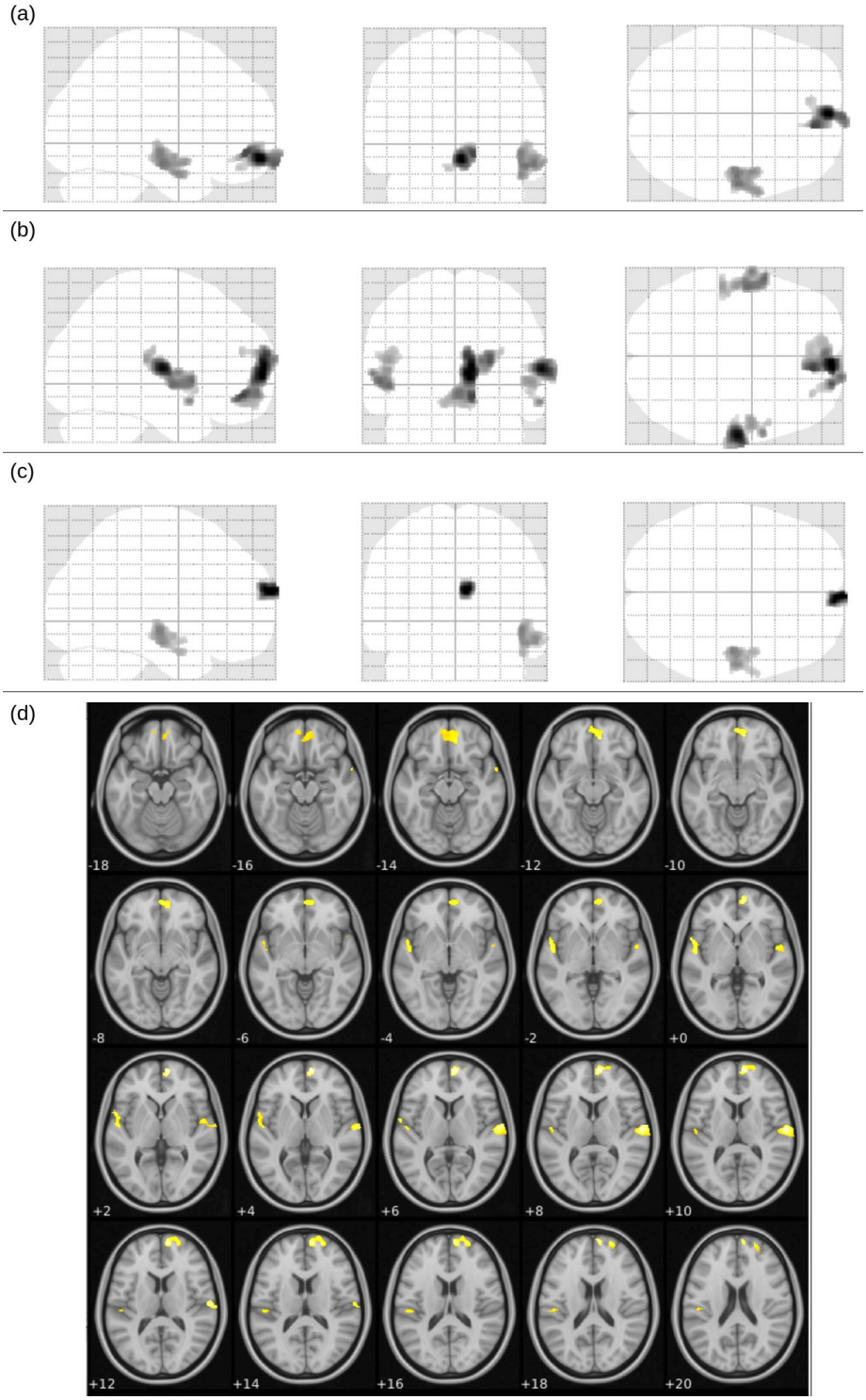


Fig. 4: Perceived stress x physical abuse interaction on gray matter volumes. (a): regions of significant interactions. (b): negative association in PA - patients. (c): positive associations in PA + patients. (d): negative association in PA – patients, slice view.

The following interactions were on the other hand found for SRE-N and SRE-S.

For Cortical thickness, SRE-S x CTQ total score (bilateral MSFG and anterior cingulate, p=0.001), with a negative association only in CTQ + subjects (p<0.001) (Fig. 5); among CTQ subscales, a similar pattern emerged for SRE-S x emotional abuse, with a statistical trend for the interaction (p=0.065) and a negative association between SRE-S and CT only in AE+ subjects (p<0.001) again in the MSFG and anterior cingulated (Tab. 3).

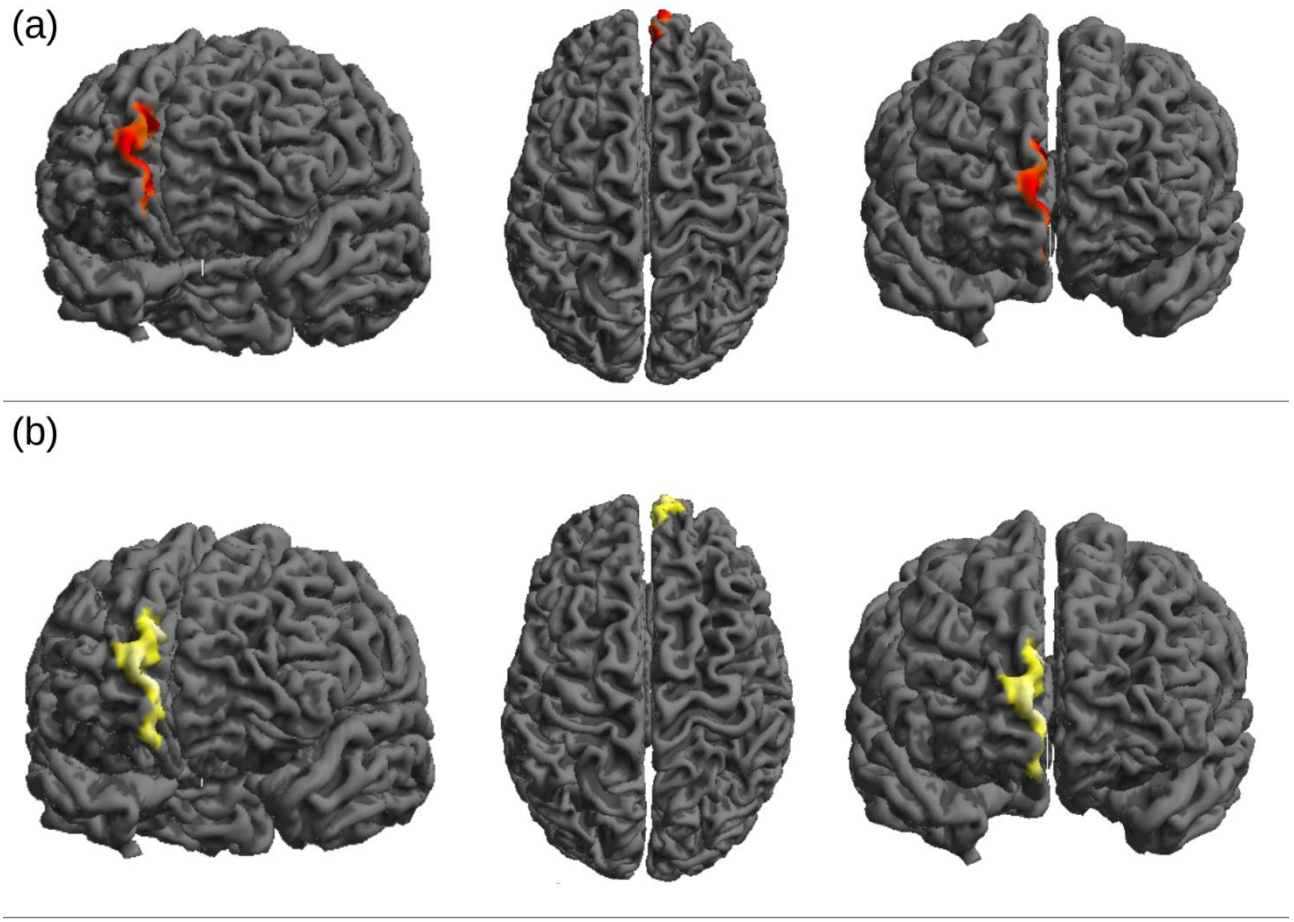


Fig. 5: SRE-S x CTQ total score interaction on cortical thickness. (a): region of significant interaction. (b): negative association between SRE-S and cortical thickness in CTQ + subjects.

Diffusion Tensor Imaging

Concerning ACEs, negative associations were found between PA and AD (p=0.047), PN and CTQ total and MD (p=0.039 and p=0.047), RFQ and AD and MD (p=0.015 and p=0.041) in widespread WM tracts (Fig. 6).

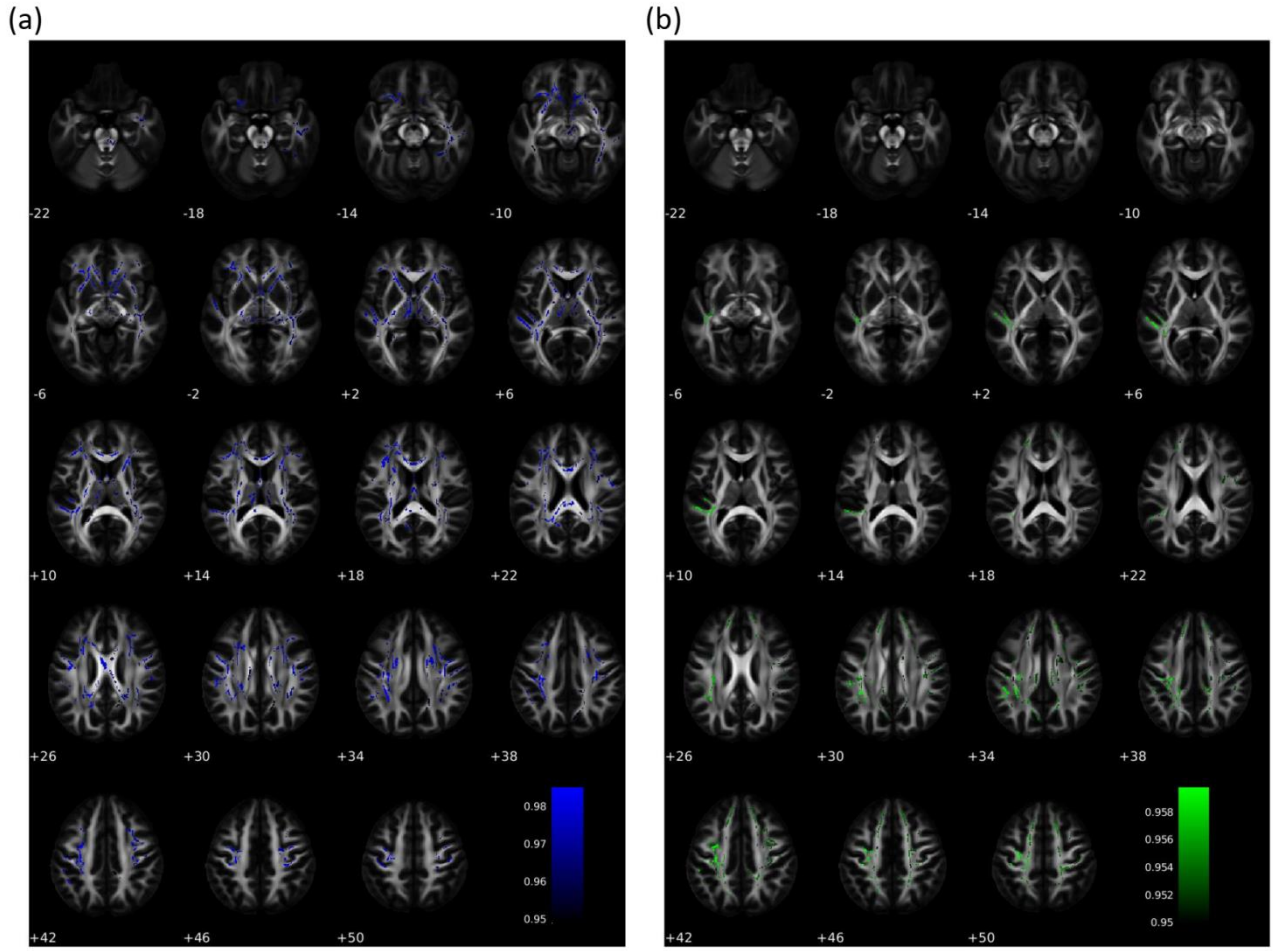


Fig. 6: association between RFQ and DTI metrics. (a): axial diffusivity. (b): mean diffusivity.

Concerning recent stress, PSS was negatively associated with FA (p=0.039), SRE-S with AD (p=0.042) and SRE-N with AD and MD (p=0.016 and p=0.038).

Significant childhood x recent stress interactions emerged. Furthermore a strong SRE-S x EA interaction emerged for FA (p=0.022) and RD (p=0.032), with widespread negative associations between SRE-S and FA (p=0.001) and positive with RD (p=0.012) only in AE + subjects (Fig. 7). A similar trend was identified for the SRE-S x CTQ total interaction (p=0.061), again with a negative association between SRE-S and FA only in CTQ + subjects (p=0.019).

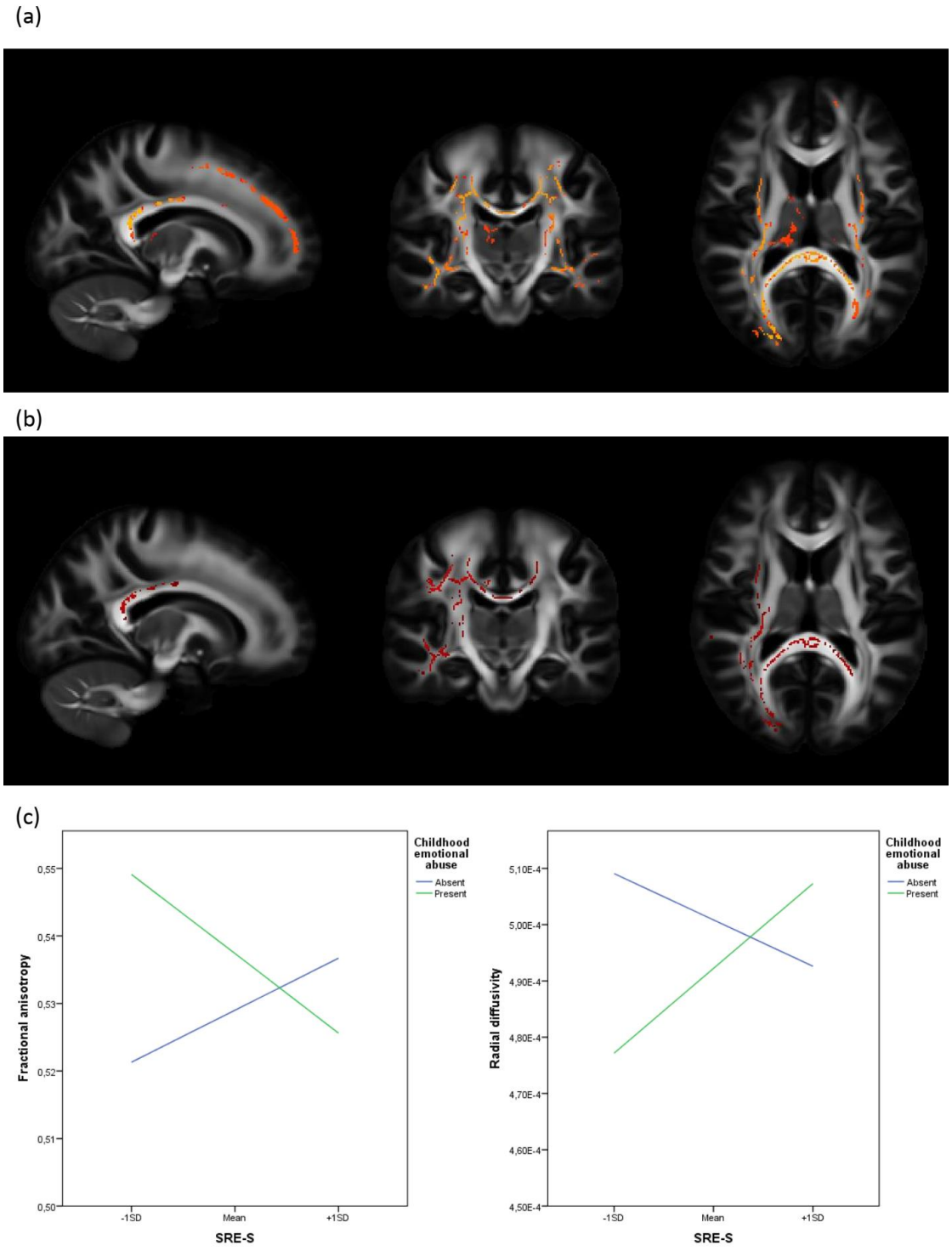


Fig. 7: SRE-S x Emotional abuse interaction. (a): white matter tracts of significant interaction for Fractional anisotropy. (b): white matter tracts of significant interaction for radial diffusivity. (c): moderation analysis.

Resting state functional connectivity

CTQ total score was negatively associated with rsFC between the right amygdala and the left opercular cortex (pFWE=0.020) (Tab. 4).

Concerning recent adversities, PSS was negatively associated to rsFC between the right hippocampus and the left Middle Frontal Gyrus (pFWE=0.009). Furthermore PSS was strongly negatively associated with fALFF in the bilateral intracalcarine cortex (pFWE<0.001).

Both SRE-N and SRE-S significantly modulated hippocampal connectivity: specifically they were negatively associated to rsFC between right hippocampus and Lateral Occipital Cortex (SRE-N pFWE=0.044; SRE-S pFWE=0.010) and positively between left hippocampus and right Paracingulate Gyrus (SRE-N pFWE=0.024; SRE-S pFWE=0.009) (Fig. 8, top).

Concerning CT x recent stress interactions, the following were identified:

PSS x PA, left amygdala with left Temporal Pole (pFWE=0.045), negative associations in PA+.

PSS x PN: right amygdala with Precuneous Cortex (pFWE=0.039), positive association in EA + subjects and negative in EA -.

A significant SRE-N and SRE-S x CTQ total interaction on fALFF in the right Middle Temporal Gyrus emerged (SRE-N: pFWE=0.034, SRE-S: pFWE=0.015), with negative associations only in CTQ + patients.

For CTQ subscales, the following interactions were found:

SRE-N x PA, left amygdala with posterior cingulate gyrus (pFWE=0.007), negative association in PA+.

SRE-S x PA, fALFF in the left frontal pole (pFWE=0.026), negative associations in PA+.

SRE-N x EN, left hippocampus with left Superior Frontal Gyrus (pFWE=0.009), negative associations in EN -, positive in EN +; fALFF in numerous temporo-parietal clusters (pFWE<0.001), positive associations in EN -.

SRE-N x EA, left amygdala with bilateral Intracalcarine Cortex (pFWE=0.008), positive association in EA + subjects and negative in EA -; fALFF in numerous temporo-parietal-occipital clusters (pFWE<0.001), positive associations in EA-, negative in EA+.

SRE-S x EA, left amygdala with bilateral Intracalcarine Cortex (pFWE<0.001), positive association in EA + subjects and negative in EA -; fALFF in numerous temporo-parietal-occipital clusters (pFWE<0.001), positive associations in EA-, negative in EA+ (Fig. 8, bottom).

SRE-N x SA, fALFF in left occipital pole (pFWE<0.001), positive association in SA+.

SRE-S x SA, left hippocampus with right Intracalcarine Cortex (pFWE=0.005), negative association in SA+; fALFF in left occipital pole (pFWE=0.013), positive association in SA+ (Tab. 4).

