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Paola Rampelotto Ziani

# EXPLORANDO A COMPLEXIDADE FISIOPATOLÓGICA NO TRANSTORNO BIPOLAR: ANÁLISE DO PERFIL TRANSCRIPTÔMICO E PROTEÔMICO

Porto Alegre

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Tese apresentada ao Programa de Pós-Graduação em Ciências Biológicas: Farmacologia e Terapêutica da Universidade Federal do Rio Grande do Sul como requisito parcial para obtenção do título de Doutora em Farmacologia e Terapêutica.

Orientadora: Profª. Drª. Adriane Ribeiro Rosa

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Aprovado em:____ de ____ de ____

BANCA EXAMINADORA

_____

Prof. Dr. Eduardo Rigon Zimmer - PPG Farmacologia e Terapêutica/UFRGS

_____

Prof. Dr. Ives Cavalcante Passos - PPG Psiquiatria e Ciências do Comportamento/UFRGS

_____

Profª. Drª. Maribel Antonello Rubin - PPG Bioquímica Toxicológica/UFSM

_____

Profª. Drª. Rosane Gomez - PPG Farmacologia e Terapêutica/UFRGS (suplente)

_____

Prof. Dr. Charles Francisco Ferreira - PPG Fisiologia/UFRGS (suplente)

_____

Profª. Drª. Adriane Ribeiro Rosa - PPG Farmacologia e Terapêutica/UFRGS (orientadora)

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# APRESENTAÇÃO

Esta tese tem como objetivo investigar biomarcadores, alvos terapêuticos e princípios biológicos do transtorno bipolar a partir de diferentes perspectivas. Para alcançar esse propósito, foram empregadas análises computacionais *in silico* utilizando dados transcriptômicos e proteômicos em nível periférico para estudar marcadores biológicos, alvos terapêuticos e fundamentos moleculares. A pesquisa por biomarcadores foi estendida ao ambiente *in vivo*, analisando o perfil proteômico no sangue de pacientes com transtorno bipolar.

Capítulo I: Resumo, Abstract, Lista de figuras, Lista de abreviaturas e siglas, Introdução e Objetivos;

Capítulo II: Metodologia e Resultados divididos em capítulos e apresentados na forma de quatro artigos científicos: o primeiro artigo, intitulado *Master Regulatory Genes in Bipolar Disorder: Employing Transcriptomics to Uncover Phase-Specific Mood Biomarkers,* utilizou uma abordagem de biologia de sistemas e transcriptômica para identificar fatores de transcrição que desempenham papel de reguladores mestres no transtorno bipolar com foco na descoberta de biomarcadores; o segundo artigo, intitulado *Drug Repurposing and Personalized Treatment Strategies for Bipolar Disorder Using Transcriptomic,* combinou dados transcriptômicos e técnicas computacionais para identificar novos compostos bioativos ou medicamentos já aprovados pela *Food and Drug Administration* para o manejo do transtorno bipolar; o terceiro artigo intitula-se *Potential Candidates for Biomarkers in Bipolar Disorder: A Proteomic Approach Through Systems Biology* e aborda uma revisão da literatura com o objetivo de encontrar biomarcadores potenciais para o transtorno bipolar através da

análise do proteoma no sangue. Além disso, o estudo visa compreender as vias moleculares disfuncionais relacionadas à fisiopatologia da doença; o quarto artigo, intitulado *Proteomic Insights into Biology of Bipolar Disorder: Implications for Health Complexity and Mortality,* aborda a análise exploratória de proteínas em sangue de pacientes do Programa de Tratamento do Transtorno de Humor Bipolar (PROTHABI) do Hospital de Clínicas de Porto Alegre (HCPA), por meio de LC-MS/MS.

Capítulo III: Discussão, Conclusão, Referências citadas na Parte I e na Parte III, e Anexo (Parecer consubstanciado do Comitê de Ética em Pesquisa).

Os trabalhos que compõem esta tese foram desenvolvidos de 2019 e 2023 no Laboratório de Psiquiatria Molecular, localizado no Centro de Pesquisa Experimental do Hospital de Clínicas de Porto Alegre, sob orientação da Dra. Adriane Ribeiro Rosa. O estudo foi financiado por CAPES, FAPERGS e FIPE-HCPA.

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# CAPÍTULO I

## Introdução e Objetivos

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## LISTA DE ABREVIATURAS E SIGLAS

**ARACNe:** algoritmo para a reconstrução de redes celulares precisas (do inglês *Algorithm for the Reconstruction of Accurate Cellular Networks*)

**BDNF**: fator neurotrófico derivado do encéfalo (do inglês *Brain-Derived Neurotrophic Factor*)

**CID-11:** Classificação Estatística Internacional das Doenças e Problemas Relacionados com a Saúde versão 11

**cMap:** Mapa de Conectividade

**DM:** Depressão Maior

**DSM-V:** Manual de Diagnóstico e Estatística dos Transtornos Mentais versão 5 (do inglês *Diagnostic and Statistical Manual of Mental Disorders*)

**ESZ:** Esquizofrenia

**FAST:** escala breve de avaliação de funcionamento (do inglês *Functioning Assessment Short Test*)

**FEA:** análise de enriquecimento funcional (do inglês *Functional Enrichment Analysis)*

**GSEA:** análise de enriquecimento de conjuntos de genes (do inglês *Gene Set Enrichment Analysis)*

**HPA:** Hipotálamo-Pituitária-Adrenal

**LINCS:** Rede Integrada de Assinaturas Celulares Baseadas em Biblioteca do NIH (do inglês *Library of Integrated Network-Based Cellular Signatures*)

**MS:** espectrometria de massas (do inglês Mass Spectrometry)

**MRA:** análise de regulador mestre (do inglês Master Regulator Analysis)

**PSA:** antígeno específico da próstata (do inglês *Prostate Specific Antigen*)

**RNA-seq:** sequenciamento de RNA

**SNC:** Sistema Nervoso Central

**TB:** Transtorno Bipolar

**WGCNA:** Análise Ponderada de Redes de Co Expressão Gênica (do inglês *Weighted Gene Co-expression Network Analysis*)

## RESUMO

A complexidade das manifestações clínicas no transtorno bipolar (TB) é frequentemente propensa a diagnósticos imprecisos ou subestimação, o que resulta no prolongamento das fases agudas, na piora do prognóstico e no comprometimento da eficácia terapêutica. Deste modo, a compreensão aprofundada da doença, aliada à busca por marcadores específicos, torna-se essencial. Assim, os estudos contidos nesta tese integraram diferentes níveis de informação, juntamente com a exploração de marcadores específicos, para aprimorar a precisão diagnóstica e promover intervenções farmacológicas mais eficazes. O primeiro artigo científico conduziu uma revisão da literatura para identificar candidatos a biomarcadores no TB por meio da análise do proteoma sanguíneo. O estudo destacou cinco proteínas distintas (IGF-1, TF, A2M, C3 e APOA1) envolvidas em diferentes processos biológicos. A análise de vias revelou associações importantes com o sistema complemento e a cascata de coagulação. No segundo artigo desta tese, a identificação de biomarcadores em pacientes com TB foi abordada com foco na análise transcriptômica. A biologia de sistemas foi utilizada para identificar fatores de transcrição que atuam como reguladores mestres no TB a partir de dados de microarranjos obtidos do banco de dados GEO. Foram identificados 59 reguladores mestres no TB, sendo que o DMTF1 estava presente em todos os estados de humor. Ademais, foram determinados conjuntos de genes enriquecidos relacionados à depressão, eutimia e mania. O terceiro artigo explorou compostos bioativos ou medicamentos aprovados pela FDA para o manejo do TB, utilizando dados transcriptômicos e técnicas computacionais. A análise de interação fármaco-gene identificou compostos relacionados às assinaturas de expressão gênica nos estados de humor do TB. Compostos promissores incluíram medicamentos aprovados pela FDA como agentes antineoplásicos (dasatinibe, sorafenibe e sunitinibe) ou agentes anti-hipertensivos (minoxidil e alisquireno), e compostos bioativos que estão sendo testados em modelos animais, como gugulsterona, ácido betulínico e ácido cafeico, sugerindo seu potencial para o tratamento do TB. O quarto artigo realizou uma análise exploratória de proteínas no sangue de pacientes do Programa de Tratamento do Transtorno de Humor Bipolar (PROTHABI) do Hospital de Clínicas de Porto Alegre (HCPA), utilizando LC-MS/MS. As proteínas estavam associadas ao metabolismo lipídico, sistema complemento e cascata de coagulação. Além disso, doenças cardiovasculares foram proeminentes, especialmente em casos de TB com baixo funcionamento, sugerindo um aumento no risco de eventos cardiovasculares. As vias identificadas estão intimamente ligadas à comorbidades no TB, que, por sua vez, estão entre as principais causas globais de morte. Portanto, uma compreensão abrangente do perfil de transcritos e proteico bem como os processos biológicos envolvidos no TB oferece insights valiosos para intervenções direcionadas, abordando aspectos de saúde mental e mecanismos subjacentes a comorbidades no TB.

