Differential Transcriptomic Profiling Between Patients With Sepsis-Induced Ards And Sepsis Without Ards

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Abstract

Introduction: Acute Respiratory Distress Syndrome (ARDS) develops in the lungs as a response to potent inflammatory triggers, and sepsis represents one of the most common causes of ARDS. It is a highly lethal syndrome with no effective therapies, and little is known about the pathways that trigger it. In this project we combine publicly available sepsis and ARDS patient datasets to identify early transcriptomic changes that may be targeted therapeutically in the future.

Hypothesis: Proinflammatory, neutrophil-centric pathways are central to the pathogenesis of ARDS in septic patients.

Methods: The GEO dataset repository was searched and available human transcriptomic data from patients with Sepsis and Sepsis+ARDS were combined and analyzed. Genes, gene sets and significant pathways that were differentially expressed or activated at a statistically significant level between the two groups were identified.

Results: No independent genes were identified to be differentially expressed across the three studies. There were 6 gene sets that were up- or down-regulated across all studies (four and two respectively), and the Sphingosine-1-Phophate (S1P) pathway was the only pathway seen to become activated in septic patients with ARDS compared to those with sepsis alone.

Conclusions: Limited consistent gene and gene set transcription hinders our ability to identify viable biomarkers or therapeutic targets in ARDS in the setting of sepsis, but S1P pathway intermediaries may be viable candidates.

Introduction

Acute Respiratory Distress Syndrome (ARDS), first described in 1967 by Ashbaugh et al,[1] develops as the lungs mount an exaggerated immune response to potent inflammatory triggers. These may be applied locally (pneumonia, pulmonary contusion, aspiration) or systemically (sepsis, multi-organ trauma, multiple transfusions). [2] Pneumonia is the most common cause of ARDS, accounting for almost 60% of all cases, while extrapulmonary sepsis is the second most common etiology for the syndrome, being responsible for 16% of all cases.[3] Although the incidence of ARDS has declined over the last decade with the widespread adoption of lung-protective mechanical ventilation,[4] it still remains very high, with an estimated 10.4% of all Intensive Care Unit (ICU) admissions meeting criteria for ARDS during their ICU stay, and an even higher 23.4% of all ICU patients on mechanical ventilation.[3] Mortality associated with the syndrome remains very high, approximating 40% across all patients with ARDS, and even exceeding 57% in the most severe cases that do not respond to supportive measures.[5] This high mortality is due to the fact that no targeted pharmacologic therapies are currently available. Unfortunately, ARDS has recently been brought to the forefront, given its sudden incidence spike from Coronavirus Disease 2019,[6] and very high related mortality that ranges from 24.5 to 96.7%.[7]

Numerous attempts to treat ARDS with steroidal[8,9] and non-steroidal[10,11] anti-inflammatories; vasodilators;[12,13] antioxidants;[14,15] exogenous surfactant;[16] statins;[17,18] and b_2 adrenergic agonists[19,20] have been made. While these therapeutic approaches showed promise in preclinical studies,[21-34] none was successful in improving survival in a clinical setting, likely due to the fact that ARDS is a syndrome of heterogeneous etiology with the involvement of various pathogenetic pathways (perhaps with the exception of early steroids in ARDS of COVID-19)[35] or other etiology.[36] Moreover, our understanding of these specific pathogenetic pathways that trigger and perpetuate the immune response that leads to lung injury remains poor.

With these limitations in mind, and with the dual goal of identifying early biomarkers that can help predict, but also serve as therapeutic targets for ARDS, new strategies aimed at highlighting the transcriptomic profile of subjects with ARDS have recently surfaced.[37,38] The ability to study whole blood gene expression in patients with certain risk factors for ARDS – here sepsis – has the potential to unveil novel mediators, biomarkers, involved pathways, and potential therapeutic targets for the syndrome.[37] Based on the key principle that differences in gene transcription precede and guide most major inflammatory processes, identifying these discrete transcriptional profiles - before the clinical manifestation of inflammation presents itself - can help differentiate early the patients likely to develop ARDS from those who will not. The goal of this project is to identify and meta-analyze publicly available [through the National Center for Biotechnology Information (NCBI) database] human transcriptomic Gene Expression Omnibus (GEO) datasets that contain information on 'Sepsis' and Sepsis+ARDS patients, and identify the genetic signature of septic patients who progress to ARDS.

Materials And Methods

Search Strategy

The NCBI GEO Dataset repository was queried for 'sepsis' and 'ARDS', and appropriate datasets were identified and downloaded. The search was conducted on September 30, 2019, and again on May 12, 2020. A summary of the search and the PRISMA flow diagram of study review and selection is presented in **Figure 1**.

Overview

Twelve different datasets that had transcriptomic data for patients with 'Sepsis' or 'Sepsis+ARDS' were evaluated for meta-analysis. Of the twelve, only three of the datasets contained both 'Sepsis' *and* 'Sepsis+ARDS' patients. The majority of the datasets contained only 'Sepsis' patients. The large variety of platforms that the 'Sepsis' only datasets utilized made it impossible to include them in the meta-analysis, as we could not control for variance from study and array design (or high-throughput sequencing). Thus, the analysis focused on three datasets: Kangelaris *et al.* 2015[37] (GSE66890), Dolinay *et al.* 2012[39] (GSE32707), and Howrylak *et al.* 2009[40] (GSE10474). All analysis, unless otherwise noted, were carried out in the R statistical programming environment (R Foundation, Vienna, Austria). Raw data and

necessary phenotype information for each dataset were downloaded from the Gene Expression Omnibus.[41]

Processing and Normalization

GSE32707

Raw data was downloaded from GEO, and only samples from Day 0, as labeled in the study, were considered for this analysis. Ten of the 48 samples were removed from subsequent analysis because of a poor detection p-value for > 75% of the probes on their array. Only genes with a good (< 0.05) detection p-value in 10% or more of the samples were considered for analysis. Quantile normalization was applied to the data to remove systematic effects and then the expression values were log2 transformed.

GSE10474

Raw data was downloaded from GEO and processed using the *affy*[42] Bioconductor[43] package. Mas5 calls were made to determine presence or absence of each probeset on the array. Only probesets present in at least 10% of the samples were considered for analysis. RMA normalization was used to eliminate systematic differences between the arrays.

GSE66890

Raw data was downloaded from GEO and processed using the *oligo*[44] Bioconductor package. The HuGene 1.0ST arrays do not contain mismatch probes, and therefore we were unable to make presence or absence calls on a gene-level. Thus, all probesets were considered for subsequent analysis. One sample that had no gender information was excluded from subsequent analysis. The data was RMA normalized to eliminate systematic differences across the arrays.

