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Biological correlates of pharmacological and non-pharmacological treatments in mood disorders

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Abstract

I disturbi dell'umore comprendono un ampio spettro di patologie psichiatriche che coinvolgono la percezione di sé, i processi di ragionamento, la sfera affettiva e cognitiva, nonché l'interazione dell'individuo con l'ambiente circostante. Nel presente lavoro di dottorato, verrà posta particolare attenzione alla depressione unipolare, patologia che mostra una prevalenza tra il 2% e il 21% dei casi depressivi, percentuale influenzata da fattori nosografici, culturali e ambientali. Verranno analizzate le principali determinanti eziopatogenetiche, inquadrate in un modello multifattoriale derivante dalla complessa interazione tra componenti genetiche, biochimiche e ambientali, tutte in grado di influenzare a diversi livelli i meccanismi di espressione genica. Particolare rilievo verrà inoltre posto alle strategie terapeutiche nei casi di depressione resistente al trattamento, condizione che interessa circa il 30% dei pazienti con diagnosi di disturbo depressivo maggiore. Nel contesto qui citato, l'obiettivo sarà quello di individuare e discutere approcci farmacologici e non farmacologici in grado di migliorare gli esiti clinici e la risposta al trattamento, in un'ottica integrata di medicina di precisione tra gli esiti clinici e i correlati biologici.

Il lavoro svolto integra le evidenze provenienti da otto studi, che includono analisi osservazionali, studi multicentrici, revisioni sistematiche e meta-analisi. Sono stati quindi esaminati: (i) fattori ambientali, quali le esperienze traumatiche precoci e gli stereotipi di genere; (ii) dimensioni cliniche, come il costrutto agitazione–ansia in coorti internazionali, in grado di influenzare gli esiti di risposta al trattamento; (iii) gli effetti molecolari e biologici dei trattamenti per la depressione farmacoresistente, con particolare riferimento alla (R/S)-ketamina; e (iv) strategie di intervento non farmacologico, tra cui la terapia elettroconvulsiva, la psicoterapia centrata sul trauma e l'attività fisica controllata. Tali approcci sono stati analizzati con l'obiettivo di individuare potenziali biomarcatori periferici ed epigenetici associati alla risposta clinica, nonché di identificare fattori predittivi utili a riconoscere i pazienti che, per una maggiore vulnerabilità biologica, risultano maggiormente esposti al rischio di ricaduta di malattia.

Dai risultati della meta-analisi condotta sulle esperienze traumatiche precoci emerge un'elevata prevalenza di neglect infantile nei disturbi psichiatrici, sottolineandone quindi il ruolo rilevante sia per lo sviluppo che per il decorso della depressione. Parallelamente, gli stereotipi di genere, tuttora ampiamente diffusi, sembrano contribuire allo sviluppo di sintomi appartenenti allo spettro depressivo, in particolare negli individui con maggiore vulnerabilità psicologica.

In aggiunta a quanto precedentemente riportato, la presenza della dimensione ansia–agitazione nei quadri di depressione unipolare si associa a una maggiore gravità clinica, a un aumentato rischio suicidario e a una ridotta risposta ai trattamenti. Per quanto riguarda la depressione farmacoresistente, invece, le evidenze emerse dalle revisioni sistematiche sull'utilizzo di ketamina ed esketamina mostrano effetti complessi e articolati su diversi pathways biologici, inclusi quelli neurotrofici, immunitari e trascrizionali.

Lo studio sull'uso della terapia elettroconvulsiva ha evidenziato che i livelli plasmatici di specifici miRNA possono ridursi in risposta al trattamento, dando un importante indizio in merito al loro ruolo come biomarcatori periferici della risposta clinica. Contemporaneamente, le evidenze derivanti dagli studi condotti sull'efficacia di psicoterapie centrate sul trauma hanno mostrato modificazioni biologiche misurabili, tra cui variazioni nei miRNA, lunghezza dei telomeri leucocitari e numero di copie del DNA mitocondriale, correlate a miglioramenti clinici. Questi interventi sembrano avere un effetto modulatore sui meccanismi di stress e sui processi di invecchiamento cellulare. In questo contesto, inoltre, l'analisi del gene MED22 suggerisce un possibile ruolo nella regolazione della vulnerabilità individuale e nella predizione degli esiti terapeutici.

Infine, la revisione sistematica sull'attività fisica come intervento non farmacologico per la depressione lieve e moderata, come raccomandato dalle linee guida, evidenzia effetti biologici complessivamente modesti ma eterogenei. Sebbene l'esercizio fisico possa contribuire infatti a modulare alcuni meccanismi biologici alla base della depressione, la significativa variabilità metodologica tra i trial randomizzati controllati limita l'estensibilità a più scendari dei dati disponibili. In conclusione, i risultati esposti supportano un modello multifattoriale della depressione, in cui fattori ambientali, clinici e biologici interagiscono nel determinare una maggiore vulnerabilità individuale e la possibile risposta ai trattamenti. L'integrazione di biomarcatori periferici ed epigenetici con le dimensioni psicopatologiche rappresenta un passo essenziale per sviluppare approcci di medicina di precisione nei disturbi dell'umore e nelle forme di depressione resistenti al trattamento. In questo contesto, la combinazione di interventi farmacologici innovativi, terapie psicologiche mirate e strategie di intervento non farmacologico costituisce la base per un modello terapeutico personalizzato, capace di adattarsi alle specifiche esigenze biologiche e cliniche del singolo individuo, migliorando gli esiti anche nei quadri di depressione più complessi e riducendo il rischio di ricaduta.

1. Introduction

In scientific literature, the term "mood disorders" refers to a broad spectrum of psychiatric disorders, such as bipolar and depressive spectrum disorders, that affect emotional tone, cognition and, the mental state of the individual. The clinical characteristics and course of mood disorders are often characterized by the presence of depressive feelings and manic or hypomanic symptoms that may disturb the perception of emotions, behaviours, sleep, appetite, and impact daily functional abilities and skills, with an increased risk of morbidity and mortality [1–3].

The wide spectrum of mood disorders ranges from bipolar disorder type I (BD-I) and II (BD-II), cyclothymic disorder (Cyclothymia), and disruptive mood dysregulation disorder (DMDD), to major depressive disorder (MDD) and persistent depressive disorder, as well as premenstrual dysphoric disorder and bipolar or depressive disorders due to medical conditions or substance abuse [1].

The global prevalence of mood disorders varies and depends on the different classification systems; for instance, the lifetime prevalence of MDD ranges widely from 2 to 21%. Relatively higher prevalence rates are found in European countries (e.g. 12.8%)[4] and in the elderly population, rather than in Asian countries [5,6], or in the United States (e.g. 7%) [1]; dysthymia's prevalence was estimated at around 8% [7], and finally the worldwide bipolar disorders (BDs) prevalence is estimated at about 2% [8]. Following the recent SARS-CoV-2 pandemic situation, due to social restrictions and the subjective experiences of social isolation, loneliness, and frustration, scientific literature highlights the role of stressors associated with pandemic-related measures in subjects with vulnerability to mental disease, leading to an increase in new diagnoses. For instance, an increase in new global cases of MDD of 27.6% has been reported, with females being more affected than males (29.8%) [9–11].

Throughout a lifetime, many factors, both endogenous and exogenous, can contribute to the development of mood disorders in individuals with underlying susceptibility. This multi-factorial hypothesis of aetiology involves genetic components, hormonal changes, epigenetic events occurring in early life, as well as psychosocial and work-related experiences, all of which may play a crucial role in the development of mood disorders [2,12–17].

The etiological hypotheses for the development of mood disorders are numerous. For instance, the first explanation of MDD was proposed as monoaminergic and neurotrophic dysfunction; afterwards, other studies have highlighted a broad spectrum of inflammatory processes involved in the development of depression, such as glutamatergic, γ-Aminobutyric acid (GABAergic)

alteration, and endocrine dysregulation related to epigenetic interactions with environmental factors. Moreover, the most recent hypotheses take into account the influence of fatty acids and gut microbiota [2,18]. Similarly, for bipolar disorder, it is not possible to explain its etiology through a single identifiable genetic trait or a single dysfunction, but rather through a complex multifactorial model that considers the evidence from epigenetic events involved in alterations of DNA methylation processes, noncoding DNA, and oxidative stress reactions [14,18].

On the basis of these etiological hypotheses, since the middle of the last century, several classes of drugs and guidelines have been developed to treat BD and MDD with specific pharmacological classes [8,19,20], such as typical and atypical antipsychotics and mood stabilizers, serotonin-norepinephrine reuptake inhibitors (SNRIs), selective serotonin reuptake inhibitors (SSRIs), tricyclic antidepressants (TCAs), noradrenaline reuptake inhibitors (NARIs), noradrenergic and selective serotonergic antidepressants (NaSSAs), and more recently experimental GABAergic and N-methyl-D-aspartate (NMDA) receptor modulators [21,22].

Despite these therapeutic advances, one of the main problems related to the treatment of mood disorders concerns the lack of effective response to first-line or subsequent pharmacological treatments within an adequate time frame, which may otherwise lead to the development of treatment-resistant depression (TRD)[23–27], a condition that presents as a resistance to improvement after two or more treatments of adequate duration and dosage of different antidepressant drug classes[23–24]. This results in a high burden of distress for the patient and an increased risk of morbidity, mortality, and psychosocial impairment, which represents one of the main challenges of personalised medical approaches.

Alternative approaches which have been proposed to tackle the lack of effectiveness of pharmacological trials include both non-pharmacological treatments with a long tradition in psychiatry and relatively novel approaches, each of them considered in isolation or in conjunction with pharmacotherapy, with four main fields of research. Belonging to the former group, one of the considered interventions is electroconvulsive therapy (ECT), which has shown in animal models an up-regulation of postsynaptic serotonin receptors and neurotrophic effects in specific human brain areas, albeit the actual physiological mechanism of this intervention remains up to debate. Other approaches include deep brain stimulation (DBS), vagus nerve stimulation (VNS), and non-invasive brain stimulation (NiBS), with different protocols of study, including transcranial magnetic stimulation (TMS), repetitive transcranial magnetic stimulation (r-TMS), transcranial direct current stimulation (tDCS), and transcranial alternating current stimulation (tACS)[2,8,28–33].

On the other hand, evidence in favour of psychological intervention administered individually or in combination with the pharmacological treatment has accumulated. For BDs, psychological interventions are recommended: in the manic phase, a predominantly supportive and psychoeducational program aimed at promoting recovery from judgment impairment, while in the depressive phase of the disease, guidelines highlight better results with the administration of cognitive behavioural therapy (CBT), interpersonal psychotherapy (IPT), mindfulness-based interventions, and computer-based cognitive remediation therapy[8,34,35]. Similarly, recent guidelines for the management of MDD recommended two different levels of intervention depending on the severity of symptoms, such as a combination of CBT with pharmacotherapy, behavioural activation intervention (BA), IPT, short-term psychodynamic psychotherapy (STPP), mindfulness, problem-solving training, and physical exercise[2,19,20].

To better understand the role of genetic and environmental factors in the development of MDD and to enable interventions that can improve treatment response, a comprehensive precision medicine approach in psychiatry requires moving beyond symptom-based disease classification and incorporating specific biological and genetic markers. A biomarker is an objectively measurable biological indicator reflecting the underlying processes of a disease or its treatment. Biomarkers can be brain-based, peripheral, genetic, epigenetic, endophenotypic, or functional [36,37].

The aim of this PhD project was to investigate the biological correlates of pharmacological and non-pharmacological treatments in mood disorders, with a focus on the correlation between psychological and functional outcomes and epigenomic markers in mood disorders. Special attention was given to unipolar depression and the phenomenology of treatment resistance, as well as to pharmacological and non-pharmacological approaches aimed at improving patient outcomes.

This thesis presents eight studies in which I actively participated, organised around three main topic areas that reflect these research objectives. The first chapter consists of a brief introduction to the field of study.

In the second chapter, I will present three studies that investigate the role of environmental factors in the social and family contexts that may contribute to a homeostatic imbalance in the individual developmental trajectory and may provide the basis for greater vulnerability to the development of various mental disorders, with particular reference to unipolar depression. I also address the question of how the presence of anxiety and agitation during MDD course plays a role in treatment response and increases the risk of maladaptive behaviours.

The third chapter, following a brief overview of current first-line treatments and other molecules under investigation, focuses on a new emerging therapy, recently approved by the Italian Medicines Agency (AIFA) in 2022 to treat patients with TRD. In particular, I present a systematic review examining the molecular effects of ketamine and esketamine. Furthermore, in this chapter, I would have liked to discuss recent evidence from the multicentric Europe-based, randomised controlled trial (RCT) INTENSIFY study (Clinical Trial ID: NCT05603104), in which I participate as a clinical assessor. This important study aims to investigate the effects of early and intensive pharmacological intervention in patients with BD and schizophrenia (SZ) over a six-week period, while in patients with MDD who have failed first-line treatment, the study evaluates the therapeutic effects of adjunctive ketamine IV or esketamine IV or intranasal spray, combined with selective serotonin reuptake inhibitor (SSRI) or with serotonin-norepinephrine reuptake inhibitor (SNRI) treatment over a four-week period [38]. However, since the recruitment and follow-up phases are still ongoing, no preliminary data are currently available.

In the fourth chapter, I discuss the evidence on non-pharmacological interventions, delivered alone or in combination with pharmacotherapy, to enhance treatment response in patients with TRD. The research presented here suggests that non-pharmacological treatments, such as ECT and specific psychotherapeutic approaches, may reduce depressive symptoms through modifications of underlying biological processes and consequently promote disease remission. Subsequently, I examine recent evidence from a systematic review of RCTs that investigates the effects of physical activity in patients with MDD, highlighting its implications for future research and clinical practice.

In the final chapter, I discuss the findings in light of the evidence provided by the eight studies included in this thesis and consider the potential implications for future research and clinical practice.

2. Endophenotype associated with poor outcomes in MDD

MDD is a diagnostic category that encompasses a wide range of signs and symptoms, which may arise from different underlying alterations throughout an individual's lifetime. Indeed, multiple factors, endogenous, exogenous, or their interaction, can contribute to the development of specific depressive phenotypes, depending on individual vulnerability. This multifactorial etiological framework can be understood more clearly by considering quantifiable intermediate traits that bridge the gap between genetic liability and clinical manifestation, which capture both downstream

expressions of genetic influences and upstream determinants of psychopathology, namely endophenotypes. Within this framework, endophenotypes are conceptualised as intermediate components in the causal pathway linking genes, environmental exposures, and disease, offering a more proximal and informative representation (in the biological sense) than syndromic diagnoses [2,12–17,39,40].

In this chapter, I described three studies investigating possible environmental factors that may contribute to the development of mental disorders, with a particular focus on depressive disorders and their outcomes.

The first study examined environmental circumstances, internalised social norms, and gender stereotypes, highlighting their role in the possible increase of individual vulnerability to MDD.

The second study focused on early childhood interactions with family members and caregivers, emphasising the impact of early neglect and its components on later mental health outcomes.

The third study explored how the presence of agitation and anxiety within unipolar depression may act as mediating factors for poor recovery and relapse with a considerably augmented suicidal risk in MDD.

2.1 The impact of societal standards in the development of MDD

In the field of the relationship between society and social health, Émile Durkheim argued that the presence of shared assumptions, characteristics, attitudes, and qualities among different cultural groups represents the basis of consensual beliefs, with specific norms that regulate the role of each member within social groups[41]. From this perspective, implicit and internalized norms, shared within social groups, are considered valid in relation to the specific attributes of women and men, based on the phenotypic expression of biological differences and the consequent sexual division into two dichotomous categories, configuring themselves as gender stereotypes, which have the function of maintaining socially shared beliefs and expectations of the respective genders[42,43]. These beliefs are very pervasive even today, and tend to influence societal behaviours and interactions, impacting relationships, workplace, and internalized roles, with consequent physical and psychological effects[44], thus tending to increase the susceptibility to develop symptoms of stress, anxiety and depression [45,46]. Indeed, these societal standards and expectations show a significant impact on mental health, especially in women's lifespan, with a rate nearly double that observed among males [47–49]. Moreover, scientific literature highlights that individuals not conforming to traditional gender roles are more likely to experience anxiety and depression compounded by identity conflict and social stigma than those who adhere to predefined gender roles [50]. Within this population, feelings of loneliness, social retirement or anxiety and depression due to being misunderstood and rejected by their social groups are particularly prevalent.

In this work, we conducted exploratory research on internalized gender stereotypes performed through an online survey using a survey of 56 questions, based on literature reviews, administered with Google Form. The questions randomly displayed in the questionnaire investigated six areas: games, profession or jobs, personality traits, home and family activities, sports, and moral judgments. For the assessment of gender stereotypes the subject was required to respond with a six points Likert scale and choose between six points ranging from 1 (completely agree) and 6 (no difference between men and women); moreover, to test the reliability of each subject's answers, we included three reversed questions.

