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Microglial function interacts with the environment to affect sex-specific depression risk

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ABSTRACT

There is a two-fold higher incidence of depression in females compared to men with recent studies suggesting a role for microglia in conferring this sex-dependent depression risk. In this study we investigated the nature of this relation. Using GWAS enrichment, gene-set enrichment analysis and Mendelian randomization, we found minimal evidence for a direct relation between genes functionally related to microglia and sex-dependent genetic risk for depression. We then used expression quantitative trait loci and single nucleus RNA-sequencing resources to generate polygenic scores (PGS) representative of individual variation in microglial function in the adult (UK Biobank; N = 54753–72682) and fetal (ALSPAC; N = 1452) periods. The adult microglial PGS moderated the association between BMI (UK Biobank; beta = 0.001, 95 %CI 0.0009 to 0.003, P = 7.74E-6) and financial insecurity (UK Biobank; beta = 0.001, 95 %CI 0.005 to 0.015, P = 2E-4) with depressive symptoms in females. The fetal microglia PGS moderated the association between maternal prenatal depressive symptoms and offspring depressive symptoms at 24 years in females (ALSPAC; beta = 0.04, 95 %CI 0.004 to 0.07, P = 0.03). We found no evidence for an interaction between the microglial PGS and depression risk factors in males. Our results illustrate a role for microglial function in the conferral of sex-dependent depression risk following exposure to a depression risk factor.

1. Introduction

In 2019 depression was the second leading contributor to years lived with disability worldwide (Ferrari, 2022), with this burden only increasing during the COVID-19 pandemic (Santomauro et al., 2021). The economic burden of depression is as dramatic, with annual costs estimated at $210.5 billion in the United States alone (Greenberg et al., 2015). These costs are disproportionately shouldered by women, with estimates of depression incidence indicating a 2-fold higher rate of depression in women over men (Ferrari, 2022; Kuehner, 2017; Seedat et al., 2009). Somewhat curiously the mechanisms behind these sex-differences are not well studied despite evidence for sex-specific pathways (Kendler et al., 2002; Silveira et al., 2023).

Even the highest estimates of overall depression heritability fall well below 50 % (Polderman et al., 2015) illustrating a strong role of the environment in depression etiology (Flint, 2023). Epidemiological studies consistently show strong links between depression and adverse experiences in both early life and adulthood, which persist across cultures, sex, gender and ancestry (Hammen, 2004; Flentje et al., 2020; Otte et al., 2016). These studies highlight the many exposures that act to increase depression risk, including perinatal exposures (e.g. exposure to prenatal maternal depression (Pearson et al., 2013; O'Donnell et al., 2014; Fitzgerald et al., 2021), adult exposures (e.g. financial insecurity (Guan et al., 2022) and physiological measures (e.g. BMI (Otte et al.,

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2016; Luppino et al., 2010).

How these diverse environmental exposures translate to increased depression risk and why this occurs with such sex-specificity is largely unclear. Much of the research in this area is performed using animal models where elegant studies have identified neuronal plasticity as an important regulator of the behavioural response to chronic stress and the subsequent therapeutic attenuation of these behaviours (Wang et al., 2022). Glial cells comprise the majority of cells in the brain and have a critical role in many aspects of neuronal plasticity (Cathomas et al., 2022), but their role in the behavioural response to adverse environments is understudied. Even fewer studies have investigated the role of glia in human depression risk. Nevertheless, several streams of evidence suggest a microglial contribution, particularly with respect to sex-dependent depression risk.

Microglia satisfy several criteria as a mechanism for sex-dependent depression risk. First, they are exceptionally responsive to the environment (Cathomas et al., 2022; Mondelli et al., 2017), with well-characterized homeostatic roles in regulating synapses (Paolicelli et al., 2011), neuronal function (Badimon et al., 2020) and myelination (Hughes and Appel, 2020). Second, in model systems microglia display sexual dimorphisms in their morphology, distribution, electrophysiology, transcriptome and proteome (Colombo et al., 2022; Lenz et al., 2013; Guneykaya et al., 2018; Villa et al., 2018). Although less studied, transcriptomic differences between male and female microglia are also seen in humans (Young et al., 2021). Third, alterations in microglia drive the behavioural response to both early life and adult stresses in animal models (Liu et al., 2019; Block et al, 2022; Calcia et al., 2016; Cao et al., 2021). Fourth, histological, *in vivo* imaging and transcriptomic approaches show microglial alterations in humans with depression (Mondelli et al., 2017; Maitra et al., 2022; Setiawan et al., 2015; Torres-Platas et al., 2014; Böttcher et al., 2020; Snijders et al., 2021). However, despite this evidence the nature of microglial involvement in sex-dependent depression risk remains unclear.

2. Methods and materials

2.1. Microglia-enriched gene identification

Microglial enriched genes for the adult PGS were obtained from Tran *et al.*, 2021. To identify enriched genes we first downloaded the normalized single-cell object (https://github.com/LieberInstitute/10xPilot_snRNAseq-human) and converted to a Seurat object. Using the cell-type assignation from Tran *et al*, we used the FindMarkers() function in Seurat v4.1.1 (Hao et al., 2021), with a minimum expression in 25 % of cells, to define genes enriched in microglia. A gene with a positive fold change and an adjusted P-value < 0.05 was defined as being enriched.

Microglial enriched genes for the fetal period were obtained from Polioudakis *et al.*, 2019 (Polioudakis et al., 2019). In their study enriched genes were defined through a linear regression of cell clusters using donor and batch as covariates in the model. Following correction for multiple comparisons using the Benjamini-Hochberg method, microglial-enriched genes were defined as those with a log fold change greater than 0.1 and an FDR < 0.05.

A full list of the genes identified by this approach and used to create all microglia PGSs are given in **Supplementary Table 1** and **Supplementary Table 7**.

2.2. Expression of microglial enriched genes in Olah et al dataset

Log normalized and filtered data from Olah *et al* (Olah et al., 2020) were downloaded from the CellXGene database. Only microglial clusters were retained and the AddModuleScore() function from Seurat (Hafemeister and Satija, 2019) was used to assess the expression of our microglial enriched genes in specific microglial states.

2.3. Gene ontology (GO) enrichment

Enrichment of gene lists for GO enrichment was done using clusterProfiler v4.2.2 (Yu et al., 2012) with the enrichGO() function. Enrichment was tested for all GO terms in the molecular function,

biological process, cellular component categories in a combined analysis. All brain expressed genes defined from the full count matrix of the relevant snRNA-seq dataset were used as background in the analysis.