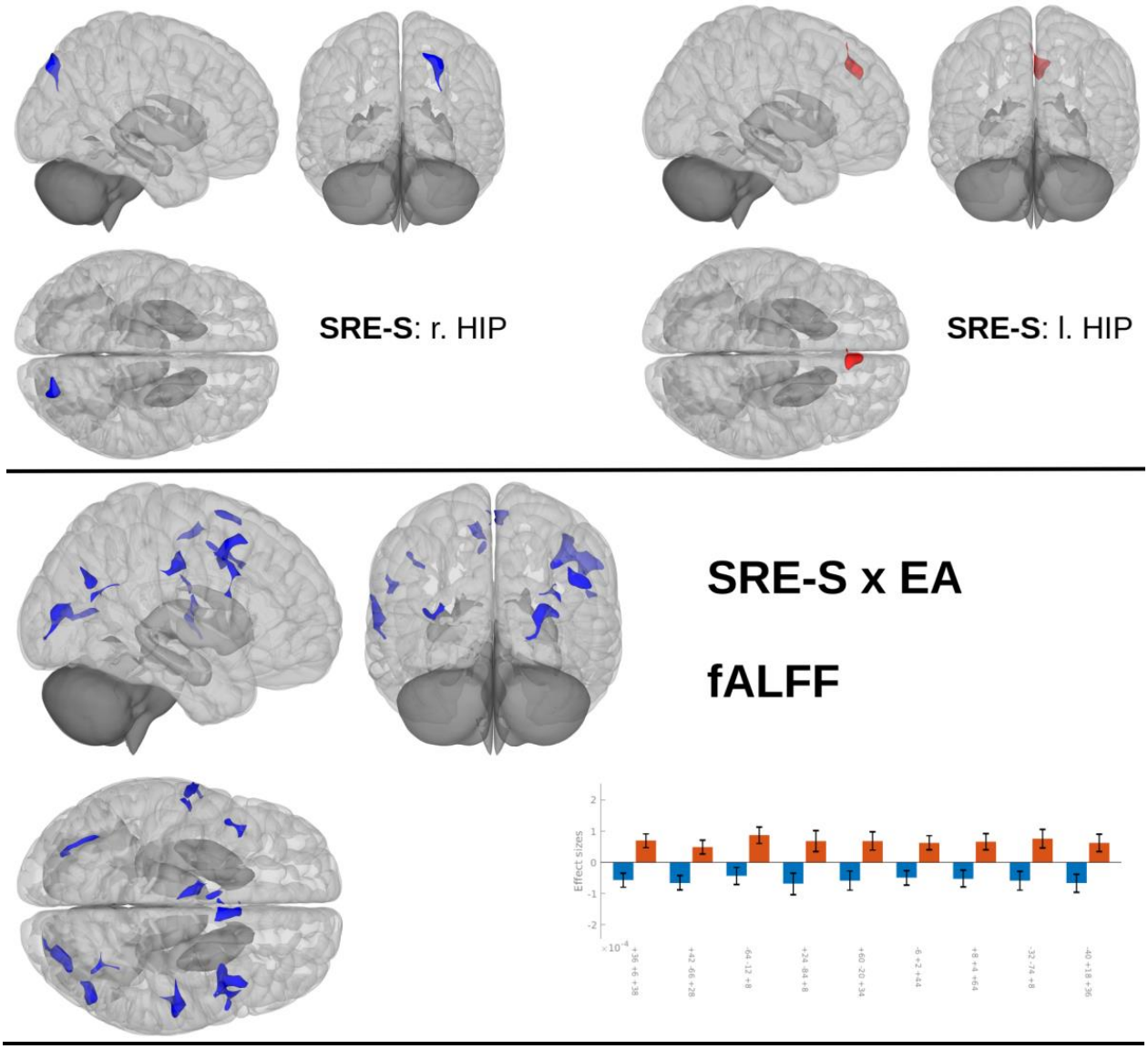
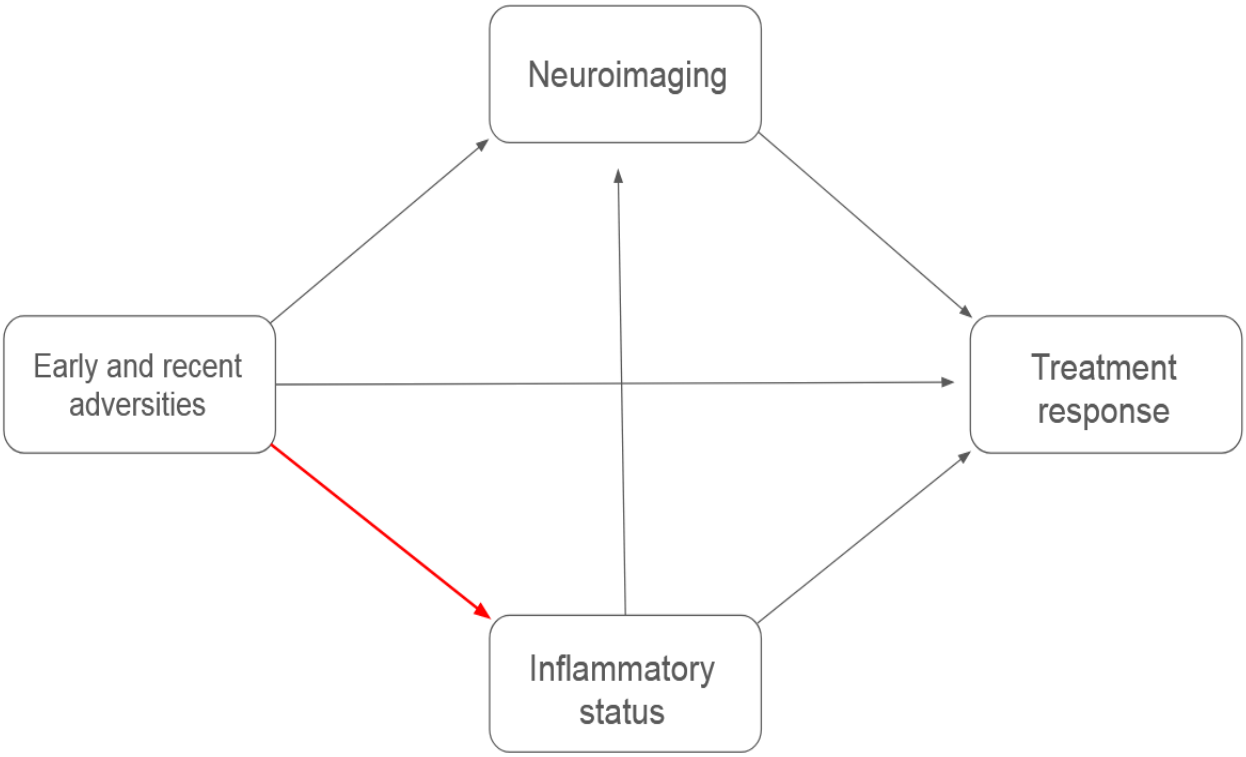


Fig. 8: association between SRE-S and resting state fMRI. Top: seed-based connectivity analysis for left and right hippocampus. Bottom: Effect of SRE-S x Emotional abuse interaction on fALFF.

3. Effect of of early life and recent adversities on inflammation



A negative association was found between RFQ scores and NLR ($p<0.001$, $\beta=-0.31$), PLR ($p=0.005$, $\beta=-0.21$), MLR ($p=0.004$, $\beta=-0.23$) and SII ($p<0.001$, $\beta=-.30$). NLR and MLR were also inversely associated with Emotional neglect scores (NLR: $p=0.044$, $\beta=-0.23$; MLR: $p<0.001$, $\beta=-0.44$); furthermore MLR was negatively associated with CTQ total ($p<0.001$, $\beta=-0.46$), physical abuse ($p=0.043$, $\beta=-0.20$) and emotional abuse ($p=0.005$, $\beta=-0.31$) (Fig. 9). No effect of recent adversities was identified.

Concerning WBC absolute values, RFQ scores were negatively associated with neutrophils ($p=0.001$, $\beta=-0.26$) and positively with lymphocytes ($p=0.013$, $\beta=0.19$). On the other hand CTQ total ($p=0.005$, $\beta=-0.35$), physical abuse ($p=0.044$, $\beta=-0.19$), emotional abuse ($p=0.020$, $\beta=-0.27$) and emotional neglect ($p=0.011$, $\beta=-0.32$) were negatively associated with monocyte count (Fig. 9).

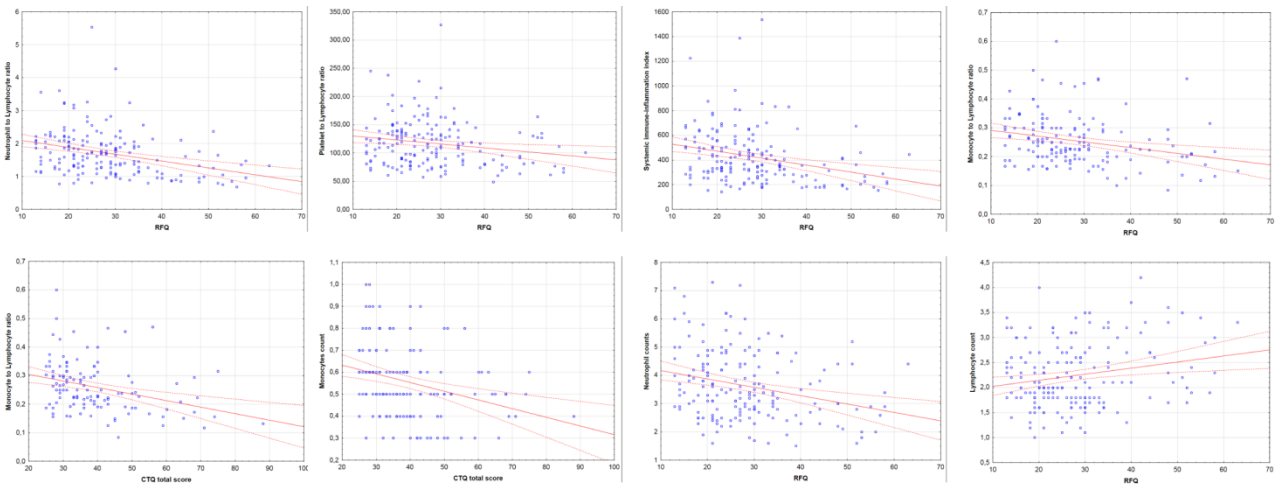


Fig. 9: association between Childhood adversities, inflammatory markers and blood cell counts.

No effect of recent stressors was identified on inflammatory ratios or WBC absolute counts.

Significant interactions were found between physical neglect and SRE-S for NLR (p=0.032) (Fig. 10), PLR (p=0.038) and SII (p=0.032). Significant positive associations between SRE-S and inflammatory ratios were identified only at higher values of childhood physical neglect. Concerning WBC absolute values, while no interactions were found between SRE-S CT and neutrophil or platelet counts, significant SRE-S x PN interactions were identified for lymphocyte count (p=0.034) (Fig. 10), with negative associations at higher values of PN.

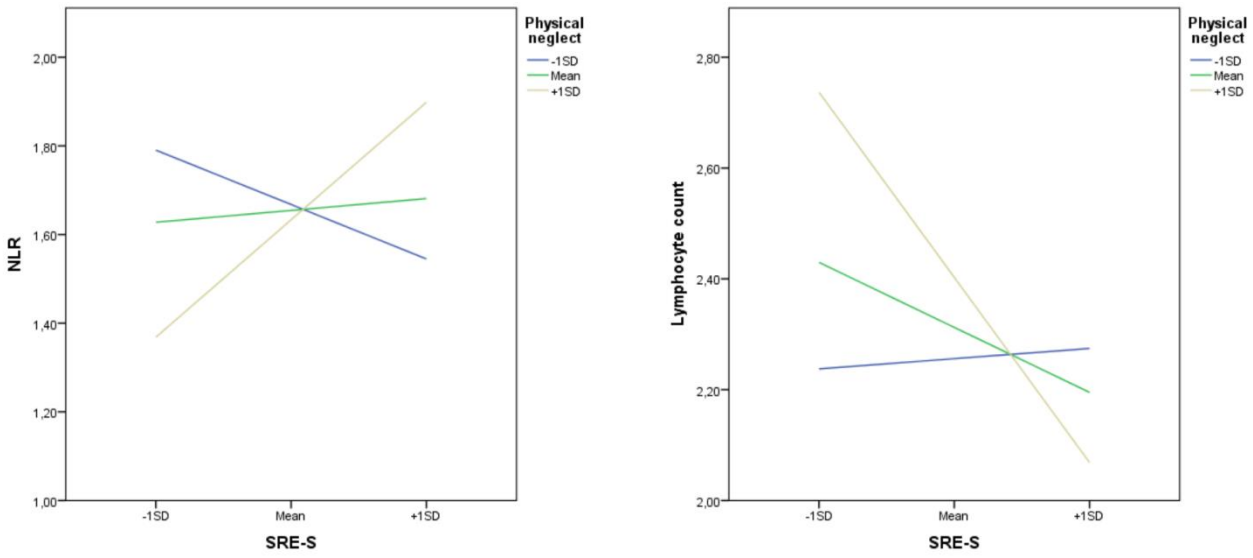


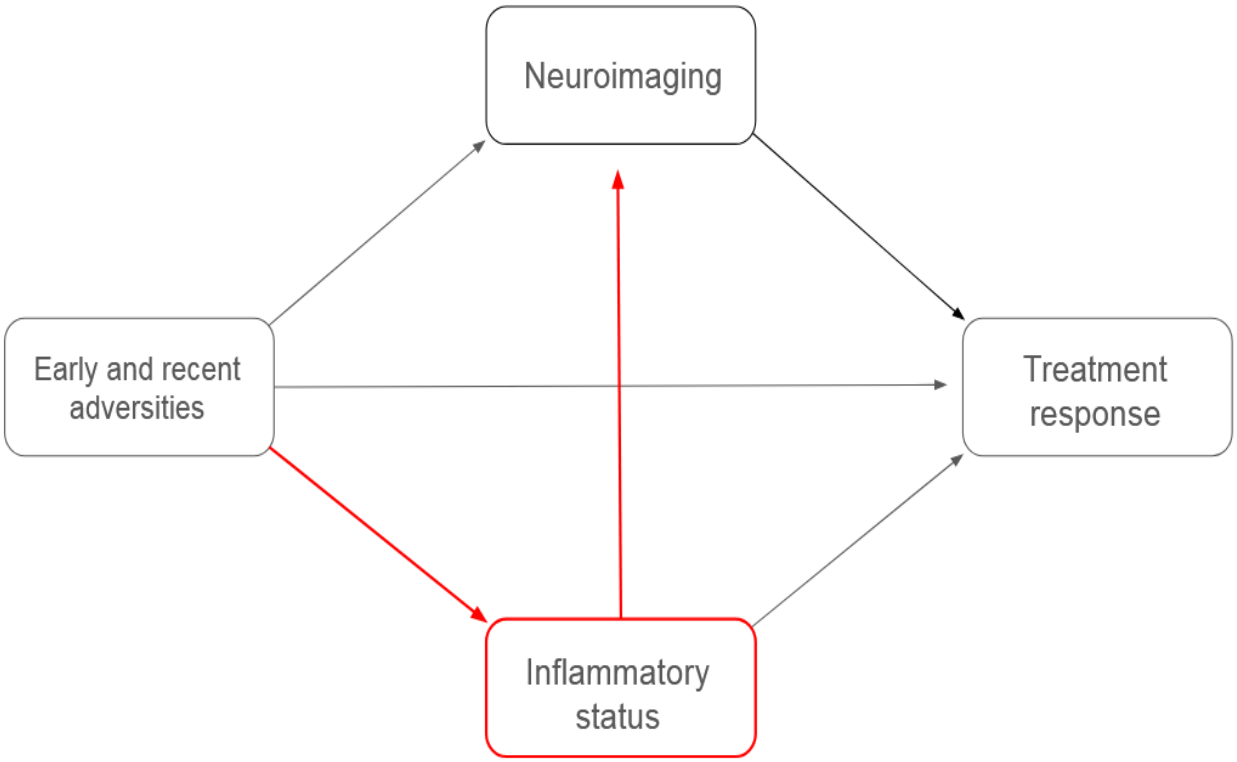
Fig. 10: SRE-S x childhood physical neglect interaction on neutrophil to lymphocyte ratio (NLR) and lymphocyte count.

CT significantly affected cytokines peripheral levels. A significant effect was found for: physical abuse ($p=0.013$), with positive associations with MIF ($p<0.001$, $\beta=0.42$), TNF-α ($p<0.001$, $\beta=0.36$), IL-1β ($p=0.017$, $\beta=0.26$); physical neglect ($p=0.008$), with positive associations with MIF ($p=0.001$, $\beta=0.42$), TNF-α ($p=0.029$, $\beta=0.28$), IP-10 ($p=0.032$, $\beta=0.24$), MCP-1($p=0.004$, $\beta=0.38$), GRO-α ($p=0.019$, $\beta=0.30$), HGF ($p=0.006$, $\beta=0.38$), TRAIL ($p=0.005$, $\beta=0.37$); sexual abuse ($p<0.001$), with positive associations with MIF ($p<0.001$, $\beta=0.45$), IL-18 ($p=0.023$, $\beta=0.25$), MCP-1 ($p=0.002$, $\beta=0.34$), HGF ($p<0.001$, $\beta=0.40$).

No effect of recent adversities on peripheral cytokines levels was identified, while significant interactions between perceived stress and childhood physical and sexual abuse emerged: PSS x physical abuse ($p=0.038$), for MIF ($p<0.001$); PSS x sexual abuse ($p=0.002$) for MIF ($p<0.001$). In both cases significant positive associations between PSS and MIF were only identified in subjects reporting childhood physical or sexual abuse.

No association was found between CRP levels and measures of childhood or recent adversities.

4. Mediation analysis: childhood trauma, inflammation and neuroimaging



A significant mediation was found for the effect of physical neglect on mean diffusivity through GRO-alpha (Fig. 11): a positive association was found between physical neglect and GRO-alpha (β=0.37, p=0.009); GRO-alpha in turn was negatively associated with MD (β=-0.24, p=0.017), with a significant indirect effect (95% BCa CI [-0.2101, -0.0023], standardized coefficients reported).

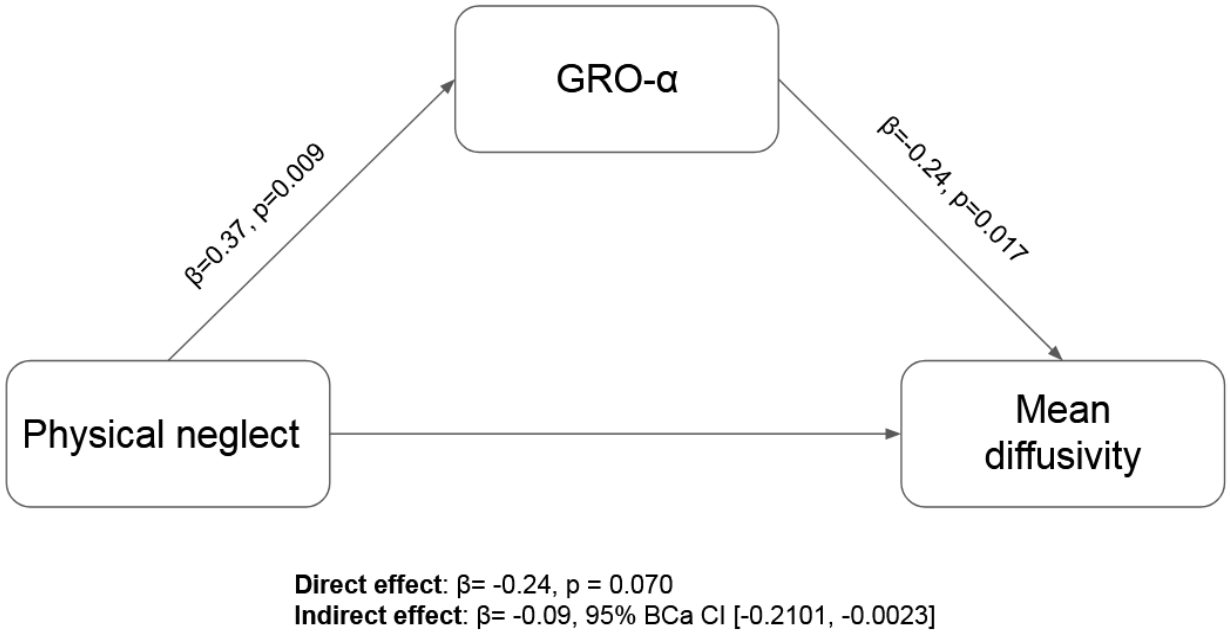


Fig. 11: mediation analysis between childhood physical neglect, GRO-α and Mean diffusivity.

5. Early and recent trauma x BD PRS interaction on treatment response, neuroimaging and inflammatory status

BD PRS significantly moderated the effect of childhood trauma on response patterns. Specifically, for remission at discharge, the following moderations were identified: CTQ total x BD PRS (p=0.008) (Fig. 12), with CTQ increasing the likelihood of remission for lower PRS scores and lowering it for higher scores; EN x BD PRS (p=0.019), positive association at low PRS scores, negative at high PRS; EA x BD PRS (p=0.011), positive association at low PRS scores, negative at high PRS; PN x BD PRS, positive association at low PRS scores. Similar results were found when using the percentage HDRS decrease from admission to discharge as dependent variable (CTQ total: p=0.018; EN: p=0.005; EA: p=0.013), again with CT associating to better response for low BD PRS and worse for high PRS (Fig. 12).

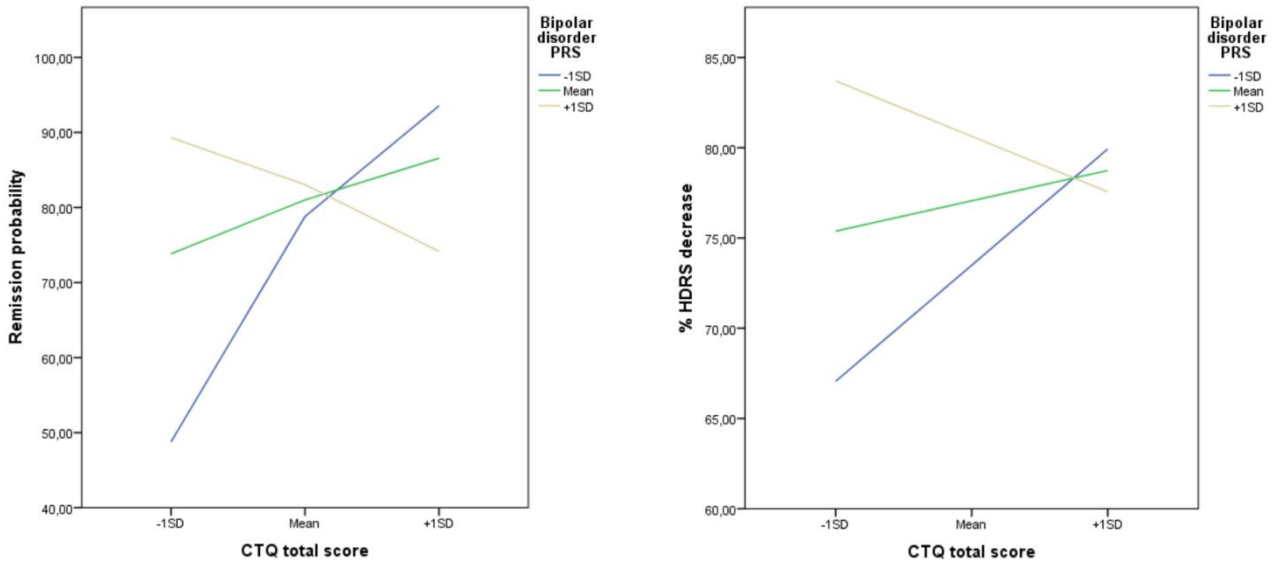


Fig. 12: Effects of childhood trauma x BD PRS interaction on response patterns. Left: remission at discharge. Right: percentage of decrease in HDRS from admission to discharge.

No significant CT x MDD PRS was identified both for treatment resistance and remission at discharge.

No significant CT x BD PRS or MDD PRS interaction on brain volumes or cortical thickness was identified.

BD PRS on the other hand significantly modulated the relation between CT and DTI metrics. Specifically, BD PRS significantly moderated the relation of CTQ total score with FA (p=0.008)(Fig. 13) and RD (p=0.013); EA with FA (p=0.001) and RD (p=0.016); PA with FA (p=0.004), MD (p=0.019) and RD (p=0.005); PN with FA (p=0.019) and RD (p=0.031); The Johnson-Neyman output showed negative associations between CT and FA and positive between CT, RD and MD for higher values of BD PRS (albeit statistically significant only for EA and with trend towards significance for CTQ, PA and PN) and an opposite trend for the lower BD PRS values.