## ABSTRACT

The complexity of clinical manifestations in bipolar disorder (BD) is often prone to inaccurate diagnoses or underestimations, leading to prolonged acute phases, worsened prognosis, and compromised therapeutic efficacy. Thus, a profound understanding of the disease, coupled with the search for specific markers, becomes essential. The studies in this thesis integrated different levels of information along with the exploration of specific markers to enhance diagnostic accuracy and promote more effective pharmacological interventions. The first scientific article conducted a literature review to identify biomarker candidates in BD through the analysis of blood proteome. The study highlighted five distinct proteins (IGF-1, TF, A2M, C3, and APOA1) involved in various biological processes. Additionally, pathway analysis revealed significant associations with the complement system and the coagulation cascade. In the second article of this thesis, the identification of biomarkers in BD patients was addressed with a focus on transcriptomic analysis. Systems biology was employed to identify transcription factors acting as master regulators in BD from microarray data obtained from the GEO database. Fifty-nine master regulators in BD were identified, with DMTF1 present in all mood states. Furthermore, sets of enriched genes related to depression, euthymia, and mania were determined. The third article explored bioactive compounds or FDA-approved medications for BD management using transcriptomic data and computational techniques. Drug-gene interaction analysis identified compounds related to gene expression signatures in BD mood states. Promising compounds included FDA-approved drugs like antineoplastic agents (dasatinib, sorafenib, and sunitinib) or antihypertensive agents (minoxidil and aliskiren), and bioactive compounds being tested in animal models, such as guggulsterone, betulinic acid, and caffeic acid, suggesting their potential for BD treatment. The fourth article conducted an exploratory analysis of blood proteins from patients in the Program for Bipolar Disorder Treatment (PROTHABI) at the Hospital de Clínicas de Porto Alegre (HCPA), using LC-MS/MS. The proteins were associated with lipid metabolism, the complement system, and the coagulation cascade. Additionally, cardiovascular diseases were prominent, especially in cases of BD with low functioning, suggesting an increased risk of cardiovascular events. The identified pathways are closely linked to BD comorbidities, which, in turn, are among the leading global causes of death. Therefore, a comprehensive understanding of the transcriptomic and proteomic profile, as well as the biological processes involved in BD, provides valuable insights for targeted interventions addressing mental health aspects and underlying mechanisms of comorbidities in BD.

# 1. INTRODUÇÃO

## 1.1 TRANSTORNO BIPOLAR

O Transtorno Bipolar (TB) é uma doença grave e altamente incapacitante entre adultos em idade produtiva (MATHERS; LONCAR, 2006; ROSA et al., 2012). Caracteriza-se por flutuações no estado de humor que levam progressivamente a prejuízos cognitivos e funcionais significativos. A doença está, inclusive, associada a altas taxas de mortalidade por causas naturais e por suicídio (CRUMP et al., 2013). Devido à sua natureza crônica e constantes mudanças no humor, o tratamento do TB é altamente desafiador, sendo que aproximadamente 37% dos pacientes recaem após 1 ano de tratamento (GITLIN et al., 1995). Em razão disso, é necessário compreender melhor os mecanismos biológicos envolvidos na doença a fim de aprimorar o desenvolvimento de fármacos mais efetivos.

O diagnóstico dos transtornos psiquiátricos é feito através de uma avaliação clínica dos sintomas, por meio de uma entrevista estruturada, baseado nos critérios estabelecidos no Manual de Diagnóstico e Estatística dos Transtornos Mentais versão 5 (DSM-V) ou pela Classificação Estatística Internacional das Doenças e Problemas Relacionados com a Saúde versão 11 (CID-11). Embora o TB se manifeste por meio de episódios maníacos, são os episódios depressivos que assumem predominância (MANNING, 2005). O TB é dividido em tipos I e II, além do Transtorno Ciclotímico e o transtorno não especificado. O tipo I caracteriza-se pela alternância de episódios maníacos e depressivos, podendo ou não envolver episódios psicóticos. Já o tipo II exige um ou mais episódios depressivos e pelo menos um episódio hipomaníaco. Os sintomas da mania incluem aumento de energia, redução do sono, impulsividade, euforia, grandiosidade, intensificação da atividade sexual, irritabilidade, agressão e comportamentos imprudentes. Nos episódios depressivos, destaca-se humor deprimido, falta de prazer em atividades, alterações no peso, insônia, retardo psicomotor e

ideação suicida (VIETA et al., 2018). Um dado preocupante é a alta prevalência (14-59%) dos indivíduos com TB que apresentam pensamentos suicidas, dos quais em torno de 20% efetivamente chegam à consumação do ato (GONDA et al., 2012). Apesar dos episódios maníacos impactarem a saúde dos pacientes com TB, muitas vezes levando a hospitalização, a fase depressiva merece destaque devido às altas taxas de resistência ao tratamento. Poucos fármacos são plenamente efetivos em tratar os episódios depressivos, e o uso de antidepressivos em monoterapia para tratar a depressão bipolar é controverso pois parece estar associado ao aumento de virada maníaca (CHEN et al., 2022). Estima-se que o diagnóstico preciso do TB ocorra, em média, cerca de 7,5 anos após o surgimento dos primeiros sintomas. Esse atraso resulta em um tratamento inadequado para muitos pacientes, que frequentemente recebem apenas antidepressivos como terapia única. Esse cenário, conforme relatado, pode aumentar significativamente o risco de episódios maníacos e deterioração no prognóstico dos pacientes (CHEN et al., 2015; STERN, 2006). A falta de precisão no diagnóstico do TB resulta em um elevado número de casos subdiagnosticados ou diagnosticados erroneamente com implementação de tratamentos inadequados que, em última instância, agravam o curso clínico da doença (CHEN et al., 2015; DE JESUS et al., 2015). Neste sentido, a busca por marcadores biológicos específicos para o TB é extremamente relevante pois poderia contribuir para aumentar a acurácia dos diagnósticos e prescrição de tratamentos efetivos.

O TB é associado a um marcado prejuízo cognitivo que afeta diferentes aspectos do funcionamento do paciente. Esses déficits não apenas persistem após o avanço da doença, mas também se manifestam após o primeiro episódio (ZANELLI et al., 2022). O TB está ligado a prejuízos em vários aspectos, tais como atenção, concentração, memória verbal e aprendizado, memória de trabalho e flexibilidade mental, prejuízos que sofrem um agravamento significativo durante as fases agudas da doença e persistem em uma trajetória de piora ao longo do curso do TB (CARDOSO et al., 2015). Segundo uma revisão sistemática recente,

pacientes eutímicos apresentaram diferenças na atividade cerebral, medida por ressonância magnética funcional, em áreas-chave como aquelas envolvidas na memória de trabalho, incluindo os córtex pré-frontal ventromedial e dorsolateral, quando comparados com participantes saudáveis (SALDARINI; GOTTLIEB; STOKES, 2022). Outra pesquisa relevante identificou três subgrupos cognitivos distintos no TB por meio de análise hierárquica de clusters. Utilizando uma bateria neurocognitiva, os pacientes foram categorizados em cognição intacta, comprometimento cognitivo seletivo e comprometimento cognitivo global. Esses achados destacam a existência de um espectro de gravidade, que vai desde pacientes sem comprometimento até aqueles com uma notável redução no funcionamento cognitivo (LIMA et al., 2019). Uma meta-análise abrangendo 6.424 pacientes com TB tipo I, 702 com Tb tipo II e 8.276 controles revelou, por meio de uma avaliação cognitiva completa, déficits em várias áreas cognitivas, em especial, na memória verbal em pacientes com TB. O TB tipo I foi associado a prejuízos mais severos em comparação ao TB tipo II em todas as funções, exceto no controle inibitório que foi mais comum no TB-II (COTRENA et al., 2020). O indivíduo com TB demonstra, inclusive, desafios na manutenção prolongada da atenção, propensão a distrações e aceleração dos pensamentos. Entre outras comorbidades, essas características podem aumentar a susceptibilidade ao desenvolvimento de transtornos de atenção e hiperatividade (SANDSTROM et al., 2021).

O impacto do TB transcende o indivíduo, afetando diversas áreas do funcionamento psicossocial incluindo o desempenho acadêmico, profissional, social e relações familiares (RICHARDSON; JANSEN; FITCH, 2018). Pacientes com TB podem experimentar variações no seu funcionamento ao longo do tempo, em decorrência das flutuações de humor características da doença e déficits cognitivos. Enquanto nos episódios de mania um indivíduo pode apresentar energia excessiva, aumento da atividade e otimismo exacerbado; durante os episódios depressivos, o funcionamento pode ser comprometido devido à falta de energia,

desânimo e dificuldade de concentração. No entanto, boa parte dos pacientes continuam apresentando dificuldades no funcionamento psicossocial mesmo durante o período de remissão, ou seja, fora dos episódios de humor. Em um estudo realizado por nosso grupo, pacientes eutímicos classificados em diferentes estágios da doença mostraram um paralelo entre o nível de funcionamento e a gravidade do curso do TB, ou seja, aqueles em estágios mais avançados da doença apresentavam maiores dificuldades no funcionamento global e em áreas específicas do comportamento (ROSA et al., 2014). Diversos fatores, como variáveis demográficas, clínicas e neurocognitivas, têm sido relacionados ao funcionamento psicossocial em indivíduos com TB (BAENA-OQUENDO et al., 2022; BONNÍN et al., 2019). Assim, a complexidade entre os déficits cognitivos e funcionais de pacientes com TB destaca a necessidade de abordagens integradas e personalizadas no tratamento. O diagnóstico precoce e a intervenção específica em áreas comprometidas podem ser promissores para melhorar a qualidade de vida dos indivíduos e atenuar o impacto do TB no seu funcionamento global.

