A Systematic Review and Meta-analysis on the Transcriptomic Signatures in Alcohol Use Disorder – a Translational Approach

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Abstract

Alcohol Use Disorder (AUD) is a complex mental disease with a large treatment need. However, the clinically available therapeutic approaches are limited and thus new treatment targets are warranted. One approach to discover new treatment targets is transcriptomic profiling of key brain regions of the addiction neurocircuitry in animal models of AUD and/or in post-mortem brain tissue from deceased AUD patients. Unfortunately, those studies suffer from large heterogeneity and low sample sizes. Here we used a cross-species meta-analysis that integrates the entire literature on this topic to make more robust conclusions on transcriptomic changes in AUD. Here, we report on differentially expressed genes (DEGs) in 108 rat brain and 380 human brain samples, respectively in one of the key regions of AUD – the prefrontal cortex. We found 50 differentially expressed genes (DEGs) in rat brain, which were mainly associated with pro-oncogenic molecules, signal transduction as well as oxidative stress and inflammation. When we compared these findings with the meta-analytic results in human post-mortem PFC, we found that across species Hsd11b1 is significantly down-regulated. This finding is also confirmed by our cross-species data integrational approach. In summary, our data suggests that down-regulation of Hsd11b1 enzyme and stress-related pathways is a conserved mechanism for the alcohol dependent phenotype across different species, which might explain increased cortisol levels in AUD and therefore serves as a new treatment target.

Keywords: transcriptomics, Meta-Analysis, Alcohol Use Disorder, alcohol, post-mortem, brain tissue, rodents, human, prefrontal cortex.

Introduction

Alcohol Use Disorder (AUD) is a complex mental disease that causes annually more than three million deaths worldwide, which represents 5.3% of deaths per year in total (WHO, 2019). Alcohol has been classified as the most harmful drug in a comparison of the most commonly abused licit and illicit drugs, considering both harm to the user and others (Nutt et al., 2010). Importantly, alcohol is acting systemically and consequently its detrimental effects affect all organ systems. In addition, there are numerous closely linked physiological and psychological comorbidities of AUD such as major depressive disorders (MDD), antisocial disorders, as well as liver and kidney injuries (McHugh et al., 2019; Rocco et al., 2014; Helle et al., 2019; Rumgay et al., 2021).

A key goal of AUD research is to identify the underlying neuroadaptations and molecular targets that lead to alcohol addiction in order to repurpose existing drugs or develop new medications (Burnette et al., 2022; Kranzler & Soyka, 2018; Witkiewitz et al., 2019). Previous studies suggested molecular pathways in diverse brain regions that might be impaired by chronic alcohol consumption and might be involved in the establishment of AUD (Abrahao et al., 2017, Gilpin& Koob, 2008). A growing body of research supports the neuroimmune hypothesis of AUD, which describes the effects of chronic alcohol intake on inflammation and neuromodulation (Mayfield & Harris, 2017, Erickson et al., 2019). Also, AUD has been associated with increased oxidative stress and impaired cell proliferation leading to increased risk for certain cancer types (Seitz & Stickel, 2007; Tsermpini et al., 2022). In addition, chronic alcohol consumption and AUD have been shown to cause disruptions in the circadian rhythm (Davis et al., 2018) and clock genes have been linked to addictive behavior (Perreau-Lenz & Spanagel, 2015). Long-term alcohol consumption, AUD and withdrawal are inducing stress-related pathways and also stressful experiences are elevating

alcohol consumption (Perreau-Lenz & Spanagel, 2015; Spanagel et al., 2014). Consequently, AUD is a common comorbidity of patients with high stress-associated psychological disorders, such as PTSD and anxiety disorders (Debell et al., 2014; Gilpin et al., 2015).

Nevertheless, even though numerous studies have been conducted to explore the effects of high alcohol consumption in general as well as compulsive alcohol drinking correlated to AUD, medical approaches for AUD therapy are still limited and only partially effective. So far, naltrexone, acamprosate and disulfiram are the only FDA-approved treatments for AUD (Witzkiewtz et al., 2019), whereby 60% of all AUD patients experience at least one relapse period during or after therapy, irrespective of the treatment itself (Mayernhoff & Durazzo, 2010; Kirshenbaum et al., 2009; Witkiewtz 2011). Furthermore, it is interesting to note that some of the most promising therapeutic mechanisms identified by preclinical research have failed in clinical trials (Schwandt et al., 2016; Heilig et al., 2019; Kwako et al., 2015; Heilig et al., 2016). As a result of these substantial translational failures, pharmaceutical industries ceased to invest efforts in the development of new possible medications (Hyman et al., 2014), leading to the still open debate on the utility of animal models for preclinical testing of novel therapies (Hyman, 2012; Heilig et al., 2021).

Animal research is characterized by several limitations, which can make the interpretation of the results obtained from the studies challenging. A major limitation is the lack of statistical power often caused by insufficient sample size, which can produce unreliable results (Button et al., 2013). Additionally, inter-study variability (due to e.g., breeding facility, experimenter, laboratory conditions, species/strain, experimental protocols) leads to diverse outcomes among published studies. These limitations impact the translatability of preclinical research. Meta-analyses are a powerful tool for at least partially overcoming such limitations (Sena et al., 2014). Hence, in this study we aim to perform systematic literature

screening and meta-analysis on transcriptome-wide studies focusing on brain samples derived from rodents exposed to ethanol. Even though no known animal model of addiction fully mimics the human condition, certain animal models are better suited to investigate specific components of the addiction process in a clinically relevant way (Lynch et al., 2010). We therefore focused our attention on the well-established post-dependent animal model, obtained via chronic intermittent alcohol exposure (CIE), that mimics the natural process in which humans develop alcohol addiction: over a prolonged period of time and after several intermittent drinking episodes interspersed with withdrawal periods (Meinhardt & Sommer, 2015). This model results in robust and stable behavioral symptoms of alcohol dependence that persist during prolonged abstinence and is characterized by long-lasting neuroadaptations that mediate such symptoms (Heilig et al., 2017). Importantly, we also applied the same procedure to transcriptome-wide studies performed on human AUD patients and healthy controls to identify commonly altered genes and gene networks among species. We chose the prefrontal cortex (PFC) as the brain region of interest for this meta-analysis. Converging lines of evidence from human and animal studies have revealed that the PFC is a key structure for performing highest-order cognitive functions (Abernathy et al., 2010; Avchalumov et al., 2021; Ball et al., 2011). Results gained over the past decade suggest that chronic alcohol exposure profoundly impairs this brain region (Abernathy et al., 2010; Pahng et al., 2017; Schacht et al., 2013; Charlton et al., 2019), leading to a dysfunctional control over compulsive behavior (Bechara, 2005; Pahng et al., 2017; Timme et al., 2022; de Paiva Lima et al., 2017). Even after abstinence, alcohol-induced cognitive dysfunctions can affect top-down inhibitory control, decision-making, and behavioral flexibility (Trantham-Davidson & Chandler, 2015; Catafau et al., 1999; Grüsser et al., 2004). In addition, as part of the extended reward system, it has a central role in the development

and maintenance of AUD (George & Koob, 2010, Goldstein & Volkow, 2011, Wise & Koob, 2014). Notably, as a stress-sensitive brain region, the PFC also plays a crucial role in the withdrawal state and relapse-like behavior (Blaine et al., 2016; Blaine & Sinha, 2017; Seo & Sinha, 2015; George et al., 2012), suggesting the functional dysregulation of this brain region as a hallmark of early relapse risk in alcoholic patients.

We believe that our findings might deliver a profound basis for further studies to understand the mechanisms of AUD and to develop new therapeutic approaches that could potentially be more successful than the currently available medications on the market.

Materials and Methods

Systematic Literature Screening

The systematic literature screening was performed as described in the PRISMA Guidelines (Page et al., 2021). Briefly, two researchers independently screened the literature with predefined keywords for PubMed and EMBASE that were determined to specify four main categories of interest: alcohol, animal model of alcohol dependence, gene expression, and brain (Fig. 1). For post-dependent rodent studies, the following keywords were used: (alcohol OR ethanol) AND (rats OR mice OR rat OR mouse) AND (ribonucleic acid OR RNA OR RNA-seq OR mRNA OR ncRNA OR miRNA OR siRNA OR piRNA OR snRNA OR microRNA OR expression OR gene OR cDNA OR transcription OR transcript OR transcripts OR transcriptional OR transcriptomic OR translation OR transcriptome OR transcriptomics OR translation) AND (post-dependent OR "post dependent" OR postdependent OR cie OR "chronic intermittent"). For human post-mortem studies, we used the following keywords: (“post-mortem” OR “postmortem” OR “human” OR “patients”) AND (“brain”) AND (“alcohol” OR “alcoholic” OR “alcohol dependence” OR “alcoholism” OR “AUD” OR “alcohol use disorder” OR “alcohol addiction”) AND (“transcriptome” OR “transcriptomic” OR “transcriptomics” OR “gene-expression” OR "gene expression" OR “mRNA” OR “messenger RNA”). The literature screening was performed using the SysRev screening tool developed by the CAMARADES research group at the University of Edinburgh (www.syrf.org.uk). After each screening step (title/abstract and full text), the researchers compared the respective resulting studies. In case of disagreement, a third independent party chose whether the study was included or excluded for the next screening step. The general structure of the predefined keywords, as well as the complete PRISMA flow diagrams with resulting number of studies for each screening step for rodent and human study screening is depicted in Fig.1.