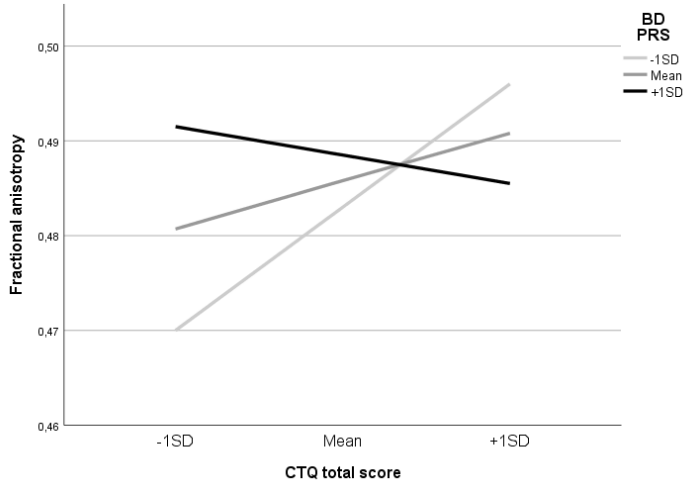
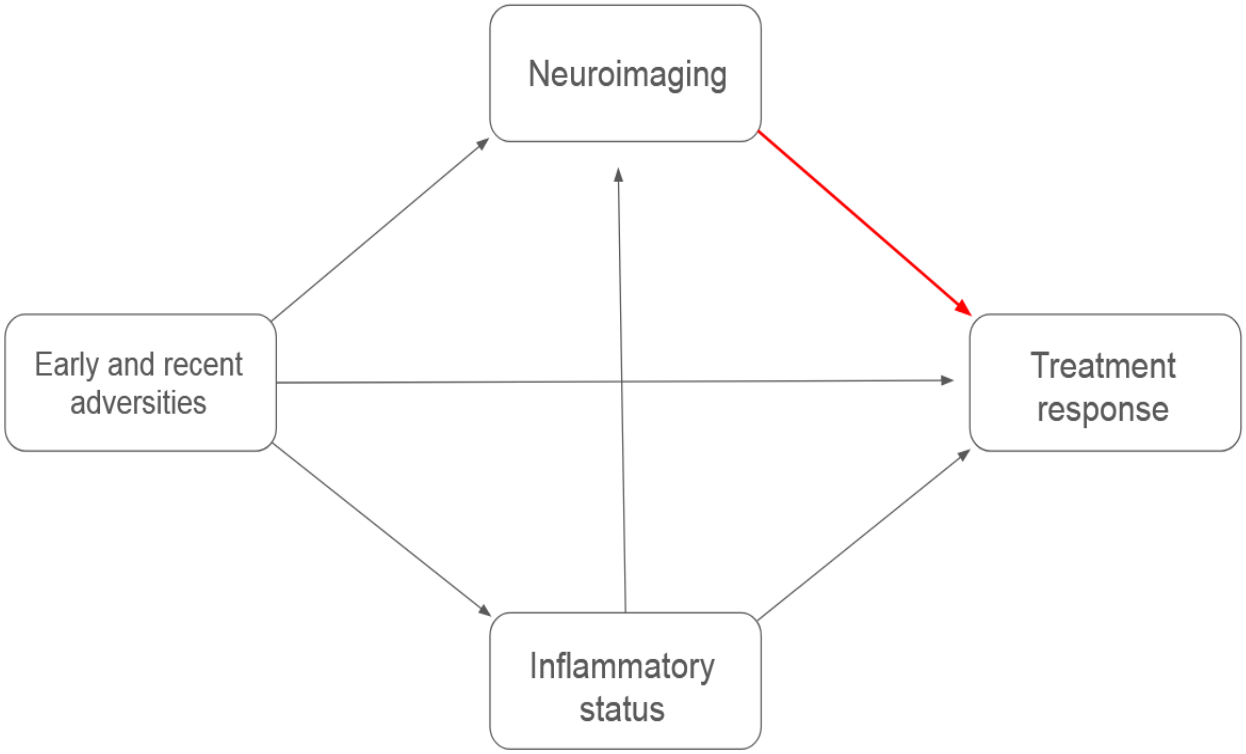


Fig. 13: Effects of childhood trauma x BD PRS interaction on fractional anisotropy.

Finally BD PRS significantly modulated the effect of physical (p=0.004) and sexual abuse (p=0.017) on peripheral cytokines. For physical abuse this was true for TNF-α (p<0.001) and IL-4 (p=0.036); for sexual abuse on the other hand significant interactions were found for Eotaxin (p=0.028), MCP-1 (p=0.021), HGF (p<0.001). In all cases positive associations between childhood stressors and peripheral cytokines were only found at higher values of BD PRS.

6. Relation between neuroimaging and treatment response and resistance



Voxel based morphometry and cortical thickness

Significant associations emerged between brain volumes, cortical thickness and both measures of treatment resistance and response.

Treatment resistant patients, compared to non-resistant ones, had significantly lower GM volumes in the right superior and middle temporal gyrus (pFWE=0.007), bilateral thalami and hippocampi (L: pFWE=0.024; R: pFWE=0.033), right inferior occipital gyrus (pFWE=0.026), left cerebellum (pFWE=0.003), right supramarginal gyrus (pFWE=0.023) and right temporal pole (pFWE=0.044) (Fig. 14) (Tab. 2).

This was paralleled by reduction in cortical thickness in the left postcentral and supramarginal gyrus (pFWE=0.008), bilateral middle and anterior cingulate gyrus (R: pFWE=0.048; L: pFWE=0.006), left fusiform and lingular gyrus (pFWE=0.043) (Fig. 15) (Tab. 3).

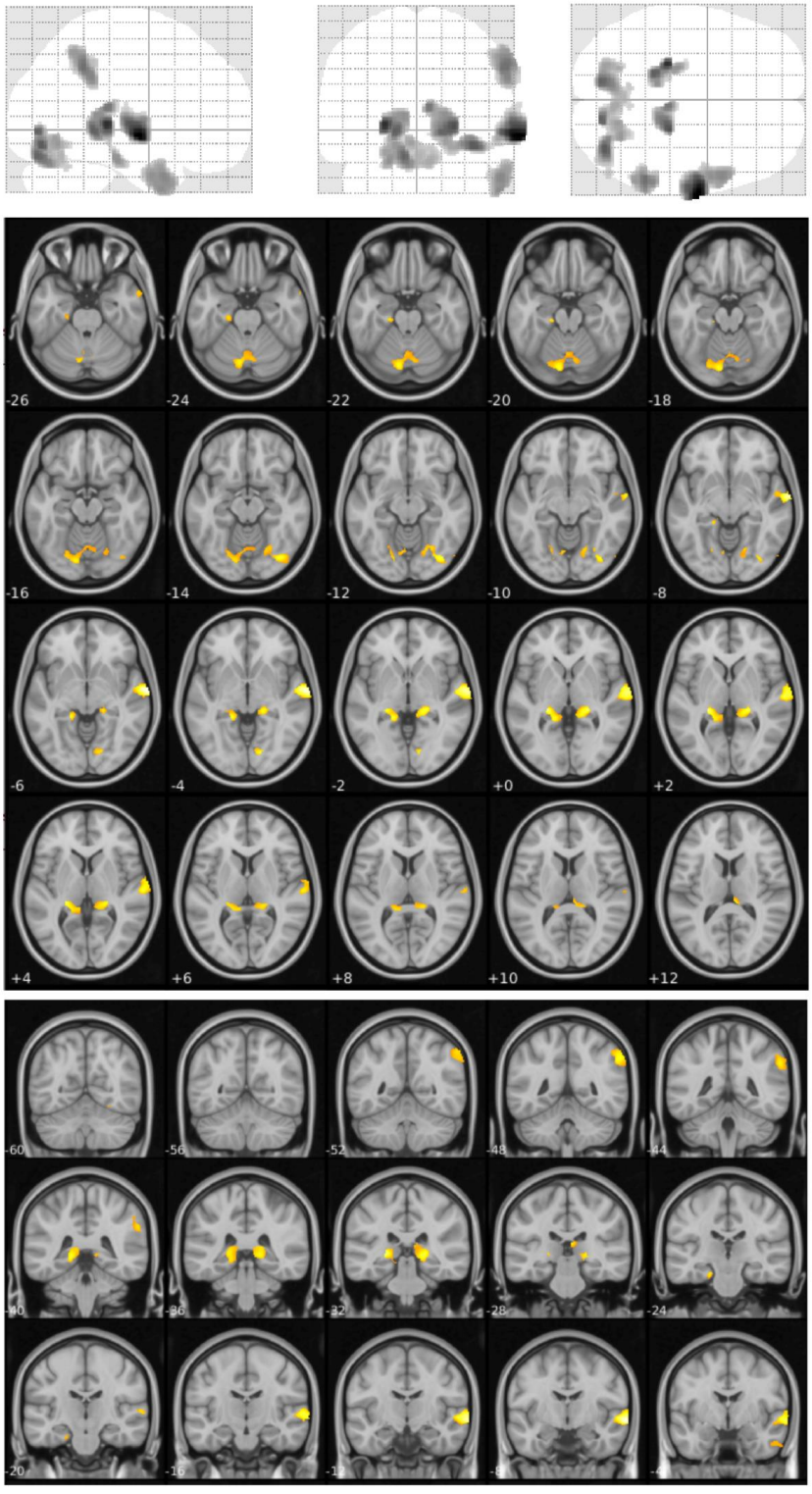


Fig. 14: brain regions of reduced grey matter volume in treatment resistant patients compared to non-treatment resistant ones.

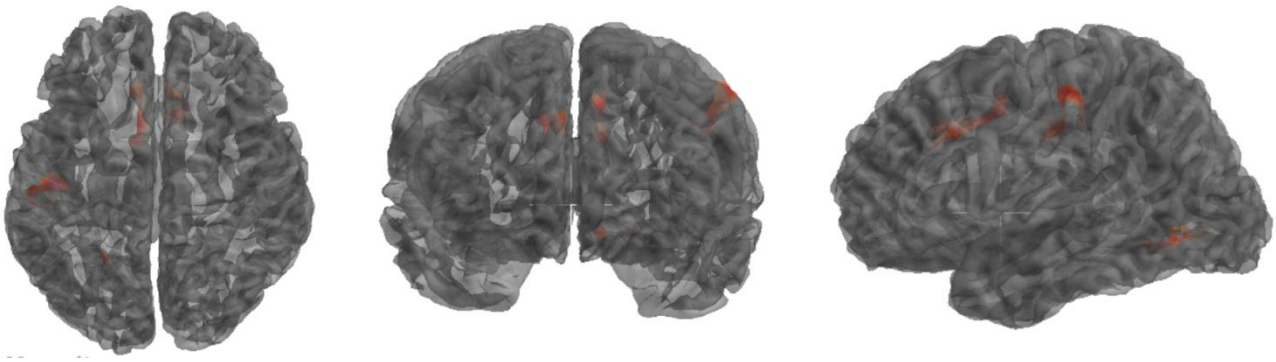


Fig. 15: brain regions of reduced cortical thickness in treatment resistant patients compared to non-treatment resistant ones.

Concerning treatment response, patients who did not achieve symptom remission upon discharge had significantly reduced cortical thickness in the right anterior cingulate gyrus (pFWE=0.006) (Fig. 16) compared to those who did. This was further confirmed exploring the effect of the percentage of reduction in HDRS scores from admission to discharge, which was positively associated with cortical thickness in the right anterior cingulate gyrus (pFWE=0.034) (Fig. 16) (Tab. 3).

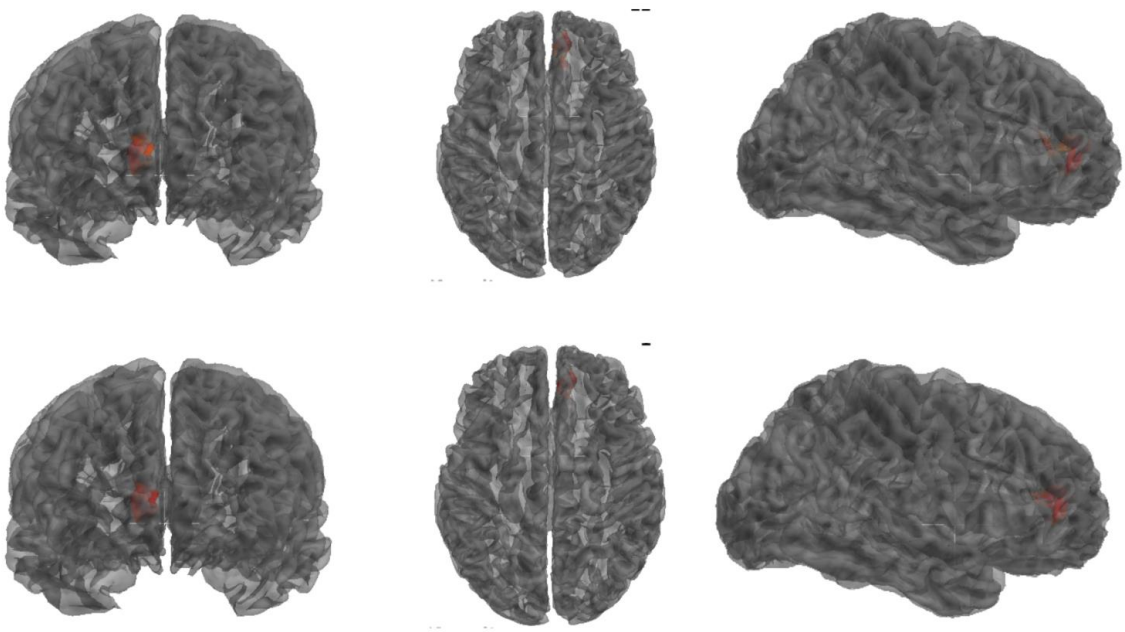


Fig 16: Top, brain regions of reduced cortical thickness in patients who did not achieve symptom remission at discharge compared to those who did. Bottom, brain regions in which

cortical thickness was positively associated with the percentage of HDRS decrease from admission to discharge.

In the ROI analysis, lower Hippocampal volumes significantly increased the risk of treatment resistance (L: p=0.006; R: p=0.011) and, for right hippocampus, decreased the possibility of achieving remission at discharge (p=0.030) (Fig. 17).

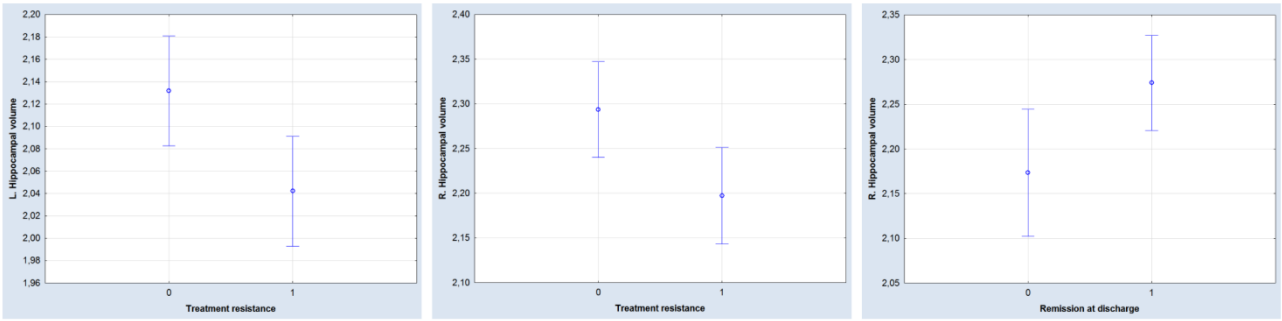


Fig. 17: relation between Hippocampal volumes and response patterns.

Exploring the effect of treatment resistance level according to Thase criteria, a progressive decrease of both right (p=0.017) and left (p=0.008) Hippocampal volumes was observed with increasing levels of resistance (Fig. 18).

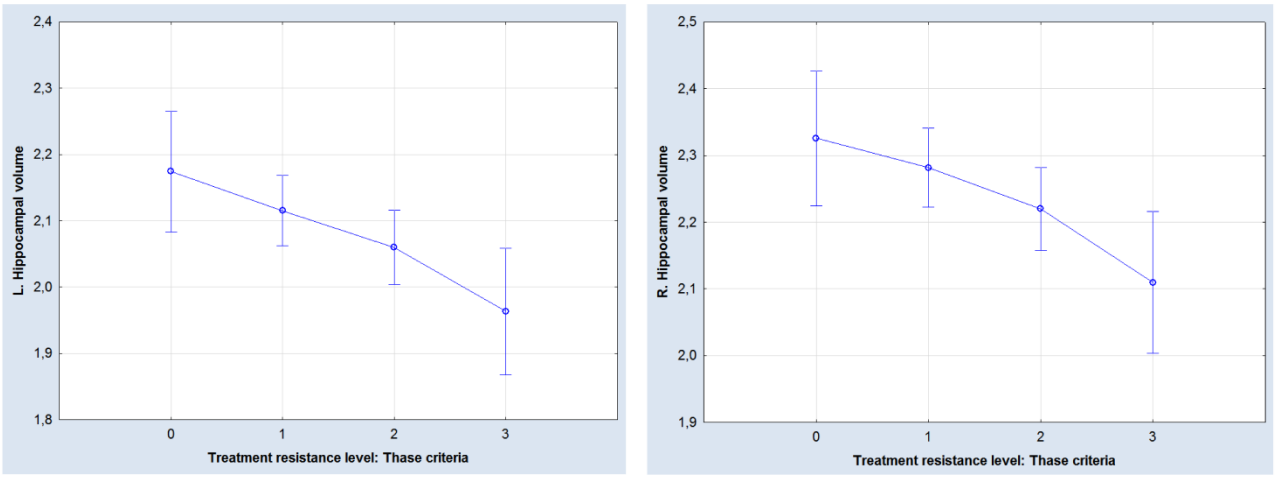


Fig. 18: relation between hippocampal volumes and Thase resistance criteria.

Diffusion Tensor Imaging

No association between treatment resistance and white matter microstructure was found.

On the other hand, exploring the associations between DTI metrics and treatment response, patients who achieved remission upon discharge had significantly higher FA ($p=0.004$) (Fig. 19) and lower RD ($p=0.001$) and MD ($p=0.006$) compared to those who did not in widespread white matter tracts. This was confirmed testing the effect of the percentage of reduction in HDRS scores, which resulted positively associated with FA ($p=0.015$) and negatively with RD ($p=0.027$).

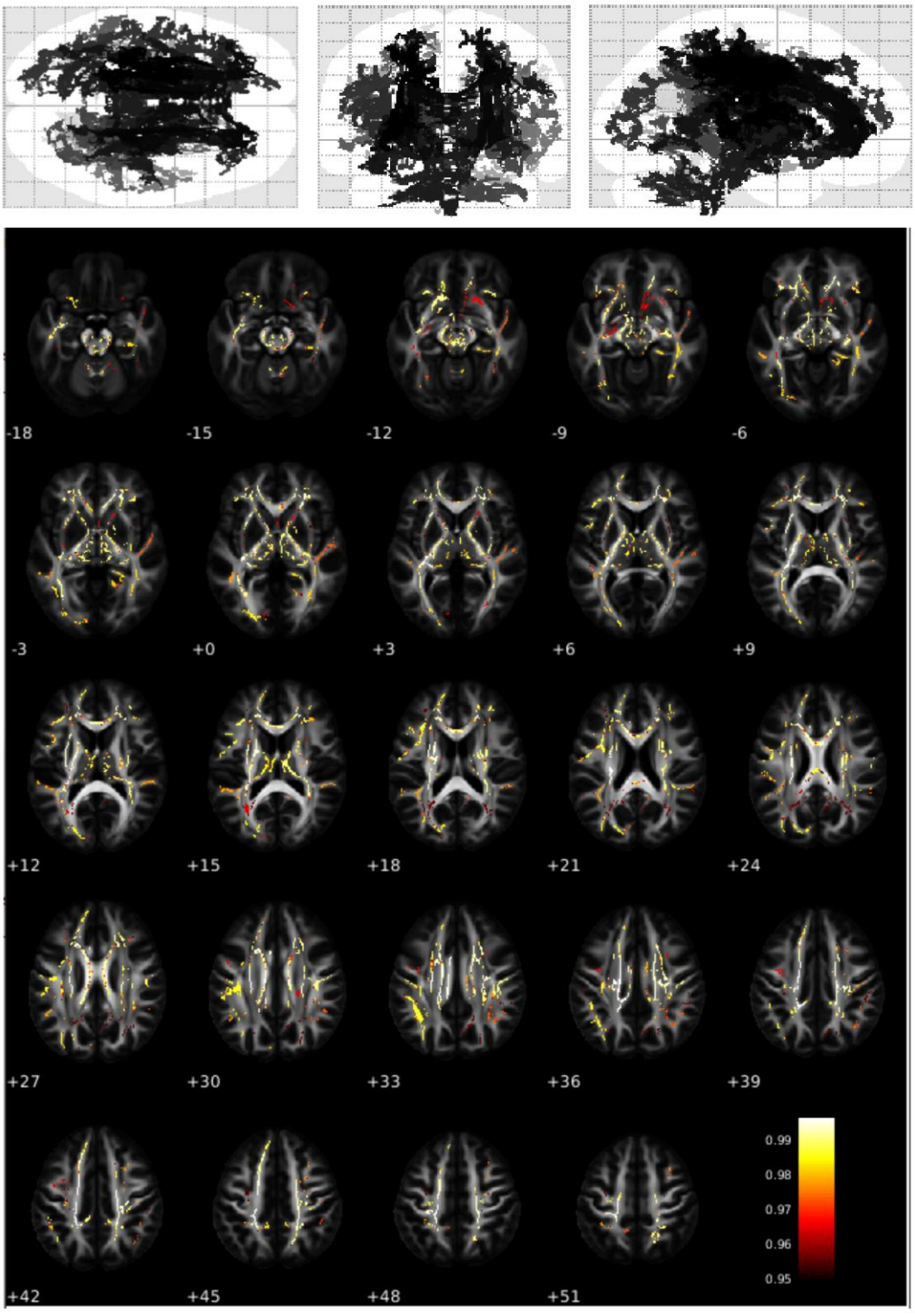


Fig. 19: white matter tracts with reduced fractional anisotropy in patients who failed to achieve symptom remission at discharge compared to those who did.

Resting state functional connectivity

Treatment resistance status significantly modulated rsFC of (1) the ACC with the right frontal pole (pFWE=0.037), with decreased connectivity in treatment resistant patients, and (Zanos & Gould) the left hippocampus with the bilateral superior frontal gyrus (pFWE=0.019), again with reduced connectivity in TRD (Fig. 20).

Concerning treatment response, patients who achieved symptom remission upon discharge had significantly lower rsFC between the left amygdala, and the left parahippocampal gyrus (pFWE=0.007) compared to those who did not (Fig. 21) (Tab. 4).