Data Analysis

Within each dataset, we used a linear model framework as deployed in the *limma*[45] package to identify genes that are associated with Sepsis+ARDS cases versus Sepsis cases while controlling for patient age and gender. Dataset GSE66890 provided batch information about their samples, so batch was included in that model as well. The false discovery rate was used to correct for multiple hypothesis testing. No genes met an FDR of 5% in any of the 3 data sets. For all subsequent analysis, a gene was considered differentially expressed if it had a nominal p-value <= 0.01.

Gene Set Enrichment Analysis[46] was employed to identify gene sets enriched for up- or down-regulated genes in each dataset. The false discovery rate was used to correct for multiple hypothesis testing. Similar to individual genes, gene sets were identified as enriched if they had a nominal p-value <= 0.01.

Ingenuity Pathway Analysis (QIAGEN Inc., https://www.qiagenbioinformatics.com/products/ingenuity-pathway-analysis) (IPA) was used to identify pathways enriched for the differentially expressed genes

from each analysis.

Results

Differential Gene Expression

The overlapping number of genes determined to be expressed in each of the individual datasets are presented in **Figure 2**. The results of the differential expression analysis for each dataset can be found in online **Online Supplement 1**, while the differentially expressed genes (p-value ≤0.01) are shown in **Online Supplements 2-4** for each of the three analyzed studies. Expression values have been z-score transformed across the rows, and both samples and genes are clustered using a correlation distance with complete linkage. The overlap of genes differentially expressed across the three analyzed studies is shown in **Figure 3**. Of note, while 6,342 genes were noted to be expressed across all three analyzed study datasets, none of the same genes were identified to be differentially expressed (up- or down-regulated) between the three datasets in septic patients that develop ARDS, compared to patients with sepsis only.

Genes found to be differentially expressed in at least two of the three datasets (p-value ≤0.01) are shown in log2 (fold-change) in **Figure 4**. Of these, FAM117A (Family With Sequence Similarity 117 Member A), ATG9A (Autophagy Related 9A), TBC1D22B (TBC1 Domain Family Member 22B), EIF3B (Eukaryotic Translation Initiation Factor 3 Subunit B), STK11 (Serine/Threonine Kinase 11), MOSPD3 (Motile Sperm Domain Containing 3), PIGQ (Phosphatidylinositol Glycan Anchor Biosynthesis Class Q), and GPS1 (G Protein Pathway Suppressor 1) were noted to all become upregulated in ARDS in septic patients compared to patients with sepsis only, while PTPRC (Protein Tyrosine Phosphatase Receptor Type C) was noted to become downregulated.

Gene Set Enrichment Analysis

The results of the Gene Set Enrichment Analysis (GSEA) for each of the three analyzed datasets are available in **Online Supplement 5**. The overlap of the gene sets enriched for up- or down-regulation for each study (p-value ≤0.01) is shown in **Figure 5**, while the normalized enrichment score for the pathways identified as enriched for changing genes in all three datasets is shown in **Figure 6**. Of note, out of the 29 gene sets assessed, 4 were noted to be consistently upregulated across all three studies: GINESTIER_BREAST_CANCER_ZNF217_AMPLIFIED_UP; NIKOLSKY_BREAST_CANCER_16P13_AMPLICON; SANSOM_APC_TARGETS_UP; VALK_AML_CLUSTER_7. Two others were downregulated across the three studies: MCLACHLAN_DENTAL_CARIES_UP; and VALK_AML_CLUSTER_5. Of note, in the remaining 22 of 23 gene sets, the Howrylak dataset typically demonstrated gene sets behaving in the opposite manner than the Dolinay and Kangelaris datasets.

Ingenuity Pathway Analysis

The overlap of significant canonical pathways identified for each of the three datasets are summarized in table format in **Online Supplement 6** and presented in heat map form in **Figure 7**. Only the Sphingosine-1-

Phophate (S1P) signaling pathway was noted to be upregulated in all three analyzed datasets.

Discussion

With the current project, we sought to identify genes, gene sets and pathways that become differentially activated or suppressed in patients with sepsis that develop ARDS compared to patients with sepsis only. We identified and combined three publicly available datasets that have examined appropriate transcriptomic changes in patients with Sepsis+ARDS vs. Sepsis only. No genes were identified to undergo up- or down-regulation across all three datasets in septic patients that develop ARDS, compared to patients with sepsis only, and only one pathway, that of S1P, was noted to be upregulated in all three datasets.

As previous studies have noted,[47] there is minimal overlap across studies aiming to identify genes that become up- or down-regulated as ARDS develops, although the project by Sweeney et al[47] attempted to compile datasets from patients with ARDS of various etiologies (sepsis, trauma, burns), which may have confounded the group's findings. In the current project, in order to minimize such confounding, we sought to include data only from studies that included both patients with Sepsis only and Sepsis+ARDS. Extra care was taken to perform quality-control assessments on the previously published datasets, as some samples, and many individual genes, were not valid to be tested for differential expression. The fact that the three studies used different array platforms could at least partly explain the noise observed in the data.

While there were not any genes that were consistent across all three datasets, there were six gene sets and one pathway that were identified as significant. However, the Howrylak dataset, typically showed the genes and pathways behaving in an opposite manner than the other two datasets. We checked multiple times that the labeling of the samples in GSE10474 was correct, and the primary investigator of the study, included on this project, confirmed appropriate and correct labeling of the raw data.

In terms of gene sets, four were identified to become upregulated in patients with Sepsis+ARDS: including one that corresponds to genes upregulated in non-metastatic breast cancer tumors having type 1 amplification in the 20q13 region and involves locus ZNF217; one that that includes genes within amplicon 16p13 identified in breast tumors)[48]; one that includes the top upregulated genes at day 5 of CreLox-induced intestinal antigen presenting cell knockouts[49]; and finally one that includes the top 40 genes from cluster 7 of the Acute Myeloid Leukemia (AML) expression profile.[50] Two other gene sets were downregulated across the three studies including one that comprises genes upregulated in dental caries[51]; and one that that encompasses the top 40 genes from cluster 5 of the AML expression profile)[50]. Interestingly none of the aforementioned gene sets have been previously associated with the development of ARDS in sepsis, or in any other etiology. Of note, in the remaining 22 of 23 gene sets, the Howrylak dataset typically demonstrated gene sets behaving in the opposite manner than the Dolinay and Kangelaris datasets.