Since the majority of the identified studies was working on tissue derived from the PFC, we decided to focus on this brain region as scope of our analysis.

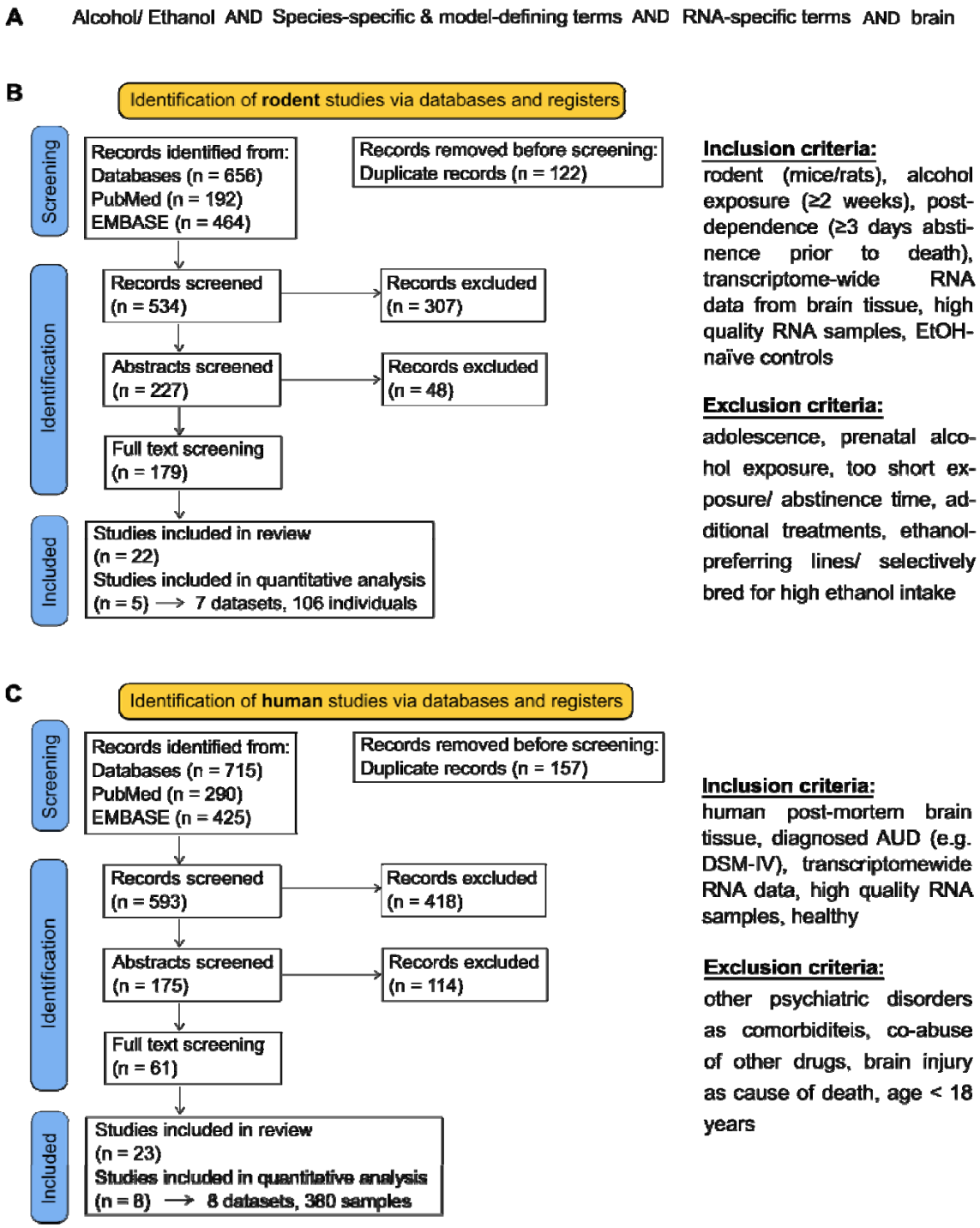

Fig. 1: General structure for keyword design for systematic literature research in PubMed and EMBASE representing the four categories based on which we specified the keywords (**A**)

and the resulting number of studies for rodent studies (n=5 studies, 7 datasets) (**B**) as well as human post-mortem brain studies (n=8 studies) (**C**). Two rodent studies each included two datasets fulfilling our criteria, which explains the higher number of datasets compared to studies. Since in both species, the PFC was predominantly used in the original studies, we focused the analysis on this brain region. Before the screening, duplicates were removed using EndNote. The template for overview of the search procedure and the resulting studies is taken from www.prisma-statement.org.

Only studies providing transcriptome-wide RNA-expression data, such as microarray or RNA-Seq data, were considered for the meta-analysis. The Summary Statistics – including the full profile of all detected genes from the sequencing experiment – of the eventually resulting studies, were either downloaded from the original publication or the corresponding authors were contacted to request the respective datasets. Details of the included studies are listed in Suppl. Tab. 1. and Suppl. Tab. 2.

As usually included in systematic reviews and meta-analyses, we aimed to perform an assessment of risk of bias (RoB) for the original studies. Unfortunately, inadequate reporting was impeding us to access full information necessary for proper RoB assessment.

Meta-Analysis

All statistical analyses were performed using the statistical program R Version 4.1.2. Rodent datasets and human post-mortem datasets were meta-analyzed separately and then compared in later steps. As reported previously, analyzing microarray datasets from scratch can be challenging due to high diversity in processing platforms, methods and analysis softwares (Kontou et al., 2018). So far, there has been no standardized analysis

methodology available to combine all these diverse datasets into one generalized analysis pipeline. Therefore, we decided to combine the summary statistics representing log2 fold-changes and corresponding p-values of all detected genes per study instead. Additionally, due to the diverse analysis platforms used, the estimation of the effect sizes was inconsistent across the original studies, which was suggesting to not perform an effect size combinatorial approach in this study. According to a previously published guideline on gene expression meta-analysis (Toro-Domínguez et al, 2021), we eventually decided to perform weighted Stouffer's p-value combination using the R package DExMA (Villatoro-Garcia et al., 2022). P values of log2-fold change calculations from original studies were used as input and studies were weighted by the square root of sample size. We set the threshold for inclusion of a gene to 60 %, meaning that each gene included in the meta-analysis was represented by at least ⅔ or 4/7 of the original studies for rodent and human datasets respectively. Before conducting the meta-analysis, all datasets were annotated with the most current databases mouse4302.db, rae230a.db, illuminaHuman4.db, org.Hs.db, EnsDb.Hsapiens.v86 to ensure comparability of the gene names. Multiple entries for one gene within a study were grouped using the collapseRows function from the R package WGCNA (Langfelder and Horvath, 2008; Miller et al., 2011). Since in the mouse and rat genome not all genes have common names, we decided to use biomaRt for annotating the rodent genes with the mouse orthologue to ensure we are not missing out on data points due to the potential divergent naming of same genes (Durinck et al., 2009). The individual analysis steps as well as the packages for human and rodent meta-analysis in use can be found in Fig. 2. Significant DEGs were defined by a threshold of $p_{corr}<0.05$.

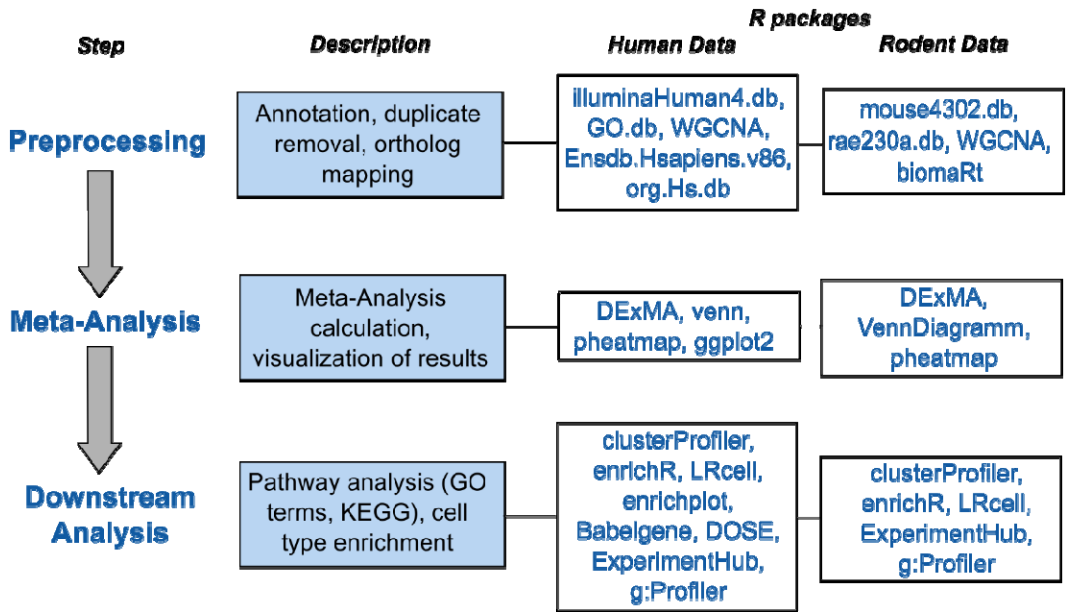

Fig. 2: Analysis pipeline for meta-analysis in animal and human post-mortem PFC tissue, including data pre-processing, meta-analysis and downstream analysis investigating potential enrichment in pathways and cell types.

