High blood levels of brain-derived neurotrophic factor (*BDNF*) mRNA in early psychosis are associated with inflammatory markers

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Abstract

The brain-derived neurotrophic factor (*BDNF*) single nucleotide polymorphism (SNP) rs6265C>T, Val66Met, affects BDNF secretion and has been related to inflammatory processes. Both the rs6265 and BDNF protein levels have been widely investigated in neuropsychiatric disorders with conflicting results. In the present study we examined *BDNF* mRNA expression in blood considering the SNP rs6265 and its relationship with inflammatory markers in the early stages of psychosis. The rs6265 genotype and blood *BDNF* mRNA levels were measured in 34 at-risk mental states (ARMS) individuals, 37 patients with first-episode psychosis (FEP) and 42 healthy controls (HCs) by quantitative PCR and reverse transcription (RT)-qPCR using validated TaqMan assays. We also obtained measures of interleukin-6 (*IL6*) mRNA levels, fibrinogen, neutrophil-to-lymphocyte ratio (NLR) and high-sensitivity C-reactive protein. We identified that *BDNF* mRNA levels were associated with the rs6265 genotype in an allele-dose-dependent manner, with low expression levels associated with the T allele (Met substitution). Thus, we controlled for the rs6265 genotype in all analyses. Blood *BDNF* mRNA levels differed between diagnostic groups: patients with FEP exhibited higher blood *BDNF* mRNA levels than ARMS individuals, and the lowest levels were observed in HC. In addition, we observed significant correlations between *BDNF* mRNA levels and inflammatory markers (*IL6* mRNA levels and NLR), controlled by the rs6265 genotype, in ARMS and FEP groups. This exploratory study suggests that the rs6265 genotype is associated with differential blood mRNA expression of *BDNF* that increases with illness progression and correlated with inflammation in the early stages of psychosis.

Key words: rs6265; mRNA expression; first-episode psychosis; at-risk mental states; inflammation; IL6

1. Introduction

Brain-derived neurotrophic factor (BDNF) is a member of the nerve growth factor family of proteins that is involved in several neuronal processes and exerts its function by binding to tropomyosin receptor kinase B (TRKB). During neurodevelopment, BDNF promotes neuronal survival and differentiation, and in the adult brain, BDNF is involved in neuroplasticity, regulation of both excitatory and inhibitory synaptic transmission and pain modulation (Pezet and McMahon, 2006). Although the highest expression is observed in the brain, BDNF is ubiquitously expressed in other nonneuronal organs and tissues (i.e., arteries, breast, colon, esophagus, heart, kidney, lung, ovary, and prostate), according to the Genotype-Tissue Expression (GTEx) Project (https://gtexportal.org/home/gene/BDNF). A wide range of endogenous and exogenous stimuli (e.g., diet, physical activity, stress, and brain injury) regulate *BDNF* gene expression in specific brain regions and peripheral tissues. Antisense RNAs and methylation mechanisms regulate *BDNF* gene expression, adding further complexity to the intricate pattern of *BDNF* gene expression (Pruunsild et al., 2007). However, among all the factors influencing *BDNF* expression, the functional single nucleotide polymorphism (SNP), rs6265 (C to T, Val66Met), located in the prodomain region of the *BDNF* gene, has been shown to disrupt the intracellular trafficking of *BDNF* mRNA and decrease neurotrophin secretion (Chen et al., 2005; Egan et al., 2003). rs6265 results in a dose-dependent decrease in the activity-dependent release of *BDNF* (Met/Met > Val/Met > Val/Val) by disrupting BDNF transport and packaging into secretory vesicles, whereas constitutive BDNF levels remain unaffected (Baj et al., 2013; Mallei et al., 2015; Wu et al., 2010). Remarkably, individuals with the CC genotype (Val) relative to those with the TT genotype (Met) have shown significantly higher plasma levels of *BDNF* mRNA and protein (Jin et al., 2015). The rs6265T allele has a prevalence of 19% in the global population, being the lowest in the African population (1%) and the highest in the Asian population (45%) (https://www.ncbi.nlm.nih.gov/snp/rs6265). Therefore, its impact has consequences for a significant part of the population.