An important finding of this project is the identification of the S1P signaling pathway being consistently upregulated across all three analyzed studies.[52] Sphingolipids are ubiquitously present in mammalian cell membranes, and are known to be rapidly metabolized to form signaling molecules. One of the most common and important such metabolite is S1P, that plays a key role in cellular homeostasis, regulating cell growth[53] and suppressing apoptosis and autophagy,[54] through inhibition of ceramide, its pro-apoptotic precursor. S1P is also involved in several intracellular messaging pathways, including indirect regulation of the innate immunity NF-kB pathway, and Histone Deacetylation. The former takes place through interaction with the TNF Receptor-Associated Factor 2 (TRAF2) that ubiquinates RIP1, a critical event in the non-canonical NF-kB activation, which in turn sets in motion the explosive transcription of several proinflammatory intermediaries that orchestrate the innate immune response.[55] Similarly, S1P is also present in significant amounts in inside the cell nucleus, where it may either directly bind with Histone Deacetylase (HDAC) 1 and 2,[56] or indirectly, through nuclear reactive oxygen species-mediated oxidative modification of HDAC1/2,[57] or aldehyde-mediated HDAC1/2 modification,[57] play an important role in epigenetic regulation of proinflammatory gene expression. This HDAC-regulated immune response is critical to the pathogenesis of ARDS, as we have previously described.[58,59]

In addition to its involvement in the regulation of the innate immune response through intracellular signaling, S1P is also a potent regulator of angiogenesis, vascular tone and permeability through paracrine messaging. TNF-α induces vasoconstriction by increasing S1P synthesis, which in turn stimulates S1PR2, whose downstream signaling leads to potent vasoconstriction and lax vascular wall integrity.[60,61] This finding has provided a potential link between inflammation and enhanced vascular permeability, both of which are hallmarks of ARDS.

The role of S1P and its polymorphisms in the pathogenesis of ARDS was further corroborated in a recent multicenter prospective study that determined serum levels of S1P in both ARDS patients and healthy volunteers.[62] S1P levels were dramatically decreased in ARDS. Also, lower levels of S1P correlated with greater organ dysfunction and higher Acute Physiology and Chronic Health Evaluation II scores, as well as worse ARDS-related clinical outcomes (length of stay and mortality). Equally importantly, certain S1P polymorphisms also conferred susceptibility to ARDS.

Combining transcriptomic data from three datasets that compared Sepsis+ARDS patients vs. Sepsis only, we were able to highlight the gene sets and one pathway that become differentially expressed or activated respectively in septic patients that develop ARDS, compared to patients with sepsis only. We were not able to determine the transcriptomic changes taking place in patients with pneumonia who go on to develop ARDS – still by far the most common cause of the syndrome, yet extrapulmonary sepsis remains the second most common cause of ARDS.[3] Another limitation of our approach, even though we attempted to utilize datasets from patients with similar clinical characteristics and similar analytical methodologies (microarray), all three studies used different array platforms, which may have compromised our results during normalization. Another limitation is the slightly different timepoints when the samples were collected between the studies (the first 24h after ICU admission in the Kangelaris

project, versus after the first 48h after ICU admission in the Dolinay and Howrylak projects). It is widely known that ARDS comprises of overlapping, yet pathologically distinct stages of progression, and it is likely that the transcriptome varies over time and, hence, across stages, even in the same patient.[63] Therefore, it is possible some va riability in our results may have been due to the varying sampling times. Last, but not least, all three projects, and as a result ours, studied the transcriptome in peripheral blood, without examining the changes occurring in the pulmonary micro-environment, or even the cell-specific transcriptomic changes to determine each cell population's contribution to lung injury. While bronchoalveolar lavage fluid would be optimal to study the regional transcriptome, which has been shown to be distinct from the peripheral one in ARDS,[64] we have already initiated an effort to collect mini-bronchoalveolar lavage samples in ARDS patients.

Declarations

Ethics approval and consent to participate

Not applicable, as ethics is a meta-analytic project and ethics approval and consent to participate had been obtained in their original primary research projects.

Consent for publication

Not applicable, as this project reports data analyses in aggregate from deidentified transcriptomics data.

Availability of data and materials

Data utilized are publicly available at https://www.ncbi.nlm.nih.gov/gds under study identifiers provided in the Methods section of the manuscript. Edits made to the combined datasets are provided as Supplemental Materials.

Competing interests

No competing interests to declare on the current project.

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Authors' contributions

GK and BS conceived the presented idea, conducted the dataset search and original analysis, and drafted the manuscript. WC and DLC conducted the statistical analyses, provided critical feedback, drafted the Methods section and generated the figures. JAH, JE and KK critically reviewed original dataset information and ensured accuracy and appropriateness for inclusion.

All authors contributed critically to design methodology, results description and explanation, manuscript editing, and approved the final manuscript.

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References

1. Ashbaugh DG, Bigelow DB, Petty TL, Levine BE. Acute respiratory distress in adults. Lancet 1967;2:319-23.
2. Matthay MA, Ware LB, Zimmerman GA. The acute respiratory distress syndrome. The Journal of clinical investigation 2012;122:2731-40.
3. Bellani G, Laffey JG, Pham T, et al. Epidemiology, Patterns of Care, and Mortality for Patients With Acute Respiratory Distress Syndrome in Intensive Care Units in 50 Countries. JAMA 2016;315:788-800.
4. Rezoagli E, Fumagalli R, Bellani G. Definition and epidemiology of acute respiratory distress syndrome. Ann Transl Med 2017;5:282.
5. Madotto F, Pham T, Bellani G, et al. Resolved versus confirmed ARDS after 24 h: insights from the LUNG SAFE study. Intensive care medicine 2018.
6. Tzotzos SJ, Fischer B, Fischer H, Zeitlinger M. Incidence of ARDS and outcomes in hospitalized patients with COVID-19: a global literature survey. Crit Care 2020;24:516.
7. Wunsch H. Mechanical Ventilation in COVID-19: Interpreting the Current Epidemiology. Am J Respir Crit Care Med 2020;202:1-4.
8. Steinberg KP, Hudson LD, Goodman RB, et al. Efficacy and safety of corticosteroids for persistent acute respiratory distress syndrome. N Engl J Med 2006;354:1671-84.
9. Bernard GR, Luce JM, Sprung CL, et al. High-dose corticosteroids in patients with the adult respiratory distress syndrome. N Engl J Med 1987;317:1565-70.
10. Kor DJ, Carter RE, Park PK, et al. Effect of Aspirin on Development of ARDS in At-Risk Patients Presenting to the Emergency Department: The LIPS-A Randomized Clinical Trial. JAMA 2016;315:2406-14.
11. Randomized, placebo-controlled trial of lisofylline for early treatment of acute lung injury and acute respiratory distress syndrome. Crit Care Med 2002;30:1-6.
12. Adhikari NK, Dellinger RP, Lundin S, et al. Inhaled nitric oxide does not reduce mortality in patients with acute respiratory distress syndrome regardless of severity: systematic review and meta-analysis. Crit Care Med 2014;42:404-12.
13. Fuller BM, Mohr NM, Skrupky L, Fowler S, Kollef MH, Carpenter CR. The use of inhaled prostaglandins in patients with ARDS: a systematic review and meta-analysis. Chest 2015;147:1510-22.