## 1.2 MARCADORES PERIFÉRICOS

Biomarcador é definido como uma substância com característica mensurável, capaz de indicar processos biológicos, discriminar entre o normal e o patológico e medir as respostas a exposições ou intervenções. Um biomarcador ideal é aquele capaz de auxiliar em áreas como diagnóstico, monitoramento, predição e prognóstico (CALIFF, 2018). Um biomarcador diagnóstico é utilizado para confirmar ou identificar a presença de uma doença ou condição específica e, também para reconhecer subtipos específicos da doença em indivíduos. No entanto, é importante definir um método de validação para medir um biomarcador de forma precisa e repetitiva a um baixo custo para garantir a sua confiabilidade. Além disso, um ponto

de grande relevância a ser considerado é o limite de detecção, o qual realizado de forma incorreta pode levar a uma significativa diferença nos cuidados médicos. Já o biomarcador de monitoramento consiste em uma substância cujas medições são repetidas, sendo empregada para avaliar continuamente o estado de uma doença ou condição médica. Os biomarcadores de monitoramento são importantes para medir os efeitos farmacodinâmicos, detectar evidências precoces de uma resposta terapêutica ou ainda para detectar complicações de uma doença ou terapia. Um exemplo clássico é o caso do tratamento da alta pressão arterial, onde uma redução na medida da pressão arterial fornece evidências de que a terapia está funcionando. O biomarcador preditivo, por sua vez, tem o propósito de identificar efeitos positivos ou negativos em resposta à exposição a um determinado tratamento ou agente ambiental. É importante para melhoria no desenho de ensaios clínicos, especialmente na fase de registo, no qual os participantes com níveis elevados de um biomarcador preditivo permite um sinal mais claro de que o tratamento realmente tem efeito. Por exemplo, pacientes com colesterol LDL elevado apresentam alto risco de desenvolver aterosclerose, uma vez diagnosticada a doença, que por consequência terão um desfecho mais pronunciado do tratamento. Por fim, o biomarcador prognóstico é utilizado para determinar a probabilidade de ocorrência de eventos clínicos, recorrência de doença ou progressão em pacientes que já têm a doença ou condição médica em questão. Distinguem-se dos biomarcadores preditivos, que identificam fatores associados ao efeito da intervenção ou exposição. Um exemplo relevante de biomarcadores prognósticos é a alocação de recursos na saúde da população: quando estratificado o risco de resultados clínicos e financeiros negativos, uma organização pode diferenciar quais os pacientes podem se beneficiar de uma avaliação mais intensiva, ao mesmo tempo que evita testes de diagnóstico desnecessários ou intervenções médicas (CALIFF, 2018; FDA-NIH BIOMARKER WORKING GROUP, 2016).

Entre diferentes fluidos biológicos, o sangue se destaca por ser de fácil acesso. Consiste em diferentes tipos celulares, proteínas e metabólitos, componentes que fornecem informações valiosas sobre os processos fisiológicos e patológicos que ocorrem no organismo. As proteínas, em particular, desempenham um papel crucial na regulação e execução de diversas funções biológicas e suas alterações podem estar associadas a condições normais, patológicas ou a respostas a intervenções específicas (LIUMBRUNO et al., 2010). Existem várias proteínas que têm grande importância clínica, como é o exemplo da troponina, um biomarcador cardíaco usado para diagnosticar e avaliar danos no músculo cardíaco (GARG et al., 2017). Algumas proteínas no sangue são conhecidas como proteínas de fase aguda (por exemplo, a proteína C-reativa), cujos níveis aumentam ou diminuem em resposta a inflamações ou lesões (DIX et al., 2022). Na oncologia, um importante exemplo de proteína biomarcadora é o PSA (do inglês *Prostate Specific Antigen*), utilizado para detectar e monitorar o câncer de próstata (SOKOLL; CHAN, 1997). Apesar do uso disseminado em Medicina, o estudo de biomarcadores sanguíneos para o diagnóstico de doenças psiquiátricas é uma área de pesquisa ainda incipiente, porém crucial dada ao potencial para aumentar a acurácia dos diagnósticos, monitorar o curso da doença e avaliar a resposta aos tratamentos. É importante ressaltar que a literatura contempla uma série de estudos envolvendo proteínas periféricas relacionadas a doenças psiquiátricas, tendo alguns autores sugerido que tais manifestações poderiam representar as alterações cerebrais (HERBERTH et al., 2011; HU et al., 2023; OLSSON et al., 2016; THE LANCET PSYCHIATRY, 2016).

## 1.3 CIÊNCIAS ÔMICAS

As ciências ômicas se referem ao estudo global de moléculas biológicas e suas interações em um organismo ou sistema biológico. A partir delas é possível explorar várias camadas de informações biológicas, como genomas, proteomas, transcriptomas, metabolomas,

entre outras (WANG; FRIDLEY, 2023). Deste modo, a cascata ômica (Figura 1) ilustra o fluxo de informações bioquímicas em diferentes níveis moleculares dentro de um organismo, desde os genes até os metabólitos. Esse processo sequencial envolve decifrar as informações genômicas, compreender a expressão gênica por meio da transcriptômica, desvendar as complexidades das proteínas por meio da proteômica e explorar o cenário metabólico por meio da metabolômica (BABU; SNYDER, 2023). Ao analisar e integrar os dados em cada nível, é possível adquirir melhor entendimento molecular de cada doença estudada. Com o objetivo de compreender melhor os mecanismos fisiológicos envolvidos no TB sob uma perspectiva integrada, nesta tese foram utilizados dados de transcriptômica e proteômica.

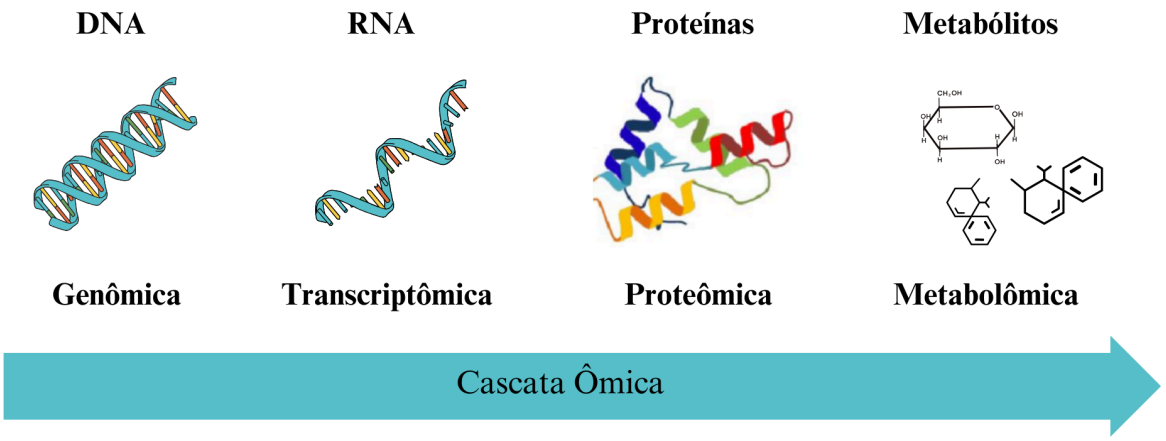


**Figura 1:** A cascata ômica ilustra os diversos níveis moleculares em um organismo. A seta simboliza os processos bioquímicos pelos quais a informação é transmitida de um nível para o seguinte. A metabolômica, como estágio final na cascata, dedica-se ao estudo de moléculas mais próximas do fenótipo.

### 1.3.1 Transcriptômica

A transcriptômica é um campo da biologia molecular que se concentra na análise abrangente dos transcritos de RNA em uma célula, tecido ou organismo específico. Os transcritos de RNA são moléculas mensageiras produzidas a partir do DNA por meio da transcrição e servem como moldes para a síntese de proteínas durante o processo de tradução

(MOROZOVA; HIRST; MARRA, 2009). A transcriptômica não se limita apenas ao RNA mensageiro, mas também abrange outros tipos de transcritos, como microRNAs e RNAs longos não-codificantes. Ela investiga não apenas a expressão desses RNAs, mas também sua função, localização e processo de degradação, tanto em condições normais quanto em situações patológicas (JIANG et al., 2015). A transcriptômica tem grande relevância pois permite estudar quais genes estão ativos e consequentemente sendo transcritos a mRNA em um determinado momento. Isso é essencial para entender as vias de sinalização celular, o desenvolvimento e as respostas a estímulos externos. A compreensão da expressão gênica regulada em condições saudáveis e patológicas pode levar à descoberta de novos alvos terapêuticos. Medicamentos podem ser desenvolvidos para modular a expressão de genes específicos (LAN et al., 2022; YI et al., 2022). Ainda, ao analisar padrões de expressão gênica em diferentes condições é possível identificar marcadores biológicos específicos para cada doença estudada (ROELANDS et al., 2023; ZENG et al., 2023).