BDNF has been studied extensively in psychiatric disorders (Miranda et al., 2019; Szarowicz et al., 2022). Specifically, two meta-analyses have confirmed that peripheral BDNF protein levels are decreased in patients with schizophrenia compared to control individuals (Fernandes et al., 2015; Green et al., 2011). One study produced similar results in patients with first-episode psychosis (FEP), reporting decreased peripheral BDNF levels in FEP compared to healthy controls (HCs), (Singh et al., 2022). Therefore, researchers aimed to investigate whether BDNF levels could be a hallmark associated with the early stages of psychotic disorders with conflicting results. Higher BDNF protein levels (Yee et al., 2018), lower levels

(Heitz et al., 2019; Sanada et al., 2018), and no differences (Counotte et al., 2019) have been reported between patients at risk of developing psychosis and control individuals. In FEP, just one study showed reduced *BDNF* gene expression in patients compared with healthy controls (Mondelli et al., 2011). Of note, none of these studies controlled BDNF levels according to the rs6265 genotype. Alternatively, the current hypothesis linking the immune system to psychotic disorders suggests that the chronic inflammatory state observed in psychotic disorders could be associated with abnormal BDNF levels (Lima Giacobbo et al., 2019).

The aim of the present study was to investigate *BDNF* blood mRNA levels in the early phases of psychosis considering the rs6265 genotype and to determine whether there was a relationship between *BDNF* and inflammatory markers.

2. Methods

2.1. Participants

This cross-sectional study included 37 patients with FEP and an illness duration of less than 2 years, 34 ARMS subjects and 42 HC individuals. Characteristics of the participants are shown in Table 1. Patients with FEP had a psychotic disorder according to the Diagnostic and Statistical Manual of Mental Disorders DSM-IV, which included the following diagnoses: schizophreniform disorder (N=6), schizoaffective disorder (N=2), schizophrenia (N=2), and psychotic disorder not otherwise specified (N=27). ARMS subjects were selected by the Risk Mental Status criteria (Yung and McGorry, 2007). Patients diagnosed with substance-induced psychosis, neurological disorders or intellectual disability were not included in the study. HC subjects were friends of the patients, nongenetic relatives, and university students with a score below 7 on the General Health Questionnaire (GHQ-28) (Artal & Pérez-Echeverría, 1986). They were screened by direct interview with an experienced psychiatrist to rule out a history of past or current psychiatric disorders. Exclusion criteria for all participants were non Caucasian ethnicity, pregnancy or puerperium, language difficulties, visual and hearing impairment, intellectual disability, severe head trauma or medical illness, treatment with corticosteroids or use of birth control pills in the previous 3 months, type 1 diabetes mellitus, and diagnosis of substance dependence (other than tobacco or cannabis) according to DSM-IV.

2.2. Clinical Assessments

All patients were assessed with the Schedules for the Clinical Assessment in Neuropsychiatry (SCAN) (Vázquez-Barquero et al., 1994) by a trained psychiatrist, and the

diagnoses of psychotic disorders were obtained with OPCRIT 4 for Windows. The ARMS group was also assessed with the Comprehensive Assessments of At-Risk Mental States (CAARMS), and all of them met the criteria for any of the three Ultra-High-Risk groups (Yung et al., 2005). Psychopathological symptoms were assessed with the Spanish adaptation of the Positive and Negative Syndrome Scale (PANNS) (Peralta and Cuesta, 1994) and depressive symptoms were assessed with the Hamilton Depression Rating Scale (HDRS) (Hamilton, 1960). A clinician administered a semistructured interview to obtain information on clinical history, substance use, and psychotropic medication use. Sociodemographic variables such as sex, age, ethnicity, and educational level were also obtained.

