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Full-length Article

Longitudinal transcriptomic profiles associated with onset and remission of adolescent depression

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ABSTRACT

Background: Depression is a leading cause of disability among adolescents, yet the biological mechanisms underlying its onset and clinical course remain poorly understood. Dysregulation of immune and metabolic pathways has been increasingly implicated in adolescent depression, but longitudinal data linking these mechanisms to clinical trajectories are still limited.

Methods: We investigated transcriptomic profiles in a three-year longitudinal study of n = 50 adolescents with major depressive disorder (MDD) and n = 50 adolescents at high sociodemographic risk (HR) for developing MDD, recruited as part of the IDEA-RiSCo cohort in Brazil. Remission (for the MDD group) or transition to depression (for the HR group) were evaluated at 3-year follow-up. Peripheral blood transcriptomics were analyzed using RNA-sequencing, and differentially expressed transcripts were subjected to pathway analysis. Baseline and 3-year follow-up transcriptomics profiles were compared between (i) adolescents with remitted versus persistent MDD, and between (ii) HR adolescents who transitioned to MDD versus those who did not.

Results: Adolescents with MDD at baseline who later remitted showed a change in activation of inflammatory pathways between baseline and 3-year follow-up when compared with those with persistent MDD, presenting an initial stronger pro-inflammatory signature which reversed at follow-up with a recovery of adaptive immune pathways, including upregulation of IL-4 signaling and downregulation of CTLA4 and neutrophil degranulation pathways. In contrast, HR adolescents who transitioned to MDD displayed early dysregulation of immune and GABAergic and glutamatergic pathways, with subsequent downregulation of cell cycle regulation, intracellular signaling, and serotonin receptor pathways at follow-up.

Conclusions: This study identifies distinct transcriptomic signatures underlying divergent trajectories of adolescent depression. A dynamic regulation of inflammatory responses appears to support remission, while progressive impairment of immune, metabolic, GABAergic, glutamatergic and serotoninergic pathways characterizes both persistence of MDD and the transition from high-risk status to disorder onset. These findings highlight potential biological targets for prevention and stratified interventions in adolescent depression.

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1. Introduction

Depression is a leading cause of disability among adolescents worldwide and a major contributor to suicide, one of the top causes of death in this age group (Thapar, 2022; Naghavi, 1990; Kieling, 2024). Despite advances in understanding the pathophysiology of depression in adults, the mechanisms underlying the development and clinical course of depression during adolescence remain poorly understood (Casey, 2019). This is particularly important to be able to develop better prevention and treatment strategies.

While treatment options for depression exist, their effectiveness in adolescents is modest (Thapar, 2022) and large-scale preventive efforts have often failed to produce robust outcomes (Luttinen, 2025). Importantly, depression in adolescence is not a static phenomenon. Longitudinal studies show that depressive symptoms can fluctuate considerably over time, with some individuals showing transient symptoms while others develop persistent or worsening depressive disorder (Chaiton, 2013); however, it remains difficult to predict these clinical trajectories and therefore plan adequate treatment strategies. We face a similar challenge when trying to predict the onset of depression. In our previous work, we have identified specific sociodemographic risk factors and developed a composite risk score which helps to identify adolescents at high risk of developing depression (Rocha, 2021). However, the biological mechanisms playing a role in the transition from high sociodemographic risk to onset of adolescent depression remain largely unclear. A better understanding of biological mechanisms involved in the development and course of adolescent depression could support the identification of targets for novel/more effective treatments or biomarker-based stratification strategies for prevention programs.

Among the emerging evidence, a dysregulation of the immune system may play a role in the pathogenesis of depression. Increased levels of immune markers, such as Tumor necrosis factor (TNF-α), C-reactive protein (CRP), and Interleukin 6 (IL-6), have been observed in adolescents with depression and to precede the onset of depression (Colasanto et al., 2020; Vöckel, 2024). Moreover, in our previous study, in a sample of adolescents stratified for sociodemographic risk of depression (IDEA-RiSCo), we identified inflammation and immune system-related pathways as leading biological mechanisms associated with the presence of adolescent depression (Zonca, 2024). This modulation was observed in adolescents with major depressive disorder (MDD) compared with control adolescents both at high risk and low risk of developing the disorder. Such activation was more evident when comparing currently depressed adolescents with the HR group, suggesting that the HR adolescents, even though they have been exposed to risk factors, might have resilience/compensatory mechanisms which may protect them from developing depression.

We have now completed a longitudinal 3-year follow-up of the original IDEA RiSCo adolescent cohort (Mondelli, 2021; Piccin, 2024). With this study we aim to investigate differences in biological pathways relevant to the follow-up clinical trajectories (remission/persistence of MDD and onset of MDD), by focusing specifically on the group of adolescents who had MDD at baseline and on the group of adolescents who were at high risk of depression (HR) at baseline (Piccin, 2024). More specifically, we aim to identify the molecular pathways relevant for: 1) remission and persistence of adolescent depression by investigating longitudinal differences in transcriptomic profiles between adolescents with MDD who go into remission *vs* those presenting with persistent depression, and 2) transition to MDD from a high risk condition, by investigating longitudinal differences in transcriptomic profiles between HR adolescents who develop MDD *vs* those who did not develop MDD.

2. Methods

2.1. Recruitment procedure

This study is part of the wider IDEA-RiSCo study, which investigates the risk factors for depression in adolescence. We used the IDEA Risk Score (IDEA-RS), originally developed to estimate the probability of the onset of depression in adolescence to screen adolescents attending public schools in Porto Alegre, Brazil, for inclusion in the IDEA-RiSCo sample (Rocha, 2021; Kieling, 2021). The baseline sample included 50 adolescents classified as high-risk for developing MDD (scoring at or above the 90th percentile on the IDEA-RS) but with no current or previous MDD diagnoses (HR group), and 50 classified with a current diagnosis of MDD and who also had high IDEA-RS (MDD group). A detailed description of the recruitment process, clinical assessment, and follow-up methods can be found in our previous publications (Zonca, 2024; Zajkowska, 2023; Nikkheslat, 2025). Participants were followed up approximately 3 years after baseline for a clinical assessment. On the basis of the clinical outcome at 3-year follow-up, the adolescents who had MDD at baseline were divided into two groups: those who no longer had MDD at the 3-year follow-up that were classified as remitted (later referred to as "adolescents with remitted MDD"), and adolescents who had MDD at both baseline and the 3-year follow-up that were classified as having persistent depression (later referred to as "adolescents with persistent MDD"). MDD status at baseline and follow-up was assessed by board-certified child and adolescent psychiatrists using the Brazilian Portuguese version of the K-SADS-PL, a semi structured diagnostic interview evaluating current and lifetime psychopathology. At baseline, lifetime MDD history was determined, while at follow-up, new or recurrent episodes since baseline were assessed (Piccin, 2025).