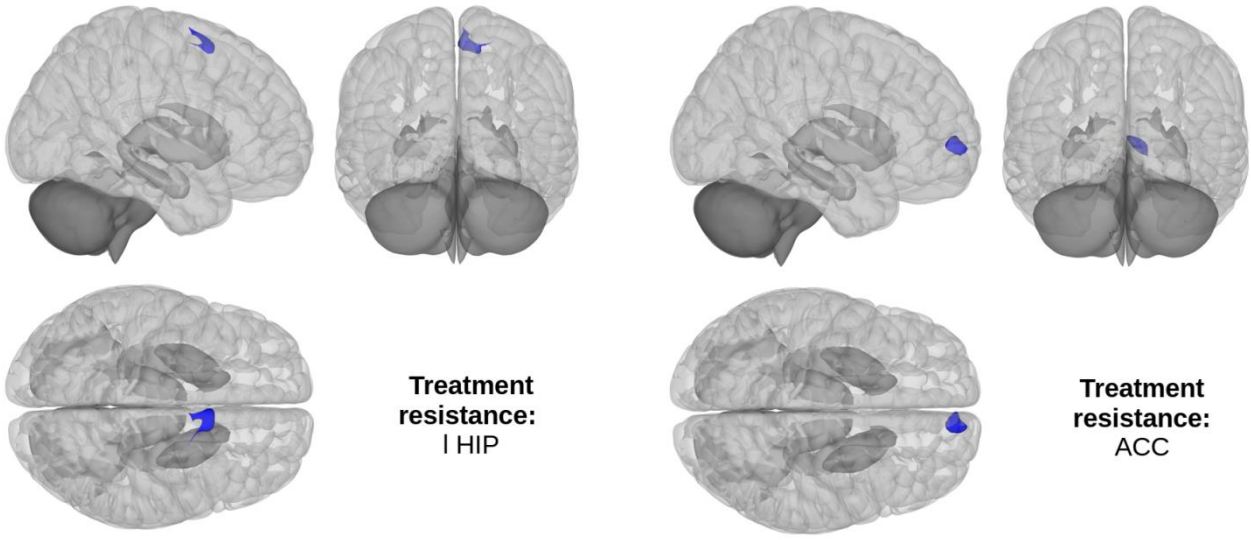


Fig. 20: brain regions of reduced functional connectivity with left Hippocampus (left) and anterior cingulate cortex in treatment resistant patients.

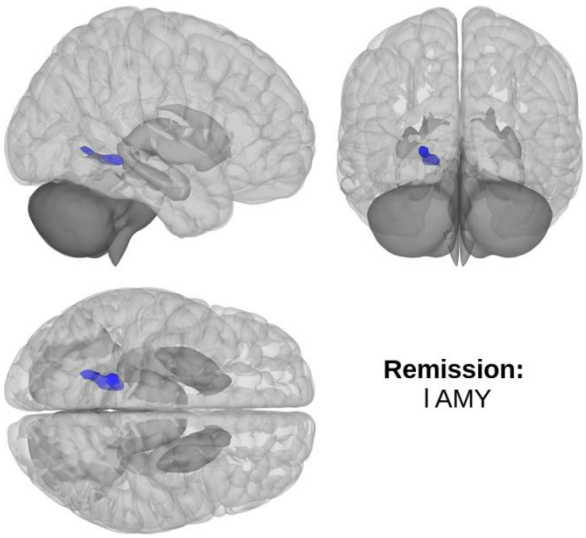
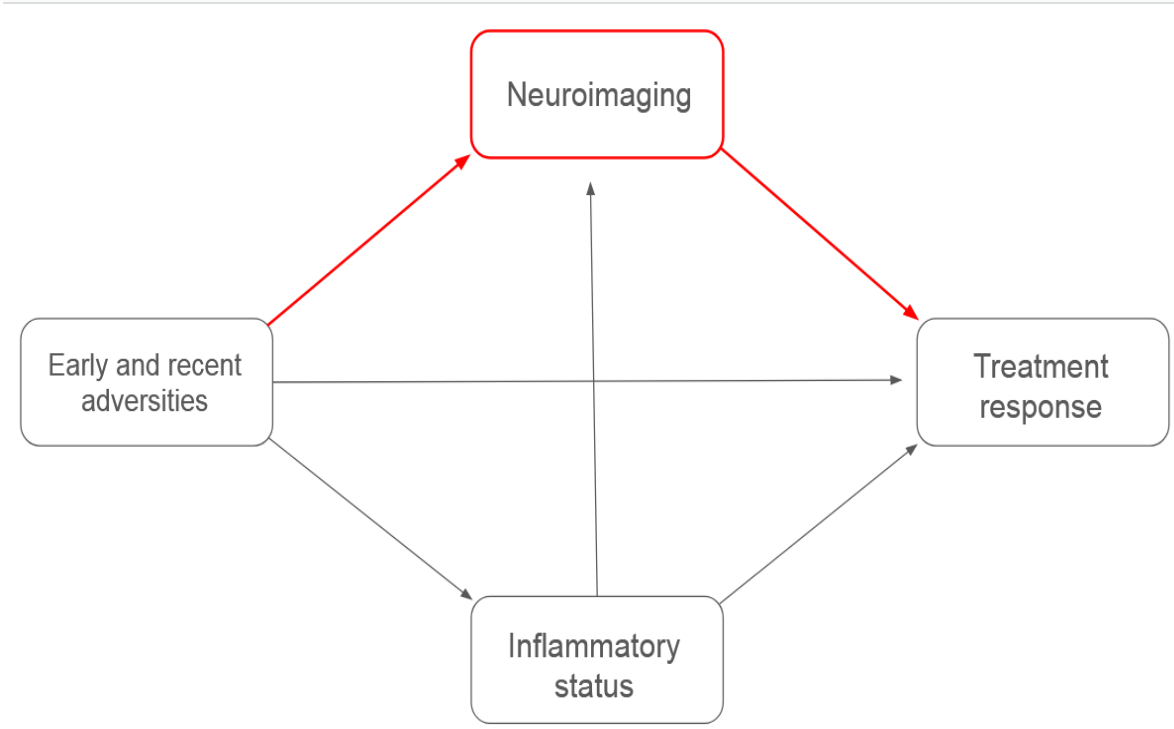


Fig. 21: brain regions of reduced functional connectivity with left Amygdala in patients who achieved symptomatic remission at discharge.

7. Moderated mediation analysis: childhood and recent adversities, neuroimaging and treatment response.



A significant moderated mediation was identified between SRE-S and emotional abuse, FA and remission at discharge (Index of moderated mediation: -0.00097, 95% BCa CI [-0.00295, -0.00003]): EA significantly moderated the effect of SRE-S on FA (p=0.019), with significant negative associations only in EA+ subjects (b=-0.00003, p=0.003); FA was significantly associated with remission at discharge (p=0.016), and significant indirect effects between SRE-S, FA and remission were identified only in EA+ subjects (95% BCa CI [-0.00201, -0.00022]) (Fig. 22).

Similar patterns were identified for SRE-S, CTQ total and FA (SRE-S x CTQ interaction on FA: p=0.041; SRE-S effect on FA in CTQ+ subjects: b=-0.0003, p<0.001; indirect effect on remission through FA in CTQ+: 95% BCa CI [-0.00213, -0.00027]); however the index of moderated mediation (IN) was not significant (95% BCa CI [-0.00276, 0.00011]).

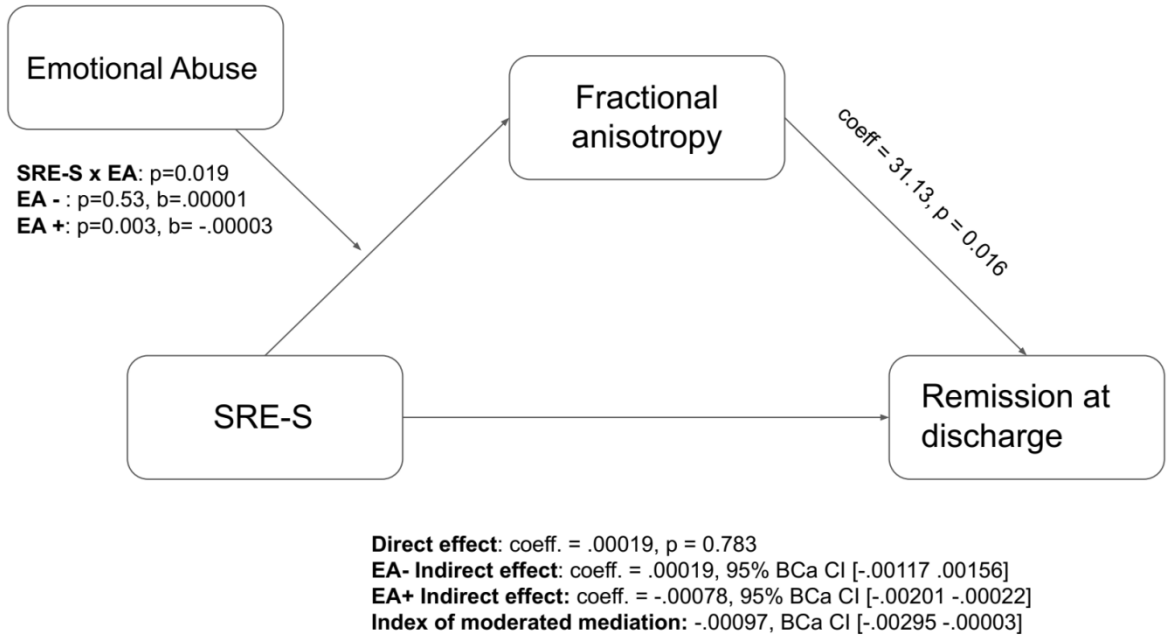


Fig. 22: moderated mediation analysis between SRE-S, childhood emotional abuse, Fractional anisotropy and remission at discharge.

8. Moderated mediation analysis: childhood trauma, BD PRS, neuroimaging and treatment response

Significant moderated mediations were identified between Childhood trauma, BD PRS, DTI metrics and treatment response. Specifically BD PRS moderated the relation between CTQ total (p=0.013), EA (p=0.006), PN (p=0.007) and FA, which in turn was positively associated with remission at discharge (p<0.001) and percentage of HDRS decrease (PERC) from admission to discharge (p<0.001). Significant indirect effects were identified of childhood trauma on response patterns through its BD PRS-moderated effect on FA. The following indexes of moderated mediation (IN) were therefore significant: CTQ total on remission, IN=-0.3850, 95% BCa CI [-1.7999, -

0.0123]; EA on remission, IN=-1.2531, 95% BCa CI [-4.3533, -0.0434]; PN on remission, IN=-1.4940, 95% BCa CI [-5.5554, -0.3779]; CTQ total on PERC, IN=-0.0192, 95% BCa CI [-0.0466, -0.0012]; EA on PERC, IN=-0.0680, 95% BCa CI [-0.1380, -0.0059]; PN on PERC, IN=-0.0703, 95% BCa CI [-0.1777, -0.0088].

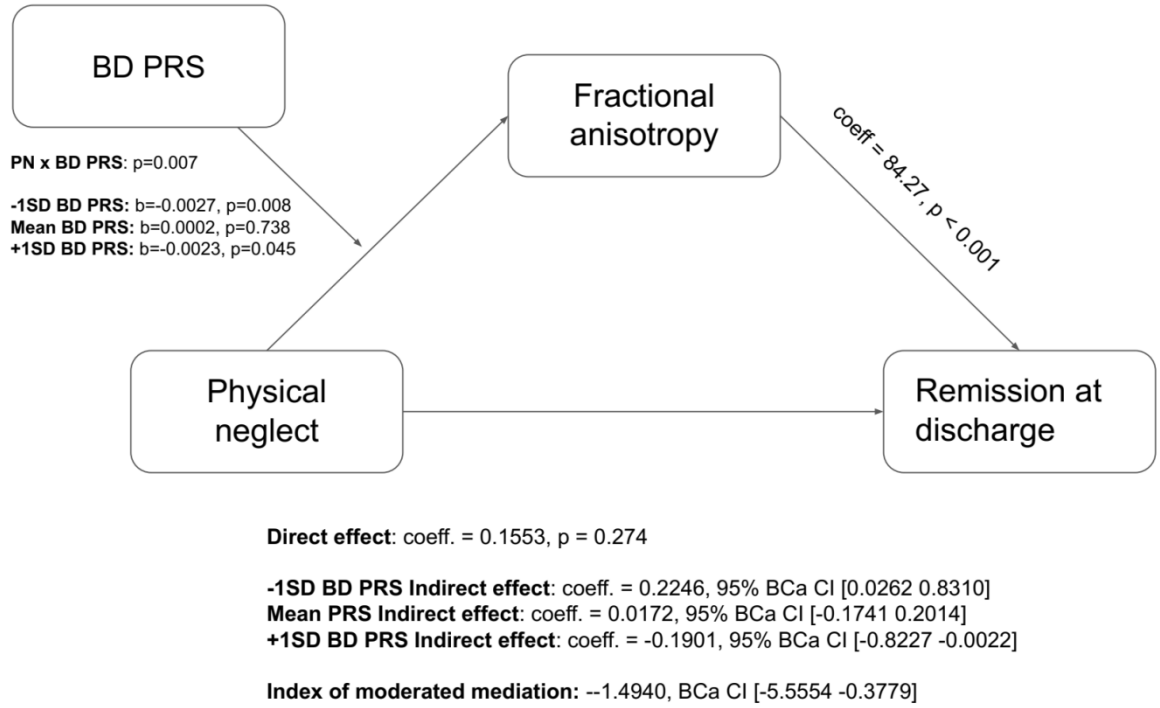
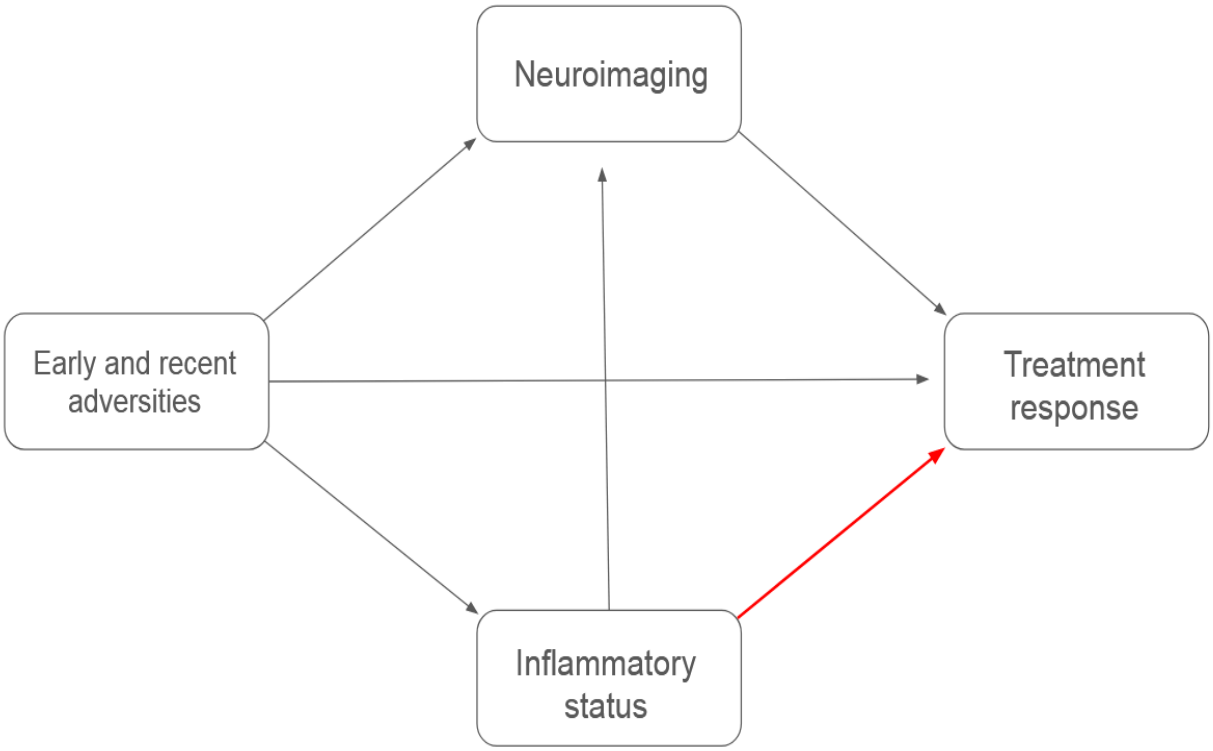


Fig. 23: Moderated mediation analysis between childhood physical neglect, bipolar disorder polygenic risk score, fractional anisotropy and remission at discharge.

9. Effect of inflammatory status on treatment response



No effect on treatment response or resistance was identified for inflammatory ratios. Monocyte absolute count was significantly associated with treatment resistance (p=0.013), with increasing levels associated with higher resistance rates (Fig. 24).

Elastic net models testing the association between peripheral cytokines and treatment resistance or remission at discharge yielded poor predictive performances (Treatment resistance: Sensitivity= 0.78; Specificity=0.28; Balance accuracy=0.53; AUC=0.52. Remission at discharge: Sensitivity=0.56; Specificity=0.59; Balance accuracy=0.58; AUC=0.53); no peripheral marker surpassed the VIP threshold of 70.

CRP levels on the other hand significantly predicted treatment resistance status (p=0.038), with higher levels associated to higher resistance rates (Fig. 24).

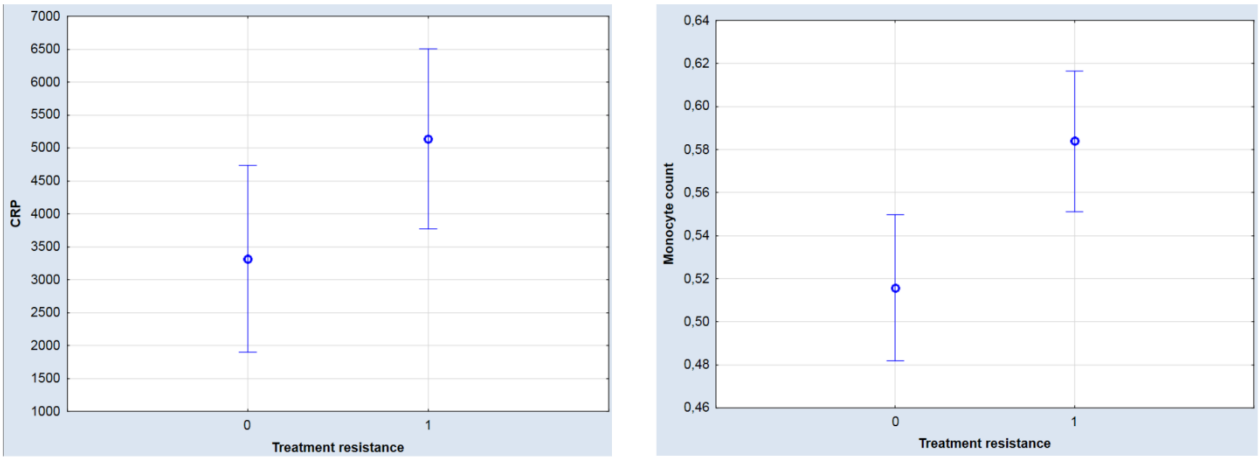
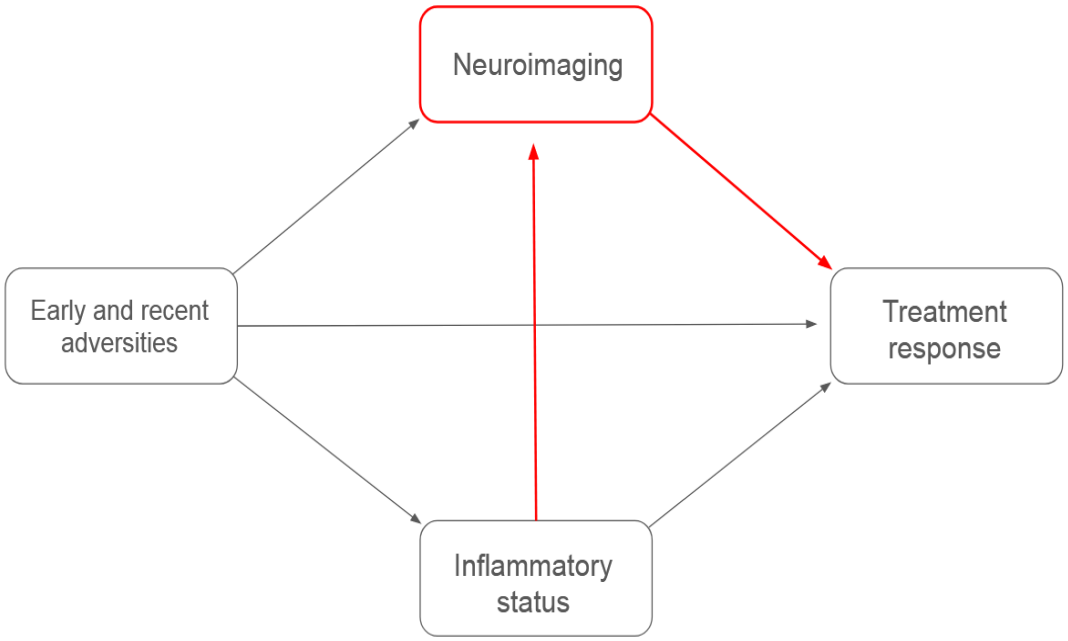


Fig. 24: association between C-reactive protein and Monocyte count and treatment resistance status.

10. Effect of CRP on neuroimaging and mediation analysis



No statistically significant result emerged when exploring the effect of CRP through VBM or cortical thickness analysis. However, in the ROI analysis, CRP levels were

negatively associated to bilateral hippocampal volumes (R: p=0.016, β= -0.31; L: p=0.011, β= -0.32).

In the mediation analysis, significant indirect effects were found for left hippocampal volumes: CRP levels affected treatment resistance status through their effect on left hippocampus (95% BCa CI [0.000001, 0.000135]) (Fig. 25).

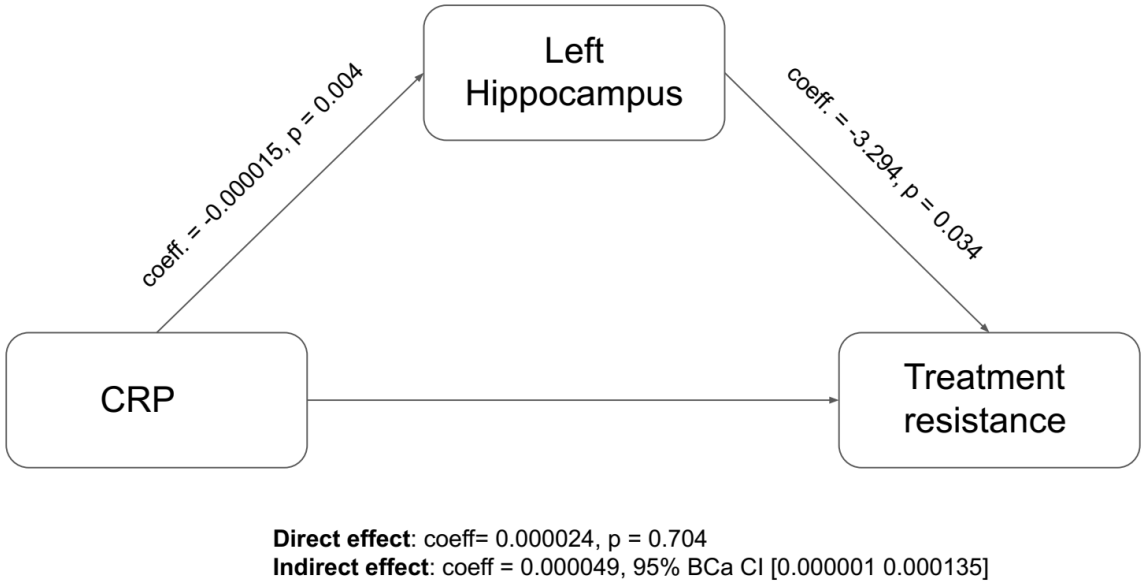


Fig. 25: mediation analysis between C-reactive protein, left hippocampal volumes and treatment resistance status.