To ensure a wide distribution of the survey link, we used a snowball sampling procedure, starting from a distribution among health professionals, university personnel and students in different regions of Italy. Furthermore, we used social networks to promote and ensure to maximize a large coverage of participants. Each survey participant was asked to answer a yes or no question confirming their voluntary willingness to participate in the survey. Exclusion criteria were participants under 15 years old and non-residents in Italy. No reward was expected and once

confirmed, participants were directed to anonymously complete a self-report questionnaire. For the online survey, no formal ethics approval was needed.

The survey was composed of two sections: first, a sociodemographic factor and second, the gender stereotypes questionnaire. The total time to complete the Italian survey required approximately 10 minutes.

For the analysis, we used chi-square tests to investigate the associations between variables and for each of the six latent constructs, Principal Component Analysis (PCA) was performed in order to reduce dimensionality and summarise constructs that were non-observable. The cumulative explained variance threshold for component retention was set at least 70%, and item loadings were assessed to evaluate the magnitude and direction of each item's contribution to the retained components.

The sociodemographic variables considered are gender, age, education, field of study, occupation, marital status, region of residence and geographical origin of family origins (Table 2.1.1).

Table 2.1.1 Sociodemographic variables and stratifications of samples (Adapted from Carvalho Silva R et al., 2024 [51])

Sociodemographic variables and stratifications of samples	
	Overall N=1844
Gender	
Female	1292 (70.1%)
Male	534 (29.0%)
Other/do not want to answer	18 (1.0%)
Age	
15–18	74 (4.0%)
19–22	223 (12.1%)
23–30	347 (18.8%)
31–40	336 (18.2%)
41–50	304 (16.5%)
51–60	296 (16.1%)
61–70	214 (11.6%)
Over 70	50 (2.7%)
Educational level	
Elementary and secondary school degrees	122 (6.6%)
High school degree	662 (35.9%)
University degree	737 (40.0%)
Post-University degrees	323 (17.5%)
Job	

Student	418 (22.7%)
Housewife	36 (2.0%)
Retired	166 (9.0%)
Unemployed	33 (1.8%)
Freelancer professionals	265 (14.4%)
Permanent worker	654 (35.5%)
Fixed-term worker	186 (10.1%)
Other	86 (4.7%)
Marital status	
Unmarried	827 (44.8%)
Married	861 (46.7%)
Divorced	132 (7.2%)
Widowed	24 (1.3%)
Family origins	
Italian descendent	1769 (95.9%)
Non-Italian descendent	75 (4.1%)
Area of residence	
N-Miss	15
North	1568 (85.7%)
Center	90 (4.9%)
South	171 (9.3%)

Summary of the main findings

1880 Italian-speaking participants took part in the online survey and 26 of them were excluded because they provided unreliable responses on the three reversed questions. The final sample was composed by 1854 participants with a gender distribution of female (70.1%), male (26.1%), and 18 subjects (1.0%) who responded others or did not provide an answer to the gender question.

The distribution of the age of participants was concentrated in the range between 23 to 40, with respectively 18.8% in the age group of 23-30 years old and 18.2% in the 31–40 range age, rather than the other age groups, which ranged from 15 to over 70. Regarding the level of education, most of the subjects had a university degree (40.0%) or a post-university degree (17.5%). 35.9% of the participants only achieved a high school degree, and 6.6% had a primary or secondary school degree.

Regarding the distribution of employment categories, the sample was mostly composed of permanent workers (35.5%), students (22.7%) and freelancer professionals (14.4%), with smaller percentages of temporary workers (10.1%), retirees (9.0%), housewives (2.0%), unemployed subjects (1.8%), and other categories (4.7%). Moreover, for marital status we find a balanced

distribution between married (46.7%) and unmarried (44.8%), with a small presence of divorced and widowed, respectively 7.2% and 1.3%.

The family origins showed that 95.5% of participants were Italian, while 4.1% were of non-Italian descent. Furthermore, 85.7% participants were residents of Northern Italy, followed by South (9.3%) and Central Italy (4.9%), with only 15 participants not reporting their area of residence.

Overall agreement on gender stereotypes

The descriptive analysis showed an overall level of agreement with statements on gender stereotypes, calculated by adding the responses "completely agree," "strongly agree," and "somewhat agree". The principal items with highest agreement (>25% of total agreement) were: "Rugby is a sport for men", "Boxing is a sport for men", "Women are more sensitive than men", "Women are more courageous than men", "Working as a babysitter is a women's job" and "The military profession is a men's job" (Table 2.1.2)[51].

Table 2.1.2. Descriptive statistics reporting the total percentage of agreement among all survey respondents, ranked in descending order

Descriptive statistics reporting the total percentage of agreement among all survey respondents, ranked in descending order	
Questions (**latent construct**)	Total % of agreement
Rugby is a sport for males (**sports**)	35.80%
Women are more sensitive than men (**personality traits**)	33.50%
Women are braver than men (**personality traits**)	30.20%
Boxing is a sport for males (**sports**)	29.00%
Working as a babysitter is for women (**profession**)	28.00%
The military profession is for men (**profession**)	26.50%
Men are more aggressive than women (**personality traits**)	23.60%
Women are more determined than men (**personality traits**)	23.40%
Playing with dolls is for girls (**games**)	22.80%
Women are cunning than men (**personality traits**)	21.40%
Men drive better than women (**home and family activities**)	20.70%
For men, more than for women, it is very important to have success in their job (**personality traits**)	20.00%
Artistic gymnastics is for females (**sports**)	19.00%
Women are more stubborn than men (**personality traits**)	17.60%
Working in a butcher shop is for men (**profession**)	17.00%
Women are more competitive than men (**personality tra**its)	16.30%
Women are kinder than men (**personality traits**)	15.70%

Football is a sport for males (**sports**)	13.80%
Ironing is an activity for women (**home and family activities**)	13.60%
Videogames are for males (**games**)	12.60%
Women are more jealous than men (**personality traits**)	12.10%
Women are more generous than men (**personality traits**)	9.80%
It is women, more than men, who must take care of the children (**home and family activities**)	9.60%
Cycling is a sport for males (**sports**)	8.60%
Being a pilot is a job for men (**profession**)	8.30%
It is men, more than women, who have to support the family (**home and family activities**)	7.50%
Nursing profession is for women (**profession**)	6.90%
Women are more aggressive than men (**personality traits**)	6.80%
Being a bus driver is a job for women (**profession**)	5.40%
At home, it is men, more than women, who command (**home and family activities**)	4.90%
Karate is a sport for males (**sports**)	4.90%
Women are nicer than men (**personality traits**)	4.70%
Women are more selfish than men (**personality traits**)	4.70%
Men are more inclined towards scientific subjects than women (**profession**)	4.70%
It is women, more than men, who have to cook at home (**home and family activities**)	4.70%
The surgical profession is a job for men (**profession**)	4.50%
It is men, more than women, who are responsible for investing the family's savings (**home and family activities**)	4.10%
The bricks are a game for boys (**games**)	4.00%
The profession of psychotherapist is for women (**profession**)	3.90%
The toy cars are a game for girls (**games**)	3.50%
The surgical profession is a job for women (**profession**)	3.50%
It is understandable that a man, more than a woman, can lose patience (**moral judgments**)	3.50%
It is men, more than women, who have to make the most important decisions concerning the family (**home and family activities**)	3.30%
Women are more disorganized than men (**personality traits**)	3.20%
Rugby is a sport for females (**sports**)	2.90%
Sewing is an activity for men (**home and family activities**)	2.50%
In a couple, female infidelity is more serious than male infidelity (**moral judgments**)	2.50%
It is men, more than women, who have to clean the house (**home and family activities**)	2.20%
It is men, more than women, who have to help the children with their homework (**home and family activities)**	1.90%
Basketball is a game for females (**sports**)	1.80%
The tricycle is a game for boys (**games**)	1.70%
Stuffed animals are a toy for boys (**games**)	1.70%
A woman who works will never be completely a good mother and partner (**moral judgments**)	1.60%
Tennis is a sport for males (**sports**)	1.50%
Skiing is a sport for males (**sports**)	1.00%
Puzzles are a game for males (**games**)	0.40%

Stratified analyses by age, gender, and educational level were performed due to the adequate numerical representation of all categories. Most items exhibited highly significant effects and supporting the strong influence of these variables on gender stereotypes. Specifically, 54 out of 56 items showed significant effects when stratified by age, 49 items when stratified by gender, and 51 items when stratified by educational level.

Principal Component Analysis (PCA) for the six areas

A preliminary analysis demonstrated a strong positive correlation among all individual items (p-values < 0.05). We conducted a PCA to identify the latent variables from the six areas below reported:

For home and family activities, four components explained at least 70% of the total variance; particularly noticeable is the first component, which accounted for 47% of the variance and was mainly driven by questions related to ironing, driving ability and childcare responsibilities.

In the game's domain, instead, three components explained more than 70% of the variance, and the first component accounted for 52%, being influenced by the item "Playing with dolls is for girls."

Regarding moral judgments, two components were extracted. The first component explained 66% of the variance, being mainly influenced by the respondents' attitudes towards male tendency to lose patience and the perceived greater severity of women's infidelity.

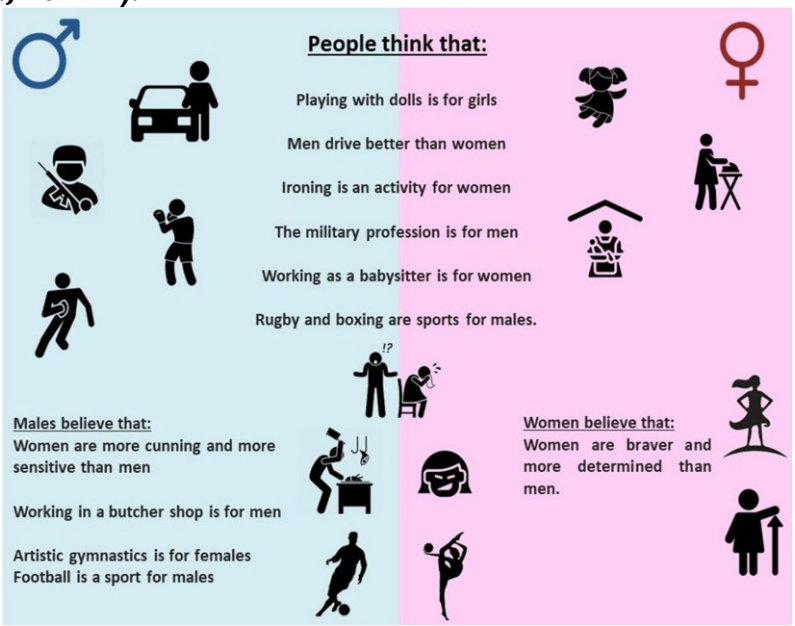
For the personality traits, the variance was mainly explained by six components, which accounted for a 70% of the total. The first component (40%) was mostly driven by items referring to women being more sensitive, determined, and cunning than men. Gender-specific analyses revealed different item loadings: among women, bravery and determination had a major impact, whereas among men, sensitivity and cunning were, remarkably, more influential.

In the jobs' domain, four components were identified. The first component explained 48% of the variance, being notably driven by the items "The military profession is for men" and "Working as a babysitter is for women." Only among male respondents, it was noted that an additional item, "Working in a butcher shop is for men", also contributed to the first component, increasing the explained variance to 52%.

Finally, in the sports domain, three components explained at least 70% of the variance, with the first component accounting for 59% of the variance. Such component was mainly influenced by the items "Boxing is a sport for males" and "Rugby is a sport for males." Among male respondents,

additional components related to artistic gymnastics (which was female-associated) and football (mostly male-associated) contributed to the explained variance (57%). A short representation is shown in Figure 2.1.

Fig. 2.1.1. Summary of the principal findings from the PCA analyses. In the figure the items most strongly associated with gender stereotypes are indicated (Adapted from Carvalho Silva R et al., 2024 [51]).



Discussion

In the present study, we conducted an exploratory research analysis through an online survey administered by Google Forms among 1854 participants residing in Italy, to describe the current prevalence of gender stereotypes. The results revealed statistically significant effects for most of the items analysed, stratified by age, gender, educational level, and demographic factors. In particular, most significant effects were observed when we divided the sample by age, educational level, and gender, accounting respectively for the 96%, 91%, and 87% of the analysed items.

These results highlight the pervasive influence of gender stereotypes on individual beliefs across diverse demographic groups, thus emphasising their capacity to form perceptions within the sample under investigation, and by extension the population at large. Moreover, the results of the Principal Component Analysis (PCA) identified latent constructs across multiple domains and therefore revealed substantial latent stereotypes within each category. Remarkably, no statistically significant gender differences were observed in the domains of "home and family activities",

"games" and "moral judgments", suggesting that these stereotypes are similarly pervasive and internalised both in men and women.

These findings are consistent with past research, suggesting that internalised gender stereotypes exert significant effects on physical and psychological health alike, increasing the vulnerability to stress, anxiety, and depressive symptoms, particularly throughout the female lifespan, with prevalence rates nearly twice as high as those observed among males[45,46,51].

One of the potential limitations of our study concerns the sample composition, as we did not take into account additional factors that may exacerbate the likelihood of developing psychological or social difficulties related to gender stereotypes. Indeed, it is more likely that certain groups of women and men, such as individuals belonging to ethnic minorities, people suffering from disabilities, those with a low socioeconomic status, members of the LGBTQIA+ population, may be disproportionately affected by gender stereotypes when these intersect with other forms of discrimination, substantially increasing the risk of social isolation and hindering the free expression of personal identity [50]. Future studies in this field will need to take into account, in addition to the deeply rooted and pervasive nature of internalised gender stereotypes at the individual level, stratification by ethnic origin, disability status, and sexual orientation.

2.2. The role of traumatic experiences in the vulnerability of mental disorder and treatment outcomes

The exposure to traumatic experiences during the early period of life, particularly in childhood, has been identified as a prominent vulnerability factor for the development of mental disorders across the lifespan. Several studies have tried to estimate the prevalence of early life stress (ELS) events, such as violence, physical or sexual abuse and others forms of maltreatment, in an overall range from 1.4% to 80.1% [52,53]. ELS has a marked association with an increased risk of developing psychiatric disorders; however, most studies addressing ELS emphasise their focus principally on sexual and physical abusers [54–61].

Nevertheless, other forms of childhood trauma and early life adversities (ELA) may contribute to vulnerability in adulthood, including neglect [52,62–64]. In the absence of a standardised definition of this construct, childhood neglect is generally referred to as a persistent and pervasive failure of the caregiver to provide a child's basic needs, whether material or psychological, thereby limiting or impairing the adequate development of physical and mental health. Several studies have also highlighted a greater association between childhood neglect and the early onset of MDD in females that reported several depressive episodes, rather than men and individuals without a history of childhood neglect [58,59,65]. Moreover, it was associated with vulnerability to other mental disorders such as anxiety disorders, bipolar disorder (BD), psychosis and schizophrenia (SCZ), post-traumatic stress disorder (PTSD), obsessive-compulsive disorder (OCD), personality disorders (PD) and eating disorders (ED) [66–76].

Furthermore, in the field of childhood neglect, it is possible to make a distinction between the caregiver's failure to provide basic physical care, such as adequate nutrition, clothing, hygiene and healthcare or appropriate living conditions, which is referred to as physical neglect (PN) and caregiver's omission or inadequacy in meeting a child's emotional needs, such as nurturance, affection and psychological support, or the persistent disregard of the child's emotional needs, defined as emotional neglect (EN) [52,77]. EN has been shown to be related to chronic MDD and TRD in young women, while PN was associated with a lower rate of remission and recovery to MDD [58].

We conducted a systematic review and meta-analysis to examine the prevalence of childhood neglect, with the aim of providing a comprehensive overview of this construct by considering unspecified neglect (Ne), PN, and EN across the most common mental disorders. Specific attention was given to differences between psychiatric diagnoses, with particular emphasis on MDD, and to the assessment of moderators influencing heterogeneity in prevalence estimates.

Our systematic review and meta-analysis were conducted in adherence to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [78] and the protocol of the systematic review was registered in the International Prospective Register of Systematic Reviews (PROSPERO, CRD42020182140).

In the literature research, we considered the studies published on PubMed, PsycINFO and Embase, between January 1, 1994 and March 24, 2023, according to the advent of the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) and subsequent editions. Also, we included the classifications based on the International Statistical Classification of Diseases (ICD). Disorders examined included PTSD, OCD, MDD, BD, ED, PD and anxiety, as well as mixed psychiatric conditions involving the aforementioned diagnoses. All of them were established according to standardised clinical diagnostic manuals and classification systems. The evaluations of childhood neglect were defined in three variables related to the exposure of interest, including EN, PN and unspecified neglect (Ne); the latter was based on the global neglect score reported in the studies, which did not differentiate between EN and PN. We considered the studies that used only validated measurement instruments or clinical interviews.

