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Article

Keywords:

Posted Date: November 11th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11516320/v1

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Yes Dr. Murrough is a full-time employee of the Icahn School of Medicine at Mount Sinai and a part-time employee of the U.S. Department of Veterans Affairs. In the past 36 months, Dr. Murrough has provided paid consultation services for Acadia Pharmaceuticals, Autobahn Therapeutics, Inc., Biohaven Pharmaceuticals, Inc., Clexio Biosciences, Definium Therapeutics, Frontier Pharma, LLC, HMP Collective, Janssen Pharmaceuticals, Merck & Co., Inc., Otsuka Pharmaceutical, Ltd, WCG Clinical, and Inc., Xenon Pharmaceuticals, Inc. Dr. Murrough is a named inventor on patent applications filed by the Icahn School of Medicine at Mount Sinai covering methods for the treatment of mood and anxiety disorders, including approaches involving KCNQ channel openers, psychedelic compounds, immunotherapeutic strategies, and digital/sensor-based interventions for emotion regulation. None of these applications has been issued as a patent or licensed, and Dr. Murrough has received no royalties or financial benefit from them.

Transcriptional profiles of CD4$^+$ and CD8$^+$ T cells in major depressive disorder

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Abstract

Major depressive disorder (MDD) has been associated with peripheral immune alterations, but transcriptional changes within the major T cell lineages remain poorly characterized. We performed bulk RNA sequencing of magnetically-enriched $CD4^+$ and $CD8^+$ T cell fractions from a cohort comprising 81 individuals with MDD and 48 healthy controls (HCs). Differential expression was assessed for protein-coding genes and long noncoding RNAs (lncRNAs), canonical T cell receptor (TCR)-segment transcripts were examined separately, and Ingenuity Pathway Analysis was used to characterize associated molecular pathways and upstream regulators. In CD4-enriched cells, 202 protein-coding genes and lncRNAs met a false discovery rate threshold of < 0.05, whereas no gene met this threshold in CD8-enriched cells. Twenty-one canonical TCR-segment transcripts, predominantly TRAJ genes, were increased in MDD. Pathway analysis implicated broad transcription- and chromatin-regulatory programs. Exploratory sex-stratified analyses identified a broader $CD4^+$ signature in males, comprising 487 differentially expressed genes and including reduced cytotoxic/natural killer-like and major histocompatibility complex class I antigen-processing programs, together with increased TRAJ transcript abundance. These findings identify an MDD-associated peripheral transcriptional signature detected primarily in CD4-enriched cell fractions and characterized by distributed regulatory-state and TCR-segment changes. Together, this dataset provides a cell-type-resolved foundation for future studies examining the clinical relevance and functional consequences of $CD4^+$ T cell transcriptional alterations in MDD.

Keywords: Major depressive disorder, adaptive immunity, T cells, transcriptomics

Introduction

Major depressive disorder (MDD) is highly prevalent and contributes substantially to global disability and socioeconomic burden[1]. Beyond functional impairment, MDD is associated with increased morbidity and mortality, including elevated suicide risk and heightened risk for several medical comorbidities[2]. Despite multiple evidence-based treatments, a substantial proportion of patients do not achieve full remission following standard antidepressant interventions[3]. MDD is etiologically, clinically, and biologically heterogeneous, and immune-metabolic abnormalities appear to characterize partially overlapping patient subgroups[4]. This heterogeneity, together with the absence of validated mechanistic stratifiers, likely contributes to variable treatment response and motivates biologically resolved studies of MDD[5].

Among peripheral systems implicated in MDD, the immune system is of particular interest because it is tightly coupled to stress physiology and can influence brain function via its effector cells and molecules[6-8]. Acute threat responses engage the autonomic nervous system and hypothalamic-pituitary-adrenal axis, coordinating sympathetic and endocrine outputs that mobilize immune cells and shape inflammatory mediator production[7,9]. In contrast, repeated or severe stress can produce context-dependent immune dysregulation, including altered leukocyte trafficking, glucocorticoid responsiveness, and affect the balance between innate and adaptive immune programs[10,11]. Peripheral immune signals can influence neural function through humoral, neural, vascular, and cellular routes[12].

The immune response comprises interconnected innate and adaptive arms: the innate immune system provides rapid pattern recognition-based responses, whereas T and B lymphocytes generate antigen-specific responses and immunological memory[13-16]. Although immunopsychiatric research has focused predominantly on cytokines and innate immune pathways, adaptive immunity has emerged as a potential contributor to the pathophysiology of depression[17]. $CD4^+$ and $CD8^+$ T cells represent the two principal conventional T cell lineages, but each encompasses heterogeneous naïve, memory, effector, and regulatory states. $CD4^+$ T cells integrate antigen-presentation cues and coordinate cellular and humoral immunity through functionally specialized helper and regulatory programs[16]. $CD8^+$ T cells classically recognize antigen in a major histocompatibility complex class I-restricted manner and eliminate infected or malignant targets through perforin- and granzyme-mediated mechanisms[16]. These lineage-associated functions are not mutually exclusive, because, for example, cytotoxic programs can also emerge within $CD4^+$ T cell compartments across multiple immune contexts[18]. T cell states integrate antigen exposure, differentiation history, cytokine and neuroendocrine signaling, and

may therefore capture persistent immune alterations not apparent from circulating inflammatory mediators alone.

Human immunophenotyping studies support alterations in both innate and adaptive immune compartments in depression, although the direction and magnitude of individual T cell findings vary according to the population, assay, and whether absolute counts or relative frequencies are analyzed. Meta-analyses report modest increases in absolute $CD4^+$ helper and activated T cell counts, as well as higher CD4/CD8 and T helper 17/regulatory T cell ratios in blood, whereas findings for relative $CD4^+$ frequencies and total $CD8^+$ cells are less consistent[19,20]. One recent cross-sectional study additionally linked altered T helper and regulatory T cell measures to depressive symptom severity[21], although subset-level findings remain heterogeneous and require replication[22]. Altered naïve T cell distributions and CD4/CD8 balance have also been reported in depressed patients with common variable immunodeficiency[23], but the specialized clinical context limits generalization to otherwise medically healthy MDD. Meta-analytic evidence for an elevated neutrophil-to-lymphocyte ratio further suggests a relative shift in innate-to-adaptive cellular balance, but this index is nonspecific and cannot localize underlying cellular processes[24]. Collectively, while cell counts and ratios provide some evidence for depression-associated changes also in the adaptive immune system, more detailed molecular and functional analyses are needed.

Transcriptomic profiling can identify coordinated molecular programs. Most peripheral gene-expression studies in depression have analyzed whole blood or total peripheral blood mononuclear cells (PBMCs) and have reported increased innate immune activity coupled to relative downregulation of adaptive immune and T cell-linked modules[25]. Whole-blood RNA sequencing has additionally linked recurrent MDD to increased expression of type I interferon pathway genes[26], and a quantitative review of available case-control datasets implicated innate immune and neutrophil-related gene networks[27]. However, findings have not been uniform. A comparatively large peripheral blood mononuclear cell RNA-sequencing study found no robust case-control differential expression after multiple-testing correction[28], whereas inflammation-stratified whole-blood RNA sequencing identified distinct immunometabolic and cell cycle-related programs across C-reactive protein and treatment-response strata[29]. Most recently, a harmonized mega-analysis of five datasets comprising 6011 participants identified few robust individual genes after adjustment for cellular composition but implicated lymphoid cell sources, including T and natural killer cells, replication-dependent histone dysregulation, and sex-related transcriptional networks[30]. These apparently divergent findings underscore both the biological heterogeneity of

MDD and the difficulty of attributing whole-blood transcriptional signals to specific immune lineages.

Because whole-blood and peripheral blood mononuclear cell transcriptomes conflate cellular composition with within-cell regulation, they cannot establish whether adaptive immune signatures arise preferentially from particular T cell lineages[25]. Cell-resolved studies have begun to address this limitation. Preliminary single-cell RNA sequencing identified immunometabolic reprogramming in monocytes and $CD4^+$ T cells from depressed participants with elevated inflammation[31]. A small single-cell study also characterized peripheral $CD8^+$ T cell transcriptional states in participants with MDD, bipolar disorder, and healthy controls[32]. Integrated single-cell RNA sequencing and assays for transposase-accessible chromatin sequencing of peripheral blood mononuclear cells subsequently identified altered abundance and differentiation of $CD4^+$ naïve T cell subsets in MDD and in relation to antidepressant treatment response[33]. Complementary immunophenotyping and repertoire-focused work suggested altered $CD4^+$ T cell receptor repertoire diversity in MDD, raising the possibility that clonal composition and antigen-exposure history differ in depression[34]. Nevertheless, it remains unclear whether MDD-associated transcriptional alterations preferentially localize to peripheral $CD4^+$ or $CD8^+$ T cell fractions.