11. Longitudinal analysis

In the VBM longitudinal analysis, patients exhibited a marked increase over time in brain volumes in a sub-cortical cluster mainly encompassing the left thalamus and caudate (pFWE=0.001) (Fig. 26) (Tab. 2); exploring TRD and non-TRD patients separately, such volumetric increase appeared evident only in treatment resistant patients (pFWE=0.003). The differential effect in TRD and non TRD for left thalamus showed a trend towards statistical significance (p=0.066) (Fig. 27).

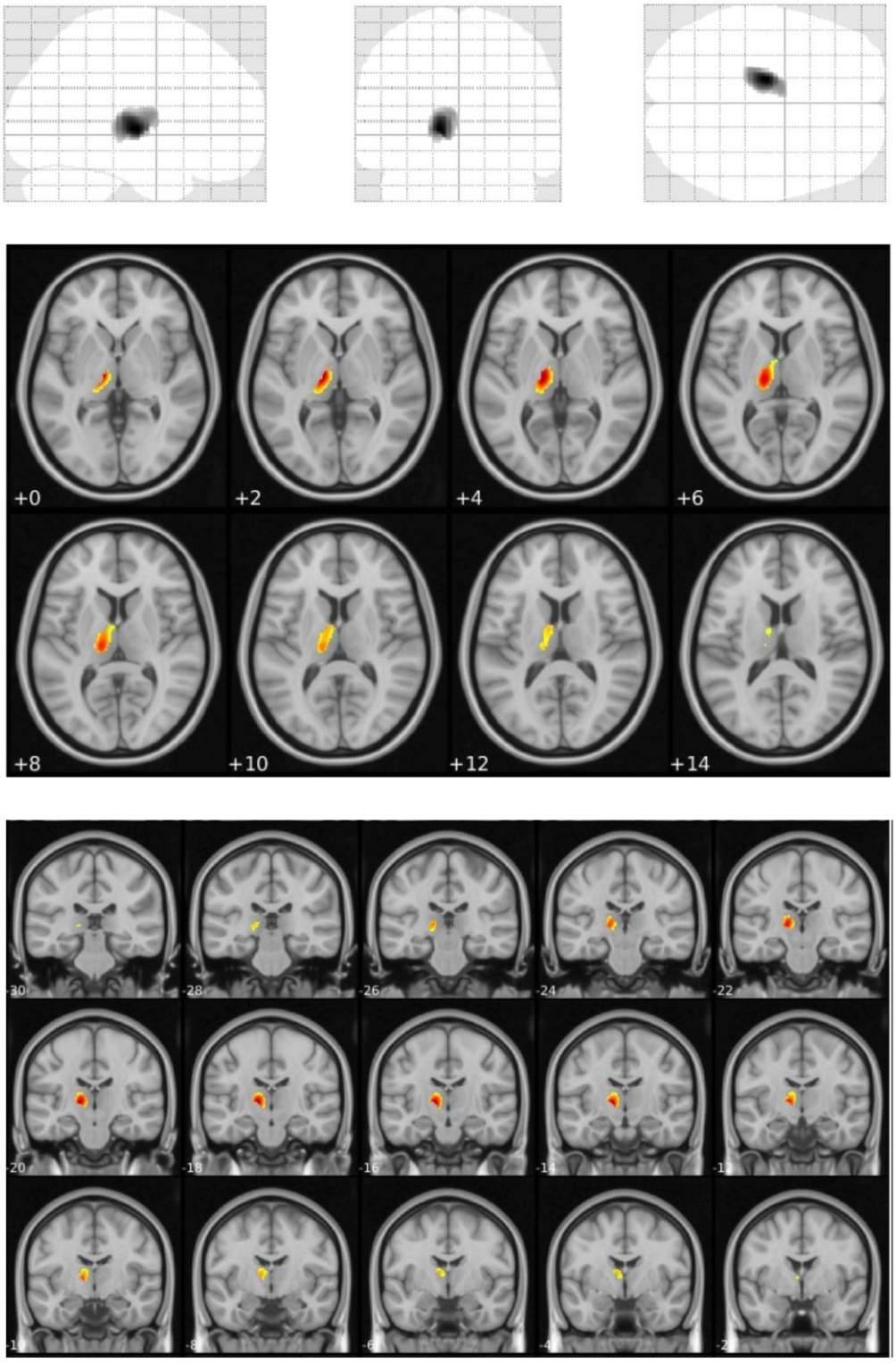


Fig. 26: Brain regions exhibiting volumetric increases after treatment.

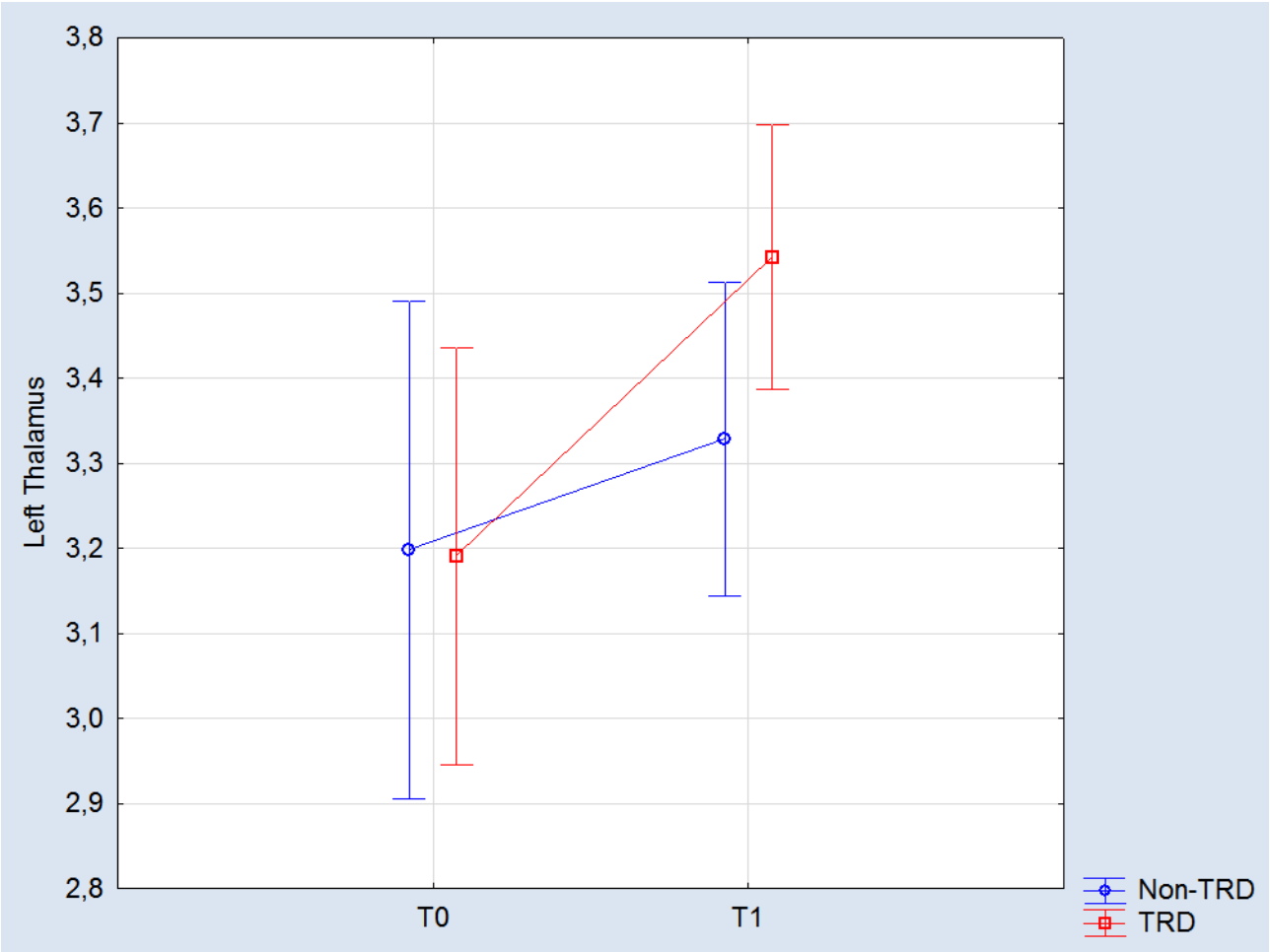


Fig. 27: longitudinal changes in left Thalamus volumes, difference between treatment resistant and treatment non-resistant patients.

Concerning cortical thickness, no statistically significant change was apparent in the whole sample. However, exploring changes separately in TRD and non-TRD, the latter showed an increase in a cluster encompassing the left fusiform and parahippocampal gyrus (pFWE=0.019) (Fig. 28). Testing the differential longitudinal changes in TRD and non TRD, significant differences appeared for both left hippocampus (0.028) and parahippocampal cortex (p=0.014), with increases only in non-TRD (significant for lPHC, p=0.006) (Fig. 29) (Tab. 3).

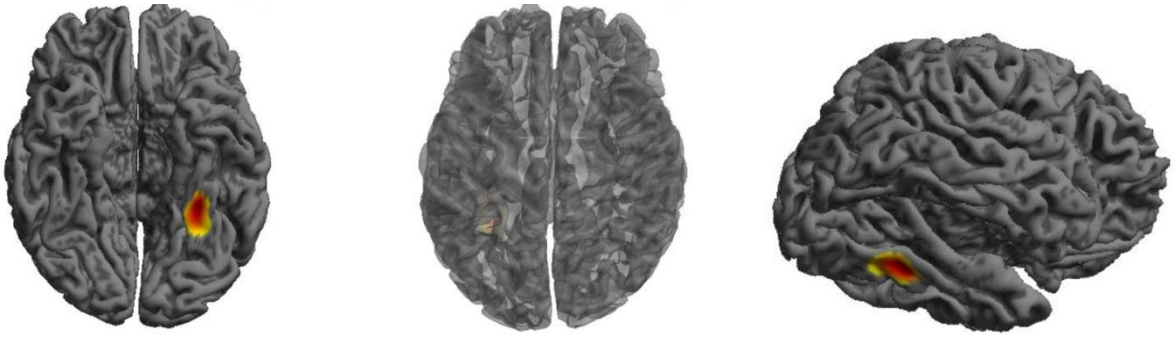


Fig. 28: longitudinal increases in cortical thickness in treatment non-resistant patients.

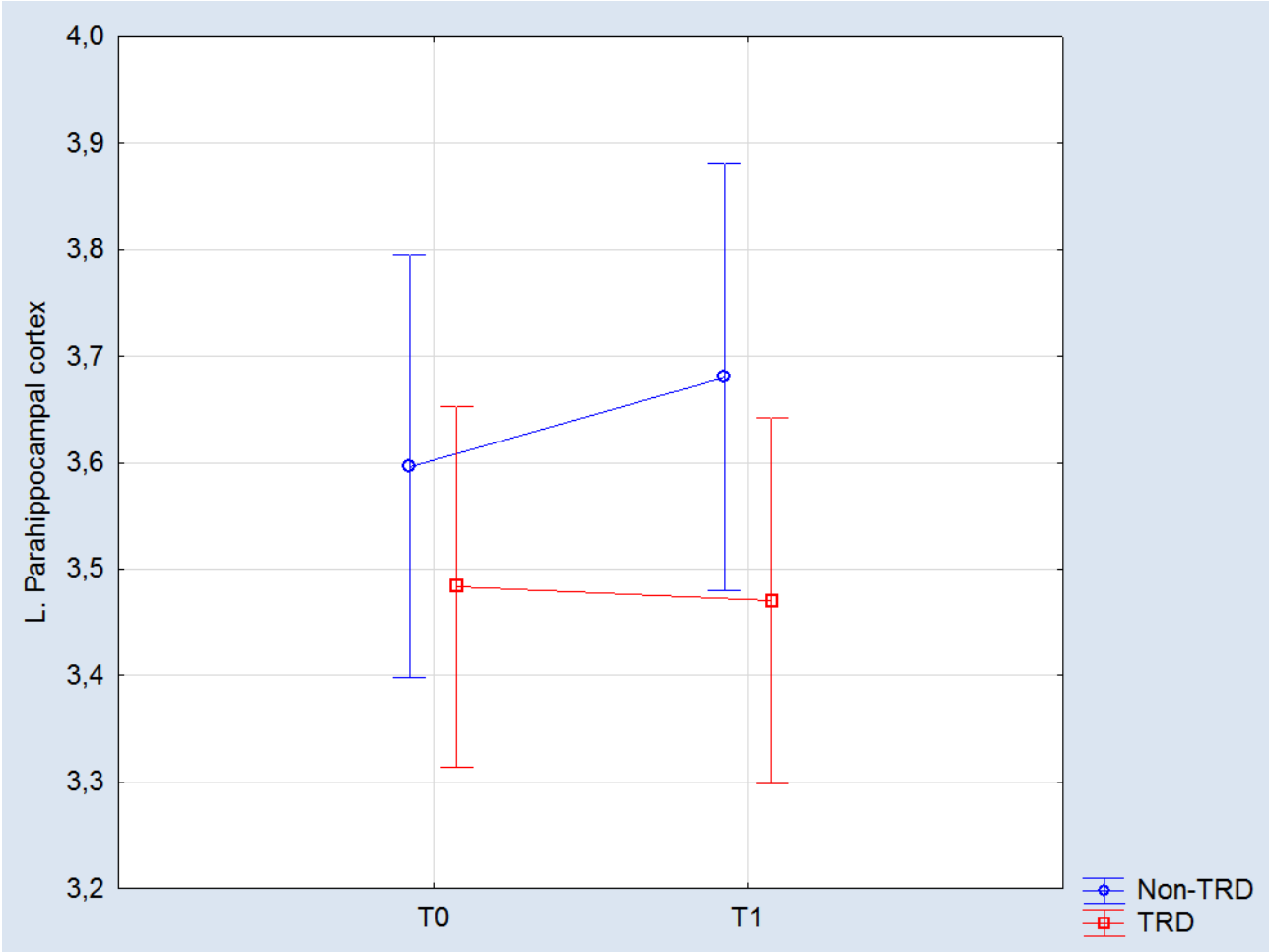


Fig. 29: longitudinal changes in left parahippocampal cortex thickness between treatment resistant and treatment non-resistant patients.

Concerning DTI metrics, the whole sample exhibited a general decrease of FA (p=0.020) and increase of RD (p=0.007) and MD (p=0.011) over time. No significant interactions were identified for TRD vs non-TRD or for remitters vs non-remitters; however, a significant differential effect over time for FA emerged comparing patients that still presented depressive symptomatology at T1 compared to remitted ones (p=0.037), with significant decreases of FA only in the first group (p=0.002) (Fig. 30).

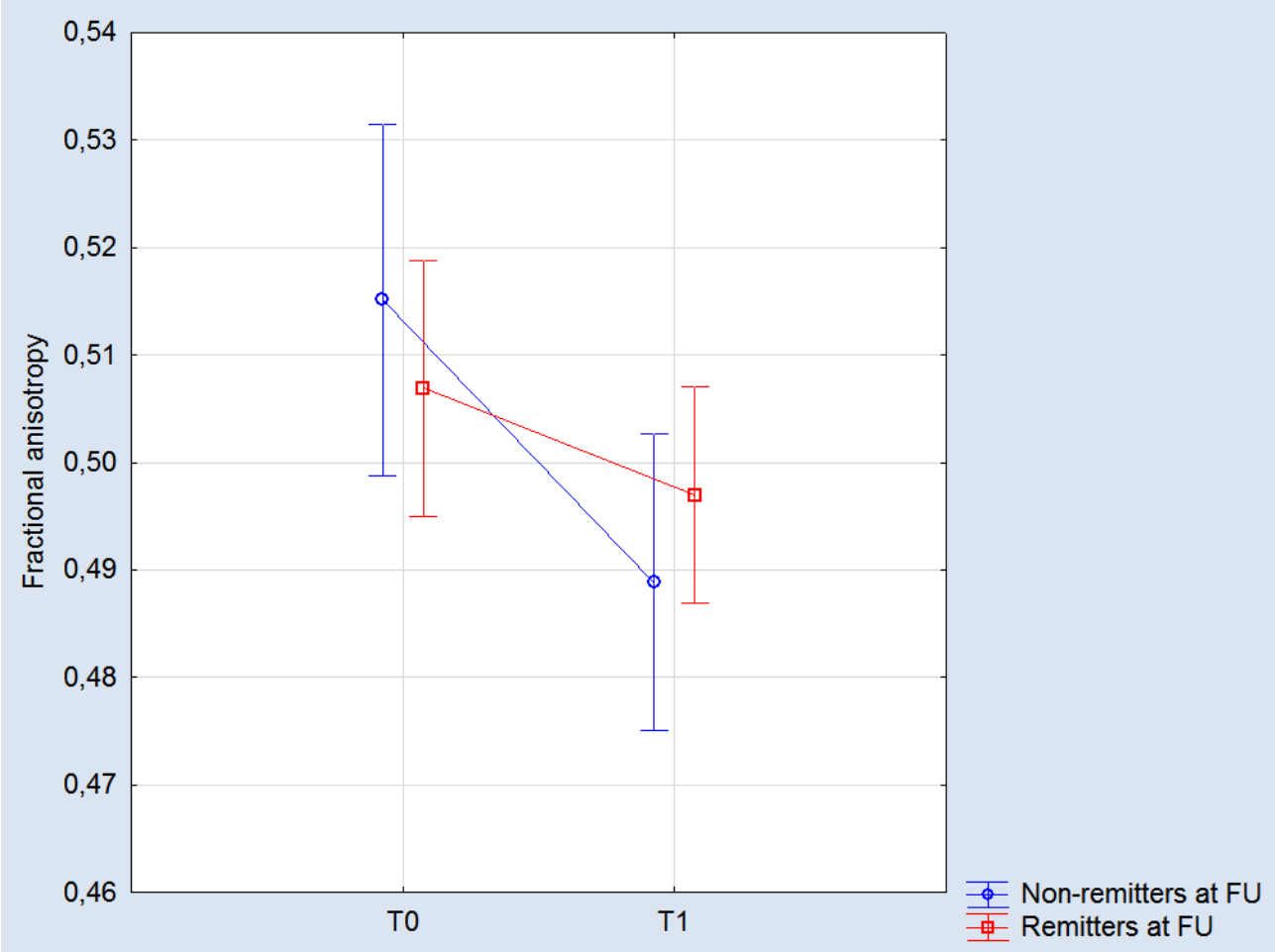


Fig 30: longitudinal changes in fractional anisotropy between symptomatic remitters and non-remitters at follow up.

For rsFC, right hippocampal connectivity with the right insula decreased over time (pFWE=0.013) (Fig. 31). Performing the analysis separately according to resistance

status, no significant changes in connectivity were identified in TRD patients. In non TRD patients on the other hand several changes in rsFC appeared: decreased connectivity FC between right and left hippocampi and posterior cingulate (pFWE=0.017); increased connectivity between left hippocampus and right superior frontal gyrus (pFWE=0.005); decreased FC between right amygdala and left frontal pole (pFWE=0.023) (Fig. 32) (Tab. 4).

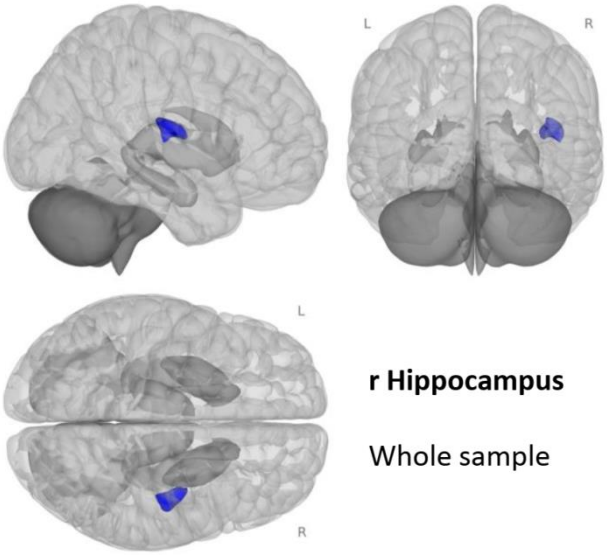


Fig. 31: right hippocampus functional connectivity longitudinal changes in the whole sample

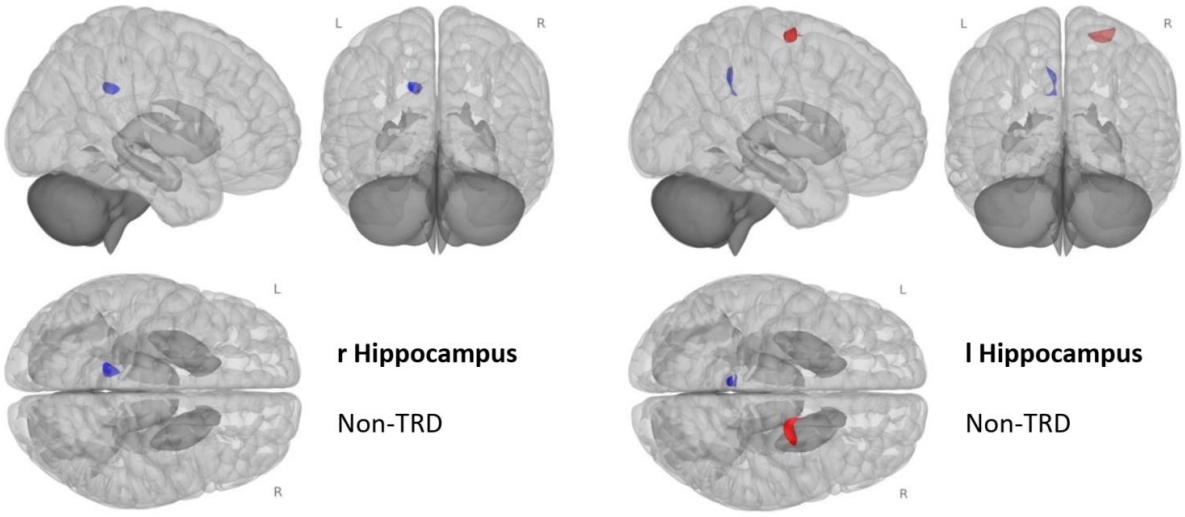


Fig. 32: right and left hippocampus functional connectivity longitudinal changes only in treatment non-resistant patients.