2.3. Biological Sampling

A fasting venous blood sample was obtained in the morning (8:00-10:00 AM) from all participants and transported to the biobank in less than one hour for rapid processing and storage. Total DNA was extracted from peripheral blood mononuclear cells using Gentra® PureGene reagent (QIAGEN, Barcelona, Spain) according to the manufacturer's instructions and quantified using a NanoDrop™ spectrophotometer (Thermo Fisher Scientific, Madrid, Spain). Total RNA was obtained from PAXgene blood collection tubes containing RNA stabilizers to prevent mRNA degradation. The tubes were kept for 2 hours at room temperature and then stored at -80°C until RNA was obtained using the PAXgene Blood RNA kit (QIAGEN, Barcelona, Spain) following the manufacturer's instructions. RNA purity and quantification were determined by spectrophotometry (NanoDrop™; Thermo Fisher Scientific, Madrid, Spain) and automated electrophoresis (Bioanalyzer; Agilent, Madrid, Spain) to obtain the RNA integrity number (RIN) ranging from 1 (lowest quality) to 10 (highest quality). The mean RIN values were 8.5, ranging from 8.2 to 9.3. Frozen aliquots of plasma and serum were stored at -80°C for less than one year before being sent to specific laboratories to measure biochemical and hematological parameters.

2.4. Genotyping

The rs6265 state was determined by quantitative real-time polymerase chain reaction (qPCR) using a TaqMan SNP genotyping assay, reference number C__11592758_10 (Thermo Fisher Scientific), on an ABI 7900HT Fast Real-Time PCR system (Thermo Fisher Scientific) according to the manufacturer's instructions.

2.5. Gene Expression Analysis

BDNF and interleukin-6 (*IL6*) mRNA expression were measured by reverse transcription qPCR (RT–qPCR) using the TaqMan RNA-to-Ct 1-Step kit with validated gene expression TaqMan assays on a 7900HT Fast Real-Time PCR system (Thermo Fisher Scientific). We used the relative quantification $2^{\Delta Cq}$ method (Expression Suite Software, Thermo Fisher Scientific) as described previously (Torrell et al., 2013), where ΔCq = Cq target gene – Cq reference gene. *BDNF* and *IL6* were the target genes. We previously identified glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), beta-actin (*ACTB*) and beta-2-microglobulin (*B2M*) as the most suitable genes for normalization in blood samples from a set of 32 reference candidate genes. Thus, we used the geometric mean of these three reference genes instead of one reference gene. Reference numbers of TaqMan assays for *BDNF*, *IL6*, *GAPDH*, *ACTB* and *B2M* were Hs02718934_s1, Hs00985639_m1, Hs0393929097_g1, Hs9999999903_m1 and Hs00187842_m1, respectively.

2.6. Biochemical Measurements

Biochemical and hematological parameters were measured spectrophotometrically. We calculated the neutrophil-to-lymphocyte ratio (NLR) as a biomarker of inflammation for psychiatric disorders (Bioque et al., 2022; Mazza et al., 2018). High-sensitivity C-reactive protein (hsCRP) and fibrinogen were measured as previously described (Stojanovic et al., 2014).

2.7. Statistical Methods

Data were analyzed in IBM SPSS Statistics 28.0 (IBM Corp., Armonk, NY, USA). Distribution of continuous variables was examined by the Kolmogorov-Smirnov test. *BDNF* and *IL6* mRNA levels, among other variables were asymmetric, so they were log-transformed (log_{10}). Chi-square tests and ANOVA were used to compare sex and age distributions among the three study groups, and *t* tests were used to compare PANSS and HDS scores and antipsychotic equivalent doses between the clinical groups (ARMS and FEP). In addition, *t* test was used to compare *BDNF* mRNA levels between patients with and without antipsychotic treatment. A general linear model was used to identify differences in gene expression between study groups adjusting for body mass index (BMI) and rs6265 genotype. Post hoc analyses were used to compare *BDNF* mRNA expression between HC and ARMS, HC and FEP and between ARMS and FEP. Partial correlations considering the rs6265 genotype as a covariate were used to explore the relationship between *BDNF* mRNA levels and inflammatory markers, clinical psychopathological symptoms, and the equivalent dose of chlorpromazine. The results of