Here, we address these gaps by performing parallel cell type-specific bulk RNA sequencing on magnetic bead-sorted $CD4^+$ and $CD8^+$ T cells from individuals with MDD and healthy controls (HCs). We sought to determine whether MDD-associated transcriptional alterations preferentially localize to either major T cell lineage, to characterize T cell receptor segment transcript abundance, and to examine these patterns in secondary, exploratory sex-stratified analyses. By defining $CD4^+$- and $CD8^+$-specific transcriptional signatures associated with MDD, this work aims to refine understanding of adaptive immune contributions to depression and provide a foundation for future biomarker and mechanistic studies of neuroimmune interactions.

Materials and methods

Study participants. Participants with MDD (n = 81) and HCs (n = 48) were recruited through the Dennis S. Charney, MD, Depression and Anxiety Discovery Center at the Icahn School of Medicine at Mount Sinai. Sample size was not based on a specific target, but was an opportunistic analysis using all available samples from a depression biomarker study. MDD diagnoses were established using the Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (SCID-5)[35], administered by trained research staff. Participants in the MDD group met criteria for a current major depressive episode. HCs were required to have no current and no lifetime DSM-5 disorder. The study was approved by the Institutional Review Board of the Icahn School of Medicine at Mount Sinai. All participants provided written informed consent before any study procedure and received financial compensation. BMI was collected, sex was determined from gene expression data based on expression of *UTY* and *XIST* for male and female participants, respectively, and agreed with clinical records, and ethnicity was self-reported using the categories Hispanic or Latino, not Hispanic or Latino, and unknown or declined to answer. Exclusion criteria included a current or previous psychotic episode, a current or previous manic episode, current substance use disorder, unstable medical illness, active infection or systemic inflammatory illness, pregnancy, and breastfeeding. Participants reported no use of medications considered to have clinically relevant immunomodulatory effects during the two weeks before blood collection. These medications included corticosteroids, biologics, and nonsteroidal anti-inflammatory drugs. Current stable psychotropic medication use was permitted and was recorded. Participants underwent biochemical and hematological laboratory testing, urine toxicology, and pregnancy testing when applicable. The Quick Inventory of Depressive Symptomatology (QIDS)-Self-Report (16-item version) was administered to all participants[36]. On the study day, participants fasted for at least 6 h before blood collection. Blood was collected in the mornings. No formal statistical method was used to predetermine the sample size.

Blood collection and clinical laboratory measurements. Venous blood was collected into Vacutainer CPT tubes (BD Biosciences, catalog no. (#) 362761) for PBMC isolation and into EDTA-containing Microtainer tubes (BD Biosciences, #365975) for complete blood counts and measurements of high sensitivity C-reactive protein (hs-CRP). Complete blood counts and differential leukocyte counts were measured using a Sysmex XN-9100™ Automated Hematology System. Hs-CRP was measured using an Abbott Alinity C analyzer. Values reported as <0.1 and <0.3 mg/L were entered as 0.1 and 0.3 mg/L, respectively, for statistical analyses.

PBMC isolation and magnetic enrichment of $CD4^+$ and $CD8^+$ cells. PBMCs were isolated from CPT tubes according to the manufacturer's instructions. Briefly, tubes were centrifuged at 1,800 × *g* for 25 min at room temperature. The PBMC layer was collected and washed twice in Dulbecco's phosphate-buffered saline (Thermo Fisher Scientific, #14190144). Cells were resuspended in autoMACS Running Buffer (Miltenyi Biotec, #130-091-221) and for each participant, equal numbers of freshly isolated PBMCs were allocated to $CD4^+$ and $CD8^+$ enrichment. Cells were incubated with human CD4 MicroBeads (Miltenyi Biotec, #130-045-101) or human CD8 MicroBeads (Miltenyi Biotec, #130-045-201) for 15 min at 4 °C. After washing, positive selection was performed using an autoMACS Pro Separator (Miltenyi Biotec, #130-092-545) with the Posseld2 program. Positive fractions were pelleted and lysed immediately in TRIzol Reagent (Thermo Fisher Scientific, #15596026). Lysates were stored at −80 °C until RNA extraction.

Flow cytometric assessment of cell enrichment. Flow cytometric quality control was performed on positive and negative fractions, which were washed in fluorescence-activated cell sorting (FACS) buffer consisting of phosphate-buffered saline, 0.5% bovine serum albumin, and 2 mM EDTA. Cells were incubated with Human TruStain FcX Fc Receptor Blocking Solution (BioLegend, #422302) and Fixable Viability Dye eFluor 780 (1:2000; Thermo Fisher Scientific, #65-0865-14) for 30 min on ice. Surface staining was performed for 30 min on ice using anti-human CD45-V500 (1:400; clone HI30; BD Biosciences, #560779), CD3-BV421 (1:400; clone UCHT1; BioLegend, #300434), CD4-APC (1:400; clone RPA-T4; #300552), and CD8-BV785 (1:400; clone SK1; BioLegend, #344740) antibodies. Data were acquired on a BD FACSymphony A5 SE Cell Analyzer. Cells were sequentially gated on forward- and side-scatter characteristics, FSC-A versus FSC-H and SSC-A versus SSC-H singlets, viability dye-negative cells, $CD45^+$ $CD3^+$ T cells, and $CD4^+$ or $CD8^+$ populations. Purity was defined as the percentage of the target $CD4^+$ or $CD8^+$ population among live $CD45^+CD3^+$ singlets. Data were analyzed using FlowJo version 10.6.2 (BD Biosciences).

RNA extraction, library preparation, and RNA sequencing. RNA was isolated in two batches using the RNeasy Micro Kit according to the manufacturer's instructions (Qiagen, #74004). RNA quality, RNA integrity number (RIN), and RNA concentrations were assessed using NanoDrop

(Thermo Fisher Scientific) and Bioanalyzer (Agilent, #5067-1513). RNA library preparations and sequencing reactions were conducted at GENEWIZ, LLC. (South Plainfield, NJ, USA). SMART-Seq HT Ultra Low Input Kit was used for full-length cDNA synthesis and amplification (Clontech, Mountain View, CA), and Illumina Nextera XT library was used for sequencing library preparation. Briefly, cDNA was fragmented and adapters were added using Transposase, followed by limited-cycle PCR to enrich and add indices to the cDNA fragments. The final library was validated on the Agilent TapeStation (Agilent Technologies, Palo Alto, CA, USA) and quantified using Qubit 2.0 Fluorometer (ThermoFisher Scientific, Waltham, MA, USA) as well as by quantitative PCR (KAPA Biosystems, Wilmington, MA, USA). The sequencing libraries were multiplexed and clustered on a flow cell on the Illumina NovaSeq instrument according to the manufacturer's instructions. The samples were sequenced using a 2x150 base pair paired-end configuration. Image analysis and base calling were conducted by the NovaSeq Control Software (NCS). Raw sequence data (.bcl files) generated from Illumina NovaSeq were converted into FASTQ files and demultiplexed using Illumina's bcl2fastq 2.20 software.

RNA-sequencing processing. FASTQC v0.12.1 was used to examine sequencing quality. Trim Galore! v0.6.6 was used to trim adapter sequences from samples. Raw sequencing reads from samples were mapped to hg38 using HISAT2[37] v2.0.1. Counts of reads mapping to genes were obtained using featureCounts v2.0.1 against Ensembl v109 annotation[38]. Most samples had alignment rates of 90% or higher, with sequencing depths ranging from 20-50 million reads. Samples that failed quality control were excluded, including 3 samples identified as PCA outliers and 4 samples with insufficient read mapping, evidenced by low gene-mapped counts in featureCounts or low total alignments in HISAT2. 129 samples for each cell type were retained. Lowly expressed genes were filtered using a threshold of at least 2 reads across 80% of the samples. Missing values in body mass index (BMI) were imputed using the missForest R package. Differential expression analyses comparing MDD vs. HC in both CD4 and CD8 were performed using the DESeq2 R package with the following linear model: log(exp) ~ Batch + BMI + Sex + Age + Group (for the sex-stratified analyses without sex). 'Batch' was included in the model to account for variation from two sequencing experiments. Canonical Pathway, Upstream Regulator, and Graphical Summary analyses were performed using QIAGEN Ingenuity Pathway Analysis (IPA; QIAGEN, Redwood City, CA, USA)[39]. Separate IPA Core Analyses were performed using the 202 combined-cohort CD4-enriched and 487 male CD4-enriched FDR-significant protein-coding genes and lncRNAs. Input data comprised Ensembl IDs, $\log_2$ fold changes, and adjusted P values.