VBM analysis					
Variable of interest	**Peak MNI**	***k***	**pFWE**	**Brain areas**	**Direction of effect**
PSS	63 -12 9	1260	0.014	r central operculum r planum temporale r STG l insula	↓ GM
	-57 6 2	1088	0.024	l central operculum l planum temporale l insula	↓ GM
PSS x PA	2 58 -14	1109	0.021	medial frontal cortex frontal pole	↓ PA - , ↑ PA +

				ACC	
	56 -14 -16	1044	0.026	r middle temporal gyrus r planum polare r insula	↓ PA - , ↑ PA +
PA -, PSS	8 60 4	2448	<0.001	medial frontal gyrus fronal pole ACC	↓ GM
	62 -14 9	1062	0.024	r planum temporale r central operculum rinsula	↓ GM
	-57 8 0	980	0.032	l planum polare l central operculum l insula	↓ GM
PA +, PSS	6 63 20	414	0.048 (peak)	r medial frontal gyrus r frontal pole	↑ GM
	46 -16 -9	714	0.085	r planum polare r sup. temporal gyrus r insula	↑ GM
Treatment resistance	69 -9 -6	1442	0.007	r middle temporal gyrus	↓ GM in TRD

				r superior temporal gyrus	
	-24 -32 2	1077	0.024	l thalamus l hippocampus	↓ GM in TRD
	22 -30 0	989	0.033	r thalamus r hippocampus	↓ GM in TRD
	38 -76 -14	1064	0.026	r occipital fusiform gyrus r inferior occipital gyrus	↓ GM in TRD
	-10 -80 -20	1721	0.003	l cerebellum	↓ GM in TRD
	58 -48 40	1095	0.023	r supramarginal gyrus	↓ GM in TRD
	56 9 -34	908	0.044	r temporal pole	↓ GM in TRD
Longitudinal analysis	-15 -18 2	1247	0.001	l thalamus and caudate	↑ GM over time

Tab. 2: Voxel based morphometry results.

Cortical thickness					
Variable of interest	**Peak MNI**	***k***	**pFWE**	**Brain areas**	**Direction of effect**
PSS	39 14 30	103	0.043	opercular part of the inferior frontal gyrus	↓ CT
	6 3 48	104	0.039	middle cingulate gyrus	↓ CT

SRE-S x CTQ	3 49 23	210	0.001	Superior medial frontal gyrus ACC	↑CTQ - , ↓ CTQ +
SRE-S, CTQ +	5 53 8	292	<0.001	Superior medial frontal gyrus ACC	↓ CT
Treatment resistance	-47 -19 31	150	0.008	left postcentral and supramarginal gyrus	↓ CT in TRD
	11 15 32	101	0.048	r middle and anterior cingulate gyrus	↓ CT in TRD
	-10 4 36	158	0.006	l middle and anterior cingulate gyrus	↓ CT in TRD
	-25 -55 -10	104	0.043	l fusiform and lingular gyrus	↓ CT in TRD
Remission at discharge	14 49 14	158	0.006	r anterior cingulate gyrus	↓ CT in non-remitters
Longitudinal analusis: TRD -	-28 -33 -31	61	0.019 (peak)	l fusiform and parahippocampal gyrus	↑ CT over time
Longitudinal analysis: TRD +	4 -28 36	96	0.032 (peak)	r middle cingulate gyrus	↓ CT over time

Tab. 3: Cortical thickness (CT) results.

Resting state functional connectivity						
Variable of interest	**Seed**	**Peak MNI**	***k***	**pFWE**	**Brain areas**	**Direction of effect**
CTQ total score	l Amygdala	-50 -16 6	183	0.020	l opercular cortex	↓ FC
PSS	r Hippocampus	-40 32 44	221	0.009	Middle Frontal Gyrus	↓ FC
SRE-S	r Hippocampus	24 -76 46	215	0.010	r Lateral Occipital Cortex	↓ FC
SRE-S	l Hippocampus	4 38 36	215	0.009	Paracingulate Gyrus	↑ FC
PSS x PA	l Amygdala	-30 -10 -42	152	0.045	l Temporal Pole	↓ FC in PA +
PSS x PN	r Amygdala	-10 -60 44	156	0.039	Precuneous Cortex	↑ FC in EA+, ↓ FC in EA -
SRE-N x PA	l Amygdala	4 -48 10	233	0.007	posterior cingulate gyrus	↓ FC in PA +
SRE-N x EN	l Hippocampus	-16 0 70	215	0.009	l Superior Frontal Gyrus	↑ FC in EN+, ↓ FC in EN -
SRE-S x EA	l Amygdala	12 -84 12	375	<0.001	bilateral Intracalcarine Cortex	↑ FC in EA+, ↓ FC in EA -

SRE-S x SA	l Hippocampus	16 -74 10	241	0.005	r Intracalcarine Cortex	↑ FC in SA+
Long. analysis: whole sample	r Hippocampus	44 -14 14	248	0.013	r insula	↓ FC over time
Long. Analysis: non TRD	l Hippocampus	-14 -42 30	110	0.017	posterior cingulate cortex	↓ FC over time
	l Hippocampus	24 -12 64	146	0.005	r superior frontal gyrus	↑ FC over time
		-6 -44 40	96	0.042	posterior cingulate cortex	↓ FC over time
	r Amygdala	-54 40 4	102	0.023	left frontal pole	↓ FC over time

Tab. 4: resting state functional connectivity (FC) results.

Discussion

1. Effect of early and recent adversities on treatment response.

1.1 Childhood adversities

In our sample childhood adversities associated with lower rates of treatment resistance and higher possibility of achieving remission upon discharge from the

psychiatric ward; this held true for both CTQ and RFQ total scores and for physical and emotional abuse and physical neglect subscales.

This result is without doubts counterintuitive; however, it must be seen in light of several elements. Existing literature on the relation between CT and treatment response and resistance in MDD is far from univocal (Giampetruzzi et al., 2023, Williams et al., 2016, Yrondi et al., 2020). A recent systematic review of environmental determinants of treatment resistance in depression did not identify ACEs as a risk factor for TRD (O'Connor et al., 2023); it did however identify higher baseline depression severity as a prominent risk factor. CT has been repeatedly associated to higher baseline depressive symptomatology (Kuzminskaite et al., 2022), and this held true also in our sample: it is possible therefore that at least part of the reported association between CT and poor response in MDD might be due to the confounding effect of higher baseline depressive scores, which on the other hand we controlled for. Indeed, a relatively large TRD outpatients study reported a weak association between CT and remission status at one year follow up, that however was no longer significant when controlling for baseline depressive scores.

A large multicentric study reported a strong negative association between childhood abuse (specifically occurring between the ages of 4 and 7) and response and remission rates at 8 weeks in depressed patients randomized to receive Sertraline, Escitalopram or Venlafaxine (Williams et al., 2016). While the authors did take into account in their analysis the effect of baseline depression severity, several other elements might underlie the inconsistency with our results: the study was performed in an outpatient setting, with a fixed therapeutic scheme employing 3 first line antidepressants and very little use of additional pharmacological treatments; furthermore mean doses of prescribed antidepressants were very close to minimum effective doses (12.3 mg for Escitalopram, 61.1 mg for Sertraline, 83.4 mg for Venlafaxine) (Taylor, Barnes et al., 2021). Our study was on the other hand performed on inpatients in a real world clinical setting, with pharmacotherapies prescribed by the ward psychiatrists according to clinical needs; antidepressants were prescribed at higher mean doses (Imipramine equivalents: 214.9, roughly equivalent to 28 mg of Escitalopram, 154 mg of Sertraline and 234 mg of Venlafaxine, values close to maximum licensed doses) (Taylor et al., 2021); add-on

treatments were frequently used, and clinicians had the possibility of treatment switch if needed. Furthermore, Williams and colleagues in their assessment of CT also recorded the age of trauma occurrence, and specifically found an effect for trauma occurring between the ages of 4 and 7, with no effects identified for trauma occurring after this window. The tools we used to assess CT on the other hand (CTQ and RFQ), while commonly used, are not sensitive to the age window in which trauma occurred, referring broadly to traumatic experiences occurring prior to age 18.

A recent meta-analysis of 20 RCTs and 9 open trials (n=6830) published on Lancet investigated the relation between childhood trauma and response patterns in MDD (Kuzminskaite et al., 2022). The authors found individuals with childhood trauma to have higher baseline depression severity, but to benefit from treatment similarly to patients without trauma. This was true regardless of trauma type or assessment tool. The authors therefore concluded that childhood trauma doesn't affect MDD treatment efficacy, stressing however the importance of controlling for baseline depression severity when assessing treatment response. Interestingly significant differences were identified based on the country of meta-analyzed study, with North American studies reporting higher improvements after treatment in individuals with childhood trauma (Kuzminskaite et al., 2022), in contrast to those done in Asia-Pacific countries. In this regard our results seem to be in line with American studies: the possible cause of this geographical discrepancy remains however to be determined.

Finally a possible explanation of our result might lie in the specificity of our study sample: indeed, while most studies investigating the effect of childhood trauma are usually performed in an outpatient setting, our sample comprised only individuals admitted to our psychiatric ward, and therefore whose depression severity or resistance to treatment didn't allow for an outpatient clinical management. Childhood trauma has been robustly associated to recurrence, chronicity and worse clinical features in MDD (as well as in other psychiatric conditions) (Negele, Kaufhold et al., 2015, Struck, Krug et al., 2020); we could therefore speculate that individuals with a depressive status severe enough to require hospitalization, but nonetheless with no history of childhood trauma, represent a specific subset of MDD patients, with reduced response to conventional treatments.

1.2 Recent stressors

Concerning the effect of recent stressors, we did not find any relation between stressful life events or perceived stress and treatment response or resistance in our sample. This is in partial contrast with results of a recent meta-analysis of 6 RCTs (n=2858) (Buckman et al., 2022), that identified an association between the occurrence of major life events in the six months prior baseline assessment and poorer prognosis at 4 months; however this association was no longer significant when taking into account depression severity and socio-demographic confounders. Furthermore, several well powered naturalistic studies, possibly closer to our experimental design, reported no association between recent stressful life events and treatment response or resistance in MDD (Allen, Harkness et al., 2021, Bock, Bukh et al., 2009, Bukh, Bock et al., 2010).

We did on the other hand find a significant interaction between childhood and recent stressors in affecting response patterns: individuals with a positive history of childhood trauma had lower probability of achieving symptomatic remission with increasing levels of recent stressors, while the opposite effect was observed for subjects with no history of CT. A large outpatient study found no interaction of childhood and recent stressors in affecting response patterns to CBT or physical exercise in mild to moderate cases of depression (Yacaman-Mendez et al., 2019), while to our knowledge no study investigated the possible interplay of CT and recent life events in affecting antidepressant response in more severe cases of depression. However, childhood and recent adversities have repeatedly been shown to interact in determining depression onset and clinical features (Hou, Shang et al., 2023, Rousson, Fleming et al., 2020, Seok, Jeon et al., 2020), suggesting their interplay to be impactful in MDD pathophysiology; our data suggest that their combined effect also impact response patterns to pharmacological treatments.

2. Effect of early and recent adversities on neuroimaging.

2.1 Grey matter volumes and cortical thickness

We did not identify any association between childhood maltreatment and GM volumes or cortical thickness. This is in contrast with various studies reporting reduced GM volumes in hippocampus and other limbic structures as well as fronto-parietal cortical thickness in depressed individuals exposed to CT (Antoniou et al., 2023); however, such studies were often performed on small samples, and are methodologically heterogeneous. On the other hand, two large ENIGMA mega-analyses exploring the effects of CT on subcortical brain volumes (n=3036) (Frodl, Janowitz et al., 2017) and of cortical thickness (n=3872) (Tozzi, Garczarek et al., 2020), report a much less straightforward picture. Concerning sub-cortical structures, the authors found no effect of CT in the whole sample; they did however find a significant CT x sex interaction on caudate volumes, with a negative association between ACEs and bilateral caudate observed only in females; similar effects were also observed for putamen and thalamic volumes, but they did not survive multiple comparison correction. Importantly, the authors did not identify any effect of CT on hippocampal volumes, despite it being previously repeatedly reported in lower powered studies (Frodl et al., 2017). Concerning cortical thickness, a significant negative association was only found between CT, superior temporal sulcus and supramarginal gyrus; interestingly again a CT x sex interaction emerged, with a negative association between ACEs and whole brain cortical thickness apparent only in females, and a positive one with anterior cingulate cortex thickness in males (Tozzi et al., 2020).

Both ENIGMA studies included MDD patients and healthy controls in their analyses, and found no specific effect of childhood trauma in depressed patients. This two large studies might help clarify the relation between CT and brain structure, and partially support our negative results, finding no association between ACEs and subcortical volumes and only a negative association with cortical thickness in two clusters; they did however find in both cases significant CT x sex interactions, strongly implying that ACEs affect GM structure in adulthood differently in the two sexes.

Concerning recent stressors, while we found no association between brain structure and number or severity of recent stressful events, we found a significant negative association between perceived stress and GM volumes in bilateral opercular and insular regions, paralleled by reductions of cortical thickness in left opercular cortex and in

middle cingulate gyrus. Reduced volumes in insular regions have been previously associated both to a higher number of recent stressful life events (Ansell et al., 2012) and to higher perceived stress (Wu, Tong et al., 2021). The insula, particularly in its anterior-ventral region, is crucial in regulating emotional experience and subjective feelings (Uddin, Nomi et al., 2017), and appears to be among the brain regions consistently shown to have lower volumes in PTSD (Meng, Jiang et al., 2016) (as it was in a study by our group on post-traumatic symptoms after COVID-19 infection) (Benedetti, Palladini et al., 2021). However reduced GM volumes and cortical thickness in bilateral temporal and insular cortex are also robust MRI correlates of major depression (Schmaal et al., 2017, Wise et al., 2017): the cross-sectional nature of our study, prevent us from drawing causal inferences, and also taking into account that we only found associations with subjectively perceived stress, we can only hypothesize that our results reflects correlates of the depressive syndrome itself, rather than morphologic consequences of stress.

Finally we identified significant interactions of recent stressful events and childhood adversities in affecting GM volumes and cortical thickness: most notably, a strong interaction was identified between recent life events and CT on cortical thickness in anterior frontal regions and ACC, with negative associations only identified in individuals with a positive history of ACEs (CTQ total scores and, among its subscales, emotional abuse). A recent study performed in a large sample (n=1465) (Ringwald et al., 2022a) identified a similar result, with a significant interaction between recent stressors and CT and a negative association between life events and orbitofrontal GM volumes only in MDD subjects with a positive history of childhood trauma. Partially confirming our result, a separate study by the same group associated stressful life events with volumetric decreases in left medial prefrontal cortex over a 2 year follow up period, more evident in subjects also reporting a positive history of ACEs (Ringwald et al., 2022b). These and our result seem to give credence to the diathesis-stress model of depression; anterior frontal regions have been robustly shown to be affected in MDD (Schmaal et al., 2017, Wise et al., 2017): individuals with a positive history of ACEs, and therefore more at risk of developing depression, appear also to be more vulnerable to brain changes induced by recent stressors in such regions, that can therefore be hypothesized to be causal in the development of the depressive syndrome; indeed a

significant stressor x CT interaction was only identified in MDD patients and not in HCs.

We identified a second interaction between perceived stress and childhood physical abuse: while in subjects with no history of physical abuse perceived stress was negatively associated to GM volumes in bilateral insular and temporal regions (akin to the whole sample) and in ACC and frontal pole cortex, a trend towards an opposite effect was identified in AF+ individuals in partially overlapping regions. While this association appears puzzling, in can be seen in light of some considerations. While perceived stress and insular volumes are negatively associated in adults, a positive association has been reported in adolescents (Wu et al., 2021); furthermore lack of perceived control over dependent stressors – that is, stressors that individuals play a role in causing, such as fighting – was associated to increased cortical thickness in adolescents and young adults in prefrontal and anterior cingulate cortex and temporal regions (Fassett-Carman, Smolker et al., 2022). It is worth noting that a child's stressor might become an adult's trauma later in life. The specific positive association between stressors and GM volumes or thickness in child and adolescents might be related to their effect on an evolving brain, subject to pruning and myelination processes, among others (Gogtay & Thompson, 2010). We could therefore see the positive associations we identified between perceived stress and brain volumes in subjects with a history of physical abuse as a consequence of brain structure natural trajectory, stemming from the moment the same subjects were experiencing physical abuse as a dependent stressor in childhood, or, to put it differently, as a biomarker of an experience-determined modality of facing stress still apparent in adulthood. As of now however this might represent a suggestive hypothesis, but further and possibly longitudinal data are needed to adequately explore it.

2.2 White matter microstructure

Childhood trauma also affected WM microstructure, as evidenced by alterations in DTI indexes: we identified negative correlations between CTQ physical abuse subscale and RFQ and axial diffusivity, as well as between CTQ total score, physical neglect and

RFQ and mean diffusivity. Existing literature on the relation between ACEs and DTI indexes is somewhat sparse (Cassiers et al., 2018, Lim et al., 2020); concerning MDD patients, a positive association between ACEs and fractional anisotropy has been reported (Graziano et al., 2019, Tatham et al., 2016).

In our sample ACEs were negatively associated with AD and MD. This is in line with a previous work by our group (Poletti, Aggio et al., 2018) that identified significant associations with the same DTI metrics. In contrast with radial diffusivity, thought to reflect myelin integrity, axial diffusivity is classically regarded as an index of axonal damage (Song, Yoshino et al., 2005). Neuroaxonal injury has been implicated in MDD pathophysiology, with increased levels of plasma neurofilament light protein reported (Chen, Liu et al., 2022, Spanier, Kilian et al., 2019). We could therefore speculate our results to be reflective of a specific axonal alteration in major depression as a consequence of childhood adversities. However, caution is warranted in interpreting this result, as the in vivo biological meaning of axial diffusivity changes is very much a matter of debate (Wheeler-Kingshott & Cercignani, 2009).

We did also identify significant effects of recent stressors on WM microstructure: recent stressful events were again negatively associated with AD and MD, while perceived stress negatively affected FA. This is in line with a recent large study associating life events perceived as "negative" by participants in the 6 months prior to reduced FA, and conversely perceived social support to increased FA (Flinkenflügel et al., 2023). These findings, besides stressing the effects of recent stressor on DTI indexes, also give a sense of the dynamical nature of WM microstructure as explored through DTI (Aggarwal, Williams et al., 2022), finding it to be robustly affected by external stressful stimuli and possibly mediating the relation between life stressors and psychiatric consequences. Indeed, a recent study found Fractional anisotropy to mediate the relation between stressful life events over the previous 12 months and depression onset and severity in a residential community sample of individuals at high-risk for depression (Wang et al., 2022b).

Finally, akin to the cortical thickness analysis, we identified a significant interaction between recent stressful events and childhood trauma in affecting WM microstructure, with negative association between life events and FA and positive with RD only in

subjects with a positive history of childhood trauma. Among the various types of trauma, this effect was again found specifically for childhood emotional abuse. The simultaneous presence of FA decreases and RD increases is usually regarded as a marker of reduced axonal and myelin integrity (Tae, Ham et al., 2018): our data therefore suggests that in MDD external stressors are associated to detrimental effects on white matter specifically in individuals with a positive history of childhood adversities, further corroborating the diathesis-stress model of depression, with a pivotal role of childhood trauma in determining future vulnerability to stress.

2.3 Resting state functional connectivity

Few results were identified for the effect of ACEs on rs-FC, with only a negative association between CTQ total score and functional connectivity between right amygdala and left opercular cortex. This is in line with previously reported reduced Amygdala volumes and connectivity in traumatized individuals (Fan, Gao et al., 2023, Kraynak, Marsland et al., 2019, Nogovitsyn, Addington et al., 2022). The amygdala (like the opercular/insular cortex with whom we identified reduced FC) is crucial in brain circuits involved in emotional processing, and specifically in emotional learning (Gallagher & Chiba, 1996); its altered rs-FC in adulthood therefore might be seen as the lasting consequence of traumatic experiences occurring during childhood neurodevelopment.

Recent stressors on the other hand had a more pronounced effect on brain activity and connectivity: this could be also related to methodological considerations, with functional neuroimaging possibly being more sensitive to state rather than trait correlates (Geerligs, Rubinov et al., 2015). Stressful life events and perceived stress both modulated hippocampal connectivity: right hippocampus connectivity with left Middle Frontal Gyrus was negatively affected by PSS, and with occipital regions by recent stressful events; life events also positively modulated left hippocampal connectivity with right Paracingulate Gyrus. Reduced hippocampal connectivity with frontal regions has been repeatedly associated to reduced FC with frontal regions (Chang, Song et al., 2023, Goldfarb, Rosenberg et al., 2020) while life stress events

have been associated to stronger functional connectivity with cingulate cortex regions (Ren, Zhao et al., 2022). The divergent effect we identified therefore on rs-FC between hippocampi and frontal regions – negative for PSS, positive for SRE-S – could be reflective of different FC patterns associated with "objective" or "subjective" stress: indeed while PSS is a measure of perceived stress, describing patients feelings and thoughts in the previous month (Cohen, Kamarck et al., 1983), the "Schedule of Recent Experience" aims at identify and quantify recent stress-producing events, regardless of the patient reaction to them (Mendels & Weinstein, 1972). It is therefore not surprising that the two measures are associated with different patterns of brain activity.

Furthermore, significant interactions between stressful events and childhood trauma were again found in affecting fALFF in several brain regions, particularly evident for childhood emotional abuse. It is striking that such results mimic the ones identified in the DTI analysis (negative association between PSS and FA and significant interaction between life events and CT). fALFF represent a relative measure of BOLD signal power, a possible proxy of the local spontaneous activity levels of the brain (Egorova, Veldsman et al., 2017, Huang, Zheng et al., 2019). Porcu and colleagues (Porcu, Cocco et al., 2021) found fALFF signals to be highly positively correlated to whole brain Fractional anisotropy; a possible explanation is that a more preserved myelin sheet (as indicated by higher FA) facilitates neural impulse transmission, resulting in diffuse brain activation (Porcu et al., 2021) it is tempting to hypothesize that the fALFF changes we identified reflect functional correlates of the already mentioned alterations in WM microstructure.