Similarly, the adolescents classified as HR at baseline were divided into two groups: those who developed MDD over the 3-year follow-up period (later referred to as "adolescents who developed MDD") and those who did not develop MDD by the time of the 3-year follow-up (later referred to as "adolescents who did not develop MDD"). Exclusion criteria, demographic and clinical description of the sample is presented in Table 1. Among the 50 adolescents with MDD at baseline, 38 were reassessed at follow-up (17 males and 21 females). Of these, 18 remitted (9 males and 9 females, 50.0% each), while 20 showed persistent MDD (8 males, 40.0%; 12 females, 60.0%). In the HR group, 41 adolescents were reassessed at follow-up (21 males and 20 females). Within this group, 13 had converted to MDD (5 males, 38.5%; 8 females, 61.5%), while 28 remained classified as HR without developing MDD (16 males, 57.1%; 12 females, 42.9%) (further details are reported in Table 1). At baseline no differences between the groups were detected in anthropometric parameters, as previously published (Zonca, 2024).

Table 1
Participant characteristics of IDEA-RiSCo cohort at baseline and 3-years follow-up, including diagnostic status at baseline, clinical course at follow-up, and biological sex. Exclusion criteria for the recruitment were: Clinical conditions (known brain malformations, epilepsy, recent traumatic brain injury, diabetes, cystic fibrosis, HIV, asthma, rheumatologic conditions such as rheumatoid arthritis, systemic lupus erythematosus, psoriasis, purpura, oncologic conditions such as cancer, lymphoma, leukaemia, and severe neurodevelopmental disorders, any recent/active infection or active inflammatory process); Use of psychotropic medication over last 30 days; Use of anti-inflammatory medication over last 14 days; Current or lifetime: Bipolar disorder, Schizophrenia or a primary psychotic disorder, Autism spectrum disorder, Substance use disorder, Eating disorder, Post-traumatic stress disorder.

	Total N	Males N (%)	Females N (%)
Baseline			
MDD Baseline	50	25 (50.0)	25 (50.0)
HR Baseline	50	25 (50. 0)	25 (50. 0)
Follow-up			
MDD (reassessed)	38	17	21
└── Remitted	18	9 (50.0)	9 (50.0)
└── Persistent	20	8 (40.0)	12 (60.0)
HR (reassessed)	41	21	20
└── converted to MDD	13	5 (38.5)	8 (61.5)
└── not converted to MDD	28	16 (57.1)	12 (42.9)

MDD: major depressive disorder; HR: high risk.

Similarly, no differences between the groups were observed at follow-up in anthropometric measures (height, weight, waist circumference, hip circumference, body temperature, and BMI), nor in socioeconomic conditions assessed using the Brazilian Criterion of Economic Classification (ABEP). Detailed results are provided in Supplementary Tables 1-4.

2.2. Biological sample collection and RNA-Sequencing analysis

Peripheral venous blood samples were collected at baseline and three-year follow-up on the same day as the clinical assessment in 2.5 ml PAXgene Blood RNA tubes (PreAnalitix, Qiagen/BD Company). Nucleic acid purification from PAXgene tubes was performed by using the PAXgene Blood miRNA kit (Qiagen, Hilden, Germany; Cat No./ID: 763134). The quality of the RNA was assessed by Tapestation 2000 (Agilent, Santa Clara, United States) with RNA ScreenTape system to measure the RNA integrity number (RIN). RNA-Seq was performed starting from 150 ng and by using the Illumina Stranded mRNA Prep Ligation Kit following the manufacturer's instruction. The libraries were sequenced on the NextSeq 2000 Illumina platform using NextSeq 2000 P3 Reagents (200 Cycles) paired-ended, read length 74.

2.3. Biostatistical analysis

RNA-Seq raw read counts were quantified at the transcript level using Salmon (version 1.4.0) in the quasi-mapping mode, consisting of two steps: i) indexing of the transcriptome and ii) quantification of the set of raw sequencing reads (FASTQ format). The first step involved constructing a decoy-aware transcriptome by integrating the entire human genome into the human transcriptome and indexing it with chromosome names following the instructions reported in https://combine-lab.github.io/alevin-tutorial/2019/selective-alignment/. Specifically, RNA-seq reads were mapped and quantified using the human reference transcriptome GRCh38. Next, paired-end reads were quantified directly against the index generated in the previous step. Salmon alignment and mapping quality metrics across samples are reported in Supplementary Table 5.

For cross-sectional analysis, transcript-level differential expression was assessed using DESeq2 (version 1.42.1) (Love et al., 2014) in R, accounting for RNA-Seq batch and sex as a covariate and correcting for unwanted variation factors estimated by RUVg (RUVseq, version 1.36.0) (Risso, 2014). Differentially expressed transcripts (DETs) were identified by applying a $|\text{fold change (FC)}| > 1.2$ cut-off and a p-value (no FDR correction applied), $p < 0.05$.

For longitudinal analysis, RNA-seq count data were analyzed using the limma package (v. 3.58.1). To assess whether temporal changes differed between groups, the group-time interaction was tested. Repeated measures from the same subjects were modeled by estimating intra-subject correlations with the function named "duplicateCorrelation" and incorporating these correlations into a linear model. Differential expression was evaluated using linear models with empirical Bayes moderation of variance estimates. Transcripts with $|\text{fold-change (FC)}| > 1.2$ and p-value < 0.05 (no FDR correction applied) were classified as differentially expressed (DETs). In addition, cross-sectional comparisons at individual time-points were performed to evaluate group differences independently of time.

Transcripts differentially expressed across groups were then imported into Ingenuity Pathway Analyses Software (IPA) (Kramer, 2014) to identify associated biological pathways ($p < 0.05$). Generally, the Ingenuity Pathway Analysis software does not apply multiple testing corrections by default, as these may lead to overcorrection and consequently increase the false negative rate, as indicated in the IPA user manual. For this reason, statistical significance of pathway enrichment is primarily based on Fisher's Exact Test p-values. However, given the recent implementation of multiple testing correction within IPA, p-values were additionally adjusted using the Benjamini–Hochberg procedure to control the false discovery rate. Both unadjusted (p-value < 0.05) and adjusted p-values (B–H adjusted p-value < 0.1) are reported.

