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A transcriptome meta-analysis of ethanol embryonic exposure: Implications in neurodevelopment and neuroinflammatory genes

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ABSTRACT

Fetal Alcohol Spectrum Disorder (FASD) comprises the phenotypes induced by prenatal alcohol exposure. Understanding the molecular mechanisms of FASD is needed since it is a public health problem. This study aimed to evaluate the impact of ethanol in the differential gene expression (DGE) of embryonic cells and fetal tissues by performing a transcriptome meta-analysis in microarrays datasets publicly available. The datasets were obtained in the GEO database and DGE was evaluated, followed by meta-analysis. DGE was also analyzed in a RNA-Seq dataset, although it was not included in the meta-analysis. To filter the main candidate genes, a database and literature review was performed, followed by ontologies enrichment analyses. In the meta-analysis, 1,938 genes were deregulated and 487 were perturbed in the RNA-Seq. Calcium homeostasis and neuroinflammation genes were overrepresented in the meta-analysis and RNA-Seq, respectively. After the database and literature review, *DOCK8*, *FOXG1*, *IL1RN*, and *PRKN* genes were proposed as new candidates for FASD; they are associated with neurodevelopment and neuroinflammation. *BDNF* and *SLC2A1*, previously associated to FASD, were also suggested in meta-analysis as candidate genes. It is known neuroinflammation reduction might help to minimize the alcohol damage. Hence, there is an urgent need to understand FASD molecular mechanisms to help in strategies aimed at preventing ethanol-induced neurologic damage.

1. Introduction

The embryonic and fetal development can be affected by the exposure to different substances. Alcohol exposure during pregnancy is a common cause of birth defects in humans and it occurs mostly through the placenta via the umbilical cord [1]. Fetal exposure also occurs through the excretion in the amniotic fluid, which acts as a reservoir for ethanol and creates a high exposure environment to the fetus [2].

Fetal Alcohol Syndrome (FAS) is the most severe outcome of a large spectrum of alcohol-induced phenotypes, named Fetal Alcohol Spectrum Disorders (FASD), and it occurs due to high doses of exposure to alcohol [3]. FAS is characterized by craniofacial dysmorphology, short palpebral fissures, thin upper lip, and absence of cupid arch of the upper lip [4]. Other features are also frequently identified, including anteverted nostrils, joint contractures, and altered palm wrinkles, although those cannot be considered for the FASD diagnosis [4]. Not all children exposed to alcohol develop the complete FAS phenotype; therefore, the term FASD includes more clinical conditions related to alcohol exposure during the prenatal period, such as minor craniofacial abnormalities, growth

Abbreviations: CTD, Comparative Toxicogenomics Database; DGE, Differential Gene Expression; FASD, Fetal Alcohol Spectrum Disorder; FAS, Fetal Alcohol Syndrome; FDR, False Discovery Rate; GEO, Gene Expression Omnibus; PCA, Principal Component Analysis; ReGEO, Restructured Gene Expression Omnibus; REM, Random Effects Model; RMA, Robust Multiarray Averaging; ROS, Reactive Oxygen Species.

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retardation, and neurological abnormalities [4]. FASD prevalence is estimated at 0.8% globally [5]. However, FAS diagnosis requires a very specialized dysmorphology evaluation, hence this prevalence is probably underestimated. In a study conducted in the United Kingdom, the weighted estimate of FASD in primary school children was 3-10% [6]. Despite the variations, FASD is considered a public health issue due to the prevalence and because it can be prevented [7].

The molecular mechanisms of FASD are not completely elucidated; however, it is believed to be related to oxidative stress. Alcohol metabolization pathway to acetaldehyde mostly occurs through cytochrome P450 2E1 (CYP2E1) [8]. In the CYP2E1 pathway, reactive oxygen species (ROS) are produced, leading to lipids and protein oxidation, therefore causing DNA damage. Those processes activate apoptosis pathways in serotonergic neurons, consequently leading to neurodegeneration [9]. Hence, excessive exposure to alcohol damages distinct cell types within the nervous system [10]. In addition, *in vivo* studies demonstrated low levels of alcohol exposure in rat fetuses lead to a unique inflammatory response in the brain, affecting cytokines, chemokines, and other inflammatory-related genes [11].

The evaluation of the whole gene expression in a cell, tissue, or organism, named transcriptome, is a recent strategy that may help to elucidate the molecular mechanisms of neurotoxicity and neuroinflammation [12,13], such as the ones suggested in FASD. Despite being widely applied in the genomics field, discrepant results are usually encountered when evaluating the same set of genes in similar experimental conditions [14]. To minimize these differences, a transcriptome meta-analysis can be applied to identify genes that are differentially expressed in several experimental studies [15].