In addition, a consistency index for the differentially expressed genes was estimated considering fold-change direction and the value of the fold-change per gene that was significant in the meta-analysis. Therefore, the index was calculated for all respective genes considering its values within each original study as described in Eq. 1. The corresponding values can be found in Suppl. Tab. 3 and Suppl. Tab. 5 in the respective column.

$$\text{Consistency index} = \left(\frac{\qquad\qquad\qquad\qquad}{\qquad\qquad\qquad\qquad}\right)$$

Eq. 1: Calculation of consistency index per gene that was detected as significant in the meta-analyses of rodent and human datasets to observe the level of consistency in the direction of dysregulation across the original publications in comparison with the meta-analysis results.

For the rodent analysis, we additionally performed subgroup analysis considering mouse and rat datasets separately to observe potential species-specific effects contributing to the main effects of the meta-analysis results.

Subgroup analysis rodent datasets

To determine the species-specific impact on the outcome of the meta-analysis of post-dependent animals, we performed a subgroup analysis by dividing the original studies according to the included species. Afterwards, we performed a meta-analysis within the subgroups and compared the results with the DEGs derived from the meta-analysis of the two rodent species together. The threshold for considering a gene for the analysis was appearance in at least 60 % of the datasets in the respective subgroup. Significant DEGs were defined by a threshold of $p_{corr}<0.05$.

Down-stream analysis

To identify potential pathways associated with the DEGs identified in the meta-analysis, we performed gene set enrichment analysis (GSEA) with GO-terms and KEGG-pathways using the R package ClusterProfiler (Wu et al., 2021) and g:Profiler (Raudvere et al., 2019). Additionally, we analyzed potential enrichment of the DEGs for specific cell-types. Therefore, we performed cell-type enrichment analysis using the R package LRcell (Ma, 2021).

Overlap between human and animal DEGs

Since animal and human genes differ in their nomenclature, we first identified the human orthologues of the animal DEGs using the Babelgene R package and then compared these

converted DEGs with the human dataset to find potential overlaps in the results. In addition, we also compared pathway analysis results for both species to identify possible commonly dysregulated pathways in the animal model as well as in the human situation. For comparison, significant DEGs were defined by a threshold of $p_{corr}<0.1$.

Cross-species data integration

The VennDiagram approach is a first step to descriptively compare and show potential overlaps of the results coming from the species-specific meta-analyses. To further investigate which genes are conserved for the alcoholic phenotype across species, we performed a cross-species integrational meta-analytic approach. Therefore, we used the "homology method", where we first transferred the human genes coming from the initial Summary Statistics per study to their respective rodent orthologues as reported previously (Schall & Latham, 2021). Afterwards, separate appearance indices for human and rodent species were calculated as the sum of the number of studies a gene occurred within one species (Eq.2 (1)). Then, the cross-species appearance indices per gene were combined to an overall appearance index as described in Eq. 2 (2). The cross-species appearance index cut-off was set to 0.8, which is representing 60% of rodent and human studies each to ensure that the genes included in the cross-species meta-analysis are included in a representative number of studies. To avoid over-representation of rodent studies, the upper limit for inclusion was set to a cross-species appearance index of 1.25.

(1) Species-specific appearance index = $\sum_{i}^{n} \quad index(i) = study1(i) + study2(i) + \ldots + studyn(i)$

(2) Cross-species appearance index = $\left(\frac{Appearance\ index\ rodents}{Appearance\ index\ humans}\right)$

Eq. 2: (1) Calculation of the species-specific appearance index considering the species separately. Let i be a gene measured in the RNA quantification experiment from the original

studies and n be the number of studies per species. If a gene was targeted in the original publication, the value 1 was assigned to the study, whereas 0 was assigned to the study, whenever the gene was not measured. The species-specific appearance index was calculated for each gene and each species separately. (2) Equation of the cross-species appearance index, which represents an overall index considering all species in combination. These indices were used as a cut-off value for integrating genes into the cross-species meta-analytical approach, considering also the proportion of rodent and human studies to avoid over-representation of one species.

Including genes with a cross-species appearance index between 0.8 and 1.25, combined meta-analysis of rodent and human datasets was performed using rodent orthologues for the human datasets and the R package DExMA for meta-analysis. The resulting significant DEGs ($p < 0.05$), were included in further downstream analyses as described in the section "down-stream analysis".

Results

Meta-Analysis in PFC of post-dependent rodents discovered six genes that were not discovered by the included original studies.

Systematic literature screening resulted in seven studies matching the research criteria for this study including the PFC as the brain region of interest (Fig. 1). Since two of these studies included more than one dataset that is matching our criteria, this was leading to nine datasets derived from seven studies. Out of those, we were able to retrieve seven datasets derived from five studies, which resulted in 106 individuals in total. Three datasets were conducted from rats (n= 42 individuals) and four datasets contain mouse tissue (n= 64 individuals). Detailed information about the included studies can be extracted from Suppl. Tab. 1 and the inclusion and exclusion criteria in Fig. 1. First, we observed the distribution of the significant DEGs per original study to get an impression of the heterogeneity across these studies (Fig. 3), where we found 11-38% of the DEGs overlapping with at least one additional original study, while the remaining 62-89% of DEGs were represented by only one original study. This finding describes a high heterogeneity across the original studies based on their reported DEGs. When we compared these genes with the outcome of the meta-analysis that resulted in 50 significant DEGs ($p_{corr}<0.05$), we observed 44 genes (corresponding to 88% of all significant DEGs of the meta-analysis) overlapping with at least one original study, while six genes (corresponding to 12% of the gene set) were found as solely significant in the meta-analysis (Fig. 3). These six genes are Rnaseh1, Dr1, Lancl2, Mc5r, Scn11a, and Tmem161b. To our knowledge, most of these genes have not been related to chronic alcohol consumption or AUD in previous studies. However, Lancl2 has been suggested as a candidate gene of interest underlying the chromosome 4 QTL for alcohol consumption in alcohol-preferring iP rats and non-alcohol-preferring iNP rats

(Spence et al., 2013). Scn11a has previously been correlated to the altered function of encoded sodium channels, leading to hyperexcitability in nociception as a response to excessive alcohol consumption (Maingret et al., 2008).

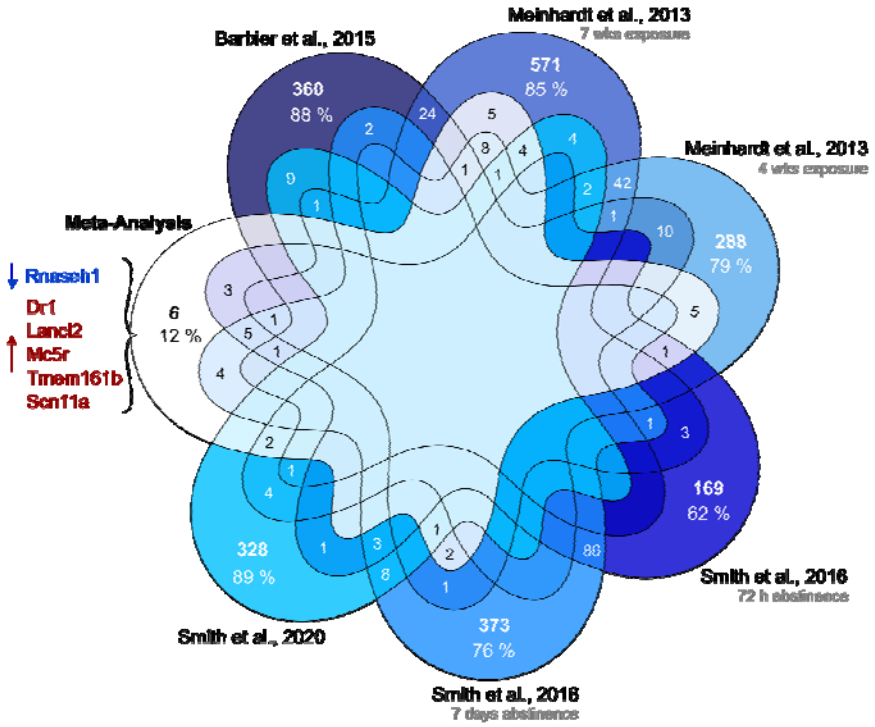

Fig. 3: Venn Diagram comparing the significant DEGs (p_{corr}<0.05) identified by the original studies and comparison of these genes with the significant DEGs derived from meta-analysis. No gene was found to be significantly dysregulated among all the datasets. This figure solely represents original studies that reported significant DEGs for PFC; for meta-analysis all datasets were included, regardless of the presence of reported significant results for this brain region. Please note that for Meinhardt et al., 2013 and Smith et al., 2016 we included two datasets per study with timepoints that were focusing on either diverse exposure length or abstinence duration. Further details about the original studies can be extracted from Suppl. Tab. 1. Colors of the ellipses were chosen randomly to provide proper visualization and comparability of the overlaps across the datasets.