Lastly, before performing the pathways analysis, the software IPA allowed to select for the cell type to be considered for the analysis; therefore, blood cells were selected as possible and unique source of results, allowing thus to take into account that a bulk RNA-seq has been performed. This selection allowed us to perform an adjustment to the analysis.

3. Results

3.1. Transcriptomics differences between adolescents with remitted MDD and adolescents with persistent MDD

Here we report the findings from the comparison of transcriptomic profiles between adolescents with MDD who went into remission (with remitted MDD) and those who continued to present with MDD (with persistent MDD) after 3 years. Firstly, we present the cross-sectional analysis showing the differences between these two groups at baseline, when both groups had current MDD, and at 3-year follow-up when one group went into remission and the other presented with persistent MDD (see Table 1 for details).

The gene expression analysis identified 516 DETs at baseline and 435 DETs at 3-year follow-up (Fig. 1A and 1B). The subsequent pathways analysis showed 60 pathways significantly modulated at baseline in the group of adolescents with remitted MDD compared with the group of adolescents with persistent MDD ($p < 0.05$) (Supplementary Table 6), and 29 pathways significantly modulated when comparing these groups at follow-up (Supplementary Table 7).

The pathway analysis (Fig. 2) showed that in adolescents with MDD at baseline who subsequently remitted, there was a downregulation of anti-inflammatory signatures, such as *IL-4 Signaling* (z-score = -1.89, p = 0.001, B-H adjusted p = 0.049) and *IL-10 Signaling* (z-score = -1.13, p = 0.049) and an up-regulation of pro-inflammatory pathways, such as the *Neutrophil degranulation* (z-score = 3.413, $p < 0.001$, B-H adjusted $p < 0.001$), the *Communication between Innate and Adaptive Immune Cells* (z-score = 2.14, $p < 0.001$, B-H adjusted p = 0.005), the *Th2 Pathway* (z-score = 2.12, p = 0.009), and the *CTLA4 Signaling in Cytotoxic T Lymphocytes* (z-score = 2.00, p = 0.009). Additionally, several inflammatory-related signatures showed small negative z-scores, including the *NF-κB signaling* (z-score = -1.13, p = 0.008), the T-*cell receptor signaling* (z-score = -0.63, p = 0.041), the *complement cascade* (z-score = -0.45, p = 0.009), and the *role of hypercytokinemia/hyperchemokinemia in the pathogenesis of influenza* (z-score = -0.45, p = 0.031).

At follow-up we observed a significant downregulation of several pathways associated with inflammation in the adolescents with remitted MDD. In particular, we observed a down-regulation of the *CTLA4 signaling in cytotoxic T lymphocytes* (z-score = -3.78, $p < 0.001$, B-H adjusted $p < 0.001$), the *role of NFAT in regulation of the immune response* (z-score = -1.34, $p < 0.001$, B-H adjusted $p < 0.001$), *T cell exhaustion signaling pathway* (z-score = -1.34, $p < 0.001$, adjusted B-H $p < 0.001$), *IL-27 signaling pathways* (z-score = -1.13, p = 0.009), *Th1 pathway* (z-score = -1.13, p = 0.001, adjusted B-H p = 0.025), *antigen presentation pathway* (z-score = -1.00, p = 0.005, adjusted B-H p = 0.081) and *complement cascade* (z-score = -1.00, p = 0.003, adjusted B-H p = 0.054). On the other hand, we observed the up-regulation of the anti-inflammatory pathway of *IL-4 signaling* (z-score = 3.80, $p < 0.001$, B-H adjusted $p < 0.001$).

Moreover, the cross-sectional results were confirmed by the longitudinal analysis, which considered the interaction between group and time (Group X Time interaction). The longitudinal analysis identified 286 DETs (p-value < 0.05, $|FC| > 1.2$), and the subsequent pathway enrichment analysis revealed 102 significantly modulated pathways ($p < 0.05$), and we also observed an overlap with those observed in the cross-sectional analysis. For example, the interaction analysis showed