3. Effect of early and recent adversities on inflammatory markers.

3.1 Inflammatory ratios

We identified negative associations between various indexes of childhood trauma and inflammatory ratios: this might appear counterintuitive, as inflammatory ratios are usually regarded as markers of inflammation (Balta & Ozturk, 2015, Buonacera, Stancanelli et al., 2022, Li, Tian et al., 2018, Xu, Zhang et al., 2021), and childhood trauma has been robustly associated to low grade chronic inflammation in adulthood

(Baumeister et al., 2016, Rehan et al., 2023). However, when examining the relation with individual blood cells counts, CT was found to be positively associated with lymphocytes and negatively with neutrophils and monocytes, thus explaining its effect on inflammatory ratios.

Furthermore, we observed a significant interaction between recent stressful events and ACEs in affecting inflammatory ratios, with positive associations between stressors and NLR, PLR and SII only in subjects reporting childhood physical neglect. Exploring blood cells counts individually, again the effect appeared to be determined by a significant interaction on lymphocytes.

A positive association between ACEs and lymphocyte count has been reported by a large population study (Surtees et al., 2003): the authors observed that lifestyle related factors, such as smoking and BMI, explained part of the association. In our analysis we controlled for BMI, but we couldn't control for smoking status as that information was unavailable to us.

Beside lifestyle-related factors, an alternative explanation can be provided for our results. A positive history of childhood trauma has been repeatedly associated with lower saliva and hair cortisol levels in adulthood (Dobernecker, Spyridou et al., 2023, Wielaard, Schaakxs et al., 2018). On the other hand, depressed patients with a positive history of CT appear to have enhanced ACTH and cortisol responses when faced with stressors, suggesting that HPA axis hyper reactivity might be a consequence of childhood adversities (Burke, Davis et al., 2005, Heim, Newport et al., 2008). Cortisol levels have profound impacts on blood cells count, being negatively associated to lymphocytes (Thomson, McMahon et al., 1980) and positively to neutrophils and possibly monocytes (Davis, Albert et al., 1991, van de Wouw, Sichetti et al., 2021, Yeager, Pioli et al., 2016). Indeed, NLR has been shown to be strongly positively associated to cortisol levels (Wang, Wang et al., 2021).

We can therefore speculate that cortisol levels mediate, at least partially, both the negative association we identified between ACEs and inflammatory ratios, as well as the significant interactions between recent stressful events and childhood physical

neglect, with a positive association between stressors and inflammatory ratios (and negative with lymphocytes) only in PN+ subjects.

3.2 Serum cytokines and mediation analysis

We found associations between physical abuse and neglect and sexual abuse and several peripheral analytes; in all cases childhood trauma increased levels of serum cytokines. no effect was found for emotional trauma.

The positive association between ACEs and inflammatory status has been repeatedly reported (Baumeister et al., 2016), and is thought to contribute to the development of depressive symptomatology (Danese & J Lewis, 2017, Flouri et al., 2019). Most studies however have investigated the effect of ACEs on few selected inflammatory markers (CRP, IL-6, TNF-α, among the most frequently reported), providing only a partial characterization of the complex immune alterations associated to CT. Among the analytes we investigated on the other hand we found significant results for TNF-α, IL-1β, MIF, IP-10, MCP-1, GRO-α, HGF, IL-18, TRAIL. While some of these analytes still have a poorly understood biological meaning, and taking into account that cytokines are by definition characterized by pleiotropy (Nicola, 1994), some conclusions can be drawn from this cytokine profile. Most of these cytokines are classically regarded as marker of monocyte M1 polarization (TNF-α, IL-1β, IP-10) (Yao, Xu et al., 2019), produced by activated macrophages (GRO-α, IL-18) (Geiser, Dewald et al., 1993, Ihim, Abubakar et al., 2022) or have regulatory functions towards monocytes-macrophages (MIF, MCP-1) (Deshmane, Kremlev et al., 2009, Grieb, Merk et al., 2010). A shift towards an M1 polarization of monocytes in MDD has been repeatedly reported (Cosma, Üsekes et al., 2021, Nowak, Grendas et al., 2019) and indeed the role of monocytes-macrophages is emerging as crucial in MDD pathophysiology (Danese & J Lewis, 2017), with some authors going as far as postulating a “macrophage theory of depression” (Dey & Hankey Giblin, 2018). Indeed, it must be stressed that microglial cells, the primary CNS immune cells, are closely related to monocytes-macrophages, can undergo M1/M2 polarization and are speculated to be crucial in MDD neuroinflammatory processes (Nakagawa & Chiba, 2014, Wang,

He et al., 2022a). While the association between peripheral inflammatory markers and brain immune status is still far from being completely disentangled (Millett, Burdick et al., 2022), our data suggest that childhood adversities might contribute to monocyte/macrophages – and possibly microglial – alterations observed in MDD patients.

A significant interaction between perceived stress and childhood physical and sexual abuse in affecting MIF levels was identified: perceived stress and MIF were positively associated only in PA+ and SA+ individuals. It is striking that among the 29 markers investigated in the multivariate analysis a significant effect was identified specifically for MIF. MIF has been robustly linked to HPA axis function and stress reactivity (Bick, Nguyen et al., 2015, Lipschutz, Bick et al., 2018); cortisol can induce MIF secretion, while MIF renders leukocytes insensitive to the anti-inflammatory effects of cortisol itself (Edwards, Bosch et al., 2010), and indeed this cytokine has been also associated to anxiety and depressive symptomatology (Edwards et al., 2010, Lipschutz et al., 2018).

The significant interaction between recent stressors and childhood abuse can be seen in light of a diathesis-stress model, with a priming effect of ACEs on immune function subsequently highlighted by recent stressors. A very similar process has already been identified in HPA axis function, with enhanced cortisol responses to stress in traumatized individuals (Burke et al., 2005, Heim et al., 2008) and in immune status, with higher suPAR levels after recent stressful events in traumatized individuals (Bourassa et al., 2021). Also, in light of the close relation between MIF and glucocorticoid signaling (Bick et al., 2015, Edwards et al., 2010, Lipschutz et al., 2018), we can hypothesize that our result can be explained by the existence of a similar process.

Finally, among the inflammatory markers found to be affected by measures of childhood trauma, GRO-α significantly mediated the relation between childhood physical neglect and mean diffusivity. GRO-α (or CXCL1) is a chemoattractant chemokine produced by various cells, including macrophages (De Filippo, Dudeck et al., 2013). Interestingly GRO-α receptor, CXCR2, is widely expressed in neurons, microglia, and endothelial cells, where it has been shown to be associated to inflammation-induced increased blood brain barrier permeability and microglia

activation (Haarmann, Schuhmann et al., 2019, Korbecki, Gąssowska-Dobrowolska et al., 2022, Serdar, Kempe et al., 2020, Wu, Chen et al., 2020). GRO-α levels have been shown to be elevated in brain and CSF in various neurological conditions, such as Alzheimer's disease, Ischemic Stroke and Multiple Sclerosis (Korbecki et al., 2022), all conditions with established alterations in DTI metrics (Tae et al., 2018). Furthermore a specific role for GRO-α in major depression pathophysiology has been hypothesized (Korbecki et al., 2022). Our data suggest that childhood stressors impact white matter microstructure in major depression (possibly contributing to the development of the disease) at least partially through their effect on GRO-α.

4. Polygenic risk score moderation

Major depressive disorder is widely considered to be a heterogeneous disorder, both from a clinical, phenomenological and physiopathological point of view (Buch & Liston, 2021, Goldberg, 2011, Lynall & McIntosh, 2023); part of this heterogeneity stem from its difficult distinction from other mood disorders, such as Bipolar disorder. A retrospective 8 years study on MDD showed that 7.6–12.1% of patients had their diagnosis changed to BD (Li, Bai et al., 2012); milder or atypical forms of (hypo)mania may be difficult to identify, and this may lead to misdiagnosis (Correa, Akiskal et al., 2010, Singh & Rajput, 2006). This difficult distinction might be related to more than just clinical and diagnostic factors, having its roots in our current nosological classification: indeed some authors criticize our current dichotomy of mood disorders, considering our current definition of MDD too wide and of BD too narrow (Ghaemi & Dalley, 2014) and propose the existence of a broader clinical entity that could comprehend bipolar and part of MDD patients (Akiskal, 2007).

The advances made by psychiatric genetic research could prove to be crucial in disentangling this heterogeneity: indeed polygenic risk scores, derived from large GWAS studies, allow for the calculation of the genetic liability for a given disorder at the individual level, thus providing information directly related to the mood disorder biology rather that to its clinical presentation (Murray et al., 2021). Wiste and colleagues investigated the effect of BD PRS on the clinical features of the STAR*D

major depression sample (Wiste et al., 2014); interestingly, they found that higher BD PRS associated with earlier age of onset, history of suicide attempt and presence of sub-clinical manic symptoms, all features classically regarded to reflect bipolar vulnerability (Frankland et al., 2018, Patella et al., 2019).

Indeed, in our study we found BD PRS to moderate the relation between childhood trauma and several clinical, biological and neuroimaging characteristics, possibly reflecting that CT has different effects depending on BD genetic vulnerability.

First, we found BD PRS to significantly moderate the relation between CT and treatment response: in individuals with low bipolar vulnerability we identified a positive association between CT and response (akin to the whole sample), while the opposite effect was identified in subjects with high BD vulnerability. It has long been speculated that a portion of MDD treatment resistant patients might be yet undiagnosed bipolar disorder patients (Correa et al., 2010, Fogelson & Kagan, 2022). Indeed, antidepressant treatment of bipolar depression, besides posing the risk of inducing manic switches, is also of dubious clinical efficacy (Ghaemi, Hsu et al., 2003, Sidor & MacQueen, 2010). While the relation between CT and treatment response in MDD is far from univocal (Kuzminskaite et al., 2022), childhood adversities have been repeatedly linked to worse clinical features in BD, including treatment resistance (Agnew-Blais & Danese, 2016), and indeed CT is among the few robust environmental risk factors for the development of the disease (Bortolato, Köhler et al., 2017). We can therefore speculate the moderating effect of BD PRS we identified to be reflective of a different impact of childhood trauma on response to antidepressants dependent on bipolar vulnerability (and possibly on bipolar spectrum status).

Second, we identified a differential effect of CT on DTI metrics depending on BD PRS level. While pre-existing literature on the effect of CT on WM microstructure in mood disorders is somewhat sparse, a possible differential effect between MDD and BD patients is beginning to emerge: indeed while ACEs have been robustly associated to reduced FA in BD (Poletti et al., 2022, Stevelink et al., 2018) some authors report an opposite effect in MDD (Graziano et al., 2019, Tatham et al., 2016), and in a previous study performed by our group we found diagnosis (MDD or BD) to significantly moderate the relation between CT and WM microstructure (Poletti et al., 2022).

Accordingly, in our MDD sample, we found the relation between ACEs and DTI metrics to mimic the one identified in BD for higher BD PRS scores (that is, negative association with FA and positive with RD), while opposite effects were found for low BD PRS scores, possibly identifying distinct biological subgroups within the MDD sample with different responses to childhood adversities. Indeed the identification of a PRS moderation might highlight the heterogeneity of the MDD diagnosis (Buch & Liston, 2021, Goldberg, 2011), with a portion of affected patients presenting brain consequences of CT similar to those of BD subjects; this might give credence to critics of the MDD/BD dichotomous conceptualization of mood disorders (Akiskal, 2007, Ghaemi & Dalley, 2014), suggesting that a subset of MDD subjects presents a bipolar-like biology in response to ACEs.

Finally, we found BD PRS to significantly affect the relation between childhood physical and sexual abuse and peripheral inflammatory markers. For physical abuse this was true for TNF-α and IL-4, while for sexual abuse a significant interaction was found for Eotaxin, MCP-1 and HGF. As already stressed CT has a strong impact on the development of the inflammatory/immune system, having been robustly associated to elevated inflammatory markers in adulthood, including upregulation of monocytes inflammation-related genes, and having been identified as a risk factor for the development of rheumatic diseases (Benedetti, Aggio et al., 2020, Brunoni, Supasitthumrong et al., 2020, Martinuzzi et al., 2021). Immune-inflammatory alterations are implicated both in MDD (Ruiz, Del Ángel et al., 2022, Simon, Schiweck et al., 2021) and BD (Benedetti et al., 2020, Rosenblat & McIntyre, 2017) pathophysiology; however distinct immunological signatures between the two disorders are beginning to emerge, including different levels of pro- and anti-inflammatory cytokines and chemokines (Brunoni et al., 2020, Martinuzzi et al., 2021, Poletti, Vai et al., 2021). Indeed, in our previous study we identified childhood trauma to affect differently inflammatory markers between MDD and BD patients (Poletti et al., 2022). We could therefore speculate that BD vulnerability changes the relation between childhood trauma and inflammatory status also within an exclusively MDD sample.

5. Relation between neuroimaging and treatment response and resistance

5.1 Gray matter volumes and cortical thickness

Patients with treatment resistant depression had lower GM volumes in several clusters including right superior and middle temporal gyrus and temporal pole, bilateral thalami and hippocampi and occipital and cerebellar regions. This was paralleled by cortical thickness reductions predominantly in middle and anterior cingulate gyrus. Reduced cortical thickness in right anterior cingulate gyrus was also identified in patients failing to achieve symptom remission at discharge, and CT in this brain region was positively associated with the percentage of decrease in HDRS from admission to discharge. This is in line with previous literature linking reduced volume or thickness, particularly in hippocampus and ACC, to reduced response and increased resistance in MDD (Enneking et al., 2020).

Reduced volumes in the hippocampus are a robust MRI finding in major depressive disorder (Gray, Müller et al., 2020, Wise et al., 2017), and appear to be linked with longer untreated episodes (Sheline, Gado et al., 2003). Hippocampal volumetric reductions could be associated to altered neuroplasticity or neurogenesis, that is to altered hippocampal microstructure or to reduced formation of new neurons in the dentate gyrus (Boku et al., 2018). Both processes could be affected by stress and elevated glucocorticoids levels.

Antidepressant medication might act, at least partly, enhancing hippocampal neuroplasticity and potentially neurogenesis, counteracting the impact of stress on the brain. Various types of antidepressant treatments have been shown to be able to enhance hippocampal neurogenesis (Malberg, Hen et al., 2021). Moreover, animal studies have shown that impaired hippocampal neurogenesis, induced by irradiation of the mouse brain, inhibits the positive effects of antidepressants (Santarelli, Saxe et al., 2003). We might therefore speculate that impaired hippocampal neuroplasticity/neurogenesis, potentially indicated by reduced hippocampal volumes, might underlie a hampered response to antidepressant treatments.

Individuals with MDD also exhibit decreased gray matter volumes in temporal regions (Gray et al., 2020, Wise et al., 2017), and such reductions have been observed in patients with treatment-resistant depression (Kang & Cho, 2020, Klok et al., 2019).

Interestingly, reduced volumes in the right superior temporal gyrus have frequently been linked to a history of suicide attempts in adolescents (McLellan, Wilkes et al., 2018, Peng, Wu et al., 2014): suicidal thoughts and behaviors are indeed a negative consequence of antidepressant treatment in adolescents and young adults (Brent, Melhem et al., 2010).

The superior and middle temporal gyri and temporal pole, traditionally associated with processing sensory information, are also crucial for emotional processing and social cognition (Takahashi, Yücel et al., 2010). Diminished volumes in these areas could thus contribute to the impaired processing of emotions and memories in depression, potentially influencing the resolution of symptoms following treatment.

Finally, reduced cortical thickness in ACC associated to treatment resistance and blunter antidepressants responses. Alterations of ACC are an established MRI correlate of major depression (Wise et al., 2017), and have been robustly linked to treatment resistance (Enneking et al., 2020). Furthermore, in TRD patients, thickness reduction in this region appears to be connected with cognitive impairments. The ACC is a crucial structure for emotion and reward, and is extensively linked to the hippocampus and to other limbic regions (Bian, Qin et al., 2019, Chen, Chang et al., 2022, Rolls, 2019). This finding therefore further stresses the importance of limbic alterations in influencing response to treatments in MDD.

5.2 White matter microstructure

While we did not find any association between treatment resistance status and DTI metrics, we found patients who failed to achieve symptom remission at discharge to have significantly lower baseline FA and higher RD and MD compared to those who did in widespread tracts of the white matter skeleton; accordingly, similar associations with FA and RD were found when investigating correlates of the percentage of HDRS decrease from admission to discharge. The simultaneous presence of reductions in FA and increases in RD and MD is usually thought to reflect dysmyelination or demyelination (Caeyenberghs & Swinnen, 2015, Song et al., 2005). Our results therefore seem to indicate that impaired integrity of myelinated white matter tracts is an

important contributor to antidepressant treatment response patterns. Indeed, similar results have repeatedly been reported in DTI studies investigating response patterns in MDD (Hoogenboom, Perlis et al., 2014, Vieira, Coelho et al., 2021) and BD individuals (Lan, Rubin-Falcone et al., 2017).

5.3 Resting state functional connectivity

Several alterations in baseline rs-FC were associated to treatment response and resistance in our sample: specifically, higher rs-FC between left Amygdala and parahippocampal gyrus was identified in patients who failed to achieve symptomatic remission at discharge, while treatment resistant patients had reduced connectivity between ACC and left Hippocampus with frontal regions.

Increased connectivity between amygdala and hippocampus/parahippocampal gyrus appears to be a relatively stable feature of Major depression, as underlined by meta-analytic evidence (Tang, Lu et al., 2018), and increased connectivity between amygdala and other limbic structures has also been identified as a correlate of antidepressant treatment resistance (Kotoula, Evans et al., 2023). The amygdala, a crucial structure for emotional processing, is deeply connected to hippocampal structures, both anatomically and functionally (Gallagher & Chiba, 1996), and the hyperconnectivity between the two in MDD is classically though to be associated to excessive concern of negative events (Tang et al., 2018).

Concerning ACC and Hippocampus, alterations in fronto-limbic connectivity patterns have long been associated to treatment resistance in MDD (Ge, Torres et al., 2019, Kotoula et al., 2023). Specifically, for left hippocampus, higher rs-FC with frontal regions has been repeatedly associated to better outcomes after antidepressant treatment (Chin Fatt, Jha et al., 2020, Xiao, Yuan et al., 2021). Fronto-limbic structures are crucial in emotion and mood regulation, and alterations in their connectivity patters have been associated to various psychiatric conditions, suggesting them to be a core feature that extends across psychopathology (Kebets, Favre et al., 2021, Mesbah, Koenders et al., 2023, Yoon, Rohrsetzer et al., 2023).

6. Moderated mediation analyses

6.1 Childhood and recent adversities, neuroimaging and treatment response.

Childhood trauma significantly modulated the relation between recent stressful events and response patterns, with higher recent stressors associated with lower remission rates at discharge for high levels of CT. A similar effect was observed for DTI metrics, with stressors exhibiting a detrimental effect on WM microstructure (decreased FA and increased RD) only in CT+ individuals. White matter microstructure, in turn, was associated with response patterns, with non-remitters presenting lower FA and higher RD values. Therefore, a significant moderated mediation emerged: white matter microstructure mediated the detrimental effect of recent stressors on treatment response only in subjects reporting childhood physical abuse. This result provides a mechanistic explanation of the hypothesized diathesis-stress model of the effect of childhood and recent stressors in MDD: our result appear to suggest that childhood stress (specifically emotional abuse, with similar results also for CTQ total score) renders WM microstructure more vulnerable to the effect of stressful events in adulthood, and this in turn associates with worse treatment outcomes.

6.2 Childhood trauma, BD PRS, neuroimaging and treatment response

BD PRS modulated the effect of childhood trauma on treatment response, with a negative association between CT and response pattern at high BD PRS, and an opposite effect for low BD PRS. At the same time BD PRS also modulated the effect of CT on white matter microstructure, with negative associations between CT and FA only for high PRS values; given the association between WM microstructure and treatment response, a significant moderated mediation was identified: for high values of BD PRS ACEs negatively impacted fractional anisotropy, which in turn affected detrimentally treatment response; the opposite was true for low BD PRS values, with a positive association between CT and FA that resulted in higher remission rates.

It has long been speculated that bipolar vulnerability might contribute to treatment resistance in major depression (Akiskal & Pinto, 1999, Correa et al., 2010, Fogelson & Kagan, 2022): our data suggest that it does so, at least partially, modulating the impact of childhood adversities on WM microstructure, which in turn will be reflected on response patterns.

On the other hand, the positive association between CT, FA and treatment response for low PRS scores appears counterintuitive; it must however be seen in light of the repeatedly reported positive association between ACEs and FA (Graziano et al., 2019, Tatham et al., 2016), and of the controversial relation between CT and treatment response in MDD (Kuzminskaite et al., 2022). Indeed, in a subset of MDD patients CT appears to be associated to improved response to antidepressants: our result might help better define this specific subset of patient and provide preliminary information on the biological basis of this unexpected phenomenon.

7. Effect of inflammatory status on treatment response and mediation analysis

In our study we found higher CRP levels to be associated to treatment resistance. This is in line with the results of a recent meta-analysis (Gasparini et al., 2022) that investigated the relation between several peripheral inflammatory markers and treatment response in MDD, finding significant results only for CRP and IL-8. Patients with MDD have long been shown to exhibit higher CRP levels (Osimo, Baxter et al., 2019), and a low-grade inflammatory status is thought to be involved in the pathogenesis of the disorder itself (Beurel et al., 2020, Kiecolt-Glaser et al., 2015).

The same biological mechanisms proposed to explain the association between low grade chronic inflammation and depression onset could also underlie its effect on treatment resistance: inflammation can induce the expression of monoamines reuptake transporters, primary biological targets of most antidepressants; it can reduce tryptophan, serotonin precursor, through IDO activation, leading to the production of quinolinic acid and enhancing glutamatergic neurotransmission; through increased glutamate neurotransmission and inflammation-induced oxidative stress it can then lead

to reduced neurogenesis and neuroplasticity (Miller et al., 2009, Miller & Raison, 2016, Zhang et al., 2016).

Indeed, neuroplasticity and neurogenesis processes are thought to play a pivotal role in antidepressant action (Harmer et al., 2017), and antidepressants are speculated to act on several neurotrophic factors, such as the BDNF-TrkB signaling pathway (Castrén & Monteggia, 2021). Reduced hippocampal volumes, a robust MRI correlate of MDD and predictor of poor antidepressant response (Enneking et al., 2020), are themselves speculated to reflect impaired neuroplasticity and neurogenesis (Boku et al., 2018).

Indeed, in our sample CRP levels were negatively associated to bilateral hippocampal volumes, possibly reflecting the detrimental effect of chronic inflammation on neuroplasticity and neurogenesis. Furthermore, we found left hippocampal volumes to significantly mediate the relation between CRP and treatment resistance status, possibly providing a mechanistic explanation of the established relation between inflammatory status and treatment response in MDD.

