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## Common genes and pathways involved in the response to stressful stimuli by astrocytes: a meta-analysis of genome-wide expression studies

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### Abstract

Astrocytes play pivotal roles in the brain and they become reactive under stress conditions. Here, we carried out, for the first time, an integrative meta-analysis of genome-wide expression profiling of astrocytes from human and mouse exposed to different stressful stimuli (hypoxia, infections by virus and bacteria, cytokines, ethanol, among others). We identified common differentially expressed genes and pathways in human and murine astrocytes. Our results showed that astrocytes induce expression of genes associated with stress response and immune system regulation when they are exposed to stressful stimuli, whereas genes related to neurogenesis are found as downregulated. Several of the identified genes showed to be important hubs in the protein-protein interaction analysis (*TRAF2*, *CDC37* and *PAX6*). This work demonstrates that despite astrocytes are highly heterogeneous and complex, there are common gene expression signatures that can be triggered under distinct detrimental stimuli, which opens an opportunity for exploring other possible markers of reactivity.

### Keywords

Astrocytes; reactivity; genome-wide expression profiling; convergence analysis; stress response.

## 1. Introduction

Astrocytes are considered the most abundant of the glial cells, followed by oligodendrocytes and microglia (Jakel & Dimou, 2017). In the entire human brain, the ratio of glia/neuron is calculated about 1:1 (von Bartheld, Bahney, & Herculano-Houzel, 2016). Approximately 20-40% of these glial cells are astrocytes (Herculano-Houzel, 2014), which are distributed for the whole brain with a diverse morphology, being the protoplasmic and fibrous astrocytes the most common. One important characteristic of these two morphological states is the function they perform; protoplasmic astrocytes are involved in synaptic processes, while the fibrous astrocytes are related to metabolic support. Moreover, these distinct shapes of astrocytes are also differentially distributed in the brain (Vasile, Dossi, & Rouach, 2017). It is important to highlight that in addition to morphological and functional heterogeneity of astrocytes, there are numerous differences at the molecular and physiological levels (Pestana, Edwards-Rinet, Belgard, Martirosyan, & Holt, 2020).

Until few years ago, astrocytes were considered as supporting cells to neurons. However, nowadays, it is well known these cells constitute a key element for the adequate functioning of the brain (Dossi, Vasile, & Rouach, 2018). Astrocytes are responsible for regulating the neurotransmitters concentrations, water, and potassium through their uptake in the synaptic cleft. In contrast, they release lactate, transmitter precursors, transmitters, purines (ATP and adenosine), growth factors, and neurosteroids. Moreover, astrocytes uptake glucose and release prostaglandins, nitric oxide, and arachidonic acid, which regulate the dilation and contraction of blood vessels in the brain-blood barrier (BBB); these blood vessels are conformed by distinct cell types, such as endothelial cells (Sofroniew & Vinters, 2010). Astrocytes are also key regulators of inflammation and respond to the injury by adopting conformational changes that may shape the microenvironment in the brain (Colombo & Farina, 2016). Likewise, astrocytes regulate proliferation, migration, and survival of oligodendrocytes, and thus, they participate in the regulation of myelination (Kiray, Lindsay, Hosseinzadeh, & Barnett, 2016). These cells also secrete molecules regulating the

microglia phenotypes, for example, monocyte chemoattractant protein (MCP)-1/CCL2 and IFN- $\gamma$  inducible protein (IP)-10/CXCL10, orosomucoid-2 (ORM2) and Lipocalin 2 (LCN2) (Jha, Jo, Kim, & Suk, 2019). Therefore, astrocytes orchestrate several cellular functions (e.g., synaptic transmission, myelination, and phagocytosis) in the brain by interaction with distinct cell types, and hence, they have been associated with cognitive impairment, development of diseases, and constitute an important therapeutic target (Dallerac & Rouach, 2016).

Astrocytes respond to any alterations in the brain homeostasis, which can be triggered by infections, inflammation, local trauma, ischemic stroke, and other diseases. This response is known as reactivity; it encompasses several morphological, physiological, and molecular changes, altering in some cases the normal function of astrocytes (Liddelw & Barres, 2017; Sofroniew & Vinters, 2010). Several studies have evaluated the transcriptional changes induced by inflammatory molecules and ischemia, in order to understand how astrocytes become reactive and how they respond against any damage (Hamby et al., 2012; Perriot et al., 2018; Zamanian et al., 2012). These works have suggested that astrocytes respond differently to several stimuli, and in addition that they can have common responses. Nevertheless, there are no clear and consistent reported transcriptomic signatures for astrocytes (Escartin, Guillemot, & Carrillo-de Sauvage, 2019). For mouse astrocytes, two phenotypes of reactivity have been proposed based on inflammatory and ischemic models, the A1 and A2, which are distinguished by the molecular changes and the response type induced. A1 is related to “harmful” responses and A2 is associated with “helpful” actions. Notably, the authors suggest there could exist other phenotypes of astrocytes reactivity, and even under the same stimulating condition two or more phenotypes might co-exist and share distinct molecular features (Liddelw & Barres, 2017). Besides, other researchers have reported that there are many differences in the response of human and mouse astrocytes to stress (J. Li et al., 2020).

Currently, there is a need to understand the common sets of genes that can be induced and downregulated in reactive astrocytes under distinct stressful or prejudicial conditions, which could be useful to find more suitable markers for detecting reactivity of these cells,

including human astrocytes (Escartin et al., 2019). One potential approach to unravel common transcriptional changes induced in astrocytes is the meta-analysis of genome-wide expression studies. This is an integrative method that allows combining data from different works to identify differentially expressed genes; this strategy also allows to increase the reliability and generalizability of results (Ramasamy, Mondry, Holmes, & Altman, 2008). Interestingly, there is a growing number of studies analyzing the transcriptomic profiling of astrocytes under several stressful and inflammatory stimuli through RNA-seq and microarray approaches using human astrocytic cells (**Table 1**) (Casselli et al., 2017; Crowe et al., 2016; Lin et al., 2015; Mense, Sengupta, Lan, et al., 2006; Mense, Sengupta, Zhou, et al., 2006; Perriot et al., 2018), and astrocytes derived from mouse models (**Table 2**) (Birck et al., 2016; Borjabad, Brooks, & Volsky, 2010; Erickson, Farris, Blednov, Mayfield, & Harris, 2018; Hamby et al., 2012; Hidano et al., 2016; Srinivasan et al., 2016; Tyzack et al., 2017; Zamanian et al., 2012). Taking into account the above, in the current study, we aimed to perform a meta-analysis of genome-wide expression studies (GWES) profiling of astrocytes exposed to molecules inducing stress or inflammation, in order to identify common genes and pathways dysregulated and to provide additional insights into the molecular mechanisms that underlie the reactivity of astrocytes.