As tecnologias transcriptômica high-throughput permitem a análise de expressão gênica em larga escala, podendo ser realizada pela técnica do microarranjo ou sequenciamento de RNA de nova geração (RNA-Seq). O microarranjo permite a investigação da expressão de milhares de genes regulados em diferentes condições biológicas, como estágios de desenvolvimento, tipos celulares ou em resposta a tratamentos específicos. Já o RNA-Seq permite a identificação e quantificação de transcritos de RNA com alta resolução e sensibilidade, fornecendo informações detalhadas sobre a diversidade de transcritos, splicing alternativo, novas isoformas de genes, e expressão diferencial de genes em diferentes condições biológicas (JIANG et al., 2015; YANG; WEI, 2015). Deste modo, a literatura contempla diferentes abordagens transcriptômicas com intuito de elucidar aspectos biológicos envolvidos nas doenças psiquiátricas (FRIES et al., 2017; HESS et al., 2020; KREBS et al., 2020; MAHMOUDI; GREEN; CAIRNS, 2021). Um recente estudo destacou o papel crucial

da microglia no envelhecimento cerebral, empregando a técnica de RNA-seq. Ao analisar o transcriptoma de 255 amostras de microglia humana obtidas post-mortem de 100 indivíduos, identificaram-se variantes associadas a doenças como Alzheimer e Parkinson. A partir daí, foi construído um catálogo abrangente dos efeitos genéticos no transcriptoma microglial, fornecendo variantes funcionais candidatas em distúrbios neuropsiquiátricos (LOPES et al., 2022). Além disso, foi realizada a integração de genótipos e sequenciamento de RNA em amostras de córtex cerebral de 1695 indivíduos com transtorno do espectro autista, esquizofrenia (ESZ), TB e controles. Foi observado que alterações em isoformas, especialmente em mais de 50 genes, mostraram impacto significativo no cérebro disfuncional, enriqueceram para risco genético e forneceram especificidade de doença em redes de coexpressão. Assim, este trabalho enfatizou a desregulação em nível de isoformas como um mecanismo crucial, que associa fatores de risco genético à fisiopatologia das doenças psiquiátricas (GANDAL et al., 2018). Considerando análises periféricas, um importante estudo combinou dados de transcriptoma e metiloma em jovens com alto risco de desenvolver TB. Utilizando microarranjo em células mononucleares do sangue de crianças e adolescentes, foram identificados 43 genes que diferenciaram pacientes com TB, jovens de alto risco e controles saudáveis. Ainda, a análise de vias indicou um enriquecimento na via do receptor de glicocorticóides, sugerindo um possível papel da resposta ao estresse no risco de TB em populações vulneráveis (FRIES et al., 2017). Hess e colaboradores realizaram uma meta-análise transcriptômica do sangue periférico de pacientes com TB e ESZ, identificando 19 genes e 4 módulos diferencialmente expressos em casos de TB. Quando combinado o grupo TB e ESZ (denominado "psicose maior"), foram identificados treze módulos gênicos ligados a apoptose, estresse oxidativo e cromatina. Utilizando aprendizado de máquina foi introduzido um método chamado "pontuação de risco poli transcriptômico" que distinguiu casos de TB e ESZ (HESS et al., 2020). A literatura explorou uma abordagem integrada para

compreender a vulnerabilidade ao estresse e a depressão, combinando a análise de características fenotípicas com o perfil de expressão gênica no sangue. Utilizando a técnica de microarranjo, foram identificados os perfis de expressão gênica em indivíduos com resiliência, vulnerabilidade e resistência ao estresse. Notavelmente, genes ribossomais foram identificados como regulados positivamente em relação à vulnerabilidade ao estresse e em pacientes deprimidos (SACKS et al., 2018). Ademais, foi realizado um estudo de caso-controle comparando perfis de expressão gênica global em células mononucleares do sangue coletadas logo após o parto. Nove mães, sem tratamento antidepressivo, que desenvolveram um episódio depressivo após o parto, foram comparadas com 10 mães que não apresentavam sintomas depressivos e foi observada uma assinatura distinta de expressão gênica. As mães que desenvolveram episódio depressivo apresentaram ativação imunológica e diminuição do envolvimento transcricional na proliferação celular e nos processos de replicação e reparo do DNA (SEGMAN et al., 2010). Apesar das importantes descobertas até o presente momento, ainda não foram encontrados biomarcadores periféricos efetivos e aplicáveis no diagnóstico de doenças psiquiátricas.

### 1.3.2 Proteômica

A proteômica, por sua vez, é uma ciência que possibilita a identificação em larga escala das proteínas envolvidas nos processos celulares e teciduais. Essa análise detalhada proporciona uma compreensão profunda dos processos biológicos em nível molecular, oferecendo entendimentos valiosos sobre as funções das proteínas e suas interações (TANGREA et al., 2004). Ao investigar os padrões de proteínas em diferentes estados patológicos, a proteômica pode auxiliar na identificação de biomarcadores. Assim como relatado acima, muitas proteínas são usadas como biomarcadores na Medicina, auxiliando na detecção precoce e o acompanhamento de doenças, possibilitando diagnósticos mais rápidos e precisos, além de facilitar o monitoramento do progresso e da resposta ao tratamento (PREECE; HAN; BAHN, 2018). Analisar a interação proteína-proteína é fundamental para entender redes de sinalização, vias e processos metabólicos de doenças de alta complexidade, como é o caso do TB (DASH ATAN et al., 2018). Assim como as proteínas no organismo humano devem variar com o estresse e outros fatores ambientais, a detecção de mudanças na expressão, na abundância, na estrutura e na função das proteínas, pode indicar anormalidades patológicas antes mesmo da propagação dos sintomas clínicos.

Deste modo, a abordagem proteômica é particularmente importante em doenças de alta complexidade como é o caso das doenças neuropsiquiátricas (SAIA-CEREDA et al., 2016; SONG et al., 2015; ZAKHAROVA et al., 2022). A literatura mostrou a relevância de realizar pesquisas sobre marcadores e elaborar estratégias para a detecção precoce de fatores de risco associados à doença de Alzheimer, utilizando como base o perfil proteômico (ZAKHAROVA et al., 2022). Uma compreensão mais aprofundada sobre a fisiopatologia da depressão maior tem sido alcançada por diferentes métodos como neuroimagem, genômica e proteômica. A identificação de biomarcadores sanguíneos por meio de tecnologias proteômicas é muito

promissora e os avanços podem abrir caminho para um diagnóstico mais preciso da depressão maior (GADAD et al., 2018; PREECE; HAN; BAHN, 2018). Análises abrangentes do plasma sanguíneo podem oferecer insights sobre vias moleculares desreguladas e sua relação com os transtornos psiquiátricos, além de fornecer informações sobre características relacionadas ao envelhecimento e ao gênero nessa condição. A pesquisa, que envolveu participantes ao longo de seis décadas de vida, revelou uma interrupção metabólica contínua e aumento de marcadores inflamatórios entre os componentes do proteoma periférico identificados nos pacientes com ESZ (CAMPEAU et al., 2022). Um estudo recente criou modelos preditivos para transtornos psicóticos em indivíduos com alto risco e indivíduos que experimentaram episódios psicóticos durante a adolescência. Esta pesquisa integrou dados biológicos, como informações sobre proteínas plasmáticas, com dados clínicos e através de técnicas de aprendizado de máquina foi possível identificar biomarcadores preditivos nessa condição (MONGAN et al., 2021). Estudos exploratórios na área da proteômica têm contribuído para uma compreensão mais aprofundada dos mecanismos moleculares relacionados ao TB. As descobertas recentes destacam a disfunção nos sistemas imunológico e inflamatório, bem como alterações em vias metabólicas cruciais, como o sistema complemento e a cascata de coagulação, como elementos-chave na fisiopatologia do TB (RODRIGUES et al., 2022; ZIANI et al., 2022). A tabela 1 mostra uma série de estudos envolvendo proteômica no TB Song e colaboradores, por exemplo, identificaram alterações em 16 proteínas plasmáticas, as quais eram específicas para cada fase do TB. Um estudo adicional diferenciou pacientes com TB de outras condições psiquiátricas ao analisar o perfil proteômico do soro sanguíneo. Os resultados apontaram diferenças nos níveis das seguintes proteínas: ApoA1, ApoE, ApoC3, ApoA4, Samp, SerpinA1, TTR, IgK, Alb, VTN, TR, C4A e C4B em pacientes com TB em comparação com pacientes com ESZ e controles saudáveis. De acordo com a rede molecular global, a maioria destas proteínas estão associadas com a resposta inflamatória (DE JESUS et

al., 2017). A literatura destaca o uso da proteômica para discriminar a depressão bipolar da depressão maior. A análise do plasma dos pacientes revelou alterações significativas em 9 proteínas entre TB e depressão maior, sendo a maioria associada ao sistema imunológico. Adicionalmente, foi realizada uma análise bioinformática que sugeriu que B2RAN2 e ENG poderiam desempenhar papéis relevantes na depressão, atuando como biomarcadores para diferenciar as duas condições (REN et al., 2017). Em conjunto, essas descobertas demonstram o valor da proteômica na compreensão de doenças, diagnóstico, monitoramento e desenvolvimento de estratégias terapêuticas mais eficazes.