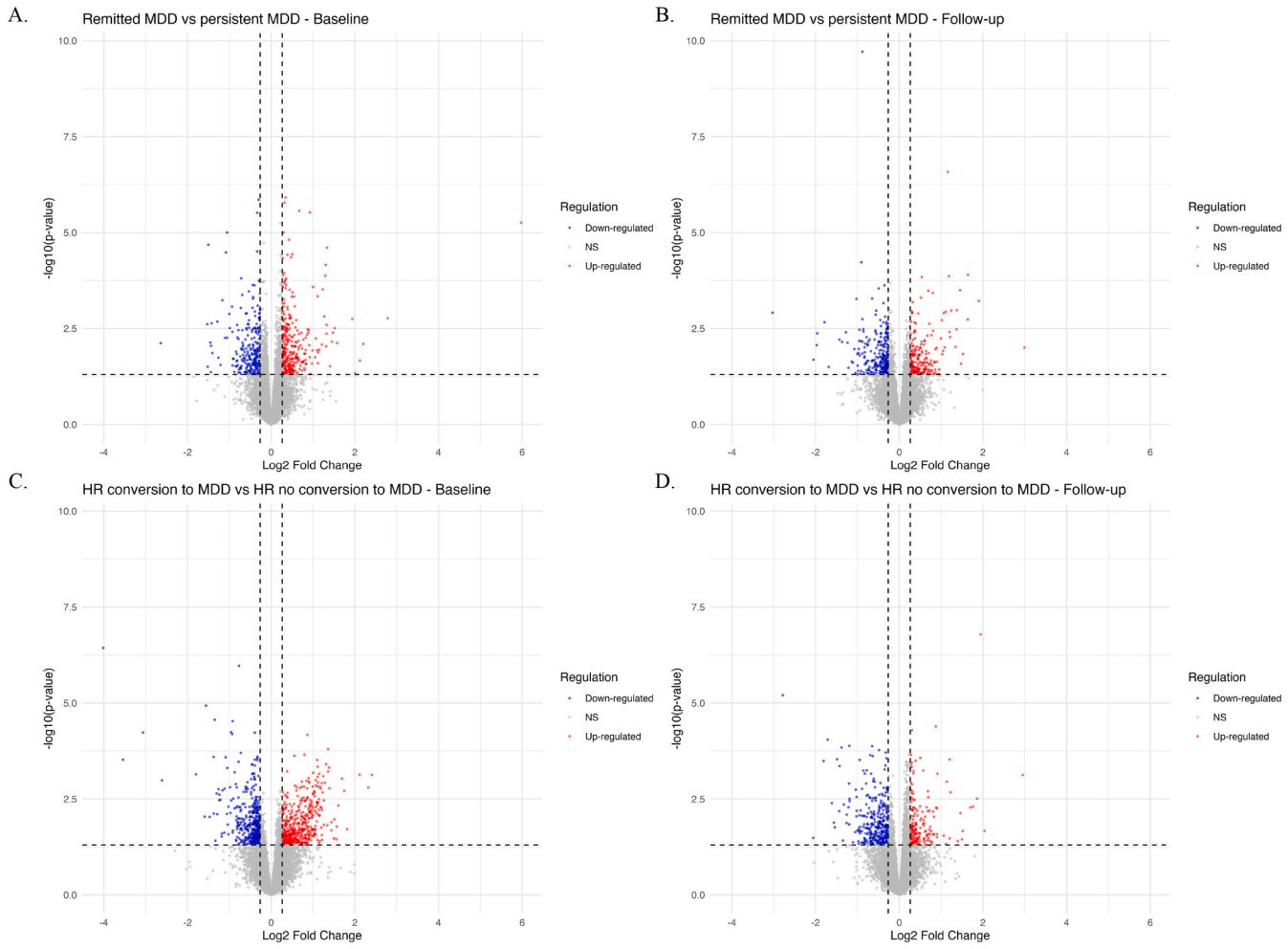

Fig. 1. Volcano plots illustrating differential gene expression across the four study comparisons. (A) Adolescents with remitted MDD *vs* Adolescents with persistent MDD – Baseline; (B) Adolescents with remitted MDD *vs* Adolescents with persistent MDD – Follow-up; (C) Adolescents HR who developed MDD at follow-up *vs* Adolescents HR who did not develop MDD at follow-up –Baseline; (D) Adolescents HR who developed MDD at follow-up *vs* Adolescents HR who did not develop MDD at follow-up –Follow-up. Each point represents an individual gene, with the x-axis indicating the $\log_2$ fold change and the y-axis showing the p-value. Cut-offs are $\log_2$ fold change $>|0.263|$ (linear FC $|1.2|$) and FDR uncorrected p-value < 0.05. Red and blue points correspond to significantly up- and down-regulated genes, respectively, based on the applied fold change and p-value thresholds (dashed lines). Grey points denote non-significant genes. MDD, major depressive disorder; HR, high risk.

differential modulation driven by the interaction between group and time for *IL-4 signaling* ($p = 0.010$), *CTLA4 signaling in cytotoxic T lymphocytes* ($p = 0.011$), *communication between innate and adaptive immune cells* ($p = 0.010$), and *role of NFAT in regulation of the immune response* ($p = 0.019$). Interestingly, the interaction analysis also revealed significant modulation of cell-cycle and eukaryotic processes, which were not detected in the cross-sectional analysis (Fig. 3). The complete list of pathways is reported in Supplementary Table 8.

3.2. Transcriptomic differences between high-risk adolescents who developed MDD and high-risk adolescents who did not develop MDD

In this section we report the findings from the comparison of those adolescents classified as high risk (HR) who developed MDD at 3-year follow up (HR who developed MDD) and those HR adolescents who did not (HR who did not develop MDD) at baseline and at 3-year follow-up (see Table 1 for details).

In the cross-sectional analysis, the gene expression analysis identified 832 DETs at baseline in HR adolescents who subsequently developed MDD *vs* HR adolescents who did not develop MDD, and 460 DETs at the follow-up (Fig. 1C and 1D). The subsequent pathways analysis showed 159 pathways significantly modulated at baseline in the HR adolescents who subsequently developed MDD *vs* HR adolescents who did not develop MDD group ($p < 0.05$) (Supplementary Table 9), and 80 pathways significantly modulated at follow-up in the same comparison (Supplementary Table 10). At baseline, HR adolescents who subsequently developed MDD already exhibited dysregulation in multiple pathways compared with the HR adolescents who did not develop MDD group. Immune-related pathways were upregulated, including *neutrophil degranulation* (z-score $= 3.81$, $p < 0.001$, B-H adjusted $p < 0.001$) and *immunoregulatory interactions between a lymphoid and a non-lymphoid cell* (z-score $= 2.50$, $p = 0.003$, B-H adjusted $p = 0.049$). GABAergic and Glutamate pathways were also significantly upregulated, such as *GABA synthesis, release, reuptake, and degradation* (z-score $= 2.45$, $p < 0.001$, B-H adjusted $p = 0.003$), *glutamate receptor signaling* (z-score $= 2.00$, $p = 0.008$, adjusted B-H $p = 0.089$), and *glutamate binding, activation of AMPA receptors, and synaptic plasticity* (z-score $= 2.00$, $p = 0.024$). In contrast, metabolic pathways and some pathways related to transcriptional regulation and inflammation were downregulated (Fig. 4A). When investigating the transcriptomic profile at follow-up, when the adolescents developed MDD, other pathways gained relevance, most of which were downregulated. Pathways involved in DNA synthesis and cell cycle regulation were suppressed, indicating inactivation of cellular stress-response and structural remodeling mechanisms. Of note, *cell*