partial correlations between *BDNF* mRNA levels and inflammatory markers were visualized using the *corrplot* package in R (https://www.r-project.org) (R Core Team, 2018). We conducted post-hoc power analysis for partial correlations between *BDNF* mRNA levels and inflammatory markers. In addition, power analyses for detecting differences between diagnostic groups were performed with the online tool developed by © 2019 HyLown Consulting LLC, Atlanta, GA (http://powerandsamplesize.com/Calculators/).

3. Results

BDNF mRNA levels did not differ between males (4110 ± 1282) and females (3769 ± 1257, t=1.326; p=0.188), nor between patients with (4259 ± 1114) and without (4147 ± 1345) antipsychotic treatment (t=-0.369, p=0.713). Furthermore, we observed no correlation between *BDNF* mRNA levels and age ($r_{partial}$=0.141, p=0.139), daily cigarette ($r_{partial}$=0.137, p=0.330), daily joint consumption referred to cannabis used ($r_{partial}$=-0.068, p=0.735), and the equivalent dose of chlorpromazine ($r_{partial}$=0.247, p=0.129). In contrast, we observed a positive correlation between *BDNF* and BMI ($r_{partial}$=0.261, p=0.007). Additionally, we observed that *BDNF* mRNA expression depends on the rs6265 genotype in a dose-dependent manner, even considering BMI as a covariate (F=6.839, p<0.001), as shown in Table 2. The highest mRNA levels were observed in CC genotype carriers (Val66 homozygotes), intermediate levels in TC carriers (Vall66Met heterozygotes) and the lowest levels in TT carriers (Met66 homozygotes) in the whole sample.

Controlling for the SNP rs6265, *BDNF* mRNA expression varied as a function of diagnostic group, as shown in Table 2 (F= 6.586, p<0.001). The highest expression levels were observed in patients with FEP (estimated marginal mean ± standard error (EMM ± SE) 4312 ± 203), intermediate levels were observed in ARMS subjects (4138 ± 211), and the lowest levels were observed in HC individuals (3734 ± 186). As shown in Table 2, *BDNF* mRNA levels also varied as a function of the rs6265 genotype in the HC and ARMS groups, where an increase in mRNA expression was observed from TT to CC genotype carriers (TT<CT<CC). However, this was not observed in the FEP group, where the highest mRNA levels were found in individuals carrying the CT genotype. Patients with FEP showed higher *BDNF* mRNA expression than HC and ARMS individuals. Statistical differences by study group were observed between HC and ARMS+FEP individuals (F=8.579; p<0.001) and between HC+ARMS and FEP patients (F=7.777; p<0.001), considering BMI and rs6265 as covariates (Table 2). Considering a type I error rate of 5%, our study had a probability of avoiding a type II error of 86% when comparing HC and patients with

FEP, 39% when comparing HC and ARMS individuals and 34% when comparing ARMS individuals and patients with FEP.

BDNF mRNA levels correlated positively with *IL6* mRNA levels ($r_{partial}$=0.387, p<0.001) and NLR ratio ($r_{partial}$=0.341, p<0.001) but not with hsCRP ($r_{partial}$=0.133, p=0.182) or fibrinogen ($r_{partial}$=0.171, p=0.082) as shown in Figure 1. Post-hoc power analyses for partial correlations between *BDNF* mRNA levels and inflammatory markers were as follows: 99% for *IL6* mRNA levels, 96% for NLR, 29% for hsCRP, and 44% for fibrinogen. In the ARMS and FEP groups, significant correlations were observed between *BDNF* mRNA levels and *IL6* mRNA levels ($r_{partial}$ =0.387, and p=0.026; $r_{partial}$ =0.444, and p=0.008; respectively), and between *BDNF* mRNA levels and NLR ($r_{partial}$=0.378, p=0.043; $r_{partial}$=0.440, p=0.012; respectively). In the diagnostic groups, post-hoc analysis for partial correlations revealed a power of 66% and 60% for *IL6* mRNA levels and NLR, respectively, in the ARMS group, and 64% and 79%, respectively, in the FEP group.