2.4. Transcription factor enrichment analysis

Transcription factor enrichment analysis was conducted using ChEA3 online portal (Keenan et al., 2019). The mean rank metric was used to infer transcription factor associations which was previously shown to have the greatest biological relevance (Keenan et al., 2019).

2.5. MAGMA and HMAGMA

We conducted GWAS enrichment using MAGMA v1.10 (de Leeuw et al., 2015), with GWAS annotated to the gene level either using H-MAGMA (Sey et al., 2020) or the standard approach using genomic windows within MAGMA. For H-MAGMA we used Hi-C data from the DLPFC (Wang et al., 2018), obtained from https://zenodo.org/record/6382668#.Y9PSG3bMJPY. For the SNP to gene annotation, we used SNP annotations from the 1000 genomes European dataset and gene annotations from the NCBI website build 38. We then used MAGMA to test for enrichment using the default settings.

2.6. Mendelian randomization

We conducted Mendelian randomization analysis using the TwoSampleMR v0.5.6 package (Hemani et al., 2018). Independent *cis*-eQTLs from the GTEx v8 anterior cingulate (BA25) were downloaded from the GTEx catalogue (Aguet et al., 2020) and SNP location was converted to rsID using the GTEx lookup table. We did not consider *trans*-eQTLs as they often have weak, indirect effects making their use as instrumental variables in Mendelian randomization analysis problematic. We used the TwoSampleMR package to harmonise effect alleles and analysed the effect of our instrumental variables on the various depression GWAS using the inverse variance weighted, weighted median, weighted mode, MR RAPS and MR Egger methods. We used the mr_leaveoneout() function to carry out leave-one out analysis, and the run_mr_presso() wrapper function to run MR-PRESSO (Verbanck et al., 2018) within the TwoSampleMR package with 10,000 bootstrap iterations.

2.7. GTEx analysis

GTEx v8 data were analyzed as described in a previous report (Fitzgerald et al., 2023). In brief GTEx v8 data were downloaded from the GTEx portal or dbGaP (phs000424.v9.p2) (Aguet et al., 2020). To facilitate cross regional comparison, genes were filtered to retain those expressed in all regions. Samples with a positive indication of the following conditions were removed from the analysis: Alzheimer's OR Dementia, Dementia With Unknown Cause, Documented Sepsis, Reyes Syndrome, Parkinson's Disease, Cerebrovascular Disease (stroke, TIA, embolism, aneurysm, other circulatory disorder affecting the brain), Amyotrophic Lateral Sclerosis, Influenza (acute viral infection including avian influenza), Bacterial Infections (including septicaemia, Unexplained seizures, Positive blood cultures, staphylococcal infection, streptococcus, sepsis), Schizophrenia, Pneumonia, Prescription pill abuse, Heroin Use, Current Diagnosis Of Cancer, Active Encephalitis, Multiple Sclerosis, Systemic Lupus, MDD, meningococcal disease or Alzheimer's. Data were then filtered (0.1 counts per million in more than 25 % of samples) and normalized using the TMM method and log normalization in DESeq2 v1.34.0. Outliers were removed if they had a connectivity Z-score greater than 3 standard deviations from the mean.

We then conducted a principal component analysis of 32 sequencing metrics and the top 10 principal components, RNA integrity number, Hardy scale, interval of onset to death, body refrigeration, sex and age were regressed from the data using a linear mixed effects model with subject as a random intercept term with the lme4 v1.1–31 package (Bates et al., 2015).

2.8. Single sample geneset enrichment analysis (ssGSEA)

We implemented ssGSEA through the Gene Set Variation Analysis (GSVA) package (Hänzelmann et al., 2013). We used the normalized and regressed GTEx v8 dataset and the microglial enriched genes from Tran *et al* as input to the analysis.

2.9. Lassosum PRS generation

Within the GTEx cohort we built polygenic scores using the lassosum package (Mak et al., 2017) and depression summary statistics from sex-specific depression GWAS (Silveira et al., 2023) or a sex-combined GWAS (Howard, 2019). PGS were generated using the standard pipeline with LD blocks from the European ancestry of the 1000 genomes dataset as defined by Berisa and Pickerall 2016 (Berisa and Pickrell, 2016). The pseudovalidate() function was then used to estimate the preferred threshold in the absence of a test population.

2.10. Cohorts

The UK Biobank is a UK-based population cohort of adults, and represented the largest cohort with relevant phenotype information in which we could test our hypothesis. Ethical approval for this cohort was obtained from the Northwest Multicentre Research Ethics Committee (REC reference 11/NW/0382), the National Information Governance Board for Health and Social Care and the Community Health Index Advisory Group. All participants provided informed written consent before data collection. The research in this manuscript was conducted under application number 41975.

ALSPAC is a UK-based longitudinal birth cohort from the southwest of England, and was also among the largest cohort with relevant phenotype and genotype data in which we could test our hypothesis. Pregnant women within the Avon area, with estimated delivery dates between April 1st, 1991 and December 31st, 1992 were invited to participate (Fraser, 2013; Boyd et al., 2013; Northstone, 2019). There were 14,541 pregnancies initially enrolled in the study, resulting in 14,676 foetuses from 14,062 live births, of which 13,988 children were alive at 1 year of age. ALSPAC data were collected and managed using REDCap electronic data capture tools hosted at the University of Bristol. REDCap (Research Electronic Data Capture) is a secure, web-based software platform designed to support data capture for research studies (Harris et al., 2009). Ethical approval for the ALSPAC study was granted by the ALSPAC Ethics and Law Committee and local research ethics committees (full list available at http://www.bristol.ac.uk/alspac/researchers/research-ethics/). Informed consent was obtained from participants in line with these approved ethical guidelines. Full details of ALSPAC data are searchable at: http://www.bristol.ac.uk/alspac/researchers/our-data/.

Throughout this study, sex was defined genetically. Analysis in ALSPAC cohorts were confined to term, singleton births. See **Supplementary Table 11** for a description of cohort demographics.

2.11. Edinburgh postnatal depression scale (EPDS)

The EPDS was used to assess levels of prenatal depressive symptoms in ALSPAC cohorts. The EPDS is a 10-point self-report questionnaire widely used to screen for perinatal depression (Paul and Pearson, 2020; Cox et al., 1987). Each item of the EPDS is scored on a 0–3 scale and the cumulative total of all responses was used as our exposure. In ALSPAC, the EPDS was administered at 18- and 32-weeks post-conception, the average of which was used in our analysis.

