

Peripheral BDNF regulation and inflammation in bipolar disorder: a multimodal study

Beatrice Feliciani

University of Teramo

Nicolaja Girone

University of Milan

Clara Cavallotto

University "G. d'Annunzio" of Chieti Pescara

Salvatore Saluzzi

ASST Papa Giovanni XXIII

Francesca Martella

University of Teramo

Martina Di Bartolomeo

University of Teramo

Emi Bondi

University of Milan

Mauro Pettorruso

University "G. d'Annunzio" of Chieti Pescara

Alberto Granzotto

University "G. d'Annunzio" of Chieti Pescara

Camilla Gesi

University of Milan

Laura Cremaschi

University of Milan

Monica Macellaro

University of Milan

Annabella Di Giorgio

ASST Papa Giovanni XXIII

Giovanni Martinotti

University "G. d'Annunzio" of Chieti Pescara

Bernardo Maria Dell'Osso

University of Milan

Claudio D'Addario

cdaddario@unite.it

Research Article

Keywords: bipolar disorder, BDNF, DNA methylation, microRNA, extracellular vesicles, cytokines, clinical staging, neuroplasticity, childhood adversity

Posted Date: October 14th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11115570/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.
[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Background

Bipolar disorder (BD) is a severe, recurrent psychiatric condition in which neuroplasticity, inflammatory and epigenetic mechanisms are implicated but incompletely characterised. Brain-derived neurotrophic factor (BDNF) is among the most investigated candidate biomarkers, yet its relationship to clinical staging, DNA methylation, microRNA regulation and peripheral inflammation remains unclear. This study integrates peripheral *BDNF* gene expression, *BDNF* promoter I and IV methylation, rs6265-CpG methylation, four *BDNF*-targeting salivary extracellular vesicle (EV)-associated miRNAs and eight peripheral cytokines in a multicentre cohort of BD patients stratified by clinical stage.

Methods

One hundred and thirty-four individuals with BD and 131 healthy controls were recruited across three Italian psychiatric centres within the BOARDING-PASS study. *BDNF* mRNA and cytokines were quantified from peripheral blood mononuclear cells (PBMCs) by qRT-PCR; *BDNF* promoter methylation was assessed by pyrosequencing; salivary EV miRNAs were profiled by qRT-PCR. Associations with clinical stage, childhood adversity (CTQ), pharmacotherapy and global functioning were examined.

Results

Peripheral *BDNF* mRNA was markedly reduced in BD patients versus controls (median 0.31 versus 1.45; $p = 5.1 \times 10^{-7}$), present from the earliest clinical stage and not deepening with progression. No *BDNF* methylation difference was detected at any of thirteen CpG sites (all $q > 0.23$). Among four pre-specified *BDNF*-targeting miRNAs, miR-16-5p was nominally elevated ($p = 0.038$) but did not survive correction. IFN- γ was the only cytokine significantly elevated after FDR correction ($q = 0.003$); IL-1 β showed a nominal increase ($p = 0.028$). Within patients, *BDNF* expression correlated positively with most cytokines (IL-2 $\rho = +0.82$), indicating shared transcriptional co-regulation. Higher childhood adversity was associated with lower methylation at the rs6265 CpG ($\rho = -0.46$, $q = 0.001$). *BDNF* expression correlated inversely with global functioning (GAF: $\rho = -0.23$, $p = 0.012$) and was selectively elevated in BD-II patients receiving antidepressants ($p = 0.009$), with no parallel effect in BD-I.

Conclusions

Peripheral *BDNF* dysregulation in BD is better described as a multilayer, largely trait-related alteration than as a linear marker of illness progression. The expression deficit, its positive coupling to peripheral inflammation, its association with functioning, and the adversity-related epigenetic mark at rs6265 constitute convergent signals that do not map onto clinical stage. A composite, rigorously replicated

biomarker strategy – integrating neurotrophic, immune and epigenetic readouts – appears more informative than any single molecule for characterising BD biology.

Background

Bipolar disorder (BD) is a severe, chronic and recurrent psychiatric disorder that affects more than 1% of the global population and is a leading cause of disability, with major effects on psychosocial functioning, cognition and life expectancy. [1–3].

BD is highly heritable, but its aetiology appears to reflect a complex interaction between genetic liability and environmental exposures, while its pathophysiology remains incompletely understood and involves disturbances in neuroplasticity, inflammatory homeostasis and related cellular pathways [1–2, 4–6].

Beyond the burden of acute mood episodes, current evidence supports the view that BD can follow a progressive course in a subset of patients, with recurrent episodes accompanied by cumulative molecular, neuroanatomical and functional changes associated with cognitive and functional decline [7–8]. At the same time, the biological literature on illness progression remains scarce and heterogeneous, and the neuroprogression construct is still debated in its scope and clinical translation [7].

Within this framework, inflammation, altered neuroplasticity, oxidative and cellular stress have been proposed as candidate pathways linking recurrence to clinical deterioration and emotion dysregulation [2, 5, 7, 9]. Nonetheless, no biomarker has yet been approved for the diagnosis or prognosis of BD, and the lack of reliable, accessible markers of illness progression remains a major obstacle to biologically anchored staging [1, 3].

Several clinical staging models have therefore been proposed to describe BD as a longitudinal trajectory from nonspecific early symptoms and at-risk states to recurrent and more persistent illness [1, 7]. However, current staging systems remain predominantly clinical rather than biological, because longitudinal clinical assessment is still the cornerstone of diagnosis and course characterization in BD [1].

Among candidate molecular markers, brain-derived neurotrophic factor (BDNF) is one of the most extensively investigated in BD [3, 10–11]. BDNF is a key regulator of neuronal survival, synaptic plasticity and neurogenesis, making it biologically plausible as an index of impaired neuroplasticity and cognitive functioning in mood disorders [10, 12–14].

Meta-analytic evidence indicates that peripheral *BDNF* levels are reduced during manic and depressive episodes and tend to normalize in euthymia, supporting a role as a state-related biomarker more clearly than as a robust marker of illness stage [8, 10, 12]. *BDNF* levels also appear to increase after successful treatment of acute mania [8, 12].

Evidence that circulating *BDNF* tracks illness stage is mixed. One meta-analysis found no significant impact of illness duration on *BDNF* levels [8], whereas another reported that longer illness duration was

associated with higher *BDNF* levels [15]. In newly diagnosed patients, *BDNF* levels were not decreased in early-stage BD and were higher than in healthy controls, further arguing against a simple linear staging interpretation [16].

At the genetic level, the rs6265 (Val66Met) polymorphism has attracted interest because it can impair activity-dependent *BDNF* release, and some studies associate it with clinical features linked to worse outcome, although evidence for a standalone diagnostic, prognostic or staging role remains limited [10, 17–18]. Beyond the polymorphism itself, the rs6265 locus carries a CpG dinucleotide whose methylation – rather than the genotype – is the focus of the present analysis at this site.

DNA methylation is especially relevant to *BDNF* regulation and has been widely investigated in BD as a mechanism through which environmental exposures may shape gene expression [3–4, 13, 19]. *BDNF* is among the most extensively studied candidate genes in this literature, and altered methylation has been reported in promoter regions and CpG sites across different BD samples [3, 11, 13, 20]. Some studies also link higher *BDNF* methylation to lower gene expression, depressive states, BD subtype differences and medication exposure [3, 10–11, 21].

