Systematic review and meta-analysis of transcriptomic studies on different brain regions reveals different sex-based profiles in schizophrenic patients

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Abstract

Schizophrenia is a severe neuropsychiatric disorder characterised by altered perception, mood and behaviour that, despite its relatively low prevalence, causes a profound impact in patients and society. Regarding genetic factors, previous studies have related dopamine receptor D2, different genes involved in glutamatergic neurotransmission, synaptic plasticity, and several immune functions. Sex differences have been clearly established in the epidemiology, symptomatology and outcome of schizophrenia. Nevertheless, the effects of schizophrenia in the transcriptome considering sex and the cerebral regions are barely explored. In this study, we performed a systematic review and sex-based meta-analysis using RNAseq studies of postmortem brain samples. Here we show that genes related to immune response functions are overexpressed in cortical regions of males and hippocampus of females, whereas cortical regions of females overexpressed genes related to neurotransmission and synapses. Regarding the meta-analysis of all brain regions available, we found again that genes related to synaptic plasticity and transmission were enriched in females, while those involved in the organisation of genetic information and protein folding were more expressed in males. Our results suggest multiple sex-based transcriptomic profiles as a consequence of schizophrenia on the brain. These results provide new insights into the role of sex in the effects of schizophrenia and facilitates the exploration of sex-specific markers of the disease.

Keywords

Systematic review, meta-analysis, schizophrenia, RNAseq, frontal cortex, hippocampus, biomarkers, personalized medicine, sex differences

Introduction

Coined in 1908, schizophrenia is a psychiatric disorder resulting from an intricate interaction of genetic and environmental factors that alter brain development, affecting less than 1% of the global population (Howes and Murray 2014; Jauhar, Johnstone, and McKenna 2022). Broadly, schizophrenia age of onset is typically described around late adolescence, symptoms are classically divided into positive, negative and cognitive categories, and its outcome ranges from total recovery to serious chronic impairment (Kahn et al. 2015; Jauhar, Johnstone, and McKenna 2022).

Regarding the molecular pathogenesis of schizophrenia, multiple studies using post-mortem brain samples have found consistently alterations in three neurotransmission systems: dopamine (Kaalund et al. 2014), glutamate (W. Hu et al. 2015) and ɣ-aminobutyric acid (GABA)(Schmidt and Mirnics 2015). Moreover, these findings were supported by GWAS (Schizophrenia Working Group of the Psychiatric Genomics Consortium 2014).

On the other hand, multiple sex differences have been described in the epidemiology of schizophrenia. The age of onset is later in women (Giordano et al. 2021), its incidence is slightly higher in males (Aleman, Kahn, and Selten 2003), and different reports describe a higher frequency of positive and affective symptoms in females and more severe negative symptoms in males (Taylor and Langdon 2006). Nevertheless, the molecular mechanisms underlying these epidemiological sex-based differences are still barely explored, hampering the identification of sex-specific biomarkers and therapeutic interventions. The use of *in silico* approaches, such as meta-analyses of transcriptomic data, can unveil new patterns of expression based on the integration of multiple data sources. Previous studies have addressed gene expression in schizophrenia examining sex differences, but mainly focused in the prefrontal cortex (PFC), finding scarce effects of the biological sex in the gene expression signature (Qin et al. 2016; Collado-Torres et al. 2019; Hoffman et al. 2022).

Thus, in order to evaluate sex-based differences in transcriptomic data from schizophrenic patients, we performed a comprehensive systematic review of available

studies in public repositories. We performed 3 meta-analyses covering alterations in gene expression of cortical, hippocampal and total brain regions, finding different gene expression signatures for each region and sex analysed and a partial overlap of schizophrenia effects between the hippocampus of females and the frontal cortex of males. Moreover, the functional enrichment of differentially expressed gene sets highlighted this overlap, raising completely different functional profiles for the rest of conditions analysed. Overall, our results suggest significant differences in the effects of schizophrenia in the gene expression of patients based on their sex, excluding the partial consensus in the gene signature between the hippocampus of females and the frontal cortex of males.

Material and methods

Systematic review

Following PRISMA Statement guidelines (Moher 2009), a systematic review was performed by searching for keywords “schizophrenia” on multiple databases during February 2022. Results were filtered by i) organism: “*Homo sapiens*”, ii) study type: “expression profiling by high throughput sequencing”, iii) sample count: at least 12 samples. The exclusion criteria applied were: i) experimental design other than patients vs. controls, ii) the absence of information on patient sex, iii) experimental samples other than postmortem brain samples, iv) the absence of female patient samples and v) experimental data excluding cellular populations. Therefore, gene expression data of 5 RNAseq datasets were retrieved: GSE174407, GSE138082, GSE80655, GSE42546, GSE78936. Additionally, we downloaded the raw sequence reads files of the PRJNA379666 study, whose expression dataset is not available.