The IPA overlap P value was calculated using a right-tailed Fisher exact test. Activation z scores were used to predict the direction of pathway or regulator activity from the concordance between observed expression changes and curated causal relationships. Pathways and upstream regulators were retained for presentation when the nominal overlap P value was < 0.05 and the absolute activation z score was ≥ 2. The male IPA Graphical Summary was generated from the same Core Analysis. For differential expression analyses and enrichment/pathway analyses, we included only the following gene biotypes: “protein coding” and “lncRNA”. T cell receptor associated genes were analyzed separately.

Statistical analysis of demographic and clinical variables. Demographic and clinical variables were analyzed using IBM SPSS Statistics for Windows, version 24.0 (IBM Corp., Armonk, NY, USA). Analyses used available observations for each variable, and variable-specific sample sizes are reported in the tables. Continuous variables are summarized as mean ± standard deviation, and categorical variables as number and percentage. Distributional assumptions were assessed using histograms and Shapiro-Wilk tests. In full-cohort comparisons between MDD and HCs, age, BMI, QIDS-SR score, and hs-CRP were compared using two-sided Mann-Whitney U tests. Absolute lymphocyte counts were compared using two-sided Welch independent-samples t tests. Sex distributions were compared using a Yates continuity-corrected χ^2 test. Ethnicity distributions were compared using Pearson χ^2. Sex-stratified continuous variables were analyzed using two-way analysis of variance with diagnosis, sex, and the diagnosis × sex interaction as fixed effects. All clinical tests were two-sided, with $P < 0.05$ considered statistically significant.

Results

Participant characteristics and validation of T cell enrichment. The study cohort comprised 81 individuals with MDD and 48 HCs (**Fig. 1A**). The groups did not differ significantly in age, ethnicity, BMI, or absolute lymphocyte count (all P ≥ 0.192). Compared with HCs, the MDD group included a higher proportion of women (60.5% versus 37.5%; P = 0.0191), had higher QIDS-SR scores (13.96 ± 4.64 versus 1.44 ± 1.43; P < 0.001), and higher hs-CRP concentrations (3.03 ± 6.20 versus 1.61 ± 2.57 mg/L; P = 0.0185) (**Table 1**). Current stable psychotropic medication use was present in 37 of 76 MDD participants with no medication in the HCs. Representative flow cytometric analyses demonstrated enrichment of the target populations in the corresponding positive fractions and depletion from the negative fractions (**Fig. 1B-C**). Consistent with the flow cytometric findings, RNA-seq data showed higher expression of *CD4* in the CD4-enriched fraction and of *CD8A* in the CD8-enriched fraction, together with low expression of the B cell and monocyte/myeloid markers *CD19* and *LILRA5*, respectively (**Fig. 1D**).

Differential expression is predominantly detected in CD4⁺ T cells. Differential-expression testing was conducted across 17,728 Ensembl features. Following Benjamini-Hochberg correction, results were restricted for primary reporting and visualization to 15,689 protein-coding genes and long noncoding RNAs (lncRNAs). In CD4-enriched cells, 202 genes met the false discovery rate (FDR) threshold of < 0.05, comprising 164 protein-coding genes and 38 lncRNAs; 135 had positive and 67 had negative $\log_2$ fold changes in MDD relative to HCs (**Fig. 2A; Supplementary File 1**). Among the strongest differentially expressed genes were *CCNK* ($\log_2$ fold change = 0.640, FDR = 6.06×10^{-8}), *CSTF2T* ($\log_2$ fold change = −0.543, FDR = 6.06×10^{-8}), *BAZ1B* ($\log_2$ fold change = 0.434, FDR = 1.37×10^{-6}), and *ACIN1* ($\log_2$ fold change = 0.287, FDR = 4.36×10^{-4}). Across all FDR-signifcant genes, $\log_2$ fold changes ranged from approximately −1.62 to +1.07. Immune-associated genes with lower expression in MDD included *KLRF1* ($\log_2$ fold change = −1.619, FDR = 7.74×10^{-4}), *KLRD1* ($\log_2$ fold change = −1.556, FDR = 0.0297), and *TSC22D3* ($\log_2$ fold change = −0.357, FDR = 0.0114). Canonical T cell receptor (TCR)-segment genes were subsequently examined as a prespecified subset of the same differential-expression results. Twenty-one TCR genes met FDR < 0.05, and all had positive $\log_2$ fold changes in MDD relative to HCs (**Fig. 2B; Supplementary File 2**). These comprised 20 TCRα joining genes and the TCRγ variable gene *TRGV1*. No

protein-coding gene or lncRNA met FDR < 0.05 in the combined-cohort CD8-enriched cell analysis (**Fig. 2C**). IPA was performed using the 202 FDR-significant CD4-enriched protein-coding genes and lncRNAs. Results were retained when the nominal IPA overlap *P* value was < 0.05 and the absolute activation *z* score was ≥2. Four canonical pathways met these criteria: Histone Modification Signaling Pathway, Generic Transcription Pathway, Cilium Assembly, and Mitotic G2-G2/M Phases (**Fig. 2D**). Five upstream regulators met the same criteria and were predicted to be activated: CLOCK (*z* = 2.00), TAL1 (*z* = 2.00), KCNJ2 (*z* = 2.00), POU5F1 (*z* = 2.18), and ESR1 (*z* = 2.22) (**Fig. 2E**).

Exploratory sex-stratified analyses identify an expanded male CD4$^+$ signature. Given established sex differences in MDD prevalence and symptomatology[40], we performed exploratory sex-stratified analyses. The cohort comprised 32 male participants with MDD and 30 male HCs, and 49 female participants with MDD and 18 female HCs (**Supplementary Table 1**). There were no differences in sociodemographic variables, hs-CRP, or lymphocyte counts between subgroups (**Supplementary Table 1**). In the male-stratified CD4-enriched analysis, 487 protein-coding genes and lncRNAs met FDR < 0.05, of which 279 had positive and 208 had negative $\log_2$ fold changes (**Fig. 3A; Supplementary File 3**). The signature included several of the principal transcription- and chromatin-associated genes detected in the combined cohort, including increased expression of *ACIN1*, *BAZ1B*, and *CCNK* and decreased expression of *CSTF2T*. The male-stratified signature additionally contained coordinated reductions in cytotoxic/NK-like genes. Among the largest immune-associated effects were *KLRD1* ($\log_2$ fold change = −2.940, FDR = 0.00257) and *KLRF1* ($\log_2$ fold change = −2.604, FDR = 1.86×10^{-4}). *GNLY*, *GZMH*, *CST7*, *HOPX*, and *HLA-E* were also significantly decreased. Genes contributing to MHC class I antigen processing and presentation, including *B2M*, *PSMB9*, *PSME2*, and *PSMA1*, likewise had negative $\log_2$ fold changes and met FDR < 0.05. *NKG7* ($\log_2$ fold change = −1.243, nominal P = 0.00258, FDR = 0.0570) and *GZMB* ($\log_2$ fold change = −1.032, nominal P = 0.00268, FDR = 0.0572) showed similar negative effect estimates but did not meet the prespecified FDR threshold. Twenty-seven canonical TCR-segment genes met FDR < 0.05 in male CD4-enriched cells, and all had positive $\log_2$ fold changes (**Fig. 3B; Supplementary File 4**). All 27 were TCRα joining genes. IPA of the 487 male differentially expressed genes in CD4-enriched cells identified both positively and negatively scored transcriptional programs (**Fig. 3C**). Pathways with positive

activation *z* scores included Generic Transcription Pathway (*z* = 4.60), Histone Modification Signaling Pathway (*z* = 3.74), and several circadian-related modules, including RORA/ RORB/ RORC-NR1D1 regulation and BMAL1:CLOCK/NPAS2-mediated circadian gene expression. Pathways with negative activation *z* scores included Class I MHC-mediated Antigen Processing and Presentation (*z* = −2.24), TNFR2 Non-canonical NF-κB Pathway (*z* = −2.24), Senescence-Associated Secretory Phenotype (*z* = −2.12), Gene Silencing by RNA (*z* = −2.00), Cellular Response to Hypoxia (*z* = −2.00), and Chemokine Signaling (*z* = −2.00). Twelve predicted upstream regulators met the nominal overlap *P* value and activation *z* score criteria (**Fig. 3D**). Five were predicted to be activated: RICTOR, ESR1, KCNJ2, BCL6, and COPS5. Seven were predicted to be inhibited: GAST, IFN ALPHA/BETA (family), CG (complex), QKI, TYK2, DDX5, and IFNG. Among the most strongly scored regulators were activated RICTOR (*z* = 3.32), ESR1 (*z* = 2.67), and BCL6 (*z* = 2.21), and inhibited IFNG (*z* = −2.27), TYK2 (*z* = −2.22), and the IFN ALPHA/BETA family (*z* = −2.00). The IPA Graphical Summary connected these expression changes and predicted regulator states to decreased cytotoxicity of cells, decreased cytotoxicity of lymphocytes and decreased lymphocyte migration (**Fig. 3E**).