Data extracted from the retrieved studies included the full bibliographic reference, study design, participants' age, proportion of female subjects, diagnosis, diagnostic category, diagnostic assessment tool, childhood neglect assessment instrument, type of neglect (Ne, EN, and/or PN), and the statistical information required for the computation of proportions, namely the number of patients exposed to neglect and the sample size of each clinical population.

When studies included a single clinical sample exposed to childhood neglect, the diagnostic category was assumed to correspond to the reported diagnosis. Conversely, when more than one clinical sample was included, and samples were comparable across different diagnoses, these were grouped under the broader category of Mood Disorders.

Accordingly, the diagnostic category was considered equivalent to the diagnosis when studies included only one clinical sample. In studies including two or more different clinical samples without a clear distinction between diagnostic groups, the number of subjects exposed to neglect was clustered under the category Psychiatric Disorders (PsychiatricD).

The risk of bias (RoB) of the studies included in the systematic review was assessed using the eight items of Joanna Briggs Institute (JBI) Critical Appraisal Checklist for Analytical Cross-Sectional Studies [79].

For the analysis of the three eligible proportions of childhood neglect (Ne, EN, PN), we used R software and its related packages, metafor and dmetar [80–82]. To calculate the overall proportion across the selected studies, a generalised linear mixed model was applied.

To estimate the variance between the studies (τ^2), we used the maximum-likelihood method and the statistical heterogeneity was assessed through Cochran's Q statistic and Higgins and Thompson's I^2 index [83]. The heterogeneity was classified as absent, low, moderate or high for I^2 values of 25%, between 25% and 50%, between 50%, 75%, and greater than 75%, respectively. The presence of outliers was investigated through the overlap between individual study confidence intervals and the pooled estimate, whereas influential studies were detected using a leave-one-out approach and influence diagnostics, including Baujat plots and the Graphic Display of Heterogeneity (GOSH) diagnostic [83–85] .

Publication bias was evaluated across contour-enhanced funnel plots and Egger's regression test. The contour-enhanced funnel plots included three shaded regions corresponding to different levels of statistical significance, within which the estimates of individual studies were located (dark blue: $0.025 < p < 0.05$; blue: $0.01 < p < 0.025$; light blue: $p < 0.01$). The Duval and Tweedie trim-and-fill method was applied to estimate bias-corrected pooled overall proportions [86–88].

Subgroup analyses were conducted using random-effects models (random-effects models within and between subgroups) to evaluate the impact of psychiatric diagnostic categories on pooled prevalence estimates data. In addition, simple meta-regression models were performed to examine moderator effects and the proportion of heterogeneity explained by selected variables, including psychiatric diagnostic category, childhood neglect assessment tool, age, sex, recruitment setting, RoB, and year of publication. Multiple meta-regression models were not conducted due to the limited number of significant moderators identified in the univariate analyses for each outcome (NE, EN, PN).

Summary of the main findings

Search results of the included studies

We identified 22564 studies on database searching (PubMed, Embase, PsycINFO) and two further studies were identified through additional sources. Once duplicates had been removed, 13258 records were left for screening based on titles and abstracts. This step led to 747 articles being selected for full-text evaluation, most of which were subsequently excluded (n = 625) due to a failure in meeting the inclusion criteria. In particular, 551 studies didn't provide the necessary

information on the primary outcome of interest, 35 records reported data which was extracted from the same sample, 32 studies were excluded for either methodological or diagnostic reasons (e.g., diagnoses not consistent with the inclusion criteria or based on DSM-III), and 7 studies could not be assessed because the whole paper was unavailable. Where possible, corresponding authors were contacted and inquired about missing articles or were asked for additional data. Ultimately, after the exclusion process,122 studies fulfilled all eligibility requirements and were included in the meta-analysis (Figure 2.2.1). The RoB analysis we performed, highlighted how forty-five studies were classified as low risk of bias (36.9%), while seventy-seven studies had a moderate risk (63.1%); none of the studies presented a high risk of bias.

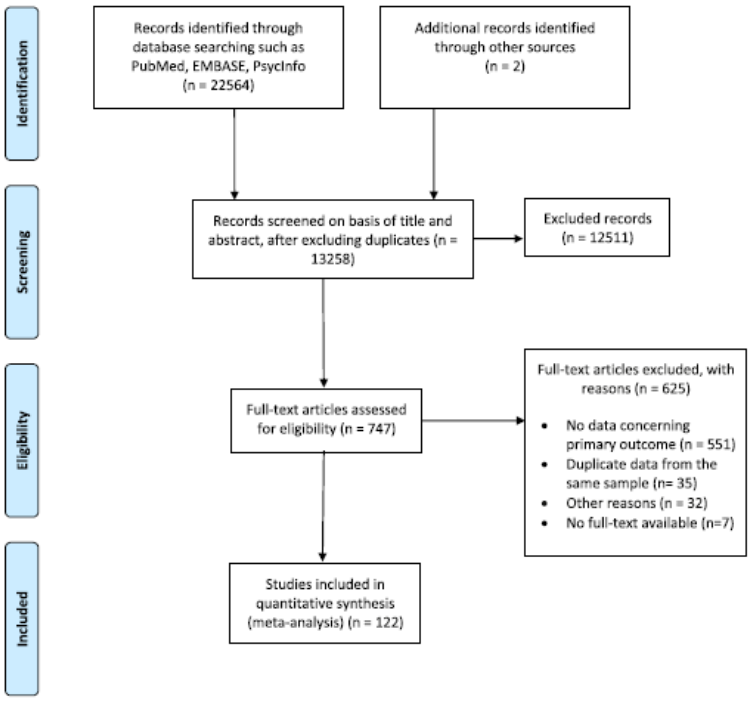
The 122 studies included in the meta-analysis comprised of clinical samples recruited across Europe, North and South America, Asia and Australia, with the sample size ranging from 19 to 9493 participants (Median=144, Interquartile Range=275.8) with a pooled age for Ne of 41.1 years (95% Confidence Interval (CI)=37.4 - 44.8), 38.9 for EN (95%CI=36.8 - 40.9) and 38.7 for PN (95% CI=36.7–40.8). The aggregate proportion of females for Ne was 60.8% (95% CI=37.9% - 79.8%), for EN was 71.1% (95%CI=62.4% - 78.4%) and 72.0% for PN (95%CI=63.2% - 79.5%).

The most commonly investigated diagnoses were MDD (33 studies), followed by BD (18 studies), unspecified mood disorders (7 studies), SCZ spectrum and other psychotic disorders (24 studies), PTSD (6 studies), ED (15 studies), PD (4 studies), OCD (2 studies) anxiety disorders (2 studies) and finally mixed psychiatric diagnoses (11 studies). Diagnoses were predominantly established according to DSM criteria, with a smaller number of studies relying on ICD-10 or structured diagnostic interviews such as the MINI or SCAN.

Childhood neglect was primarily evaluated using the Childhood Trauma Questionnaire (CTQ), while alternative instruments included the Childhood Experiences of Care and Abuse Interview (CECA), Adverse Childhood Experiences Questionnaire (ACE-Q), Adverse Childhood Experiences International Questionnaire (ACQ-IQ), Childhood Experiences Questionnaire (CEQ), Traumatic Antecedents Questionnaire (TAQ), Infancy Trauma Interview (ITI), Adult Attachment Interview (AAI), Early Life Stress Questionnaire (ELSQ) and the Life Stressor Checklist (LSC).

Out of 122 studies, 55 reported prevalence rates for Ne (n = 3417; 46.6%, 95% CI = 34.5 - 59.0), while 107 studies reported rates for EN (n = 50373; 43.1%, 95% CI = 39.0 - 47.4) and PN (n = 51807; 34.8%, 95% CI = 30.6 - 39.2) among patients with any psychiatric diagnosis of interest.

Figure 2.2.1. PRISMA flow diagram of the study selection procedure, including identification, screening, eligibility and inclusion stages (Adapted from Carvalho Silva et al. 2024 [58]).



The high heterogeneity in the estimated proportions led to the isolation and identification of several outliers, 7 for Ne, 52 for EN and 50 for PN. Sensitivity analyses performed removing these outliers showed a decrease in prevalence for Ne (42.2%, 95% CI = 33 - 52), EN (41.7%, 95% CI = 39.7 - 43.7), while PN slightly increased (35.2%, 95% CI = 33.2 - 37.2). While reduced, heterogeneity remained substantial for all outcomes even after the outlier removal: Ne (I^2 = 79.8%; Q = 34.7, df=7, p < 0.001), EN (I^2 = 71.4%; Q = 188.9, df=54, p < 0.001) and PN (I^2 = 71.2%; Q = 194.7, df=56, p < 0.001).

Moreover, while the Influential analysis identified a few key studies (2 for Ne, 3 for EN, and 4 for PN), excluding them had minimal impact on pooled prevalence and heterogeneity, except for Ne, which exhibited a slightly lower prevalence (41.3%, 95% CI = 30.2 - 53.3; 93.2%, Q = 177.2, df=12, p < 0.001).

For the assessment of publication bias the funnel plots yielded some noteworthy results, with small studies tending to report lower Ne prevalence and higher EN and PN prevalence. Egger's test confirmed bias only for EN. Applying the trim-and-fill method reduced the pooled prevalence of EN (28.5%) and PN (25.1%) but didn't influence the high heterogeneity.

The subgroup analyses by psychiatric diagnosis showed that prevalence rates varied across disorders: for Ne, the small number of studies per group hindered interpretation, although differences between groups were significant. For EN, BD and MDD were associated with lower prevalence, while for PN, MDD, BD, ED, and broader psychiatric disorders predicted lower rates. However, heterogeneity within groups remained high for most outcomes. Moreover, the meta-regression analyses revealed that the type of psychiatric diagnosis did not explain the heterogeneity for any form of neglect. In contrast, the assessment tool influenced the prevalence estimates for Ne and PN. Indeed, ELSQ, ITI and CECA yielded lower Ne rates, while CEQ and CTQ reported higher rates; PN prevalence was lower when measured with ACE-IQ, ACE, or CTQ. Finally, other sociodemographic factors, recruitment setting, RoB and year of publication of the study did not significantly affect prevalence or heterogeneity.

Discussion

Our meta-analysis provides a comprehensive assessment of neglect prevalence across a range of psychiatric disorders, based on evidence from 122 studies including a total of 57638 patients and demonstrated the importance of investigating childhood neglect, as shown in scientific literature on ELA, in relation to vulnerability to the development of mental disorders. Previously, studies highlighted how Ne was more firmly related to possibly MDD in adulthood, especially for EN, which has evidenced a stronger positive association with MDD diagnosis rather than other maltreatment categories. Another meta-analysis underlined that EN and PN were associated with depression in adults with pre-existing childhood maltreatment in the 43.2% and 36.1% of cases, respectively [60]. Also, other previous research has shown how adults with a history of childhood neglect presented a 2.75 times higher risk of developing MDD compared to non-exposed adults [59,60,65,89].

In the studies considered for our meta-analysis, the prevalence of Ne was 46.6%, based on 15 studies, whereas for EN and PN, each examined in 107 studies, the prevalence rates highlighted was 43.1% and 34.8%, respectively; across studies, PN seemed to be lower than the Ne and EN. This divergence in the accounting of studies on childhood neglect or its different forms raises questions about the importance of distinguishing among the neglect subtypes, as each form is known to be associated with specific and distinct effects on mental health outcomes [52,53]. As a matter of fact, there is a notable absence of a standardised definition of childhood neglect in literature, and this may contribute to the heterogeneity of the results that emerged, explaining

how in previous studies we found a large prevalence rate ranging from 1.4% to 80.1% depending on the identified sample [52].

We carried out a subgroup analysis to disentangle whether childhood neglect prevalence differed across psychiatric diagnoses. Concerning Ne, we found a lower prevalence rate in the MDD groups (26%, 2 studies), whereas EN was underlined in the BD (37%, 15 studies) and MDD (42%, 30 studies) sample of patients, rather than other considered diagnoses. Comparable findings were observed for PN in patients with MDD (28%, 30 studies), BD (33%, 15 studies), ED (32%, 14 studies) and psychotic disorders (31%, 8 studies). Conversely, a high prevalence of Ne values was found in the PTSD samples (61%, 2 studies) and a high prevalence of EN emerged from the PTSD (66%, 3 studies), OCD (50%, 2 studies) and PD category sample (60%, 3 studies); on the other hand, PN was present in OCD (74%, 2 studies), PD (50%, 4 studies), PTSD (47%, 5 studies) and psychotic disorders (44%, 22 studies). A preceding systematic review of different psychiatric diagnoses highlighted that Ne was predominantly associated with mood disorders, anxiety, and PD. EN was found to be correlated with mood disorders, anxiety, ED, PD, and SCZ, whereas PN was associated with PD and SCZ [90]. This systematic review found that PN was the maltreatment subtype with the fewest positive associations with psychiatric disorders, consistent with our findings showing a lower prevalence of PN in clinical samples compared with Ne and EN.

For these reasons, a moderating effect of the psychiatric diagnostic category could not be definitively confirmed, as observed differences may rather be the reflection of the intrinsic heterogeneity of the included studies than true diagnostic effects. It has to be noted that only a limited number of meta-analyses have focused on childhood neglect as the primary topic within clinical samples, rather than on specific clinical diagnoses or in the general population, and this may be a relevant contribution to the observed heterogeneity of results [58].

As previously identified, the different outcomes from previous meta-analyses can be explained by three main limitations in childhood neglect research: first, the lack of a shared consensus definition for the subject matter, which may have contributed to heterogeneity among samples; second, the absence of standardized methods and measures to assess the dimensional aspects of neglect; and finally, the difficulty of accounting for additional biopsychosocial factors that may have interacted to sustain a functional development trajectory.

2.3. The role of agitation–anxiety symptoms in MDD

The term depression typically refers to a broad spectrum of symptoms encompassing emotional tone, cognition, physical health, and socio-occupational functioning. Accumulating evidence suggests that MDD should be conceptualised not as a single nosological entity, but as a syndromic constellation of symptoms that leads to heterogeneity in clinical presentation, illness course, recovery trajectories, and treatment response at the individual level [91–93]. A prior cluster analysis of 10158 individuals drawn from a large mental health platform revealed that there are five principal symptom components in MDD, such as anxious distress, core emotional symptoms, agitation/irritability, insomnia and anergic/apathy domains [92].

The presence of psychomotor agitation symptoms during depressive episodes appears to be associated with an increased risk of suicide in patients treated with antidepressants. Similarly, the presence of anxiety increases the risk of suicidal ideation in patients with unipolar and bipolar depression [94–96]. Several studies had underlined that anxious depression has been suggested to be associated with a distinct neurobiological profile, including immune and HPA-axis dysregulation, as well as brain alterations such as cortical thinning and increased corticolimbic functional connectivity, when compared with patients without anxious depression. The presence of anxiety in depression appears to be more prevalent in women than in men [97,98].

Moreover, previous research has shown that the co-occurrence of anxiety in depression is associated with poorer recovery outcomes, impairment in social and occupational dysfunction, lower quality of life, greater frequency of side-effects and increased risk of suicide attempts compared with non-anxious depressed subjects [99–101]. Furthermore, several studies suggest that the presence of comorbid anxiety in MDD is associated with an increased risk of developing treatment-resistant depression (TRD) [102].

The magnitude of this phenomenon is non-negligible in clinical practice, as the prevalence of lifetime co-occurring anxiety among individuals with MDD ranges from 16% to 72%, depending on the reference sample and the diagnostic criteria used [103–106].

In this study, we examined the construct of agitation–anxiety in unipolar depression across three independent samples (PANDORA, GSRD, and STAR*D), focusing on its association with overall depression severity and its relationships with other clinical variables, such as illness duration and treatment response.

Sociodemographic and clinical data were extracted from three trials: the Group for the Study of Resistant Depression" (GSRD)[107], “PANDORA trial”[108] and “Sequenced Treatment Alternatives

to Relieve Depression" (STAR*D) [109]. Each study was approved by the relevant Ethics Committee, and all participants provided written informed consent. Agitation–anxiety was assessed using different instruments across the studies: the retrospective Montgomery–Åsberg Depression Rating Scale (rMADRS), the 17-item Hamilton Depression Rating Scale (HAM-D), and the 30-item Inventory for Depressive Symptomatology, Clinician-Rated (IDS-C30), respectively. For each subject of the dataset, a composite agitation–anxiety cluster score was calculated by summing the scale items that best represented psychomotor activation and anxious distress. The median cluster score was calculated and patients were classified into individuals with (AA+ = above the median cluster score) and without agitation-anxiety (AA- = equal to or less than the median). In relation to the extraction of treatment response, we used a cut-off of ≥50 % improvement in depressive symptoms following adequate antidepressant treatment.

All datasets were analysed using STATA v.18 [110]. A t-test and chi-square tests were used to examine the continuous and categorical variables. A multivariate analysis was performed to discover the potential associations between agitation features and suicidal thoughts or attempts, disease duration, and treatment response.