Analysis

The workflow for each of the selected studies was: i) data download and normalisation, ii) exploratory analysis of data, iii) DGE analysis, iv) integration of DGE results

using meta-analysis, and v) functional enrichment of meta-analyses data. Studies were grouped based on the region of study, allowing us to explore common sex-based differences throughout multiple brain regions, as well as those specifically present in neocortex and hippocampus. Bioinformatics analysis was carried out using R 4.1.2.

Data download and processing

Expression data of selected studies were downloaded and standardized as follows: conversion and updating (if necessary) gene names to Ensembl gene ID nomenclature, calculation of the median expression in the case of multiple values for a gene and normalization of expression data by Log2 on those studies with raw data. In those studies that included multiple regions (GSE138082 and GSE80655), we splitted expression data to get one data frame per region. For the PRJNA379666 study, we downloaded raw FASTQ files, performed the control quality with FastQC (Andrews 2010), aligned them against the reference human genome GRCh38.p13 using Hisat2 (Kim, Langmead, and Salzberg 2015). Then, we sorted the results with SAMtools (Danecek et al. 2021) and obtained raw counts with HTSeq (Anders, Pyl, and Huber 2015). Then, expression data was standardised as previously described. Regarding metadata, labels used for the sex of patients were homogenized, and individuals were grouped into ‘Control’ or ‘Schizophrenia’. After normalization of the data, possible batch effects or anomalous behavior of the data were analyzed using clustering and principal component analysis (PCA).

Differential Gene Expression and meta-analyses

Differential gene expression (DGE) analyses for each selected study were performed using the function voom() of the R package limma (Anders, Pyl, and Huber 2015; Ritchie et al. 2015), adding the control factor ‘age’ to the model. Specifically, we analysed the schizophrenia sex-differential impact (SDID) through the comparison ((Schizophrenia.Female - Control.Female) - (Schizophrenia.Male - Control.Male)). The logarithm of the fold change (LFC) was used to measure the statistical effect in DGE

analysis. Thus, genes with positive LFC are increased in females, while genes with a negative LFC are increased in males. After calculating the differential expression statistics, p-values were adjusted using the Benjamini & Hochberg (BH) method. Then, we integrated the DGE results of each study, using the metafor R package as previously described (Viechtbauer 2010). We meta-analysed the SDID comparison and brain region of study (frontal cortex, hippocampus and all of them), using a random-effects model for each gene (DerSimonian and Laird 1986). The meta-analyses provide a p-value adjusted by the BH method. The significance cutoff was set to p_value < 0.05 and absolute LFC > 0.5. Finally, we explored the number of differentially expressed genes previously related to schizophrenia in the Open Targets database (Ochoa et al. 2021).

Functional Enrichment Analysis

First, Protein-protein interaction (PPI) networks were analysed using the STRING web tool for each subset of differentially expressed genes (Szklarczyk et al. 2019). The total number of edges was examined, and PPI enrichment was assessed using the following parameters: 1) for active interaction sources, we excluded the options 'Text Mining' and 'Databases' and 2) minimum required interaction score was set to 0.7 (high confidence). The rest of the parameters were unaltered.

Then, to detect sex-specific and sex-differential functions affected by schizophrenia, a gene set analysis (GSEA) of the 3 meta-analyses was performed using the logistic regression model implemented in the mdgsa() R package (Montaner and Dopazo 2010). The functional annotation of biological processes was obtained from the Gene Ontology (GO) database (Montaner and Dopazo 2010; Ashburner et al. 2000). Due to their hierarchical structure, the gene annotations were propagated with GO terms. Therefore, excessively specific or generic annotations were filtered out (blocks smaller than 10 or larger than 500 genes). Finally, functions p-value was adjusted with the Benjamini & Yekutieli (BY) method, the logarithm of the odds ratio (LOR) was used to measure the statistical effect. The significance cutoff was set to p_value < 0.05.

Metafun-SCZ web tool (to be completed)

All data and results generated in the different steps of the meta-analysis are available on the Metafun-SCZ platform (http://bioinfo.cipf.es/metafun-SCZ), accessible to any user, allowing the confirmation of obtained results and the exploration of other results of interest. This easy-to-use resource is divided into 5 sections: i) the summary of analysis results in each phase, followed by detailed results for the ii) exploratory analysis, iii) meta-analysis, and iv) functional profiling for each study. The user can interact with the web tool through graphics and tables and explore information associated with specific genes or biological functions.