Tabela 1: Estudos proteômicos identificando biomarcadores periféricos no TB.

| **Autor** | **Fração** | **Grupo** | **Amostra** | **Técnica** | **Resultados** |
|---|---|---|---|---|---|
| Herbert h *et al.* (2011) | Soro | TB<br>C<br>TB<br>C | 32<br>32<br>16<br>15 | LC-MS<br><br>Multiple x | 22 analitos diferencialmente expressos em comparação com controles |
| Alsaif *et al.* (2012) | Soro<br>Plasma | TB<br>C<br>TB<br>C | 24<br>21<br>24<br>21 | Multiple x | 6 (soro) e 10 (plasma) proteínas diferencialmente expressas no TB comparado com controle<br><br>2 proteínas alteradas em ambos os fluidos |
| Haenisch *et al.* (2014) | Plasma | TB<br>C | 17<br>46 | Multiple x | 26 analitos diferencialmente expressos em comparação com controles |
| Chen *et al.* (2015) | Plasma | TB<br>C | 20<br>30 | 2-DE/MS<br>Multiple x | 3 proteínas diferencialmente expressas no TB comparado com controle |
| Song *et al.* (2015) | Plasma | Eutímia<br>C<br>Depressão<br>C<br>Mania<br>C | 10<br>20<br>20<br>20<br>15<br>20 | 2-DE/MS | 32 proteínas diferencialmente expressas no TB comparado com controle;<br>16 proteínas diferencialmente expressas no TB independente da fase;<br>16 proteínas associadas a um estado de humor específico |

| | | | | | |
|---|---|---|---|---|---|
| de Jesus *et al.* (2017) | Soro | TB<br>CNF<br>TB<br>CF | 14<br>9<br>14<br>3 | LC-MS/ MS | 6 proteínas diferencialmente expressas no TB comparado com controle |
| Ren *et al.* (2017) | Plasma | TB<br>C | 30<br>30 | LC-MS/ MS | 54 proteínas diferencialmente expressas no TB comparado com controle |

TB, transtorno bipolar; C, controle; CNF, controle não-familiar; CF, controle familiar; LC, cromatografia líquida; MS, espectrometria de massas; 2-DE, eletroforese bidimensional.
Adaptado de Ziani et al., 2022.

Apesar do que foi mencionado anteriormente, a pesquisa em proteômica na área da psiquiatria ainda é limitada. É mais comum encontrar estudos que avaliam marcadores individuais em vez de investigar redes de proteínas e sua relação com a doença. Dessa forma, a análise de proteínas agindo individualmente aumenta a chance de que se perca uma informação fundamental: a interação entre milhares de proteínas nos sistemas biológicos em um determinado momento e sua importância sobre a fisiopatologia do TB. Nesse cenário, é fundamental se apropriar de metodologias inovadoras, como é o caso, da técnica de espectrometria de massas (MS, do inglês *Mass Spectrometry*) que permite a análise de redes de proteínas em larga escala. A MS é uma técnica fundamental para a caracterização de proteínas, permitindo a identificação da sequência de aminoácidos de uma proteína. O princípio básico da MS envolve a medida da massa das moléculas ionizadas em um processo que consiste em três etapas principais: ionização, análise de massa e detecção. Após a separação, os íons são detectados e registrados, produzindo um espectro de massas. Este espectro mostra a intensidade dos íons em função de sua razão massa-carga. Cada composto possui uma massa molecular única, o que permite a identificação e quantificação precisa das substâncias na amostra (DE HOFFMANN; STROOBANT, 2007). Em suma, a MS assume protagonismo na proteômica, especialmente por estar envolvida nas inovações tecnológicas e proporcionar avanços científicos. Ela tem funcionado como uma importante ferramenta na

identificação e quantificação de proteínas, bem como no mapeamento de interações proteicas e na compreensão das modificações pós-traducionais. Esse detalhamento de informações reveladas nas análises proteômica, por meio de MS, tem sido fundamental para descobertas e avanços no entendimento da fisiopatologia do TB.

Assim, as tecnologias transcriptômica e proteômica high-throughput são ferramentas fundamentais e sólidas na obtenção de dados para entender a fisiopatologia do TB. As informações oriundas dessas disciplinas constituem peças-chave na construção de uma visão mais abrangente da biologia de sistemas. Deste modo, buscamos avaliar, *in silico*, o transcriptoma e proteoma de pacientes com TB a fim de identificar um perfil que discrimine casos de controles, podendo contribuir na descoberta de biomarcadores capazes de auxiliar nas questões diagnósticas. Além disso, a pesquisa por biomarcadores foi estendida ao ambiente *in vivo*, analisando o perfil proteômico no sangue de pacientes com TB.

## 1.4 BIOLOGIA DE SISTEMAS

A biologia de sistemas é uma abordagem interdisciplinar que busca compreender e modelar os sistemas biológicos complexos como uma entidade integral, priorizando uma visão abrangente em vez de se concentrar exclusivamente em componentes isolados. Ela emprega técnicas experimentais e computacionais quantitativas para interpretar o fluxo de informações proveniente de genes, proteínas e outros componentes presentes em vias de sinalização, regulação e funcionalidade. Essa ferramenta tem como objetivo o estudo das interações moleculares em diferentes níveis, permitindo a caracterização da maquinaria subcelular responsável pelas unidades funcionais em células, tecidos e sistemas de órgãos, resultando em comportamentos fisiológicos (TAVASSOLY; GOLDFARB; IYENGAR, 2018; ZUPANIC; BERNSTEIN; HEILAND, 2020). A biologia de sistemas aborda sistemas biológicos em diferentes níveis hierárquicos, nos quais a interação entre componentes diversos leva à

cooperação para atingir estados estáveis de homeostase (NIJHOUT; BEST; REED, 2019). A hierarquia vai desde a representação tradicional da organização funcional da célula: genoma, transcriptoma, proteoma e metaboloma até a arquitetura hierárquica de organização em larga escala. Mesmo com a diversidade de sistemas, há princípios comuns que regem a organização e a conectividade nas redes. Esses princípios podem ser aplicados de maneira ampla, transcendendo as diferenças específicas entre os organismos, sugerindo uma espécie de universalidade nas leis subjacentes à formação e estrutura das redes (OLTVAI; BARABÁSI, 2002). Portanto, a compreensão dessas interações pode fornecer insights valiosos para a medicina, desafiando abordagens tradicionais ao reconhecer a importância da interconexão entre diferentes componentes moleculares para decifrar os sistemas biológicos.

### 1.4.1 Redes regulatórias transcricionais

No contexto da biologia de sistemas, as redes regulatórias transcricionais referem-se a sistemas de interações entre genes e proteínas que controlam a expressão gênica em uma célula. Dentro da rede regulatória transcricional, compreendem-se quatro elementos fundamentais: os fatores de transcrição; seus motivos de ligação ao DNA; os genes-alvo; e os genes expressos. O fator de transcrição associa-se ao seu motivo específico de ligação ao DNA, desencadeando a transcrição e expressão subsequente do gene-alvo na forma de RNA mensageiro (LIN; ZACK; QIAN, 2006). Essas redes desempenham papel fundamental na regulação dos processos biológicos, permitindo que as células respondam a sinais ambientais e mantenham a homeostase. Diversos métodos computacionais podem ser utilizados para criar redes regulatórias transcricionais, incluindo o algoritmo para a reconstrução de redes celulares precisas (ARACNe, do inglês *Algorithm for the Reconstruction of Accurate Cellular Networks*). Ele identifica interações transcricionais entre produtos gênicos ao analisar dados

de perfis de expressão de microarranjos, ao mesmo tempo em que prevê associações funcionais entre genes ou propõe novas funções para genes ainda não caracterizados (LEE; TZOU, 2009; MARGOLIN et al., 2006). Sob a orientação dos fatores de transcrição, cada gene exerce influência sobre a atividade celular ao produzir RNA mensageiro, que por sua vez guia a síntese de proteínas pelos ribossomos no citoplasma, onde ocorrem reações bioquímicas. A representação da regulação da expressão gênica como uma rede surge da necessidade dos estudiosos de deduzir essas redes a partir de informações disponíveis, visando descrever os complexos fenômenos naturais (LEE; TZOU, 2009). A Figura 2 ilustra a inferência de Redes de Regulação Gênica a partir de dados de expressão gênica, representando a obtenção da rede regulatória de transcrição. Esta representação refere-se à organização estrutural e aos padrões de interconexão entre os elementos constituintes da rede. Cada vértice ou nó, na representação gráfica, simboliza a presença de um gene específico, ao passo que cada aresta estabelece visualmente as interações entre esses genes, proporcionando uma visão abrangente e detalhada das conexões na rede.