Summary of the main findings

The total sample included 3364 patients with MDD, of whom 2163 (64.3%) were female. The mean age was 47.3 ± 14.3 years. No significant differences were observed within the samples, except in the GSRD cohort, where AA+ patients were slightly older ($p = 0.005$).

Table 2.3.1. summarises the main demographic and clinical characteristics of the three MDD cohort studies, PANDORA, GSRD and STAR*D. For each dataset, the total number of participants, the number and percentage of females, the mean age with standard deviation (SD), and the mean disease duration with SD are reported. Additionally, the mean and median values of the agitation–anxiety cluster scores are shown, along with the number of patients classified as AA+ (those with a cluster score above the median). Overall, the samples were predominantly female, with mean ages ranging from 43.1 to 51.4 years and mean disease durations between 15.1 and 17.6 years. The proportion of AA+ patients varied across studies, from 21% in the GSRD cohort, 39% in PANDORA and 43% in STAR*D.

Table 2.3.1. Clinical and sociodemographic features of the three MDD cohorts

Dataset	Total Number	Females n (%)	Mean age ± SD	Mean disease duration ± SD	Agitation-anxiety cluster mean ± SD	Cluster median	AA+ n
PANDORA	244	172 (70.5%)	46.8 ± 14.5	15.3 ± 13.1	3.9 ± 1.5	4	95
GSRD	1588	1029 (64.9%)	51.4 ± 14.1	15.1 ± 13.0	3.6 ± 1.2	4	335
STAR*D	1532	962 (62.8%)	43.1 ± 13.2	17.6 ± 14.0	3.2 ± 1.7	3	663

Patients with agitation–anxiety (AA+) showed higher baseline depression severity, independent of age and sex, across all three datasets (PANDORA, p =0.003; GSRD and STAR*D, p <0.001). AA+ status was also strongly associated with suicidal ideation and attempts in STAR*D and GSRD (p <0.001). Additionally, AA+ patients in GSRD and STAR*D had longer disease duration (p =0.001) and poorer treatment response (p <0.001), even after adjusting for age and sex. No association was found between agitated depression and a family history of bipolar disorder (p >0.05).

Discussion

In this study, we analysed the clinical construct of agitation-anxiety in three cohorts of MDD patients, who took part in international studies, as a predictor of poor recovery of the disease. In this way, we have placed our focus on the associations between the severity of depression assessed by standardised rating scales and the clinical disease duration. In the total sample of 3364 patients, no statistical difference was found for the mean age of subjects, except for a slightly older sample in the GSRD cohort, which does not invalidate the analysis.

Moreover, the presence of agitation–anxiety (AA+) showed a higher baseline depression severity, independent of age and sex, across all three datasets, with a strong association with suicidal behaviour for the cohort of STAR*D and GSRD (p <0.001). In line with the other studies, anxious depression shows an increased risk of suicidal behaviour with a specific phenotype [94–96]. Additionally, AA+ patients in GSRD and STAR*D had longer disease duration (p =0.001) and poorer treatment response (p <0.001), even after adjusting for age and sex. No association was found between agitated depression and a family history of bipolar disorder (p >0.05), in previous studies [95].

Despite methodological limitations, including heterogeneity in rating scales (HAM-D, rMADRS, IDS-C30) and the proxy nature of the agitation–anxiety construct, the present study supports the clinical relevance of considering AA+ as a combined dimension in unipolar depression. Across

three large and independent cohorts, this dimension was consistently associated with greater depression severity, poorer outcomes, and augmented risk of suicidal risk, highlighting its potential value for clinical assessment and future biological and psychological research in MDD.

3. New Insights into Pharmacological Treatments of Mood Disorders

The prevalence of mood disorders in the world varies in accordance with the diagnostic classification system employed by each examiner. Even though the pathophysiological mechanisms underlying unipolar depression, or MDD, as well as the mechanisms of action for antidepressant pharmacological treatments are highly complex and not yet fully explained, the reason why some patients respond to first-line therapies, whereas others fail to achieve an adequate clinical response after two or more treatment trials, even when different classes of antidepressant medications are used, remains unclear. These patients are commonly classified as TRDs, for which additional and more precise diagnostic and therapeutic strategies are still to be defined.

At the moment of this analysis, SSRIs and SNRIs are all considered first-line pharmacological treatments for MDD, thanks to both their favourable efficacy–tolerability balance and their safety profile. In contrast, tricyclic antidepressants are generally considered second- or third-line options because of their higher incidence of adverse effects and increased toxicity risk. In addition, other antidepressant agents are commonly used either as first-line alternatives or as augmentation strategies in patients with MDD: these antidepressants include mirtazapine (NaSSA), trazodone (SARI), vortioxetine (a multimodal serotonergic antidepressant), and bupropion (NDRI), which all belong to a heterogeneous group of antidepressants characterized by pharmacological mechanisms which are distinct from those of SSRIs and SNRIs [111–113].

Furthermore, some studies have shown that the effect of endogenous l-acetylcarnitine (LAC) has been suggested to exert antidepressant effects as well, by supporting mitochondrial energy metabolism while modulating neurotransmitter systems, and promoting neuroplasticity via epigenetic acetylation of histones and transcription factors, including NF-κB, that enhances mGlu2 receptor expression. In a similar fashion, N-acetylcysteine (NAC), administered as monotherapy or in combination with other antidepressant treatments, has clearly demonstrated a reduction in depressive symptoms compared with placebo, with its mechanism of action partially involving, according to different studies, the alleviation of oxidative stress through increased glutathione synthesis [114–116]. However, this evidence is still limited and unclear and has not yet received approval for MDD.

In this section, I analyse and discuss the recent insights highlighted in literature on the use of ketamine and esketamine, the latter recently approved by AIFA for clinical use (AIFA Resolution No. 334/2022), as a combination treatment with SSRIs or SNRIs for patients with TRD.

3.1. Evidence from the literature on the use of ketamine and esketamine in TRD patients

Repeated failures in the treatment of depressive symptoms represent a substantial challenge in the development of effective therapeutic strategies, since patients with TRD exhibit substantially reduced quality of life, diminished work productivity, and an increase in the utilisation of healthcare resources. An in-depth literature analysis shows that TRD can affect up to 30% of patients, even after adequate trials of conventional antidepressant therapies for MDD [117,118]. These treatment-resistant cases emphasise the pressing need to develop novel therapeutic approaches that both involve alternative neurobiological pathways and deliver faster, more robust, and sustained clinical benefits.

Recent evidences have highlighted that an innovative therapeutic strategy for the treatment of TRD patients has emerged with the use of intravenous ketamine and intranasal esketamine, which are, respectively, R and S-enantiomers of racemic ketamine. Both the approaches have demonstrated faster antidepressant effects when compared to classical antidepressant drugs, due to their action as non-competitive antagonists of the NMDA (N-methyl-D-aspartate) receptor in patient's brain [36]. Moreover, the duration of therapeutic effects seems to be linked to the type of substance used and to the clinical or hospital context in which it is administered.

From a molecular standpoint, ketamine is thought to exert pleiotropic effects on multiple interconnected biological systems, influencing immune, inflammatory, and neurotrophic pathways alike. Although the exact molecular mechanisms underlying esketamine's effects remain to be fully clarified, current evidence suggests its involvement in the modulation of immune pathways, interferon signalling, and the upregulation of brain-derived neurotrophic factor (BDNF), all processes that are closely linked to neuroplasticity-related mechanisms [119].

As of the time of this paper's finalisation, there is no clear consensus in the literature regarding optimal biomarkers for evaluating the effects of esketamine treatment. Several studies have examined peripheral inflammatory and immune biomarkers (e.g., interleukin-6 [IL-6], tumour necrosis factor-α [TNF-α], and C-reactive protein [CRP]). Conversely, other investigations have focused on metabolic pathways, particularly the kynurenine pathway, as well as neurotrophic factors such as BDNF. The latter has been proposed as a potential marker of neuroplasticity-related response [118–120].

In addition to biological markers, several studies have also investigated clinical variables that may help to predict and model responses to ketamine treatment, although with mixed and conflicting results. The most frequently examined clinical markers include a family history that presents alcohol use disorder or dependence, concomitant use of benzodiazepines, premedication with naltrexone, a diagnosis of unipolar depression, male biological sex, higher body mass index (BMI), a positive history of childhood trauma, comorbid anxiety, higher levels of anhedonic symptoms, and the presence of dissociative symptoms following intravenous (IV) ketamine administration. It must be noted that, of all of the above listed markers, only a positive family history of alcohol use disorder has consistently emerged as statistically significant [36,121–123]. This body of evidence reflects the heterogeneity of ketamine's biological effects and emphasises the importance of developing alternative and more integrative approaches to this novel treatment strategy.

In this work, we conducted a systematic review of the molecular effects of ketamine and esketamine treatment on RNA expression, with the aim of identifying the transcriptional changes associated with their use during MDD episodes in unipolar and bipolar disorders. This review is focused exclusively on studies conducted on human subjects, as well as on in vitro experiments using human-derived cells, in order to better clarify the potential molecular biomarkers and mechanisms underlying the antidepressant properties of ketamine and esketamine.

Our systematic review was conducted in adherence to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [78] and the protocol of the systematic review was registered in the International Prospective Register of Systematic Reviews (PROSPERO, CRD420250654037). In the literature search, we considered studies published in English up to February 2025, which were indexed in PubMed and Scopus. The original search strings used were: ("ketamine " OR " esketamine " OR " s-ketamine") AND (miRNA* OR microRNA * OR miRNA * OR transcript OR "gene expression" OR non-coding). Exclusion criteria were studies in which participants were not adults with unipolar or bipolar depression, diagnosed according to recognised criteria, studies in which ketamine or esketamine treatment was not administered, studies which did not evaluate the expression in peripheral blood or in human-derived cells, preclinical studies, reviews, case reports, and in silico studies.

The titles and abstracts of eligible articles were screened independently by two authors. In cases of disagreement, potential conflicts were resolved through a discussion and separate evaluation by a third author. The methodological quality of the selected studies was assessed using a modified version of the Downs and Black checklist [124], following an approach analogous to the one used in

previous research [125,126]. This tool consists of 27 items designed to evaluate multiple aspects of study quality, including the clarity and completeness of reporting, external validity, internal validity with respect to study bias, confounding and selection bias, as well as the statistical power of the study.

Summary of the main findings

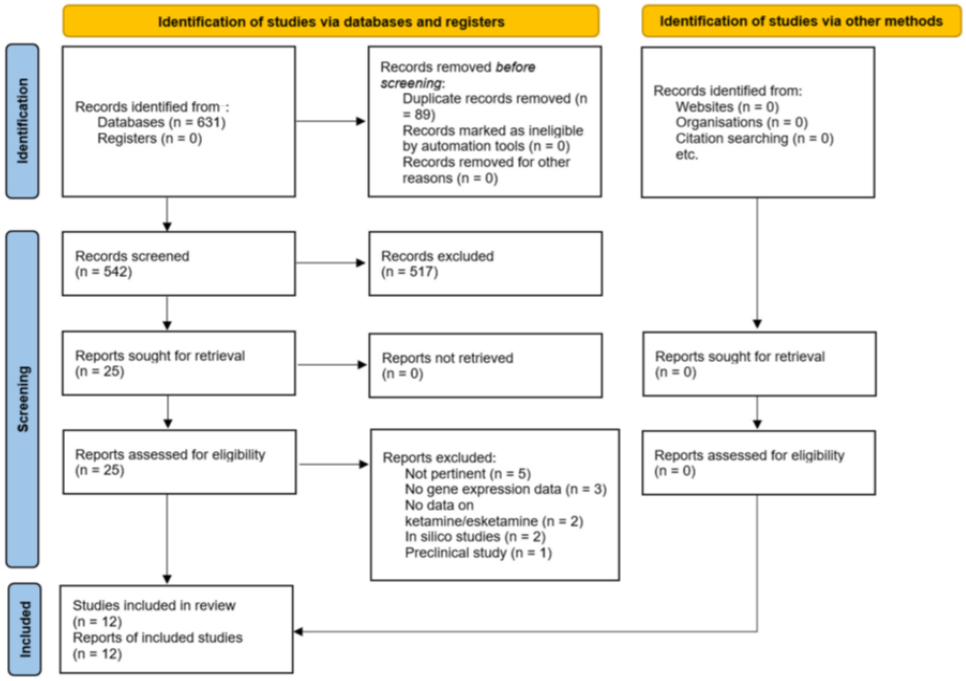
Search results of the included studies

We identified 631 studies on database searching (PubMed, Scopus, not including additional studies retrieved through complementary sources. Once duplicates had been removed (N=89), 542 records were left for screening based on titles and abstracts. 517 of these records were excluded after title and abstract evaluations. The full texts of 25 articles considered potentially relevant were obtained and reviewed.

Thirteen articles were excluded for various reasons, including irrelevance (n = 5), lack of gene expression data (n = 3), absence of ketamine or esketamine treatment information (n = 2), in silico experiments (n = 2), or preclinical studies (n = 1). Ultimately, after the exclusion process, 12 studies fulfilled all eligibility requirements and were included in this review (Figure 3.1.1).

Among the twelve studies investigating the link between blood RNA levels and response to ketamine, three applied a genome-wide approach, analysing either the whole blood transcriptome [127], the miRNome [128], or both [129]. Two additional studies focused on specific candidate genes, measuring mRNAs [130] or lncRNAs [131]. All in vitro studies assessed mRNA expression, with two following a genome-wide strategy [132,133], and five examining selected candidate genes [134–138] .

Figure 3.1.1. PRISMA flow diagram of the study selection procedure, including identification, screening, eligibility and inclusion stages (Adapted from Pisanu et al., 2025 [118]).



Blood RNA levels and the response to ketamine

Within the existing literature on genome-wide evaluations of the association between blood RNA levels and response to ketamine, one of the studies eligible for this systematic review included 26 individuals with TRD at the time of recruitment. Each recruited subject in the study was naïve to ketamine and did not use other antidepressant or anxiolytic medications. Eighteen of them showed a clinical improvement 24 hours after a single IV ketamine medication (0.5 mg/kg over 40 minutes), measured with a reduction of 50% minimum in MADRS total score compared to baseline. Furthermore, the authors' analysis of responders and non-responders highlighting the presence of 331 genes that were nominally differentially expressed at baseline and enriched in signalling cascades associated with cAMP activity and neuropathic pain transmission in dorsal horn neurons. Moreover, genes associated with changes in MADRS scores were found to be enriched for the cAMP signalling pathway [127].

Another study evaluated whole-genome mRNA and miRNA expression in peripheral blood mononuclear cells (PBMCs) in a sample of TRD patients which were divided as such: 14 receiving intravenous or intranasal ketamine, 17 undergoing ECT, and 16 receiving treatment-as-usual

(TAU). A significant reduction in FKBP Prolyl Isomerase 5 (FKBP5) gene levels, which encodes a protein capable of inhibiting the glucocorticoid receptor, was observed only in the ECT group, but not in the other two groups [139].

A final study investigating genome-wide miRNA profiles in TRD patients who underwent intravenous ketamine, compared to ECT and healthy controls (HC), did not find a significant association between the collected miRNAs and response to ketamine treatment at both one day and one week after administration [128].

Evidences from peripheral blood analysis

We identified several studies investigating candidate genes in the blood of patients with TRD: the first one that was examined reported a change in the expression levels of the long non-coding RNA FEDORA (LncFEDORA) in female TRD patients, but notably not in males, when compared to HC before the treatment with a single intravenous ketamine infusion at a dose of 0.5 mg/kg. The author noticed that this change in LncFEDORA expression in blood following a single treatment was also correlated with a change of depression severity, as measured by the clinical scale Quick Inventory of Depressive Symptomatology (QIDS) [131].

Another study investigated peripheral blood mRNA expression of vascular endothelial growth factor A (VEGFA) and pigment epithelium-derived factor (PEDF; SERPINF1) in 25 MDD patients who presented a Hamilton Depression Rating Scale (HDRS) total score ≥21, which is consistent with moderate to severe depression. Gene expression levels were evaluated twice, 4 hours before and 4 hours after a single intravenous infusion of ketamine (0.5 mg/kg) or midazolam (0.045 mg/kg); a statistically significant increase in peripheral VEGFA mRNA expression was observed specifically and exclusively in the ketamine group, whereas no significant changes were detected for PEDF. It has to be noted that the increase in VEGFA expression was not associated with a parallel reduction in depressive symptom severity [130]

In vitro human cell studies and models

This systematic review also included an in vitro study, which investigated the impact of ketamine on pro-inflammatory cytokines present in plasma and circulating monocytes, which were derived from 33 MDD patients reporting a positive history of suicide attempts. The authors managed to demonstrate how knockdown of Insulin-like Growth Factor 2 (IGF2) decreased ketamine's ability

to induce neural progenitor cells (NPCs) proliferation, suggesting a possible role for ketamine in regulating the expression of IGF2 [136]. An additional test found that both ketamine and esketamine in commercial human cell lines enter the pressures of genetic expression signal regulation to enhance of DNA Damage Inducible Transcript 3 (DDIT3), whereas escitalopram and amitriptyline correlated negatively. Furthermore, authors showed how peripheral blood mononuclear cells (PBMCs) derived from MDD patients generated activated macrophage differentiation in vitro with different doses of ketamine administration. The cohort analysis with The Cancer Genome Atlas (TCGA) demonstrated elevated expression of C-C Motif Chemokine Ligand 22 (CCL22) and Transglutaminase 2 (TGM2), as well as showed an upregulation of genes associated with the mechanistic Target of Rapamycin (mTOR) pathway, including Serum/Glucocorticoid Regulated Kinase 1 (SGK1), Eukaryotic Translation Initiation Factor 4B (EIF4B), and Forkhead Box O1 (FOXO1) [136].