Results

Data acquisition and preliminary analysis

Our systematic review screened 96 publications, which rendered a total of 6 studies eligible for our analyses (**Figure 1**). In turn, 2 studies contained expression data from 3 different regions. Therefore, we splitted these data frames by region, obtaining 10 studies: 4 of different cortical regions, 4 of hippocampal regions, 1 of amygdala and 1 of nucleus accumbens (**Table 1**).

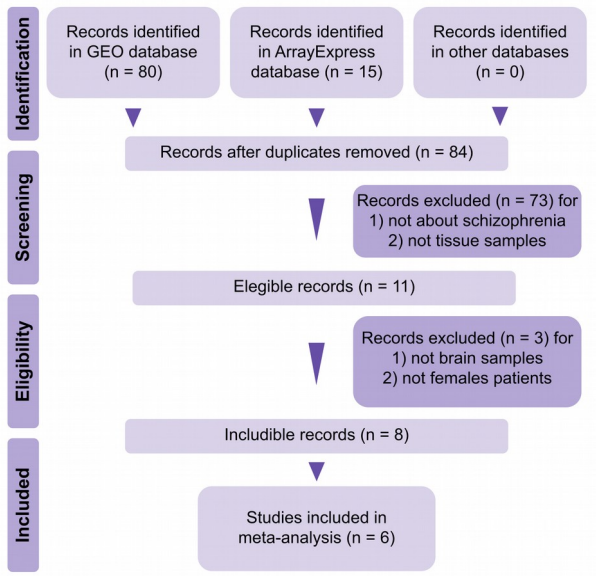

Figure 1. Prisma diagram of the systematic review.

The selected studies contained 400 samples of 252 patients (97 control males, 90 schizophrenic males, 34 control females and 30 schizophrenic patients) (**Supplementary table 1**). We performed an exploratory analysis that revealed the absence of biases or batch effects in the samples from all studies except for GSE42546, in which we found one outlier in the schizophrenic male group.

Accession ID	Platform	Brain areas	Reference
GSE174407	Illumina HiSeq 4000	FC	(Zhu et al. 2021) (preprint)
GSE138082	Illumina HiSeq 2500	CA1, CA3, DG	(Perez et al. 2021)
GSE80655	Illumina HiSeq 2000	ACC, DLPFC, NA	(Ramaker et al. 2017)
GSE42546	AB SOLiD 4 System	DG	(Kohen et al. 2014)
GSE78936	Illumina HiSeq 2000	OFC	(J. Hu et al. 2016)
PRJNA379666	Illumina HiSeq 2000	Amygdala	(Chang et al. 2017)

Table 1. Summary of selected studies. FC, frontal cortex; CA1, cornus ammonis 1; CA3, cornus ammonis 3; DG, dentate gyrus; ACC, anterior cingulate cortex; DLPFC, dorsolateral prefrontal cortex; NA, nucleus accumbens; OFC, orbitofrontal cortex.

Meta-analysis and functional enrichment

1. *Cortical regions*

We meta-analysed 43.309 genes of cortical regions (**Figure 2A**) that revealed the altered expression of 265 genes (44 increased in females and 221 increased in males). From them, we found 90 genes (34%) previously related to schizophrenia in the Open Targets database (**Supplementary table 2**).

Then, we explored the PPI networks for differentially expressed genes using the STRING web tool. In males, the network was significantly enriched (p_value < 1.0^{-16}), with two prominent clusters (**Figure 2C**): the central cluster (7 nodes) related to immune response and other cluster (3 nodes) involved in protein processing. Besides, we found multiple independent pairs of interacting proteins (**Supplementary table 3**). Regarding

females, the resulting network was not significantly enriched.

Finally, we performed a GSEA on the significantly altered genes to assess the functional role of these genes in the biological process ontology of GO. We found 1.149 functions significantly affected (**Figure 2D**). From them, 56 were significantly increased in females, mainly related to synapse regulation. On the other hand, 1.085 BP were increased in males, being involved in immune system stimulation, extracellular matrix and cell adhesion (**Supplementary table 4**).