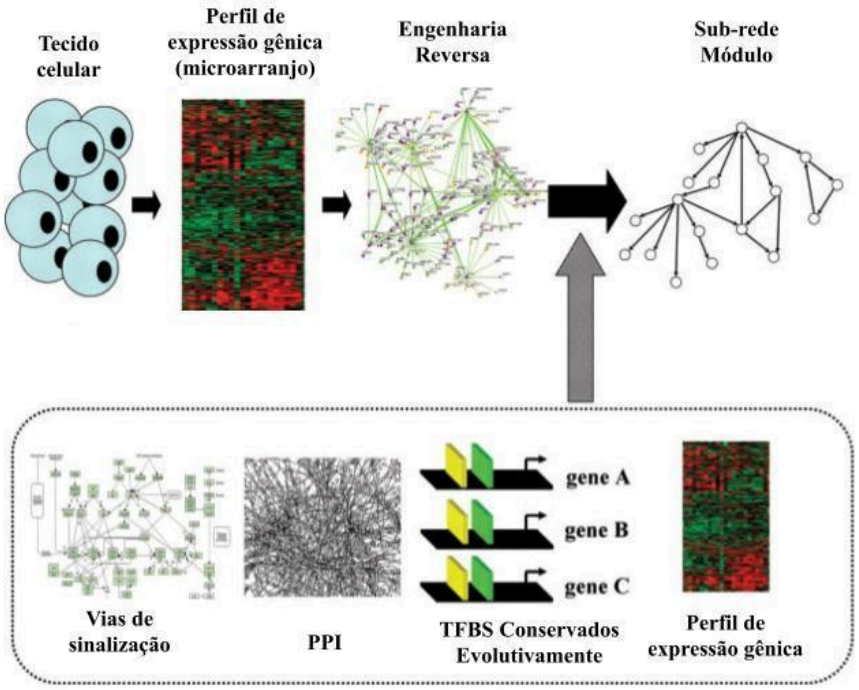


**Figura 2**: Inferência de Redes de Regulação Gênica. O mRNA é obtido de células ou tecidos, seguido por experimentos de microarranjo. Os resultados desses experimentos geram um conjunto de dados de perfil de expressão gênica, utilizado por métodos computacionais na inferência da Redes de Regulação Gênica. Em algumas situações, os biólogos precisam consultar bases de dados de natureza diversa (como caminhos biológicos, interações proteína-proteína, sítios de ligação conservados evolutivamente de fatores de transcrição ou perfil de expressão gênica) para limitar o escopo da pesquisa. A expectativa é que uma sub-rede ou módulo se manifeste, servindo como um modelo final para os biólogos, permitindo a formulação de hipóteses e o planejamento de experimentos.

PPI, do inglês Protein-Protein Interaction; TFBS, do inglês Transcription Factors Binding Sites.

Adaptado de Lee e Tzou, 2009.

### 1.4.2 Análise de regulador mestre

A análise de regulador mestre (MRA, do inglês *Master Regulator Analysis*), por sua vez, é uma abordagem na biologia de sistemas que visa identificar genes ou fatores de transcrição que desempenham um papel crucial na regulação de redes gênicas. Neste contexto,

certos fatores de transcrição atuam como reguladores mestres coordenando o comportamento celular e exercendo influência sobre um fenótipo específico (CAI et al., 2020). O emprego de reguladores mestres como biomarcadores tem sido objeto de investigação em diferentes condições, incluindo câncer (TOVAR et al., 2015), asma (DO et al., 2021) e doença de Alzheimer (VARGAS et al., 2018). A literatura enfatiza, inclusive, a aplicação deste método na investigação da regulação transcricional de doenças psiquiátricas. Por meio da MRA, o gene EGR3 foi reconhecido como um potencial alvo chave, mostrando-se reprimido no córtex pré-frontal de indivíduos com TB (PFAFFENSELLER et al., 2016). Em seguida, um estudo adicional investigou fatores de transcrição atuando como reguladores mestre no TB, na depressão maior (DM) e na ESZ. Os resultados indicaram que a supressão do gene EGR3 foi predominante no TB e na ESZ, com uma presença menos pronunciada na DM (LIN; ZACK; QIAN, 2006). Embora diversos estudos tenham explorado a expressão gênica diferencial em distúrbios psiquiátricos em estruturas cerebrais, até o momento desconhecemos estudos usando o método MRA para avaliar fatores de transcrição no sangue de pacientes com TB. Este enfoque é particularmente relevante, uma vez que os reguladores mestres desempenham um papel central na regulação gênica global e podem ter implicações significativas na compreensão dos processos patológicos, assim como no desenvolvimento de estratégias terapêuticas.

### 1.4.3 Análise de enriquecimento funcional

A análise de enriquecimento funcional (FEA, do inglês *Functional Enrichment Analysis*) é uma técnica utilizada para interpretar grandes conjuntos de genes ou proteínas, comumente derivados de experimentos de expressão gênica. O objetivo principal é entender os processos biológicos, funções celulares ou vias metabólicas que estão significativamente

representadas nos conjuntos de genes em análise. O processo geral da FEA envolve comparar o conjunto de genes de interesse com anotações funcionais previamente conhecidas, como termos de ontologia gênica, vias metabólicas ou funções celulares (WEBBER, 2011). Para facilitar a identificação de pacientes com características fenotípicas semelhantes, diversos bancos de dados foram desenvolvidos, permitindo uma rápida disseminação dos dados genéticos associados a esses pacientes. As fontes de informações funcionais frequentemente utilizadas compreendem anotações na literatura como Gene Ontology, KEGG, REACTOME, entre outros (WEBBER, 2011).

A análise de enriquecimento de conjuntos de genes (GSEA, do inglês *Gene Set Enrichment Analysis*), por sua vez, é uma técnica amplamente reconhecida para identificar genes enriquecidos em amostras de diferentes grupos. Este método compara dois grupos de amostras para identificar se os genes em um conjunto pré-definido estão distribuídos de maneira não aleatória entre essas amostras. Deste modo, a GSEA é realizada para entender se esse conjunto de genes está correlacionado com alguma característica fenotípica ou condição experimental (REIMAND et al., 2019; SUBRAMANIAN et al., 2005). A relação entre o genótipo e o fenótipo de um indivíduo torna-se mais robusta e confiável quando são encontradas variações genéticas comuns que são exclusivas para um grupo de pacientes que compartilham sintomas semelhantes (WEBBER, 2011). Assim, analisar as características compartilhadas entre os genes candidatos pode fornecer insights valiosos sobre a origem e desenvolvimento de uma doença, contribuindo para uma compreensão mais aprofundada de sua etiologia.

### 1.4.4 Reposicionamento de fármacos

O reposicionamento de fármacos trata da identificação de novos usos terapêuticos para medicamentos existentes que foram inicialmente desenvolvidos para tratar outras condições. Em vez de criar novos medicamentos a partir do zero, o reposicionamento de fármacos explora a possibilidade de reutilizar medicamentos já aprovados para outras finalidades, aproveitando seu perfil de segurança conhecido e acelerando o processo de desenvolvimento (PUSHPAKOM et al., 2019). Dessa forma, o Connectivity Map (cMap) destaca-se como uma perspectiva promissora para a reutilização de medicamentos. Este banco de dados abrange perfis transcriptômicos de compostos bioativos, como medicamentos e substâncias químicas experimentais, capazes de impactar a expressão gênica celular. Ao facilitar a análise e comparação desses perfis, o cMap possibilita uma compreensão mais profunda das relações entre doenças, processos fisiológicos e os mecanismos de ação de agentes (LAMB, 2007). Juntamente com o cMap é possível utilizar dados da Rede Integrada de Assinaturas Celulares Baseadas em Biblioteca do NIH (do inglês LINCS). O LINCS é uma iniciativa do Instituto Nacional de Saúde dos Estados Unidos que visa compreender e catalogar as respostas celulares a diversas perturbações, como drogas, agentes genéticos e outras intervenções. O objetivo é criar uma biblioteca de assinaturas celulares que representem as mudanças nas expressões genéticas e nas vias biológicas induzidas por essas perturbações (KEENAN et al., 2018). Ao contrário da abordagem convencional da farmacologia, os perfis transcriptômicos disponibilizados pelo cMap e pelo LINCS empregam estratégias de biologia de sistemas nos níveis de vias e redes, em oposição à focalização em um único alvo. Assim, ao integrar a transcriptômica high-throughput e a análise de redes, é possível identificar potenciais fármacos específicos para o tratamento do TB, fundamentados na assinatura de expressão

gênica. Deste modo, podemos melhorar nossa compreensão da biologia do TB e facilitar a elaboração de estratégias terapêuticas personalizadas para esse transtorno complexo.

## 2. HIPÓTESE

Hipotetizamos que pacientes com TB apresentam um perfil molecular distinto daqueles encontrados em pessoas saudáveis. Tais padrões poderão fornecer insights sobre mecanismos patológicos subjacentes, contribuindo para a identificação de novos alvos terapêuticos. Acreditamos que a compreensão aprofundada dessas assinaturas moleculares não apenas aprimora a estratificação de pacientes com TB, mas também abre caminho para intervenções terapêuticas mais direcionadas e eficazes.

# 3. OBJETIVO

## 3.1 Objetivo Geral

O objetivo central deste estudo é identificar um padrão molecular, por meio da análise do proteoma e do transcriptoma, no sangue periférico de pacientes com TB, bem como avaliar a associação destas medidas com a progressão da doença e identificar novos alvos terapêuticos.

## 3.2 Objetivos Específicos

a) Examinar os níveis séricos de transcritos em pacientes nos estados maníaco, depressivo, eutímico do TB, a partir de bancos de dados, com o intuito de identificar potenciais biomarcadores para cada fase da doença;

b) Identificar, por meio de dados transcriptômicos e técnicas computacionais, com base em assinaturas de expressão gênica, novos compostos bioativos ou medicamentos aprovados pela FDA para o tratamento do TB;

c) Comparar os proteomas sanguíneos de indivíduos com TB e indivíduos saudáveis reportados na literatura utilizando interação proteína-proteína, a fim de identificar e propor vias biológicas disfuncionais associadas envolvidas na doença;

d) Caracterizar assinaturas moleculares, vias de sinalização e doenças relacionadas ao TB utilizando análise integrativa de proteômica e bioinformática em amostras de sangue de pacientes em comparação com controles.