## 2. Methods

### 2.1. Dataset identification and inclusion criteria

To perform this integrative meta-analysis, we searched in the Gene Expression Omnibus (GEO) database studies that have analyzed genome-wide expression profiling of astrocytes under any stimuli of activation, stress condition or injury, up to March 2020. The following combination of terms was used to find the potential studies: Astrocytes; transcriptomic; stress condition; treatment; inflammation; in-vitro and in-vivo models for humans and mice. The information of datasets selected is shown in **Tables 1** (Humans) and **2** (Mouse). We only included studies with control and stimulated samples, for at least 2 biological replicates and having the raw data available. Further, we selected studies where the expression analysis had been performed in purified astrocytes, primary astrocyte, pluripotent stem cell-derived astrocytes, or astrocyte cell lines.

## 2.2. Meta-analysis

The NetworkAnalyst program, an online tool to perform comprehensive gene expression analysis, meta-analyses and network biology studies (Zhou et al., 2019), was used to carry out the current meta-analysis of GWES for astrocytes. This analysis encompassed several steps. First, the count and intensity tables derived from RNA-seq or microarray analysis for each dataset were extracted to start the process. For microarrays, the series matrix files containing the intensity tables were downloaded from GEO, and for RNA-seq analysis, the normalized count tables were obtained through the GREIN tool, a Web platform for re-analyzing GEO RNA-seq data (Mahi, Najafabadi, Pilarczyk, Kocani, & Medvedovic, 2019).

Second, once the count and intensity tables were obtained, in the module of multiple gene expression data tables from the NetworkAnalyst program, the following processes were performed for every dataset: I) upload and integrity check of raw data II) identifiers conversion, III) normalization and IV) differential expression analysis, which was performed comparing the treatment versus control sample in astrocytes from the same source; this avoids any interference by basal levels of gene expression. Finally, after the data were integrated, the combining  $p$  value approach (Stouffer's method) was selected to perform the meta-analysis. A False Discovery Rate (FDR) of 0.05 was used to correct for multiple testing. Meta-analyses were carried out independently for human and mouse.

## 2.3. Convergence and Enrichment analysis

An online tool (<http://bioinformatics.psb.ugent.be/webtools/Venn/>) was used to perform the convergence analysis using the differentially expressed (DE) genes identified in the meta-analyses of human and mouse samples, as previously employed (Forero & Gonzalez-Giraldo, 2020; Forero, Guio-Vega, & Gonzalez-Giraldo, 2017). The ToppFun application, which is located in the Toppgene suite, was employed to perform the enrichment analysis, (Chen, Bardes, Aronow, & Jegga, 2009); this analysis was carried out for upregulated and downregulated DE genes independently. Functional categories such as Gene ontology (GO), which includes molecular functions, biological process and cellular components were analyzed. Other categories were signaling pathways (using the KEGG: Kyoto Encyclopedia of Genes and Genomes), Transcription Factor Binding Sites (TFBS), MicroRNAs (using

the mirTarbase database) and diseases. Statistical significance was determined by the hypergeometric distribution and the Benjamini–Hochberg FDR of 0.05 was used to correct for multiple testing.

#### 2.4. Analysis of Protein–Protein Interaction (PPI) Networks

To perform the protein-protein interaction networks, we used the NetworkAnalyst program through the HuRI (human binary protein interactions) interactome database (Luck et al., 2020), which contains experimentally validated binary human PPI data. We employed the first-order interaction network to create the PPI. Hub analysis (important node identification) was carried out considering two topological measures, Degree and Betweenness. Module analysis was performed using the connection-first approach to identify and extract functional modules within the network through the Walktrap algorithm (Xia, Benner, & Hancock, 2014). A *p* value of 0.05 was considered significant.

### 3. Results

In the current work, we identified 26 datasets for genome wide expression (GWE) profiling of astrocytes exposed to inflammatory molecules, insecticides, virus and bacterial infection, stress, ethanol, and others. However, the raw data for several studies were not available in GEO or GREIN, and others did not meet the inclusion criteria. Therefore, in total sixteen datasets were included in the final analyses for humans and mice.

#### 3.1. Meta-analysis of human astrocytes

We identified five datasets derived from microarray studies of human astrocytes, which analyzed the following stimuli: Hypoxia (GSE4483, GSE145935); the cyfluthrin and chlorpyrifos insecticides (GSE5023); harmane, a tremorogenic chemical (GSE5080); and H5N1 virus (GSE66597). For RNA-seq, we included three datasets with the following treatments: hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) (GSE58910); *Borrelia burgdorferi* infection (GSE85143); Interleukin 1 beta (IL1B), Interleukin 6 (IL6), Tumor necrosis factor (TNF) and TNF+IL1B (GSE120411). For each experimental group, their respective controls were also included. Most studies used primary fetal human astrocytes (**Table 1**). These 12 experimental groups were used to carry out the meta-analysis and 1625 genes were detected

as differentially expressed; 740 were downregulated and 885 were upregulated. The top fifteen for upregulated and downregulated DE genes are presented in **Figure 1A**. Growth Differentiation Factor 10 (*GDF10*) and Cadherin 6 (*CDH6*) were among the most significant genes identified in this meta-analysis, being both the most consistent since in 11 of 12 studies their expression was downregulated. In contrast, DExH-Box Helicase 58 (*DHX58*) and Hexokinase 2 (*HK2*) were the top upregulated genes, having a consistent expression in 9 and 10 studies, respectively. The complete list of identified genes is shown in **Table S1**.