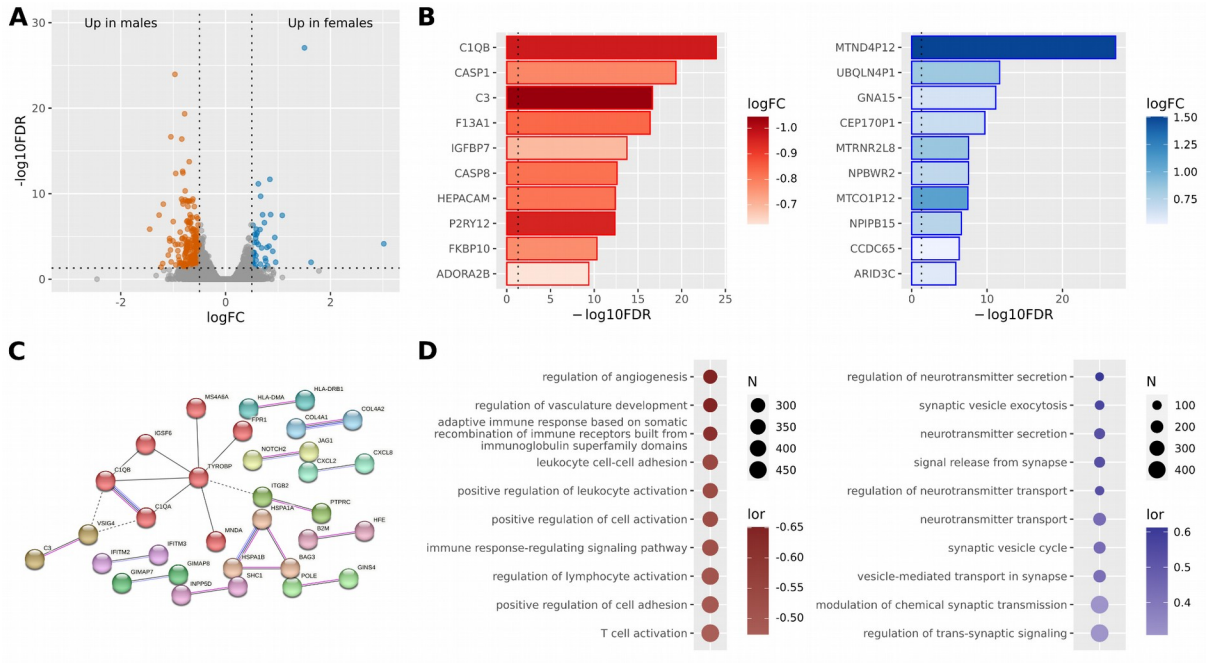

Figure 2. Meta-analysis of cortical regions. **A**) Volcano plot showing the genes analyzed. Vertical dashed lines are set al logFC 0.5 and -0.5, horizontal dashed line set at 1.3 -log10FDR. Red dots are genes overexpressed in males while blue dots are genes overexpressed in females. **B**) Bar plots showing the top ten genes overexpressed in females (blue) and males (red). Vertical dashed lines are set at p_value = 0.05. **C**) PPI network generated using genes overexpressed in males. **D**) Dot plots showing the top ten enriched functions in females (blue) and males (red).

2. *Hippocampal regions*

We compared 15.679 genes of 4 data sets, finding 1.414 altered genes (765 increased in females and 649 increased in males - **Figure 3A**). From them, 478 genes (34%) were previously related to schizophrenia in the Open Targets plattform (**Supplementary table 5**).

Regarding PPI networks for differentially expressed genes, we found they were

significantly enriched both in males (p_value < 1.0^{-16}) and females (p_value < 2.0^{-4}), with two prominent clusters (**Figure 3C**): the central cluster (7 nodes) related to immune response and other cluster (3 nodes) involved in protein processing. Besides, we found multiple independent pairs of interacting proteins (**Supplementary table 6**). Regarding females, the resulting network was not significantly enriched (**Supplementary table 7**).