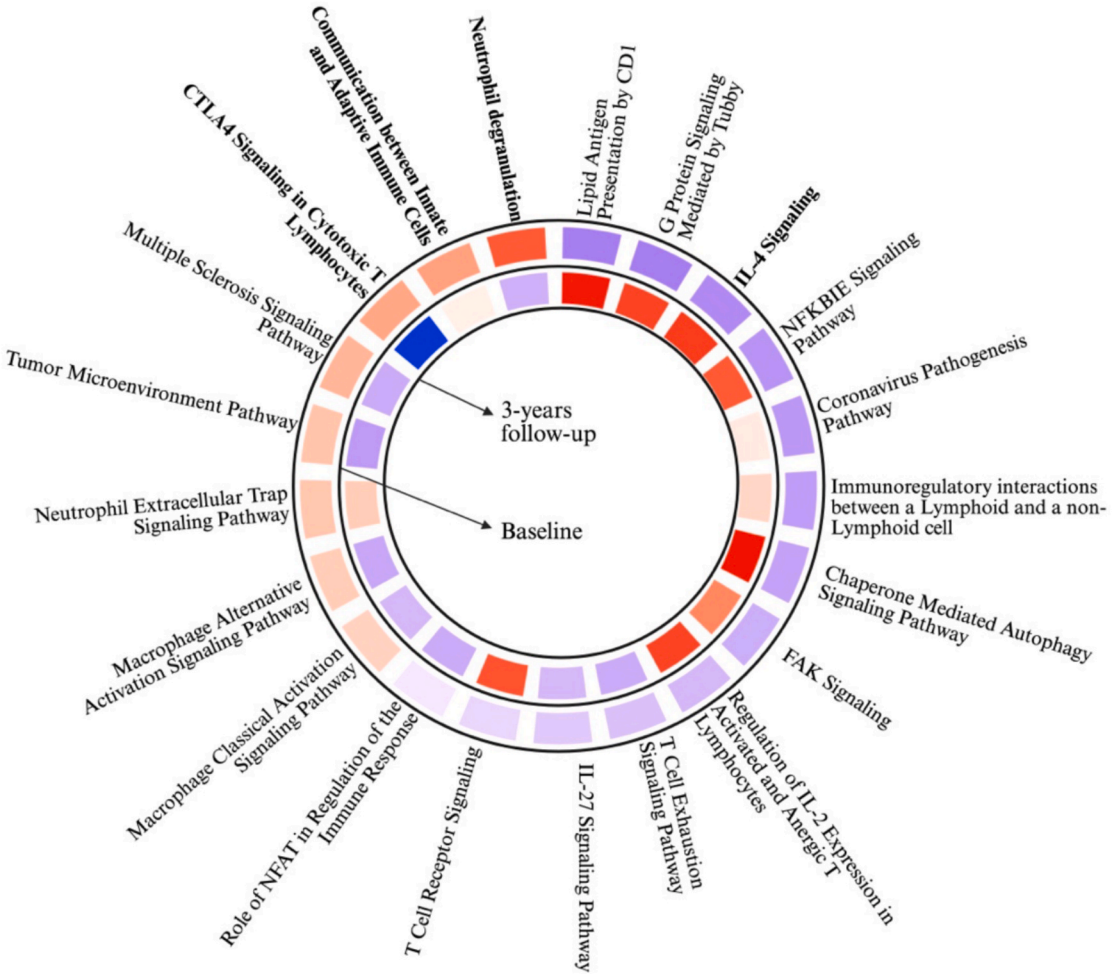

Fig. 2. Circular heatmap comparing shared biological pathways between MDD remitters *vs* MDD non-remitters at baseline and three-year follow-up. Pathways with a positive Z-score (red) indicate increased activity in adolescents MDD remitted *vs* adolescents with persistent MDD, whereas pathways with a negative Z-score (blue) reflect decreased activity. The outer circle represents the baseline, whereas the inner circle corresponds to the follow-up.

cycle checkpoints (z-score = -3.32, p = 0.015), *synthesis of DNA* (z-score = -2.45, p = 0.026), and *DNA replication pre-initiation* (z-score = -2.45, p = 0.014), along with pathways linked to intracellular signaling. Notably, we found a strong downregulation of *serotonin receptor signaling* (z-score = -3.36, p = 0.026) at follow-up, whereas no modulation was observed at baseline (z-score = 0, p < 0.001, B-H adjusted p = 0.013) (Fig. 4B). Lastly, the comparison analysis of the same pathways differently modulated and in common between baseline and follow-up are reported in Supplementary Fig. 1. For both baseline and follow-up, the complete list of pathways is reported in Supplementary Tables 9 and 10 respectively.

The longitudinal analysis considering the interaction between group and time identified 363 DETs (p-value < 0.05, |FC|>1.2) and revealed 38 pathways differentially modulated (p < 0.05), predominantly related to cell-cycle and eukaryotic processes, together with mitochondrial function (Fig. 5). These findings are consistent with the cross-sectional analysis at the follow-up time-point, where modulation over time was observed for *cell cycle checkpoints* (p = 0.036) and *mitotic prometaphase* (p = 0.018). Notably, we also detected significant modulation of several eukaryotic-related mechanisms, including *elongation* (p < 0.001, B-H adjusted p < 0.001), *termination* (p < 0.001, B-H adjusted p < 0.001), and *initiation* (p < 0.001, B-H adjusted p < 0.001), alongside mitochondrial-related signatures such as *mitochondrial translation* (p = 0.003, adjusted B-H p = 0.098), *biogenesis* (p = 0.010), *mitochondrial calcium ion transport* (p = 0.012), and the *mitochondrial L-carnitine shuttle pathway* (p = 0.025). The complete list of pathways is reported in Supplementary Table 11.

4. Discussion

This longitudinal study provides a unique transcriptomic perspective on the biological trajectories differentiating the development and course of MDD in adolescents. By comparing individuals based on their clinical outcome over a 3-year period, we identified distinct and dynamic immune and metabolic pathway signatures that not only distinguish persistent from remitting MDD but also predict the transition from an high-risk status to disorder onset. Our central finding is that a dysregulated, yet dynamic, inflammatory response is a key biological feature associated with MDD recovery, while a progressive impairment across immune, GABAergic, glutamatergic and metabolic systems characterizes the persistence of the disorder.

The most striking finding pertains to the divergent inflammatory trajectories between adolescents who remitted from MDD and those with persistent illness. At baseline, future remission is associated with a complex inflammatory signature, represented by a pronounced upregulation of pro-inflammatory pathways (e.g., neutrophil degranulation, communication between innate and adaptive immune cells) co-occurring with a downregulation of key anti-inflammatory pathways (IL-4 and IL-10 signaling). This profile may not represent simple increased inflammation, but rather a state of significant immune dysregulation. Indeed, this is in line with our previous published results, showing an overall upregulation of inflammatory related pathways in