The enrichment analysis demonstrated that downregulated and upregulated genes participate in distinct functions; upregulated genes were associated with processes such as defense response, immune effector process and cell activation (**Figure 1B**), whereas, downregulated genes were related to cell cycle, neurogenesis, and organelle fission processes (**Figure 1C**). Other GO terms were significantly observed, for instance, several molecular functions and cellular components, which were also different between up and downregulated genes (**Figure 1B and C**). Our enrichment analysis also involved the identification of pathways, TFBS, microRNAs and diseases, where we observed that some of the induced pathways were oligomerization domain (NOD)-like receptor signaling pathway, TNF signaling pathway and cytokine-cytokine receptor interaction. Few transcription factors binding sites were significantly enriched for DE genes, being interferon-regulatory factor 1 (IRF1) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF- $\kappa$ B) the most important for upregulated genes, whereas, for downregulated genes, nuclear transcription factor Y (NFY) and Forkhead box protein O4 (FOXO4) showed to regulate a larger number of genes. On the other hand, several microRNAs were found in this analysis, the most important for up and downregulated genes were hsa-miR-26b-5p and hsa-miR-192-5p, respectively. Finally, we also found different diseases, such as multiple sclerosis, and substance use disorders associated with up and downregulated genes (**Table 3**). The top 50 of significantly enriched categories are shown in **Table S2**.

### 3.2. Meta-analysis for mouse astrocytes

We identified eight datasets for mouse astrocytes, five were derived from microarray analysis, which involved the following treatments: HIV infection (GSE17383), transforming growth factor beta 1 (TGF- $\beta$ 1) for 24 h followed by lipopolysaccharide (LPS) plus interferon gamma (IFN $\gamma$ ) for 8 hours (GSE36089), middle cerebral artery occlusion (MCAO), LPS (GSE35338), TNF (GSE73022), IFN $\gamma$  and interferon alpha A (IFN $\alpha$ ) (GSE67137). For RNA-seq studies, we included the following groups: LPS (GSE75246), IL6 (GSE102903) and ethanol (GSE92457). In total, ten experimental groups with their respective controls were meta-analyzed and 3809 DE genes were identified: 1943 were downregulated genes and 1867 were upregulated genes (**Table S2**); the top 15 for each group are shown in **Figure 2A**. The upregulated genes: C-X-C Motif Chemokine Ligand 9 (*Cxcl9*) and DNA Damage Regulated Autophagy Modulator 1 (*Dram1*), and the downregulated genes: Formin 2 (*Fmn2*) and Adaptor Protein, Phosphotyrosine Interacting with PH Domain and Leucine Zipper 2 (*Appl2*), were the most significant in this analysis. The most consistent gene was *Fmn2*, its expression was decreased in all treatments with a Log Fold Change of -0,07921 to -2,9912.

For all categories analyzed in the enrichment, we found significant results (**Figures 2B, C**). Kinase activity was one of the up and downregulated molecular functions. For upregulated genes, there was a predominance of functions associated with nucleotide binding (**Figure 2B**). Interestingly, we found that downregulated genes were associated with biological processes such as neurogenesis and gliogenesis (**Figure 2C**). Similar to human astrocyte meta-analysis, in the mouse astrocytes meta-analysis upregulated genes were involved in inflammation and defense response. However, differences were observed in the cellular component terms (**Figure 2**). The metabolic and carbon metabolism pathways were significantly enriched for downregulated genes. In contrast, for upregulated genes, the most important pathways were the TNF signaling pathway, apoptosis and nucleotide-binding and NOD-like receptor signaling pathway. Additionally, results for microRNAs, TFBS and diseases are shown in **Table 3**. Diseases such as Alzheimer's Disease, multiple sclerosis and schizophrenia were among the significantly enriched for the DE genes. Further significant categories are shown in **Table S4**.

### 3.3. Convergence functional analysis

Once the DE genes were identified in the meta-analyses for human and mouse astrocytes, we carried out a convergence analysis to evaluate whether there were common genes between both species, using the Venn diagram tool. We identified 323 genes that were DE in both humans and mice (**Figure 3A**): 140 were downregulated genes and 183 genes were upregulated. Although there were another 75 convergent genes, these were expressed differently in opposite directions, for example, in human 46 genes were upregulated whereas in mouse these were downregulated (**Table S5**). In order to prioritize the most important convergent genes that were up and downregulated in both species, we compared which of those genes had in both groups the highest and lowest levels of expression, finding that among the top 50, only 15 genes were similarly upregulated in human and mouse astrocytes, and 14 were within the most downregulated (**Table 4**).

As it was observed in the findings of each meta-analysis, we identified that the convergent upregulated genes are associated with identical protein binding and defense response (**Figure 3B**). Similarly, for convergent downregulated genes, significant enrichment for neurogenesis was observed (**Figure 3C**). In contrast to the results of each meta-analysis, drug and ATP binding were the most important molecular functions for convergent downregulated genes. On the other hand, it was evidenced a significant enrichment of pathways for upregulated genes and a similar result was observed for diseases (**Table 5**). Regarding factors that regulate the gene expression, such as transcription factors and microRNAs, we found that the most important TFBS were IRF (interferon-regulatory factor) and E2F Transcription Factor 1 (E2F1), for up and downregulated genes, respectively. Finally, hsa-miR-124-3p and hsa-miR-26b-5p were among the most significant microRNAs associated with upregulated genes. Other microRNAs were also identified for downregulated genes (**Table 5**). Additional significant categories are shown in **Table S6**.

Further, we compared the DE genes identified in human and mouse astrocytes with the previously proposed markers of A1 and A2 phenotypes of reactive astrocytes (Zamanian et al., 2012), and markers for pan-reactive astrocytes, which correspond to genes induced by

both phenotypes. For human astrocytes, only two genes of A1 and A2 markers were found in the DE gene list. For pan-reactive astrocytes, we found three genes in common, however, one of them, glial fibrillary acidic protein (GFAP) was found as downregulated. For mouse astrocytes, most of the markers of A1, A2, and pan-reactive astrocytes were identified in the DE gene list (**Figure 4**).