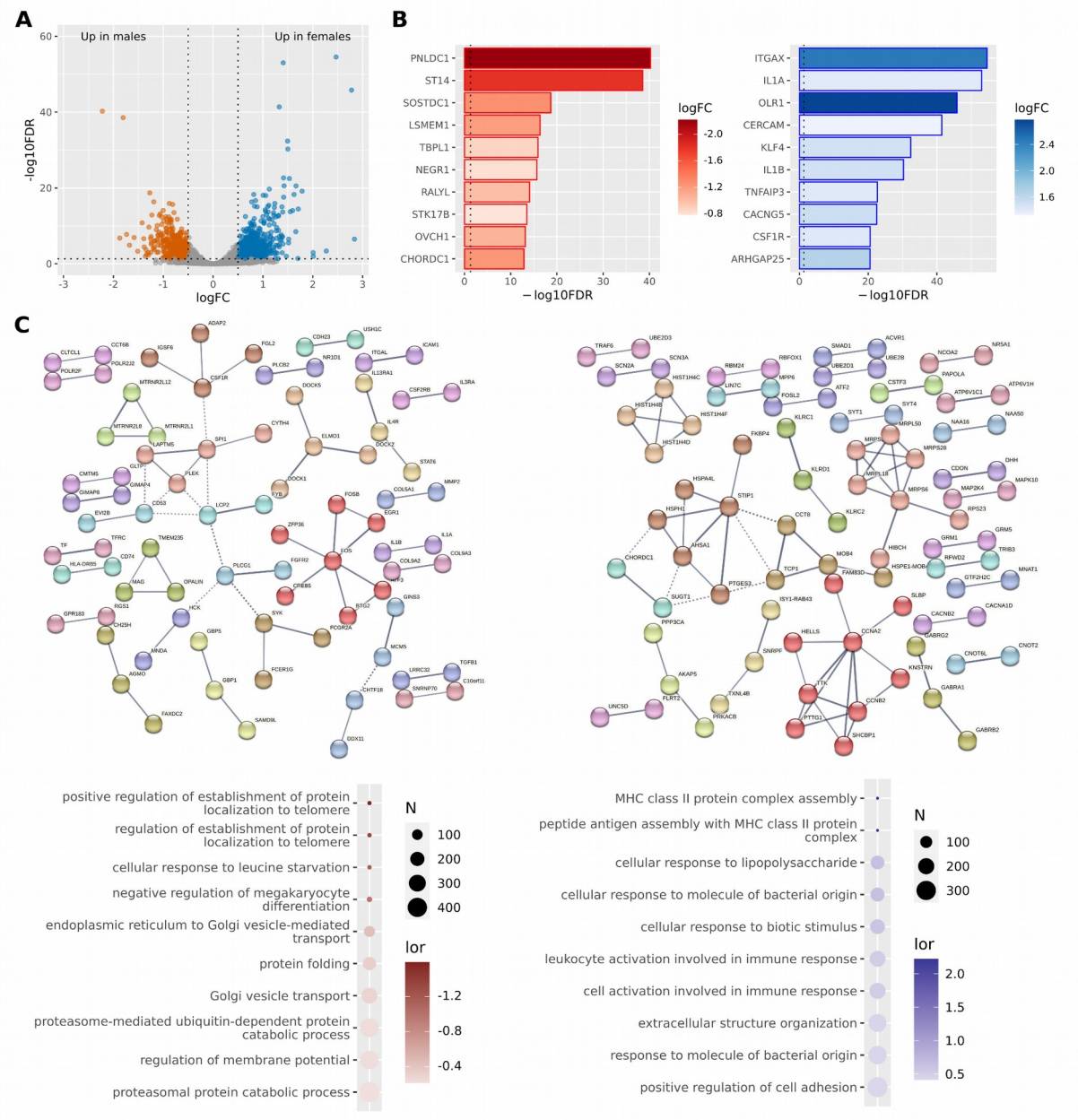

Figure 3. Meta-analysis of hippocampal regions. **A**) Volcano plot showing the genes analysed. Vertical dashed lines are set al logFC 0.5 and -0.5, horizontal dashed line set at 1.3 -log10FDR. Red dots are genes overexpressed in males while blue dots are genes overexpressed in females. **B**) Bar plots showing the top ten genes overexpressed in females (blue) and males (red). Vertical dashed lines are set at p_value = 0.05. **C**) PPI network generated using genes overexpressed in males (left panel) and females (right panel). **D**) Dot

plots showing the top ten enriched functions in females (blue) and males (red).

Then, we explored the functional effects of schizophrenia on 5.650 GO terms related to the DGE, finding 225 altered GO terms (197 increased in females and 28 increased in males) in the SDID comparison (**Figure 3D, supplementary table 8**). Conversely to the results obtained in cortical regions, in the hippocampus those BP related immune system activation, cell adhesion and extracellular matrix were increased in females, pointing to opposite scenarios as a consequence of schizophrenia in the neocortex and the hippocampus. On the other hand, BP terms increased in males were primarily related to the processing, location and regulation of protein synthesis and folding.

3. *All studies*

We performed a global meta-analysis of 47.786 genes including all data sets. We found 66 genes with altered expression (25 increased in females and 41 increased in males) in the SDID comparison (**Figure 4A**), 17 of them (26%) previously related to schizophrenia in Open Targets (**Supplementary table 9**). The evaluation of these genes in the STRING database did not reveal enriched PPI.