14. Bernard GR, Wheeler AP, Arons MM, et al. A trial of antioxidants N-acetylcysteine and procysteine in ARDS. The Antioxidant in ARDS Study Group. Chest 1997;112:164-72.
15. Rice TW, Wheeler AP, Thompson BT, et al. Enteral omega-3 fatty acid, gamma-linolenic acid, and antioxidant supplementation in acute lung injury. JAMA 2011;306:1574-81.
16. Meng H, Sun Y, Lu J, et al. Exogenous surfactant may improve oxygenation but not mortality in adult patients with acute lung injury/acute respiratory distress syndrome: a meta-analysis of 9 clinical trials. J Cardiothorac Vasc Anesth 2012;26:849-56.
17. National Heart L, Blood Institute ACTN, Truwit JD, et al. Rosuvastatin for sepsis-associated acute respiratory distress syndrome. N Engl J Med 2014;370:2191-200.
18. McAuley DF, Laffey JG, O'Kane CM, et al. Simvastatin in the acute respiratory distress syndrome. N Engl J Med 2014;371:1695-703.
19. Gao Smith F, Perkins GD, Gates S, et al. Effect of intravenous beta-2 agonist treatment on clinical outcomes in acute respiratory distress syndrome (BALTI-2): a multicentre, randomised controlled trial. Lancet 2012;379:229-35.
20. National Heart L, Blood Institute Acute Respiratory Distress Syndrome Clinical Trials N, Matthay MA, et al. Randomized, placebo-controlled clinical trial of an aerosolized beta(2)-agonist for treatment of acute lung injury. Am J Respir Crit Care Med 2011;184:561-8.
21. Al-Harbi NO, Imam F, Al-Harbi MM, et al. Dexamethasone Attenuates LPS-induced Acute Lung Injury through Inhibition of NF-kappaB, COX-2, and Pro-inflammatory Mediators. Immunol Invest 2016;45:349-69.
22. Grommes J, Vijayan S, Drechsler M, et al. Simvastatin reduces endotoxin-induced acute lung injury by decreasing neutrophil recruitment and radical formation. PLoS One 2012;7:e38917.
23. Jacobson JR, Barnard JW, Grigoryev DN, Ma SF, Tuder RM, Garcia JG. Simvastatin attenuates vascular leak and inflammation in murine inflammatory lung injury. Am J Physiol Lung Cell Mol Physiol 2005;288:L1026-32.
24. Leite-Junior JH, Garcia CS, Souza-Fernandes AB, et al. Methylprednisolone improves lung mechanics and reduces the inflammatory response in pulmonary but not in extrapulmonary mild acute lung injury in mice. Crit Care Med 2008;36:2621-8.
25. Panka BA, de Grooth HJ, Spoelstra-de Man AM, Looney MR, Tuinman PR. Prevention or Treatment of Ards With Aspirin: A Review of Preclinical Models and Meta-Analysis of Clinical Studies. Shock 2017;47:13-21.
26. Perkins GD, Gao F, Thickett DR. In vivo and in vitro effects of salbutamol on alveolar epithelial repair in acute lung injury. Thorax 2008;63:215-20.
27. Perkins GD, McAuley DF, Richter A, Thickett DR, Gao F. Bench-to-bedside review: beta2-Agonists and the acute respiratory distress syndrome. Crit Care 2004;8:25-32.
28. Silva PL, Garcia CS, Maronas PA, et al. Early short-term versus prolonged low-dose methylprednisolone therapy in acute lung injury. Eur Respir J 2009;33:634-45.

29. Takano K, Yamamoto S, Tomita K, et al. Successful treatment of acute lung injury with pitavastatin in septic mice: potential role of glucocorticoid receptor expression in alveolar macrophages. J Pharmacol Exp Ther 2011;336:381-90.
30. Tilgner J, von Trotha KT, Gombert A, et al. Aspirin, but Not Tirofiban Displays Protective Effects in Endotoxin Induced Lung Injury. PLoS One 2016;11:e0161218.
31. Tu GW, Shi Y, Zheng YJ, et al. Glucocorticoid attenuates acute lung injury through induction of type 2 macrophage. J Transl Med 2017;15:181.
32. Tuinman PR, Muller MC, Jongsma G, Hegeman MA, Juffermans NP. High-dose acetylsalicylic acid is superior to low-dose as well as to clopidogrel in preventing lipopolysaccharide-induced lung injury in mice. Shock 2013;40:334-8.
33. Yeo CD, Rhee CK, Kim IK, et al. Protective effect of pravastatin on lipopolysaccharide-induced acute lung injury during neutropenia recovery in mice. Exp Lung Res 2013;39:99-106.
34. Yu Y, Jing L, Zhang X, Gao C. Simvastatin Attenuates Acute Lung Injury via Regulating CDC42-PAK4 and Endothelial Microparticles. Shock 2017;47:378-84.
35. Group RC, Horby P, Lim WS, et al. Dexamethasone in Hospitalized Patients with Covid-19 - Preliminary Report. N Engl J Med 2020.
36. Villar J, Ferrando C, Martinez D, et al. Dexamethasone treatment for the acute respiratory distress syndrome: a multicentre, randomised controlled trial. Lancet Respir Med 2020;8:267-76.
37. Kangelaris KN, Prakash A, Liu KD, et al. Increased expression of neutrophil-related genes in patients with early sepsis-induced ARDS. Am J Physiol Lung Cell Mol Physiol 2015;308:L1102-13.
38. Englert JA, Cho MH, Lamb AE, et al. Whole blood RNA sequencing reveals a unique transcriptomic profile in patients with ARDS following hematopoietic stem cell transplantation. Respir Res 2019;20:15.
39. Dolinay T, Kim YS, Howrylak J, et al. Inflammasome-regulated cytokines are critical mediators of acute lung injury. Am J Respir Crit Care Med 2012;185:1225-34.
40. Howrylak JA, Dolinay T, Lucht L, et al. Discovery of the gene signature for acute lung injury in patients with sepsis. Physiol Genomics 2009;37:133-9.
41. Clough E, Barrett T. The Gene Expression Omnibus Database. Methods Mol Biol 2016;1418:93-110.
42. Gautier L, Cope L, Bolstad BM, Irizarry RA. affy--analysis of Affymetrix GeneChip data at the probe level. Bioinformatics 2004;20:307-15.
43. Gentleman RC, Carey VJ, Bates DM, et al. Bioconductor: open software development for computational biology and bioinformatics. Genome Biol 2004;5:R80.
44. Carvalho BS, Irizarry RA. A framework for oligonucleotide microarray preprocessing. Bioinformatics 2010;26:2363-7.
45. Ritchie ME, Phipson B, Wu D, et al. limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res 2015;43:e47.