Similarly, in vitro studies have shown the ability of ketamine and its two metabolites 6R-hydroxynorketamine (6R-HNK) and 2S,6S-hydroxynorketamine (2S,6S-HNK), to modulate type I interferon pathway genes through the activation of the STAT3 transcription factor [140]. Another study by the same authors reported that ketamine and its metabolites upregulated AMPA-type glutamate receptor subunit (GRIA1, GRIA2, GRIA4) mRNA expression in U251 malignant glioblastoma (MG) cells as well as in human iPSC-derived astrocytes.[118,141]

Another study performed in vitro on astroglial cells demonstrated that ketamine can also suppress the production of pro-inflammatory cytokines, Interleukin-6 (IL-6) and Tumour Necrosis Factor alpha (TNFα) [142].

Discussion

Our systematic review analysed the available evidence from both human and in vitro studies on the transcriptional impact of ketamine and esketamine to elucidate the molecular mechanisms that may underlie their antidepressant effects in studies focused on TRD. Only 12 out of 631 identified studies met the eligibility criteria and were included after the selection process, including genome-wide and candidate-gene approaches.

Peripheral blood analyses of 33 MDD patients with suicide attempts revealed a modulation of gene expression associated with clinical depression severity. For instance, the long non-coding RNA LncFEDORA was upregulated in female TRD patients and its change post-ketamine treatment

correlated with improvements in depressive symptoms; this change was not observed in males with TRD. Similarly, VEGFA mRNA increased after ketamine administration, although this did not correspond directly to symptom reduction, suggesting a dissociation between molecular changes and short-term clinical outcomes [130].

In vitro studies showed that the administration of ketamine enhanced the proliferation of NPCs through IGF2, while knockdown of IGF2 attenuated this effect, suggesting a direct role of IGF2-mediated pathways in neuroplasticity. Ketamine and esketamine also increased DDIT3 expression in commercial cell lines, whereas conventional antidepressants such as escitalopram and amitriptyline did not. Furthermore, ketamine promoted macrophage differentiation in PBMCs, increasing expression of CCL22, TGM2, and genes associated with the mTOR pathway (SGK1, EIF4B, FOXO1), highlighting its influence on both immune and intracellular signalling pathways [130,136].

Additional in vitro studies in U251 glioblastoma cells and iPSC-derived astrocytes demonstrated that ketamine and its metabolites, 6R-HNK and 2S,6S-HNK, modulated genes in the type I interferon pathway through STAT3 activation, while also increasing mRNA expression of AMPA receptor subunits GRIA1, GRIA2, GRIA4, but not in GRIA3 [138]. Additionally, dose-dependent effects of ketamine were found in the suppression of pro-inflammatory cytokine production, including IL-6 and TNFα, in astroglial cells, supporting its anti-inflammatory and neuroprotective effects.

The findings of our systematic review indicate that ketamine exerts a broad transcriptional effect, influencing networks related to neuroplasticity, synaptic signalling, immune regulation, endocrine function and metabolic pathways. These effects appear to be pleiotropic and cell-type-specific, reflecting ketamine's complex impact on brain neuroplasticity and immunomodulation. However, these findings should be interpreted with caution, particularly when considering the type of ketamine studied, as most human studies have focused on racemic ketamine, while esketamine remains underrepresented in the literature.

Another hurdle for the relevance of this evidence may come from the limited number of studies that met the inclusion criteria of the systematic review. Furthermore, the large heterogeneity in study design, patient populations, and gene expression quantification methods further limits the generalizability of the results.

In addition, in vitro models cannot fully capture the complexity of neurobiological processes occurring in vivo, especially when studies rely on commercial human cell lines or cells derived from HC, which may not adequately represent the molecular characteristics of individuals with TRD. This currently remains a limitation of the translational relevance of these findings; therefore future research should prioritise the use of patient-derived TRD cells, which could provide more clinically meaningful and biologically representative insights.

Albeit easy to access and commonly used in clinical research, peripheral blood may not be the best tissue to represent central nervous system processes. Neural and glial cell models, on the other hand, can yield more specific mechanistic insights into brain-derived processes, but they are still limited by experimental culture conditions that may not fully replicate relevant physiological processes. The use of genome-wide approaches in peripheral blood can overcome some limitations by allowing unbiased discovery of molecular pathways.

Finally, future research should take into account the temporal dynamics of ketamine-induced transcriptional changes, because it remains unclear whether these transcriptional changes are sustained over time, reversible, or predictive of long-term treatment outcomes.

4. Exploring the Evidence from Non-Pharmacological Treatments of Mood Disorders

As addressed in the preceding paragraphs, MDD is a highly prevalent psychiatric disorder with a high rate of lifetime disability, family burden, and healthcare costs. Despite the availability of various antidepressants, about one-third of patients fail to achieve symptom remission after treatment with two or more drug classes at adequate doses and duration and develop TRD.

In this chapter, I present four studies focusing on evidence derived from non-pharmacological treatments of depressive symptoms, delivered either as augmentation to previous pharmacotherapy or as stand-alone interventions.

The first study explored the plasma miRNA transcriptomic profiles in MDD-TRD patients who underwent ECT treatment and aimed to clarify the potential molecular modifications occurring during the treatment and their association with treatment outcomes through plasma miRNA analysis.

The second study aimed to explore the efficacy of TF-CBT and eye movement desensitization and reprocessing (EMDR) in individuals with TRD who have experienced ELA, through the analysis of leukocyte telomere length (LTL) and mitochondrial DNA copy number (mtDNAcn).

The third study focused on clarifying the role of Mediator Complex Subunit 22 (MED22) in the relationship between childhood trauma (CT) and the development of MDD. We investigated how genetic and environmental factors influence MED22 expression by examining potential associations between MED22 single nucleotide polymorphisms (SNPs) and neuroticism in an independent cohort of healthy volunteers, and by conducting a longitudinal analysis in a separate cohort of MDD patients with a history of CT who underwent trauma-focused psychotherapy (TFP).

The final study aimed to investigate the biological mechanisms underlying MDD outcomes in response to physical activity through a systematic review of randomised controlled trials (RCTs).

4.1. Analysing the response of TRD patients through plasma miRNA levels after Electroconvulsive Therapy (ECT)

MDD is the most prevalent psychiatric disorder with high lifetime disability, substantial healthcare costs, and significant familial burden. Despite the availability of various antidepressant treatments,

approximately one-third of the people affected, presents TRD, which represents a major challenge in clinical psychiatry and psychology.

In recent years, the emergence of a great interest in the development of complementary non-pharmacological interventions has led to the promotion of upregulation of postsynaptic serotonin receptors and has shown neurotrophic effects in specific human brain areas. The use of ECT for the treatment of TRD has shown quick and sustained clinical responses, with a positive impact on neurotransmission mechanisms, neurogenesis, BDNF, VEGF, and other neurobiological processes involved in the modulation of the inflammatory-immune response [143–145]. Additionally, ECT administered in combination with other antidepressant medications has been shown to be effective in prolonging the remission phase and has been associated with a reduction in suicidal risk following discharge [146].

Despite different studies in scientific literature having highlighted in the past the potential involvement of miRNAs in mechanisms targeting BDNF during ECT, their applicability was limited as they were based on rodent models [147,148]. Recent research in human epigenetics has led to a growing interest in the crucial role miRNAs can play in regulating gene expression, neuronal development, neurogenesis, and synaptic plasticity. The alteration of circulating miRNAs detected in biological fluids, plasma or saliva, has been observed in several studies on patients with mental illness, particularly in MDD, and they could be used as biomarkers for the diagnosis, assessment, and monitoring of disease course [149–153].

In this study, we explored plasma miRNA transcriptomic levels in 27 MDD-TRD patients who underwent ECT to clarify the potential modifications during the treatment and their associations with treatment outcomes. The diagnosis of MDD was performed through the Structured Clinical Interview for DSM-IV Axis I Disorders in accordance with the Diagnostic and Statistical Manual of Mental Disorders (Fourth Edition). Exclusion criteria included pregnancy, cognitive impairment, intellectual disability, history of neurological illness, bipolar disorder, obsessive-compulsive disorder, schizophrenia, personality disorders, comorbidities with a diagnosis of post-traumatic stress disorder, eating disorders, severe medical illnesses, autoimmune diseases, primary diagnosis of substance and/or alcohol abuse. The study protocol received approval from the local Ethics Committee of the Provinces of Verona and Rovigo (4997/09.11.01), and all participants provided written informed consent in accordance with the Declaration of Helsinki.

All subjects were assessed at two time points, at the baseline (T0) and 1 month after the end of ECT administration (T1): at each point, depressive symptoms were assessed with the MADRS and miRNA profiling was performed from plasma samples [154]. The improvement of depressive

symptoms was calculated as a percentage reduction of MADRS score (Δ MADRS %). Patients who achieved a reduction of 50% were considered responders to the treatment.

The ECT sessions were delivered 3 times per week with standard settings, using bitemporal electrode placement and a bipolar brief pulse square wave, through the Thymatron DG machine (Somatics, Inc., Lake Bluff, IL) [155–157]. Each patient was anaesthetised for ECT session by IV thiopental sodium (3.0 mg/kg IV for males and 2.5 mg/kg IV for females), and muscle relaxation was obtained with IV succinylcholine (0.7 mg/kg IV). Patients were also premedicated with atropine sulfate (0.5 mg IV).

For all patients, the isolation and profiling of circulating miRNA were both obtained by extracting plasma from peripheral blood samples collected in K2 EDTA-coated tubes; such sample was subsequently centrifuged at 1620 rcf for 15 minutes, and stored at −80°C. For miRNA extraction 200 microliters of each plasma sample at T0 and T1 were used. The NucleoSpin miRNA Plasma Mini kit (Macherey-Nagel, DEU) was used for total miRNA isolation. A panel of 188 target miRNAs was analysed through the use of TaqMan Advanced miRNA Human Serum/Plasma Card (ThermoFisher) and miRNA plasma levels were calculated using the $2^{-\Delta Ct}$ method, whereas fold changes between T0 and T1 for each miRNA were determined using the $2^{-\Delta\Delta Ct}$ method.

Statistical analysis was conducted using the SPSS software package, version 21[158]. A paired-sample t-test was applied to evaluate changes in depressive symptoms and miRNA expression levels from T0 to T1. Nominal significance was set at $p < 0.05$, and p-values were corrected for multiple comparisons using the False Discovery Rate (FDR) approach according to the Benjamini-Hochberg method [159]. Only changes in miRNA expression with a fold change (FC) below 0.9 or above 1.1 were included in the analysis. Pearson's correlation coefficient was used to examine the relationship between miRNA alterations and clinical improvement. Longitudinal differences in miRNA $2^{\wedge-\Delta Ct}$ values between responders and non-responders were analysed with a repeated-measures general linear model, including group and the group x time interaction as fixed factors[160].

Summary of the main findings

ECT effects on the reduction of depressive symptoms in the MADRS score

The clinical group, composed by 27 patients, received a mean of 7.9 ± 2.7 ECT sessions. ECT treatment prompted a notable reduction ($p < 0.01$; $t = 8.633$) in MADRS score at T1 (mean: 11.0 ± 12.2) compared to T0 (mean: 34.2 ± 5.4). The Δ MADRS % mean was 66.1%. Two-thirds of patients (n = 18) achieved a reduction of 50% or more and were classified as responders, while 9

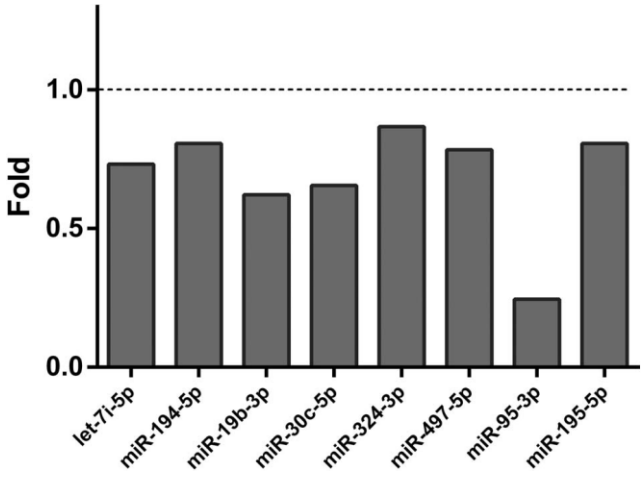
patients were classified as non-responders (Table 4.1.1). Demographical and clinical characteristics of the sample are shown in Table 4.1.1.

Table 4.1.1. Demographic and clinical characteristics of the sample (Adapted from Galbiati et al, 2025 [160]).

VARIABLES	PATIENTS (N = 27)
Female	18 (67%)
Male	9 (33%)
Age, mean (SD)	58.1 (11.2)
Age of onset, mean (SD)	39.2 (14.5)
BMI, mean (SD)	26.5 (4.5)
Smoking	8 (29%)
Psychotic symptoms	17 (63%)
Alcohol abuse	1 (4%)
Comorbid anxiety disorders	6 (22%)
Comorbid personality disorders	6 (22%)
ECT sessions, mean (SD)	7.9 (2.7)
MADRS T0, mean (SD)	34.1 (5.4)
MADRS T1, mean (SD)	11.0 (12.2)
Δ MADRS T0-T1, mean (SD)	66.1 (38.4)
Responders, N	18 (67%)
SD=standard deviation; BMI=body mass index; ECT=electroconvulsive therapy; MADRS=Montgomery-Asberg Depression Rating Scale; Δ MADRS= percentage reduction of MADRS Montgomery-Asberg Depression Rating Scale between T0-T1	

Of the 188 miRNAs present in the TaqMan Advanced miRNA Human Serum/Plasma Card panel, 150 passed the quality of control procedure and were considered for subsequent analysis. Data analysis revealed a downregulation of 8 miRNAs at T1 (hsa-miR- 95-3p, p = 0.003; hsa-miR-194-5p, p = 0.018; hsa-miR-324-3p, p = 0.019; hsa-miR-195-5p, p = 0.022; hsa-miR-19b-3p, p = 0.025; hsa-miR-30c-5p, p = 0.036; hsa-let-7i-5p, p = 0.040; hsa-miR-497-5p, p = 0.042), with hsa-miR-95-3p showing the highest significance level (p = 0.003) and the lowest fold change (FC = 0.245) (Figure 4.1.1).

Figure 4.1.1. Fold changes between T0 and T1 for the 8 significant miRNAs after ECT treatment (Adapted from Galbiati et al, 2025 [160]).



Gene Target Prediction and KEGG Pathways Analysis

The confounding factors (age, sex, BMI, smoking), after adjusting p-values for multiple analyses using the Benjamini-Hochberg correction, did not show statistically significant changes in miRNA modification. We uncovered an interesting direct correlation between symptom improvement, measured by Δ MADRS %, and the analysis of miR-324-3p, miR-30c-5p in the responder patients ($r = 0.473$, $p = 0.048$, $r = 0.616$, $p = 0.006$, respectively; $N = 18$). Additionally, only miR-324-3p showed a correlation with clinical improvement in the whole group ($r = 0.460$, $p = 0.016$; $N = 27$). No other significant association was observed between the two time points (T0-T1) in plasma miRNA related to ECT response. Furthermore, to identify the biological process involved in ECT response, a target gene prediction pathway analysis was conducted using the Kyoto Encyclopedia of Genes and Genomes (KEGG), with target genes provided by the Diana Lab Mited database, the DIANA-microT-CDS algorithm, or experimentally validated interactions derived from DIANA-TarBase. Based on KEGG analysis of 34 enriched pathways of miRNA candidates, we predicted of the target gene involved in various biological processes related to the pathophysiology and treatment response of MDD (e.g., mTOR, TGF-beta, Wnt, Axon Guidance, PI3K-Akt, Neurotrophin, and Glutamatergic Synapse signalling pathways).