Finally, we observed a positive correlation between *BDNF* mRNA levels and the PANSS negative subscale score ($r_{partial}$=0.395, p=0.001) and, similarly, between *IL6* mRNA levels and the PANSS negative subscale score (r=0.288; p=0.025).

4. Discussion

BDNF is indispensable during embryogenesis and postnatally for the normal development and function of both the central and peripheral nervous systems (Nguyen et al., 2023). Its role is decisive from the beginning of life until adulthood, and although mainly expressed in the nervous system, BDNF is present in several tissues and is known to be involved in a variety of other biological functions, including the regulation of glucose metabolism (Briana and Malamitsi-Puchner, 2018). Lower BDNF levels have been associated with neurodegenerative and psychiatric disorders, and BDNF-targeted therapies have recently been proposed for the rehabilitation of these disorders (Szarowicz et al., 2022). In this study, we first explored the expression levels of *BDNF* mRNA in blood in the early stages of psychosis considering the rs6265 variant. We found that the rs6265 genotype influences *BDNF* mRNA levels in a dose-dependent manner. Therefore, our results are in line with the conception that rs6265 is an expression quantitative trait locus (eQTL) influencing the amount of *BDNF* mRNA expression through the *BDNF-AS* gene, a long non-coding RNA encoded on the opposite strand to the BDNF gene (https://www.gtexportal.org/home/snp/rs6265). Consequently, it can be expected that lower BDNF secretion influenced by the rs6265 genotype is associated with the clinical progression and severity of the disease. If we consider the diagnosis group, both healthy

individuals and ARMS subjects follow the same pattern as described above consisting of lower *BDNF* mRNA in those carrying the rs6265TT genotype, although a significant effect of the genotype was observed only in the ARMS group. Conversely, in the individuals with FEP, we observed a different pattern. Although the lowest *BDNF* mRNA levels were found in those with the rs6265TT genotype, the levels among the 3 genotypes were very similar. Thus, it can be speculated that this polymorphism would not have the regulatory effect of BDNF production due to the establishment of the disease, as it has been previously suggested in schizophrenia (kumar et al., 2020). The mechanism by which rs6265 has lost its regulatory function in patients with FEP is unknown, although several factors could influence BDNF production, including a sedentary lifestyle (Chan et al., 2021; Walsh and Tschakovsky, 2018), smoking and obesity (Halldén et al., 2013) that could be mediated by epigenetic modifications associated with the rs6265 variant (Lin and Huang, 2020). Our study suggests that the effect of the rs6265T allele variant contributes to the reduced levels of *BDNF* mRNA levels in people at risk for psychosis, but this effect is lost in people with an established psychotic disorder.

Second, we explored the *BDNF* mRNA values in the study groups (HC, ARMS and FEP). Contrary to what was expected, the lowest values were found in the healthy population, intermediate values in ARMS subjects and the highest values were found in patients with FEP. Our results obtained from whole blood conflict with a previous study that reported lower levels of *BDNF* mRNA in leukocytes from patients with FEP compared to HC (Mondelli et al., 2011). However, inconsistent results have also been found in studies measuring protein levels, with higher, lower, and no differences in the BDNF protein levels reported between ARMS and FEP subjects compared to HC (Counotte et al., 2019; Heitz et al., 2019; Sanada et al., 2018; Yee et al., 2018). It is worth mentioning that most BDNF found in serum, although originally secreted by neuronal cells, is released from platelets after storage. Therefore, it has been suggested that serum BDNF levels may be a less accurate marker of acute changes in CNS than plasma BDNF (Fernandes et al., 2015). According to our results, it is possible that the high *BDNF* mRNA expression observed in blood in FEP is due to low BDNF protein levels in the brain, as the brain contributes to a large amount of circulating BDNF in blood and is considered its major source (Fernandes et al., 2015). It can be speculated that dysregulation in the BDNF production process, transport, or storage leads to the synthesis of more *BDNF* mRNA in blood.