Limited differential expression in the remaining sex- and cell-type-stratified analyses. In male CD8-enriched cells, *PDCD11* was the only protein-coding gene or lncRNA meeting FDR < 0.05 and showed higher expression in MDD ($\log_2$ fold change = 0.236, FDR = 0.0187; **Supplementary Fig. 1A; Supplementary File 5**). In female CD4-enriched cells, *CCNK* was the only protein-coding gene or lncRNA meeting FDR < 0.05 and was more highly expressed in MDD ($\log_2$ fold change = 0.716, FDR = 0.0134; **Supplementary Fig. 1B; Supplementary File 6**). No canonical TCR-segment gene met FDR < 0.05 in female CD4-enriched cells (**Supplementary Fig. 1C**). In female CD8-enriched cells, *CRTAP* ($\log_2$ fold change = 0.305, FDR = 0.0181) and *PRC1-AS1* ($\log_2$ fold change = 1.362, FDR = 0.0311) were more highly expressed in MDD (**Supplementary Fig. 1D; Supplementary File 7**).

Discussion

In this cell type-resolved transcriptomic study, the primary combined-cohort analysis identified an MDD-associated expression signature in magnetically-enriched $CD4^+$ T cell fractions, whereas no protein-coding gene or lncRNA met the prespecified FDR threshold in $CD8^+$ T cell fractions. The CD4-enriched cell signature comprised 202 protein-coding genes and lncRNAs, together with increased abundance of a set of canonical TCR-segment transcripts. Exploratory male-stratified analyses revealed a broader CD4-enriched cell signature that combined the transcriptional and chromatin-regulatory features of the primary analysis with reduced cytotoxic/NK-like and MHC class I antigen-processing programs.

These findings extend previous whole-blood and PBMC transcriptomic studies reporting increased innate immune activity together with relative attenuation of adaptive immune and T cell-linked expression modules in MDD[25]. By profiling magnetically-enriched $CD4^+$ and $CD8^+$ fractions separately, the present study more directly localizes a case-control expression signal to the CD4-enriched compartment. This conclusion is consistent with emerging cell-resolved work showing that MDD-associated immune alterations may be concentrated in particular $CD4^+$ cell states. Integrated single-cell RNA-seq and ATAC-seq profiling identified altered abundance and differentiation of naïve $CD4^+$ T cell populations in MDD and in relation to antidepressant response[33], while preliminary single-cell work in inflammation-associated depression implicated immunometabolic changes in $CD4^+$ T cells and monocytes[31]. A recent peripheral-blood transcriptomic mega-analysis also implicated lymphoid cell sources and replication-dependent histone dysregulation in depression[30].

The combined-cohort CD4-enriched cell signature was not dominated by a single canonical inflammatory pathway. Instead, it included multiple chromatin-associated genes, including *BAZ1B*, *ASH1L*, *KMT2E*, *BPTF*, *HIRA* and *DNMT3B*, together with genes involved in transcription and RNA processing, including *CCNK*, *CSTF2T*, *ACIN1*, *SON*, *TAF3*, *SRSF4* and *MED1*. The presence of coordinated, mostly modest expression differences across these functions is consistent with a distributed change in transcriptional state rather than a large alteration in one isolated immune-effector pathway. The contribution of lncRNAs, 38 of the 202 significant features, is also noteworthy, although their regulatory functions cannot be inferred from expression data alone.

The IPA Generic Transcription and Histone Modification findings provide a higher-order summary of this regulatory pattern. Chromatin remodeling is central to $CD4^+$ T cell

differentiation[41] and also shapes differentiation and persistence in $CD8^+$ T cells[42]. Furthermore, genetic risk variants for depression and other psychiatric disorders have been reported to be enriched at epigenetically-active sites in lymphoid cells, particularly stimulated $CD4^+$ T cells[43]. This provides an independent rationale for examining $CD4^+$ regulatory programs in psychiatric disease. Nevertheless, the present study measured RNA abundance rather than chromatin accessibility, DNA methylation or histone modifications. Accordingly, these results should be described as altered expression of chromatin-regulatory and transcriptional programs, not as evidence that epigenetic modifications themselves occurred.

The interferon-related findings were similarly more complex than a uniformly activated type 1 inflammatory response[44]. In the combined CD4-enriched cell analysis, *IFNAR2* and *IFIH1* were significantly increased, whereas *IFNG-AS1* did not meet FDR significance and became significant only in the male-stratified analysis. IFIH1 encodes MDA5, an innate sensor of long double-stranded RNA, including viral and endogenous RNA species. Earlier work reported increased IFNγ secretion from stimulated PBMCs in MDD[45], and whole-blood RNA-seq implicated type I interferon-stimulated genes in recurrent MDD[26]. In contrast, the male IPA analysis predicted inhibited activity of IFNG, TYK2 and the IFN ALPHA/BETA regulator family. Increased expression of interferon receptors or sensors alongside reduced inferred upstream activity could reflect altered responsiveness, compensatory feedback or chronic exposure history. These data therefore support altered interferon-related regulation but do not establish uniform activation or suppression of interferon signaling. The simultaneous IPA prediction of reduced IFNG activity and increased quantity of Th1 cells also illustrates that IPA disease/function annotations should not be equated with measured cytokine activity or cell abundance.

TSC22D3, encoding glucocorticoid-induced leucine zipper (GILZ), was significantly decreased in the combined and male CD4-enriched cell analyses. GILZ mediates several anti-inflammatory actions of glucocorticoids in immune cells[46], making lower *TSC22D3* expression compatible with an altered glucocorticoid-responsive transcriptional state. It does not, however, directly demonstrate glucocorticoid resistance, which would require functional assessment of glucocorticoid receptor signaling and downstream responses[47]. Interestingly, a previous whole-blood study associated reduced expression of glucocorticoid-inducible genes including GILZ with smaller hippocampal volume in MDD[48], which is notable as reduced hippocampal volume has been reported in depression[49,50].

A particularly coherent immune-effector feature emerged in the male CD4-enriched cell analysis. *KLRD1* and *KLRF1*, which were already significant in the combined cohort, showed substantially larger negative effect estimates in males. These findings were accompanied by decreased *GNLY*, *GZMH*, *CST7*, *HOPX* and *HLA-E*, as well as coordinated reductions in MHC class I antigen-processing and presentation genes including *B2M*, *PSMB9*, *PSME2* and *PSMA1*. *NKG7* and *GZMB* showed concordant decreases with FDR values immediately above 0.05. Conversely, IPA predicted decreased cytotoxicity of CD4-enriched cells. Cytotoxic programs are not exclusive to $CD8^+$ T cells, and circulating cytotoxic $CD4^+$ populations can express killer-cell receptors, perforin, granzymes and NK-associated transcriptional markers[51,52]. Earlier studies reported altered NK-cell cytotoxicity in MDD and changes in NK function in association with antidepressant exposure[53,54]. Single-cell RNA-seq or CITE-seq combined with markers of naïve, memory, effector-memory and cytotoxic $CD4^+$ states will be required to distinguish a compositional shift from within-state transcriptional regulation. Chronic viral exposures, particularly cytomegalovirus, are also strong determinants of cytotoxic $CD4^+$ expansion and should be considered in future studies of this signature[55].

The increased abundance of TCR-segment transcripts represents a second coherent component of the findings in CD4-enriched cells. Twenty-one canonical TCR genes were increased in the combined analysis, including 20 TRAJ genes and *TRGV1*, while all 27 significant male TCR genes were TRAJ segments. Prior work reported reduced $CD4^+$ TCR repertoire diversity in MDD[34], providing a precedent for altered adaptive immune receptor structure in depression. However, increased abundance of individual TRAJ transcripts does not directly replicate reduced repertoire diversity. A less diverse or clonally-skewed repertoire can produce increased representation of selected segments, but bulk gene-level RNA abundance can also vary with T cell subset composition and activation state. Dedicated repertoire-sequencing methods are required to quantify clonotype abundance and diversity[56,57].

The substantially larger number of discoveries in the male-stratified analysis is potentially important but requires cautious interpretation, because the more balanced male comparison, 32 MDD and 30 HCs, may have had different effective power and dispersion characteristics than the female comparison of 49 MDD participants and 18 HCs. Prior brain transcriptomic studies have identified substantial sex divergence in MDD-associated molecular signatures[58], and sex is known to influence immune cell composition and function[59]. The predicted activation of ESR1 likewise should not be interpreted as evidence that estrogen signaling explains the male-stratified signature. IPA infers regulator activity from concordance among downstream target

genes; it does not measure receptor expression, circulating hormone concentrations or receptor activation. ESR1 signaling can affect immune cells in both sexes[59]. Similarly, predicted RICTOR and BCL6 activation identifies plausible regulatory nodes because mTORC2 and BCL6 influence $CD4^+$ T cell metabolism, differentiation and effector state specification[60-63], but these predictions require further validation.