By the time of the completion of this manuscript, transcriptome meta-analyses regarding alcohol exposure in human fetuses or cells were not encountered in the scientific literature. Hence, this study aimed to fill this gap by performing a transcriptome meta-analysis in human embryonic cells or fetal tissues, using publicly available gene expression datasets. In addition, a database and literature review was performed, as well as gene ontology assessment, to propose new candidates and the role of these genes in FASD.

2. Material and methods

2.1. Expression datasets selection

Gene expression studies were selected in the Gene Expression Omnibus (GEO) repository and Restructured Gene Expression Omnibus (ReGEO) databases [16]. The terms used in the query were: (a) “alcohol AND fetal”, (b) “alcohol AND embryo”, (c) “ethanol AND fetal”, (d) “ethanol AND embryo”, (e) “alcohol AND pregnancy”, and (f) “ethanol AND pregnancy”. Filters were applied to meet the inclusion criteria of expression studies of human embryonic cells or fetal tissue exposure to alcohol, conducted through microarray or RNA-Seq methodologies, and with raw data available in GEO. In summary, no dataset from other species except *Homo sapiens* were included. Studies in adult cells exposed to alcohol or performed in tumor cells were excluded. In addition, datasets without duplicate or triplicate samples in the control and/or exposure group were not considered, because of the lack of reproducibility in the experimental assay.

2.2. Datasets processing and meta-analysis

The data extraction for microarray studies was conducted manually through GEO, and the robust multiarray average (RMA) normalization was applied in R v.3.6.2 with the *affy* package [17]. For RNA-Seq assays, raw data was manually downloaded from GEO and processed using the Galaxy server [18].

Differential gene expression (DGE) analysis was performed using the *limma* package [19] for microarray data and *edgeR* package [20] for the RNA-Seq dataset. Principal Component Analysis (PCA) was performed to assess the heterogeneity of the samples for all datasets. Differentially expressed genes with logFC ≥ 1 and adjusted P-Value for false discovery rate (FDR) ≤ 0.05 were considered upregulated; genes with logFC ≤ -1 and the same adjusted P-Value were considered downregulated.

Meta-Analysis was performed with the *MetaVolcanoR* package [21] including only microarray studies. The Random Effects Model (REM) approach was used to combine the differential gene expression results. This method is indicated for meta-analysis comprising expression assays which were all performed in the same platform, but when the samples are heterogeneous; this method is robust for identifying the biological profile that leads to the differential gene expression [14].

In summary, the gene expression analysis provided two lists of genes: (A) differentially expressed in RNA-Seq; and (B) microarray meta-analysis genes.

2.3. Genomic databases research

The Comparative Toxicogenomics Database (CTD) [22] was accessed to search for curated genes previously related to ethanol exposure. Only FASD-related neurophenotypes were included: attention deficit disorder with hyperactivity, cognition disorders, developmental disabilities, intellectual disability, and learning disabilities. This search resulted in the third list of genes (list C).

A Venn Diagram was drawn in the Bioinformatics and Evolutionary Genomics webtool (Ghent University), to evaluate the common genes resulting from RNA-Seq (list A), meta-analysis (list B), and CTD search (list C).

2.4. Ontology assessment

Ontologies comprise the biological processes the genes are involved and/or their associated phenotypes. To assess the ontologies of the genes obtained from the previous lists, an enrichment analysis was performed in R, using the *clusterprofileR* package [23]. Biological processes were assessed through the Gene Ontology consortium (AmiGO). Phenotype associations were performed using the DisGeNet database, also included in the same R package. For all the enrichment analyses, an adjusted P-Value ≤ 0.05 was considered significant; adjustment was performed by the FDR method.

2.5. Candidate genes prioritization

A literature review was performed for the common genes between CTD and meta-analysis or CTD and RNA-Seq. The literature review comprised the terms “ethanol” combined with the name of the gene. If many results were encountered, other keywords such as “prenatal” or “brain” were added to filter for studies that potentially evaluated prenatal ethanol exposure and the neurological effects induced.

The genes selected for the literature review were also assessed regarding their Gene Ontology and annotations in the DisGeNet database. In sequence, we combined the results from the literature review and DisGeNet associated neurophenotypes in a plot, drawn with R package *ggplot2* [24]. Genes were separated into four categories: (i) studied in prenatal ethanol exposure, with an evaluation of the brain damages; (ii) studied regarding ethanol effects in the adult brain; (iii) studied, but not associated with ethanol effects; and (iv) not studied regarding ethanol exposure. Aiming to propose different genes that could explain ethanol teratogenesis

than the ones already presented in literature reports, we selected genes with more associations with phenotypes compatible with FASD in DisGeNet. These genes do not have studies regarding prenatal ethanol exposure but are relevant in FASD scenario due to the neurobiological processes they are involved.