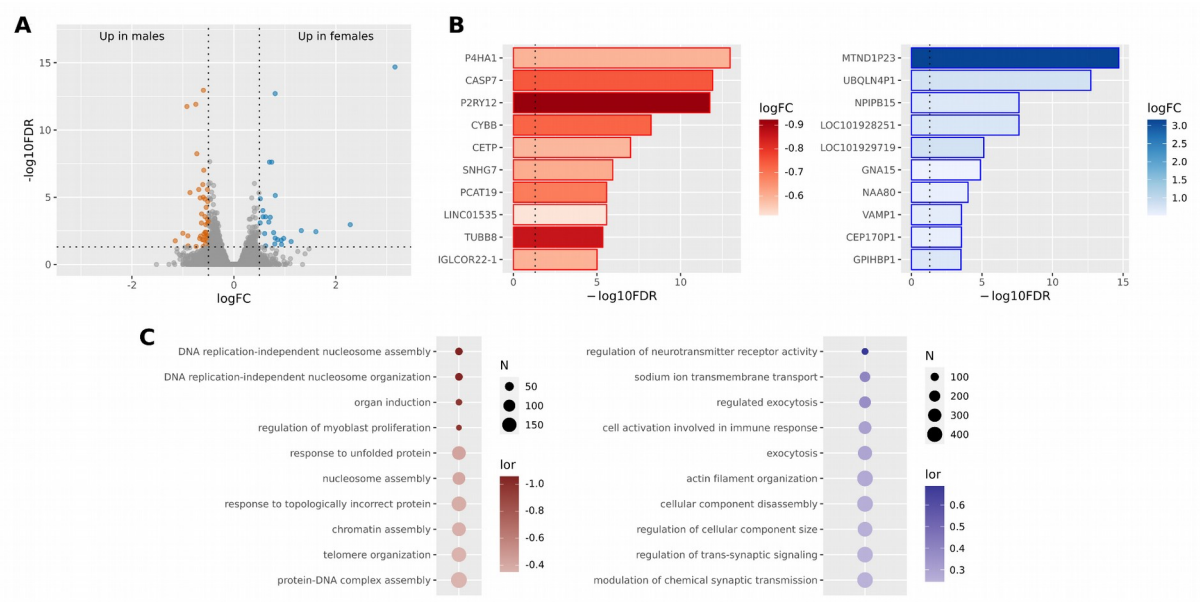

Figure 4. Meta-analysis of all regions. **A**) Volcano plot showing the genes analysed. Vertical dashed lines are set al logFC 0.5 and -0.5, horizontal dashed line set at 1.3 -log10FDR. Red dots are genes overexpressed in males while blue dots are genes overexpressed in females.

B) Bar plots showing the top ten genes overexpressed in females (blue) and males (red). Vertical dashed lines are set at p_value = 0.05. **C**) Dot plots showing the top ten enriched functions in females (blue) and males (red).

For the functional enrichment, we checked 6.291 GO terms, finding 42 significantly affected (32 increased in females and 10 increased in males) in the SDID comparison (**Figure 4C**). Recapitulating findings in the cortical regions, most of BP increased in females were related to synaptic transmission, whilst the BP terms increased in males pointed to protein folding and nucleosome organisation (**Supplementary table 10**).

Meta-analyses intersection

We explored the overlapping of those genes and functions differentially expressed in the meta-analyses performed. Regarding the intersection of genes (**Figure 5A**), we found that most intersections were produced between different meta-analyses that showed genes overexpressed in the same group (i.e. genes increased in males in hippocampus and cortical regions). Nevertheless, the largest overlapping was produced between those genes overexpressed in hippocampal regions of females and cortical regions of females (*VEGCF, FYCO1, GIMAP8, SLC2A5, ARHGDIB, CMTM7, CPQ, IGS6, ELK3, RNF122, TNFRSF1B, ARHGAP29, HEY2, ACSBG1, MNDA*), highlighting the common effects of schizophrenia in the transcriptomic profile of these regions. We explored the potential interaction of these genes using the PPI enrichment, but we did not find significant networks. Regarding functional enrichment (**Figure 5B**), the groups with more overlapping BP terms were those increased in the hippocampus of females (**Supplementary table 11**), supporting the idea of common effects of schizophrenia in these two regions.