46. Mootha VK, Lindgren CM, Eriksson KF, et al. PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. Nat Genet 2003;34:267-73.
47. Sweeney TE, Thomas NJ, Howrylak JA, Wong HR, Rogers AJ, Khatri P. Multicohort Analysis of Whole-Blood Gene Expression Data Does Not Form a Robust Diagnostic for Acute Respiratory Distress Syndrome. Crit Care Med 2018;46:244-51.
48. Nikolsky Y, Sviridov E, Yao J, et al. Genome-wide functional synergy between amplified and mutated genes in human breast cancer. Cancer Res 2008;68:9532-40.
49. Sansom OJ, Reed KR, Hayes AJ, et al. Loss of Apc in vivo immediately perturbs Wnt signaling, differentiation, and migration. Genes Dev 2004;18:1385-90.
50. Valk PJ, Verhaak RG, Beijen MA, et al. Prognostically useful gene-expression profiles in acute myeloid leukemia. N Engl J Med 2004;350:1617-28.
51. McLachlan JL, Smith AJ, Bujalska IJ, Cooper PR. Gene expression profiling of pulpal tissue reveals the molecular complexity of dental caries. Biochim Biophys Acta 2005;1741:271-81.
52. Maceyka M, Harikumar KB, Milstien S, Spiegel S. Sphingosine-1-phosphate signaling and its role in disease. Trends Cell Biol 2012;22:50-60.
53. Olivera A, Spiegel S. Sphingosine-1-phosphate as second messenger in cell proliferation induced by PDGF and FCS mitogens. Nature 1993;365:557-60.
54. Cuvillier O, Pirianov G, Kleuser B, et al. Suppression of ceramide-mediated programmed cell death by sphingosine-1-phosphate. Nature 1996;381:800-3.
55. Xia P, Wang L, Moretti PA, et al. Sphingosine kinase interacts with TRAF2 and dissects tumor necrosis factor-alpha signaling. J Biol Chem 2002;277:7996-8003.
56. Hait NC, Allegood J, Maceyka M, et al. Regulation of histone acetylation in the nucleus by sphingosine-1-phosphate. Science 2009;325:1254-7.
57. Fu P, Ebenezer DL, Ha AW, Suryadevara V, Harijith A, Natarajan V. Nuclear lipid mediators: Role of nuclear sphingolipids and sphingosine-1-phosphate signaling in epigenetic regulation of inflammation and gene expression. J Cell Biochem 2018;119:6337-53.
58. Kasotakis G, Galvan MD, Osathanugrah P, et al. Timing of valproic acid in acute lung injury: prevention is the best therapy? J Surg Res 2017;220:206-12.
59. Kasotakis G, Kintsurashvili E, Galvan MD, et al. Histone Deacetylase 7 Inhibition in A Murine Model of Gram-Negative Pneumonia-Induced Acute Lung Injury. Shock 2019;53:344-51.
60. Camerer E, Regard JB, Cornelissen I, et al. Sphingosine-1-phosphate in the plasma compartment regulates basal and inflammation-induced vascular leak in mice. The Journal of clinical investigation 2009;119:1871-9.
61. Scherer EQ, Yang J, Canis M, et al. Tumor necrosis factor-alpha enhances microvascular tone and reduces blood flow in the cochlea via enhanced sphingosine-1-phosphate signaling. Stroke 2010;41:2618-24.

62. Zhao J, Tan Y, Wang L, Su X, Shi Y. Serum sphingosine-1-phosphate levels and Sphingosine-1-Phosphate gene polymorphisms in acute respiratory distress syndrome: a multicenter prospective study. J Transl Med 2020;18:156.
63. Tomashefski JF, Jr. Pulmonary pathology of the adult respiratory distress syndrome. Clin Chest Med 1990;11:593-619.
64. Morrell ED, Radella F, 2nd, Manicone AM, et al. Peripheral and Alveolar Cell Transcriptional Programs Are Distinct in Acute Respiratory Distress Syndrome. Am J Respir Crit Care Med 2018;197:528-32.

Figures

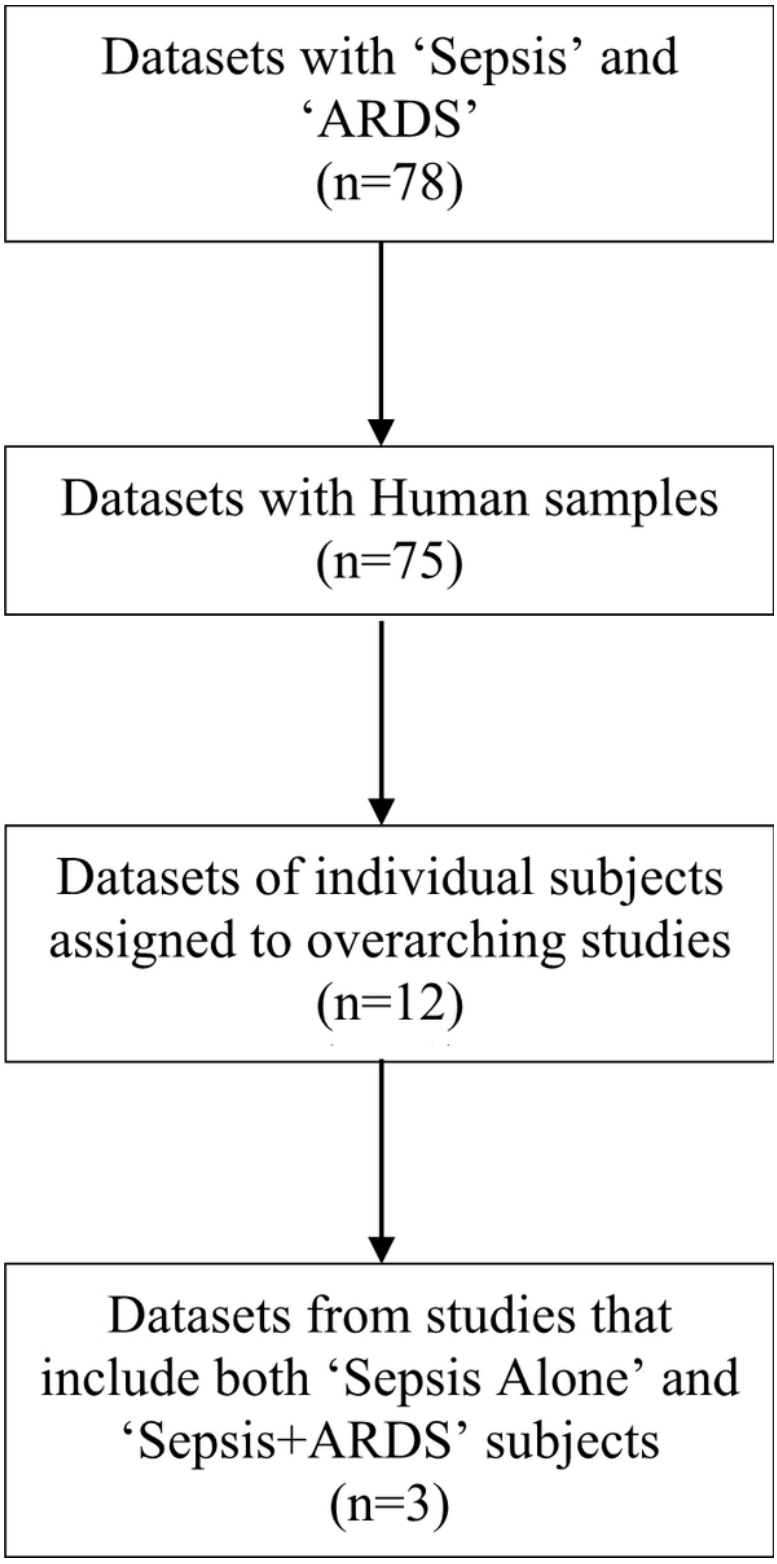

Figure 1

Prisma flow diagram of study selection for analysis.