Although observation of the heterogeneity of DEGs across the original studies did not suggest species-specific differences in the distribution of the transcriptomic patterns, we performed subgroup analysis, considering mouse and rat studies as respective subgroups (Fig.4, Suppl. Tab. 4). Interestingly, we did not find any DEG overlapping between the species that were not found in the meta-analysis results as well. One gene, namely Nidogen-2 (Nid2) (up-reg., cell adhesion), appeared significant across all three datasets: mouse subgroup, rat subgroup and the meta-analysis. In the main analysis, we identified six DEGs that were not significant in any of the original datasets. In the subgroup analysis, we found eleven DEGs that did appear significant only in the meta-analysis dataset, but not in the subgroup analyses (Fig. 4). Despite Tmem116, which was significant in the rodent subgroup analysis and in the meta-analysis, the previously identified unique DEGs of the meta-analysis remained unique by this type of analysis as well (Fig.4). Additionally, six DEGs appeared as unique for the meta-analysis results, which are Birc7 (up-reg., apoptosis), Dio1 (down-reg., thyroid hormone activity), Itga7 (up-reg., autophagy), Pnpla3 (down-reg., balance of energy usage/storage in adipocytes), Tat (down-reg., tyrosine metabolism) and Ttc7b (up-reg., plasma membrane). For rat datasets, 30 genes (corresponding to 4 % of all DEGs of this dataset) overlapped with the results of the main analysis, whereas for mice 13 genes (corresponding to 6% of all DEGs of this dataset) overlapped with the main analysis. When comparing the number of DEGs from the mice subgroup analysis (n=4 datasets, all J57BL/6 strain) to the rat subgroup analysis (n=3 datasets, all Wistar strain), the mice subgroup shows less than ⅓ of the number of DEGs derived from the rat subgroup.

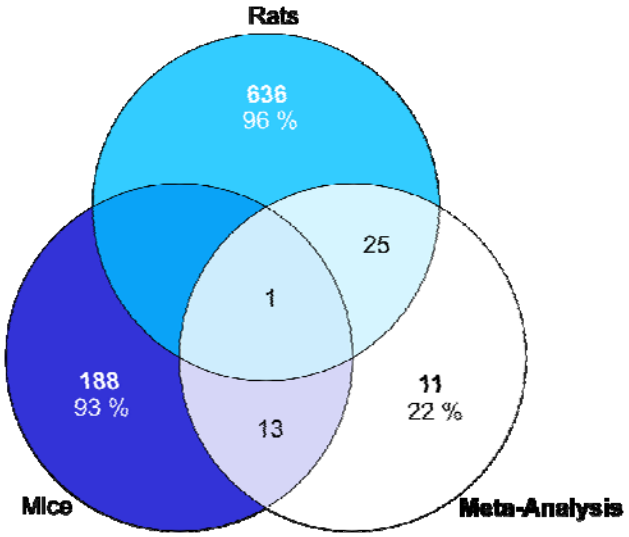

Fig. 4: Subgroup analysis considering mouse and rat studies as individual subgroups and comparison of the resulting genes with the main findings of the meta-analysis. Venn diagram is depicting the significant DEGs (p_{corr}<0.05) of the separate analyses per species and of the meta-analysis. Rat subgroup analysis revealed 715 DEGs, mouse subgroup analysis resulted in 202 DEG, and meta-analysis across both species led to 50 DEGs in total. One gene, Nid-2, is commonly significant in all three datasets. The percentage of genes per overlay is calculated with respect to the total number of DEGs detected across all three datasets (n= 972 genes). All included mouse studies worked with the J57BL/6 strain, whereas all rat studies work with the Wistar strain. Additional information can be found in Suppl. Tab. 4. Colors of the circles were chosen randomly to provide proper visualization and comparability of the overlaps.

Thus, in total our analysis of rodent PFC identified 50 DEGs, we next were interested whether these genes were enriched for specific pathways. Therefore, we performed pathway enrichment analyses using mouse orthologs and mouse terms derived from the

most commonly used databases (Supplementary). Signal transduction-related pathways as well as nitrogen metabolic pathways were among the most significant pathways.

Additionally, if we consider individual genes and their involvement in pathways based on the literature, it seems that apoptosis and general cell maintenance controlling genes are downregulated whereas genes that have previously been correlated to cancer, such as Fgf16, Cabin1, Usp35 and Atg101 (He et al, 2021; Zhang et al., 2018; Wang et al., 2022; Zhang et al., 2022) were up-regulated. This finding suggests a higher prevalence of tumor development in ethanol-exposed rodents. In addition, the gene Tat – encoding for Tyrosine aminotransferase –, which is associated with tyrosine breakdown, is up-regulated. Tyrosine is directly correlated to dopamine biosynthesis. Therefore, enhanced tyrosine breakdown might lead to significant dopamine decrease. Previous studies have shown that alcohol-dependent animals as well as AUD patients have decreased dopamine levels, which were associated with withdrawal and the likelihood of relapse (Dahchour & Ward, 2022; Hansson et al., 2019).

In addition, we observed potential enrichment of the DEGs derived from rodent meta-analysis in PFC for cell-type specific patterns, which resulted in enrichment for layer 6 neurons (Suppl. Fig. 1).

Meta-Analysis in PFC of human post-mortem samples results in 278 DEGs enriched in immune- regulatory and pro-oncogenic pathways.

Systematic literature screening resulted in ten studies for quantitative analysis for human post-mortem PFC, where we were able to conduct eight datasets in total, resulting in 380 samples (Fig. 1). Since all included studies were using samples derived from the NSWBTRC at

the University of Sydney, there might be an overlap among the patient samples, which might lead to over-representation of certain individuals. To date, unfortunately, we were not able to correct for this potential bias in our analysis and therefore we were considering each sample as unique.

When we observed the total distribution of significant DEGs ($p < 0.1$) across the original studies, we found that 50-84 % of DEGs represented solely by the original studies and therefore were only significant in one publication (Fig. 5). When additionally considering the DEGs derived from the meta-analysis, the identified significant DEGs ($p < 0.1$) had an overlap of 37 % with at least one of the original studies and one gene – Mt1x, encoding for Metallothionein 1X – was overlapping with two original datasets. Fig. 5 shows the Venn Diagram representing the overlap of all significant DEGs reported in the original studies as well as the significant DEGs derived from the meta-analysis.

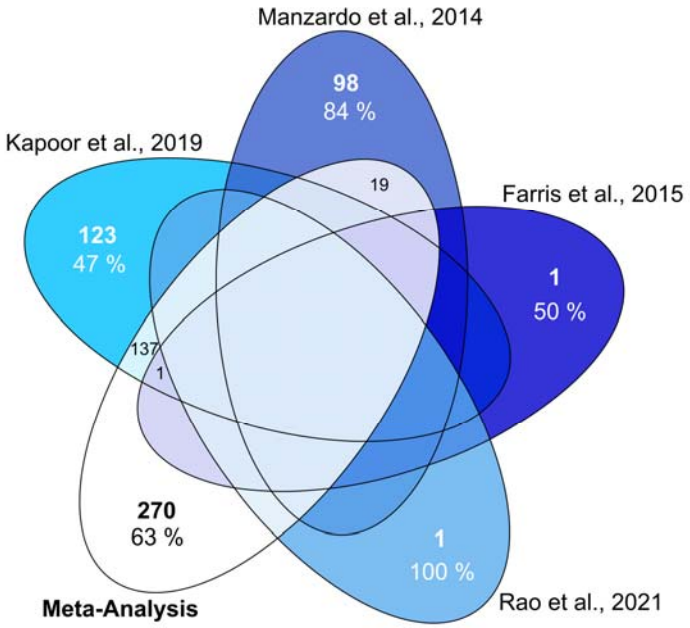

Fig. 5: Venn Diagram representing the overlap of significant DEGs reported in the original studies ($p<0.1$) and DEGs derived by meta-analysis ($p<0.1$). No gene was found to be significantly dysregulated among all the datasets. This figure solely represents original

studies that reported significant DEGs for PFC (n=4), while for meta-analysis all PFC datasets (n=8) were included, regardless of the number of reported significant results in the original publication. Detailed information about the included publications can be found in Suppl. Tab. 2. Colors of the ellipses were chosen to provide proper visualization and comparability of the overlaps coming from different studies.

Next, we tested the significant DEGs ($p<0.05$) of the meta-analysis for enrichment in specific pathways (Suppl. Tab. 6). Generally, DEGs were enriched in GO-terms associated with inflammation, cell adhesion, and signal transduction, whereas enriched KEGG-pathways were associated with apoptosis, cell differentiation, and inflammatory pathways. When we then ordered the DEGs according to their fold-change direction into activated and repressed genes, we found activated NF-κB expression, and toll-like receptor signaling, as well as cytokine storm and pro-oncogenic pathways (Suppl. Tab. 6). Down-regulated DEGs were enriched for suppressed metabolic processes, neuroactive ligand-receptor interaction and glycerolipid metabolism, as well as signal transduction-related pathways and cellular membrane stability (Suppl. Tab. 6).

Cell-type specific enrichment of the DEGs derived from meta-analysis in human post-mortem brain tissue resulted in a tendency for enrichment in oligodendrocytes ($p= .12$; Suppl. Fig. 2).

Overlap DEGs derived from a meta-analysis in rodents exposed to ethanol and human post-mortem brain tissue suggests a conserved mechanism for the alcoholic phenotype.