However, the epigenetic literature remains methodologically heterogeneous and poorly harmonized, limiting direct comparison across cohorts and molecular layers [4, 10, 15]. Peripheral *BDNF* methylation has drawn interest because some evidence suggests a correlation between post-mortem peripheral and brain tissue methylation, supporting its potential as a proxy for central alterations [3, 13].

A second epigenetic layer involves non-coding RNAs, which are considered plausible regulators of neuroplasticity-related transcripts in BD, although the field is still developing [4, 11, 22–23]. This supports multimodal designs that assess DNA methylation, gene expression and RNA-based regulation jointly rather than in isolation [4–5, 24].

The use of peripheral blood mononuclear cells (PBMCs) for *BDNF* expression and DNA methylation, together with salivary extracellular vesicles (EVs) for miRNA profiling, is biologically plausible because extracellular vesicles can cross the blood–brain barrier, are detectable in peripheral fluids including saliva, and carry cargo relevant to neuroinflammation and synaptic function [25–28]. EVs are increasingly studied as candidate biomarkers in neurological and psychiatric disorders, including bipolar disorder, but their classification, isolation and functional interpretation remain under active investigation, so they are best framed as promising rather than established biomarkers [25–27].

Building on this rationale, the present study integrates *BDNF* gene expression, DNA methylation (assessed at *BDNF* promoter I and promoter IV, and at the CpG overlapping the rs6265/Val66Met locus), salivary EV-associated miRNAs and peripheral inflammatory markers in patients with BD stratified according to a clinical staging framework. Genotyping of rs6265 was not performed, so this locus is examined at the level of DNA methylation rather than allele status. Given the current state of the literature, the most defensible objective is to test whether convergent transcriptional, epigenetic and inflammatory signals related to *BDNF* are associated with BD features and with stage-defined clinical

profiles, rather than to presuppose validated biomarkers of clinical stage [7–8, 17]. Overall, a composite biomarker strategy integrating the interplay between neurotrophic, immune and neuroendocrine systems appears more plausible than any single molecule for capturing the biological complexity of BD [8, 17, 29].

Materials and methods

Participants and clinical staging

Patients with BD and healthy controls were recruited within the Bipolar Disorder Integrative Staging: Incorporating Biomarkers into Progression Across Stages (BOARDING-PASS) study across three centres. Diagnoses were established by trained psychiatrists according to DSM-5 criteria [30]. Each patient was assigned a clinical stage on an ordinal framework (stages 0–4) intended to capture illness progression from at-risk / prodromal states to recurrent and more persistent illness [31]. Baseline clinical characterisation included age, sex, age at onset, duration of illness, number of lifetime episodes and hospitalisations, global functioning (GAF), symptom severity (MADRS, YMRS, HAM-D, HAM-A), current pharmacotherapy (lithium, antiepileptics, antidepressants, antipsychotics) and early-life adversity (Childhood Trauma Questionnaire, CTQ) [32]. As reported in Table 1, the study enrolled 134 individuals including subjects at high risk for BD and patients with BD (66 female, 68 male), and 131 healthy controls (56 female, 75 male) without current or lifetime psychiatric conditions or a positive family history of psychiatric disorders in their first-degree relatives. BD patients were recruited from three secondary-level psychiatric services in Italy: 42 from ASST Papa Giovanni XXIII in Bergamo, 43 from ASST Fatebenefratelli-Sacco in Milan and 47 from the ASL 2 Lanciano-Vasto-Chieti in Chieti. Biological samples were collected between October 2023 and December 2025. Inclusion criteria comprised age between 18 and 70 years and the ability to provide informed consent. Exclusion criteria included major neurological or systemic medical conditions, intellectual disability, current or recent substance use disorder [30]. All participants provided written informed consent to participate in the study and to allow the use of their anonymized data for research purposes. The study was conducted in accordance with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975 [30], as revised in 2008. The Ethics Committee of Milan, Area 1, reviewed and approved the study protocol (approval number 0008265/2023, dated 24 February 2023).

Table 1
Socio-demographic, clinical, psychometric, and pharmacological characteristics across healthy controls and patient groups.

Variable	Controls (n = 131)	BD–0 (n = 23)	BD–I (n = 59)	BD–II (n = 42)	P value
Socio–demographic features					
Gender: Female (%)	56 (42.7%)	13 (54.2%)	27 (45.8%)	23 (54.8%)	0.480 ^a
Male (%)	75 (57.3%)	11 (45.8%)	32 (54.2%)	19 (45.2%)	
Age at recruitment (years, mean $\pm$ SD)	40.7 $\pm$ 12.0	46.8 $\pm$ 14.9	43.8 $\pm$ 12.5	45.8 $\pm$ 13.2	0.061 ^b
Married / Engaged (%)	—	17 (70.8%)	24 (40.0%)	22 (51.2%)	0.037 ^{*a}
Education: High School / Degree (%)	—	19 (79.2%)	52 (86.7%)	37 (86.0%)	0.667 ^{*a}
Clinical features					
Age at onset (years, mean $\pm$ SD)	—	24.2 $\pm$ 14.2	26.4 $\pm$ 10.9	27.2 $\pm$ 10.1	0.793 ^{*b}
Duration of illness (years, mean $\pm$ SD)	—	19.5 $\pm$ 17.1	17.4 $\pm$ 12.0	18.8 $\pm$ 13.0	0.834 ^{*b}
Family history of mood disorders (%)	—	23 (95.8%)	35 (58.3%)	27 (62.8%)	0.003 ^{*a}
Psychiatric comorbidity (%)	—	2 (9.5%)	18 (30.0%)	22 (51.2%)	0.003 ^{*a}
Medical comorbidity (%)	—	6 (26.1%)	16 (26.7%)	10 (23.3%)	0.923 ^{*a}
Psychometric variables					
GAF score (mean $\pm$ SD)	—	92.2 $\pm$ 7.8	79.7 $\pm$ 12.8	78.1 $\pm$ 14.2	< 0.00 ^{*b}
MADRS score (mean $\pm$ SD)	—	4.4 $\pm$ 3.5	7.3 $\pm$ 7.3	10.5 $\pm$ 10.0	0.009 ^{*b}
YMRS score (mean $\pm$ SD)	—	2.2 $\pm$ 2.6	2.5 $\pm$ 3.5	3.0 $\pm$ 3.9	0.620 ^{*b}
HAM–A score (mean $\pm$ SD)	—	3.0 $\pm$ 2.1	3.8 $\pm$ 3.2	7.6 $\pm$ 7.4	< 0.001 ^{*b}
HAM–D score (mean $\pm$ SD)	—	3.0 $\pm$ 2.8	4.3 $\pm$ 4.2	7.2 $\pm$ 6.2	< 0.001 ^{*b}
Current treatment (%)					

Variable	Controls (n = 131)	BD–0 (n = 23)	BD–I (n = 59)	BD–II (n = 42)	P value
Lithium	—	1 (4.8%)	41 (68.3%)	20 (47.6%)	< 0.001 ^{*a}
Anticonvulsants / Mood stabilizers	—	3 (14.3%)	26 (43.3%)	21 (50.0%)	0.021 ^{*a}
Antidepressants	—	6 (28.6%)	15 (25.0%)	28 (66.7%)	< 0.001 ^{*a}
Atypical Antipsychotics (AP2)	—	1 (4.8%)	32 (53.3%)	17 (40.5%)	< 0.001 ^{*a}
Long–Acting Injectable (LAI)	—	0 (0.0%)	12 (20.0%)	4 (9.5%)	0.045 ^{*a}

Data are presented as mean $\pm$ standard deviation (SD) for continuous variables and as frequency (n) and percentage (%) for categorical variables. Statistical methodology: Differences in age and gender across all 4 groups were tested using one-way ANOVA (F) and Chi-square (χ^2) tests, respectively. Clinical, psychometric, and treatment comparisons across patient groups (BD-I, BD-II) were conducted using ANOVA (F) ^(b) for continuous measures and Pearson's Chi-square (χ^2) ^(a) test for categorical distributions.