The study has several strengths, including parallel profiling of the two principal T cell lineages in the same clinically characterized cohort, careful evaluation of lineage enrichment by flow cytometry and marker gene expression, inclusion of relevant demographic and technical covariates, and genome-wide control of the false discovery rate before biotype-specific reporting. The relatively large CD4-enriched dataset also enabled detection of a distributed transcriptional signature that would be difficult to identify using targeted candidate-gene approaches. Several limitations should nevertheless be considered. First, the cross-sectional design cannot distinguish whether the observed transcriptional differences precede MDD, result from the disorder or its treatment, or reflect correlated behavioral and physiological exposures. Second, bulk RNA-seq of magnetically-enriched $CD4^+$ and $CD8^+$ fractions cannot distinguish changes in subpopulation abundance from regulation within a given cell state. Third, TCR loci are highly homologous and rearranged, and gene-level alignment to a standard reference is not equivalent to clonotype-resolved repertoire analysis. Fourth, the sex-stratified analyses were exploratory and were affected by unequal case-control balance in the female stratum. Fifth, the IPA analyses used thresholded DEG lists, nominal overlap *P* values and curated knowledge-base relationships; their pathway, regulator and functional predictions are hypothesis-generating and were not independently validated. Finally, current psychotropic medication use was present in a substantial subset of MDD participants and absent in HCs. Although medication exposure is difficult to disentangle from clinical diagnosis in observational MDD cohorts, it remains a potential confounder and should be addressed in future studies.

Collectively, the findings identify an MDD-associated transcriptional signature in peripheral $CD4^+$ T cell fractions involving transcription/RNA-processing and chromatin-regulatory genes, increased canonical TCR-segment transcript abundance, and, in exploratory male-stratified analyses, a reduced cytotoxic/NK-like and MHC class I antigen-processing program. These results propose $CD4^+$ T cell state heterogeneity and regulatory organization as candidate components of peripheral immune variation in MDD, but require replication, formal sex- and cell-type-interaction testing, cell state-resolved profiling, clonotype-level TCR analysis and functional validation.

Data/code availability. RNA sequencing data have been deposited in the Gene Expression Omnibus under accession number (GSE ID: GSE345973, token: oxqjiymybjgdzij).

Acknowledgements

We thank the study participants for their participation in our study.

Funding

This study was supported by NIH grants R01MH114882-01 (S.J.R.), R01MH104559 (S.J.R.), R01MH127820 (S.J.R.), R01MH141038 (J.W.M. and S.J.R.), K99DK137037 (K.L.C.), R00DK137037 (K.L.C.) and grants or fellowships from the Swiss National Science Foundation (P400PM_186708 to F.C.), Brain & Behavior Research Foundation (29104 to F.C., 30894 to K.L.C., and 31194 to L.F.P), Helmut Horten Foundation (2024-YIG-036) (F.C.), University Medicine Zurich (F.C.), a Postdoctoral Fellowship from the Canadian Institutes of Health Research (201811MFE-414896-231226) (K.L.C). This work was supported in part through the computational and data resources and staff expertise provided by Scientific Computing and Data at the Icahn School of Medicine at Mount Sinai and supported by the Clinical and Translational Science Awards (CTSA) grant UL1TR004419 from the National Center for Advancing Translational Sciences. Additional support was provided by the Ehrenkranz Center for Human Resilience at the Icahn School of Medicine at Mount Sinai and by generous support from the Gottesman Foundation, Great Hill and the Molly Lou Foundation.

Competing interests

Dr. Murrough is a full-time employee of the Icahn School of Medicine at Mount Sinai and a part-time employee of the U.S. Department of Veterans Affairs. In the past 36 months, Dr. Murrough has provided paid consultation services for Acadia Pharmaceuticals, Autobahn Therapeutics, Inc., Biohaven Pharmaceuticals, Inc., Clexio Biosciences, Definium Therapeutics, Frontier Pharma, LLC, HMP Collective, Janssen Pharmaceuticals, Merck & Co., Inc., Otsuka Pharmaceutical, Ltd, WCG Clinical, and Inc., Xenon Pharmaceuticals, Inc. Dr. Murrough is a named inventor on patent applications filed by the Icahn School of Medicine at Mount Sinai covering methods for the

treatment of mood and anxiety disorders, including approaches involving KCNQ channel openers, psychedelic compounds, immunotherapeutic strategies, and digital/sensor-based interventions for emotion regulation. None of these applications has been issued as a patent or licensed, and Dr. Murrough has received no royalties or financial benefit from them.

References

1 Whiteford, H. A. *et al.* Global burden of disease attributable to mental and substance use disorders: findings from the Global Burden of Disease Study 2010. *Lancet (London, England)* **382**, 1575–1586 (2013). https://doi.org/10.1016/s0140-6736(13)61611-6

2 Otte, C. *et al.* Major depressive disorder. *Nature Reviews Disease Primers* **2**, 16065 (2016). https://doi.org/10.1038/nrdp.2016.65

3 Gaynes, B. N. *et al.* What did STAR*D teach us? Results from a large-scale, practical, clinical trial for patients with depression. *Psychiatric services (Washington, D.C.)* **60**, 1439–1445 (2009). https://doi.org/10.1176/ps.2009.60.11.1439

4 Milaneschi, Y., Lamers, F., Berk, M. & Penninx, B. Depression Heterogeneity and Its Biological Underpinnings: Toward Immunometabolic Depression. *Biological psychiatry* **88**, 369–380 (2020). https://doi.org/10.1016/j.biopsych.2020.01.014

5 Drevets, W. C., Wittenberg, G. M., Bullmore, E. T. & Manji, H. K. Immune targets for therapeutic development in depression: towards precision medicine. *Nat Rev Drug Discov* **21**, 224–244 (2022). https://doi.org/10.1038/s41573-021-00368-1

6 Cathomas, F., Murrough, J. W., Nestler, E. J., Han, M. H. & Russo, S. J. Neurobiology of Resilience: Interface Between Mind and Body. *Biological psychiatry* **86**, 410–420 (2019). https://doi.org/10.1016/j.biopsych.2019.04.011

7 Herman, J. P. *et al.* Regulation of the Hypothalamic-Pituitary-Adrenocortical Stress Response. *Comprehensive Physiology* **6**, 603–621 (2016). https://doi.org/10.1002/cphy.c150015

8 Miller, A. H., Haroon, E., Raison, C. L. & Felger, J. C. Cytokine targets in the brain: impact on neurotransmitters and neurocircuits. *Depression and anxiety* **30**, 297–306 (2013). https://doi.org/10.1002/da.22084

9 Smith, S. M. & Vale, W. W. The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress. *Dialogues in clinical neuroscience* **8**, 383–395 (2006).

10 Miller, A. H. & Raison, C. L. The role of inflammation in depression: from evolutionary imperative to modern treatment target. *Nature reviews. Immunology* **16**, 22–34 (2016). https://doi.org/10.1038/nri.2015.5

11 Wohleb, E. S., Franklin, T., Iwata, M. & Duman, R. S. Integrating neuroimmune systems in the neurobiology of depression. *Nature reviews. Neuroscience* **17**, 497–511 (2016). https://doi.org/10.1038/nrn.2016.69

12 Cathomas, F. *et al.* Beyond the neuron: Role of non-neuronal cells in stress disorders. *Neuron* **110**, 1116–1138 (2022). https://doi.org/10.1016/j.neuron.2022.01.033

13 Chaplin, D. D. Overview of the immune response. *The Journal of allergy and clinical immunology* **125**, S3–S23 (2010). https://doi.org/10.1016/j.jaci.2009.12.980

14 Medzhitov, R. & Janeway, C., Jr. Innate immunity. *The New England journal of medicine* **343**, 338–344 (2000). https://doi.org/10.1056/nejm200008033430506

15 Mogensen, T. H. Pathogen recognition and inflammatory signaling in innate immune defenses. *Clinical microbiology reviews* **22**, 240–273, Table of Contents (2009). https://doi.org/10.1128/cmr.00046-08

16 Chi, H., Pepper, M. & Thomas, P. G. Principles and therapeutic applications of adaptive immunity. *Cell* **187**, 2052–2078 (2024). https://doi.org/10.1016/j.cell.2024.03.037