### 3.4. Protein-protein interaction analysis for convergent genes

In this work, we performed a network analysis for convergent genes using the NetworkAnalyst program. The first-order interaction generated a network with 1427 nodes, 1981 edges and 130 seeds (genes in the query list) (**Figure 5**). The main hub proteins identified in this network were TNF receptor-associated factor 2 (TRAF2), cell division cycle 37 HSP90 cochaperone (CDC37), paired box 6 (PAX6) and VPS37C subunit of ESCRT-I (VPS37C), which had the highest degree (**Table S7**). Additionally, the module analysis identified 38 significant modules (**Table S8**). The first module had a size of 164 nodes, 15 queries and a  $p$ -value of  $1.21e-12$ . The main hubs in this module were dystrobrevin beta (DTNB) and C-X-C motif chemokine ligand 16 (CXCL16) (**Figure 6A**). The second module was constituted by 136 nodes and 11 queries ( $p$  value:  $7.66e-27$ ). The main hubs were epithelial membrane protein 3 (EMP3), small integral membrane protein 3 (SMIM3) and three prime repair exonuclease 1 (TREX1) (**Figure 6B**). Four additional modules are shown in **Table S8**, which includes the most important hubs in the PPI network such as *VPS37C*, *TRAF2*, *CDC37* and *PAX6*. These modules had a greater size and number of queries (**Table S8**).

## 4. Discussion

Here, we carried out, for the first time, an integrative meta-analysis of genome-wide expression profiling of astrocytes from human and mouse under different stressful stimuli, such as hypoxia, infections by virus and bacteria, cytokines, ethanol, among others. We identified 323 differently expressed genes that were common in both species, involved in processes such as the defense response, regulation of immune system (upregulated genes),

and neurogenesis (downregulated genes). Several of these genes showed to be important hubs in the protein-protein interaction network analysis.

We found that astrocytes from human and mouse respond differently to stressful conditions, as previously reported (J. Li et al., 2020). We identified genes upregulated in human astrocytes but downregulated in mouse (**Table S5**). Furthermore, several categories in the enrichment analysis were different between both species. For instance, molecular functions such as membrane and organelle fusion and fission were only enriched for genes detected in human astrocytes. In contrast, gliogenesis was a biological process only associated with downregulated genes in mouse. Additionally, several transcription factors were distinct in both species, with cyclic AMP-responsive element-binding protein 1 (CREB) only enriched in human cells (**Table S2**). However, we also showed that several genes and functional categories can be similar in astrocytes from human and mouse. For instance, we observed a significant enrichment for the NOD-like receptor signaling pathway, which plays an important role in the innate immune response (Gharagozloo et al., 2017), and transcription factors such as IRX4 and NF- $\kappa$ B. It has been suggested that NF- $\kappa$ B is a key transcription factor involved in detrimental actions of astrocytes (Liddel et al., 2017).

Our results confirm the pivotal role that astrocytes perform in the brain, as we observed that one of the processes identified in the enrichment analysis for upregulated genes was the defense response. Interestingly, most of the top genes presented in (**Table 4**) are associated with this process (**Table S6**). Downregulated genes were mainly associated with neurogenesis. These results suggest that astrocytes can lose their ability to perform some functions, while they attempt to mitigate any injury, damage or alterations triggered in the brain. Notably, this is a mechanism for which reactive astrocytes are proposed to be involved in the development of neurodegenerative diseases (Casse, Richetin, & Toni, 2018).

It is important to highlight that several of the convergent genes are more expressed in astrocytes in comparison to neurons and microglia, for example, the top upregulated genes

such as *ANGPTL4* (Angiopoietin like 4), *DHX58*, *TAPBP* (TAP binding protein) and *TREX*. There are also top downregulated genes such as *AASS* (Aminoadipate-semialdehyde synthase), *CC2D2A* (Coiled-coil and C2 domain containing 2A) and *EXD2* (Exonuclease 3'-5' domain containing 2) that are highly expressed in astrocytes (Zhang et al., 2014; Zhang et al., 2016). This suggests that the expression of several of these convergent genes can be exclusively altered in astrocytes under stressful stimuli. Nevertheless, there may be many other genes dysregulated in a similar fashion in distinct cell types, such as microglia. Remarkably, by comparing our convergent lists with those genes identified in a transcriptomic study of microglia stimulated with LPS, we observed that a small percentage of genes were similar, and these included the inflammatory genes *NFKBIA*, *TNF* and *CXCL10*, (Gerrits, Heng, Boddeke, & Eggen, 2020).

The unknown function of some markers proposed for phenotypes of astrocyte reactivity is a limitation discussed previously by (Sofroniew, 2010). Interestingly, certain genes identified in the current work have been well characterized in reactive astrocytes. A study found that *ANGPTL4* is increased in reactive cortical astrocytes, promoting endothelial cell migration (Chakraborty et al., 2018). Another convergent gene identified in the present work was the *NFE2L2* (nuclear factor, erythroid 2 like 2), which encodes the transcription factor NRF2, a protein involved in anti-inflammatory and antioxidant actions (Stelzer et al., 2016). These anti-inflammatory actions have been corroborated in astrocytes stimulated with IL-1 $\beta$  and TNF (Wheeler et al., 2020). However, there is a bulk of genes highly expressed in astrocytes that have not been explored in these cells under stressful stimuli yet. For instance, *DHX58*, a gene related to the RIG-I-like receptor signaling pathway (Stelzer et al., 2016), was significantly enriched in our analysis (**Table S6**). Although this gene has not been deeply analyzed in astrocytes, its expression was found as upregulated in the brain of AD patients (Twine, Janitz, Wilkins, & Janitz, 2011).