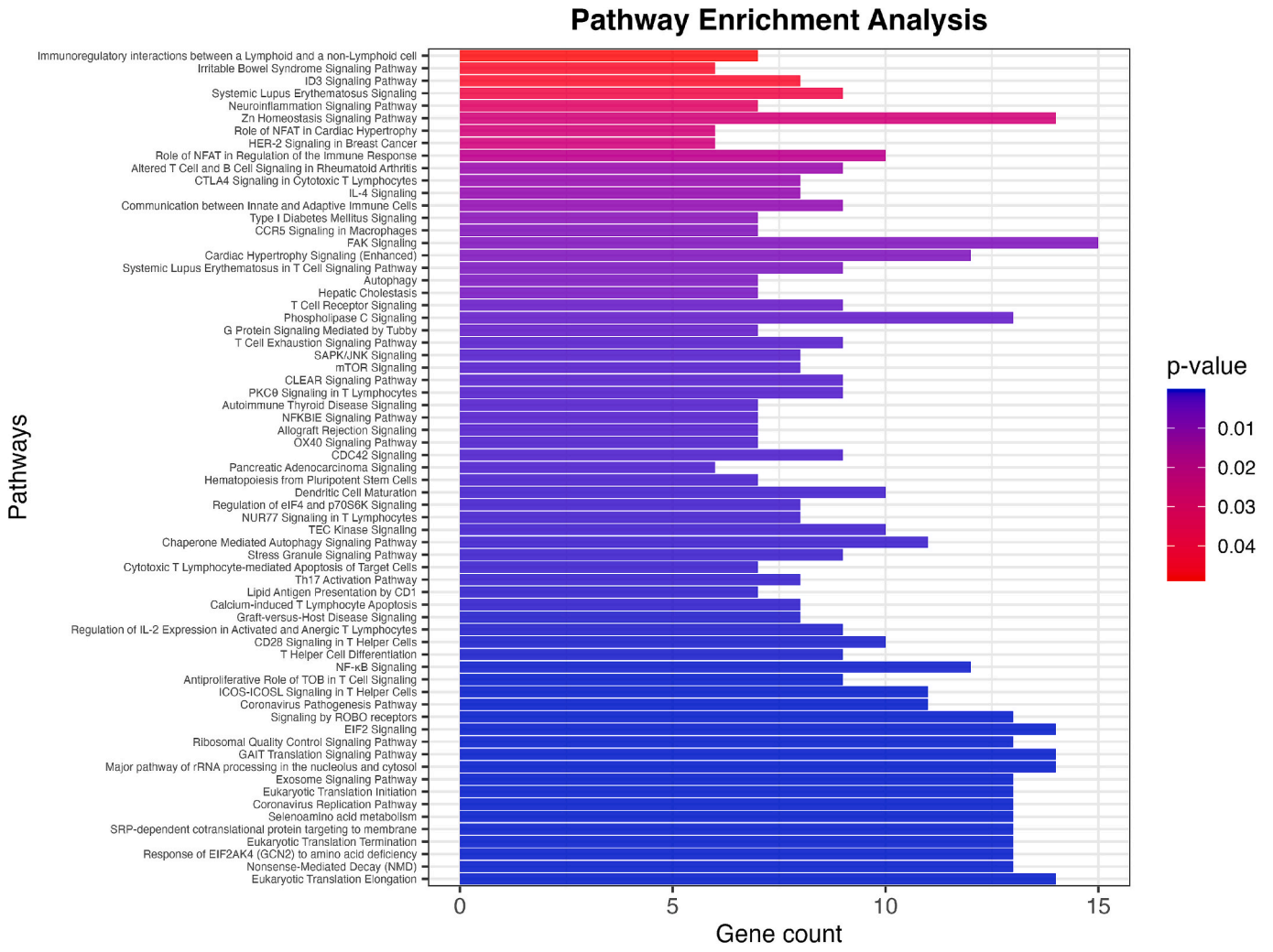

Fig. 3. Differential longitudinal modulation of biological pathways between MDD remitters and MDD non-remitters identified through group X time interaction analysis. The figure illustrates pathways showing significantly distinct temporal trajectories between the two groups ($p < 0.05$), highlighting biological processes associated with symptom remission versus persistence over time. For graphical representation, a gene count cut-off > 5 was applied.

depressed adolescents (both males and females) compared with non-depressed individuals (Zonca, 2024). Our 3-year follow-up data further support the role of immune dysregulation in MDD. In the remitted group, we observed a clear normalization of the immune profile, characterized by a marked downregulation of the previously elevated cytotoxic T-cell and pro-inflammatory pathways (e.g., CTLA4, NFAT, T cell exhaustion pathways) and a robust upregulation of the anti-inflammatory IL-4 signaling pathway. The reversal in the z-scores for pathways like IL-4 signaling and CTLA4 Signaling from baseline to follow-up might suggest a recovery of adaptive immune function and a resolution of inflammation in the adolescents who go into remission. This suggests that clinical remission may be underpinned by a biological process of immune rebalancing, moving from a dysregulated, pro-inflammatory state towards a regulated, anti-inflammatory homeostasis (Nikkheslat, 2025; Sampson, 2025). Nevertheless, our longitudinal analysis supports this hypothesis, as inflammation-related pathways were differentially modulated over time between the two groups when considering the group X time interaction, further highlighting the role of inflammatory processes in MDD remission. Overall, our findings might also align with emerging concepts of depression subtypes, where an "inflammatory phenotype" may be particularly relevant for prognosis and treatment response (Sforzini, 2025; Osimo, 2020; Beurel et al., 2020).

In our second set of analyses in relation to the onset of MDD, our findings reveal a dynamic shift in molecular pathways associated with the onset of depression in high-risk adolescents. At baseline, before the emergence of MDD, adolescents who later developed MDD already exhibited significant dysregulation in multiple pathways compared with HR adolescents who did not develop MDD, and the majority were upregulated. Notably, immune-related pathways were upregulated, including neutrophil degranulation and immunoregulatory interactions between lymphoid and non-lymphoid cells. This early immune activation aligns with growing evidence that inflammation and immune system dysregulation contribute to depression vulnerability, possibly by influencing neurodevelopment or stress reactivity (Palmer, 2024; Jiao, 2025). Simultaneously, GABAergic, glutamatergic, and serotonin-related pathways were also modulated at baseline in adolescents who later developed MDD. While these pathways are traditionally associated with neurotransmission in the nervous system, in leukocytes they have diverse roles, including immune regulation, inflammatory modulation, and chemotaxis. In contrast, metabolic pathways and pathways involved in transcriptional regulation were downregulated, suggesting a complex pre-symptomatic imbalance between immune, metabolic, and regulatory processes.

At follow-up, when MDD was clinically manifest, the overall pattern shifted toward a widespread downregulation of the transcriptomic expression. Pathways involved in DNA synthesis, cell cycle regulation, and intracellular signaling were suppressed, reflecting a potential