Our study also measured inflammatory markers, including *IL6* mRNA levels, NLR, hsCRP and fibrinogen, as there is evidence for immune-related alterations in the early stages of psychosis (Bocchio-Chiavetto et al., 2018). We observed significant differences in the *IL6* mRNA levels between diagnostic groups, with the lowest levels in the HC group, intermediate levels in the

ARMS group and the highest levels in the FEP group, but no differences were observed in the other inflammatory markers. Remarkably, *BDNF* mRNA expression was positively correlated with *IL6 mRNA* and NLR in the ARMS and FEP groups. As psychotic episodes are associated with increased neuroinflammation, it can be hypothesized that proinflammatory cytokines like IL6 may be modulating *BDNF* mRNA expression (Lima Giacobbo et al., 2019). The higher the inflammation is, the higher the levels of *IL6* mRNA and NLR, which could signal the need to synthesize more BDNF to deal with the inflammatory process, as one of the main consequences of dysregulation of proinflammatory cytokine levels would be the impairment of neurogenesis and neuroplasticity through BDNF depletions (Calabrese et al., 2014). Thus, BDNF production mechanisms would be activated and *BDNF* mRNA levels increased in cases where there is more inflammation (ARMS and FEP) with the intention of normalizing or attenuating the inflammation-dependent decrease of BDNF. Both, neuroinflammation and altered BDNF expression are common phenomena in the early stages of psychosis and a better understanding of the relationship between BDNF and neuroinflammation could open new avenues for the therapeutic management.

Finally, at the clinical level, we observed that *BDNF* mRNA levels positively correlated with the PANSS negative subscale score, indicating that high mRNA *BDNF* levels were associated with the presence of negative symptomatology. No studies have assessed the relationship between mRNA *BDNF* levels and clinical symptomatology measured with the PANSS in FEP patients or ARMS individuals. Some studies have found a negative relationship between serum BDNF protein values and PANSS scores in FEP patients (Aydın et al., 2020) and in patients with chronic schizophrenia (Xu et al., 2021). Recently, low serum BDNF protein levels, decreased left amygdala brain volume and more severe negative symptomatology have been identified as predictors of negative symptomatology in antipsychotic naïve FEP patients (Toll et al., 2022). The positive association between *IL6* mRNA and PANSS negative subscale scores could indicate that IL6 may be involved in the clinical symptomatology of schizophrenia through immunological mechanisms, as some studies have also shown in FEP patients (Dunleavy et al., 2022; Hatziagelaki et al., 2019) and ARMS (Stojanovic et al., 2014). In our study, we did not observe any relationship between positive or depressive symptoms and *BDNF* mRNA levels, indicating that the dysregulated process would be related to the lessening or absence of normal behaviors and functions related to motivation and interest or emotional expression when compared to symptoms of other domains (positive symptoms, depressive symptoms).

4.1. Strengths and limitations

Some strengths of this study are the inclusion of three different groups of subjects (ARMS, FEP and HC), the genotyping of one of the most relevant genetic variants in the *BDNF* gene and the inclusion of immunological markers, since a proinflammatory phenotype has been related to psychotic disorders. In addition, our study reports results of blood levels of *BDNF* mRNA, of which there are very few studies, and not of protein levels, which are the most frequently reported. In contrast, the main limitations of this study include the small sample size and the absence of BDNF protein values in serum or plasma, so we could not assess the association between *BDNF* mRNA values in blood and protein values in serum or plasma. Additionally, the study had substantial statistical power to detect differences between HCs and FEP patients, whereas this was low in the comparison of both groups with ARMS individuals; however, the results of the ARMS subjects were between HCs and FEPs as expected. Finally, although we found no relationship between antipsychotic drug treatment and *BDNF* mRNA expression, we cannot rule out that some antipsychotic treatments may have an effect on *BDNF* expression, due to the diversity of drug treatments and the small size of our sample.