In this work, we also carried out an analysis of the protein-protein interaction network for convergent genes, where we observed that several of the up and downregulated genes showed to be important nodes (hubs) within the network (**Figure 5**). The hub analysis is useful to identify biomarkers or therapeutic targets (Xia et al., 2014). TRAF2 was the most

important upregulated hub gene in this network and it is involved in the activation of NF-kappa-B and Jun N-Terminal Kinase (JNK); moreover, TRAF2 plays a central role in the regulation of cell survival and apoptosis (Stelzer et al., 2016). Previously, it has been found that this is an important regulator of NF-kB pathway in astrocyte cells (S. Li, Wang, Berman, Zhang, & Dorf, 2006). Another important downregulated hub identified was PAX6, a key transcription factor involved in the generation of neurons (Mo et al., 2018). Besides, two of the prioritized convergent genes (Table 4) were found within the most 50 important hubs (Table S6): TREX1 and basic leucine zipper ATF-like transcription factor 2 (BATF2). TREX1 is a protein with exonuclease activity involved in DNA repair (Stelzer et al., 2016) and associated with Aicardi-Goutieres syndrome (Cuadrado et al., 2013).

It is well recognized that astrocytes are widely heterogeneous, and they can have diverse states of reactivity, which differ by their molecular expression, cell morphological, and their interactions with other cell types. However, it is still not well known the functions and the molecular differences in those reactivity states (Linnerbauer, Wheeler, & Quintana, 2020; Sofroniew, 2020). Numerous studies have determined some of these differences in several contexts, for instance, by comparing astrocytes from several species (J. Li et al., 2020), astrocytes from distinct brain regions (Oliveira et al., 2018), and by evaluating multiple inducers of reactive-like phenotypes (Zamanian et al., 2012). Here, we compared our results with the markers for A1 and A2 astrocyte subtypes (Clarke et al., 2018; Zamanian et al., 2012), evidencing that most genes were found in common with mouse astrocytes, but not with human astrocytes. These results support previous findings that showed that those markers are not suitable for cells derived from humans (Perriot et al., 2018). In mouse models, several studies have found that A1 astrocytes are induced in normal aging (Clarke et al., 2018), and that it can be triggered by activated microglia (Liddelow et al., 2017). However, it has been corroborated that the induction of these phenotypes in human astrocytes differ greatly of those from mouse cells (Tarassishin, Suh, & Lee, 2014). Interestingly, a meta-analysis of GWES for aged human prefrontal cortex found that DE genes were compatible with A1 and A2 phenotypes (Payan-Gomez, Rodriguez, Amador-Munoz, & Ramirez-Clavijo, 2018), suggesting the need to explore other molecules or models to induce these phenotypes in human astrocytes.

Meta-analyses have an important power to detect more reliable DE genes through integrating several studies, as this integration allows to increase the sample size, which is a limitation to identify DE genes in individual studies (Wang, Ning, & Guo, 2015). However, some limitations can be present in this type of work, such as the lack of raw data available. We observed that some studies reported the analysis of global gene expression in GEO but did not include the values of intensity for each sample. Moreover, we identified other studies in PubMed that analyzed GWE profiling by RNA-seq in astrocytes under stressful conditions, but the authors did not report the raw data in any database. Therefore, it is crucial that all studies report the raw data of gene expression profiling, which will greatly open new avenues for the use of these public datasets for further analysis.

Altogether, our results demonstrate that despite astrocytes are highly heterogeneous and complex, there are common genetic signatures that can be triggered by them under distinct stressful stimuli. It is noteworthy that the functional roles for several of the top genes identified in our convergent analysis have not been studied in astrocytes. It is known that these genes are expressed in these cells and dysregulated in samples from patients with different neurological diseases and disorders. Therefore, this opens an opportunity for exploring new possible markers of reactive astrocytes, including human astrocytes. We also demonstrate that astrocytes of both species have different molecular signatures, and therefore, it is important to be cautious with the conclusions from studies of animal models, because they might have limitations in their translation to humans. Notably, we also observed that several pathways and biological processes were modulated similarly in both species. It suggests that *in vitro* models of human astrocytes are suitable to analyze the transcriptional responses to stressful stimuli, which will be useful for future studies aiming at evaluating molecular mechanisms or possible therapies.

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#### Author Statement

All authors contributed to the study conception and design. Data collection and analysis were performed by Yeimy González-Giraldo, and Diego A. Forero. The first draft of the manuscript was written by Yeimy González-Giraldo and all authors commented and contributed on previous versions of the manuscript. All authors read and approved the final manuscript.

#### Conflict of interest

The authors declare no conflict of interest.

Supplementary data

Supplementary material

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**Table 1.** Genome-wide expression studies included in the meta-analysis for human astrocytes exposed to stressful stimuli

GE O ID	Datas et	Treatment (n)	Control (n)	Platform	Astrocyte/Source/ Age	PM ID
GS E44 83	Mense , 2006	Hypoxia (1% O <sub>2</sub> -5% CO <sub>2</sub> , 94% N <sub>2</sub> ) for 24 h (3)	Normoxia (95% air- 5% CO <sub>2</sub> ) (3)	Affymetrix Human Genome U133 Plus 2.0 Array	Human fetal astrocytes/Whole brain/14-19 week	165 077 82
GS E50 23	Mense , 2006	25 µM cyfluthrin for 7 d (3) 25 µM chlorpyrifos for 7 d (3)	1% DMSO (3) 1% DMSO (3)	Affymetrix Human Genome U133 Plus 2.0 Array	Human fetal astrocytes/Whole brain/14-19 week	167 904 87
GS E50 80	Mense , 2007	1 µM harmaline for 7 d (3)	1% DMSO (3)	Affymetrix Human Genome U133 Plus 2.0 Array	Human fetal astrocytes/Whole brain/14-19 week	NR
GS E66 597	Lin, 2016	H5N1 infection (MOI: 1.0) for 24 h (3)	not infected (3)	Agilent-014850 Whole Human Genome Microarray 4x44K G4112F	U-251 MG human cell line	260 087 03
GS E14 593 5	Heath, 2020	Hypoxia (1% O <sub>2</sub> , 5% CO <sub>2</sub> , 94% N <sub>2</sub> ) for 24 h (3)	Normoxia (95% air- 5% CO <sub>2</sub> ) (3)	Affymetrix Human Genome U133 Plus 2.0 Array	Human fetal astrocytes	NR
RNA-seq						
GS E58 910	Grego ry, 2014	200 µM H <sub>2</sub> O <sub>2</sub> for 2 h (2)	Untreated (2)	Illumina HiSeq 2000	Human Fetal astrocytes/ Fetal cortex /20-22 weeks	276 305 59
GS E85 143	Dhasa rathy, 2017	<i>Borrelia burgdorferi</i> infection (MOI: 10:1) for 24 h (39)	Untreated (3)	Illumina HiSeq 2000	Human astrocytes	281 353 03
GS	Perrio	10 ng/mL IL1B for	Untreated	Illumina HiSeq 2500	Pluripotent stem	304