* P-values for variables with data available only in the clinical sample were calculated comparing the three patient groups (BD-0, BD-I, and BD-II).

Abbreviations: Controls, Healthy Controls; BD-0, increased risk, having a

first-degree relative with BD, without psychiatric symptoms [30], not included in the analyses; BD-I, Bipolar Disorder Type I; BD-II, Bipolar Disorder Type II; GAF, Global Assessment of Functioning; MADRS, Montgomery–Åsberg Depression Rating Scale; YMRS, Young Mania Rating Scale; HAM-A, Hamilton Anxiety Rating Scale; HAM-D, Hamilton Depression Rating Scale; AP2, Second-Generation (Atypical) Antipsychotics; LAI, Long-Acting Injectable Antipsychotics.

***BDNF* and cytokines gene expression**

BDNF as well as eight cytokines (IL-6, IL-19, TNF- α , IL-1 β , IFN- γ , IL-2, IL-4, IL-8) mRNA levels were quantified from PBMCs by quantitative real-time PCR (qRT-PCR). Differences in threshold cycle values were used to quantify the relative amounts of PCR targets in each sample. Following PCR, a dissociation (melting) curve was generated from 60 to 95 °C [33] to assess the specificity of the amplified products. Total RNA was extracted using Qiazol[®] Reagent (Qiagen, Hilden, DE, Germany) following the manufacturer's instructions. The concentration and quality of the extracted RNA were evaluated using a NanoSNAP spectrophotometer (Spex, Metuchen, NJ, United States). RNA was reverse-transcribed into

complementary DNA (cDNA) using the SensiFAST™ cDNA Synthesis Kit (Bioline Reagents, London, UK), starting from 500 ng of total RNA per sample. Gene expression analysis was performed by qRT-PCR using the SensiFAST™ SYBR Lo-ROX Kit (Bioline Reagents, London, UK) on a QIAquant 96 5plex instrument (Qiagen, Hilden, Germany). *BDNF* transcript levels were normalized to the arithmetic mean of the Ct values of two reference genes: glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and beta-actin (β -ACT), which were selected based on their stable expression across experimental groups. The primer sequences used for amplification were as follows: GAPDH, forward 5'-CAGCCTCAAGATCATCAGCA-3' and reverse 5'-TGTGGTCATGAGTCCTTCCA-3'; β -actin, forward 5'-GACCCAGATCATGTTTGAGACCT-3' and reverse 5'-CCATCACGATGCCAGTGGT-3'; *BDNF*, forward 5'-AAGAAGCAAACATCCGAGG-3' and reverse 5'-AAGGCACTTGACTACTGAGC-3'. Relative expression was computed by the $\Delta\Delta C_t$ method and expressed as $2^{(-\Delta\Delta C_t)}$ [1].

DNA methylation analysis by pyrosequencing

Genomic DNA was extracted from PBMCs using Qiazol® Reagent (Qiagen, Hilden, DE, Germany). Each purified DNA (500 ng) sample was subjected to bisulfite modification using the EZ DNA Methylation-Gold™ Kit (Zymo Research, Orange, CA, USA) according to the manufacturer's instructions. Bisulfite-converted DNA was then amplified with a biotin-labelled primer using the PyroMark PCR kit (Qiagen, Hilden, Germany) in accordance with the manufacturer's guidelines. PCR conditions were as follows: 95 °C for 15 min, followed by 45 cycles of 94 °C for 30 s, 56 °C for 30 s, 72 °C for 30 s, and, finally, 72 °C for 10 min. The specificity of PCR products was then verified by electrophoresis. Pyrosequencing was performed on a PyroMark Q48 Autoprep using PyroMark Q48 Advanced Reagents (Qiagen). *BDNF* promoter I sequence was analysed with a Qiagen commercial assay (Hs_BDNF_08, PM00155540), PCR amplification and sequencing primers for *BDNF* promoter IV and IX were designed using PyroMark Assay Design Software version 2.0 (Qiagen, Hilden, Germany). Methylation was quantified at every individual CpG sites of *BDNF* promoter I (four CpGs) and promoter IV (six CpGs), and at the CpG overlapping the rs6265 (Val66Met) locus; promoter averages were computed as the mean of the constituent CpGs. The DNA methylation level was analyzed through the PyroMark Q48 Autoprep 2.4.2 software, which calculates the methylation percentage $mC/(mC + C)$ (mC = methylated cytosine, C = unmethylated cytosine) for each CpG site, allowing quantitative comparisons. Primers, analysed sequences and genome localisation are reported in Supplementary Table S1 and Figure 1. The rs6265 assay was designed to quantify methylation and the dispensation order did not interrogate the rs6265 polymorphic base (see Statistical analysis).

Extracellular vesicle (EV) isolation and microRNA analysis

EVs were isolated from 1 mL of saliva using Total Exosome Isolation Reagent (Invitrogen, Thermo Fisher Scientific), and miRNAs were extracted with the Total Exosome RNA and Protein Isolation kit (Invitrogen) according to the manufacturer's protocol, in agreement with the Minimal Information for Studies of Extracellular Vesicles (MISEV2023) guidelines [47]. miRNA expression was assessed by qRT-PCR after

reverse transcription using miRCURY LNA RT kit (Qiagen, Hilden, Germany). qPCR reaction was performed using sensiFAST SYBR[™] Green on a QIAquant 96 5 plex RealTime PCR System (Qiagen, Hilden, Germany). Four *BDNF*-targeting miRNAs were analyzed using a Qiagen assay: miR-16-5p YP00205702, miR-29a-3p YP0020469, miR-191-5p YP00204306, miR-202-3p YP00205990. Expression levels were normalised using two endogenous references small RNAs: U6 small nuclear RNA (U6 snRNA) YP02119464 and small nucleolar RNA 48 (SNORD48) YP00203903. For each sample, the normalisation reference was calculated as the arithmetic mean of the mean Ct values obtained for the two reference genes. Relative expression was calculated using the $2^{(-\Delta\Delta Ct)}$ method [1], with the control group serving as calibrator. To assess the robustness of the miRNA case–control comparison to reference-gene instability, we repeated it under alternative normalisation strategies (SNORD48 alone, U6 alone, and a reference-free global-mean normalisation); results are reported in Table S-norm.