4.2. Conclusions

The present study identified that the rs6265 genotype is associated with differential *BDNF* mRNA expression. *BDNF* mRNA blood levels increase with disease progression, and this may indicate that patients with FEPs require more BDNF protein production in response of low BDNF protein levels. Finally, our results add further evidence for the involvement of *BDNF* mRNA in the inflammatory processes in the early stages of psychosis. However, these results require further validation on larger population.

Author contributions. LM, JL: Conceptualization, Methodology; IM, AS-P: Biological Data curation, Writing- Original draft preparation; VS-G, MJA, LO: Clinical Data curation, Visualization, Investigation; EV: Supervision; GM: Data analysis, LM: Writing, Reviewing, Editing. All authors have contributed in multiples roles and have approved the final version.

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Conflicts of interest. The authors declare no known conflicts of interest.

Ethical standards. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

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Sample Data Availability

The data underlying this article will be shared on reasonable request to the corresponding author.

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Figure 1 legend

Partial correlation between *BDNF* mRNA expression levels and inflammatory markers

HC: healthy controls; ARMS: at-risk mental states; FEP: first-episode psychosis; ALL: whole sample; *BDNF*: brain-derived neurotrophic factor mRNA levels; *IL6*: interleukin-6 mRNA levels; NLR: neutrophil-to-lymphocyte ratio; hsCRP: high-sensitivity C-reactive protein. The strength and the direction of the Pearson partial correlation coefficients ($r_{partial}$) between each pair of variables are displayed by color intensity (red and blue for positive and negative correlations, respectively). Crosses indicate statistically nonsignificant correlations. The legend of the Pearson coefficients (ρ2) is shown at the bottom of the graphs.

Estimates and p values of statistically significant correlations:
HC: *IL6*–NLR: $r_{partial}$=0.409, p=0.010; PCR–fibrinogen: $r_{partial}$=0.320, p=0.042.
ARMS: *BDNF*–*IL6*: $r_{partial}$=0.387, p=0.026; *BDNF*–NLR: $r_{partial}$=0.378, p=0.043; *IL6*–NLR: $r_{partial}$=0.384, p=0.040; PCR–fibrinogen: $r_{partial}$=0.551, p=0.002.
FEP: *BDNF*–*IL6*: $r_{partial}$=0.444, p=0.008; *BDNF*–NLR: $r_{partial}$=0.440, p=0.01; *IL6*–NLR: $r_{partial}$=0.552, p=0.001; NLR–fibrinogen: $r_{partial}$=0.496, p=0.004; PCR–fibrinogen: $r_{partial}$=0.497, p=0.004.
ALL: *BDNF*–*IL6*: $r_{partial}$=0.386, p<0.001; *BDNF*–NLR: $r_{partial}$=0.341, p<0.001; *IL6*–NLR: $r_{partial}$=0.474, p<0.001; NLR–fibrinogen: $r_{partial}$=0.257, p=0.009; PCR–fibrinogen: $r_{partial}$=0.407, p<0.001.

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Table 2. Blood *BDNF* mRNA expression levels, expressed as relative expression ratio (RER), observed in the study groups and the total sample according to rs6265 genotype.

	N	**HC**	**N**	**ARMS**	**N**	**FEP**	N	**Total**
rs6265		Mean ± SD		Mean ± SD		Mean ± SD		Mean ± SD
CC	21	3993 ± 1553	21	4534 ± 1087	20	4335 ± 1156	62	4287 ± 1283
CT	19	3335 ± 1067	11	3424 ± 1093	14	4525 ± 1067	44	3736 ± 1182
TT	2	2744 ± 455	2	2063 ± 558	3	3982 ± 1221	7	3042 ± 1138
Lineal general models in each study group adjusted by BMI		F=2.630; p=0.065 r^2=0.111 p_{BMI}=**0.045** p_{rs6265}=0.629		F=7.712; p=**0.001** r^2=0.394 p_{BMI}=0.123 p_{rs6265}<**0.001**		F=0.524; p=0.669 r^2=-0.044 p_{BMI}=0.421 p_{rs6265}=0.551		F=6.839; p<**0.001** r^2=0.142 p_{BMI}= **0.004** p_{rs6265}= **0.005**
Total	**42**	**3636 ± 1352**	**34**	**4029 ± 1268**	**37**	**4371 ± 1109**	**113**	**3995 ± 1278**