E12	t,	24 h (3)	(3)	cell-derived	095
041	2018	100 ng/mL IL6 for		astrocytes	08
1		24 h (3)			
		10 ng/mL TNF for			
		24 h (3)			
		TNF + IL1B for 24			
		h (3)			

**Abbreviations:** DMSO (Dimethyl sulfoxide); H<sub>2</sub>O<sub>2</sub> (hydrogen peroxide); IL1B (Interleukin 1 beta); IL6 (Interleukin 6); TNF (Tumor necrosis factor- $\alpha$ ); h (hours) and d (days); NR (No reported); n (sample size), PMID (PubMed identifier); MOI (Multiplicity of infection).

**Table 2.** Genome-wide expression studies included in the meta-analysis of mouse astrocytes exposed to stressful stimuli

GEO ID	Dataset	Treatment (n)	Control (n)	Platform	Astrocyte/Source/ Age	PMID
GSE17383	Borjabad, 2009	HIV infection (MOI: 1.0) for 24 h (3)	Mock virus (MOI: 1.0) for 24 h (3)	Affymetrix Mouse Genome 430 2.0 Array	NR	19697136
GSE36089	Coppola, 2012	3ng/ml TGF- $\beta$ 1 for 24 h + 2 $\mu$ g/ml LPS + 3ng/ml IFN $\gamma$ for 8h (4)	Basal condition (growing medium) for 32 hours (4)	MouseRef -8 v2.0 expression beadchip	Primary astrocytes/Cortex/1-3-day-old	23077035
GSE35338	Zamani, 2012	MCAO for 1 d (5) LPS (5 mg/kg) for 1 d (5)	Sham for 1 d (4) Saline for 1 d (4)	Affymetrix Mouse Genome 430 2.0 Array	NR	22553043
GSE7	Glaab,	50 ng/mL TNF for 24 h	untreated	Affymetrix	Primary	2698

3022	2015	(3)	(3)	x Mouse	astrocytes/Cortex/postnatal	1349
				Gene 1.0	, P0–P2	
				ST Array		
GSE6	Beiting,	10ng/ml IFN $\gamma$ for 12 h	unstimula	llumina		
7137	2016	(2)	ted (2)	MouseRef	Primary	2783
		10ng/ml IFN $\alpha$ for 12 h	unstimula	-8 v2.0	astrocytes/Cortex/1–3-day-	4206
		(2)	ted (2)	expression	old	
				beadchip		
RNA-seq						
GSE7	Friedma	10 mg kg <sup>-1</sup> LPS for 24 h		llumina		
5246	n, 2016	(4)	Saline (4)	HiSeq	Primary astrocytes	2709
				2500		7852
GSE1	Sibley,	50 ng ml <sup>-1</sup> IL6 for 11 h	untreated	llumina	Purified astrocytes/	
02903	2017	(3)	(3)	HiSeq	Cerebral cortices/P1	2907
				2500		9839
GSE9	Erickso	15% (v/v) ethanol every	Water for	llumina	Purified astrocytes/	
2457	n, 2018	other day for 72 d (10)	72 d (11)	HiSeq	Prefrontal cortex/ adult, 8	2930
				4000	weeks	5589

**Abbreviations:** TGF- $\beta$ 1 (Transforming growth factor beta 1); LPS (lipopolysaccharide); IFN $\gamma$  (Interferon gamma); MCAO (Middle cerebral artery occlusion); TNF (Tumor necrosis factor-alpha); IFN $\alpha$  (Interferon alpha A); IL6 (Interleukin 6); h (hours) and d (days); NR (Not reported); PMID (PubMed identifier); MOI (Multiplicity of infection).

**Table 3.** Significant categories in the functional enrichment analysis of DE genes identified in the meta-analyses for human and mouse astrocytes exposed to stressful stimuli. The statistical significance was analyzed using the hypergeometric distribution followed by a correction for multiple testing (Benjamini–Hochberg False Discovery Rate) in the Toppgene portal.

Category	Upregulated genes	FDR	Downregulated genes	FDR
Human astrocytes				
Pathways	NOD-like receptor signaling pathway	3,32E-12		
(KEEG)	TNF signaling pathway	1,20E-07	NS	
	Cytokine-cytokine receptor interaction	4,33E-05		

	IRF	8,20E-06	FREAC2	7,60E-06
TFBS	REL	2,01E-04	NFY	1,38E-04
	NFKB	2,26E-04	FOXO4	1,76E-04
	hsa-miR-26b-5p	3,03E-27	hsa-miR-192-5p	2,70E-63
MicroRNAs	hsa-miR-124-3p	3,03E-27	hsa-miR-215-5p	1,45E-57
(miRTarBase)	hsa-miR-16-5p	8,25E-19	hsa-miR-193b-3p	4,20E-33
	Multiple Sclerosis	1,61E-07	Organic Mental Disorders, Substance-Induced Substance-Related Disorders	1,72E-05 2,12E-05
Diseases				
	TNF signaling pathway	4,83E-17	Metabolic pathways	3,17E-06
Pathways	Apoptosis	1,61E-10	Carbon metabolism	2,68E-03
	NOD-like receptor signaling pathway	2,09E-13	Lysosome	2,92E-03
	IRF	4,47E-14	LEF1	2,62E-05
TFBS	NF-KB (p65)	1,31E-07	FOXO4	9,76E-05
	ELK1	1,97E-06	E12	1,24E-03
	hsa-miR-16-5p	2,91E-144	hsa-miR-26b-5p	5,37E-79
MicroRNAs	hsa-miR-124-3p	3,85E-137	hsa-miR-93-5p	2,32E-61
	hsa-miR-12a-5p	1,22E-123	hsa-miR-20a-5p	2,73E-54
	Multiple Sclerosis	1,56E-14	Schizophrenia	7,75E-03
Diseases		1,90E-10	Alzheimer's Disease	3,04E-02

**Abbreviations:** KEGG (Kyoto Encyclopedia of Genes and Genomes); TFBS (Transcription factor binding sites); IRF (Interferon Regulatory Factor); REL (REL Proto-Oncogene, NF-KB Subunit); NF-KB (Nuclear Factor Kappa B Subunit); FREAC2 (Forkhead Box F2); NFY (Nuclear Transcription Factor Y); FOXO4 (Forkhead Box O4); ELK1 (ETS Transcription Factor ELK1); LEF1 (Lymphoid Enhancer Binding Factor 1); E12 (TCF3: transcription factor 3); FDR (False discovery rate); NS (Not significant); DE (differentially expressed).