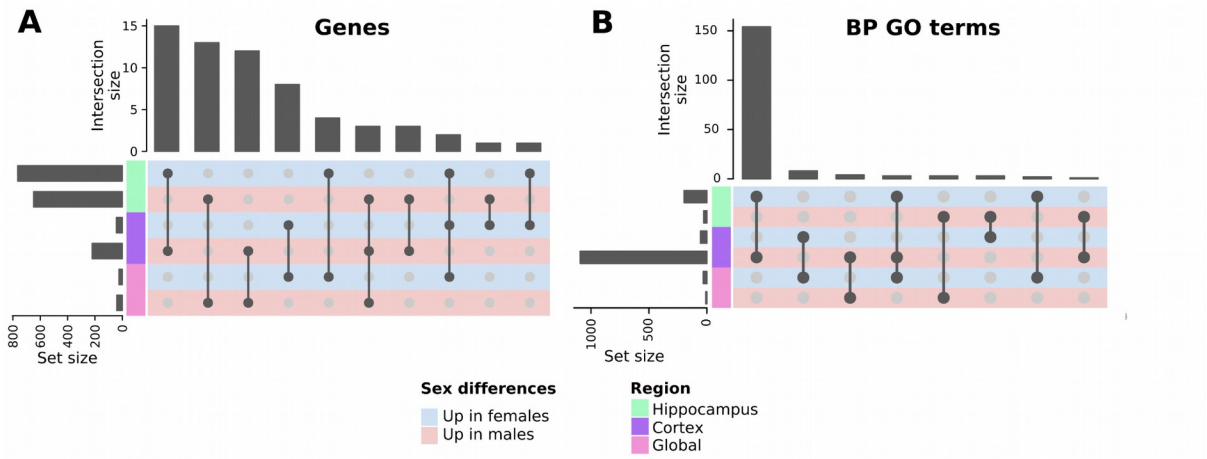

Figure 5. Intersection of meta-analyses and comparisons performed. **A**) Upset plot showing the intersection of genes differentially expressed between regions and comparisons. The groups with more genes commonly affected are the hippocampus of females and the cortical regions of males. **B**) Upset plot showing the intersection of GO terms (biological process ontology) commonly dysregulated, showing the highest number of common terms between the hippocampus of females and the cortex of males.

Discussion

Despite multiple sex differences described in the onset and symptomatology of schizophrenia, the molecular mechanisms originating these differences are barely explored, remaining most of the research conducted primarily focused on the DLPFC of men. To unveil this gap, we reviewed the gene expression studies in schizophrenia and performed three meta-analyses on frontal cortex, hippocampus and total brain regions, applying a sex-based approach.

First, we determined the sex-based differential effect of schizophrenia on frontal cortex meta-analysis, we found that most genes were overexpressed in males and a third of them have been previously related to schizophrenia in Opentargets, contrasting a recent study that used two cohorts of RNAseq data in DLPFC in which only the ALKBH3 gene was close to exhibit sex-based expression difference (Hoffman et al. 2022). Regarding the last systematic review focused on schizophrenia (Merikangas et al. 2022), they found 160 genes differentially expressed including multiple technologies (microarray and RNAseq) and tissues (blood, brain and induced stem pluripotent cells, among others), without sex perspective. Ten of those genes are also differentially expressed in our frontal cortex meta-analysis

(CASP1, GBP2, HSPA1A, IFITM2, IFITM3, LRRC37A2, NAGA, PDGFRB RERGL and ZC3HAV1), all of them up in males compared to females, and mainly related to immune function, cortical immune activation and inflammation in schizophrenia (Merikangas et al. 2022; Lanz et al. 2019). Moreover, we found an increased expression of component 4 (C4B), as well as C1QA and C1QB in males, previously related to schizophrenia vulnerability (Sekar et al. 2016; Purves-Tyson et al. 2020). Besides, most of the top biological process GO terms differentially increased in males were related to immune system activation and neuroinflamation. On the other hand, none of the genes increased in females was previously described in a sex-based analysis and most of their top enriched biological process GO terms are related to synaptic function. A recent meta-analysis focused on synaptic protein changes in schizophrenia, without sex perspective, found a decrease of synaptophysin (SYP) in DLPFC and cingulate cortex (Osimo et al. 2019). Here we show that SYP expression is comparatively increased in females. Moreover, another gene related with synaptic function (SV2C) was also increased in females. A reduction of SV2A, a member of the same family protein, has been described using PET imaging (Onwordi et al. 2020). Connecting gene sets differentially expressed in males and females, it has been shown that increased C4 expression leads to microglia-mediated engulfment in mice (Comer et al. 2020). Altogether, our results suggest a scenario of major inflammatory impairment and synaptic loss in males compared to females, not previously described to our knowledge.