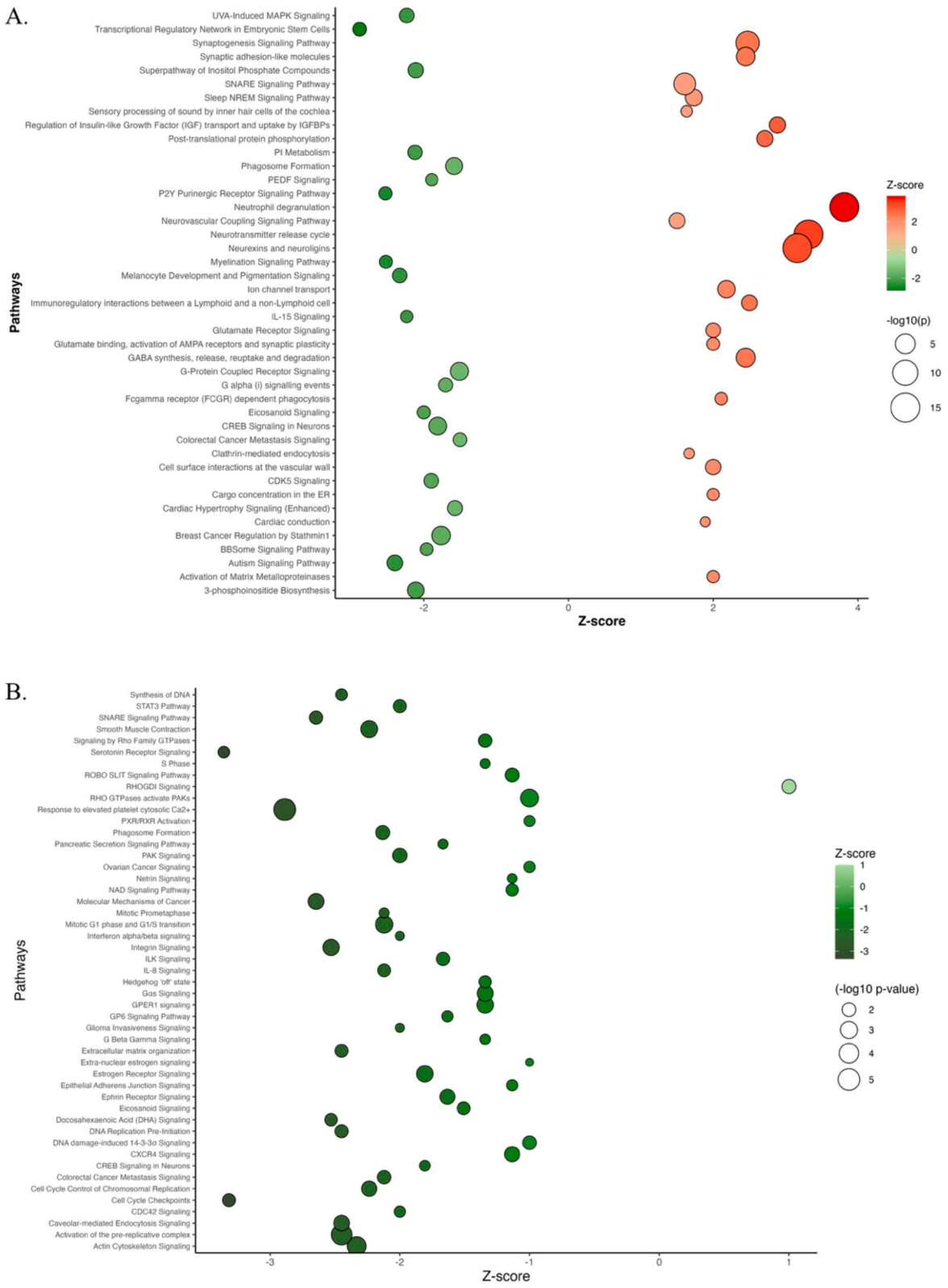

Fig. 4. Bubble charts showing biological pathways between Adolescents HR who developed MDD *vs* Adolescents HR who did not develop MDD at baseline (A) and follow-up (B). Pathways with a positive Z-score (red) indicate increased activity in HR who develop MDD *vs* HR who did not develop MDD, whereas pathways with a negative Z-score (green) reflect decreased activity. For graphical representation, only pathways with a Z-score greater than |2| were reported in figure 4A, whereas only pathways with a Z-score greater than |1| were reported in figure 4B.