**Table 4.** The top genes with the highest and lowest fold change that were found convergent in humans and mouse astrocytes exposed to stressful stimuli. Convergent genes were ranked according to their fold changes and *p* values. Meta-analyses were performed using the combining *p* value approach (Stouffer's method).

Symbol	Gene name	Humans		Mouse	
Upregulated genes		<b>Fold Change</b>	<b>Combined <i>p</i> value</b>	<b>Fold Change</b>	<b>Combined <i>p</i> value</b>
<i>ANGP</i>	Angiopoietin like 4	5,2261	7,46E-05	7,1395	6,78E-11
<i>TL4</i>					
<i>BATF2</i>	Basic leucine zipper ATF-like transcription factor 2	5,1831	8,41E-05	7,2298	3,75E-11
<i>CEBPB</i>	CCAAT enhancer binding protein beta	4,7624	0,0037013	7,9013	3,07E-13
<i>CXCL10</i>	C-X-C motif chemokine ligand 10	5,0375	0,0013848	13,503	0,0
<i>DHX58</i>	DExH-box helicase 58	6,6561	2,14E-07	9,0421	0,0
<i>GBP3</i>	Guanylate binding protein 3	4,1340	0,0022819	11,564	0,0
<i>ISG20</i>	Interferon stimulated exonuclease gene 20	5,1644	8,62E-05	8,7571	0,0
<i>LY6E</i>	Lymphocyte antigen 6 family member E	5,1893	8,41E-05	8,1376	5,60E-14
<i>NFKB1A</i>	NFKB inhibitor alpha	5,7119	1,41E-05	7,3598	1,55E-11
<i>OGFR</i>	Opioid growth factor receptor	5,3186	6,15E-05	8,2593	2,94E-14
<i>PSMB10</i>	Proteasome 20S subunit beta 10	4,7436	0,00038791	9,2874	0,0
<i>RELB</i>	RELB proto-oncogene, NF-kB subunit	4,4145	0,0010154	7,9292	2,58E-13
<i>TAPBP</i>	TAP binding protein	4,9857	0,00015798	7,0425	1,30E-10
<i>TREX1</i>	Three prime repair exonuclease 1	4,04	0,0030374	7,8513	4,62E-13
<i>TRIM25</i>	Tripartite motif containing 25	4,4372	0,00095469	7,1574	6,09E-11
Downregulated genes		<b>Fold Change</b>	<b>Combine d <i>p</i> value</b>	<b>Fold Change</b>	<b>Combined <i>p</i> value</b>
<i>AASS</i>	Amino adipate-semialdehyde synthase	-3,6861	0,0073272	-5,1716	4,55E-06
<i>ABHD</i>	Abhydrolase domain containing 3,	-3,9493	0,0038359	-6,0076	6,69E-08

3	phospholipase				
<i>ATE1</i>	Arginyltransferase 1	-3,7647	0,0061334	-4,6034	5,53E-05
<i>ATP1B1</i>	ATPase Na <sup>+</sup> /K <sup>+</sup> transporting subunit beta 1	-3,678	0,0074308	-5,5076	9,17E-07
<i>CC2D2A</i>	Coiled-coil and C2 domain containing 2A	-3,8199	0,0054082	-5,3283	2,19E-06
<i>DHRS11</i>	Dehydrogenase/reductase 11	-3,8972	0,0044207	-4,4402	0,00010721
<i>DUSP19</i>	Dual specificity phosphatase 19	-4,1044	0,0024874	-5,0724	7,16E-06
<i>EXD2</i>	Exonuclease 3'-5' domain containing 2	-3,8073	0,0055302	-5,6028	5,65E-07
<i>FGF12</i>	Fibroblast growth factor 12	-3,6987	0,007099	-4,579	6,12E-05
<i>GLRB</i>	Glycine receptor beta	-4,0051	0,0033505	-4,7484	3,01E-05
<i>LUZP2</i>	Leucine zipper protein 2	-3,9572	0,0037911	-5,6477	4,53E-07
<i>PPM1E</i>	Protein phosphatase, Mg <sup>2+</sup> /Mn <sup>2+</sup> dependent 1E	-3,968	0,0037007	-5,884	1,31E-07
<i>SLC12A6</i>	Solute carrier family 12 member 6	-4,4495	0,00092028	-5,3792	1,71E-06
<i>ZIC3</i>	Zic family member 3	-3,7176	0,0067904	-5,0066	9,63E-06

**Table 5.** Significant functional categories derived from the enrichment analysis for convergent genes. The statistical significance was analyzed using the hypergeometric distribution followed by a correction for multiple testing (Benjamini–Hochberg False Discovery Rate) in the TopGene portal.