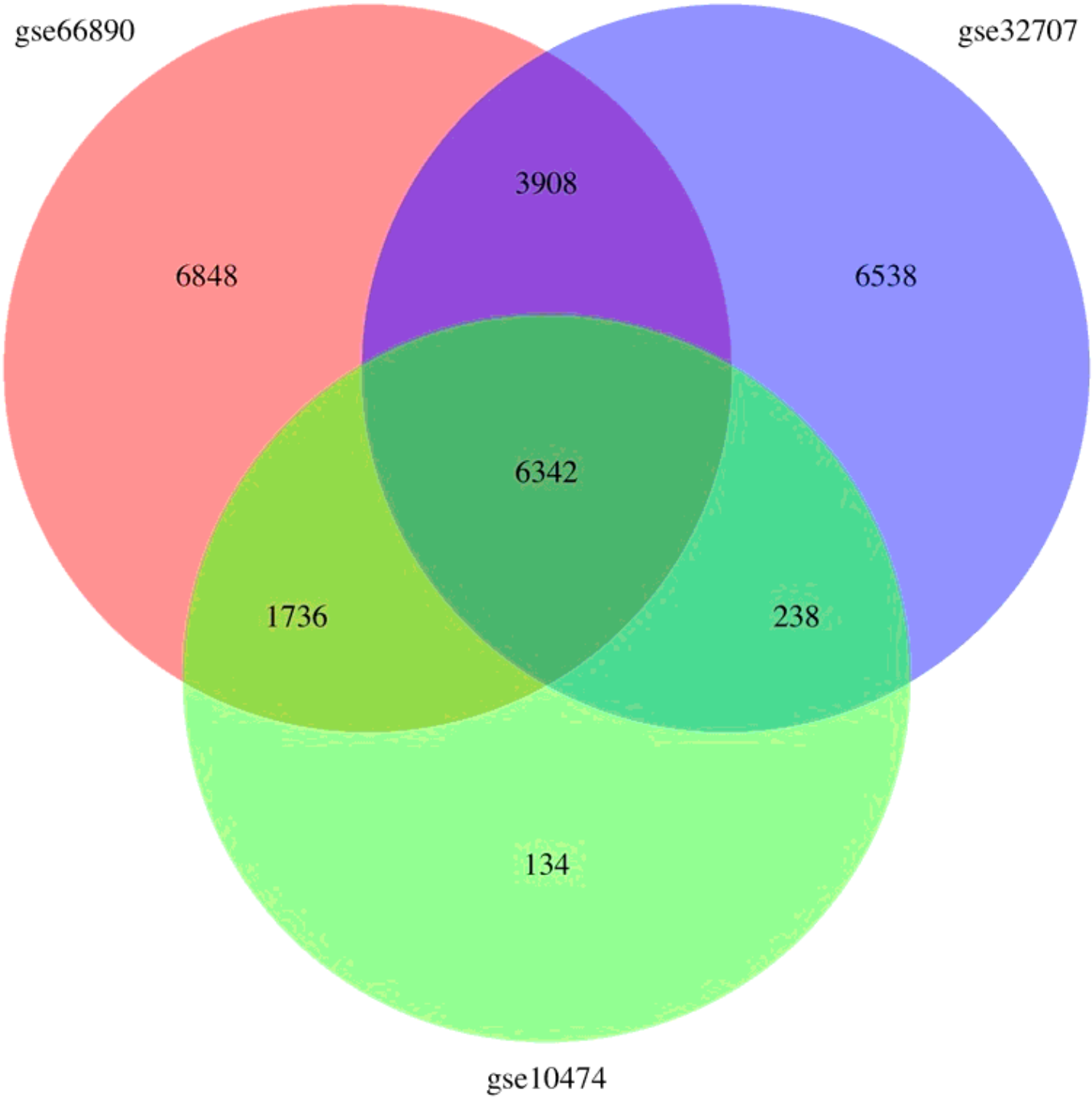

Figure 2

The overlapping number of genes determined to be expressed in each of the individual datasets.