Table 4.1.2. Summary of the KEGG Pathway Enrichment Analysis of biological processes persistently involved in the pathogenesis and treatment of MDD, p-value < 0.01 (Adapted from Galbiati et al, 2025 [160]).

KEGG Pathway	P-value	n. miRNAs involved
mTOR signaling pathway	8.33E − 05	6
TGF-beta signaling pathway	1.32E − 04	6
Wnt signaling pathway	4.08E − 04	7
Axon guidance	2.15E − 03	7
Neurotrophin signaling pathway	6.58E − 03	7
PI3K-Akt signaling pathway	6.95E − 03	6
Glutamatergic synapse	7.48E − 03	6

Discussion

Our results highlighted the presence of eight miRNAs that appeared downregulated in the plasma of TRD patients who underwent ECT treatment, albeit at a nominal level (miR-95-3p, miR-194-5p, miR-324-3p, miR-195-5p, miR-19b-3p, miR-30c-5p, let-7i-5p, miR-497-5p). Nevertheless, a more in-depth investigation, including target gene prediction and KEGG pathway enrichment analyses of the eight nominally downregulated miRNAs, revealed that miR-30c-5p and miR-324-3p may be involved in key biological mechanisms underlying the clinical response to both ECT and antidepressant treatments.

In contrast to previous studies conducted on whole blood and PBMCs from patients with TRD, our findings revealed a significant correlation between the two miRNAs and clinical improvement [160]. Specifically, the reduction in their plasma levels was associated with symptom improvement in patients who responded to treatment, supporting their potential role as candidate biomarkers of treatment response.

The specific mechanism of miR-30c-5p has previously been highlighted in electroconvulsive seizure (ECS) animal models in the angiogenesis mediated by VEGFa, which appears to be involved in a range of brain processes related to neuronal growth, cell differentiation and neuroprotection [161,162]. Other studies have underlined the relevance of VEGFa concentrations at the pretreatment stage as biomarkers of clinical outcome and treatment response [163–165], and, additionally, miR-30c-5p has been suggested to regulate SIRT1, a nicotinamide adenine dinucleotide (NAD^+)-dependent deacetylase that contributes to the pathophysiology of depression by modulating both

inflammation and neurogenesis in MDD subjects and animal models of the disease [166–168]. It should also be noted that, for the miR-324-3p, different studies have highlighted its relationship with VEGFa in the mediation of angiogenic and neuronal growth factors after neurological injuries in several conditions and in animal models of stress responses [169–171].

Despite some limitations, mainly due to the small sample size, which may affect the generalizability of the results, a key strength of this unique study design was the identification of potential molecular biomarkers, focusing on plasma miRNA levels in TRD patients, their mechanisms of action, and their correlation with the outcomes of ECT treatment. Moreover, these findings may pave the way for the development of a rapid and minimally invasive framework to identify plasma biomarkers for personalised treatment of TRD and other mood disorders resistant to first-line therapies.

4.2. Investigating the effect of Trauma-Focused Psychotherapies on leucocyte telomere length (LTL) and mitochondrial DNA copy number (mtDNAcn) in TRD patients

As highlighted in previous research presented in chapter 2, ELA have often been related to a significant risk for developing MDD and/or TRD; however, the molecular mechanisms underlying this relation remains unclear. One explanation for the influence of ELA influences on the development of mental illness is its role in disrupting the body's allostatic function, promoting oxidative stress and inflammatory response [172,173]. This maladaptive coping response may lead to behaviours and biological dysregulations, particularly affecting the serotonergic, dopaminergic, and oxytocin systems; as an additional point, it can interfere with neurotrophic activity and glucocorticoid-related stress responses in the hypothalamic-pituitary-adrenal (HPA) axis [172,173].

The dysregulation of glucocorticoid systems, combined also with elevated blood glucose levels, alter mitochondrial morphology, therefore leading to mitochondrial fragmentation and increased production of reactive oxygen species (ROS) rather than enhancing antioxidant defence capacity [173,174]. These allostatic imbalances, together with the reduction of telomere length, contribute to accelerated biological ageing. Indeed, telomere shortening, linked to ageing and mental illness, can be accelerated by stress and inflammation, connecting ELA to poor health outcomes [175,176].

This is particularly relevant because telomeres are non-coding DNA sequences consisting of repetitive TTAGGG nucleotide sequences located at the ends of chromosomes which serve to safeguard genomic integrity during cell division and naturally shorten with each cellular replication and as part of the physiological ageing process [177]. However, previous findings emerging from several meta-analyses showed inconsistent evidence [178–181]. Similarly, an increased mitochondrial DNA copy number (mtDNAcn) was associated with early stress and psychopathology, but its role and relationship with telomere length require further research, since only one research showed the presence of higher levels of mtDNAcn in subjects with ELA rather than HC [182,183].

An evidence-based non-pharmacological approach recommended for individuals with MDD-TRD who have experienced ELA includes trauma-focused psychotherapies, particularly trauma-focused cognitive behavioural therapy (TF-CBT) and eye movement desensitization and reprocessing (EMDR) [184,185].

Due to limited scientific literature on LTL and mtDNAcn in TRD subjects, we evaluated the relationship between symptom improvement and changes in LTL and mtDNAcn in TRD-affected patients treated with TF-CBT and EMDR. Additionally, we explored whether treatment response

in TRD patients was associated with LTL and mtDNAcn by comparing results with a cohort of HC.

A total of 30 TRD patients, with a diagnosis of MDD based on the Diagnostic and Statistical Manual of Mental Disorders-IV (DSM-IV) criteria, were enrolled. Each participant had failed to respond to at least two or more classes of antidepressant drugs for an adequate period and was classified as TRD [25]. The exclusion criteria were cognitive impairment, dementia, bipolar disorders, schizophrenia and schizoaffective disorders, primary diagnosis of alcohol and substance abuse, obsessive-compulsive disorder, personality disorders, PTSD, pregnancy, severe medical illness, neurological and severe autoimmune diseases. A second sample of 65 healthy subjects, aged 18 to 65, without a diagnosis of psychiatric disorders, was recruited and matched for the variables age and sex. Patients were recruited at Villa Santa Chiara Hospital in Verona. All participants were completely informed about study procedures and signed the consent to participate in the study. The study protocol was approved by both local Ethics Committees (Province of Verona and Rovigo N: 234777/11.05.16; University Hospital Agency of Cagliari, PG/2019/6277) [178].

Clinical assessments of ELA and depressive symptoms were collected by clinicians with the Italian version of the Childhood Experience of Care and Abuse Questionnaire (CECA.Q) and MADRS [154,186]. All 30 TRD patients, in addition to their drug treatment, participated in trauma-focused psychotherapy interventions, with a blind assignment to EMDR or TF-CBT arms. Each patient received 24 sessions, corresponding to 3 individual sessions of TF-CBT or EMDR per week, each lasting 60 minutes. Clinical depressive symptoms were assessed at baseline (T0), after four (T4) and eight (T8) weeks of treatment, and four weeks after the end of treatment at follow-up (T12). Blood samples collections occurred at T0, T8, and T12 using EDTA Tubes. DNA was extracted from whole blood samples using the Gentra Puregene Blood kit (Qiagen). DNA quantification and quality evaluation were performed using spectrophotometric analysis (NanoDrop, 2000; Thermo Scientific). Quantitative polymerase chain reaction (qPCR) was performed, in accordance with previous literature, to evaluate LTL, using a StepOnePlus™ Real-Time PCR System (Thermo Fisher Scientific) [187,188]. A control sample was included on each plate as a calibrator: LTL and mtDNAcn were calculated using the 2 - ΔΔCT method.

The statistical analyses were performed through SPSS software package, version 28 and GraphPad Prism version 9[189,190]. Data distribution normality was evaluated by means of the Kolmogorov–Smirnov test, and outliers were tested with the Grubbs' test. As appropriate, we used a chi-square test or independent-samples t-test; differences in demographic and clinical characteristics across the study groups were assessed with the chi-square test or independent-samples t-test, as

appropriate. A Pearson's correlation was employed between continuous variables, with partial correlations performed when adjusting for age. Comparisons between the TRD group and HC were conducted using a general linear model including age and BMI as covariates.

The evaluation of the relationship between longitudinal changes in LTL or mtDNAcn and psychotherapy response from baseline to T12 was conducted with a repeated-measures general linear model. Spearman's rank correlation was used to test the association between variations in LTL and mtDNAcn and changes in MADRS scores over the same time interval.
As a final step, the predictive potential of LTL and mtDNAcn was assessed through binary logistic regression analysis, and a Receiver Operating Characteristic (ROC) curve was generated for mtDNAcn to evaluate its ability to predict diagnosis and treatment response.

Summary of the main findings

Significant differences in BMI score between TRD patients and controls

The mean age of subjects was 51.9 years (SD±8.9), with 76.7% of participants being female. The mean age of participants was 52.5 (SD±6.2) for TF-CBT and 51.4 (SD±10.7) for EMDR. The mean age of healthy control group was 52.6 (SD±6.8). The randomisation process assigned 12 patients to the TF-CBT and 18 patients to the EMDR group. A statistical difference was in BMI between patients and healthy controls, with the BMI being significantly higher in the patient groups compared to the healthy controls ($t = 3.105$, $p = 0.003$). No statistical differences were found for age and sex (Table 4.2.1).

Table 4.2.1. Socio-demographic and clinical characteristics of the two patient groups and healthy controls (Adapted from Minelli et al., 2025 [178]).

Variable	TF-CBT group (n = 12)	EMDR group (n = 18)	HC group (n = 65)	p-value
Age (mean ± SD)	52.5 (± 6.2)	51.4 (± 10.7)	52.6 (± 6.8)	n.s.
Sex (M/F)	5/7	2/16	24/41	n.s.
Onset age (mean ± SD)	33.3 (± 14.4)	31.4 (± 13.6)	-	n.s.
T12 response (Y/N)	6/6	14/4		n.s.
Psychotic symptoms (Y/N)	3/9	2/16	-	n.s.
Personality disorders comorbidity (Y/N)	9/3	13/5	-	n.s.
Anxiety disorders comorbidity (Y/N)	11/1	14/4	-	n.s.

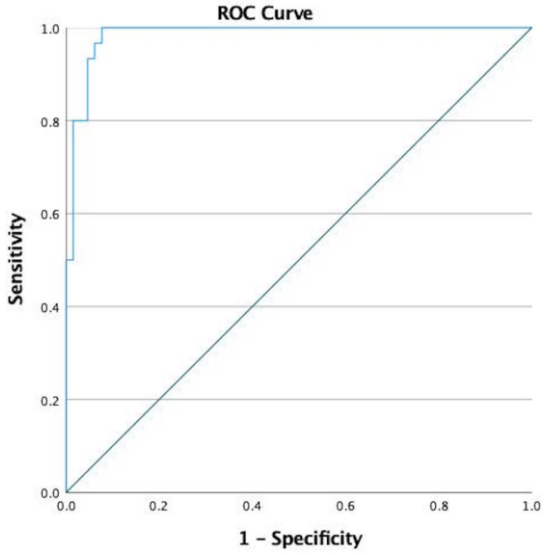
Other medical disorders comorbidity (Y/N)	5/7	11/7	-	n.s.
BMI (mean ± SD)	25.2 (± 3.1)	26.7 (± 5.2)	23.7 (± 3.1)	**0.006**
Smoke (Y/N)	6/6	6/12	35/30	n.s.
MADRS baseline (mean ± SD)	26.8 (± 12.1)	26.1 (± 10.5)	-	n.s.
MADRS T12 (mean ± SD)	16.9 (± 3.9)	8.9 (± 10.9)	-	n.s.
Neglect	10/2	16/2	-	n.s.
Sexual abuse	5/7	9/9	-	n.s.
Physical abuse	6/6	9/9	-	n.s.
Emotional abuse	8/1	11/7	-	n.s.
Antipathy	10/0	11/7	-	n.s.

TF-CBT=Trauma-focused cognitive behavioural therapy; EMDR=Eye Movement Desensitization and Reprocessing; HC=healthy controls; SD=standard deviation; M=male; F=female; Y=yes; N=no; BMI=body mass index; MADRS= Montgomery-Asberg Depression Rating Scale; T12= time point after twelve weeks to started Trauma-focused psychotherapies; n.s.= not statistical significative p-value. The statistical significative p-value was reported in bold.

BMI relation with mitochondrial DNA copy number as a predictor of diagnosis

LTL and mtDNAcn were not normally distributed and were therefore log-transformed. Neither variable was correlated with age or with each other, although both were correlated with BMI (LTL, Pearson's r = -0.254, p = 0.013; mtDNAcn, Pearson's r = 0.277, p = 0.007). A logistic regression model including BMI and mtDNAcn, but not LTL, was a significant predictor of diagnosis (model chi-square 96.220, p = 1.01e-20; contribution of mtDNAcn, B = - 42.797, p = 0.001; contribution of LTL, B = 9.996, p = 0.089, Table 2). The ROC curve for mtDNAcn had an area under the curve (AUC) of 0.99 (C.I. 0.97–1.00), with a sensitivity of 100% and a specificity of 97% (Figure 4.2.1).

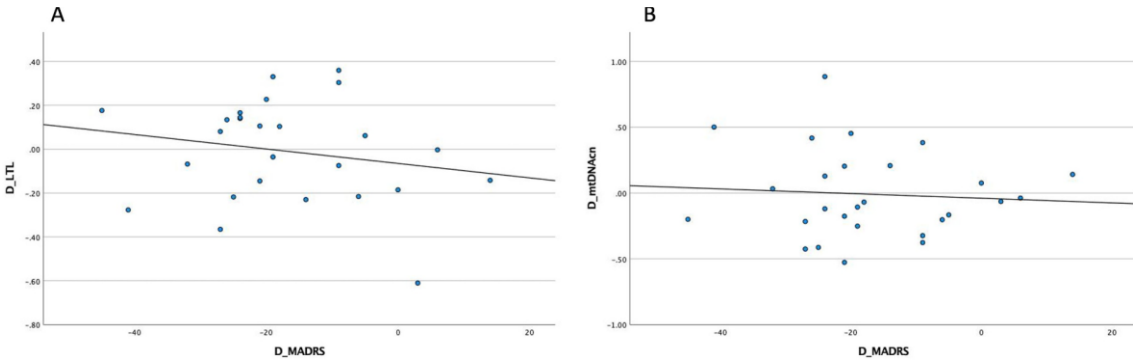
Figure 4.2.1. ROC curve analysis for mtDNAcn (Adapted from Minelli et al., 2025 [178]).



Analysis of the correlation between LTL, mtDNAcn, and response to trauma-focused psychotherapies

No influence of the investigated clinical variances was observed on LTL and mtDNAcn in TRD patients, except for an inverse correlation between LTL and mtDNAcn and MADRS score at T0 (LTL, Pearson's r = - 0.478, p = 0.008; mtDNAcn, Pearson's r = - 0.656, p = 0.00008). Similarly, no differences were found in LTL and mtDNAcn between TF-CBT and EMDR treatment groups. Patients defined as responders and non-responders at T12 did not differ for age, onset age disease, BMI, sex, smoking, ELA, comorbidities with personality, anxiety, or medical disorders, except for the presence of psychotic symptoms (Chi-Square = 5.880, p = 0.031). Furthermore, a repeated measures model with age, BMI, and the presence of psychotic symptoms as covariates did not show a significant difference in response outcome between LTL and mtDNAcn. The significant interaction between BMI and mtDNAcn did not show a significant link with treatment response. Likewise, LTL and mtDNAcn did not present a significant correlation with MADRS scores at T0 and T12 in the regression model analysis (Figure 4.2.2).

Figure 4.2.2. Correlation analysis of LTL (A) and mtDNAcn (B) with MADRS scores across two measured time points baseline (T0) and four weeks after end of treatments (T12) (Adapted from Minelli et al., 2025 [178]).



Discussion

One of the strengths of our study is that it represents the first investigation of LTL and mtDNAcn in TRD patients undergoing trauma-focused psychotherapies (TF-CBT and EMDR).

Our results showed that mtDNAcn levels were significantly higher in TRD patients compared to HC, highlighting a biological difference between groups. However, neither mtDNAcn nor LTL were significantly associated with treatment response or participants' age, indicating that these markers cannot be considered predictive of psychotherapy outcomes.

Although our results are in line with previous empirical evidence, they should be interpreted with caution due to the modest sample size and the potential influence of other factors not considered (e.g. oxidative stress, inflammation markers, blood cell count) that can interfere with LTL and mtDNAcn [191–193].

Even though several gaps remain in our understanding of the exact role of ELA, including its heterogeneity and the fact that it encompasses several types of traumatic events, and the mechanisms linking ELA to telomere shortening in adulthood, two recent meta-analyses support the association between traumatic events experienced in early life and allostatic imbalance in adulthood [194–196]. Furthermore, one study suggested a possible co-regulation process, in which telomere shortening is accompanied by compensatory higher levels of mtDNAcn in subjects who experienced ELA and subsequently developed depressive and/or anxiety disorders [182].