2.6. Ethical statement

All the data here evaluated are retrieved from public genomic repositories. The study strategy here conducted was approved by the Ethical Committee of Research of the Hospital de Clínicas de Porto Alegre, no. 20190792, CAAE no. 39155220900005327.

3. Results

3.1. Five microarrays and one RNA-Seq dataset were included in the study for differential gene expression evaluation and meta-analysis

Nineteen datasets were identified in the GEO repository. According to the inclusion criteria, seven datasets were selected to assess gene expression alterations in neural cells and tissues with alcohol prenatal exposure [25–31]. The following datasets are all microarrays. Study GSE98853 was excluded since it presents only one sample for both control and exposure groups. We also excluded study GSE141253 because it includes duplicate samples from GSE71127. Studies GSE56906 and GSE45036 were subsetted because of sample heterogeneity presented in PCA (Supplementary Figures S1 and S2). For GSE56906, subsets GSE56906_H1 and GSE56906_NPC included neural stem cells and neural progenitor cells, respectively. For GSE45036, subsets GSE45036_Undif and GSE45036_EB comprise undifferentiated cells and embryoid bodies, respectively. Additional studies details are available in Supplementary Table S1.

Differential gene expression analysis was conducted separately in the seven selected datasets (Fig. 1A). For the RNA-Seq dataset (GSE86006), 487 genes were perturbed by alcohol exposure during pregnancy. This analysis reveals 293 downregulated and 194 upregulated genes. Meta-analysis containing only microarray datasets showed 4,081 genes perturbed by fetal alcohol exposure (Fig. 1B). The raw output of the meta-analysis is available in Supplementary Table S2, and an interactive version of the volcano plot is available as Supplementary Material 1. We did not observe any clustering pattern of the controls and alcohol exposure samples included in the meta-analysis (Supplementary Figure S3). We observed 1,938 downregulated and 2,143 upregulated genes in the meta-analysis.

3.2. Thirty-nine genes differentially expressed were also included in the CTD as related to ethanol exposure

CTD research resulted in 149 genes related to FASD-related neurophenotypes (Supplementary Table S3). Of these, 15 genes were associated with attention deficit disorder with hyperactivity; 28 genes to cognition disorders; 21 genes to developmental disabilities; 80 genes to intellectual disability; and 25 genes to learning disabilities.

The Venn Diagram revealed an intersection of 31 genes resulting from the meta-analysis and CTD research; 8 genes shared by both RNA-Seq and CTD analysis; and 91 genes overlapped between RNA-Seq and meta-analysis (Fig. 2 and Supplementary Material 2).

In the PubMed literature review, 18 genes had no literature association to ethanol, all of them from the intersection of CTD and meta-analysis; 16 genes with articles in fetal alcohol exposure and brain; 7 genes with literature only in prenatal alcohol exposure effects; 13 genes with manuscripts only for brain effects after alcohol exposure; and 6 genes did not present literature for alcohol exposure effects in the fetus or brain (Supplementary Table S4). To better comprehend these genes' roles, an ontology enrichment analysis was performed.

3.3. CTD-proposed genes are related to cognition and neurotransmission, whilst the RNA-Seq differentially expressed genes are associated with neuroinflammation

First, ontology enrichment analysis was performed in the lists of genes obtained from the RNA-Seq dataset evaluation (list A) and the microarray meta-analysis (list B). When evaluating the 91 common genes between both lists, no ontology enrichment was obtained. RNA-Seq ontologies were mainly related to inflammatory response, including leukocyte migration and chemotaxis, as well as positive regulation of the inflammatory response (Fig. 3A; Supplementary Table S5). For the microarray meta-analysis genes, few ontologies were enriched, probably because of the big number of genes proposed (4,081 genes); the enrichments encountered were associated with calcium homeostasis (Fig. 3B; Supplementary Table S6).

Since it was intended to provide a biological sense to the candidate genes proposed, a third ontology enrichment analysis was performed, using the 39 genes differentially expressed that were also encountered in the CTD review (31 from the meta-analysis and eight from the RNA-Seq evaluation; Fig. 2). In the Gene Ontology analysis, biological processes such as cognition and learning were enriched; many synaptic processes were also overrepresented (Fig. 3C; Supplementary Table S7). When performing DisGeNet analysis, the same neurophenotypes selected in the CTD were enriched (Fig. 3D; Supplementary Table S8). This validation confirmed the genes selected in CTD are good candidates for explaining the ethanol exposure effects in neural cells.