8. Longitudinal analyses

In our longitudinal analysis we detected several changes in MRI markers occurring after major depressive episode treatment.

First, we found volumetric increases following treatment in a cluster encompassing left thalamus and basal ganglia; stratifying patients according to treatment resistance status, this effect was only observed in TRD subjects. Basal ganglia volumetric increases have not been reported in longitudinal studies of antidepressant treatments, but on the other hand have been repeatedly observed following ECT (Enneking et al., 2020). Patients who receive ECT are by definition severely resistant to treatments (Hermida, Glass et al., 2018), and this might shed light also on our result: we can indeed hypothesize that basal ganglia volumetric increases are not a consequence of ECT per se, but rather a specific brain change of TRD patients after treatment.

Our result concerning hippocampal and para-hippocampal structures might have a more straightforward interpretation: indeed, we identified volumetric increases in left

hippocampus and parahippocampal gyrus only in non-TRD patients. As already stressed, antidepressants are thought to exert at least partially their activity restoring neuroplasticity and neurogenesis, either enhancing monoaminergic neurotransmission or directly affecting BDNF-TrkB signaling (Björkholm & Monteggia, 2016, Castrén & Monteggia, 2021). The adult hippocampus is a primary site of neuroplasticity, and is among the few regions in adult brain where neurogenesis occurs (Tatu & Vuillier, 2014): volumetric increases in this region could therefore reflect an antidepressant-induced restoration of both processes, and indeed appear to be a relatively robust finding in longitudinal studies of depression treatment (Enneking et al., 2020). Further, the notion that we identified such volumetric increases only in non-TRD patients might provide significant information on the biology underlying treatment resistant depression.

Concerning DTI metrics, we identified significant decreases in FA after treatment. This finding is of difficult interpretation, also because studies on longitudinal consequences of antidepressant therapy on white matter microstructure are somewhat sparse. Some studies reported detrimental effects of antidepressants on white matter in older samples (Grool et al., 2013, Steffens et al., 2008). However, when dividing patients according to remission status at follow up, the decrease in FA from baseline was apparent only in those with residual symptomatology, possibly suggesting it to be related to the detrimental effects of a prolonged depressive status rather than to the effects of drug treatment itself.

Finally, in the resting state functional connectivity analysis, several longitudinal changes in rs-FC patterns were identified. Hippocampal connectivity with limbic-related cortical structures decreased over time (between right hippocampus and insula in the whole sample and between bilateral hippocampi and posterior cingulate in the non-TRD group); furthermore, for non-TRD patients, frontal connectivity increased with left hippocampus and decreased with right amygdala. Existing literature on longitudinal changes in rs-FC after antidepressant treatment is somewhat sparse and heterogeneous, employing different methodologies of functional connectivity analysis and investigating correlates of numerous antidepressant interventions (Gudayol-Ferré, Peró-Cebollero et al., 2015, Kotoula et al., 2023).

Our results seem to point towards a reduction in hippocampal connectivity with limbic structures and an increase with frontal regions after antidepressant treatment: indeed, increased connectivity between hippocampal and frontal regions has been repeatedly associated to favorable outcomes after antidepressant treatment, and this held true also in our sample (Chin Fatt et al., 2020, Xiao et al., 2021). The opposite effect was found concerning amygdala, with decreases in rs-FC after antidepressant treatment: increased amygdala activity and connectivity is a relatively robust correlate of MDD diagnosis and of poor treatment response, and it has been shown to normalize after successful treatment (Helm, Viol et al., 2018).

9. Limitations

Our study had several limitations: patients were recruited in a single psychiatric hospital, thus limiting the generalizability of our findings. Given the real world clinical setting of our study, patients were prescribed various psychotropic medications: we tried to control for the dose of prescribed antidepressants converting them into imipramine equivalents, but we couldn't account for the wide variety of prescribed pharmacotherapies. Treatment response wasn't assessed at fixed time intervals, but rather at admission and discharge from the hospital, while treatment resistance status was assessed retrospectively from the clinical charts. Only a subset of patients had neuroimaging, peripheral inflammatory markers and genetic data available; likewise only for a small subsample longitudinal MRI data was available.

Childhood trauma was retrospectively assessed: this might be subject to recollection bias and potential under-reporting; we tried to account for this using the CTQ "minimization" subscale as a nuisance covariate. Furthermore the tools we used to assess childhood trauma, while largely employed in other studies, aren't sensible to the age period in which traumatic experiences occurred, which on the other hand could be a determining factor in influencing adverse childhood experiences impact on psychopathology and biological markers.

10. Conclusions

Our work provided several results. Our original hypothesis – that childhood trauma would impact inflammatory status which would in turn negatively affect treatment response - was not confirmed. Indeed, while childhood stressors associated with higher levels of inflammatory cytokines, they were not associated with response patterns. CRP levels were on the other hand associated with treatment resistance, but not affected by childhood stressors in our sample. Furthermore adverse childhood experiences were associated to better treatment response patterns: as noted by a recent large meta-analysis (Kuzminskaite et al., 2022), adverse childhood experiences, while associated to higher baseline depression severity, don't appear to influence treatment response in major depression, and in north American studies a positive association with response is reported. As already discussed several elements could underlie our result, including the specificity of our inpatient clinical setting.

We repeatedly observed significant interactions between childhood and recent stressors in affecting response patterns, inflammation status and brain MRI correlates. Furthermore the interaction between early and recent adverse experiences was found to affect white matter microstructure, which in turn influenced treatment response. This gives credence to the notion of a diathesis-stress model explaining the effect of childhood and recent traumatic experiences on psychopathology: subjects with a positive history of childhood trauma might be more susceptible to the detrimental effects of life stressors, both in their immune-inflammatory status, in their brain structure and function and ultimately in their response to treatment.

Our study also highlighted the heterogeneity of "major depression", providing a possible tool to disentangle such heterogeneity by taking into account genetic liabilities. Indeed if we accept the notion that a portion of major depression patients might have a bipolar disorder vulnerability, be yet unrecognized bipolar patients or fall within a broader clinical entity encompassing bipolar disorder and a portion of major depression, genetic data could provide informations directly related to the underlying biology of the disease rather than to its phenotypic presentation. In a previous study we found childhood trauma to affect DTI metrics differently in unipolar and bipolar depressed patients, and bipolar disorder (or bipolar vulnerability in major depression) has long

been recognized to be associated with poor and inconsistent responses to antidepressants; in our study major depression patients with high bipolar disorder PRS had associations between childhood trauma and DTI metrics similar to those found in bipolar samples, and also exhibited a negative association between childhood adversities and response patterns. Furthermore bipolar disorder PRS moderated the effect of childhood trauma on white matter microstructure, which in turn affected treatment response, thus providing a mechanistic explanation of the impact of bipolar genetic liability on major depression treatment response.

Finally treatment response patterns were associated to several MRI findings across neuroimaging modalities. Some of these associations replicated existing literature on brain correlates of antidepressant treatment response, however rarely performed in real world clinical settings. Of particular interest, we found a robust association between reduced hippocampal and parahippocampal volumes and treatment resistance, paralleled by longitudinal increases in the same regions only in treatment non-resistant patients. This might give credence to the neurotrophic theory of depression and of antidepressant action, being the hippocampus a primary site of adult neurogenesis, and also shed light on the biology underlying treatment resistance in depression. If antidepressant acts ultimately enhancing BDNF-TrkB signaling, thus restoring impaired brain neurogenesis and neuroplasticity (signaled by reduced hippocampal volumes and by longitudinal increases in such structures), then treatment resistant patients might be immune to this drug induced biological effect, exhibiting no longitudinal change in hippocampal structures after treatment.

Materials and Methods

1. Participants

Our study was performed on a maximum sample of 220 MDD patients admitted to our psychiatric ward during a major depressive episode. Exclusion criteria were: any additional psychiatric diagnosis, intellectual disability, pregnancy, major medical and neurological disorders; conditions known to affect the immune-inflammatory system, such as rheumatic or autoimmune diseases, chronic inflammatory diseases,

malignancies, other hematological conditions, chronic or acute infections were also exclusion criteria.

All patients were prescribed pharmacotherapies according to their clinical needs, and were discharged from the hospital when their clinical conditions allowed for the continuation on treatment at home. From antidepressant drug treatment at discharge the equivalent dose of Imipramine was calculated (Bollini, Pampaliona et al., 1999).

After a complete description of the study, written informed consent was obtained. All research activities have been approved by the local Ethical Committee.

2. Clinical and psychometric assessment

Childhood trauma (CT) and family environment were retrospectively assessed via the 28-item Childhood Trauma Questionnaire (CTQ) (Bernstein, Stein et al., 2003) and the 13-item Risky Families Questionnaire (RFQ) (Taylor, Lerner et al., 2004). CTQ scores of physical (PA), emotional (EA) and sexual abuse (SA), and physical (PN) and emotional neglect (EN) were obtained for each patient; CTQ total score was also obtained from the sum of the 5 subscales; furthermore CTQ minimization score was calculated, to denote potential under-reporters of trauma.

Recent stress (RT) perception was assessed via the Perceived Stress Scale (PSS) (Cohen et al., 1983), while the recent occurrence of stress-producing events was explored through the Schedule of Recent Experience (SRE) (Mendels & Weinstein, 1972): from SRE both the absolute number of recent stressful events (SRE-N) and a score accounting for the relative stress contribution of each event (SRE-S) were obtained.

Depression severity was assessed upon admission and at discharge from the hospital by a trained psychiatrist via the 21-item Hamilton depression rating scale (HDRS) (Hamilton, 1960); as per standard HDRS scoring, only the first 17 items of the scale were used to determine HDRS final score. At baseline, self-report Beck Depression Inventory was also administered (Richter, Werner et al., 1998).

3. Treatment response determination

Treatment response patterns were assessed exploring treatment resistance status and remission upon discharge. Treatment resistance was extracted by the clinical charts by a trained psychiatrist: patients were defined treatment resistant if, during the current episode, failed to respond to at least two separate antidepressant treatments with different mechanisms of action, administered to an adequate dose for at least 4 weeks (Gaynes et al., 2020, Sforzini et al., 2022).

Given the real world nature of our study, all patients were discharged only after a significant clinical improvement; however only a portion of patients achieved full symptomatic remission at discharge (HDRS $\leq$ 7), while in others residual depressive symptomatology was still present. Remission at discharge was therefore considered as an indicator of treatment efficacy, as well as the percentage of HDRS decrease from admission to discharge.

4. Inflammatory markers

Patients underwent routine venous blood sampling upon admission. From the clinical charts neutrophils, lymphocytes, monocytes, and platelets absolute values were obtained. Neutrophil to lymphocyte ratio (NLR), monocyte to lymphocyte ratio (MLR), platelet to lymphocyte ratio (PLR), and systemic inflammatory index (neutrophils x platelets / lymphocyte) (SII) were calculated (we will refer to the results of such calculations collectively as inflammatory ratios). For patients whose blood cell counts were outside normal ranges (neutrophils: 1500-7000; lymphocytes: 1000-4500; monocytes: 200-1000; platelets: 150.000-450.000) such ratios were not calculated, as they could be indicative of an inflammatory or pathological condition.

Furthermore a second venous blood sampling was performed the day of the MRI acquisition for 99 patients: Bio-Plex Pro Human Cytokine 48-Plex and Bio-Plex Pro Human Acute Phase 4-Plex Panel assays (BIO-RAD) were used to detect plasma concentrations of immune analytes, through the bead-based Luminex system according

to xMAP technology (Luminex 200 system, Merck Millipore). Analyses were performed on observed concentrations (pg/ml) calculated using Belysa Immunoassay software (version 1.2).

Analites with more than 10% missing values were excluded from the analysis. Therefore analyses were conducted on the following analytes: IL-2R- α, MIG, MIP-1 β, IFN- γ, SDF-1- α, IL-1ra, IL-16, TNF- β, MIF, TNF- α, RANTES, IL-1 β, IL-18, Eotaxin, Basic FGF, PDGF-BB, IP-10, IL-13, IL-4, MCP-1, MIP-1- α, GRO- α, HGF, SCF, TRAIL, M-CSF, CTACK, IL-9, SCGF- β, CRP.

5. MRI acquisition and preprocessing

140 patients underwent 3 tesla MRI acquisition. For 31 patients a second follow up MRI scan was also performed. The mean time between baseline and follow-up MRI acquisition was 99.3 ± 13.4 days.

Patients were scanned on a Ingenia CX scanner, Philips, using a 32-channel head coil. The following parameters were used for T1 sequences: TR 8.00 ms, TE 3.7 ms, in-plane resolution 1 × 1 mm, yielding 182 transversal slices with a thickness of 1 mm. For the voxel-based morphometry, cortical thickness and ROI analyses, the images underwent the Computational Anatomy Toolbox (CAT12) preprocessing pipeline (Gaser, Dahnke et al., 2022) within statistical parametric mapping 12 (SPM12) framework (https://www.fil.ion.ucl.ac.uk/spm/) on Matlab R2016b. This pipeline also allowed the extraction of tissue volumes for regions of interest (ROIs). Additionally, measures for total intracranial volume (TIV) were obtained. Details on the preprocessing pipeline can be found here (Paolini, Harrington et al., 2023).

DWI was executed using SE Eco-planar imaging and the following parameters: scanner TR/TE=5900/78 ms, FoV (mm) 240 (ap), 129 (Negele et al.), 232 (rl); acquisition matrix 2.14×2.73×2.30; 56 contiguous, 2.3mm thick axial slices reconstructed with in-plane pixel size 1.88×1.88x2.30 mm; SENSE acceleration factor=2; 1 b0 and 40 non-collinear directions of the diffusion gradients; b value=1000 s/mm2.

DTI analysis and tensor calculations were carried out using the "Oxford Center for Functional Magnetic Resonance Imaging of the Brain Software Library" (FSL 6.0; www.fmrib.ox.ac.uk/fsl/index.html) (Smith, Jenkinson et al., 2006). Details on DTI sequences and TBSS preprocessing can be found here (Poletti et al., 2022).

In relation to the resting-state functional data, the fMRI images comprised 200 consecutive T2*-weighted volumes. These were acquired using an echo planar imaging pulse sequence, encompassing interleaved ascending transverse slices covering the entire brain. The parameters for image acquisition were as follows: Repetition time (TR) = 2000 ms, echo time (TE) = 30 ms, field of view (FOV) = 192 mm, slice thickness = 3.7 mm

Images were preprocessed using the CONN toolbox (www.nitrc.org/projects/conn), within SPM12. The standard preprocessing pipeline was followed. Details on preprocessing steps can be found here (Paolini et al., 2023).

6. Genetic sequencing and polygenic risk scores calculation

Genetic data were available for 127 subjects. Genotyping was carried out via Infinium PsychArray 24 BeadChip (Illumina, Inc., San Diego), a microarray developed in collaboration with the Psychiatric Genomics Consortium (https://www.illumina.com/products/by-type/microarray-kits/infinium-psycharray.html). Quality ceck was performed with PLINK1.9 (Purcell, Neale et al., 2007). Individuals with discrepant genotyped sex information, genotype rate < 95%, or outlying autosomal heterozygosity (Fhet > ±0.2) were removed. Markers with minor allele frequency (MAF) < 1%, call rate < 95%, or deviant from Hardy-Weinberg equilibrium at $p < 10^{-6}$ were excluded. Relatedness of participants was checked and individuals with a degree of recent shared ancestry (identity by descendent, IBD) > 0.1875, were excluded (Anderson, Pettersson et al., 2010). Finally, European population ancestry of the sample was confirmed with a principal component analyses (PCA). Michigan Imputation Server (https://imputationserver.sph.umich.edu/index.html) was used for genotype imputation and PRS calculation, using the 1000 Genomes Project V5 as reference panel and Eagle V2.3 algorithm for genotype phasing. MDD PRS and BD

PRS were calculated using weights derived from Gui and colleagues (Gui, Zhou et al., 2022).

7. Statistical analysis

Multiple regression, binary logistic regression, multivariate analysis of covariance (MANCOVA) and repeated measures ANOVA analyses were performed via StatSoft Statistica 12. Mediation and moderation analyses were performed via SPSS "Process" Macro 4.0 (Hayes, 2017) in IBM SPSS statistics 26.0.

The effect of early life and recent adversities on treatment response was assessed through binary logistic regressions, entering resistance status or remission state at discharge as binary dependent variables. CTQ total and subscales scores and RFQ for childhood trauma, PSS and Schedule of Recent Experience (SRE) scores for recent trauma were tested as independent variables; interactions between childhood and recent stressful events were also tested. Age, sex, baseline depression severity and antidepressant dose equivalents were used as nuisance covariates in all analyses. In analyses involving CTQ and its subscales, the "minimization" scale was also used as a covariate to account for trauma under-reporting.

VBM and cortical thickness statistics were carried out within the general linear model framework, as implemented in SPM12: measures of childhood or recent stress were entered as variables of interest, and age, sex, baseline depression severity and antidepressant dose equivalents as nuisance covariates. In VBM analyses TIV was also used as a nuisance covariate. To test possible childhood x recent stress interactions, a continuous covariate interaction model was implemented, testing the possible differential effects of PSS, SRE-N and SRE-S in subjects with a positive or negative history of various types of childhood trauma. Subjects were divided according to established cut-off scores for CTQ (EA: ≥9; PA: ≥8; SA: ≥6; EN: ≥10; PN: ≥8) (Bernstein et al., 2003).

The effect of childhood and recent stressors on DTI metrics was tested in analogous models performing voxelwise DTI analyses using nonparametric permutation-based

testing (Nichols & Holmes, 2002) as implemented in Randomise in FSL. Threshold-free cluster enhancement (TFCE) was used. The data were tested against an empirical null distribution generated by 5000 permutations for each contrast.

Finally fALFF maps were computed at the single-subject level. Second-level analyses were performed using general linear models (GLM), with analogous variables on interest and nuisance covariates used in the GM and DTI analyses. The variable of interest effect was tested on fALFF maps and on rs-FC pattern on selected ROIs (bilateral hippocampi and amygdale, ACC) in a seed to voxel analysis.

The effect of childhood and recent adversities and a possible CT x RT interaction on inflammatory ratios and blood cell counts were tested in the context of general linear models, entering the latter as dependent variables and CTQ total and subscales scores, RFQ, PSS, SRE absolute number and score as independent variables. The effect on peripheral cytokines levels was assessed in a MANCOVA statistical design, entering analytes as dependent variables, CTQ total and subscales scores, RFQ, PSS, SRE-N and SRE-S as independent variables. Again possible CT x RT interactions were tested. Again age, sex, baseline depression severity and antidepressant dose equivalents were used as nuisance covariates; given its possible effect on inflammatory markers, BMI was also used as a covariate.

After reviewing the results of such analyses, we tested a possible indirect effect of childhood physical abuse on axial diffusivity and physical neglect on mean diffusivity via the peripheral cytokines they affected. Measures of childhood trauma were used as independent variables, DTI metrics as dependent variables, cytokines as moderators and age, sex, baseline depression severity, antidepressant dose equivalents and BMI as nuisance covariates. The indirect effect was assessed through the bootstrap method (number of bootstrap samples = 5000) and 95% confidence intervals were estimated.

A possible interactions between childhood trauma and polygenic risk scores was tested in moderation analyses, entering measures of childhood stress as independent variables and MDD or BD PRS as moderators. For response patterns, treatment resistance or remission at discharge were used as dependent variables. For neuroimaging we used as dependent variables the average values of FA, RD and MD of

the WM tracts found to be differently affected by ACEs in a previous analysis. This was achieved creating binary masks from the voxels where a significant ACEs x diagnosis effect on DTI metric was identified, and then applying such masks to the FA, RD and MD skeletonized data. For inflammatory markers, a possible interaction was tested in the context of a GLM or MANCOVA design.

Associations between neuroimaging and response patterns were tested in t-test in the GLM context. Patients were divided according to treatment resistance and remission at discharge status. Statistical analyses were analogous to those employed to test the effect of stressors on neuroimaging, and included VBM, cortical thickness, DTI TBSS, resting state fALFF and seed to voxel analyses. Furthermore hippocampal volumes and cingulate cortex thickness were estimated and their effect on response patterns was stested via binary logistic regressions. Hippocampal volumes were converted into percentage of total intracranial volume (TIV) with the formula: (Hippocampal Volume × 100)/TIV.

After reviewing the results of such analyses, we tested a moderated mediation model, entering SRE-S as dependent variable, childhood emotional abuse or CTQ total score as moderators, mean value of FA were a significant SRE-S x CT interaction was found as mediator, and remission at discharge as dependent variable.

A second moderated mediation model was tested entering measures of childhood trauma as independent variables, BD PRS as moderator, mean values of FA where a significant ACEs x diagnosis effect on DTI metric was identified as mediator, and remission at discharge as dependent variable.

In both cases the indirect effect was assessed through the bootstrap method.

The effect of inflammatory ratios and blood cell count on response patterns was tested in binary logistic regression, entering resistance status or remission at discharge as dependent variables, inflammatory ratios or cell counts as independent variables, age, sex, baseline depression severity, antidepressant dose equivalents and BMI as nuisance covariates. An analogous statistical design was used when testing the effect of CRP on response patterns.

The impact of peripheral cytokines on response was investigated using Elastic net penalized logistic regression (Bunea, She et al., 2011, Poletti et al., 2021), using resistance status or remission at discharge as the outcomes (dependent variables) and peripheral cytokines as predictors (independent variables). Elastic net models, employing regularization techniques like L1 and L2 penalties, were utilized to shrink the coefficients, potentially estimating them as zero. To ensure robustness, a 10-fold nested cross-validation was conducted. This process involved splitting the sample into training and test sets: the former for model estimation and the latter for assessing predictive accuracy. To estimate coefficients, a non-parametric bootstrap procedure was employed. This yielded mean log Odds Ratios for each predictor, along with related 95% confidence intervals and variable inclusion probability (VIP). A 70% threshold for VIP was set for predictors.

After reviewing the results of such analyses, we tested a possible indirect effect of CRP levels on treatment resistance through hippocampal volumes, entering CRP as independent variable, hippocampal volumes (expressed as percentage of TIV) as mediators, and response patterns as dependent variables. Again the indirect effect was assessed through the bootstrap method.

Finally voxelwise longitudinal analyses were conducted for VBM, cortical thickness, DTI and resting state data in the GLM context. Longitudinal changes in gray matter volumes, cortical thickness, DTI metrics, fALFF or seed connectivity were explored in the whole sample and in treatment resistant and treatment non-resistant patients separately. Furthermore for left thalamus, hippocampus, parahippocampal cortex, fractional anisotropy, radial and mean diffusivity, the differential longitudinal changes according to treatment resistance and remission at follow up status (defined as HDRS $\leq$ 7) was tested via repeated measures ANOVA.

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