Lineal general model for predicting *BDNF* mRNA levels in each study group adjusted by BMI and rs6265: F= 6.586, **p<0.001**; Adjusted r^2=0.174 (p_{BMI}=**0.018**, p_{rs6265}=**0.003**, $p_{diagnosis}$=**0.037**). Group comparisons: HC vs. ARMS: p=0.107; HC vs. FEP: p=**0.012**; ARMS vs. FEP: p=0.410

ARMS: at-risk mental states for psychosis; *BDNF*: brain-derived neurotrophic factor; FEP: first-episode psychosis; HC: healthy controls; N: number of subjects; SD: standard deviation.

Table 1. Characteristics of the study sample.

	Healthy controls (HC)	At-Risk Mental States (ARMS)	First-Episode Psychosis (FEP)	P value
N	42	34	37	
Females, N (%)	15 (36)	11 (32)	12 (32)	0.937
Age (years, M ± SD)	23.0 ± 4	23.1 ± 5	24.6 ± 5	0.198
Education level (years in school)	13.0 ± 2.5	10.7 ± 2.3	11.4 ± 3.1	**0.002**
BMI (kg/m^2, M ± SD)	22.7 ± 3.6	22.0 ± 3.7	24.1 ± 3.6	**0.045**
BDNF mRNA levels (RER, M ± SD)	3636 ± 1352	4029 ± 1268	4371 ± 1109	**0.036**
Inflammatory markers				
IL6 mRNA levels (RER, M ± SD)	9690 ± 4340	10502 ± 6833	14177 ± 9330	**0.041**
NLR ratio	1.44 ± 0.67	1.46 ± 0.69	1.71 ± 0.80	0.269
Fibrinogen (mg/L, M ± SD)	270 ± 65	275 ± 65	272 ± 72	0.942
hsCRP (mg/L, M ± SD)	2.44 ± 2.80	4.50 ± 10.80	2.42 ± 3.97	0.510
Substance use				
Daily tobacco, N (%)	12 (29)	15 (44)	28 (76)	**0.011**
Daily cannabis, N (%)	4 (9.5)	5 (15)	20 (54)	**0.001**
Psychopathology				
PANSS (M ± SD)	-	55.0 ± 14.2	57.7 ± 18.1	0.660
Positive subscale		9.4 ± 2.8	11.9 ± 5.5	**0.044**
Negative subscale		12.9 ± 5.7	14.4 ± 6.5	0.353
General subscale		32.7 ± 9.8	31.4 ± 10.1	0.562
HDS (M ± SD)	-	15.7 ± 10.4	9.7 ± 8.0	**0.022**
Pharmacological treatment				
Antipsychotics, N (%)	-	10 (29)	35 (95)	**< 0.001**
CPZ equivalent doses (mg/d, M ± SD	-	162 ± 114	415 ± 266	**0.004**
Antidepressants, N (%)	-	17 (50)	6 (16)	**0.006**
Mood stabilizers, N (%)	-	2 (6)	7 (19)	0.085

BMI: body mass index; CPZ: chlorpromazine; HDS: Hamilton depression scale; hsCRP: high-sensitive C-reactive protein; *IL6*: interleukin-6; M: mean; N number of subjects; NLR: neutrophil-to-lymphocyte ratio; PANSS: positive and negative syndrome scale; RER: relative expression ratio; SD: standard deviation.

Figure 1