Finally, all these analyses were combined in a single image to better visualize the candidate genes proposed (Fig. 4). Some genes that are widely known in the FASD etiology were identified, including Brain-derived neurotrophic factor (*BDNF*), and Solute Carrier Family 2 Member 1 (*SLC2A1*). Other genes that were not previously associated with prenatal ethanol exposure are proposed in this analysis: Dedicator Of Cytokinesis 8 (*DOCK8*), Forkhead Box G1 (*FOXG1*), Interleukin 1 Receptor Antagonist (*IL1RN*), and Parkin RBR E3 Ubiquitin Protein Ligase (*PRKN*). These genes' individual expression in each dataset here evaluated is presented in Supplementary Figures S4.

4. Discussion

In this study, a transcriptome meta-analysis was performed to identify potential genes that could help to comprehend FASD molecular mechanisms and phenotypes in humans. To accomplish this, microarray studies performed in human embryonic cells or fetal tissues were evaluated, through a random effect model, to estimate the effect of the differential gene expression associated. In addition, one RNA-Seq study, performed in human fetal brains, was also evaluated and the candidate genes annotated; these genes were related to inflammatory ontologies. To filter the potential genes, an evaluation in the CTD was performed, and only genes previously associated with ethanol-induced FASD phenotypes were considered. Validation was performed by a gene ontology enrichment analysis and literature review. Finally, genes *BDNF, SLC2A1, DOCK8, FOXG1, IL1RN*, and *PRKN* are proposed (Table 1). According to our ontology enrichment analysis, these genes have been mainly associated with neurodevelopment and neurophenotypes, such as learning and cognition.

The increased popularity of the transcriptome meta-analyses is justified by the need to integrate data from different experiments to understand the whole scenario of a biological system