The four microRNAs studied (miR-16-5p, miR-29a-3p, miR-191-5p, miR-202-3p) were pre-specified rather than selected by discovery screening, on the basis of a documented relationship with *BDNF* and with mood disorders. miR-16-5p and miR-191-5p are among the microRNAs experimentally and computationally identified as post-transcriptional repressors of *BDNF* in human prefrontal cortex [34] and recur in curated inventories of *BDNF*-modulating microRNAs [35]. miR-16-5p additionally targets the serotonin transporter and participates in adaptive responses to antidepressants [36], while miR-202-3p and miR-29a-3p are predicted *BDNF* regulators with reported roles in stress- and mood-related neuroplasticity. Importantly, these four microRNAs emerged as the *BDNF*-targeting candidates of interest in our group's earlier miRNome analysis in related affective and obsessive-compulsive conditions [35,37]; the present study therefore constitutes a focused, hypothesis-driven test of these specific molecules in bipolar disorder rather than an untargeted survey.

Statistical analysis

Because none of the measured variables was normally distributed, between-group comparisons used the Mann–Whitney U test and associations were quantified with Spearman rank correlation; ordinal trends across clinical stages were tested with Spearman correlation on the ordinal stage. *BDNF*, cytokines and miRNAs expression was analysed as $2^{(-\Delta\Delta Ct)}$ values and displayed on a log2 scale. Multiplicity was controlled with the Benjamini–Hochberg false-discovery rate, applied within each family of tests (the 13 *BDNF* methylation measures, the 8 cytokines, the stage-trend analyses and the stratification analyses). For null findings, the smallest difference detectable at 80% power ($\alpha = 0.05$) is reported so that absence of evidence is distinguished from evidence of absence.

One aspect of the methylation data warrants note. Of 131 enrolled healthy controls, only those with methylation samples processed contemporaneously with patient batches were included in the methylation case–control comparison, to minimise inter-run pyrosequencing variability (Promoter I: $n = 49$; Promoter IV: $n = 18–21$; rs6265 CpG: $n = 62$). In addition, the CpG assayed at rs6265 lies on a polymorphic site and the assay did not genotype it, so the value there reflects both methylation and allele

dosage and is interpreted accordingly; where an rs6265 association is examined within patients, it is additionally tested in a subgroup homogeneous for methylation level, in which allele dosage is constant by construction.

Results

***BDNF* gene expression is reduced in bipolar disorder**

BDNF mRNA was markedly lower in patients than in controls (median 0.31 versus 1.45, a 4.7-fold reduction; $p = 5.12 \times 10^{-7}$; Figure 2A). The difference held within the single centre contributing both groups (median 0.33 versus 1.35; $p = 0.020$; Figure 2B), excluding a centre-composition artefact. Expression did not differ across clinical stages (Kruskal–Wallis $p = 0.17$; Figure 2C), so the deficit was present from the earliest stage and did not deepen with progression.

***BDNF* methylation does not differ between patients and controls**

Across all thirteen *BDNF* measures (promoter-I and promoter-IV CpGs, the two promoter averages and the rs6265 CpG), no site differed between patients and matched controls, and none survived FDR correction (smallest $q = 0.23$; Figure 3, Table S2). Effect sizes were negligible ($|d| \leq 0.24$) and inconsistent in direction. The study had 80% power to detect differences of ~ 2.3 –5 percentage points, so moderate and large differences are excluded. Methylation at the rs6265 CpG site is reported without genotype, the assay having measured methylation only (Supplementary Methods).

***BDNF*-targeting microRNAs in salivary extracellular vesicles**

None differed between patients and controls after correction for multiple testing (all $q > 0.24$). miR-16-5p was nominally higher in patients (median 1.89 versus 0.31; $p = 0.038$), in the direction reported in prior mood-disorder studies, although it did not survive FDR correction across the four miRNAs ($q = 0.15$) and was sensitive to the choice of normalisation reference (Table S-refgenes); the remaining three miRNAs did not differ (Figure 4). This instability reflects a systematic technical offset between the two sample sets: neither reference small RNA was invariant across groups, both showing lower Ct in patients (Table S-refgenes), so between-group miRNA comparisons could only be partially normalised. Within patients, the miRNAs did not correlate consistently with *BDNF* expression: associations were weak, inconsistent in sign, and did not survive correction, as expected for salivary EV-derived species measured in a different compartment from PBMCs-derived *BDNF* mRNA. Correlations with promoter and rs6265 methylation, cytokines and childhood adversity yielded no association surviving FDR (0 of 28 tests; the strongest nominal signals were miR-16-5p with IL-6, $\rho = +0.33$, $q = 0.05$, and with the inflammatory

composite, $\rho = +0.25$). No miRNA expression was modified by sex, clinical stage or pharmacotherapy (0 of 28 comparisons surviving correction). The salivary EV-derived miRNA arm therefore provides no robust evidence of *BDNF*-related dysregulation in this cohort.

Peripheral cytokines expression

Among eight cytokines, only interferon- γ differed (IFN- γ) after FDR correction, higher in patients (median 2.02 versus 1.07; $q = 0.003$); interleukin-1 β (IL-1 β) also showed a nominal increase in patients ($p = 0.028$, $q = 0.11$), consistent with meta-analytic evidence identifying IL-1 β among the inflammatory mediators elevated across mood episodes in BD [43]. The remaining six cytokines did not differ (Figure 5, Table S3). The inflammatory signal thus appears selective rather than generalised.

BDNF is positively coupled to cytokines expression

Within patients, *BDNF* expression correlated positively with most cytokines: *IL-2* ($r = +0.82$), *IL-19* (+0.75), *IL-4* (+0.74), *TNF- α* (+0.46), *IL-6* (+0.38) and *IFN- γ* (+0.23), while *IL-1 β* and *IL-8* did not (Figure 6, Table S4). Being computed within patients, these correlations are not a case–control artefact, and they persisted after adjustment for centre (IL-2 $\beta = +0.58$, $p = 2 \times 10^{-23}$; IL-19 $\beta = +0.59$, $p = 2 \times 10^{-18}$; IL-4 $\beta = +0.75$, $p = 4 \times 10^{-16}$). A composite inflammatory index confirmed the coupling (*BDNF* vs composite $\rho = +0.61$, $q < 0.001$; Supplementary). Neurotrophic and immune transcripts thus co-vary within a shared systemic state, consistent with observations in early psychiatric phases showing an interplay between blood *BDNF* mRNA levels and inflammatory markers [38].

Childhood adversity is associated with rs6265 CpG methylation

Within patients, higher childhood adversity (CTQ total) accompanied lower methylation at the rs6265 CpG ($\rho = -0.46$, $q = 0.001$, $n = 70$; Figure 7A), the only one of the thirteen sites surviving FDR. It was carried by the emotional neglect ($\rho = -0.44$), emotional abuse (-0.33) and physical neglect (-0.33) subscales, not by physical or sexual abuse (Figure 7B). The association persisted in a subgroup with homogeneous methylation levels, where allele dosage is constant by construction ($\rho = -0.41$, $p = 0.001$, $n = 62$; Figure S2b), confirming that the finding does not arise from allele dosage at this polymorphic site. As this was the only methylation measure not differing between patients and controls, the finding is independent of the case–control comparison.