2.12. Clinical interview Schedule (CIS-R)

The CIS-R is a self-administered computer-based questionnaire, which serves as a proxy for a clinical interview in the diagnosis of mental health disorders (Lewis et al., 1992; Patton et al., 1999). It was administered to ALSPAC offspring participants at 24 years of age during an in-person visit between June 2015 and October 2017. The CIS-R can assess a range of mental health outcomes, including anxiety and depression. In the CIS-R the frequency and severity of outcomes are assessed, permitting the classification of mild, moderate, or severe depression (corresponding to variables FKDQ1000, FKDQ1010 and FKDQ1020 in the ALSPAC catalogue). In our study a positive indication in any of these depression criteria warranted "case" status, with other individuals designated as controls.

2.13. UK Biobank exposures and outcomes

The UK Biobank mental health follow-up questionnaire was administered in 2016, by August 2017 157,366 individuals had responded. It consisted of a series of questionnaires based around both risk factors and outcomes for mental health (described by Davis *et al.*, 2020). We used the answers to 3 of these questionnaires as part of our study:

1. The post-traumatic stress disorder checklist (civilian short version; PCL-6; UK Biobank ID f20526-f20531) (Ruggiero et al., 2003), to assess traumatic life events. Responses to these questions were used as exposures in our study. The questionnaire consists of 6 questions, with "Yes, but not in the last 12 months", "Yes, within the last 12 months", "Never" or "Prefer not to answer" being the possible answers. Any answer of Yes was coded as 1, with Never being 0 and "Prefer not to say" was coded as NA.
2. A custom questionnaire designed to assess stressful life events (UK Biobank ID f20521-20525). Responses to these questions were used as exposures in our study. The questionnaire consists of 5 questions with "Very often true", "Often", "Sometimes true", "Rarely true", "Never true" or "Prefer not to answer" as possible answers. We coded these outcomes so 4 was the objectively more stressful outcome and "Prefer not to say" was coded as NA. For instance, in response to "There was money to pay the rent or mortgage when I needed it", the response "Never" was coded 4 and "Very often true" was coded 0. Whereas responses to "A partner or ex-partner repeatedly belittled me to the extent that I felt worthless" were coded in reverse, with the response "Never" coded as 0 and "Very often true" coded as 4.
3. The patient health questionnaire 9 (PHQ9; UK Biobank ID f20507, f20508, f20510, f20511, f20513, f20514, f20517- f20519), which measures current symptoms of depression based on the symptoms described in the DSM (Levis et al., 2019). The response to each question was used as an outcome in the analysis. Possible answers to questions were "Prefer not to answer", "Not at all", "Several days", "More than half the days" or "Nearly every day" and were coded as NA ("Prefer not to say") or from 0 to 3, respectively. A cumulative score was generated by summing an individual's coded response to each question.

A causal role for body mass index (BMI) in depression has previously been described (Tyrrell et al., 2019). Therefore we also including BMI as an exposure in our analysis. This was measured on the first in person visit from 2006 to 2010, where participants also gave consent. BMI was calculated based on height and weight measurements at this visit and we use BMI with the units Kg/m^2.

2.14. Genotyping

Genotyping was performed in ALSAPC using the Illumina HumanHap550 quad-chip genotyping platform by the Wellcome Trust Sanger Institute, Cambridge, UK and the Laboratory Corporation of America, Burlington, NC, US (Richmond et al., 2017). Samples discordant for reported and genetic sex, those with abnormal heterozygosity, > 3 % missingness or inadequate sample replication (IBD < 0.8) were removed from the analysis. Any SNP with a minor allele frequency < 1 %, evidence of Hardy-Weinberg equilibrium violation (P-value threshold of p < 5e-7), those with evidence for cryptic relatedness or a call rate < 95 % were removed from the analysis. Imputation was conducted with Impute v3 and Haplotype Reference Consortium (HRC) imputation reference panel (release 1.1). The population structure of the ALSPAC cohort was evaluated with PLINK 1.9 using PCA, which was conducted on the genotyped autosomal SNPs with MAF > 5 %, not in high linkage disequilibrium $r^2 < 0.20$ across 100 kilobases region, a 5 SNPs step size and a threshold of 1.01 for variance inflation factor. To account for population stratification, the first ten PCs were included in the ALSPAC analyses. Gestational age at birth was also included as a covariate in the ALSPAC analyses.

In the UK Biobank genotyping was conducted with the Applied Biosystems UK Biobank Axiom Array or the closely related Applied Biosystems UK BiLEVE Axiom Array. A comprehensive description of the genotyping and quality control for the UK Biobank is available in Bycroft et al. (2018). Participants that withdrew consent, without genotyping data, genetic/reported sex mismatch, had a genetic kinship to another participant >0.044 or that were an outlier for heterozygosity were removed. A single participant from each genetic kinship group was then returned to the analysis (genetic relatedness <0.025 calculated using Genome-wide Complex Trait Analysis GCTA 1.93.2). The population structure in UK Biobank was assessed using fastPCA method (Galinsky et al., 2016) for principal component analysis and the first 40 PCs were included as covariates in all UK Biobank analyses. Other measured included as covariates in the UK Biobank analysis include participant age, assessment centre attended, and genotyping array used.

2.15. Microglial polygenic score (PGS) generation

We generated cell-type PGS by identifying eQTLs associated with microglia-enriched genes (described in "*Microglia-enriched gene identification*"). We reasoned those genes expressed at higher levels in microglia compared to other cell-types, would be involved in microglial-specific functions and that variation in the expression of these genes would, therefore, be a proxy of microglial function. We used eQTL data, from either Walker et al., 2019 (fetal cortex) or Aguet et al. (2020) (adult anterior cingulate cortex) to identify functional variants associated with variation in the expression of these microglial enriched genes. We identified these eQTLs in the individuals and weighted them based on their effect size for the relevant gene (as identified in the original eQTL study), before summing them to create a PGS for each individual.