In summary, our study represents the first longitudinal examination of changes in LTL and mtDNA in relation to trauma-focused psychotherapies (TF-CBT, EMDR) outcomes in patients experiencing ELA. While our results support the involvement of mtDNAcn in the biology of depression, they indicate that neither marker appears suitable as a predictive biomarker of

therapeutic response to the psychotherapies considered. Further research with larger samples is needed to explore these associations in greater depth.

4.3. Observing the role of MED22 in predicting response to Trauma-Focused Psychotherapies in TRD patients

Exposure to ELS is known to increase the risk of developing severe mental illnesses in adulthood, particularly MDD. Several studies have shown the contribution of different type of childhood trauma (CT) in the imbalance of the hypothalamic-pituitary-adrenal (HPA) axis, the dopaminergic system, the serotonergic system and the immune/inflammatory system to increasing the vulnerability of developing MDD [58,197]. In the context of vulnerability, specific personality traits characterised by high levels of emotional instability and stress sensitivity, such as neuroticism, substantially increase the risk of developing MDD through the influence of particular biological processes, gene expression and stress-response systems [198–201].

Considering the substantial impact of these adverse experiences in childhood, several non-pharmacological approaches have been developed for individuals with a history of maltreatment, among which trauma-focused psychotherapies have been specifically proposed for MDD patients with CT [160,184,185,202–204]

In this field, our group has previously conducted a genome-wide study that highlighted an association between exposure to CT and the Mediator Complex Subunit 22 (MED22), alias Surfeit Locus Protein 5 (SURF5), a transcriptional factor gene known to be involved in various human diseases [204,205].

Our goal was to examine three research questions, while expanding on this previous research linking MED22 to CT in MDD,: (1) the role of MED22 in mediating the relationship between CT and MDD, considering genetic (GReX) and environmental (EReX) components of gene expression regulation; (2) the associations between MED22 genetic variations, specifically single nucleotide polymorphisms (SNPs) and personality traits, including neuroticism, in 177 healthy volunteers; and (3) longitudinal changes in MED22 expression in an independent cohort of 22 MDD patients who experienced CT and underwent trauma-focused psychotherapy, with clinical and blood assessments performed at baseline (T0), 4 weeks (T4), 8 weeks (T8), 12 weeks (T12), and 24 weeks (T24).

Our study was performed in compliance with the most recent revision of the Declaration of Helsinki. Ethical approval was granted by the Ethics Committee for Clinical Trials of the Provinces of Verona and Rovigo (N: 234777/11.05.16). All participants provided written informed consent after receiving a comprehensive explanation of the study procedures. HC subjects were enrolled

through a separate protocol approved by the Local Ethics Committee of CEIOC IRCCS Istituto Centro San Giovanni di Dio Fatebenefratelli, Brescia (N: 50/2008).

Summary of the main findings

Analysis of EReX and GrEX factors was performed using mRNA sequencing data from 368 MDD patients and 344 healthy controls with PrediXcan software [206].

The analysis of MED22 expression profiles revealed a significant association between neglect maltreatment and both EReX ($p = 3.65 \times 10^{-4}$) and GReX components of MED22 expression ($p = 2.56 \times 10^{-4}$). Moreover, the GreX component also showed significant associations with sexual abuse ($p = 2.12 \times 10^{-3}$) and emotional abuse ($p = 2.17 \times 10^{-5}$), while the global MED22 expression values were significantly correlated with emotional abuse ($p = 0.03$) and with neglect maltreatment ($p = 1.11 \times 10^{-6}$).

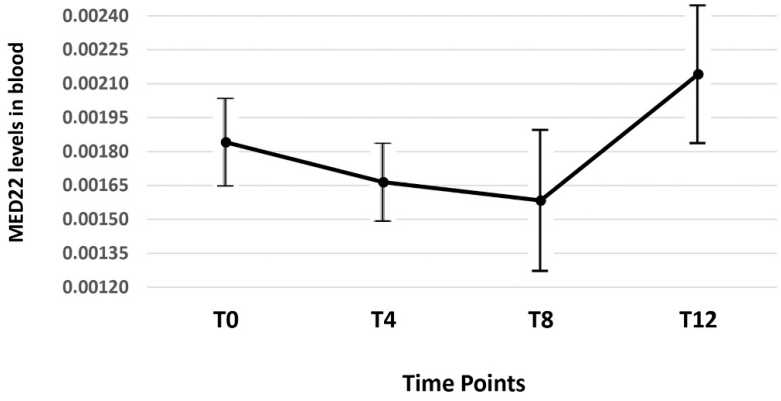
Assessment of MED22 SNPs associated with personality traits

A significant association emerged between cis-eSNPs of MED22 (SNPs influencing gene expression) and higher neuroticism scores (best p-value = 0.008), suggesting decreased resilience to stressors in the cohort of 177 healthy volunteers. Most significant results were observed with the SNP rs12335 within the allele A (Beta value = 0.2284; p = 0.0085) and the SNP rs484248 within the allele A (Beta value = 0.1964; p = 0.0291).

Longitudinal evaluation of MED22 expression in MDD patients undergoing trauma-focused psychotherapy

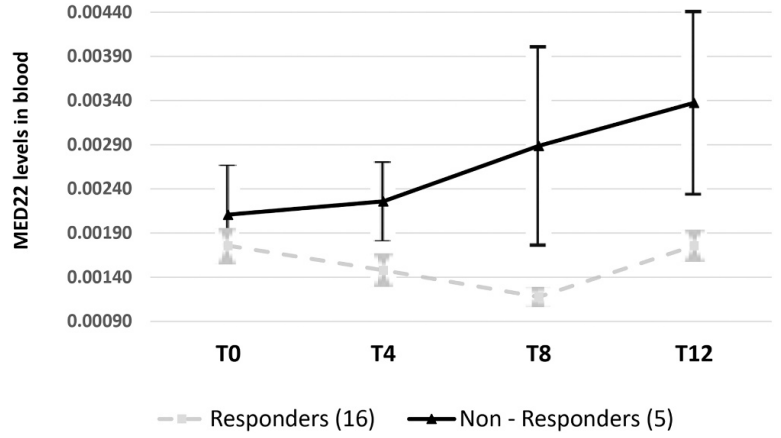
The comparison between baseline (T0) and follow-up visits (T12) of MDD patients who underwent trauma-focused psychotherapy demonstrated a reduction in depressive symptoms ($p < 0.001$), with 73% of patients showing improvement at the follow-up visit (T12). Figure 4.3.1 shows a significant change observed over time in blood mRNA levels of MED22 ($F = 3.05$; $p = 3.05 \times 10^{-2}$). Expression reductions were noted from baseline to T8 ($p = 0.07$), with the alterations being restored at T12 ($p = 0.003$).

Figure 4.3.1. The graphic illustrates the overall expression of MED22 across the four measured time points. MED22 expression exhibited a significant decrease during trauma-focused psychotherapy (Adapted from Carvalho Silva et al., 2025 [205]).



MED22 expression during trauma-focused psychotherapy exhibited a significant decrease ($F = 3.05$; $p = 3.43 \times 10^{-2}$), with an interaction effect with treatment response ($F = 3.44$; $p = 2.13 \times 10^{-2}$). Additionally, the results indicated a response effect ($F = 18.09$; $p = 2.37 \times 10^{-4}$). As shown in Figure 4.3.2, both responder and non-responder patients exhibited significant differences in MED22 expression during trauma-focused psychotherapy at T4 (z_value=-2.13; p = 0.04), T8 (end of treatment; z_value=-3.85; p=0.0004), and T12 (follow-up; z_value=-2.93; p = 0.007), but not at baseline (p = 0.96).

Figure 4.3.2. Evolution of MED22 expression across the four measured time points, comparing responders (n = 16) and non-responders (n = 5) (Adapted from Carvalho Silva et al., 2025 [205]).



As a further point of interest, higher transcript levels of MED22 at baseline showed a positive association with disease relapse at T24 ($\tau = 0.390$; $p = 0.037$): significantly higher baseline transcript levels of MED22 ($p = 0.026$) were found in non-remitters compared to remitters, suggesting its predictive power to detect disease relapse.

Discussion

The aim of our study was multidimensional: specifically, we aimed to investigate whether both genetic and environmental factors influence the dysregulation of MED22 expression. To achieve this goal, we examined the role of MED22 and its association with CT and MDD, with a particular focus on the relationship between MED22 SNPs and neuroticism in HC. Additionally, we conducted a longitudinal analysis at four (T4), eight (T8), twelve (T12) and 24 (T24) weeks in MDD patients with CT who underwent TF-CBT or EMDR.

Our findings emphasise the interaction between biological and psychological factors in stress-related disorders, including MDD. We observed a significant association between overall MED22 expression levels and measures of emotional abuse and neglect. In particular, the GReX component of MED22 showed positive correlations with neglect, sexual abuse, and emotional abuse scores, whereas the EReX component was linked exclusively to neglect-related maltreatment scores.

These results illustrate how both genetic and environmental factors contribute to a predisposition for reduced resilience to stressors. Indeed, neglect and emotional abuse represent forms of trauma often considered less severe trauma, yet they constitute vulnerability factors comparable to other types of abuse and are significant risk factors for the development of psychiatric disorders [58,59,196,207].

Previous genome-wide association studies have identified a shared genetic influence between neuroticism and MDD, underscoring neuroticism's role as a predispositional factor that interacts with CT to increase vulnerability to MDD [206]. Reduced resilience to stressors has also been linked to MED22 cis-eSNPs associated with higher neuroticism scores, also in HC, suggesting a potential molecular mechanism underlying this predisposition. This may help explain, in accordance with previous evidence, why MDD patients with elevated neuroticism at baseline appear less likely to respond to pharmacological treatment following negative life events [208].

Furthermore, we found that MED22 expression in the blood of MDD patients decreased during trauma-focused psychotherapy, and this decrease was associated with a significant reduction in

depressive symptoms as measured by MADRS score at weeks 4, 8, and 12, with a distinction between responders and non-responders. Additionally, baseline MED22 levels were significantly higher in patients not in remission compared to those in remission, and this may suggest a potential role for MED22 in predicting relapse rather than treatment response. This result is clinically meaningful and could provide valuable insights for forecasting disease outcomes, particularly given that standardized clinical assessments were conducted before and after the non-pharmacological intervention, as well as throughout the entire follow-up period.

Finally, the discovery of predictive biomarker of treatment response is particularly important in MDD research, as it can guide treatment strategies and support personalised therapeutic interventions.

Although the aforementioned evidence supports our findings, this study has several limitations. First, the sample sizes for both the HC cohort and the longitudinal MDD cohort were relatively small, which may limit the generalizability of the results. Second, the use of pharmacological treatments and other psychosocial interventions in the hospital setting where participants were enrolled may have influenced the observed outcomes, even though medications remained stable during psychotherapy interventions. Finally, the absence of a control group prevents causal conclusions regarding the relationship between trauma-focused psychotherapy and changes in MED22 expression, which could also be affected by unmeasured factors in this research.

4.4. Findings on biological outcomes in MDD patients undergoing Physical Activity

Major depressive disorder and other depressive disorders are typically characterised by changes in cognitive functioning, mood disturbances, diminished interest or pleasure in activities, feelings of guilt, chronic fatigue and altered perception of pain, reduced self-esteem and self-confidence, and behavioural alterations that may affect sleep quality, appetite, and suicidal ideation. Moreover, they often impair an individual's occupational and social functioning, as well as negatively impact the family environment, resulting in a substantial overall burden [111,209].

s mentioned in the previous chapters, several studies have highlighted the involvement of neurobiological pathways, chronic inflammation, immune responses, endocrine function and neurotrophic signalling [111,210,211]. First-line treatments recommended for the management of MDD include SSRIs and SNRIs due to their relatively low incidence of adverse effects and their favourable efficacy and tolerability profiles. Furthermore, depending on patient-specific factors and regional guidelines, first-line treatment may include agomelatine, bupropion (NDRI), or mirtazapine (NaSSA). However, a significant proportion of patients do not respond adequately to these treatments and are therefore transitioned to second- and third-line therapeutic options.[111–113,212,213].

Physical activity is a non-pharmacological intervention recommended by guidelines to alleviate mild to moderate MDD symptoms in combination with antidepressants or with other treatments, to improve therapeutic effectiveness. Indeed, regular physical activity of low to moderate intensity is recommended, lasting about 30–40 minutes three to four times per week, for a total of approximately 150 minutes, maintained for at least nine weeks [214–216]; notably, numerous systematic reviews and meta-analyses showed a potential effect of physical activity to ameliorate depressive symptoms [217–220].

The neurobiological mechanism underlying the antidepressant effects of exercise remain unclear, as current evidence highlights the neurobiological effects of physical exercise in patients with MDD, but the predominant focus of most studies has been on inflammatory and immune pathways, such as IL-6 and BDNF, while other biological mechanisms have only rarely been considered or appear inconsistent [221,222].

It appears that novel, updated and comprehensive research is required to elucidate the mechanisms by which different forms of physical exercise contribute to the treatment of MDD. Based on this, our group aimed to conduct a systematic review of RCTs investigating the biological mechanisms underlying the effects of physical exercise in patients with MDD, aiming to investigate not only the immune-inflammatory and neurotrophic pathways that have already been the focus

of previous studies, but also to consider oxidative stress, monoamine neurotransmitters (such as adrenaline, dopamine, noradrenaline and serotonin), and stress-response mechanisms.

Our systematic review was conducted in adherence to PRISMA[223] guidelines and the protocol was registered on PROSPERO (CRD42024548511) [221]. Study identification was conducted using major scientific databases such as PubMed, EMBASE, and Cochrane Central. The selection process was carried out independently by two reviewers who screened titles and abstracts, with a third independent reviewer resolving any disagreements. The inclusion criteria were: 1) Biomarkers' evaluations through blood marker tests conducted before and after an exercise intervention; studies comparing two or more different interventions or including a control group were also considered eligible; 2) Participants aged 18 years or older with a diagnosis of MDD according to DSM-IV or subsequent editions, or according to ICD-10 or ICD-11; 3) Articles written in English. The exclusion criteria were: 1) studies including children or adolescents, or those investigating conditions other than MDD; 2) non-RCTs or 3) RCTs that did not report biomarker assessments both before and after the intervention.

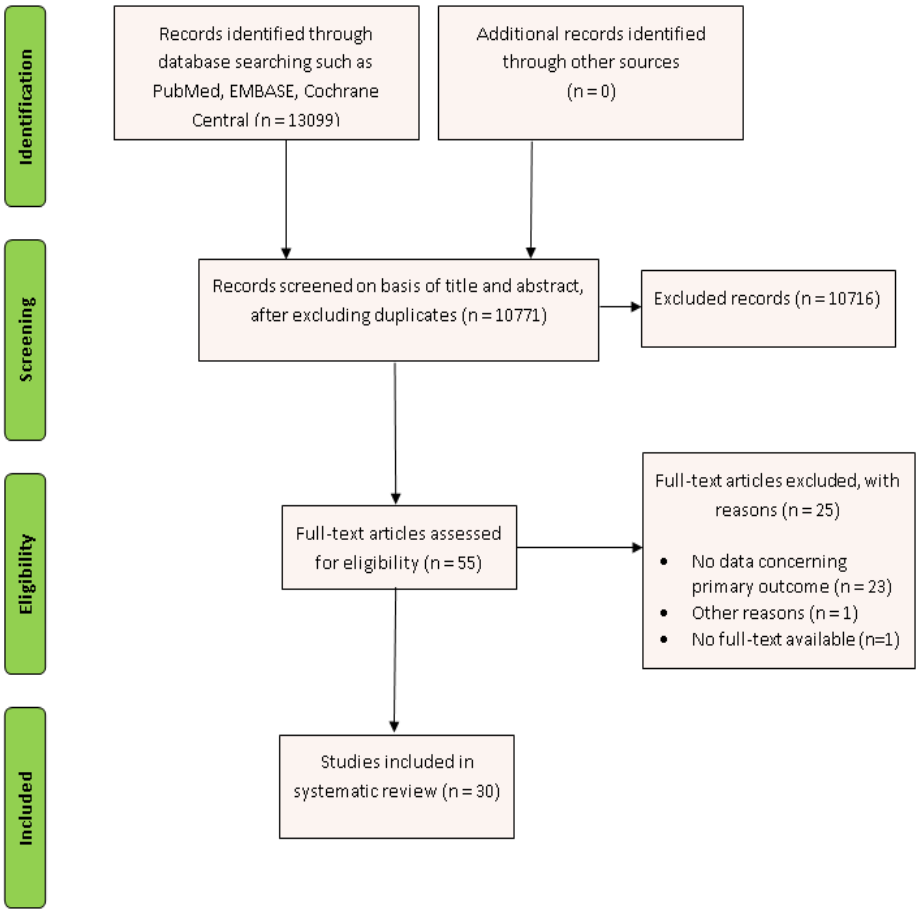
To assess methodological quality and internal validity, we used the Revised Cochrane Risk of Bias tool for randomised trials (RoB 2), which allows the assessment of risk of bias across five domains: the randomization process, possible deviations from intended interventions, missing outcome data, measurement of outcomes, and selective reporting of results. The results allow the overall study quality to be categorized as low risk, some concerns, or high risk [224] .