To identify common alterations in gene expression across species, we first determined the human orthologues of the DEGs derived from the rodent datasets. Fig. 6 depicts the results from the comparison of these DEGs across human and rodent species. The overlap between human and rodent meta-analysis results in six commonly dysregulated genes: Adcy3, Hsd11b1, Itm2c, Oga, Rab26 and Rnf122. Interestingly, these genes were at least partially also dysregulated in the same direction in both human and rodent meta-analysis results. However, when we consider the subgroup analysis from the rodents, Hsd11b1 was only significant in the rats and pointing towards an up-regulation, whereby a combination of the rodent datasets is still remaining significant, but leading to a total down-regulation of Hsd11b1 (Suppl. Tab. 4). Interestingly, to our knowledge these six overlapping genes have so far not been studied with an intense interest in the context of alcohol consumption and AUD in the brain.

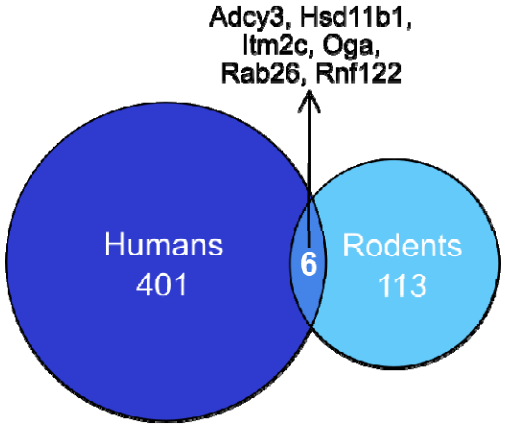

Fig. 6: Venn Diagram representing the comparative overlap of significant DEGs (p_{FDR}<0.1) derived from the meta-analysis in PFC of post-dependent rodents and AUD patients respectively results in six genes being significantly dysregulated. Across these species, Hsd11b1 and Rnd122 were consistently down-regulated, and Itm2 was consistently up-

regulated. Further information can be extracted from Suppl. Tab. 4. Colors of the circles were chosen randomly to provide proper visualization and comparability of the overlaps.

Cross-species data integration confirms the importance of dysregulation in the cortisol pathway for the alcoholic phenotype.

Eventually, we aimed to find genes that are conserved for the alcoholic phenotype across species. Therefore, we performed a cross-species integrational meta-analytic approach using cross-species appearance indices to ensure equal contribution of the species (Eq. 2). After applying the cross-species appearance indices for every homologous gene detected in the original experiments, 8513 genes from eight human and seven rodent original datasets were included. Meta-analysis resulted in 31 significant DEGs ($p < 0.05$), 22 up-regulated and 9 down-regulated. Hsd11b1 – the gene that showed highest significance in the comparison between the species-specific meta-analyses (Fig. 6) – also appeared in this cross-species meta-analysis as the most significantly down-regulated gene (Suppl. Tab. 7).

Down-stream pathway enrichment analysis using the 31 DEGs resulting from cross-species data integration resulted in enrichment in signal transduction-related pathways, such as glutamatergic signal transduction, signal receptor binding, and focal adhesion (Supplementary). Additionally, enrichment in general biological processes involving protein biosynthesis and metabolism was detected.

Cell-type enrichment analysis resulted in significant enrichment for endothelial cells, astrocytes, oligodendrocytes and polydendrocytes (p_{FDR}=0.05; Suppl. Fig. 3).

Discussion

We performed a meta-analysis on transcriptome-wide datasets derived from post-dependent rodent and post-mortem human brain tissue in the context of alcohol dependence and AUD in a comprehensive manner. (i) Meta-analysis in post-dependent rodent PFC led to 50 DEGs, whereby six of these genes were not identified before in the original studies included in the analysis. (ii) The DEGs in rodents were mainly related to immune-regulatory and cancer-related pathways and showed enrichment in layer 6 neurons. (iii) Meta-analysis in human post-mortem PFC resulted in more than 278 DEGs, which showed association with inflammatory, cell adhesion and apoptosis pathways and enrichment in oligodendrocytes. (iv) Cross-species comparison and integration suggest a novel mechanism to explain increased cortisol levels in alcohol addiction via down-regulation of Hsd11b1 as a conserved mechanism for the alcohol dependent phenotype.

The observation of the DEGs identified in the respective original rodent studies pointed out a high heterogeneity, since 62-92% of the identified significant genes were found dysregulated in only one original study. One of the general aspects of performing meta-analyses is to observe an effect across several original studies, considering heterogeneity introduced by e.g., diverse study design, and including a higher sample size for the analysis to increase statistical power. The varying study design, such as the differences in length of alcohol exposure and duration of abstinence before sacrifice of the animals, might be a potential explanation for the heterogeneous DEG distribution among the original studies. Also, it is known that mice and rats show differing transcriptomic patterns in response to alcohol exposure (Contet et al., 2012). Additionally, individuals derived from different breeders can differ genetically and physiologically. It has been shown in the past, that the

microbiome has an impact on gene expression in the brain via the gut-brain-axis (Margolis et al., 2021) and that the microbiome of laboratory animals is influenced by a variety of factors (Ericsson & Franklin, 2021). Nevertheless, this heterogeneity across the original studies points out the importance of our meta-analysis: genes being identified as significantly dysregulated despite these inter-study variances might represent targets of particular interest, as their dysregulation was detected regardless of the chronic alcohol treatment paradigm. Also, in the clinical situation of human AUD patients and the post-mortem brain samples included in the pre-clinical analyses, the background of the patients varies in terms of drinking duration and relapse phases, environment, genotype, period of abstinence before death and psychological aspects that influence the transcriptomic profile. Therefore, observing transcriptomic changes on a heterogeneous background might deliver promising targets that could be representative for the general alcohol dependent phenotype that might be useful for treatment of a broad spectrum of AUD patients.

In the meta-analysis of alcohol-dependent rodent PFC, we found 50 DEGs ($p<0.05$), including six genes that do not overlap with the previous findings of any of the original studies. Interestingly, to our knowledge, most of these uniquely identified genes have not yet been addressed in the alcohol field. Tmem161b has previously been associated with reward processing in alcohol dependence in a GWAS on patients with major depressive disorder (Muench et al., 2018). Lancl2 was suggested as a candidate gene of interest underlying the chromosome 4 QTL for alcohol consumption in iP and iNP rats (Spence et al., 2013). In addition, Scn11a has been found to cause altered functions of encoded sodium channels that are correlated with hyperexcitability and nociception in response to alcohol consumption (Maingret et al., 2008; Yang et al., 2020). However, when we performed subgroup analysis considering the rodent species, mouse and rat as separate subgroups, we

identified eleven unique DEGs that were represented by the meta-analysis results solely and not in any of the subgroup-specific results. Within these eleven genes, five out of the six unique genes from the previous comparison of the meta-analysis with the results of the original studies were replicated. Also, the number of non-overlapping DEGs in the meta-analysis decreased when comparing these resulting genes with the DEGs from subgroup analyses. One gene was found to be significant in both of the subgroups as well as in the meta-analysis, which is Nid2, a protein-coding gene that is involved in cell adhesion. Nid2 is essential for the plasticity and excitability of neurons, neuronal network establishment and maintenance (Vasudevan et al., 2010). In our analysis, Nid2 was significantly up-regulated in post-dependent rodents, which therefore might lead to increased neuronal plasticity and excitability. Also, an increase in Nid2 due to aging has been previously found in humans (Keable et al., 2016). Nid2 has been suggested as a potential tumor marker for ovarian, esophagus and colon cancer (Kuk et al., 2010; Chai et al., 2018; Yu et al., 2019) and therefore, its over-expression might also give evidence for an elevated risk for tumor development in these animals.

In general, we observed differences between the results coming from subgroup analysis and meta-analysis. Subgroup-specific differences leading to stronger effects coming from one subgroup only and potentially evening-out effects coming from opposite effects in mice and rats are enlightened by this type of additional analysis. The different outcomes of the subgroup analyses compared to the results from the meta-analysis in terms of the number of DEGs and the general overlap might be due to the settings of the analysis. To avoid the bias caused by genes that might have strong effects, but were only detected in a small number of studies, we set the threshold for including a gene in the analyses to 60%.

Pathway analysis of the DEGs related to rodent PFC meta-analysis resulted in enrichment for dysregulated basic cellular regulatory pathways, such as general cellular catabolic processes, voltage-gated channel regulatory activity, cell membrane regulatory aspects and signal transduction. In addition, the DEGs of the rodent PFC were enriched for pathways related to nitrogen biosynthesis. Nitrogen is an essential element for biosynthesis of amino acids and therefore general protein synthesis. Also, nucleotides –the smallest compartment of DNA and RNA molecules– central coenzymes such as NAD, and hormones have nitrogen implemented as an essential element. Therefore, impairment of nitrogen biosynthesis is supposedly having a broad influence on cellular mechanisms and metabolisms. As central molecule of the urea cycle, nitrogen biosynthesis dysregulation is not only having impact on digestion-related pathways across the whole organism, but also urea cycle disorders have previously been found to impair brain functions, mainly on astrocytes, and therefore lead to disabilities in cognitive functions (Gropman et al., 2013). When we then split the DEGs of the meta-analysis according to the direction of the fold-change, we found a potentially elevated risk for cancer development, since some genes that are known to be pro-oncogenic molecules were up-regulated. This finding is adding to the previous studies that found an increased risk for cancer in alcohol-exposed rodents and AUD patients (Chan et al., 2014; Rumgay et al., 2021).