In brief, SNPs associated with microglia enriched gene lists were identified using the biomaRt package (Durinck et al., 2009; Durinck et al., 2005) and the GRCh37.p13 assembly. SNPs common with the eQTL study (Walker et al., 2019 or Aguet et al., 2020) and each cohort were then retained and subjected to linkage disequilibrium clumping ($r^2 < 0.2$). The number of effect alleles at a particular locus was weighted by the effect size on expression from the eQTL study. This was done at all loci in a given gene list that were retained after clumping, before summation to generate the PGS.

2.16. Statistical analysis

All analyses, unless otherwise stated, were conducted with R v4.1.1 (R Core Team, 2021) and RStudio v1.4.1717 (RStudio Team, 2020). Linear and logistic regression analyses were performed using the lm() or glm() functions. We used the influence.measures() function in R to identify samples with undue influence on the regression results. Any samples with excessive influence were removed and a new regression model was calculated. This was not done in the UK Biobank analysis due to the large sample size. Specific covariates and sample size for each comparison are mentioned in the results. If a significant interaction was found in the regression model, a simple slopes analysis was performed using the Interactions package (Bauer et al., 2021). PGS were treated as continuous variables in all analyses. Permutation testing was conducted using 1000 iterations, samples were shuffled using the sample() function in R. Where appropriate correction for multiple comparisons was done using the Benjamini- Hochberg method (false discovery rate; FDR). Plotting was done using either the Interactions package or ggplot2 (Wickham, 2016). An alpha level of 0.05 for statistical significance was used throughout.

3. Results

3.1. No evidence for a direct genetic association between microglial function and depression

We hypothesised microglial function associates with sex-dependent depression risk (Fig. 1). First, we tested for a direct association of microglial function with sex-dependent depression risk. We utilized a scRNA-seq dataset from the subgenual anterior cingulate cortex (Tran et al., 2021) (sgACC) to study the association between microglial function and depression. Although the neurobiology of depression certainly encompasses many brain regions, we selected the sgACC as studies using deep brain stimulation provide convincing evidence for a causal role in depression (Alagapan et al., 2023; Drevets et al., 2008; Morris et al., 2020). We structured our approach around the consistent finding from many elegant studies showing that genes enriched in a particular cell type are involved in specific functions that are characteristic of that cell type (Scala et al., 2020; Siletti, et al., 2022). We reasoned that if there were a direct relation between microglial function and depression risk, there would be an association between microglia enriched genes and depression GWAS. Accordingly, we identified 923 genes enriched in sgACC microglia (**Supplementary Table 1**), of the Tran *et al* dataset, for further analysis. These genes were expressed broadly across microglial subsets (**Supplementary** Fig. 1), enriched for inflammation related gene ontology terms (**Supplementary** Fig. 2) and transcription factors (**Supplementary Table 2**). Together these data suggest the genes are generally representative of microglial function rather than a specific microglial state.

We first asked if these microglial-enriched genes were associated with depression in a sex-specific (Silveira et al., 2023) or sex-combined (Howard, 2019) GWAS. We used MAGMA (de Leeuw et al., 2015) to annotate GWAS loci to the gene level using 3 different genetic windows (+/-0Kb, +/-20 Kb or +/-50 Kb) or using chromatin interaction (Hi-C) data from the frontal cortex (as implemented in H-MAGMA (Sey et al., 2020). We then asked if the sgACC microglial enriched genes were associated with any of these GWAS, but did not find evidence for an enrichment in either the sex-specific or sex-combined depression GWAS (Fig. 2**A**).

We next used Mendelian randomization, a widely used method for causal inference (Davies et al., 2018), to estimate the association between microglial-enriched genes and the sex-specific or sex-combined depression GWAS. We used *cis*-eQTLs for the microglial-enriched genes from the ACC of the GTEx v8 study as instrumental variables. We used five distinct, complementary methods of Mendelian randomization to estimate the effect of these instrumental variables on sex-specific and sex-combined depression GWAS and found no evidence for an association (Fig. 2**B**). Leave-one out analysis and MR-PRESSO suggested these results were not affected by instrument heterogeneity or horizontal pleiotropy (**Supplementary** Fig. 3; **Supplementary Table 3**).

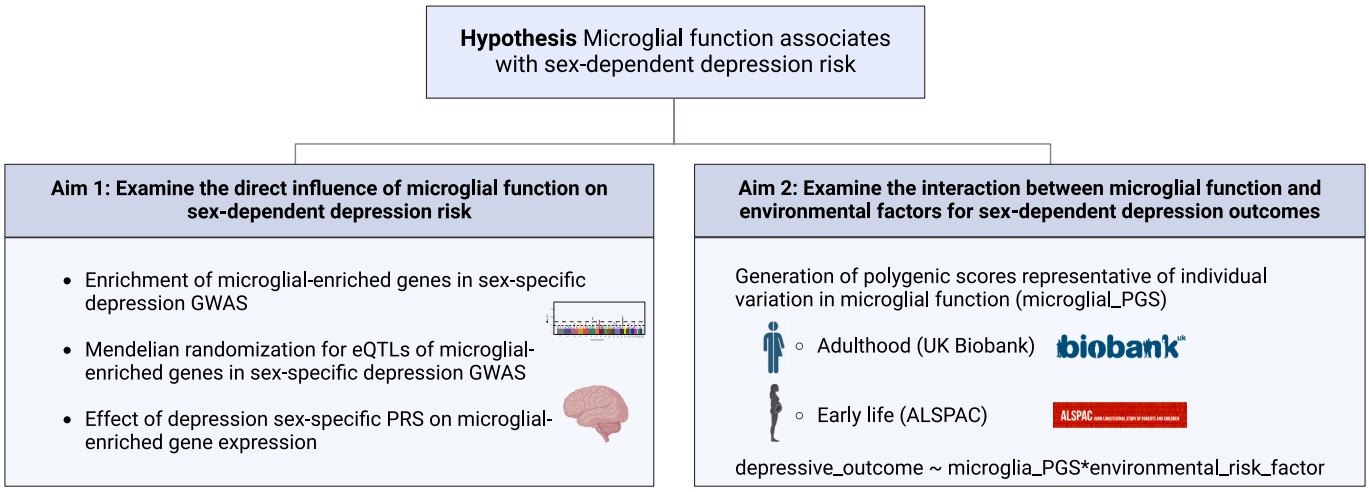

Fig. 1. Schematic of study outline.