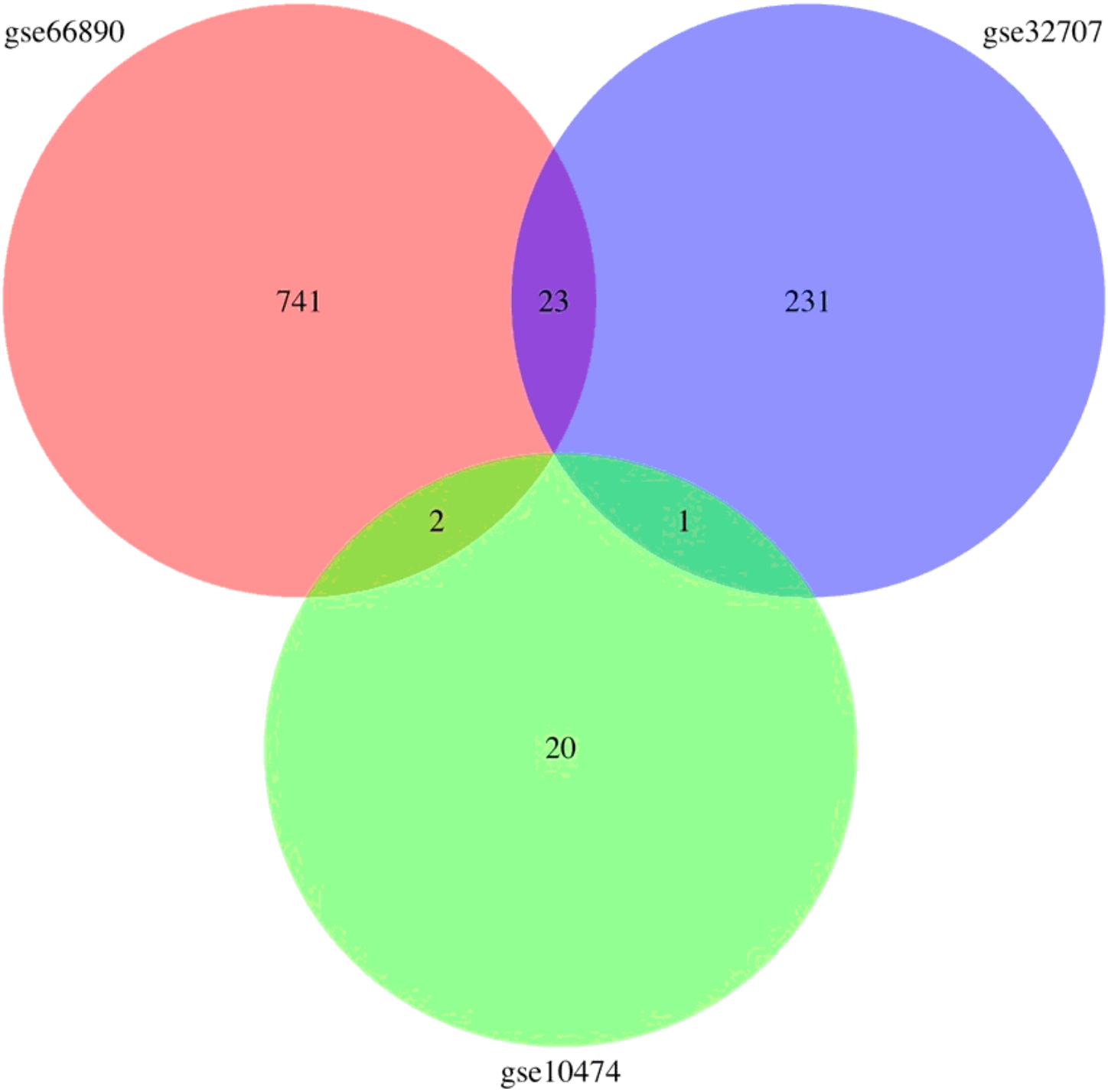

Figure 3

The overlap of genes across the three data sets that were considered differentially expressed (p-value <= 0.01).

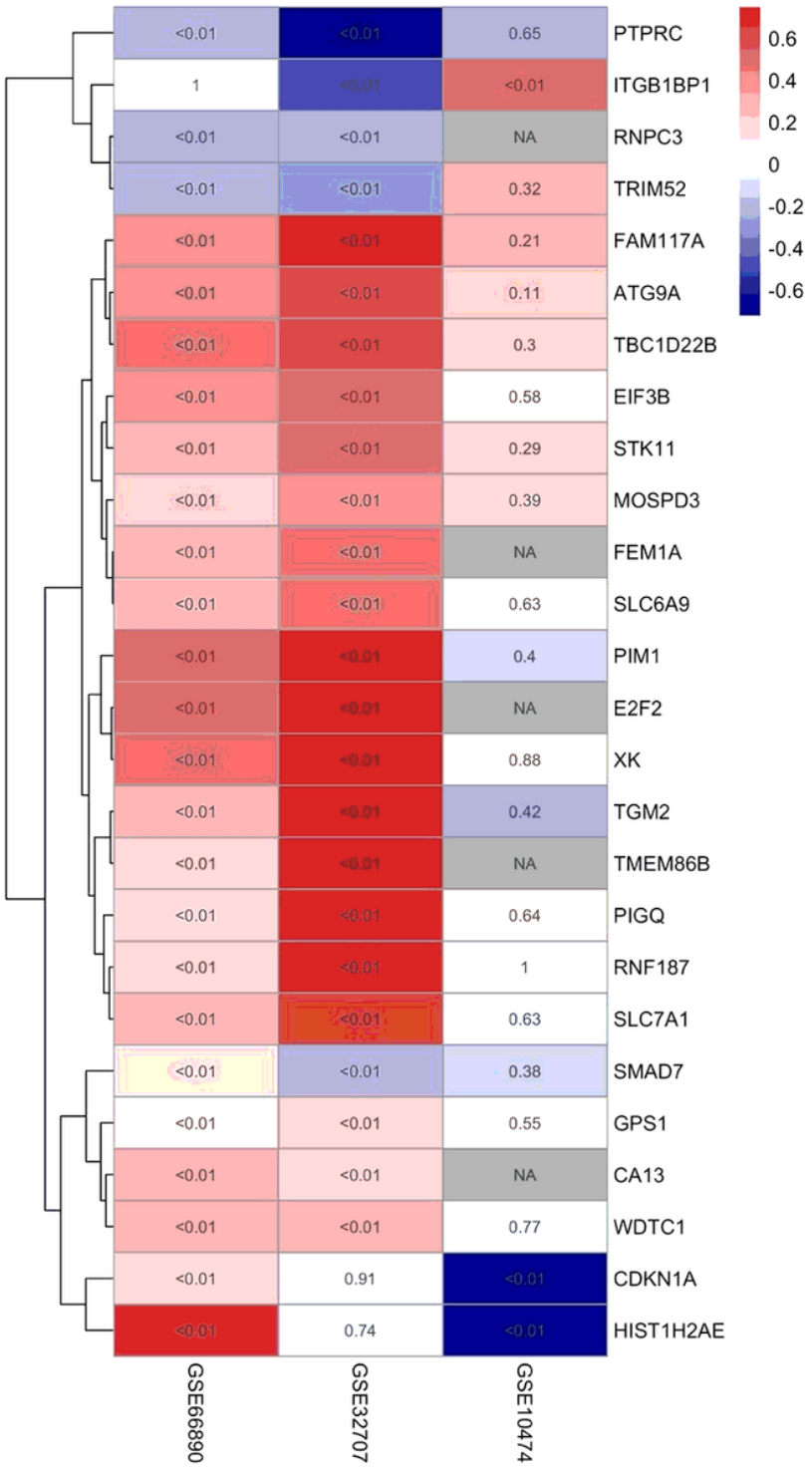

Figure 4

The log2(fold-change) of genes found differentially expressed (p-value <= 0.01) in at least two of the three datasets. The differential expression p-value is listed in each cell.