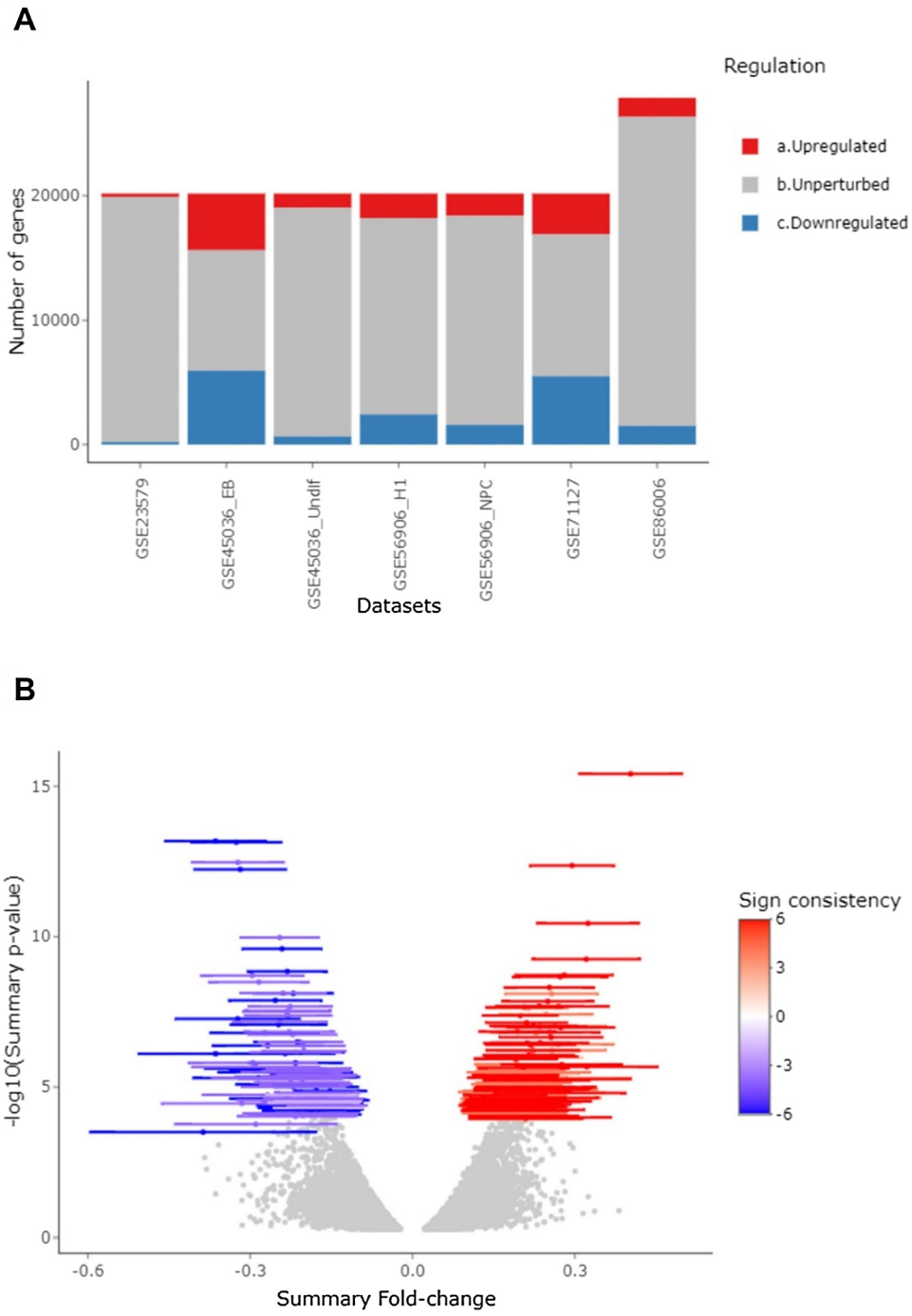

Fig. 1. Upregulated and downregulated genes evaluated in this study for each dataset. **(A)** Genes upregulated (red), downregulated (blue), and unperturbed (gray) in each dataset; GSE23579 (microarray), GSE45036 (microarray), GSE56906 (microarray), GSE71127 (microarray), and GSE86006 (RNA-Seq). **(B)** Volcano plot of the microarray meta-analysis performed, with a meta |logFC| > 1 (x-axis) and a -log10(p-value) > 5 (y-axis), assuming an adjusted P-Value < 0.05. The Volcano Plot can be accessed as an interactive plot through Supplementary Material 1.

Table 1
Candidate genes, their groups and regulatory pathways.

Gene	Group[1]	Pathways[2]
BDNF	Neurotrophins	GPCR PathwayTNF Superfamily - Human Ligand-Receptor Interactions and their Associated Functions
DOCK8	Armadillo like helical domain containing DOCK family Rho GEFs	Response to elevated platelet cytosolic Ca2+Signal Transduction
FOXG1	Forkhead boxes	Packaging Of Telomere EndsGene expression (Transcription)
IL1RN	Interleukins	IL-1 Family Signaling PathwaysCytokine Signaling in Immune system
PRKN	RBR E3 ubiquitin ligases	Mitophagy Metabolism of proteins
SLC2A1	Solute carriers	Lactose synthesis Validated targets of C-MYC transcriptional activation

[1] HGNC Database.
[2] GeneCards Database.

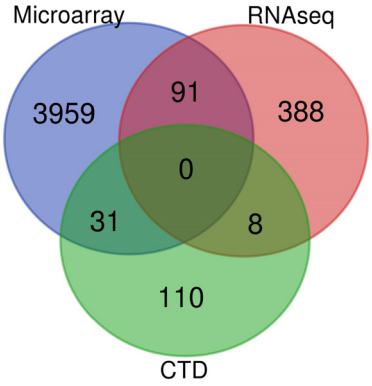

Fig. 2. Venn Diagram including the number of differentially expressed genes resulting from the meta-analysis with microarray studies (blue circle), RNA-Seq (red circle), and Comparative Toxicogenomics Database (CTD) research (green circle). The intersections reveal the number of genes included in both analyses.

[32]; it is even recommended to assess external databases, in addition to the meta-analysis results [32]. Different neurological conditions have been evaluated through the transcriptome meta-analysis strategy, including Alzheimer's disease [33], psychiatric disorders [34], and alcohol abuse [35]. However, by the completion of this manuscript, microarrays evaluating prenatal alcohol exposure have not been integrated with a meta-analysis strategy. In mouse and rat models, a transcriptome meta-analysis has identified genes associated with chromatin organization and transcription/translation processes [36]; despite the importance of these findings, these pathways are not very informative regarding the biological context of the neurological findings in FASD. A better understanding on comparative transcriptomics is necessary to properly provide a link between human *in vitro* and animal *in vivo* studies, hence providing the genes that might lead to FAS and FASD occurrence in humans.

In the microarray meta-analysis, 4,081 genes were identified by the random effect model applied. There was an overrepresentation of only one gene ontology, related to calcium homeostasis. Chronic alcohol consumption is known to alter calcium homeostasis, both in hepatocytes [37] and in the brain [38]. The alcohol-induced alterations in calcium signaling disturb neuronal homeostasis and it is believed to cause several neuropsychiatric symptoms commonly identified in alcoholism and in alcohol withdrawal in adults [38]. In a study evaluating chicken neural crest cells exposed to acute doses of ethanol, it was observed calcium deregulation impairs the Snai2/p53 apoptosis pathway, an essential signaling mechanism in neurodevelopment [39]. Alcohol-induced deregulation of calcium homeostasis is also proposed to affect fetal skeleton development [40].