We next approached this question from a different perspective and asked if PGSs generated from the sex-specific or sex-combined GWAS associated with expression of the microglial enriched genes. We postulated that if a relation existed, a PGS generated from a depression (sex-combined or sex-specific) GWAS would correlate with the expression of microglial-specific genes in a sex-specific fashion. We tested this hypothesis by constructing a PGS using the sex-specific or sex-combined depression GWAS for all subjects of the GTEx v8 study with a penalized regression method (Mak et al., 2017). We used single sample geneset enrichment analysis (ssGSEA) to evaluate the expression of the microglial enriched genes. This method allowed us to create a comparable expression metric for the microglial-enriched genes within each sample. We then correlated the sex-specific or sex-combined PGS and the ssGSEA scores for each sample, grouping our analysis by brain region, PGS and sex. No correlations passed correction for multiple comparisons (Fig. 2**C**), suggesting a negligible relation between depression PGS and the expression of microglial enriched genes.

Together these results provide little evidence for a direct association between microglial-enriched genes and sex-specific or sex-combined depression GWAS. However, a fundamental role of microglial function is in response to the environment. We therefore hypothesized that rather than a direct effect, microglial function may interact with depression risk factors to modify depression outcomes in a sex-specific manner.

3.2. A microglial PGS moderates the relation of depression risk factors on depressive symptoms

Adult exposures such as high BMI (Tyrrell et al., 2019; Speed et al., 2019) or stressful life events (Otte et al., 2016) in adulthood are well-characterized risk factors for depression. To test the hypothesis that microglial function moderates this association, we turned to expression-based polygenic scores (Silveira et al., 2017). This method integrates genotype data (e.g. from the UK Biobank) and eQTL data (e.g. from GTEx), to create a genetic surrogate for the expression of a set of genes. We previously showed this method captures individual variation in gene expression (Fitzgerald et al., 2023) and, therefore, reasoned that the adult microglial PGS would serve as a genetic surrogate for the expression of microglial enriched genes and downstream microglial function.

Our analysis required the risk factor (e.g., a stressful experience) to occur before the evaluation of depression, this precluded us from using the most common definition of depression in the UK Biobank, lifetime incidence. We instead used answers to the patient health questionnaire 9 (PHQ9) a well-validated (Davis et al., 2020; Sun et al., 2020; Levis et al., 2019) measure of current depression, as our outcomes of interest. This measure was advantageous for two primary reasons. First, it was administered concurrently with the questionnaires querying stressful life exposures providing the required temporal ordering of events. Second, it enabled an analysis of the nine DSM symptoms of depression. We conducted multivariable regression to estimate the interaction between BMI or stressful life events and the adult microglial PGS with the first 40 principal components, age, assessment center attended, and genotype array used as covariates in the analysis. All analyses were stratified by sex and we used the individual answers to the nine questions of the PHQ9 and their cumulative score as continuous outcomes.

Four exposures in females showed a significant interaction (FDR P-value < 0.05) with the microglial PGS (Fig. 3**A**; **Supplementary Table 4**). In contrast, no interactions passed the correction for multiple comparisons in males (**Supplementary Table 5**). Of the significant female interaction effects, BMI was the exposure for the significant interactions, when anhedonia (β coefficient $= 0.001$, 95 %CI 0.0009 to 0.003, P = 7.74E-6, FDR P value $= 0.004$) or tiredness (β coefficient $=$ 0.002, 95 %CI 0.0008 to 0.003, P $= 0.001$, FDR P value $= 0.04$) were the outcomes. An ability to pay rent or mortgage was the exposure for the remaining two significant interactions, when anhedonia (β coefficient $=$ 0.01, 95 %CI 0.006 to 0.016, P = 7.74E-5, FDR P value $= 0.007$) or depressed mood (β coefficient $= 0.001$, 95 %CI 0.005 to 0.015, P = 2E-4, FDR P value $= 0.008$; Fig. 3**A**) were used as the outcomes. Permutation testing suggested these associations were robust (**Supplementary Fig. 4**).

A simple slopes analysis was then conducted on the four regression models with a significant interaction (Fig. 3**B-E**), which showed significant associations for both the higher and lower PGS slopes in all four models (see Fig. 3**B-E** and **Supplementary Table 4**; **Supplementary Table 6**). These results suggest that adult microglia bidirectionally modulate the risk of specific depressive symptoms in the presence of specific risk factors only in females.

3.3. A microglia PGS moderates the association of prenatal maternal depressive symptoms on offspring depressive outcomes at 24 years

Depression has a peak onset in late adolescence to early adulthood (Ferrari, 2022), and many of the most potent depression risk factors are early life events (O'Donnell and Meaney, 2017). Prenatal maternal depression is one such major risk factor for offspring depression (Pearson et al., 2013; O'Donnell and Meaney, 2017), which has a stronger association in daughters (Quarini et al., 2016). We hypothesized that microglial function would also moderate the relation between prenatal maternal depressive symptoms and offspring depression in a sex-specific manner. To test this hypothesis, we identified microglial-enriched genes using a scRNA-seq study of the mid-gestational fetal cortex (gestational