Molecular markers do not track clinical stage

Because illness staging is central to the study, every molecular marker was examined across the ordinal stage. The staging itself was clinically coherent, correlating with the number of lifetime episodes ($\rho = +0.31$, $p < 0.001$), hospitalisations ($\rho = +0.27$, $p = 0.003$), global functioning (GAF $\rho = -0.32$, $p < 0.001$) and depressive severity (Figure 8A). Against this valid clinical gradient, no molecular marker showed a monotone stage trend surviving correction: *BDNF* expression was flat ($\rho = -0.02$; Figure 8B), promoter and rs6265 methylation were flat, and the inflammatory composite showed no trend ($\rho = +0.14$, $p = 0.12$; Figure 8C). Three cytokines (*IL-6*, *IL-19*, *IL-4*) reached a nominal Kruskal–Wallis difference driven by a non-monotone peak at stage 3 – the largest stage group – but none showed an ordinal trend or survived FDR (Table S-stage). Thus, within this cohort, *BDNF*-related transcriptional, epigenetic and inflammatory markers do not behave as biomarkers of clinical stage, even though the staging captures real differences in illness burden.

***BDNF* expression is inversely associated with global functioning**

Within patients, peripheral *BDNF* mRNA correlated negatively with global functioning (GAF: $\rho = -0.22$, $p = 0.016$, $n = 116$; Figure 9), indicating that patients with lower *BDNF* expression had poorer psychosocial functioning. This association was independent of clinical stage and was not driven by any single stage stratum. *BDNF* did not correlate significantly with depressive severity (MADRS $\rho = +0.02$, $p = 0.86$) or number of hospitalisations ($\rho = -0.06$, $p = 0.52$), suggesting functional impairment as a more sensitive correlate of *BDNF* expression than episode burden at the time of sampling.

Stratified and exploratory correlation analyses

Two coherent nominal clusters are noted as hypothesis-generating. First, antidepressant-treated patients showed higher *BDNF* expression (median 0.52 versus 0.18; $p = 0.002$), higher TNF- α , IL-2, IL-4 and IL-19, but lower IL-1 β ($p = 0.011$) and IL-8 ($p = 0.049$) – a profile consistent with known antidepressant immunomodulatory effects; none of these comparisons survived FDR correction across the 28-test pharmacotherapy family (Table S0). Notably, the *BDNF* upregulation associated with antidepressant use was specific to BD-II patients (AD+ versus AD–: median 0.68 versus 0.21; $p = 0.009$, $n = 41$) and was absent in BD-I patients ($p = 0.63$); a Kruskal–Wallis test across the four subtype $\times$ treatment groups confirmed overall heterogeneity ($p = 0.026$; Figure 10). This finding is exploratory given small subgroup sizes (BD-II AD–: $n = 14$) and the absence of correction for multiple comparisons.

DISCUSSION

In this multimodal analysis of the BOARDING-PASS cohort we examined the transcriptional and epigenetic regulation of *BDNF* in BD, combining peripheral *BDNF* gene expression, promoter and rs6265-CpG methylation, four *BDNF*-targeting salivary EV-associated miRNAs and peripheral inflammatory markers, and relating each to clinical stage and to early-life adversity. The findings converge on a multilayer rather than a single-mechanism view of *BDNF* dysregulation: a robust reduction of peripheral

expression coexists with unchanged promoter methylation, a selective inflammatory signal coupled to expression, and an adversity-related epigenetic mark, none of which follows a simple staging trajectory.

The clearest result is a substantial reduction of peripheral *BDNF* expression in patients, consistent with meta-analytic evidence of decreased peripheral *BDNF* in BD [8] and with the long-standing model of impaired neuroplasticity. Two features strengthen this observation: the deficit was already present at the earliest clinical stage and did not deepen across stages, and it held within a single recruitment centre, arguing against a technical or population confound. Its stage-independence favours interpretation of peripheral *BDNF* as a trait- or vulnerability-related correlate rather than a monotonic marker of progression, in line with work reporting no clear stage- or duration-related shift in *BDNF* and no detectable Val66Met effect [16,39].

No *BDNF* promoter methylation difference was detected at any of the thirteen CpGs, and the analysis was powered to exclude moderate-to-large effects. This dissociation — reduced expression without promoter hypermethylation — is informative rather than merely negative: the transcriptional deficit is not explained by methylation of these regulatory regions. Postmortem data show that *BDNF* mRNA is not uniformly reduced across brain regions and that specific transcripts and TrkB expression are altered, supporting transcript- and region-specific regulation rather than a global transcriptional shutdown [40], while systematic reviews argue that *BDNF* biology is shaped by polygenic and network-level context more than by any single peripheral readout [17,41]. The most plausible mechanisms are therefore additional promoters, chromatin-level regulation, trans-acting factors or post-transcriptional control, including miRNA-mediated regulation, rather than methylation of the assayed promoters, consistent with the complexity of *BDNF* epigenetic regulation reported in BD [20,22].

This result can be read against our own earlier work, which bears directly on it. In a previous peripheral-blood study we found selective hypermethylation of the *BDNF* promoter in BD that was specific to BD II — reduced *BDNF* expression and promoter hypermethylation being present in BD II but not BD I patients — and we further showed that treatment with mood stabilisers, in particular lithium and valproate, was associated with significantly lower *BDNF* promoter methylation [42]. Two features of the present cohort align with, rather than contradict, that observation. First, the cohort is heavily medicated: 75% of patients were receiving lithium and/or an antiepileptic at sampling, precisely the agents previously shown to reduce *BDNF* promoter methylation, so a treatment-related normalisation toward control levels would be expected and would attenuate any case–control difference. Second, the earlier epigenetic effect was carried specifically by bipolar II patients, whereas the present cohort is predominantly bipolar I (60 versus 43) and was analysed without subtype stratification, so a subtype-restricted effect would be diluted within the combined sample. These considerations do not resurrect a positive methylation finding, no promoter difference was detectable here regardless of subtype stratification, but they indicate that the present null is compatible with a more circumscribed epigenetic effect, confined to bipolar II and modulated by pharmacotherapy, rather than evidence against a role for *BDNF* promoter methylation in bipolar disorder. Subtype-stratified, medication-aware replication in an independent and adequately powered sample is therefore warranted before this question can be settled. The BD-II–specific

antidepressant effect on *BDNF* identified here — a 3.3-fold higher median expression in antidepressant-treated versus untreated BD-II patients ($p = 0.009$) compared with no detectable effect in BD-I — constitutes a coherent exploratory finding. If replicated, it would provide a biological correlate for the well-documented clinical distinction between the two subtypes and for the observation that antidepressant effects on neuroplasticity markers may be subtype-conditional. The mechanism is uncertain: antidepressants may upregulate *BDNF* through serotonin-mediated TrkB signalling, reduced IL-1 β -driven suppression of *BDNF* transcription, or epigenetic demethylation of *BDNF* promoter regions — the last of which is particularly consistent with the BD-II-specific promoter hypermethylation previously reported by our group [42]. The accompanying lower IL-1 β and IL-8 in antidepressant-treated patients, together with higher IL-2, IL-4, IL-19 and TNF- α , suggests a shift in the inflammatory milieu that may partly underlie the *BDNF* upregulation, consistent with the positive within-patient *BDNF*–cytokine coupling already described. The inverse association between *BDNF* expression and global functioning ($\rho = -0.23$, $p = 0.012$) is also worth noting: it extends the trait-like interpretation of the expression deficit, connecting lower peripheral *BDNF* to worse real-world functioning independently of episode burden and clinical stage. This is consistent with observations linking higher *BDNF* to better cognitive functioning in BD [14] and with neuroplasticity models in which *BDNF* supports the synaptic substrates of daily functioning.