A contrastingly set of genes differentially expressed was proposed when evaluating the RNA-Seq study. By assessing the ontology enrichment, several inflammatory processes were shown to be affected by ethanol exposure. The neuroinflammatory potential of alcohol is currently proposed as one of the main mechanisms to explain FASD [41]. Previously, it was believed that ethanol-induced neuroinflammation occurred through the reprogramming of astrocyte and microglial cells; more recently, it has been demonstrated ethanol alters the profile of cytokines and chemokines in the fetal brain, including TNFα, TNFβ, IFNγ, and IL-10 increase [42,43]. In addition, according to studies performed in mouse and rat fetuses, even a low to moderate prenatal alcohol exposure might be sufficient to impair neuroimmune functions in adulthood; hence, prenatal alcohol exposure can be considered a major risk factor for aberrant neuroimmune function regardless of the age [44].

It is important to emphasize the different results obtained from the microarray meta-analysis and the RNA-Seq dataset. Both strategies comprised biologically plausible processes, although separately the assays and even the methods were not sufficient to provide the scenario of calcium homeostasis and neuroimmune deregulation. These results confirm the need to assess different experimental conditions, methodologies, and datasets for a full comprehension of the actual cellular transcriptome status. Therefore, a filtering strategy was proposed, by assessing the CTD data on ethanol exposure. By integrating this external database, as it is recommended [32], some genes previously known to be deregulated in ethanol exposure were encountered, such as *BDNF* and *SLC2A1*, and other candidates were proposed, such as *DOCK8, FOXG1, IL1RN*, and *PRKN*.

Neurotrophins, such as BDNF, are known to play a pivotal role in neurodevelopment and in developmental disturbances as FASD; BDNF decreased expression is associated with cognitive abnormalities, increased neuronal apoptosis, upregulation of pro-inflammatory cytokines in the brain tissue, and downregulation of other neurodevelopment genes [45]. BDNF also plays a role in glucose transportation in the placenta [45]. In the brain, SLC2A1, also known as GLUT1, is one of the main glucose transporters; ethanol is known to decrease SLC2A1 expression, leading to blood-brain barrier damage and neuronal degradation [46].

On behalf of our proposed candidates, DOCK8 downregulation results in combined immunodeficiency with malignancy predisposition associated; more recently, the protein was demonstrated to be pivotal in cytokine production and immune cells polarization [47]. Another candidate involved in inflammation, IL1RN was studied in mice and it has been associated with predisposition to alcohol consumption [48,49]. Contrastingly, FOXG1 is a transcription factor with an integral role in forebrain development; it is known that the altered expression of *FOXG1* is related to neurodevelopmental disorders [50]. Finally, PRKN is a ubiquitin ligase with pathogenic variants in this gene associated with dopaminergic neuronal loss, hence being implicated in Parkinson's etiology [51]. Recently, *PRKN* mutations were also implicated in astrocyte reduced reactivity [52].

The lack of validation of the candidate genes proposed is the main limitation of the present study. Such validation should be carefully evaluated, taking into account genes, tissues, and the