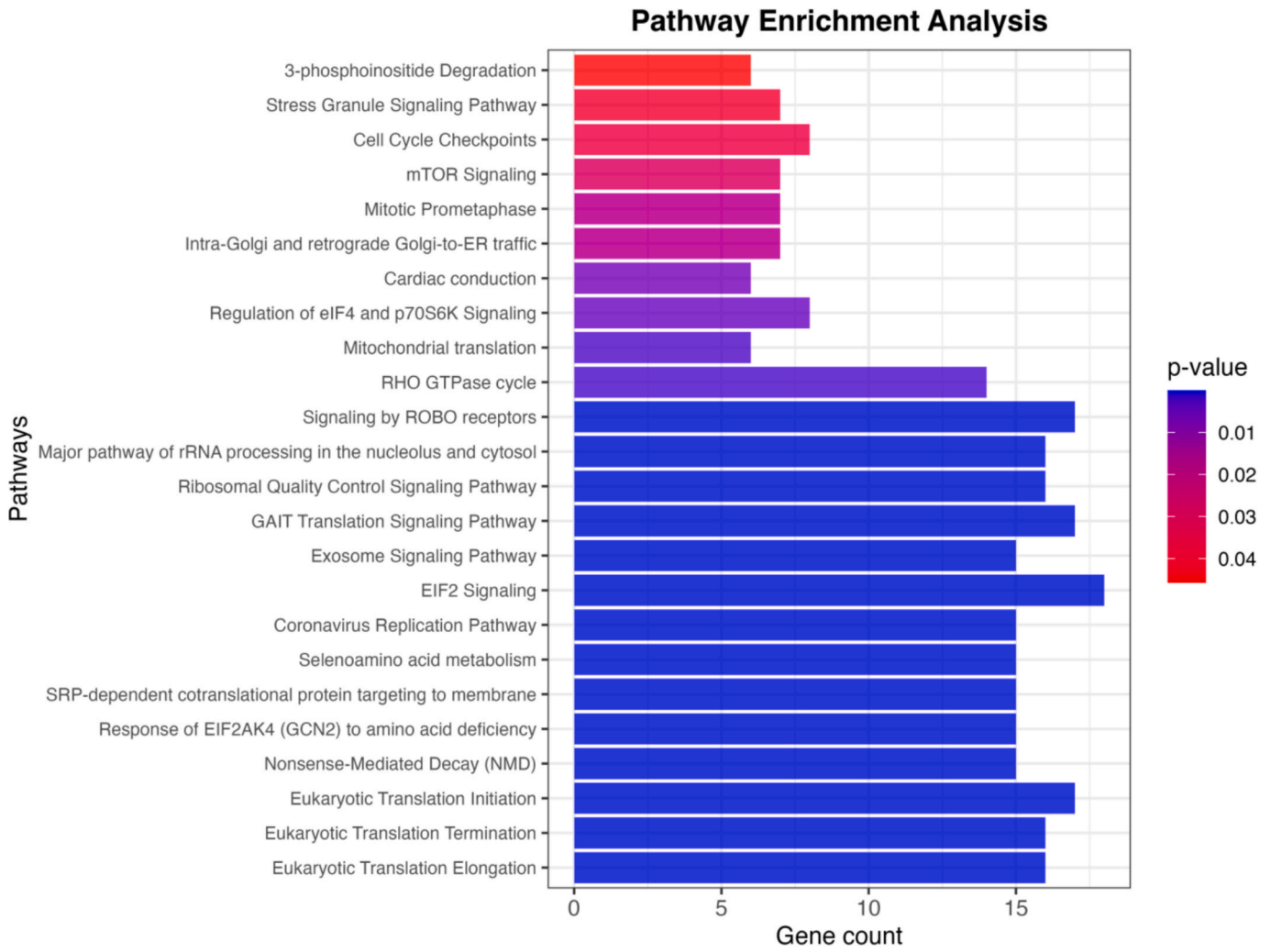

Fig. 5. Differential longitudinal modulation of biological pathways between high-risk (HR) individuals who developed MDD and those who did not, identified through group X time interaction analysis. The figure illustrates pathways showing significantly distinct temporal trajectories between the two groups ($p < 0.05$) over time. For graphical representation, a gene count cut-off > 5 was applied.

impairment in cellular stress responses, structural remodeling, and serotonin signaling. The pronounced downregulation of serotonin receptor signaling is particularly notable, highlighting the critical role of serotonergic dysfunction in the pathophysiology of adolescent depression. Importantly, this downregulation was absent at baseline, suggesting it may emerge because of disease progression rather than as a predisposing factor. Moreover, the longitudinal analysis examining the group X time interaction revealed modulation of eukaryotic-related mechanisms, indicating that fundamental biological processes change over time and are associated with the onset of depression in high-risk adolescents. Interestingly, similar modulations were also observed in relation to MDD remission, suggesting that both high-risk status and depression may induce sustained alterations in basal cellular processes that, over time, could underlie the additional molecular changes observed in this cohort. Overall, our results indicate that the onset of depression in high-risk adolescents involves a temporal evolution from early immune and serotonin dysregulation to later impairment in cellular maintenance and serotonergic signaling.

Our study presents various strengths, including the longitudinal design of the study and the opportunity to analyze transcriptomic profiles at both baseline and follow-up in a very well characterized sample of adolescents. However, the study presents also a few limitations. First, transcriptomic data from peripheral blood may not fully reflect changes occurring in the central nervous system, although increasing evidence supports strong immune and metabolic parallels (Ciobanu, 2016). A second limitation is that an insufficient number of transcripts survived the FDR correction and thus were not suitable for the subsequent pathway analysis. Therefore, for the pathway analysis we used DE transcripts with an unadjusted p-value. However, it is important to clarify that it is not unusual for such a small number of DE transcripts to survive the FDR correction in genome-wide gene expression analysis, specifically when such expression analyses are performed in clinical cohorts. Then, as few or no transcripts survived the FDR correction, it is possible to expect some false positive DE transcripts. A further limitation concerns the pathway-level results, as some pathways that were statistically significant based on Fisher's Exact Test ($p < 0.05$) did not remain significant after multiple testing correction using the Benjamini–Hochberg approach (B-H adjusted $p>0.1$). While the main pathways discussed in the manuscript retained significance after correction, these discrepancies highlight the need for cautious interpretation of pathway enrichment results. Accordingly, findings related to pathways not surviving FDR correction should be considered exploratory and hypothesis-generating rather than definitive evidence. Moreover, unlike our previous baseline analysis (Zonca, 2024), we were unable to analyze males and females separately in this study because of lack of statistical power and therefore this would need to be addressed in future larger studies. Then, as all participants in this study had IDEA-RS scores at or above the 90th percentile, the sample represents adolescents at the highest

sociodemographic risk for depression, which may limit the generalizability of our findings to the broader adolescent population. A further limitation is also that all results are correlational, and consequently no causal conclusions can be drawn regarding the relationship between blood gene regulation and MDD outcomes. It is possible that the observed transcriptional alterations are a consequence of MDD or related neural and endocrine changes, rather than contributing causally to the disorder. Future studies using interventions that can influence directly the immune system might clarify the directionality of these associations. Lastly, we must acknowledge that the adolescents of the IDEA-RiSCo are all based in Brazil, and thus the findings may not generalize to other parts of the world.

5. Conclusion

In conclusion, this longitudinal transcriptomic study highlights the dynamic biological processes underpinning both recovery from and persistence of adolescent MDD, as well as the transition from being at high risk to disorder onset. Our findings underscore immune dysregulation as a central feature, with remission associated with immune rebalancing and persistence marked by impairments in immune and metabolic pathways. Moreover, the early immune and GABAergic, glutamatergic and serotoninergic alterations observed in high-risk adolescents who later developed MDD suggest potential pre-symptomatic markers of vulnerability, while the emergence of widespread downregulation at follow-up points to mechanisms of MDD progression. Together, these results provide new insights into the molecular trajectories of adolescent MDD, supporting the relevance of immune–metabolic interactions as targets for prognosis and intervention strategies.

CRediT authorship contribution statement

Valentina Zonca: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Samantha Saleri:** Writing – review & editing, Methodology, Formal analysis. **Moira Marizzoni:** Writing – review & editing, Formal analysis, Data curation. **Pedro H. Manfro:** Writing – review & editing, Methodology, Data curation, Conceptualization. **Naghmeh Nikkheslat:** Writing – review & editing, Data curation, Conceptualization. **Jader Piccin:** Writing – review & editing, Methodology, Data curation, Conceptualization. **Laila Souza:** Writing – review & editing, Methodology, Data curation, Conceptualization. **Anna Viduani:** Writing – review & editing, Methodology, Data curation, Conceptualization. **Zuzanna Zajkowska:** Writing – review & editing, Methodology, Data curation, Conceptualization. **Johnna R. Swartz:** Writing – review & editing, Funding acquisition, Conceptualization. **Helen L. Fisher:** Writing – review & editing, Funding acquisition, Conceptualization. **Brandon A. Kohrt:** Writing – review & editing, Funding acquisition, Conceptualization. **Christian Kieling:** Writing – review & editing, Funding acquisition, Conceptualization. **Annamaria Cattaneo:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Data curation, Conceptualization. **Valeria Mondelli:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Disclosures.

The authors declare that they have no known conflict of interest that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbi.2026.106566.

Data availability

Data will be made available on request.

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