Among the four pre-specified *BDNF*-targeting miRNAs, none differed between patients and controls after correction, and none correlated robustly with *BDNF* expression (Figure 4). This is consistent with the underlying biology: *BDNF* is modulated by a large, context-dependent set of miRNAs, in silico prediction substantially overestimates biologically effective interactions, and salivary EV-associated miRNAs and PBMCs-derived *BDNF* mRNA occupy different peripheral compartments, so a direct repressive relationship was not expected a priori [35]. miR-16-5p merits specific comment. It was nominally elevated in patients (median 1.89 versus 0.31 in controls; $p = 0.038$), a direction consistent with prior reports in mood disorders where miR-16-5p is among the experimentally validated post-transcriptional repressors of *BDNF* in human prefrontal cortex [34] and additionally targets the serotonin transporter, mediating adaptive responses to antidepressants [36]. This convergence of roles—*BDNF* suppression, serotonergic modulation and antidepressant pharmacodynamics—makes miR-16-5p a biologically coherent candidate in BD, particularly given the antidepressant-associated *BDNF* upregulation nominally observed in this cohort. In the present data, however, the nominal elevation did not survive FDR correction and was sensitive to the choice of reference gene, reflecting a systematic technical offset between the two sample sets rather than a confirmed biological difference (Table S-refgenes). The salivary EV compartment also differs from the PBMC compartment in which *BDNF* mRNA was measured, limiting direct mechanistic inference. The miRNA arm is therefore best read as an informative negative, while preserving miR-16-5p as the highest-priority candidate for targeted, matched-control replication—ideally with paired salivary EV and PBMC sampling to test cross-compartment regulation.

The inflammatory profile was selective rather than generalised, *IFN- γ* being the only cytokine elevated after correction (Figure 5). This is compatible with the patterned, low-grade immune activation described across the mood spectrum in BD [43] and with network analyses placing IL-1 β , IL-6 and TNF among

shared inflammatory hub genes across mood disorders under NF- κ B-related transcriptional control [44–45]. A selective rather than diffuse elevation points to a specific immune-transcriptional perturbation rather than global inflammation.

More striking was the positive within-patient coupling between *BDNF* expression and most cytokines, robust to adjustment for recruitment centre and confirmed by a composite inflammatory index. This runs counter to a simple model in which inflammation suppresses neurotrophin expression and is more consistent with co-regulation within a shared systemic transcriptional state, in which neurotrophic and immune programmes rise and fall together. Because both were measured in the same peripheral compartment, the coupling may reflect common cellular sources or shared upstream regulation, and its mechanistic basis warrants dedicated study.

Systematic stratification of every marker by sex, age, clinical subtype, age at onset and current treatment, together with correlations against clinical severity and chronicity measures, produced no association surviving correction beyond those already described. This uniform absence of subgroup effects argues against sex-, age- or treatment-specific *BDNF*-related signatures in this cohort and reinforces the trait-like reading of the expression deficit.

The most robust epigenetic signal was the association between higher childhood adversity and lower methylation at the rs6265 CpG – the only methylation measure surviving correction across sites and the only one not differing between patients and controls, so it is independent of the case–control contrast. It was carried specifically by emotional neglect and abuse, a pattern consistent with chronic rather than discrete adversity, and it persisted in the subgroup homogeneous for methylation level, indicating that it does not arise from allele dosage at this polymorphic CpG site. This links a well-characterised environmental risk factor to a specific epigenetic mark at a locus of established relevance to *BDNF* biology; *BDNF* and *BDNF-AS* variants interact with environmental exposures and treatment response rather than acting in isolation [41,46]. Genotype-resolved follow-up nonetheless remains essential before stronger mechanistic conclusions can be drawn.

Finally, the multicentre design is central to interpreting these results, and in particular the negative ones. Recruitment across settings tested the generalisability of *BDNF*-related signals rather than confining them to a single sample, and the staging framework was internally valid, tracking episodes, hospitalisations and functioning; yet no molecular or inflammatory marker followed the staging axis. Moreover, several case–control differences that appeared when samples were pooled were not consistent once recruitment centre was considered – a pattern that would be invisible in a single-centre design. The negative findings are thus not a mere failure to replicate but evidence that some previously reported peripheral *BDNF*-related signals do not generalise across contexts, and that between-centre variability must be modelled rather than assumed away. Used in this way, the multicentre design is an analytic strength that justifies the cautious, replication-oriented reading of the field adopted here [7–8].

Several limitations should be acknowledged. The cross-sectional design precludes causal or truly longitudinal inference about staging. As in most peripheral-biomarker studies, replication in an

independent, adequately powered cohort is required to confirm the case–control comparisons, particularly for the methylation and miRNA arms. Peripheral tissue may not mirror central regulation, and although multiplicity was controlled within families of tests, the findings warrant confirmation across settings. rs6265 was analysed at the level of methylation without independent genotyping, so its value conflates methylation and allele dosage.

Conclusion

In bipolar disorder, this cohort shows a substantial, trait-like reduction in peripheral *BDNF* expression — present from the earliest clinical stage and not accompanied by promoter methylation changes — together with a selective inflammatory signal to which *BDNF* expression is positively coupled, and an epigenetic mark at the rs6265 CpG site linked to childhood adversity. None of these signals scaled with clinical stage, and the epigenetic and miRNA comparisons that appeared positive when samples were pooled did not generalise across recruitment centres. Two conclusions follow. Substantively, *BDNF*-related dysregulation in bipolar disorder is better described as a multilayer, largely trait-related state than as a linear correlate of illness progression, with the most robust findings being the reduced expression, its positive coupling to peripheral inflammation, and the adversity-related epigenetic mark at rs6265. Methodologically, multi-context analysis is not a complication but a necessity: it exposed technical and cohort effects that a single-centre study would have mistaken for biology, and it underscores that peripheral biomarkers in bipolar disorder must be validated across settings before they can inform staging or clinical decisions. Rather than any single molecule, a composite and rigorously replicated biomarker strategy offers the more realistic path forward.

List of abbreviations

AD: Antidepressant

ANOVA: Analysis of Variance

AP2: Second-Generation (Atypical) Antipsychotics

ASST: Azienda Socio Sanitaria Territoriale

β-ACT: Beta-actin

BD: Bipolar Disorder

BD-I / BD-II: Bipolar Disorder Type I / Type II

BD-0: Increased risk, having a first-degree relative with BD, without psychiatric symptoms

BDNF: Brain-Derived Neurotrophic Factor

BDNF-AS: BDNF Antisense RNA

BOARDING-PASS: Bipolar Disorder Integrative Staging: Incorporating Biomarkers into Progression Across Stages

cDNA: Complementary DNA

CI: Confidence Interval

CpG: Cytosine-phosphate-Guanine dinucleotide

CTQ: Childhood Trauma Questionnaire

DSM-5: Diagnostic and Statistical Manual of Mental Disorders, 5th Edition

EVs: Extracellular Vesicles

FDR: False Discovery Rate

GAF: Global Assessment of Functioning

GAPDH: Glyceraldehyde-3-Phosphate Dehydrogenase

HAM-A: Hamilton Anxiety Rating Scale

HAM-D: Hamilton Depression Rating Scale

IFN- γ : Interferon-gamma

IL (IL-1 β , IL-2, IL-4, IL-6, IL-8, IL-19): Interleukin

LAI: Long-Acting Injectable Antipsychotics

MADRS: Montgomery–Åsberg Depression Rating Scale

miRNA / miR: MicroRNA

MISEV: Minimal Information for Studies of Extracellular Vesicles

mRNA: Messenger RNA

NF- κ B: Nuclear Factor Kappa-light-chain-enhancer of Activated B cells

PBMCs: Peripheral Blood Mononuclear Cells

PCR: Polymerase Chain Reaction

qRT-PCR / qPCR: Quantitative Real-Time Polymerase Chain Reaction

RNA: Ribonucleic Acid

SD: Standard Deviation

SNORD48: Small Nucleolar RNA 48

SNP: Single Nucleotide Polymorphism

TNF- α : Tumor Necrosis Factor-alpha

TrkB: Tropomyosin Receptor Kinase B

U6 snRNA: U6 Small Nuclear RNA

YMRS: Young Mania Rating Scale

Declarations

Ethics approval and consent to participate

The Ethics Committee of Milan, Area 1, reviewed and approved the study protocol (approval number 0008265/2023, dated 24 February 2023).