Category	Upregulated genes	FDR	Downregulated genes	FDR
Pathways (KEGG)	NOD-like receptor signaling pathway	4,19E-11		
	TNF signaling pathway	5,27E-09		
	NF-kappa B signaling pathway	6,42E-09		
TFBS	IRF	5,56E-12	E2F1	3,63E-02
	ISRE	1,46E-07	CAC-binding protein	3,63E-02
	NFKB	1,62E-05	CP2	4,82E-02

MicroRNAs	hsa-miR-124-3p	1,53E-14	hsa-miR-192-5p	1,55E-10
(miRTarBase)	hsa-miR-26b-5p	1,06E-13	hsa-miR-193b-3p	1,11E-07
	hsa-miR-1-3p	1,56E-13	hsa-miR-215-5p	1,59E-07
Diseases	Multiple Sclerosis	2,20E-08		
	Alzheimer's Disease	2,39E-04		

**Abbreviations:** KEGG (Kyoto Encyclopedia of Genes and Genomes); TFB $\beta$  (Transcription factor binding sites); IRF (Interferon Regulatory Factor); ISRE (interferon-sensitive response element); NF-KB (Nuclear Factor Kappa B Subunit); E2F1 (E2F Transcription Factor 1); CP2 (Transcription Factor CP2); FDR (False discovery rate); NS (Not significant).

**Figure 1.** The most consistent genes identified in human astrocytes under different stimuli and the top terms derived from gene ontology analysis.

**A.** Heatmap for the top 15 upregulated (red) and downregulated (green) genes obtained through the meta-analysis of genome-wide expression studies of human astrocytes under different stressful stimuli. The combining  $p$  value approach (Stouffer's method) was used to perform the meta-analysis in the NetworkAnalyst program. After a correction for multiple testing, a  $p$  value  $<0.05$  was considered significant.

**B and C.** These figures show the most significant cellular component, biological process, and molecular function identified in the gene ontology (GO) analysis, which was performed for 885 upregulated and 740 downregulated genes derived from meta-analysis of human astrocytes. The exploration of GO is part of the enrichment analysis carried out in the Toppgene portal. The statistical significance was determined by hypergeometric distribution followed by a correction for multiple testing (Benjamini–Hochberg False Discovery Rate).

**Abbreviations:** H<sub>2</sub>O<sub>2</sub> (hydrogen peroxide); IL1B (Interleukin 1 beta); IL6 (Interleukin 6); TNF (Tumor necrosis factor-alpha).

**Figure 2.** Top 15 of differentially expressed genes identified in the meta-analysis for mouse astrocytes under different stimuli and the most significant terms found by the gene ontology analysis.

**A.** Heatmap shows the expression levels for the top 15 upregulated and downregulated genes, which were identified by the meta-analysis using the combining  $p$  value approach (Stouffer's method) in the NetworkAnalyst program. This analysis was carried out using datasets of genome wide expression studies derived from astrocytes stimulated at *in vitro* and *in vivo* conditions. After a correction for multiple testing, a  $p$  value  $<0.05$  was considered significant.

**B and C.** Gene ontology analysis identified several cellular components, biological processes and molecular functions associated to 1867 upregulated and 1943 downregulated genes in mouse astrocytes. The statistical significance was determined by hypergeometric distribution followed by a correction for multiple testing (Benjamini–Hochberg False Discovery Rate).

**Abbreviations:** TGF+L+I: TGF- $\beta$ 1 (Transforming growth factor beta 1), LPS (lipopolysaccharide), IFN $\gamma$  (Interferon gamma); MCAO (Middle cerebral artery occlusion); TNF (Tumor necrosis factor-alpha); IFN $\alpha$  (Universal Type I Interferon); IL6 (Interleukin 6).

**Figure 3.** Convergence analysis of DE genes identified in the meta-analysis for human and mouse astrocytes exposed to stressful stimuli and gene ontology analysis.

**A.** Venn diagram shows the convergent genes (upregulated and downregulated) derived from each meta-analysis for human and mouse astrocytes.

**B and C.** Gene ontology analysis identified molecular functions, biological processes and cellular components associated to 323 common genes in human and mouse astrocytes under stressful stimuli. The statistical significance was determined by hypergeometric distribution followed by a correction for multiple testing (Benjamini–Hochberg False Discovery Rate).

**Abbreviations:** MDG: downregulated genes in Mouse. MUG: Upregulated genes in mouse; HDG: downregulated genes in Human; HUD: Upregulated genes in Human.

**Figure 4.** Comparison of A1 and A2 markers with DE genes identified in the meta-analysis of human and mouse astrocytes exposed to stressful stimuli.

Lists of DE genes from human and mouse astrocytes identified by the meta-analyses were compared with the A1 and A2 markers suggested by Zamanian et al., 2012. PAN (pan-reactive) corresponds to markers induced by both type of astrocytes. Red: upregulated expression. White: the gene was not identified in the DE gene lists. Green: downregulated expression.

**Figure 5.** First-order protein-protein interaction network for convergent genes.

Graph shows a network of 1427 nodes, 1981 edges and 130 seeds (genes in the query list). Red represents upregulated nodes; green represents downregulated nodes. Node sizes represent the degree with larger nodes are the most important hubs in the network: TRAF2, CDC37, PAX6, VPS37C, and AP1M1. The protein-protein interaction network was built using the HuRI (human binary protein interactions) interactome database in the NetworkAnalyst program. Two topological measures (Degree and Betweenness) were considered to identify important nodes (hub analysis).

**Figure 6.** Module analysis of protein-protein interaction network.

The connection-first approach was used to identify and extract the significant modules within the first-order protein-protein interaction network by means of the Walktrap algorithm. Red nodes represent the upregulated genes and green nodes show the downregulated genes. The node size represents the importance within the module.

**A** This graph depicts the significant module 1. Size of 164 nodes, 15 queries and a  $p$  value of  $1.21e-12$ . The main hubs in this module were dystrobrevin beta (DTNB) and C-X-C motif chemokine ligand 16 (CXCL16).

**B.** The second module was constituted by 136 nodes and 11 queries ( $p: 7.66e-27$ ). The main hubs were epithelial membrane protein 3 (EMP3), small integral membrane protein 3 (SMIM3) and three prime repair exonuclease 1 (TREX1).

### Highlights

- This meta-analysis identified common differentially expressed genes in human and mouse astrocytes in 16 datasets of genome-wide expression (microarrays and RNA-seq) studies.
- Astrocytes induce expression of genes associated with stress response and immune system regulation when they are exposed to stressful stimuli, whereas genes related to neurogenesis are found downregulated.
- Several of the genes identified showed to be important hubs in the Protein-Protein Interaction network (TRAF7, CDC37 and PAX6).