Summary of the main findings

Search results of the included studies

Preliminary screening yielded 30 eligible studies involving 2194 participants. Participants had a mean age of 42.3 years, and 68,5% were female, compared to 42,8 years and 65% female in the control groups (Figure 4.4.1). Information regarding illness duration and clinical course could not be systematically reported across the 30 studies. All subjects included in the systematic review received a diagnosis of depression based on standardised diagnostic instruments administered by the authors of the respective studies.

Figure 4.4.1. PRISMA flowchart illustrating the study selection process (Adapted from Carvalho Silva et al., 2025[221]).



The **ROB** assessment showed that 29 studies had an intermediate risk of bias and one study had a high risk. 26 studies had some concerns in bias arising from the randomization process, while 29 studies had some concerns regarding deviation from intended intervention. One study showed concerns on missing outcome data and 4 studies for outcome measurement (Figure 4.4.2).

Figure 4.4.2. Summary of RoB assessments for the thirty eligible studies (Adapted from Carvalho Silva et al., 2025[221]).

Main results concerning inflammation and immune response

The main findings concerning the inflammatory and immune systems derived from 15 studies appear to be inconsistent: one study on MDD patients that combined CBT and physical exercise showed an increase in anti-inflammatory IL-10 levels [225]; however, other studies that examined the IL-10 response did not find this effect when comparing MDD group undergoing physical

activity with the control group [226,227], and the same results were observed for studies that took into account pro-inflammatory IL-6 levels [228–231], and other interleukins, such as IL-12, IL-8 and IL-1β, in MDD patients following physical activity [226–228,232].

Evidence on the effect of physical exercise on CRP levels in patients with MDD remains inconclusive, with studies reporting mixed results, particularly in specific patient subgroups [225,229,231,233,234]. Comparable results were reported for TNF-α, indicating that both biomarkers may serve as potential indicators for assessing the effects of physical exercise in MDD. Nevertheless, their response seems to vary according to the characteristics and duration of the intervention [227,228,231,235,236].

Evidence on neurotrophic system

Overall evidence from 12 studies showed inconsistent effects on VEGF and insulin-like growth factor 1 (IGF-1), despite some evidence on cognitive and behavioural aspects, with other neurotrophic factors being unaffected [227,237].

In contrast, aerobic and mind–body interventions appear to increase BDNF levels with a time-dependent effect [235,238,239]. Moreover, in some studies, BDNF levels appeared to be linked to the possibility of predicting remission or symptom improvement [230,240].

Data obtained from stress-response mechanisms

Eight studies investigating changes in the stress-response system showed mixed effects of physical activity on cortisol regulation and cortisol awakening response (CAR) beyond standard pharmacological treatment, whereas other studies did not show any effect on HPA axis reactivity [236,241–243].

Insights derived from the monoaminergic system

Only four studies focused on the monoaminergic and noradrenergic systems, and the results were not entirely consistent, including three studies on peripheral blood candidate protein biomarkers and only one genetic study. The latter investigated, in MDD patients undergoing Yoga training, the role of the serotonin transporter-linked polymorphic region (5-HTTLPR) within the SLC6A4

gene, along with the MTHFR 677C>T, which showed increased odds of remission, independent of genotype, compared with drug treatment [230].

The remaining three studies concerning peripheral plasma biomarkers showed mixed effects on catechol-O-methyltransferase and monoamine neurotransmitters (serotonin, dopamine, noradrenaline, adrenaline) [242,244,245].

Data concerning endocrine activity, oxidative stress markers, and β-endorphin responses

A total of two studies investigated the endocrine system and showed that physical activity may promote metabolic improvements without consistent antidepressant effects[234,246].

Only one RCT investigated β-endorphin levels in patients with MDD undergoing physical exercise combined with antidepressant therapy and reported a correlation between increased serum β-endorphin levels and a reduction in depressive symptoms compared with the control group [247].

The final two studies focused on oxidative stress changes in MDD patients after physical activity, and showed reduced thiobarbituric acid-reactive substances (TBARS) and other markers with exercise and yoga interventions [248] .

Discussion

Our review aimed to broaden the analysis of the effects of physical activity in MDD and to explore less-studied biological systems. Of the 13,099 studies identified, only 30 met the inclusion criteria and were analysed for this systematic review, involving 2194 participants. The intensity of physical exercise was distributed as follows: six studies examined low-intensity exercises, all non-aerobic, such as Tai Chi and yoga; eighteen studies involved moderate-intensity exercises, mainly aerobic; while five studies employed moderate-to-high or high-intensity exercises, including aerobic or combined interventions (aerobic and non-aerobic).

Findings from the included studies were heterogeneous and suggest an interdependence between biomarker responsiveness, intervention type and duration. For example, for the inflammatory-immune system, specific subgroups of MDD patients showed increased IL-10 and decreased IL-6 and CRP levels[225,228–231]. In contrast, no consistent evidence was found for physical exercise effects on IL-12, IL-8, IL-1β and TNF-α in MDD patients following physical activity [225–228,231–237].

Furthermore, the changes in neurotrophic systems following physical exercise in MDD suggest that they may contribute to cognitive improvements associated with exercise: in fact, effects on BDNF levels were time-dependent and appeared to be associated with the potential to predict

symptom improvement or remission [230,235,238–240], while VEGF and IGF-1may not represent primary targets for exercise response [227,237]. Additionally, the evidence from eight studies on the stress-response system indicates that physical activity does not exert additional effects on CAR expression beyond antidepressant therapy [243] .

Findings on the monoaminergic system suggest that genetic factors, such as 5-HTTLPR and MTHFR 677C>T polymorphisms, may interact with ELS to influence treatment outcomes of physical exercise in MDD[230]. Moreover, physical activity appears to affect catecholamine metabolism and noradrenergic activity, but its effects on serotonin and other monoamines are inconsistent across studies taken into account [242,244]. Overall, exercise shows some potential for modulating monoaminergic biomarkers in MDD, yet the evidence remains limited and variable, highlighting the need for further investigation [221].

In other biological systems, exercise influenced metabolic markers, but effects on hormones such as growth hormone were not consistent. Evidence emerged underlining a possible effect of physical exercise in increasing plasma levels of β-endorphin, and this may enhance clinical outcomes [247]. Also, Yoga and meditation appeared to play a role in the reduction of oxidative stress damage in MDD patients [230,248].

Our findings highlight the need for further research to determine the conditions in which exercise most effectively influences these biological markers. Unlike previous systematic reviews and meta-analysis, a strong point of our study was the adoption of a broader approach by including additional biological systems, enabling a more comprehensive examination of the effects of physical exercise. This approach allows for the identification of interactions and mechanisms that have been insufficiently addressed in previous literature. Specifically, our review extends beyond earlier analyses by exploring less-studied systems, such as oxidative stress, monoaminergic pathways, and stress-response mechanisms, alongside the well-established immune-inflammatory and neurotrophic pathways.

As an additional point that both strengthens our analysis and sheds light on previous studies, the performed RoB assessment allowed us to determine the risk profile of all the investigated studies. The results showed that twenty-nine were rated as having a moderate risk of bias, while one study was classified as high risk. Common concerns among most of the papers were identified in domains related to the randomisation process, minor or major deviations from intended interventions, and selective reporting, with many studies showing a moderate risk of bias in these areas. Notably how these findings emphasise the need for methodological consensus and improvements to enhance the reliability and validity of randomised trial outcomes. It should be noted that this review has

some limitations: the small sample sizes of the included studies may have been a significant contributor to the observed inconsistencies in the findings and, additionally, the predominant focus on biochemical markers highlights the need for further research that can both increase the sample sizes considered and yield more comprehensive, multi-dimensional approaches for a more in-depth understanding of the biological effects of physical activity in MDD.

5. Conclusion

The present thesis, carried out over the course of three years, aimed to provide an integrated overview of the biological correlates, endophenotypes and environmental factors associated with both pharmacological and non-pharmacological treatments in mood disorders, with a special focus on MDD and TRD.

Through the eight complementary studies presented, the thesis emphasises the relevance of social, psychosocial, and biological factors that can interact during the first years of life and across lifespan, shaping biological vulnerability, clinical presentation, treatment course, and treatment response in mood disorders. Rather than focusing on a single biomarker for clinical application, the primary goal of these works was to identify shared biological pathways and endophenotypic profiles that could help better understand the wide heterogeneity of depressive disorders.

The first part of the dissertation investigated the potential role of factors that predispose individuals to early vulnerability and their pervasiveness through changes in developmental trajectories.

The first study presented concerned the presence of internalised gender stereotypes and demonstrated that socially shared norms remain deeply rooted and still present in the age groups studied, influencing beliefs and expectations regarding gender roles. These findings are consistent with previous evidence demonstrating that rigid gender norms increase exposure to chronic stressors, which can affect the biological homeostasis of individuals who do not adhere to stereotypes. A large body of literature has been produced on ethnic minorities, social groups, and sexual orientation. This study fits within this perspective by highlighting that the presence of internalised gender stereotypes can also increase an individual's susceptibility to the onset of depressive disorders, as observed in women throughout their lifespan.

In addition to the influence of the social environment in adulthood, the results of our systematic review and meta-analysis investigating the presence of childhood neglect during early life adds evidence to the importance of adverse environmental elements, especially when occurring early in life, in mental health. Despite our analysis taking into consideration 122 studies and 57,638 patients, the heterogeneity of the included studies, the lack of consensus on the definition of neglect, and the absence of shared standardised method and measures to assess the dimensional aspects of neglect made it difficult to generalise the results. However, we observed low prevalence rates of Ne and PN in subjects diagnosed with MDD, while high prevalence rates of EN were

found in subjects with BD and MDD, with the latter showing the highest prevalence. Furthermore, PN and EN were also related to anxiety disorders and PTSD in psychiatric populations. Evidence from these studies highlights that childhood neglect represents a transdiagnostic vulnerability factor that contributes to cumulative biological burden and increases individual susceptibility to developing dysfunctional and pathological trajectories in adulthood. Taken together, these studies emphasise how vulnerability to mood disorders is not solely a consequence of genetic predisposition, but rather emerges from the interaction between multiple factors, including social context, early environmental exposures, and individual biological sensitivity.

The third study examined the clinical endophenotypes associated with the presence of agitation–anxiety symptoms in MDD, and raised an important question regarding whether the presence of these symptoms may modulate individual vulnerability and treatment response. Indeed, in the three large cohort studies reviewed, the presence of agitation–anxiety symptoms was consistently associated with greater severity of depression, increased suicidal risk, longer duration of illness, and reduced response to antidepressant treatments. These findings highlight the importance of identifying such phenotypes in order to better understand treatment resistance and to guide a stratified therapeutic approach, moving beyond categorical diagnoses and focusing instead on biologically and clinically informed subtypes of depression.

Studying specific endophenotypes in mental disorders, particularly MDD, may allow for improved pharmacological and non-pharmacological interventions, with the potential to enhance response rates and prevent relapse, thereby reducing patient distress as well as the burden on work and society. This is particularly relevant in the case of TRD, where repeated treatment failures require a shift in perspective and pose new challenges for increasingly precise clinical treatment.

As highlighted by study presented in the third chapter of this thesis, the Italian Medicines Agency (AIFA) has approved the use of the s-enantiomer formulation of ketamine, administered as a nasal spray in a controlled clinical setting, for the treatment of TRD since 2022. Our systematic review of the literature on the molecular mechanisms of TRD treatment with ketamine and esketamine highlighted the complexity of the molecular processes underlying their rapid antidepressant effects. Analysing the twelve studies that met the selection criteria, current evidence from both human and in vitro molecular studies on human cells suggests that the mechanism of action of ketamine's R–S enantiomers contribute to the modulation of neuroplasticity, immune-inflammatory pathways, and intracellular signalling cascades, including mTOR-related pathways and glutamate transmission. However, the full mechanism remains only partially understood and appears to be time-dependent

following a single dose of treatment. Indeed, a dissociation emerged between the evidence from the molecular findings identified in their transcriptional changes and the clinical outcome in terms of short-term reduction of depressive symptoms, highlighting the current difficulty in translating molecular findings into biomarkers of potential clinical response. The results presented, although showing molecular effects in the short-term following ketamine treatment, suggest that the antidepressant effects are likely mediated by dynamic biological processes that interact with other factors involved in the homeostatic regulation of the body, reinforcing the importance of integrative models of investigation.

A possible integrated treatment and research strategy for depression and TRD was explored in the works presented in the fourth chapter, which focused on non-pharmacological treatments used either in addition to pharmacological therapy or as stand-alone interventions, while analysing changes in specific immunological, inflammatory, and genetic biomarkers in relation to clinical outcomes.

The study of plasma miRNA transcriptomic levels in twenty-seven patients with MDD-TRD undergoing ECT as a complementary approach demonstrated potential changes during treatment and their association with treatment outcomes. Specifically, two miRNAs, miR-30c-5p and miR-324-3p, were found to be downregulated following ECT treatment and appeared to correlate with significant modulation of depressive symptoms. Previous studies have demonstrated the role of miR-30c-5p in the regulation of VEGFα and SIRT1, which are respectively involved in several brain processes related to neuronal growth, cell differentiation, and neuroprotection through the modulation of pro-inflammatory pathways.

Similarly, in the study examining the effects of TF-CBT and EMDR on leukocyte telomere length (LTL) and mitochondrial DNA copy number in TRD patients with a history of ELS, we identified significantly higher mtDNAcn levels in TRD patients compared with healthy controls. Nevertheless, neither mtDNAcn nor LTL was significantly associated with clinical treatment response. Exposure to ELS may be involved in a co-regulation process in which telomere shortening is accompanied by compensatory increases in mtDNAcn. Our findings therefore support the involvement of ELS in the biological basis of depression, particularly in relation to stress exposure and accelerated biological ageing; however, changes in these markers cannot be considered predictive of psychotherapy outcomes.

The investigation of how environmental factors and genetic influences affect the dysregulation of MED22 expression in MDD patients with a history of childhood trauma highlighted the influence

of early experiences on gene expression profiles and the involvement of MED22 in stress-response vulnerability and emotional regulation in individuals exposed to emotional abuse and neglect, as well as in healthy controls with high levels of neuroticism. Our study further underlined the importance of non-pharmacological interventions, such as TF-CBT and EMDR, in MDD patients, revealing decreasing levels of MED22 expression in blood that were associated with a significant reduction in depressive symptoms during the twelve weeks of psychotherapeutic treatment, with a clear distinction between responders and non-responders. Furthermore, we observed a correlation between higher baseline levels of MED22 and patients who did not achieve remission, suggesting a potential role for MED22 in predicting relapse rather than treatment response. Once again, these findings suggest that non-pharmacological interventions such as trauma-focused psychotherapy may exert their effects through complex biological adaptations that cannot be fully captured by single static biomarkers.

Finally, in the last study developed during this doctoral program, we conducted a systematic review on the effects of physical activity in MDD patients and the associated biological outcomes. Our results, based on the thirty studies included after the selection process, demonstrated modest but heterogeneous effects on biological systems involved in depression, including neurotrophic, immune, and stress-response pathways. Evidence from non-pharmacological interventions such as physical activity indicated modulation of immunoinflammatory and neurotrophic systems, including increases in IL-10 and BDNF levels and reductions in IL-6 and CRP. Other studies included in the review focused on the effects of physical activity on the monoaminergic and catecholaminergic systems as well as on β-endorphin levels. However, although physical exercise appears to influence several biological processes, variability in study design and sample characteristics limits definitive conclusions, emphasising the need for more standardized research.

In summary, the findings presented in this thesis highlight the multifactorial and dynamic nature of depressive disorders, emphasising that vulnerability, clinical factors, and treatment response emerge from a complex interaction between biological, psychological, and environmental factors across the lifespan. The studies included in this thesis support the view that early-life experiences, social context, and individual biological sensitivity contribute to shaping risk trajectories for mood disorders, while specific clinical endophenotypes may help explain the marked heterogeneity observed in MDD and TRD. At the same time, the investigation of both pharmacological and non-pharmacological interventions demonstrates that therapeutic effects are likely mediated by interconnected biological pathways involving neuroplasticity, immune-inflammatory responses,

stress regulation, and genetic and epigenetic mechanisms. However, the variability of findings across studies and the limited predictive value of individual biomarkers highlight the current challenges in translating molecular evidence into reliable clinical tools. Taken together, these results reinforce the need for integrative and multidimensional approaches that combine biological, clinical, and environmental perspectives in order to better characterise depressive disorders and support the development of more personalised and effective treatment strategies.

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