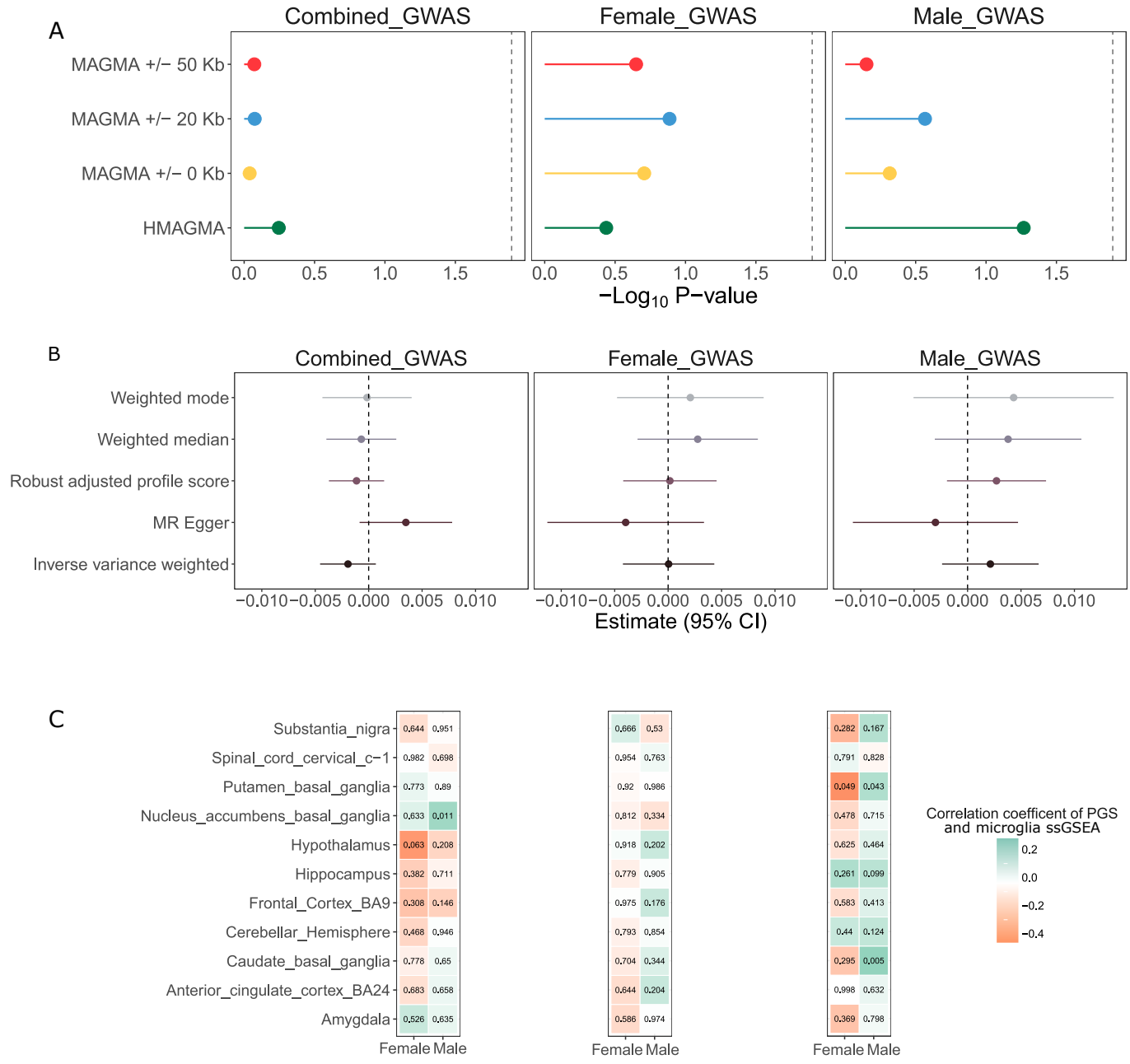

Fig. 2. There is little evidence for a direct association between microglial function and depression GWAS. A) Enrichment of a microglial expression signature from the subcallosal anterior cingulate cortex in combined (males and females), female-specific or male-specific depression GWAS. GWAS were annotated to the gene level using either a window approach implemented in MAGMA (using windows of +/-0, +/-20 or +/-50 Kb; red, blue or yellow colors, respectively) or by H-MAGMA using Hi-C data from the human frontal cortex (green). The x-axis represents the −log10 (P-value). B) Mendelian randomization for an association of eQTLs for microglial enriched genes with combined (males and females), female-specific or male-specific depression GWAS. The x-axis shows the estimate of the association with accompanying 95 % confidence intervals. The y-axis shows five distinct methods for Mendelian randomization. C) Polygenic scores (PRS), generated from sex-combined, female-specific or male-specific GWAS of depression, were correlated with the single sample gene set enrichment score of microglial enriched genes. Correlations are split by sex (x-axis) and specific GTEx CNS region (y-axis). The tiles are colored by the correlation coefficient. The unadjusted P-value is presented within each tile. No correlations passed correction for multiple comparisons. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

week 17–18; (Polioudakis et al., 2019) **Supplementary Table 7**) and used eQTL data from Walker *et al* (an eQTL study of mid-gestational cortex) to generate a PGS representative of individual variation in microglial-enriched gene expression for offspring of the ALSPAC cohort (Fraser, 2013; Boyd et al., 2013; Northstone, 2019). These fetal microglial genes showed moderate overlap with the adult enriched genes (280 common genes; 480 unique fetal enriched genes; **Supplementary Figure 5**). Go terms related to cytokine secretion and response to the environment were enriched in the fetal microglial enriched genes (**Supplementary Figure 6**). These genes were also enriched for several transcription factors involved in homeostatic function of microglia (**Supplementary Table 8**). Therefore, these data suggest that the fetal microglia enriched genes, like the adult enriched genes, were broadly representative of general microglial functionality.

We explored the interaction of the fetal microglial PGS with prenatal maternal depressive symptoms, measured using the Edinburgh postnatal depression scale (EPDS; in which a score above 10 indicates clinically relevant depressive symptoms (O'Connor et al., 2016). We used