17 Beurel, E., Medina-Rodriguez, E. M. & Jope, R. S. Targeting the Adaptive Immune System in Depression: Focus on T Helper 17 Cells. *Pharmacol Rev* **74**, 373–386 (2022). https://doi.org/10.1124/pharmrev.120.000256
18 Takeuchi, A. & Saito, T. CD4 CTL, a Cytotoxic Subset of CD4(+) T Cells, Their Differentiation and Function. *Frontiers in immunology* **8**, 194 (2017). https://doi.org/10.3389/fimmu.2017.00194
19 Foley É, M., Parkinson, J. T., Mitchell, R. E., Turner, L. & Khandaker, G. M. Peripheral blood cellular immunophenotype in depression: a systematic review and meta-analysis. *Molecular psychiatry* (2022). https://doi.org/10.1038/s41380-022-01919-7
20 Sørensen, N. V. *et al.* Immune cell composition in unipolar depression: a comprehensive systematic review and meta-analysis. *Molecular psychiatry* **28**, 391–401 (2023). https://doi.org/10.1038/s41380-022-01905-z
21 Zhang, W., Wang, C., Fu, T., Zhang, S. & Tian, F. CD4 + T lymphocyte and cytokine profiles in depressive disorders. *BMC Psychiatry* **25**, 911 (2025). https://doi.org/10.1186/s12888-025-07344-8
22 Wang, F. *et al.* Peripheral CD4(+) T helper lymphocytes alterations in major depressive disorder: A systematic review and meta-analysis. *Neuroscience* **555**, 145–155 (2024). https://doi.org/10.1016/j.neuroscience.2024.07.027
23 Manusama, O. *et al.* Reduced numbers of naïve CD4 + T cells and an altered CD4/CD8 balance in depressed common variable immune deficiency (CVID) patients. Is thymosin-α1 a possible treatment? *Int Immunopharmacol* **119**, 110168 (2023). https://doi.org/10.1016/j.intimp.2023.110168
24 Bhikram, T. & Sandor, P. Neutrophil-lymphocyte ratios as inflammatory biomarkers in psychiatric patients. *Brain, behavior, and immunity* **105**, 237–246 (2022). https://doi.org/10.1016/j.bbi.2022.07.006
25 Leday, G. G. R. *et al.* Replicable and Coupled Changes in Innate and Adaptive Immune Gene Expression in Two Case-Control Studies of Blood Microarrays in Major Depressive Disorder. *Biological psychiatry* **83**, 70–80 (2018). https://doi.org/10.1016/j.biopsych.2017.01.021
26 Mostafavi, S. *et al.* Type I interferon signaling genes in recurrent major depression: increased expression detected by whole-blood RNA sequencing. *Molecular psychiatry* **19**, 1267–1274 (2014). https://doi.org/10.1038/mp.2013.161
27 Wittenberg, G. M., Greene, J., Vértes, P. E., Drevets, W. C. & Bullmore, E. T. Major Depressive Disorder Is Associated With Differential Expression of Innate Immune and Neutrophil-Related Gene Networks in Peripheral Blood: A Quantitative Review of Whole-Genome Transcriptional Data From Case-Control Studies. *Biological psychiatry* **88**, 625–637 (2020). https://doi.org/10.1016/j.biopsych.2020.05.006
28 Cole, J. J. *et al.* No evidence for differential gene expression in major depressive disorder PBMCs, but robust evidence of elevated biological ageing. *Translational psychiatry* **11**, 404 (2021). https://doi.org/10.1038/s41398-021-01506-4
29 Sforzini, L. *et al.* Transcriptomic profiles in major depressive disorder: the role of immunometabolic and cell-cycle-related pathways in depression with different levels of inflammation. *Molecular psychiatry* **30**, 1308–1318 (2025). https://doi.org/10.1038/s41380-024-02736-w
30 Erady, C., Bethlehem, R., Bullmore, E. & Lynall, M. E. Systematic Review and Mega-Analysis of the Peripheral Blood Transcriptome in Depression Implicates Dysregulation of Lymphoid Cells and Histones. *Biological psychiatry* **99**, 1130–1140 (2026). https://doi.org/10.1016/j.biopsych.2025.09.008
31 Bekhbat, M., Ulukaya, G. B., Bhasin, M. K., Felger, J. C. & Miller, A. H. Cellular and immunometabolic mechanisms of inflammation in depression: Preliminary

findings from single cell RNA sequencing and a tribute to Bruce McEwen. *Neurobiology of stress* **19**, 100462 (2022). https://doi.org/10.1016/j.ynstr.2022.100462

32 Lu, J. *et al.* Linking peripheral CD8(+) single-cell transcriptomic characteristics of mood disorders underlying with the pathological mechanism. *Clin Transl Med* **11**, e489 (2021). https://doi.org/10.1002/ctm2.489

33 Sun, Z. *et al.* Integrated Single-Cell RNA-seq and ATAC-seq Reveals Heterogeneous Differentiation of CD4(+) Naive T Cell Subsets is Associated with Response to Antidepressant Treatment in Major Depressive Disorder. *Adv Sci (Weinh)* **11**, e2308393 (2024). https://doi.org/10.1002/advs.202308393

34 Patas, K. *et al.* T Cell Phenotype and T Cell Receptor Repertoire in Patients with Major Depressive Disorder. *Frontiers in immunology* **9**, 291 (2018). https://doi.org/10.3389/fimmu.2018.00291

35 First, M. B. in *The Encyclopedia of Clinical Psychology* 1–6.

36 Rush, A. J. *et al.* The 16-Item Quick Inventory of Depressive Symptomatology (QIDS), clinician rating (QIDS-C), and self-report (QIDS-SR): a psychometric evaluation in patients with chronic major depression. *Biological psychiatry* **54**, 573–583 (2003). https://doi.org/10.1016/s0006-3223(02)01866-8

37 Kim, D., Langmead, B. & Salzberg, S. L. HISAT: a fast spliced aligner with low memory requirements. *Nat Methods* **12**, 357–360 (2015). https://doi.org/10.1038/nmeth.3317

38 Cunningham, F. *et al.* Ensembl 2022. *Nucleic Acids Res* **50**, D988–d995 (2022). https://doi.org/10.1093/nar/gkab1049

39 Kramer, A., Green, J., Pollard, J., Jr. & Tugendreich, S. Causal analysis approaches in Ingenuity Pathway Analysis. *Bioinformatics (Oxford, England)* **30**, 523–530 (2014). https://doi.org/10.1093/bioinformatics/btt703

40 Salk, R. H., Hyde, J. S. & Abramson, L. Y. Gender differences in depression in representative national samples: Meta-analyses of diagnoses and symptoms. *Psychol Bull* **143**, 783–822 (2017). https://doi.org/10.1037/bul0000102

41 Wei, G. *et al.* Global mapping of H3K4me3 and H3K27me3 reveals specificity and plasticity in lineage fate determination of differentiating CD4+ T cells. *Immunity* **30**, 155–167 (2009). https://doi.org/10.1016/j.immuni.2008.12.009

42 Henning, A. N., Roychoudhuri, R. & Restifo, N. P. Epigenetic control of CD8(+) T cell differentiation. *Nature reviews. Immunology* **18**, 340–356 (2018). https://doi.org/10.1038/nri.2017.146

43 Lynall, M. E. *et al.* Genetic variants associated with psychiatric disorders are enriched at epigenetically active sites in lymphoid cells. *Nature communications* **13**, 6102 (2022). https://doi.org/10.1038/s41467-022-33885-7

44 Petermann, F. *et al.* The Magnitude of IFN-γ Responses Is Fine-Tuned by DNA Architecture and the Non-coding Transcript of Ifng-as1. *Molecular cell* **75**, 1229–1242.e1225 (2019). https://doi.org/10.1016/j.molcel.2019.06.025

45 Maes, M. *et al.* Increased neopterin and interferon-gamma secretion and lower availability of L-tryptophan in major depression: further evidence for an immune response. *Psychiatry research* **54**, 143–160 (1994). https://doi.org/10.1016/0165-1781(94)90003-5

46 Beaulieu, E. & Morand, E. F. Role of GILZ in immune regulation, glucocorticoid actions and rheumatoid arthritis. *Nat Rev Rheumatol* **7**, 340–348 (2011). https://doi.org/10.1038/nrrheum.2011.59

47 Cohen, S. *et al.* Chronic stress, glucocorticoid receptor resistance, inflammation, and disease risk. *Proceedings of the National Academy of Sciences of the United*

States of America **109**, 5995–5999 (2012). https://doi.org/10.1073/pnas.1118355109

48 Frodl, T. *et al.* Reduced expression of glucocorticoid-inducible genes GILZ and SGK-1: high IL-6 levels are associated with reduced hippocampal volumes in major depressive disorder. *Translational psychiatry* **2**, e88 (2012). https://doi.org/10.1038/tp.2012.14