When we looked into a potential cell type-specific enrichment of the DEGs identified by meta-analysis, we found significant enrichment for layer 6 neuronal cells in post-dependent rodents. These days, more and more single-cell sequencing data are published, which are pointing out that different cell types play an important role in health and disease. Neurons in general, and especially cortical neurons, have been previously shown to be associated

with immune system deficiency caused by alcohol use disorders (Erickson et al., 2019). Layer 6 is the deepest layer of the mammalian cerebral cortex and contains the highest variety of neuronal cells, including pyramidal, inhibitory and excitatory neurons. Previous studies reported the effects of ethanol on layer 6 neurons mainly in the context of alcohol-exposure during brain development and fetal ethanol exposure. Therefore, a generally reduced size and altered morphology of prefrontal cortex layer 6 neurons were found in mice exposed to ethanol during fetal development (Louth et al., 2018). In addition, the synaptic activity of layer 6 neurons of the somatosensory cortex is altered by prenatal ethanol exposure in mice (Delatour et al., 2020). However, even though this specific type of neuronal cells seems to have been only in the scope of studies focusing on the influence of ethanol exposure during the developmental stage, our data suggest that alcohol consumption might have an impact on this cell type also at the adult stage.

In the meta-analysis of human post-mortem brain samples derived from the PFC, we identified a high heterogeneity among the reported DEGs from the original studies and the meta-analysis results, as well. This heterogeneity might at least partially be explained by the inclusion of different subjects per original study, which are potentially representing diverse genotypes, drinking patterns, and environmental aspects. In addition, the time span in which these data were obtained is more than 20 years and therefore RNA detection methods as well as analysis methods have changed throughout the years, which might introduce a technical bias into our analyses. However, since we analyzed Summary Statistics from the original studies and all human samples included in our analysis were originally derived from the NSWBTRC at the University of Sydney, Australia, we were not able to balance out the bias due to the potential over-representation of specific individuals that

have been analyzed in more than one of the original studies. Therefore, we have to consider that potentially patients are over-represented in our analysis.

Our analysis of human PFC resulted in a total number of 268 DEGs ($p < 0.05$), where more than half of these genes were uniquely identified by the meta-analysis and did not appear as significant in the original studies. Downstream analysis showed enrichment in several pathways. GO-term and KEGG pathway analyses were associated with inflammation, cell adhesion and differentiation as well as apoptosis. When we ordered the DEGs from the meta-analysis by their fold-change direction, pathways for neuro-inflammation such as NF-κB and cytokine storm as well as pro-oncogenic pathways were associated with the up-regulated DEGs. Suppressed pathways associated with the DEGs are e.g., neuroactive ligand-receptor interaction, signal transduction and cellular membrane stability processes. GSEA led to enhanced glutamatergic signal transmission, which in the past has been shown to be associated with enhanced drug seeking (Koob & Volkow, 2010). The NMDA receptor is one of the ionotropic glutamate receptors and is deactivated by acute alcohol consumption (Hoffman et al., 1989), but activated by chronic alcohol exposure and withdrawal (Smothers et al., 1997). Enhanced NMDA-induced calcium invasion is hypothesized to contribute to alcohol-related neuronal loss in the PFC due to increased excitotoxicity (Iorio et al., 1993; Bliss et al., 2014). Up-regulation of the GABA-ergic synapses in our meta-analysis also contributes to previous findings, which state that the GABA(A)-receptors might enhance tolerance to anxiolytic, sedative and positive reinforcing effects during chronic alcohol consumption (Kumar et al., 2004; Kumar et al., 2009). Dopamine (DA) and related signal transmission and pathways have been the scope of alcohol-related research for decades, where it was identified as directly affected by alcohol consumption and these impairments of the DA system were associated with impaired cognitive function due to alcohol addiction

(Trantham-Davidson & Chandler, 2015). In this scenario, our results point towards increased dopaminergic synaptic transmission in AUD patients (Suppl. Tab. 6). Furthermore, we were also able to obtain findings that were not studied before: we observed down-regulation of steroid-related metabolic pathways and receptor binding. Neurosteroids have potentially inhibitory functions leading to anxiolytic, neuroprotective, memory-related and cognitive effects (Baulieu, 1991; Rupprecht & Holsboer, 1999; Reddy, 2010). Impairment of neurosteroids in the PFC have been associated with mood disorders (Qi et al., 2018), particularly in depression (Romeo et al., 1998). Even though neurosteroids have not been studied extensively in the field of AUD, we believe that they represent potential targets for future research, especially when considering the high proportion of AUD patients with additional psychiatric comorbidities (Swendsen and Merikangas, 2000).

As already found in the rodent data, an elevated risk for cancer development has been confirmed also in the human analysis. In addition, several studies have shown that the immune system is strongly impaired by long-term alcohol consumption and neuro-inflammation has been stated as a risk factor for additional psychiatric disorders in AUD patients (Erickson et al., 2019). Also, NF-κB and cytokine storm-related genes have previously been described to be enhanced during chronic alcohol consumption (Pascual et al., 2021; Mayfield et al., 2013).

In human post-mortem AUD tissue, we found cell type-specific enrichment for oligodendrocytes, which is supporting previous findings from other labs (Lewohl et al., 2000; Mayfield et al., 2002; Brenner et al., 2020). Impairment of oligodendrocytes in human AUD patients has been associated with reduced white matter volume and cognitive malfunction (Xin et al., 2020). This cell type is mainly regulating myelinating processes. In terms of AUD,

myelin damage and impairment of oligodendrocytes have been identified in the past and the potential correlating risk for AUD patients to develop comorbid neurodegenerative diseases such as Parkinson's Disease or Alzheimer's Disease has been discussed (Rice & Gu, 2019; Guo et al., 2021).

In this comparative study, we aimed to observe potential commonalities between the post-dependent animal model of alcohol dependence (chronic intermittent ethanol exposure; CIE) and the patient situation, where we found six significant DEGs overlapping in the direct comparison: Adcy3, Hsd11b1, Itm2c, Oga, Rab26 and Rnf122. Among these six genes, Hsd11b1 showed the highest significance in the comparative approach and is also appearing as the most significantly down-regulated gene in the cross-species data integrative approach. Hsd11b1 is encoding a protein that is involved in the catabolism of cortisol. Down-regulation of this gene might lead to an increase in cortisol levels and therefore potentially induce chronic stress reaction. Furthermore, the transcript encoding CRH (Corticotropin-releasing hormone) was significantly downregulated in human PFC and also in the integrative cross-species meta-analysis. CRH is the central activator of the HPA (hypothalamic-pituitary-adrenal) axis, which is the main regulatory system of the body's response to stress. Accumulating evidence confirms an increase of CRF in the brain after acute alcohol exposure. The effect of chronic alcohol exposure has not been so clear, but several studies support decrease of CRF mRNA in the brain after chronic alcohol exposure (Schreiber and Gilpin, 2018). The current study supports the cross-species effect of decreased CRF in the PFC after chronic alcohol exposure that might be resulted from negative feedback of chronically increased cortisol in long-term excess alcohol use. Since the PFC is generally known to be a stress-sensitive brain region, these dysregulations might even

have a broader impact on the whole individual. Stress and increased cortisol levels are implemented in the process of establishing AUD and are also one of the key aspects of acute and long-term withdrawal (Anderson et al., 2016; Becker, 2017; Heilig & Koob, 2007). Previous studies have suggested that increased cortisol concentrations are induced by increased cortisol biosynthesis (Lu & Richardson, 2014), whereas our results suggest a new pathway of escalated alcohol consumption leading to reduced cortisol catabolism via reduced Hsd11b1 presence and an increased cortisol level as a consequence.

On a genome-wide level, human GWAS studies support significant association between excessive alcohol use and the corticotropin receptor gene *Crhr1* (Sanchez-Roige et al., 2018; Gelernter et al, 2019; Brazel et al, 2019) but no GWAS evidence for alcohol use has been currently found for the Hsd11b1 gene in these recent high throughput studies. However, an SNP in the promoter region of the Hsd11b1 gene, rs10779511, is significantly associated with Aspartate aminotransferase (AST) levels (Sakaue et al, 2021). Elevated AST levels in the serum, have been shown to be one of the strongest diagnostic markers for history of excess alcohol consumption as elevated AST to alanine transferase (ALT) ratio is a useful index for distinguishing nonalcoholic steatohepatitis from alcoholic liver disease (Sorbi et al, 1999). Also, in the liver of AUD patients, down-regulation of Hsd11b and its analogs has been shown to induce a hypercortisolism state, which was suggested to be associated with alcoholic liver disease (Ahmed et al., 2008).

Adcy3 encodes for adenylate cyclase 3 and has previously been found to be strongly associated with obesity (Wu et al., 2016). In addition, it was suggested to have a regulatory function in the immune system, as it has been found to be dysregulated in systemic inflammatory diseases, such as inflammatory bowel disease and ulcerative colitis (Hulur et al., 2015). Even though, so far there has been found no association of Adcy3 with AUD or

related phenotypes, its importance to obesity and immune regulatory function links to well-known comorbidities of AUD. The finding of Adcy3 dysregulation is also supported by the cross-species data integrative approach, where Adcy3 is significantly up-regulated.