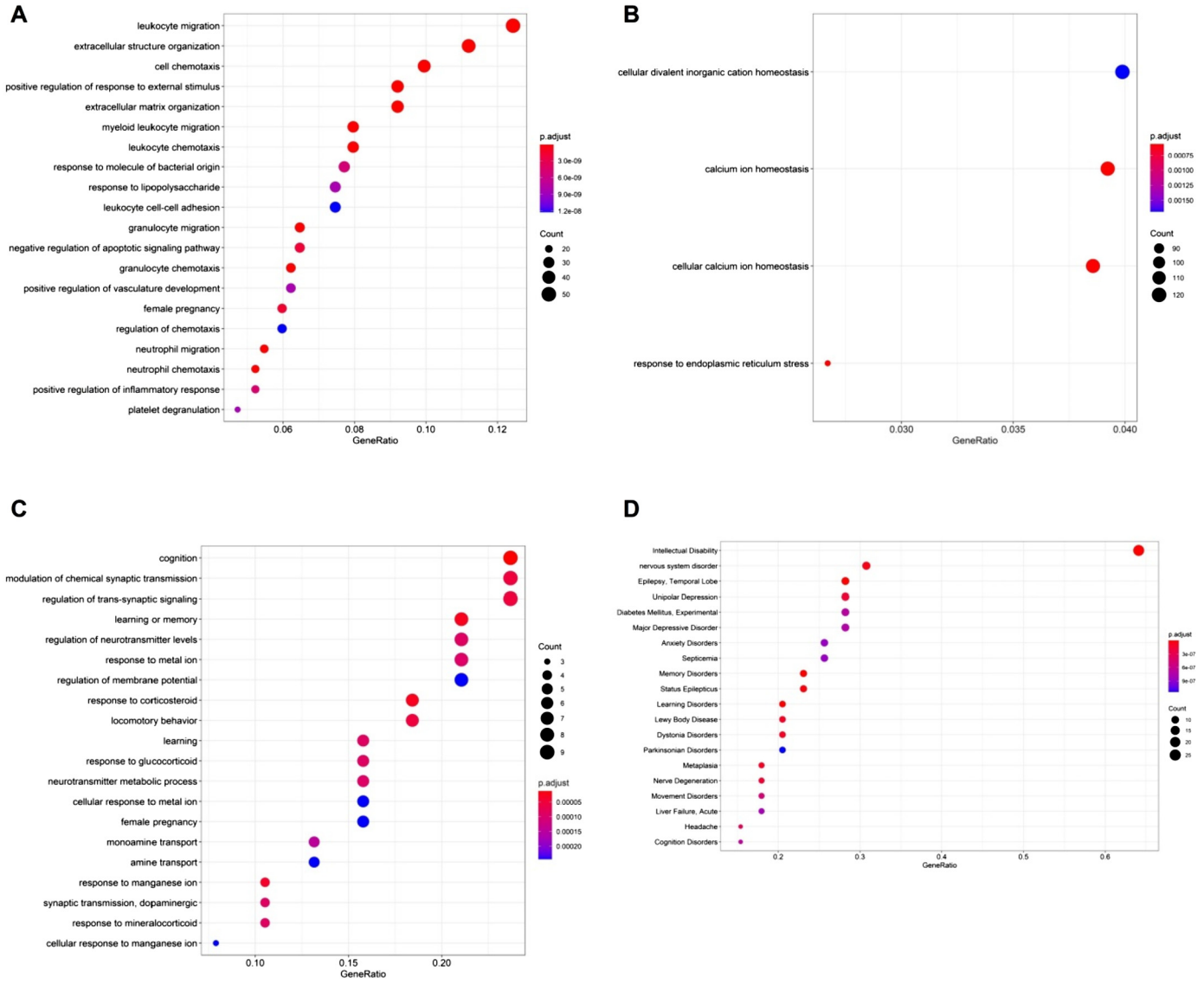

Fig. 3. Ontology enrichment analysis of the differentially expressed genes. **(A)** RNA-Seq genes were mostly associated with inflammatory responses; **(B)** Meta-analysis including microarray studies reveal genes mainly related to calcium homeostasis. The ontologies related to the genes resulting from the Comparative Toxicogenomics Database (CTD) research **(C)** and DisGeNet analysis **(D)** indicate distinct neurophenotypes as enriched biological processes.

moment of the targets' evaluation. Once defined these parameters, simple assays, as a qPCR, for example, could be performed to measure the gene expression, in order to filter-out false positive results. However, our study presents an integrative, exploratory analysis to better assess both microarray and RNA-Seq studies with a robust, consolidated statistical method. However, this research employs an integrative, exploratory approach to better assess both microarray and RNA-Seq studies with a robust, consolidated statistical method. Sample heterogeneity was probably a limiting aspect for a better specification of the candidate genes in the microarray meta-analysis, a limitation we tried to equalize by using only microarray assays performed in the same platform. We also emphasize the importance of studying the human fetal brain samples available in two of the included datasets [27,31]. Another limitation of our study is the lack of single-cell RNA-seq (scRNA-seq) evaluation. By the time of this study completion, there was only one bulk RNA-seq, which is GSE86006 (included in our study), and no scRNA-seq that matched our inclusion criteria. This single-cell approach could be very relevant to evaluate small patterns of cell differentiation, a relevant process in neurogenesis, consequently affected in FAS/FASD [4].

Prenatal alcohol exposure in humans and its consequences, FAS or FASD, is a public health issue [7]. The global prevalence of alcohol consumption in pregnancy is estimated at 9.8% [53]. In addition to FAS and FASD congenital anomalies, alcohol consumption in pregnancy is associated with future mental health issues in the offspring [54]. Low awareness is related to the mother's drinking behavior, although education does not explain the whole scenario [55,56]. Prevention campaigns and epidemiological surveillance are urgently needed [53], but other strategies might help to mitigate the problem, from basic research to prenatal care.