49 Videbech, P. & Ravnkilde, B. Hippocampal volume and depression: a meta-analysis of MRI studies. *The American journal of psychiatry* **161**, 1957–1966 (2004). https://doi.org/10.1176/appi.ajp.161.11.1957

50 Schmaal, L. *et al.* Subcortical brain alterations in major depressive disorder: findings from the ENIGMA Major Depressive Disorder working group. *Molecular psychiatry* **21**, 806–812 (2016). https://doi.org/10.1038/mp.2015.69

51 Juno, J. A. *et al.* Cytotoxic CD4 T Cells-Friend or Foe during Viral Infection? *Frontiers in immunology* **8**, 19 (2017). https://doi.org/10.3389/fimmu.2017.00019

52 Cenerenti, M., Saillard, M., Romero, P. & Jandus, C. The Era of Cytotoxic CD4 T Cells. *Frontiers in immunology* **13**, 867189 (2022). https://doi.org/10.3389/fimmu.2022.867189

53 Blume, J., Douglas, S. D. & Evans, D. L. Immune suppression and immune activation in depression. *Brain, behavior, and immunity* **25**, 221–229 (2011). https://doi.org/10.1016/j.bbi.2010.10.008

54 Frank, M. G., Hendricks, S. E., Johnson, D. R., Wieseler, J. L. & Burke, W. J. Antidepressants augment natural killer cell activity: in vivo and in vitro. *Neuropsychobiology* **39**, 18–24 (1999). https://doi.org/10.1159/000026555

55 Lai, L. *et al.* Cytotoxic CD4(+) T cells: origin, biological functions, diseases and therapeutic targets. *Signal Transduct Target Ther* **11** (2026). https://doi.org/10.1038/s41392-025-02533-z

56 Bolotin, D. A. *et al.* Antigen receptor repertoire profiling from RNA-seq data. *Nat Biotechnol* **35**, 908–911 (2017). https://doi.org/10.1038/nbt.3979

57 Barennes, P. *et al.* Benchmarking of T cell receptor repertoire profiling methods reveals large systematic biases. *Nat Biotechnol* **39**, 236–245 (2021). https://doi.org/10.1038/s41587-020-0656-3

58 Labonté, B. *et al.* Sex-specific transcriptional signatures in human depression. *Nature medicine* **23**, 1102–1111 (2017). https://doi.org/10.1038/nm.4386

59 Kovats, S. Estrogen receptors regulate innate immune cells and signaling pathways. *Cell Immunol* **294**, 63–69 (2015). https://doi.org/10.1016/j.cellimm.2015.01.018

60 Van de Velde, L. A. & Murray, P. J. Proliferating Helper T Cells Require Rictor/mTORC2 Complex to Integrate Signals from Limiting Environmental Amino Acids. *The Journal of biological chemistry* **291**, 25815–25822 (2016). https://doi.org/10.1074/jbc.C116.763623

61 Delgoffe, G. M. *et al.* The kinase mTOR regulates the differentiation of helper T cells through the selective activation of signaling by mTORC1 and mTORC2. *Nature immunology* **12**, 295–303 (2011). https://doi.org/10.1038/ni.2005

62 Mohammad, I. *et al.* Estrogen receptor α contributes to T cell-mediated autoimmune inflammation by promoting T cell activation and proliferation. *Sci Signal* **11** (2018). https://doi.org/10.1126/scisignal.aap9415

63 Hatzi, K. *et al.* BCL6 orchestrates Tfh cell differentiation via multiple distinct mechanisms. *The Journal of experimental medicine* **212**, 539–553 (2015). https://doi.org/10.1084/jem.20141380

Figure Legend

Figure 1. Study overview and validation of magnetically-enriched CD4$^+$ and CD8$^+$ T-cell fractions. **A** Overview of the study cohort and experimental workflow. Peripheral blood mononuclear cells (PBMCs) were isolated from participants with major depressive disorder (MDD) and healthy controls (HCs), followed by magnetic bead-based enrichment of CD4$^+$ and CD8$^+$ cells and cell type-specific bulk RNA sequencing (RNA-seq). **B** Representative flow cytometric gating strategy. Cells were sequentially selected on the basis of forward- and side-scatter characteristics, singlet gates based on FSC-A versus FSC-H and SSC-A versus SSC-H, exclusion of viability dye-positive cells, selection of CD45$^+$ CD3$^+$ T cells, and separation of CD4$^+$ and CD8$^+$ populations. **C** Representative CD4 versus CD8 staining in total PBMCs, the CD4-depleted and CD8-depleted negative fractions, and the positively-selected CD4$^+$ and CD8$^+$ fractions. **D** Expression of the lineage-associated marker genes *CD4*, *CD8A*, *CD19* and *LILRA5* across the plotted CD4-enriched and CD8-enriched RNA-seq samples. Each column represents one sample, and color indicates $\log_2$(normalized count + 1), as shown in the figure. *CD4* and *CD8A* represent T cell lineage markers, whereas *CD19* and *LILRA5* represent B cell and monocyte/myeloid lineage markers, respectively. Abbreviations: FSC-A, forward-scatter area; FSC-H, forward-scatter height; HC, healthy control; MDD, major depressive disorder; PBMC, peripheral blood mononuclear cell; RNA-seq, RNA sequencing; SSC-A, side-scatter area; SSC-H, side-scatter height.

Figure 2. Differential-expression and pathway analyses of CD4- and CD8-enriched cells in the combined study cohort. A Volcano plot of protein-coding genes and long noncoding RNAs (lncRNAs) in CD4-enriched cells comparing participants with major depressive disorder (MDD) with healthy controls (HCs). A total of 202 genes met the false discovery rate (FDR) threshold of FDR < 0.05, of which 135 had positive and 67 had negative $\log_2$ fold changes. The 25 most significant genes with available gene symbols are labeled. **B** Volcano plot of canonical T cell receptor segment genes in CD4-enriched cells. The displayed subset comprised genes annotated as TR_V_gene, TR_J_gene or TR_C_gene; TCR pseudogenes were excluded. 21 TCR genes met FDR < 0.05, all had positive $\log_2$ fold changes. **C** Volcano plot of protein-coding genes and long noncoding RNAs in CD8-enriched cells; no gene met FDR < 0.05. In panels A-C, positive $\log_2$ fold changes indicate higher expression in MDD than in HCs. Red and blue points indicate significantly increased and decreased expression, respectively; gray points did not meet FDR < 0.05. The dashed horizontal line denotes FDR = 0.05, and the vertical line denotes a $\log_2$ fold

change of zero. Differential expression was assessed using DESeq2 with a negative-binomial generalized linear model including batch, body mass index, sex, age and diagnostic group. Two-sided Wald-test P values were adjusted using the Benjamini-Hochberg procedure. **D** Canonical pathways identified by Ingenuity Pathway Analysis of the 202 FDR-significant protein-coding/lncRNA genes in CD4-enriched cells. **E** Predicted upstream regulators identified from the same gene set. Pathways and upstream regulators are displayed when the IPA overlap P value was < 0.05 and the absolute activation z-score was ≥ 2. In panel D, bar length represents -$\log_{10}$(overlap P value), bar color indicates the predicted activation z-score, and the number at the end of each bar indicates the number of input differentially expressed genes mapped to that pathway. In panel E, tile color represents the predicted activation z-score, with positive and negative scores indicating predicted activation and inhibition, respectively. Abbreviations: FDR, false discovery rate; HC, healthy control; IPA, Ingenuity Pathway Analysis; lncRNA, long noncoding RNA; MDD, major depressive disorder; TCR, T-cell receptor.