Itm2c encodes for integral membrane protein 2 c, which is inhibiting the proliferation and projection development of neuronal cells. Previous studies have shown interaction of this gene in Alzheimer's Disease (Dolfe et al., 2018) and it seems to be highly expressed in the rodent brain (Choi et al., 2001). However, to our knowledge, this gene and the corresponding protein have not been studied extensively in general and have also not been addressed in the field of alcohol research so far.

The Oga gene – encoding for O-GlcNAcase – has previously been suggested to have an emerging role in several cancer types via an atypical gene expression alteration mechanism (Parker et al., 2021). This finding is contributing to the previously reported increased risk of AUD patients to develop several cancer types (Cao & Giovannucci, 2016), even though this gene has not been found to be associated with alcohol abuse or dependence so far. In brain-specific disorders, Oga has been found to be associated with intelligence (Savage et al., 2018), cognitive performance (Lee et al., 2018), Alzheimer's disease (Kulminski et al., 2022), Educational attainment (Okbay et al., 2016), ADHD and autism spectrum disorder (Rao et al, 2022). Additionally, Oga was also significantly dysregulated in the cross-species integrative meta-analysis, underlining its potentially important role in AUD and the development of common AUD- related comorbidities.

Also the recently discovered gene Rab26, encoding for the Ras-related protein Rab-26, is contributing to the increased risk for cancer development in AUD patients, based on our analysis. Rab26 is a member of the Ras protein family, which is generally categorized as an oncogene family. Ras proteins are generally involved in regulation of endothelial cell

permeability and induction of autophagy and therefore, when dysregulated, contribute to cancer development and progression (Dong et al., 2018).

Rnf122 was characterized as a ubiquitin ligase and therefore it is involved in protein degradation, including its own degradation process. In addition, it is associated with the calcium-modulating cyclophilin ligand (Peng et al., 2010). Previous Genome-wide association study (GWAS) identified SNPs in Rnf122 as susceptibility markers for attention deficit hyperactivity disorder (ADHD) and also found up-regulation of the Rnf122 RNA levels in the respective patient group (Garcia-Martinez et al.,2017). However, based on our literature research it seems like Rnf122 just recently got attention from researchers and therefore this gene also requires further investigation into its involvement in physiological and mental diseases, including AUD.

Even though these genes identified by cross-species overlap were the only ones identically labeled across the species, there are also genes coming from similar gene families that are commonly altered in the rodent as well as the human meta-analysis e.g., up-regulation of genes encoding for integrins and Lancl gene family members.

In the cross-species integrative approach, we aimed to observe the commonly dysregulated transcriptomic changes in a combined analysis. Here, we observed 31 DEGs, which also partially confirmed previously identified DEGs coming from species-specific meta-analyses, considering rodent and human data separately. The increased number of DEGs compared to the comparative approach of the separate species-specific meta-analyses (Fig. 6) could be explained by increased statistical power due to the strongly increased input. While for species-specific meta-analyses seven rodent and eight human datasets were used respectively, in the cross-species approach the input increased to 15 studies in total.

Pathway enrichment analysis pointed towards signal transduction-related pathways, including glutamatergic signaling. Alcohol is known to impact the glutamatergic system and glutamatergic neurons have been suggested as a promising target for psychopharmacotherapy of AUD patients (Holmes et al., 2013; Alasmari et al., 2018).

This translational approach comparing the effects of chronic alcohol consumption on transcriptomic signatures in animal models of alcohol dependence and AUD patient confirms previous findings and also enlightens potentially new targets for further study in the alcohol addiction field. Based on our results that point towards an overlay of transcriptomic signatures between the animal model of alcohol dependence and human AUD brain tissue, we suggest further investigation into stress-related pathways involving Hsd11b1 in the brain. Our results suggest a new mechanism to explain increased cortisol levels in the alcohol addicted phenotype via down-regulation of Hsd11b1. Therefore, manipulation of Hsd11b1 expression and activity in the AUD patient might lead to long-lasting decrease in relapse risk, which so far is the biggest challenge in AUD psychopharmacotherapy.

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Authors contribution

MMF, AMB, FG and RS designed the study. MMF, AMB and FG designed keywords for the systematic literature screening. MMF, ECT, AMB and FG performed systematic literature screening and data extraction. MMF and WHS contacted the authors in case datasets were not published including all necessary information. MMF and ECT designed the analysis pipeline. MMF, ECT and MJWH performed data analysis. ACH and MAP provided previously unpublished data. MAP provided scientific support. MMF, AMB, WHS, and RS wrote the manuscript. All authors have read and approved the manuscript.

Data Availability Statement

The meta-analysis in rodents (Open Science Framework: https://doi.org/10.17605/OSF.IO/TF8R4) as well as in human post-mortem brain tissue (Prospero: CRD42020192453) has been pre-registered in advance. As mentioned in the methods section, the analysis strategy was deviating from the pre-registered methods, since the obtained data were not enabling us to perform the initially planned random effect size combination.

Datasets as well as analysis pipelines will be publicly available from the day of publication onwards.

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GO:MF

Term name	Term ID	stats p_{adj}	$-\log_{10}(p_{adj})$
myosin VI light chain binding	GO:0070858	2.858×10^{-2}	
corticotropin-releasing hormone activity	GO:0017045	2.858×10^{-2}	
proteoglycan sulfotransferase activity	GO:0050698	2.858×10^{-2}	
7-beta-hydroxysteroid dehydrogenase (NADP+) activity	GO:0047022	2.858×10^{-2}	
oxytocin receptor activity	GO:0004990	2.858×10^{-2}	
11-beta-hydroxysteroid dehydrogenase (NADP+) activity	GO:0070524	2.858×10^{-2}	
[protein]-3-O-(N-acetyl-α-D-glucosaminyl)-L-serine O-N...	GO:0102167	2.858×10^{-2}	
[protein]-3-O-(N-acetyl-α-D-glucosaminyl)-L-threonine ...	GO:0102166	2.858×10^{-2}	
cortisol dehydrogenase activity	GO:0102196	2.858×10^{-2}	
[protein]-3-O-(N-acetyl-α-D-glucosaminyl)-L-serine/L-th...	GO:0102571	2.858×10^{-2}	
11-beta-hydroxysteroid dehydrogenase [NAD(P)] activity	GO:0033845	3.174×10^{-2}	
beta-N-acetylglucosaminidase activity	GO:0016231	3.174×10^{-2}	
formyltetrahydrofolate dehydrogenase activity	GO:0016155	3.174×10^{-2}	
chondroitin 6-sulfotransferase activity	GO:0008459	3.174×10^{-2}	
chylomicron binding	GO:0034378	3.174×10^{-2}	
beta-3 adrenergic receptor binding	GO:0031699	3.174×10^{-2}	
myosin VI binding	GO:0070853	3.174×10^{-2}	
ornithine decarboxylase activity	GO:0004586	3.174×10^{-2}	
signaling receptor binding	GO:0005102	3.772×10^{-2}	
aldehyde dehydrogenase (NADP+) activity	GO:0033721	4.078×10^{-2}	
RNA polymerase I core binding	GO:0001042	4.078×10^{-2}	
corticotropin-releasing hormone receptor 2 binding	GO:0051431	4.389×10^{-2}	
corticotropin-releasing hormone receptor 1 binding	GO:0051430	4.389×10^{-2}	
thrombospondin receptor activity	GO:0070053	4.389×10^{-2}	
calcium- and calmodulin-responsive adenylate cyclase ...	GO:0008294	4.389×10^{-2}	
very-low-density lipoprotein particle receptor binding	GO:0070326	4.389×10^{-2}	
lipoprotein lipase activity	GO:0004465	4.752×10^{-2}	
chondroitin sulfotransferase activity	GO:0034481	4.752×10^{-2}	
G protein-coupled receptor binding	GO:0001664	4.752×10^{-2}	
heparan sulfate binding	GO:1904399	4.752×10^{-2}	
ErbB-3 class receptor binding	GO:0043125	4.885×10^{-2}	
N-acetylglucosamine 6-O-sulfotransferase activity	GO:0001517	4.885×10^{-2}	
beta-N-acetylhexosaminidase activity	GO:0004563	4.885×10^{-2}	
corticotropin-releasing hormone receptor binding	GO:0051429	4.885×10^{-2}	
neuregulin binding	GO:0038132	4.885×10^{-2}	