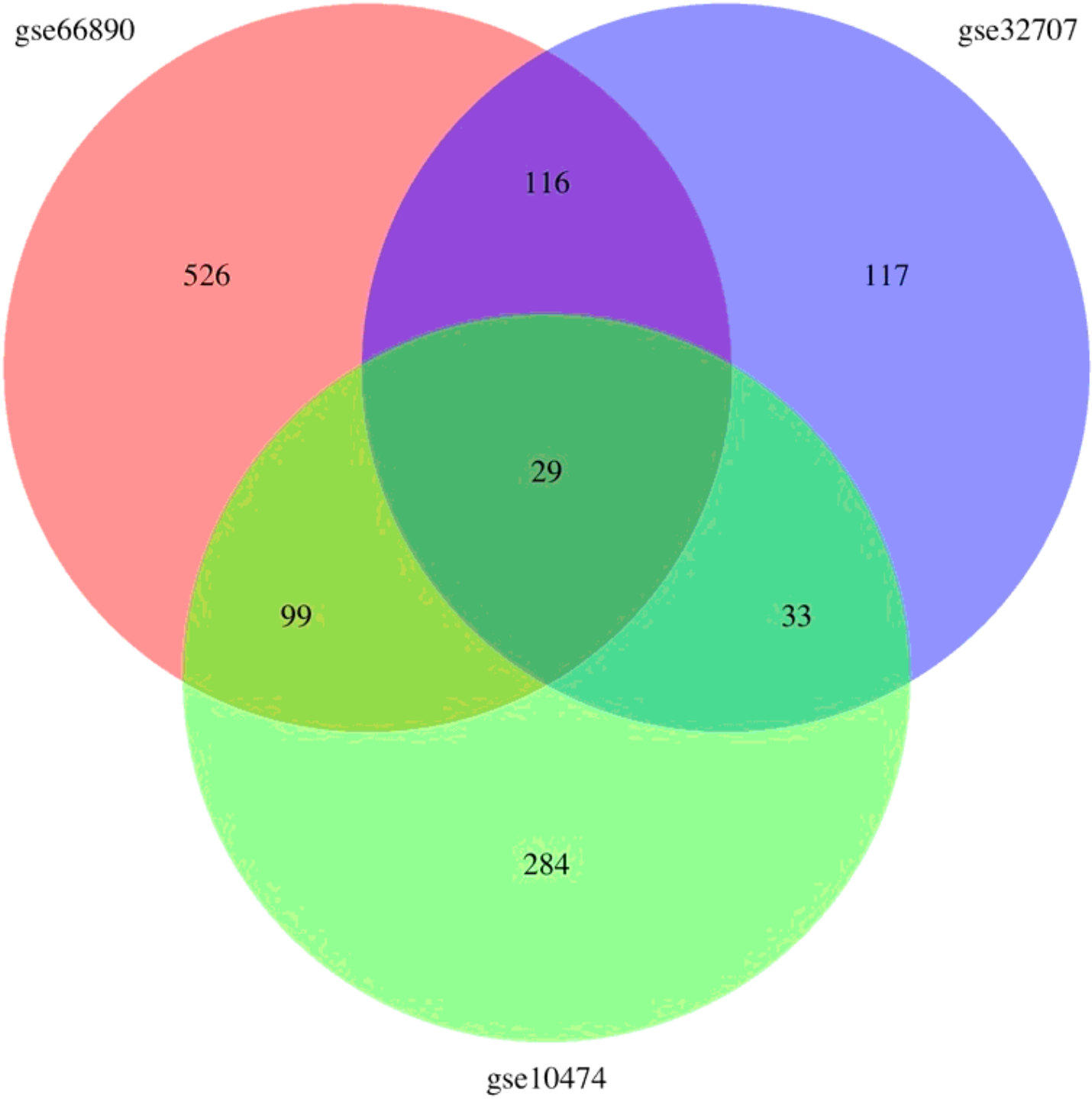

Figure 5

The overlap of gene sets enriched (p-value <= 0.01) for up- or down-regulated genes from each analysis.

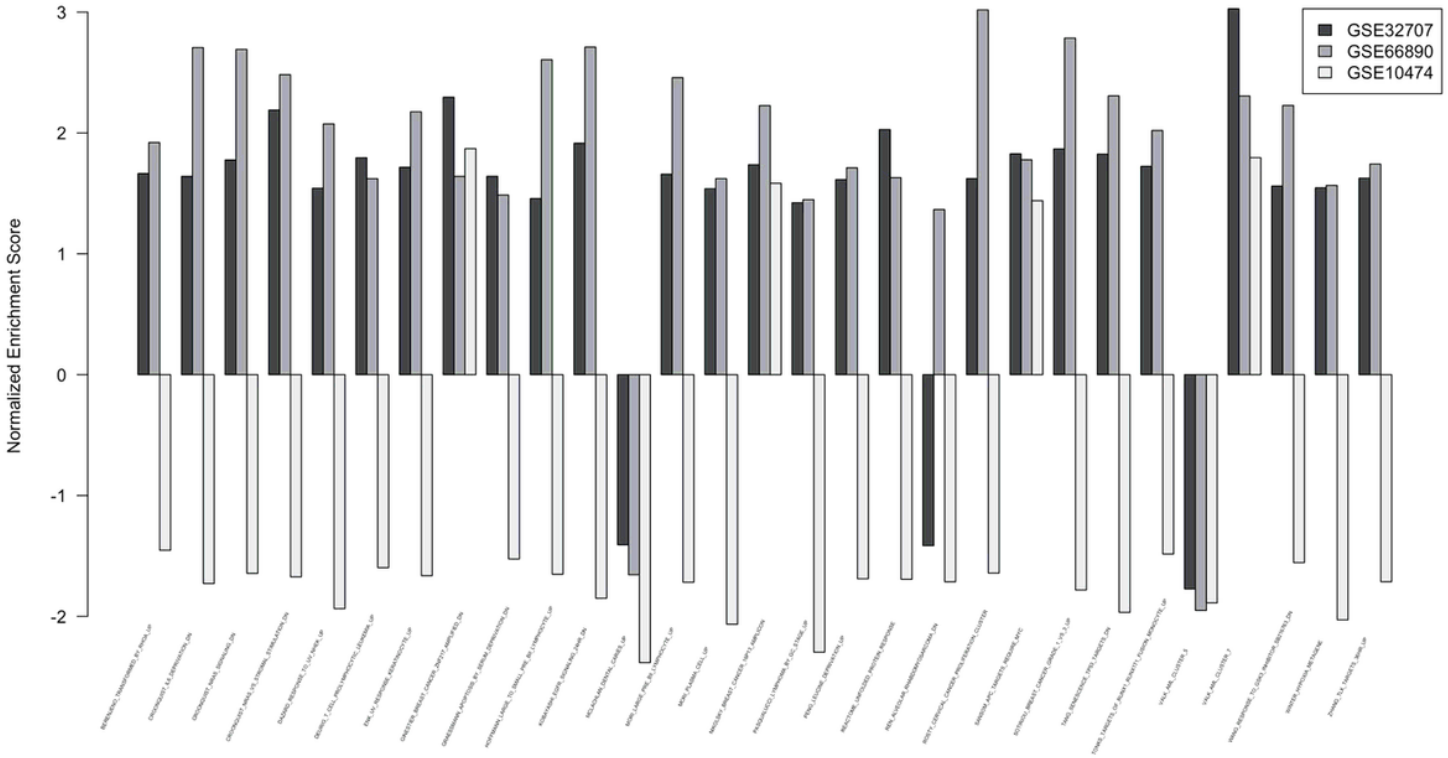

Figure 6

The normalized enrichment score for the pathways that were identified as enriched for changing genes (p-value <= 0.01) in all 3 of the data sets.

Figure 7

The overlap of significant canonical pathways identified for each dataset.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplement.docx