FASD prevention interventions, such as the use of antioxidants, vitamins, and neuroprotective peptides during pregnancy have been applied [57,45]. All these interventions try to reverse the alcohol-induced neurotoxicity to the fetal brain when there is no success in reducing maternal alcohol consumption [57]. To establish an efficient intervention, however, it is first necessary to determine the molecular mechanisms responsible for a disorder [58]. Therefore, the candidate genes here presented might be another small clue to finally minimize FASD prevalence and its damaging effects. The main challenge regarding FASD is the underestimated prevalence, due to the lack of proper diagnosis, especially because

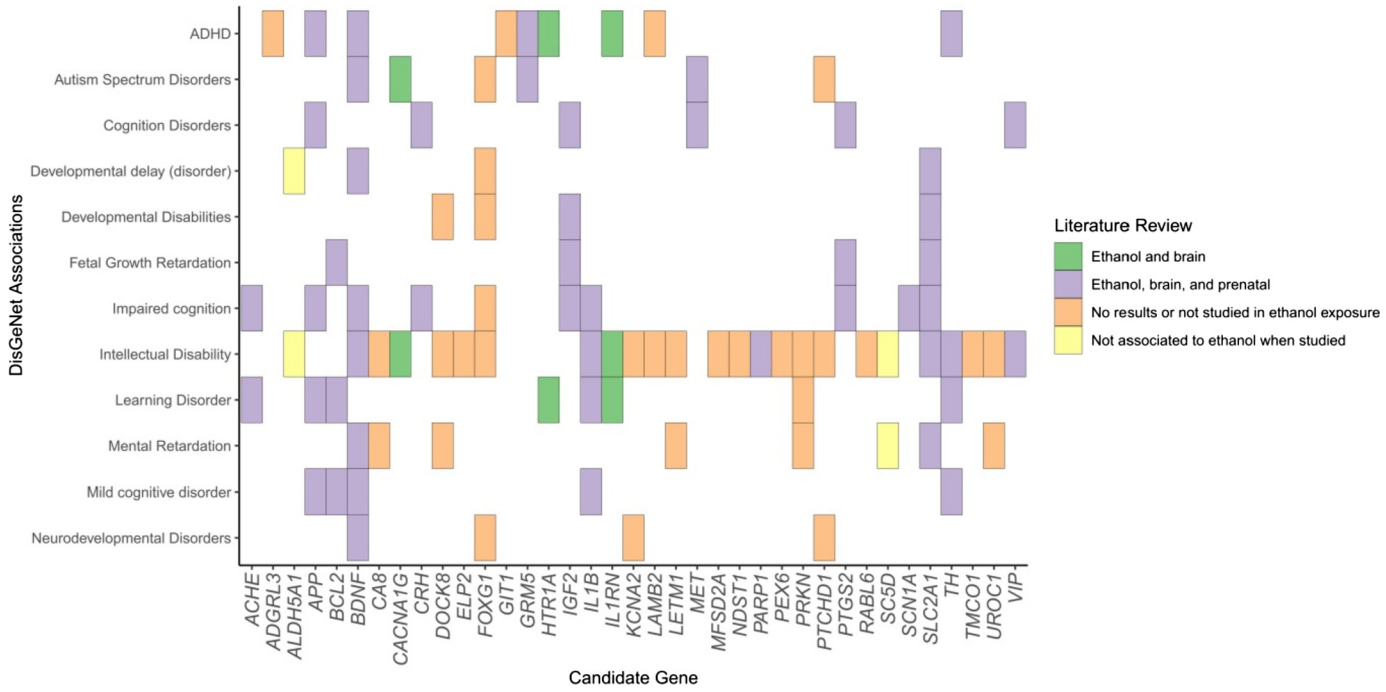

Fig. 4. Candidate genes prioritization combining the common genes resulting from the meta-analysis, RNA-Seq, and Comparative Toxicogenomics Database (CTD) research. Genes were separated into four categories: (i) studied in prenatal ethanol exposure, with an evaluation of the brain damages (purple); (ii) studied regarding ethanol effects in the adult brain (green); (iii) studied, but not associated with ethanol effects (yellow); and (iv) not studied regarding ethanol exposure (orange).

it is a neurobehavioral disorder, many times without dysmorphic phenotype, and due to the lack of self-report of maternal drinking [4]. Studies using meta-analysis of transcriptomic data are relevant to propose candidate genes that can be further assessed as possible FAS or FASD biomarkers. To the present moment, there are no biomarkers validated for these disorders [4]. Identifying these biomarkers is a necessary step in the area to provide a better diagnosis and, consequently, follow-up, and care of the affected children.

Human and animal rights

The authors declare that the work described has not involved experimentation on humans or animals.

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Author contributions

All authors attest that they meet the current International Committee of Medical Journal Editors (ICMJE) criteria for Authorship. Individual author contributions are as follows:

TWK, FSLV, and MRM contributed to the study concept and design. VOL, GCG, and TWK contributed to data acquisition, analysis, and interpretation. TWK and FSLV contributed to study coordination. All authors contributed to drafting and revising the manuscript. All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial or personal relationships that could be viewed as influencing the work reported in this paper.

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Appendix A. Supplementary material

Supplementary material related to this article can be found online at https://doi.org/10.1016/j.neuri.2022.100094.

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