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GO:BP

Term name	Term ID	stats p_{adj}	$-\log_{10}(p_{adj})$
estrous cycle	GO:0044849	1.537×10^{-3}	
learning or memory	GO:0007611	1.537×10^{-3}	
cognition	GO:0050890	1.545×10^{-3}	
phosphorus metabolic process	GO:0006793	1.626×10^{-3}	
phosphate-containing compound metabolic process	GO:0006796	1.626×10^{-3}	
regulation of behavior	GO:0050795	1.628×10^{-3}	
regulation of protein metabolic process	GO:0051246	3.572×10^{-3}	
behavior	GO:0007610	3.572×10^{-3}	
negative regulation of multicellular organismal process	GO:0051241	4.919×10^{-3}	
tube development	GO:0035295	5.178×10^{-3}	
memory	GO:0007613	5.178×10^{-3}	
biological regulation	GO:0065007	5.178×10^{-3}	
regulation of protein modification process	GO:0031399	5.933×10^{-3}	
organonitrogen compound metabolic process	GO:1901564	6.314×10^{-3}	
multicellular organismal process	GO:0032501	7.133×10^{-3}	
ovulation cycle	GO:0042698	1.382×10^{-2}	
regulation of catalytic activity	GO:0050790	1.382×10^{-2}	
glucocorticoid biosynthetic process	GO:0006704	1.382×10^{-2}	
positive regulation of protein modification process	GO:0031401	1.382×10^{-2}	
regulation of biological quality	GO:0065008	1.385×10^{-2}	
transport	GO:0006810	1.385×10^{-2}	
positive regulation of protein metabolic process	GO:0051247	1.385×10^{-2}	
NADPH regeneration	GO:0006740	1.385×10^{-2}	
glial cell differentiation	GO:0010001	1.385×10^{-2}	
regulation of multicellular organismal process	GO:0051239	1.385×10^{-2}	
regulation of norepinephrine secretion	GO:0014061	1.385×10^{-2}	
N-acetylglucosamine metabolic process	GO:0006044	1.385×10^{-2}	
multicellular organismal response to stress	GO:0033555	1.385×10^{-2}	
norepinephrine secretion	GO:0048243	1.385×10^{-2}	
dentate gyrus development	GO:0021542	1.385×10^{-2}	
digestive system process	GO:0022600	1.385×10^{-2}	
positive regulation of neuron migration	GO:2001224	1.627×10^{-2}	
regulation of biological process	GO:0050789	1.527×10^{-2}	
associative learning	GO:0008306	1.595×10^{-2}	
regulation of molecular function	GO:0065009	1.596×10^{-2}	
establishment of localization	GO:0051234	1.651×10^{-2}	
digestion	GO:0007586	1.743×10^{-2}	
glucocorticoid metabolic process	GO:0008211	1.743×10^{-2}	
limbic system development	GO:0021761	1.775×10^{-2}	
negative regulation of T cell activation	GO:0050868	1.775×10^{-2}	
norepinephrine transport	GO:0051574	1.820×10^{-2}	
protein metabolic process	GO:0019538	1.820×10^{-2}	
response to ketone	GO:1901654	1.820×10^{-2}	
glucosamine-containing compound metabolic process	GO:1901071	1.864×10^{-2}	
positive regulation of ion transport	GO:0043270	1.910×10^{-2}	
regulation of NMDA receptor activity	GO:2000310	1.913×10^{-2}	
negative regulation of leukocyte cell-cell adhesion	GO:1903038	2.093×10^{-2}	
adrenal gland development	GO:0030325	2.097×10^{-2}	
animal organ development	GO:0048513	2.103×10^{-2}	
homeostatic process	GO:0042592	2.274×10^{-2}	
carbohydrate derivative biosynthetic process	GO:1901137	2.284×10^{-2}	
gliogenesis	GO:0042063	2.294×10^{-2}	
positive regulation of secretion by cell	GO:1903532	2.294×10^{-2}	
response to pain	GO:0048265	2.458×10^{-2}	
acylglycerol homeostasis	GO:0055090	2.458×10^{-2}	
negative regulation of lymphocyte activation	GO:0051250	2.458×10^{-2}	
positive regulation of metabolic process	GO:0009893	2.458×10^{-2}	
locomotion	GO:0040011	2.458×10^{-2}	
organophosphate metabolic process	GO:0019637	2.458×10^{-2}	
triglyceride homeostasis	GO:0070328	2.458×10^{-2}	
positive regulation of synaptic transmission, glutamater...	GO:0051968	2.482×10^{-2}	
response to stimulus	GO:0050896	2.888×10^{-2}	
regulation of phosphate metabolic process	GO:0019220	2.539×10^{-2}	
regulation of phosphorus metabolic process	GO:0051174	2.539×10^{-2}	
positive regulation of motor neuron migration	GO:1905485	2.582×10^{-2}	
regulation of motor neuron migration	GO:1905483	2.582×10^{-2}	
positive regulation of lateral motor column neuron migr...	GO:1902078	2.582×10^{-2}	
negative regulation of heat generation	GO:0031651	2.582×10^{-2}	
regulation of lateral motor column neuron migration	GO:1902076	2.582×10^{-2}	
positive regulation of secretion	GO:0051047	2.582×10^{-2}	
positive regulation of transport	GO:0051050	2.582×10^{-2}	
obsolete regulation of histone phosphorylation	GO:0033127	2.582×10^{-2}	
carbohydrate derivative metabolic process	GO:1901135	2.582×10^{-2}	
positive regulation of biological process	GO:0048518	2.582×10^{-2}	
response to stress	GO:0006950	2.582×10^{-2}	
regulation of locomotion	GO:0040012	2.582×10^{-2}	
regulation of primary metabolic process	GO:0080090	2.582×10^{-2}	
protein unfolding	GO:0043335	2.582×10^{-2}	
learning	GO:0007612	2.637×10^{-2}	
developmental process	GO:0032502	2.651×10^{-2}	
NADP metabolic process	GO:0006739	2.692×10^{-2}	
amino sugar metabolic process	GO:0006040	2.692×10^{-2}	
localization	GO:0051179	2.692×10^{-2}	
negative regulation of leukocyte activation	GO:0002695	2.719×10^{-2}	
forebrain development	GO:0030900	2.764×10^{-2}	
organic substance transport	GO:0071702	2.764×10^{-2}	
anatomical structure development	GO:0048856	2.772×10^{-2}	
cellular response to ketone	GO:1901655	2.772×10^{-2}	
regulation of ion transport	GO:0043269	2.789×10^{-2}	
cell junction assembly	GO:0034329	2.814×10^{-2}	
cell junction organization	GO:0034330	2.844×10^{-2}	
negative regulation of T cell differentiation	GO:0045581	2.844×10^{-2}	
negative regulation of cell-cell adhesion	GO:0022408	2.855×10^{-2}	
regulation of protein phosphorylation	GO:0001932	2.855×10^{-2}	
behavioral fear response	GO:0001662	3.059×10^{-2}	
regulation of neuron migration	GO:2001222	3.059×10^{-2}	
behavioral defense response	GO:0002209	3.083×10^{-2}	
negative regulation of cell activation	GO:0050866	3.083×10^{-2}	
regulation of digestive system process	GO:0044058	3.083×10^{-2}	
positive regulation of protein phosphorylation	GO:0001934	3.083×10^{-2}	

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GO:CC

Term name	Term ID	stats p_{adj}	$-\log_{10}(p_{adj})$
microvillus	GO:0005902	1.152×10^{-2}	
actin-based cell projection	GO:0098858	1.398×10^{-2}	
endomembrane system	GO:0012505	2.139×10^{-2}	
early endosome	GO:0005769	3.058×10^{-2}	
rasfn complex	GO:0110157	3.058×10^{-2}	
cytoplasm	GO:0005737	3.058×10^{-2}	
integral component of plasma membrane	GO:0005887	3.101×10^{-2}	
intrinsic component of plasma membrane	GO:0031226	3.285×10^{-2}	
cell projection	GO:0042995	3.285×10^{-2}	
apical part of cell	GO:0045177	3.496×10^{-2}	
ERBB3-ERBB2 complex	GO:0038143	3.496×10^{-2}	
extracellular region	GO:0005576	3.593×10^{-2}	
ciliary membrane	GO:0060170	3.593×10^{-2}	
plasma membrane region	GO:0098590	4.265×10^{-2}	
basal plasma membrane	GO:0009925	4.265×10^{-2}	
cell periphery	GO:0071944	4.717×10^{-2}	
plasma membrane bounded cell projection	GO:0120025	4.717×10^{-2}	
basal part of cell	GO:0045178	4.717×10^{-2}	

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REAC

Term name	Term ID	stats p_{adj}	$-\log_{10}(p_{adj})$
Syndecan interactions	REAC:R-MMU-...	2.832×10^{-2}	
Assembly of active LPL and LIPC lipase complexes	REAC:R-MMU-...	2.832×10^{-2}	

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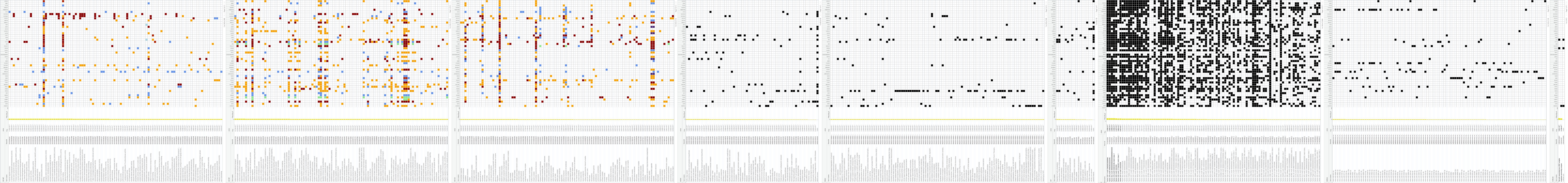
CORUM

Term name	Term ID	stats p_{adj}	$-\log_{10}(p_{adj})$
S4-syntectin-Rho(GDI) complex	CORUM:6227	5.566×10^{-3}	

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