Considering the hippocampus meta-analysis, we found the largest set of differentially expressed genes (649 increased in males and 765 increased in females). Comparing these results with the meta-analysis previously described (Merikangas et al. 2022), we found 16 common genes, 3 increased in males (FAM8A1, SNCA and SPR) and 13 increased in females (ANXA4, CD82, CDKN1A, GNG7, HIPK2, ITGA5, NR4A1, NUAK2, PILRB, PPP1R15A, RRBP1, SRGN and TBX2), most of the latter set related to immune system process in the biological process ontology. Additionally, the gene set analysis results show that top biological process terms increased in females are related to immune response. Therefore, our results suggest the opposite scenario than frontal cortex meta-analysis, an

increased activation of immune system activation and neuroinflammation in females compared to males. Hippocampal neuroinflammation has been previously described in schizophrenia (Merikangas et al. 2022; Lanz et al. 2019), but not analysed based on the sex of patients. Conversely, our results show that top biological process terms increased in males are related to protein folding and ubiquitin-proteasome system (Luza et al. 2020). Previous studies have reported an increase in protein insolubility in schizophrenia (Nucifora et al. 2019) and an increase in ubiquitin immunoreactivity in the hippocampus from patients with schizophrenia, most of them males (Nishimura et al. 2000). Specifically, we found an increase in males compared to females in the expression of four ubiquitin-conjugating enzymes (E2): UBED1, UBED3, UBEB and UBE2U. Altered gene expression related to E2 activity in the hippocampus has been previously linked to schizophrenia, without sex perspective (Schubert, Föcking, and Cotter 2015; Altar et al. 2005). Therefore, our results support previous findings about the effects of schizophrenia in the hippocampus and furthermore identify differentiated outcomes based on the sex of patients.

The global meta-analysis include, besides those data sets used in frontal cortex and hippocampus meta-analyses, two studies of amygdala and nucleus accumbens. We found 41 genes more expressed in males and 25 in females, overlapping the heat shock protein 70 (HSPA1A, increased in males) with a previous systematic review (Merikangas et al. 2022), highlighting the impairment of schizophrenia on protein processing. Interestingly, 11 (16%) of differentially expressed genes were pseudogenes, arising two main groups: mitochondrial pseudogenes increased exclusively in females (MTND1P23, MTCO1P12, MTCO1P40 and MTND2P28) and ribosomal pseudogenes increased in males (RPS4XP22, RPS16P4 and RPS28P7). Although the role of pseudogenes in schizophrenia is barely explored (Bergman et al. 2020), these two clusters suggest characteristic sex-based effects. On the other hand 15 (23%) of differentially expressed genes were non-coding RNA (ncRNA), 12 of them increased in males and none of them previously described in schizophrenic patients (Ghafouri-Fard et al. 2021). Although scarce, previous studies link ncRNA increased expression with the inflammatory process and immune system activation in schizophrenia (J.

Hu et al. 2016).

Strengths and limitations

The systematic review and meta-analysis strategy used integrates multiple data sets, allowing to increase statistical power and find more subtle effects than individual studies. Other studies have previously analysed bulk RNAseq sex-differences in schizophrenia, but limited to DLPFC (Hoffman et al. 2022). Our systematic review allowed us to include studies carried on hippocampus, nucleus accumbens and amygdala, regions vastly affected by schizophrenia (Mikell et al. 2009; Rajarethinam et al. 2001). On the other hand, our analysis presents some limitations, such as the bias in the subjects, with far more male than females, which constrains the statistical power of the analysis. Furthermore, bulk RNAseq studies do not allow to assign the identified differences to specific cell populations, limiting the deep understanding of their consequences. Finally, important covariates such as medication usage, smoking status and postmortem interval were not included in the metadata of most studies, limiting again the statistical power. The addition of further data, especially from female donors and the expansion of single cell RNAseq technology, would improve the power to detect cell type-specific sex differences in schizophrenia.

Conclusions

In the current study we present, to the best of our knowledge, the first systematic review and meta-analysis of transcriptomic studies in schizophrenia with sex-based approach. Our results show the different effects of schizophrenia in the transcriptomic profile of males and females based on the brain region analysed, highlighting the overlapping processes of immune activation in the frontal cortex of males and the hippocampus of females. Moreover, the global meta-analysis manifests a large number of non protein coding genes differentially altered in males and females, pointing to their hypothetical role in the disease and barely explored in the current research.

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