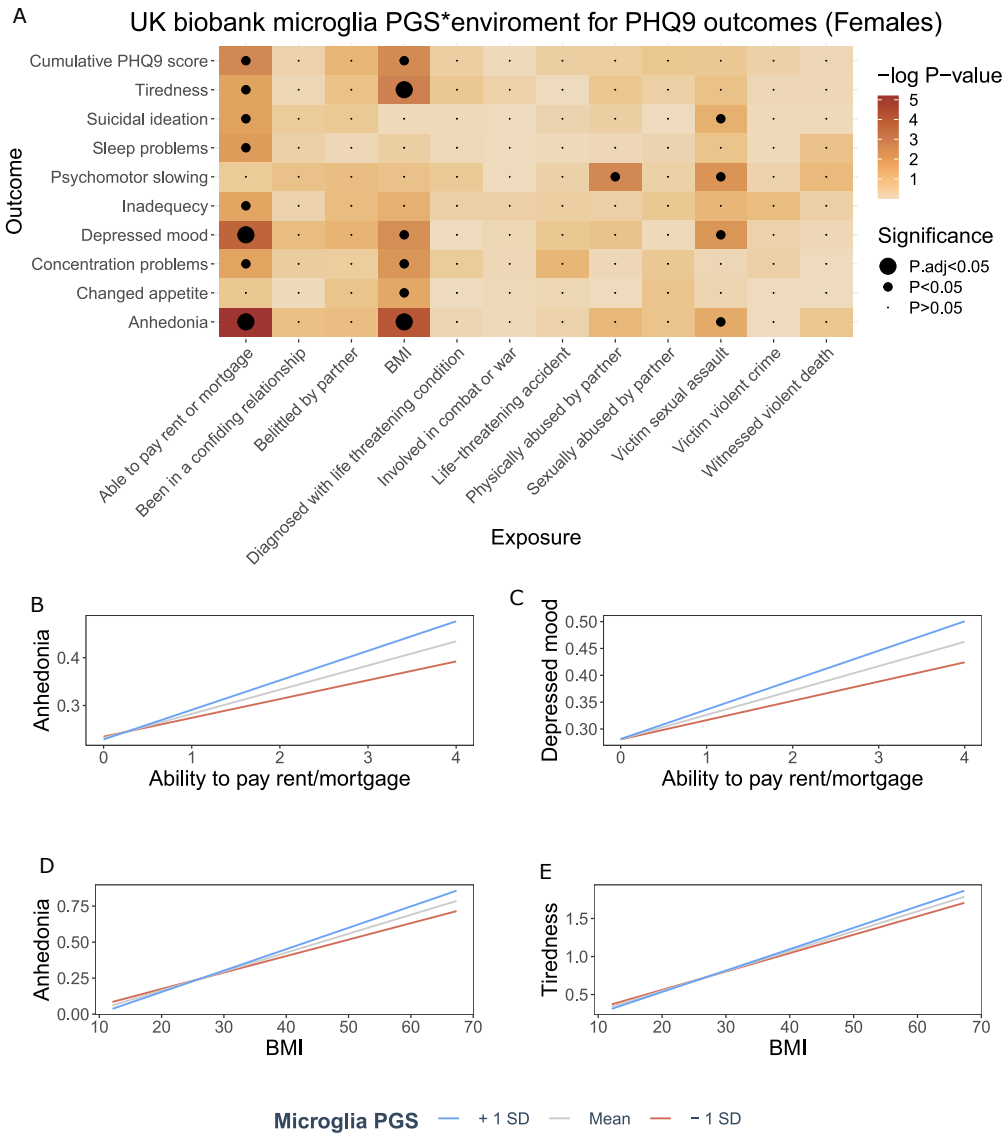

Fig. 3. An adult microglial PGS moderates the association of risk factors on depressive symptoms in females of the UK Biobank. A) Heatmap illustrating the results from 120 multivariate linear regressions investigating the interaction between an adult microglial PGS and 12 depression risk factors (x-axis) using answers to the PHQ9 as outcomes (y-axis). Tiles are coloured by the −log of the interaction P-value, with a darker colour indicated a lower P-value. The largest dot indicates an FDR adjusted P-value < 0.05, the intermediate sized dot indicates nominal P < 0.05 and the smallest dot indicates nominal P > 0.05. There were 4 interactions that passed FDR correction. A simple slopes analysis for these interactions is plotted in B-E. In B-E, the exposure and outcome are plotted on the x- and y-axis, respectively. The relationship is stratified by higher (+1 SD; blue line), lower (−1 SD; red line) or mean (grey line) adult microglial PGS. B) Relationship between an ability to pay rent/mortgage and anhedonia in females is shown, with significant differences seen for both the higher and lower PGS slopes (N = 71438; lower PGS- slope = 0.04, SE = 0.004, P = 6.38E-28; higher PGS- slope = 0.06, SE = 0.003, P = 7.37E-70). C) Relationship between an ability to pay rent/mortgage and depressed mood in females is shown, with significant differences seen for both the higher and lower PGS slopes (N = 71379; lower PGS- slope = 0.04, SE = 0.004, P = 1.58E-22; higher PGS- slope = 0.05, SE = 0.004, P = 6.53E-70). D) Relationship between BMI and anhedonia in females is shown, with significant differences seen for both the higher and lower PGS slopes (N = 72529; lower PGS- slope = 0.1, SE = 0.0006, P = 4.28E-76; higher PGS- slope = 0.01, SE = 0.0006, P = 3.64E-127). E) Relationship between BMI and tiredness in females is shown, with significant differences seen for both the higher and lower PGS slopes (N = 72439; lower PGS- slope = 0.02, SE = 0.0009, P = 7.77E-164; higher PGS- slope = 0.03, SE = 0.0009, P = 1.06E-220). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

offspring depressive symptoms at 24 years as our outcome of interest. Depressive symptoms were assessed using the Clinical Interview Schedule- Revised edition (CIS-R), a validated proxy for a clinical interview in the assessment of mental health outcomes. We used this dichotomized (case, control) data as outcomes in a logistic regression analysis, which was stratified by sex, including the first 10 genetic principal components and gestational age at birth as covariates in the analysis.

We found significant interactions between the fetal microglial PGS and EPDS in females when depression at 24 years (assessed with the CIS-R) was considered (1273 controls, 179 cases; coefficient = 0.04, 95 % CI 0.004 to 0.08, P = 0.02). Simple slopes analysis showed a significant association for the higher PGS, which was consistent (Fig. 4; **Supplementary Table 9**) with results from the UK Biobank analysis. There were no significant interaction effects when equivalent analyses were conducted in males (**Supplementary Table 10**). Furthermore, a sensitivity analysis with the adult microglial PGS, as used in the UK Biobank analysis, did not find an association (P-value = 0.6, for an interaction in females between the adult microglial PGS and maternal prenatal EPDS, when depressive outcomes at 24 years were used), demonstrating the

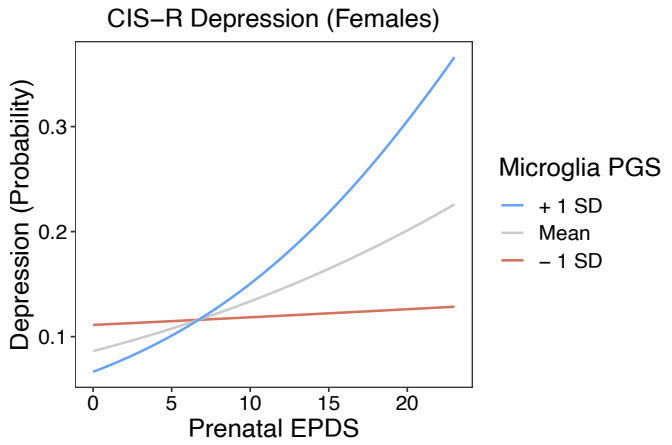

Fig. 4. A prenatal microglia PGS moderates the relationship between prenatal maternal EPDS and offspring depressive outcomes at 24 years in females of the ALSPAC cohort. Data are the result of a simple slopes analysis. The relationship between prenatal maternal EPDS (x-axis) and offspring depression (y-axis; measured by the CIS-R) is stratified by higher (+1 SD; blue line), lower (−1 SD; red line) or mean (grey line) microglial PGS in offspring. The microglial PGS moderates the relationship between prenatal maternal EPDS and depression at 24 years of age in females (N = 1452; lower PGS- slope = 0.007, SE = 0.02, P = 0.79; higher PGS- slope = 0.09, SE = 0.03, P = 7.82E-4). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

importance of considering developmental stage in the analysis. Together, these data suggest that a higher fetal microglia PGS is associated with increased risk of adult depression in females, in the context of high prenatal maternal depressive symptoms.