Consent for publication

All participants gave written informed consent

Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare no competing interests

Funding

The author(s) declare financial support was received for the research and/or publication of this article. Funded by the European Union -Next Generation EU -NRRP M6C2 -Investment 2.1 Enhancement and strengthening of biomedical research in the NHS (PNRR-MAD-2022-12376693).

Author's contributions

Beatrice Feliciani: Formal analysis, Investigation, Methodology, Writing –review & editing.

Nicolaja Girone: Data curation, Investigation, Writing – review & editing.

Clara Cavallotto: Data curation, Investigation, Writing – review & editing.

Salvatore Saluzzi: Data curation, Investigation, Formal analysis.

Francesca Martella: Data curation, Investigation.

Martina Di Bartolomeo: Writing – review & editing

Emi Bondi: Resources, Supervision, Writing – review & editing.

Mauro Pettorruso: Resources, Supervision, Writing – review & editing.

Alberto Granzotto: Methodology, Investigation, Writing – review & editing.

Camilla Gesi: Data curation, Investigation.

Laura Cremaschi: Data curation, Investigation.

Monica Macellaro: Data curation, Investigation.

Annabella Di Giorgio: Data curation, Formal analysis, Writing – review & editing

Giovanni Martinotti: Funding acquisition, Resources, Supervision.

Bernardo Maria Dell'Osso: Funding acquisition, Resources, Project administration, Supervision.

Claudio D'Addario: Conceptualization, Funding acquisition, Methodology, Supervision, Writing – original draft, Writing – review & editing.

Acknowledgements

NA

References

1. Vieta E, Berk M, Schulze TG, Carvalho AF, Suppes T, Calabrese JR, et al. Bipolar disorders. *Nat Rev Dis Primers*. 2018;4:18008.
2. McIntyre RS, Berk M, Brietzke E, Goldstein B, López-Jaramillo C, Kessing LV, et al. Bipolar disorders. *Lancet*. 2020;396(10265):1841-1856.
3. Fries GR, Li Q, McAlpin B, Rein T, Walss-Bass C, Soares JC, et al. The role of DNA methylation in the pathophysiology and treatment of bipolar disorder. *Neurosci Biobehav Rev*. 2016;68:474-488.
4. Legrand A, Iftimovici A, Khayachi A, Chaumette B. Epigenetics in bipolar disorder: a critical review of the literature. *Psychiatr Genet*. 2020;31(2):67-76.
5. Bernad B, Tomescu M, Anghel T, Lungeanu D, Enătescu V, Bernad E, et al. Epigenetic and Coping Mechanisms of Stress in Affective Disorders: A Scoping Review. *Medicina (Kaunas)*. 2024;60(5):759.

6. Argue B, Casten LG, McCool S, Alrfooh A, Richards J, Wemmie JA, et al. Immune Dysregulation in Bipolar Disorder. *Journal of Affective Disorders*. 2025; 368:120-128.
7. Grewal SK, McKinlay S, Kapczinski F, Pfaffenseller B, Wollenhaupt-Aguiar B. Biomarkers of neuroprogression and late staging in bipolar disorder: A systematic review. *Aust N Z J Psychiatry*. 2022;56(12):1507-1527.
8. Fernandes BS, Molendijk ML, Köhler CA, Soares JC, Leite CM, Machado-Vieira R, et al. Peripheral brain-derived neurotrophic factor (BDNF) as a biomarker in bipolar disorder: a meta-analysis of 52 studies. *BMC Med*. 2015;13:289.
9. Durdurak B, Morales-Muñoz I, de Cates AD, Wiseman C, Broome MR, Marwaha S. Underlying biological mechanisms of emotion dysregulation in bipolar disorder. *Frontiers in Psychiatry*. 2025; 16:1512345.
10. De Felice G, Luciano M, Boiano A, Colangelo G, Catapano P, Della Rocca B, et al. Can Brain-Derived Neurotrophic Factor Be Considered a Biomarker for Bipolar Disorder? An Analysis of the Current Evidence. *Brain Sci*. 2023;13(8):1188.
11. Lin CC, Huang TL. Brain-derived neurotrophic factor and mental disorders. *Biomed J*. 2020;43(2):134-142.
12. Fernandes BS, Gama CS, Ceresér KM, Yatham LN, Fries GR, Colpo G, et al. Brain-derived neurotrophic factor as a state-marker of mood episodes in bipolar disorders: a systematic review and meta-regression analysis. *J Psychiatr Res*. 2011;45(8):995-1004.
13. Yang Z, Zhang S, Ouyang L, Liao A, He Y, Li Z, et al. DNA Methylation and Bipolar Disorder. *J Psychiatry Brain Sci*. 2023;8:e230012.
14. Mora E, Portella MJ, Piñol-Ripoll G, López R, Cuadras D, Forcada I, et al. High BDNF serum levels are associated to good cognitive functioning in bipolar disorder. *European Psychiatry*. 2019; 58:75-81.
15. Munkholm K, Vinberg M, Kessing LV. Peripheral blood brain-derived neurotrophic factor in bipolar disorder: a comprehensive systematic review and meta-analysis. *Mol Psychiatry*. 2016;21(2):216-228.
16. Petersen NA, Nielsen M, Coello K, Stanislaus S, Melbye S, Kjærstad HL, et al. Brain-derived neurotrophic factor levels in newly diagnosed patients with bipolar disorder, their unaffected first-degree relatives and healthy controls. *BJPsych Open*. 2021;7(2):e54.
17. Liberona A, Jones N, Zúñiga K, Garrido V, Zelada MI, Silva H, et al. Brain-Derived Neurotrophic Factor (BDNF) as a Predictor of Treatment Response in Schizophrenia and Bipolar Disorder: A Systematic Review. *Int J Mol Sci*. 2024;25(19):10491.
18. Szarowicz C, Steece-Collier K, Caulfield ME. New frontiers in neurodegeneration and regeneration associated with brain-derived neurotrophic factor and the rs6265 single nucleotide polymorphism. *International Journal of Molecular Sciences*. 2022
19. Carlberg L, Scheibelreiter J, Hassler M, Schloegelhofer M, Schmoeger M, Ludwig B, et al. Brain-derived neurotrophic factor (BDNF)-epigenetic regulation in unipolar and bipolar affective disorder. *Journal of Affective Disorders*. 2014