# CAPÍTULO II

Artigos científicos

## 4. ARTIGO CIENTÍFICO 1

Artigo científico intitulado: Master Regulatory Genes in Bipolar Disorder: Employing Transcriptomics to Uncover Phase-Specific Mood Biomarkers

Artigo a ser submetido.

**Master Regulatory Genes in Bipolar Disorder: Employing Transcriptomics to Uncover Phase-Specific Mood Biomarkers**

Paola Rampelotto Ziani1,2, Marco Antônio de Bastiani2, Pedro Henrique da Rosa1,2, Tainá Schons1, Giovana Mezzomo1,2, Gabriel Henrique Hizo4, Quênia de Carvalho1, Adriane R Rosa1,2,3

1 Molecular Psychiatry Laboratory, Clinics Hospital of Porto Alegre, Porto Alegre, Brazil.

2 Postgraduate Program in Biological Sciences: Pharmacology and Therapeutics - Institute of Basic Health Sciences, Federal University of Rio Grande do Sul, Porto Alegre, Brazil.

3 Department of Pharmacology, Institute of Basic Science Health, Federal University of Rio Grande do Sul, Porto Alegre, Brazil.

4 Postgraduate Program in Gastroenterology and Hepatology Sciences, Federal University of Rio Grande do Sul, Porto Alegre, Brazil.

Corresponding author: arrosa@hcpa.edu.br

Rua Ramiro Barcelos, 2350, Santa Cecília, Hospital de Clínicas de Porto Alegre, Porto Alegre, RS, Brazil - Postal Code: 90410-000

ORCID: 0000-0001-8629-4625

Abstract

Bipolar disorder (BD) is a complex mood disorder affecting approximately 2.4% of the global population. Distinguishing bipolar depression from regular depression poses a significant challenge in clinical diagnosis. Omics sciences, particularly transcriptomics, have emerged as essential tools in understanding BD. Transcriptomics, focusing on messenger RNAs, aids in tracking disease progression and identifying biomarkers for personalized treatment. Master Regulatory Genes play a vital role in cellular regulation and are categorized into family MRGs and signaling pathway MRGs. Gene Set Enrichment Analysis identifies genes involved in specific biological processes. This study utilized a systems biology approach, employing Microarray data from BD patients' blood samples. We identified 59 Master Regulatory Genes in BD, with DMTF1 identified across all mood states. Enrichr analysis further delineated key biological processes associated with each BD phase. Transcriptomic tools enhance our understanding of BD and improve diagnostic accuracy, paving the way for targeted treatments.

Keywords: Transcriptomic, Biomarkers, Mood Disorder, Systems Biology, Bioinformatics.

## 5. ARTIGO CIENTÍFICO 2

Artigo científico intitulado: Drug Repurposing and Personalized Treatment Strategies for Bipolar Disorder Using Transcriptomic

Artigo aceito para publicação na revista: Brazilian Journal of Psychiatry

Fator de impacto: 5.5

E-mail de confirmação:

Dear Dr. Rosa:

It is a pleasure to accept your manuscript entitled "Drug Repurposing and Personalized Treatment Strategies for Bipolar Disorder Using Transcriptomic" in its current form for publication in the Brazilian Journal of Psychiatry. The comments of the reviewer(s) who reviewed your manuscript are included at the foot of this letter.

Thank you for your fine contribution. On behalf of the Editors of the Brazilian Journal of Psychiatry, we look forward to your continued contributions to the Journal.

Sincerely,
Andre Brunoni
Editor-in-Chief, Brazilian Journal of Psychiatry
brunoni@usp.br

Associate Editor
Comments to the Author:
(There are no comments.)

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Original Article

# Drug Repurposing and Personalized Treatment Strategies for Bipolar Disorder Using Transcriptomic

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# Drug Repurposing and Personalized Treatment Strategies for Bipolar Disorder Using Transcriptomic: an exploratory study

Paola Rampelotto Ziani[1,2], Marco Antônio de Bastiani[2], Ellen Scotton[1,2], Pedro Henrique da Rosa[1,2], Tainá Schons[1], Giovana Mezzomo[1,2], Quênia de Carvalho[1,2], Flávio Kapczinski[1,4,5,6], Adriane R. Rosa[1,2,3]

[1] Laboratory of Molecular Psychiatry, Hospital Clinic of Porto Alegre, Porto Alegre, Brazil.

[2] Postgraduate Program in Biological Sciences: Pharmacology and Therapeutics - Institute of Basic Health Sciences, University Federal of Rio Grande do Sul, Porto Alegre, RS, Brazil.

[3] Department of Pharmacology, Institute of Basic Science Health, University Federal of Rio Grande do Sul, Porto Alegre, RS, Brazil.

[4] Department of Psychiatry, University Federal of Rio Grande do Sul, Porto Alegre, RS, Brazil.

[5] National Institute of Translational Science and Technology in Medicine, Porto Alegre, RS, Brazil.

[6] Department of Psychiatry and Behavioral Neurosciences, McMaster University, Hamilton, ON, Canada.

**Previous Presentations:** This manuscript was presented as a poster presentation at the XVI Congresso Gaúcho de Psiquiatria in 2023 and Escola Gaúcha de Bioinformatica in 2023.

**Corresponding author**: Adriane R. Rosa
E-mail: arrosa@hcpa.edu.br
Rua Ramiro Barcelos, 2350, Santa Cecília, Hospital de Clínicas de Porto Alegre, Porto Alegre, RS, Brazil - Postal Code: 90410-000

**Abstract**

**Objective:** The present study combined transcriptomic data and computational techniques based on gene expression signatures to identify novel bioactive compounds or FDA-approved drugs for the management of Bipolar Disorder (BD).

**Methods**: Five transcriptomic datasets, comprising a total of 165 blood samples from BD case-control, were selected from the Gene Expression Omnibus repository (GEO). The number of subjects varied from 6 to 60, with a mean age ranging from 35 to 48, with a gender variation between them. Most of the patients were on pharmacological treatment. Master Regulator Analysis (MRA) and Gene Set Enrichment Analysis (GSEA) were performed to identify statistically significant genes between BD and HC and their association with the mood states of BD. Additionally, existing molecules with the potential to reverse the transcriptomic profiles of disease-altered regulons in BD were identified using the LINCS and cMap databases.

**Results:** MRA identified 59 potential MRs candidates modulating the regulatory units enriched with genes altered in BD, while the GSEA identified 134 enriched genes, and a total of 982 regulons had their activation state determined. Both analyses showed genes exclusively associated with mania, depression, or euthymia, and some genes were common between the three mood states. We identified bioactive compounds and licensed drug candidates, including antihypertensives and antineoplastics, as promising candidates for treating BD. Nevertheless, experimental validation is essential to authenticate these findings in subsequent studies.

**Conclusion:** Although preliminary, our data provides some insights regarding the biological patterns of BD into distinct mood states and potential therapeutic targets. The combined transcriptomic and bioinformatics strategy offers a route to advance

drug discovery and personalized medicine by tapping into gene expression information.

**Keywords:** Computational biology, personalized medicine, drug repurposing, psychiatry pharmacology.

## 1. Introduction

Bipolar disorder (BD) is a chronic mental health condition that affects over 40 million individuals globally [1]. It is a highly disabling disease associated with elevated rates of premature mortality from suicide and medical comorbidities [2]. The complexity and heterogeneity of BD represent a challenge in understanding the fundamental causes and implementing consistent treatment strategies [3]. Patients often demonstrate insufficient improvement even after undergoing a series of medication protocols, showing the low effectiveness of usual treatments [4]. One possible approach to accelerate the discovery of pathological mechanisms and novel therapeutic targets is to utilize high-throughput-omics.

Data generated by omics experiments combined with a sophisticated computational analysis may provide a comprehensive understanding of the systemic alterations that occur in diseases, allowing the exploration of multifaceted molecular changes rather than being restricted to singular genes or proteins[5]. This methodology has streamlined identifying innovative molecular mechanisms and potential therapeutic targets. In Psychiatry, it would result in a paradigm shift in understanding its biological basis and allow the identification of more homogeneous

biological subtypes, specific biomarkers, and personalized treatments. This innovative approach aligns with the Research Domain Criteria (RDoC) goals.

In this context, transcriptomics, which comprehensively assesses the entirety of transcripts and their abundance within a given sample, assumes a pivotal role in elucidating the functional constituents of the genome. Transcriptomic data enables the identification of putative functional mechanisms through which genetic sequence variations and alterations in gene expression can precipitate a specific state or disease [6]. An additional advantage conferred by transcriptome analysis pertains to the capacity to dissect the intricate interplay involving transcription factors (TFs) and DNA. Thus, TFs modulate overall gene expression and play a crucial role in transcriptional regulatory networks [7].

The Connectivity Map (cMap), a database containing transcriptomic profiles of the entire genome of bioactive compounds, allows the establishment of relationships between diseases, physiological processes, and the mechanism of action of therapeutic agents [8]. Using cMaps is advantageous as it does not necessitate a detailed mechanism of action or prior knowledge of the molecules' targets to function, thus more effectively achieving the goal of predicting therapeutic compounds for a specific condition[5]. In contrast to classical pharmacology, the transcriptomic profiles provided by cMap use the systems biology approaches at the pathway and network levels rather than focusing on one target. Thus, combining high-throughput transcriptomics and network analysis, we aimed to identify potential targeted drug candidates for BD treatment based on the gene expression signature (GES). Through this approach, we seek to advance our comprehension of BD

mechanisms and facilitate the development of personalized therapeutic strategies for this complex disorder.