Figure 3. Male-stratified differential-expression and pathway analyses of CD4-enriched cells. A Volcano plot of protein-coding genes and long noncoding RNAs (lncRNAs) in CD4-enriched cells from male participants with major depressive disorder (MDD) and male healthy controls (HCs). A total of 487 genes met FDR < 0.05, of which 279 had positive and 208 had negative $\log_2$ fold changes. The 25 most significant genes with available gene symbols are labeled. **B** Volcano plot of canonical T cell receptor segment genes in male CD4-enriched cells. The displayed subset comprised genes annotated as TR_V_gene, TR_J_gene or TR_C_gene; TCR pseudogenes were excluded. Twenty-seven TCR genes met FDR < 0.05, all had positive $\log_2$ fold changes, and the 25 genes with the smallest P values are labeled. In panels A and B, positive $\log_2$ fold changes indicate higher expression in MDD than in HCs. Red and blue points denote significantly increased and decreased expression, respectively; gray points did not meet FDR < 0.05. The dashed horizontal line denotes FDR = 0.05, and the vertical line denotes a $\log_2$ fold change of zero. Male-stratified differential expression was assessed using DESeq2 with a negative-binomial generalized linear model including batch, body mass index, age and diagnostic group. Two-sided Wald-test P values were adjusted using the Benjamini-Hochberg procedure. **C** Canonical pathways and **D** predicted upstream regulators identified by Ingenuity Pathway Analysis (IPA) of the 487 FDR-significant male protein-coding/lncRNA genes in CD4-enriched cells. Results are displayed when the IPA overlap P value was < 0.05 and the absolute activation z-score was ≥2. In panel C, bar length represents $-\log_{10}$(overlap P value); orange and blue

indicate positive and negative activation z-scores, respectively; and the number at the end of each bar indicates the number of input differentially expressed genes mapped to that pathway. In panel D, color represents the predicted activation z-score, with orange indicating predicted activation and blue indicating predicted inhibition. **E** IPA graphical summary integrating selected predicted upstream regulators, canonical pathways and disease/function annotations from the male CD4-enriched cell analysis. Orange and blue nodes denote predicted activation and inhibition, respectively. Orange and blue edges indicate predicted activating and inhibitory relationships; solid and dashed lines denote direct and indirect relationships, respectively; and dotted gray lines denote inferred relationships. Abbreviations: FDR, false discovery rate; HC, healthy control; IPA, Ingenuity Pathway Analysis; lncRNA, long noncoding RNA; MAPK, mitogen-activated protein kinase; MDD, major depressive disorder; MHC, major histocompatibility complex; NF-κB, nuclear factor κB; SASP, senescence-associated secretory phenotype; TCR, T cell receptor; TNFR2, tumor necrosis factor receptor 2.

Supplementary Figure 1. Sex-stratified differential-expression analyses of CD4- and CD8-enriched cells. Volcano plots show protein-coding genes and long noncoding RNAs (lncRNA) in **A** CD8-enriched cells from male participants, where *PDCD11* was the only gene meeting FDR < 0.05, **B** CD4-enriched cells from female participants, with *CCNK* as the only protein-coding/lncRNA gene meeting FDR < 0.05, and **D** CD8-enriched cells from female participants, where *CRTAP* and *PRC1-AS1* met FDR < 0.05. **C** T cell receptor (TCR) segment genes in CD4-enriched cells from female participants; no TCR gene met FDR < 0.05. Sex-stratified differential expression was assessed using DESeq2 with a negative-binomial generalized linear model including batch, body mass index, age and diagnostic group. Two-sided Wald-test P values were adjusted using the Benjamini-Hochberg procedure. The TCR subset in panel C comprised genes annotated as TR_V_gene, TR_J_gene or TR_C_gene; TCR pseudogenes were excluded. Positive $\log_2$ fold changes indicate higher expression in MDD than in HCs. Red and blue points denote significantly increased and decreased expression, respectively; gray points did not meet FDR < 0.05. The dashed horizontal line denotes FDR = 0.05, and the vertical line denotes a $\log_2$ fold change of zero. Abbreviations: FDR, false discovery rate; HC, healthy control; lncRNA, long noncoding RNA; MDD, major depressive disorder; TCR, T cell receptor.

Table 1 Clinical and Sociodemographic Characteristics

	MDD n = 81	**HC n = 48**	**Group tests (Statistics and p-values)**
Age (years) mean (±SD)	33.84 (±11.40)	33.62 (±9.48)	U/W = 2015.5, p = 0.729
	n = 81	n = 48	
Sex n (%)	Females: 49 (60.49%)	Females: 18 (37.50%)	χ^2 = 5.5, p = 0.0191
	n = 81	n = 48	
Ethnicity n (%)	Hispanic: 14 (17.5%) Not Hispanic: 63 (78.8%) Unknown: 3 (3.8%)	Hispanic: 11 (22.9%) Not Hispanic: 33 (68.8%) Unknown: 4 (8.3%)	χ^2 = 2.00, p = 0.367
	n = 80	n = 48	
Current psychotropic medication n (%)	Yes: 37 (48.68%)	Yes: 0 (0%)	**χ^2 = 33.31, p < 0.001**
	n = 76	n = 48	
BMI (kg/m^2) mean (±SD)	26.65 (±6.07)	25.52 (±3.77)	U = 1745, p = 0.802
	n = 78	n = 46	
QIDS mean (±SD)	13.96 (±4.64)	1.44 (±1.43)	**U = 4, p < 0.001**
	n = 73	n = 48	
hs-C-reactive protein (mg/L) mean (±SD)	3.03 (±6.20)	1.61 (±2.57)	**U/W = 1403.5, p = 0.0185**
	n = 78	n = 48	
Lymphocyte # (x10^3 cells/μL) mean (±SD)	1.89 (±0.59)	1.97 (±0.60)	t = 0.77, p = 0.445
	n = 76	n = 47	

BMI = body mass index; F = F statistic from two-way ANOVA; HC = healthy controls; hs = high-sensitivity; MDD = major depressive disorder; QIDS = Quick Inventory of Depressive Symptomatology; SD = standard deviation; χ^2 = Pearson's chi-square statistic. All tests are two-tailed; significant results (p < 0.05) are shown in bold.

Supplementary Table 1. Comparison of clinical and sociodemographic characteristics between study participants with major depressive disorder (MDD) and healthy controls (HC) by sex.

	MDD Male n = 32	HC Male n = 30	MDD Female n = 49	HC Female n = 18	Group tests (Statistics and p values)
Age (years) mean (±SD)	36.22 (±13.23)	34.10 (±9.02)	32.29 (±9.87)	32.83 (±10.43)	Group F(1,125) = 0.012, p = 0.913; Sex F(1,125) = 2.332, p = 0.129; Interaction F(1,125) = 0.442, p = 0.507
	n = 32	n = 30	n = 49	n = 18	
Ethnicity n (%)	Hispanic: 2 (6.45%) Not Hispanic: 27 (87.10%) Unknown: 2 (6.45%)	Hispanic: 8 (26.67%) Not Hispanic: 18 (60.00%) Unknown: 4 (13.33%)	Hispanic: 12 (24.5%) Not Hispanic: 36 (73.5%) Unknown: 1 (2.0%)	Hispanic: 3 (16.7%) Not Hispanic: 15 (83.3%) Unknown: 0 (0%)	$\chi^2 = 11.362$, p = 0.078
	n = 31	n = 30	n = 49	n = 18	
Current psychotropic medication n (%)	Yes: 12 (41.38%)	Yes: 0 (0%)	Yes: 25 (53.19%)	Yes: 0 (0%)	$\chi^2 = 34.50$, p < 0.001 Male MDD vs. Female MDD: $\chi^2 = 1.00$, p = 0.317
	n = 29	n = 30	n = 47	n = 18	
BMI (kg/m^2) mean (±SD)	26.33 (±4.81)	26.61 (±3.23)	26.85 (±6.78)	23.48 (±3.95)	Group F(1,120) = 1.305, p = 0.256; Sex F(1,120) = 0.647, p = 0.423; Interaction F(1,120) = 3.167, p = 0.078
	n = 30	n = 30	n = 48	n = 16	
QIDS mean (±SD)	13.37 (±4.66)	1.53 (±1.50)	14.30 (±4.64)	1.28 (±1.32)	Group F(1,117) = 326.477, **p < 0.001;** Sex F(1,117) = 0.431, p = 0.513; Interaction F(1,117) = 0.689, p = 0.408
	n = 27	n = 30	n = 46	n = 18	
hs-C-reactive protein mean (±SD)	1.881 (±3.30)	1.08 (±1.17)	3.79 (±7.47)	2.50 (±3.82)	Group F(1,122) = 2.288, p = 0.133; Sex F(1,122) = 3.415, p = 0.067; Interaction F(1,122) = 0.062, p = 0.804
	n = 31	n = 30	n = 47	n = 18	
Lymphocyte # (x10^3 cells/µL) mean (±SD)	1.86 (±0.55)	1.81 (±0.61)	1.90 (±0.61)	2.23 (±0.50)	Group F(1,119) = 0.615, p = 0.434; Sex F(1,119) = 3.167, p = 0.078; Interaction F(1,119) = 2.920, p = 0.090
	n = 30	n = 29	n = 46	n = 18	

BMI = body mass index; F = F statistic from two-way ANOVA; HC = healthy controls; hs = high-sensitivity; MDD = major depressive disorder; QIDS = Quick Inventory of Depressive Symptomatology; SD = standard deviation; χ^2 = Pearson's chi-square statistic. All tests are two-tailed; significant results (p < 0.05) are shown in bold.

Supplementary Files

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- AdaptiveImmuneSupplementaryFile21.xlsx
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- AdaptiveImmuneSeqTable1TableS11.docx
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- AdaptiveImmuneSupplementaryFile11.xlsx
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