4. Discussion

Understanding the mechanisms of depression risk is critical for both the development of novel treatment strategies and population-based initiatives aimed at promoting mental health. We used a novel functional genomics approach in multiple independent cohorts of distinct life stages to identify microglial function as a female-specific mechanism linking adverse environmental exposures to depression risk. We found that a PGS that reflected the expression of microglial-enriched genes significantly moderated the association between established risk factors and depression in a sex-specific manner. However, we note that these results pertain primarily to mechanistic inference, rather than genetic prediction of depression risk. As such, our results identify microglial function as a potential target to mitigate the risk of depression associated with particular environmental exposures and provide a credible sex-specific regulator of depression risk. With the increasing diversity of scRNA-seq datasets, our approach also provides a method to delineate cell-specific contributions to disease in large human cohorts.

There is an increasingly appreciated role for inflammation in depression, and microglia as the primary immune-related cell within the brain, are an attractive candidate to mediate these effects. Immune responses differ by sex (Beydoun et al., 2019; Lombardo et al., 2021), and many homeostatic microglial functions, such as synaptic pruning, are governed by pathways traditionally associated with inflammation. Indeed, microglia mediated synaptic remodelling is a key mechanism in the response to chronic stress in animal models (Wang et al., 2023), and previous studies have demonstrated that microglial function is integral to the sex-dependent response to stress (Liu et al., 2019; Block et al., 2022; Cao et al., 2021).

Single nucleus RNA-sequencing studies show profound differential expression in microglia in the dorsolateral prefrontal cortex of depressed female, but not male, suicides (Maitra et al., 2023). Pervasive differential expression in the microglia of females was also observed using a naturalistic paradigm of social stress in macaques, suggesting that microglia may have a conserved role in depression or depression −like behaviours (Wu et al., 2023). Interestingly, the bulk RNA-seq study by Scheepstra *et al* in which microglia were isolated from post-mortem depression tissue (Scheepstra et al., 2023) found notable differential expression patterns in human grey matter microglia related to synaptic pruning. Together these data suggest synaptic pruning as a candidate mechanism which may underly the results we describe, but further functional studies designed to assess sex-differences in microglia are needed. Technical and tissue limitations have limited our ability to infer such functional relations in humans, but as functional genomic resources expand, we believe studies such as ours will provide a fruitful approach for further mechanistic inference in large human cohorts.

The role of microglia in depression risk and progression is becoming more widely appreciated (Yirmiya et al., 2015), but several studies have also shown microglial function is involved in the anti-depressant response. For instance, microglia are necessary for the anti-depressant response to both electroconvulsive therapy (Rimmerman et al., 2021) and ketamine (Yao et al., 2021) in mice, and the most common anti-depressant drugs, selective serotonin reuptake inhibitors, modulate microglial function (Nicolai et al., 2023; Mariani et al., 2022). There is a huge demand for novel anti-depressants, as they may be of use for individuals resistant to current approaches or those who suffer side-effects from traditional anti-depressants (Cipriani et al., 2018). Therefore, direct targeting of microglial function may be an attractive therapeutic approach. Minocycline, a well-described modulator of microglial function has anti-depressant effects in a mouse model of chronic stress (Bassett et al., 2021), and several anti-inflammatory agents show an ability to modulate both depressive-like behaviours and microglial function in animal models (Yirmiya et al., 2015). Together with these studies, our work suggests designing therapeutic strategies around microglial homeostatic function may be a novel therapeutic strategy in depression.

It should be noted that our study should be viewed in the context of the following considerations. Our study was limited by the availability of eQTL resources and as functional genomic resources expand, the discovery power of studies such as ours will increase enormously. An analysis in GTEx showed few sex-biased brain eQTLs (Oliva et al., 2020), therefore we do not expect sex differences in the genomic architecture of gene expression to significantly bias our findings. However, with the increasing sample size of functional genomic resources, using sex-specific functional variants in future studies will be an interesting avenue. Furthermore, increasing the size of functional genomic resources will increase the power for methods like Mendelian randomization. In our study, despite using methods to account for the use of many weak instrumental variables it is possible we were underpowered to detect a direct effect of the eQTLs on depression risk. Our study also only included males and females that identified with their sex at birth. We also acknowledge that our results using BMI as a risk factor in the UK Biobank show small effect sizes within the healthy BMI range, which may in part be attributable to our use of genetically regulated gene expression. As such, an important component of future studies will be to assess the therapeutic potential of targeting microglial in heterogenous populations. Finally our study is not intended to directly inform clinical prediction of depression risk, but rather to inform mechanisms of sex-dependent depression risk.

In conclusion, our study provides a framework to assess cell-specific involvement in human health and disease and our results suggest microglia are an important player in the sex-specific translation of environmental exposures into depression risk.

Author contributions

EF planned the study, conducted the analysis, interpreted the data, and wrote the manuscript, with editing from MJM. IP and SP contributed to the analysis and genetic scores. SYC, APT, PPS and MJM aided in

study design. All authors approved the final version of the manuscript.

CRediT authorship contribution statement

Eamon Fitzgerald: Conceptualization, Formal analysis, Project administration, Visualization, Writing – original draft, Writing – review & editing. **Irina Pokhvisneva:** Data curation, Formal analysis, Writing – review & editing. **Sachin Patel:** Formal analysis. **Shi Yu Chan:** Conceptualization. **Ai Peng Tan:** Conceptualization. **Helen Chen:** Resources. **Patricia Pelufo Silveira:** Conceptualization, Methodology, Resources, Writing – review & editing. **Michael J. Meaney:** Conceptualization, Funding acquisition, Writing – review & editing.

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.

Data availability

Access to data from the ALSPAC and UK Biobank are dependent on approved application to the respective data access committees.

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The schematic in Figure 1 was creating using BioRender.com.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbi.2024.04.030.

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