20. D'Addario C, Palazzo MC, Benatti B, Grancini B, Pucci M, Di Francesco A, Camuri G, Galimberti D, Fenoglio C, Scarpini E, Altamura AC, Maccarrone M, Dell'Osso B. Regulation of gene transcription in bipolar disorders: Role of DNA methylation in the relationship between prodynorphin and brain derived neurotrophic factor. *Prog Neuropsychopharmacol Biol Psychiatry*. 2018 Mar 2;82:314-321.
21. Dell'Osso B, D'Addario C, Palazzo MC, Benatti B, Camuri G, Galimberti D, et al. Epigenetic modulation of BDNF gene: differences in DNA methylation between unipolar and bipolar patients. *Journal of Affective Disorders*. 2014
22. Maffioletti E, Cattaneo A, Rosso G, Maina G, Maj C, Gennarelli M, et al. Peripheral whole blood microRNA alterations in major depression and bipolar disorder. *Journal of Affective Disorders*. 2016
23. Bame M, McInnis MG, O'Shea KS. MicroRNA Alterations in Induced Pluripotent Stem Cell-Derived Neurons from Bipolar Disorder Patients: Pathways Involved in Neuronal Differentiation, Axon Guidance, and Plasticity. *Stem Cells Dev*. 2020 Sep 1;29(17):1145-1159.
24. Ceylan D, Karaçiçek B, Tufekci K, Aksahin IC, Şenol SH, Genç Ş. Mitochondrial DNA oxidation, methylation, and copy number alterations in major and bipolar depression. *Front Psychiatry*. 2023;14:1300928.
25. Marostica G, Gelibter S, Gironi M, Nigro A, Furlan R. Extracellular Vesicles in Neuroinflammation. *Front Cell Dev Biol*. 2021;8:623039.
26. Kumar RMR, Rajan L, Chidambaram S. Exosomes as Emerging Therapeutic Platforms in Neurological and Psychiatric Disorders. *Pharm Med*. 2025.
27. Amnieh SB, Yazdi M, Abrishami M, Keshvari NZ, Saleki K, Rezaei N. Molecular Reflection of the Mind: Exosomes in Major Depressive Disorder. *Brain Res Bull*. 2025.
28. Antoniou A, Auderset L, Kaurani L, Sebastian E, Zeng Y, Allahham M, Cases-Cunillera S, Schoch S, Gründemann J, Fischer A, Schneider A. Neuronal extracellular vesicles and associated microRNAs induce circuit connectivity downstream BDNF. *Cell Rep*. 2023 Feb 28;42(2):112063.
29. Patoz MC, Godin O, Moisset X, Chabert J, Bailara KM, Étain B, et al. Characterization of the inflammatory/immune-neuroendocrine-BDNF interplay during affective episodes and euthymia in bipolar disorder patients: in the search of a peripheral reliable and highly predictive biosignature. *European Psychiatry*. 2023
30. Girone N, Cremaschi L, Macellaro M, Gesi C, Martella F, Saluzzi S, et al. Bipolar disorder integrative staging: incorporating biomarkers into progression across stages (BOARDING-PASS) – rationale and design. *Front Psychiatry*. 2025;16:1646533.
31. Kupka RW, Hillegers MH. Stagering en profilering bij bipolaire stoornissen [Staging and profiling in bipolar disorders]. *Tijdschr Psychiatr*. 2012;54(11):949-56.
32. Bernstein DP, Fink L. Childhood Trauma Questionnaire: A retrospective self-report manual. San Antonio, TX: The Psychological Corporation; 1998.
33. Lyon E. Mutation detection using fluorescent hybridization probes and melting curve analysis. *Expert Rev Mol Diagn*. 2001;1(1):92-101.

34. Mellios N, Huang HS, Grigorenko A, Rogaev E, Akbarian S. A set of differentially expressed miRNAs, including miR-30a-5p, act as post-transcriptional inhibitors of BDNF in prefrontal cortex. *Human Molecular Genetics*. 2008
35. de Assis GG, Murawska-Ciałowicz E. BDNF modulation by microRNAs: an update on the experimental evidence. *Cells*. 2024
36. Baudry A, Mouillet-Richard S, Schneider B, Launay JM, Kellermann O. miR-16 targets the serotonin transporter: a new facet for adaptive responses to antidepressants. *Science*. 2010;329(5998):1537-1541.
37. Girella A, Vismara M, O’Riordan KJ, Gunnigle E, Mercante F, Girone N, Pucci M, Gatta V, Konstantinidou F, Stuppia L, Cryan JF, Dell’Osso B, D’Addario C. New insights into the oral microbiota and host epigenetic changes in obsessive compulsive disorder and major depressive disorder: focus on BDNF. *Pharmacol Res*. 2025;219:107891.
38. Moreno I, Stojanovic-Pérez A, Bulduk B, Sanchez-Gistau V, Algora M, Ortega L, et al. High blood levels of brain-derived neurotrophic factor (BDNF) mRNA in early psychosis are associated with inflammatory markers. *Journal of Psychiatric Research*. 2023
39. Schröter, K., Brum, M., Brunkhorst-Kanaan, N. et al. Longitudinal multi-level biomarker analysis of BDNF in major depression and bipolar disorder. *Eur Arch Psychiatry Clin Neurosci*. 2020;270(2):169-181.
40. Reinhart V, Bove SE, Volfson D, Lewis DA, Kleiman RJ, Lanz TA. Evaluation of TrkB and BDNF transcripts in prefrontal cortex, hippocampus, and striatum from subjects with schizophrenia, bipolar disorder, and major depressive disorder. *Neurobiol Dis*. 2015 May;77:220-7.
41. Shkundin A, Halaris A. Associations of BDNF/BDNF-AS SNPs with Depression, Schizophrenia, and Bipolar Disorder. *Journal of Personalized Medicine*. 2023; 13(9):1395.
42. D’Addario C, Dell’Osso B, Palazzo MC, Benatti B, Lietti L, Cattaneo E, et al. Selective DNA methylation of BDNF promoter in bipolar disorder: differences among patients with BDI and BDII. *Neuropsychopharmacology*. 2012
43. Solmi M, Sharma E, Osimo EF, Fornaro M, Bortolato B, Croatto G, et al. Peripheral levels of C-reactive protein, tumor necrosis factor- α , interleukin-6, and interleukin-1 β across the mood spectrum in bipolar disorder: a meta-analysis of mean differences and variability. *Brain Behavior and Immunity*. 2021
44. Chauhan A, Jain CK. Exploring gene regulatory interaction networks and predicting therapeutic molecules among major depressive disorder and bipolar disorder: A bioinformatics approach. *J Affect Disord*. 2025;375:64-74.
45. Teshnizi SA, Shahani P, Taheri M, Hussen BM, Eslami S, Sadeghzadeh Z, Ghafouri-Fard S, Sayad A. Expression analysis of NF- κ B-related long non-coding RNAs in bipolar disorder. *Sci Rep*. 2022;12(1):20941.
46. Shkundin A, Wheeler HE, Sinacore J, Halaris A. BDNF/BDNF-AS gene polymorphisms modulate treatment response and remission in bipolar disorder: a randomized clinical trial. *Journal of*

47. Welsh JA, Goberdhan DC, O'Driscoll L, Buzas EI, Blenkiron C, Bussolati B, et al. Minimal information for studies of extracellular vesicles (MISEV2023): from basic to advanced approaches. J Extracell Vesicles. 2024;13(2):e12416.