## 2. Material and methods

### 2.1 Data Acquisition and Differential Expression Analysis

Data sets were searched on the Gene Expression Omnibus (GEO) repository in July 2023 using the following search terms: ("bipolar disorder"[MeSH Terms] OR bipolar disorder[All Fields]) AND ("blood"[Subheading] OR "blood"[MeSH Terms] OR blood[All Fields]). In addition, “homo sapiens” “expression profiling by array” and “expression profiling by high throughput sequencing” filters were applied, resulting in 32 databases. The inclusion criteria consisted of a) case-control studies in which the mood state of BD was previously characterized, b) transcription factors assessed by microarray analysis, and c) studies conducted on human blood samples. We excluded studies that utilized brain tissue, cell culture, redundant databases, RNA-Seq analysis, and patients without disease state stratification (Supplementary Table 1). Hence, five microarray transcriptomics datasets representing different phases of BD (euthymia, mania, depression) were selected from the GEO repository: [GSE121963, GSE46416, GSE45484, GSE39653, GSE23848] (Figure 1) and downloaded using the GEO query. The dataset was then filtered and batch-corrected using the *virtualArrayComBat* function [9–11], retaining 165 samples. Differential expression analyses were performed using the *limma* package, applying multiple linear models and moderated t-statistics for identifying differentially expressed genes (DEGs) with empirical Bayes moderation [10]. Herein, altered genes with unadjusted p-

value < 0.05 and a fold change over 15% were considered DEGs (Supplementary Table 2).

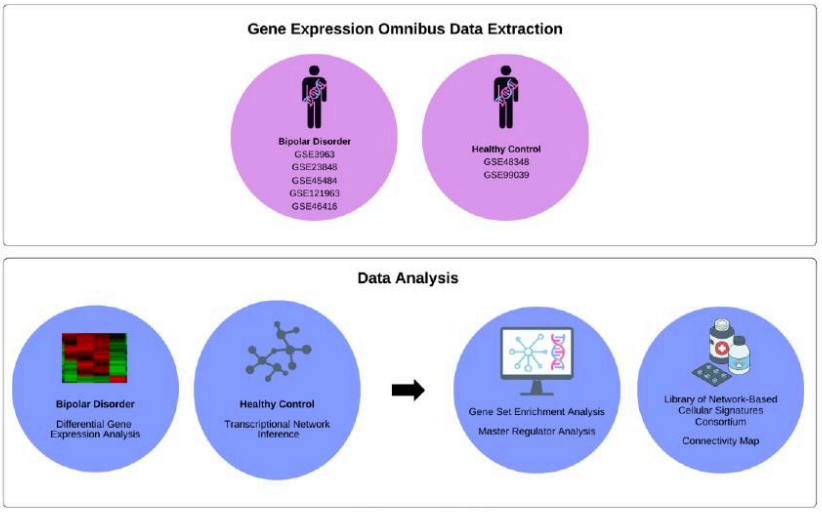


Figure 1: Methodology flowchart. BD and health control data were obtained from the Gene Expression Omnibus database. Healthy human blood gene expression data was used to reconstruct a normal blood regulatory network centered on transcription factors. Differential Expression Genes were computed from controls and each phase of BD patients' data. The convergence of Master Regulator Analysis, one-tailed and two-tailed Gene Set Enrichment Analysis results were used to provide the input to unveil bioactive compounds. To identify potential drug repurposing candidates in BD, we utilized the LINCS and cMap databases.

Healthy human blood samples were collected from two large microarray datasets [GSE48348 and GSE99039] obtained from the GEO repository. The datasets were combined and processed using the GEOquery and virtualArray packages, including a batch correction to address batch effects [9–11]. The final dataset contained 967 healthy blood samples used for transcriptional network inference (Figure 1).

2.2 Reverse Engineering of Transcriptional Network

A transcriptional reference network (TN) centered on TFs and their predicted target genes were inferred using a large cohort of healthy blood samples and merged as described in "Data Acquisition". Herein, "regulatory unit" or "regulon" describes the groups of inferred genes and their associated TFs. RTN (v2.16.1) package was used to reconstruct and analyze TNs based on the mutual information (MI) using the Algorithm for the Reconstruction of Accurate Cellular Networks (ARACNe) method [12]. In summary, the regulatory structure of the network is derived by mapping significant associations between known TFs and all potential targets. A curated list of genes was used to annotate TF eligibility for TN inference input[7]. To create a consensus bootstrap network, a permutation step eliminates the interactions below a minimum MI threshold, and unstable interactions are additionally removed by bootstrap. Finally, the data processing inequality algorithm is applied with null tolerance to eliminate interactions likely to be mediated by a third TF. The reference blood TN was built using the package's default number of 5000 permutations and 100 bootstraps (p-value < 0.001).

2.3 Master Regulator Analysis and Gene Set Enrichment Analysis

We conducted the master regulator analysis (MRA) described by Carro and colleagues [13]. For each regulatory unit in the blood TN, the algorithm computes the statistical overrepresentation (calculated by a modified Fisher's exact test) of genes obtained from differential expression analyses (unadjusted p-value < 0.05 and a fold change over 15%). Additionally, one-tailed Gene Set Enrichment Analysis (GSEA) was used to evaluate whether members of a gene set are associated with phenotypic class distinctions [14]. Finally, two-tailed GSEA was also performed using the RTN package (v2.16.1), with a p-value cutoff set to 0.05 and using 1000 permutations. Briefly, Pearson's correlation was used to split the regulatory units into two subgroups: positively associated targets (A) and negatively associated targets (B). Afterward, the GSEA [14] statistics tested the phenotype association of each subgroup, resulting in independent enrichment scores (ES) for each subset. An additional step was carried out to test the differential enrichment ($ES_A - ES_B$), considering that a maximum deviation from zero near opposite extremes and a good separation of the two distributions are desirable for a clear association. Thus, a high negative differential score implies that the regulon is repressed in the disorder phenotype. In contrast, a high positive differential score indicates that the regulon is induced in the disorder phenotype.

2.4 Drug Repurposing

We used the Library of Network-Based Cellular Signatures Consortium (LINCS) [15] and the Broad Institute Connectivity Map Drug Repurposing Database (cMap) [16] to identify existing molecules reversing the transcriptomic profiles of the disease-altered regulons found in each BD phase. In this study, we used the "qSig" (method = "LINCS") function from the "signatureSearch" R package [17] for performing gene GES searches and then interpreting the results functionally using specialized enrichment methods [17]. In drug discovery, these tools can identify novel mechanisms of action through which bioactive compounds.

**3. Results**

3.1 Sample Characterization

Table 1 shows the sample characteristics. The number of subjects (n) varies between 6 and 60. Four studies used whole blood, while one specifically employed peripheral blood mononuclear cells (PBMC) for analysis. Furthermore, two studies scrutinized manic versus euthymic states, whereas three studies concentrated on assessing the depressive state within the context of BD. The mean age of the subjects ranged from 35 to 48.3 years in the five studies. The gender distribution within the studies exhibited significant variations: one study predominantly comprised male subjects, three comprised female subjects, and a single study had an even distribution of 50% for both genders. Regarding pharmacological interventions, one

study did not specify the medication used. In contrast, the second study detailed patients receiving a combination of antipsychotics, mood stabilizers, anticonvulsants, antidepressants, and benzodiazepines. One study mentioned that the subjects in the depression-focused sets did not use any medication. Meanwhile, the other two studies indicated the utilization of antipsychotics, mood stabilizers, and anticonvulsants.

Table 1: Sample Characterization.

| GEO ID | PMID | Mood state | Age | Gender | n | Tissue | Medication |
|---|---|---|---|---|---|---|---|
| GSE121963 | 31118907 | Mania x Euthymia | 45,2 ± 9,9 | 50% male | 6 | Whole blood | No individual information |
| GSE46416 | 25136889 | Mania x Euthymia | 48,3 ± 12,0 | 100% male | 11 | Whole blood | Antipsychotics, Mood stabilizers, Anticonvulsants, antidepressants, Benzodiazepines |
| GSE45484 | 23670706 | Depression | 39,45 ± 12,2 | 31,6% male | 60 | Whole blood | Mood stabilizers |
| GSE39653 | 23064081 | Depression | 35 ± 10,0 | 32% male | 29 | PBMC | No medication |
| GSE23848 | 21176028 | Depression | 38.4 ± 9,5 | 30% male | 20 | Whole blood | Antipsychotic, Mood stabilizers, Anticonvulsants |

3.2 Master Regulator Analysis

The MRA is an approach that aims to identify the transcription factors acting as master regulators (MRs) responsible for changes in gene expression between different conditions. In our study, the MRA identified 59 potential MRs candidates modulating the regulatory units enriched with genes altered in BD. We observed specific MRs in patients during depressive and euthymic phases, but no exclusive MRs were identified during mania. Additionally, we found 12 MRs that were common between euthymia and depression and two MRs that were common between depression and mania (Supplementary Table 3).

3.3 Gene Set Enrichment Analysis (GSEA)

One-tailed GSEA evaluates whether a pre-defined set of genes, based on biological information, is enriched among a set of DEGs across various conditions. In our dataset, GSEA identified 134 enriched gene sets (regulatory units), among which two were exclusively associated with bipolar depression, three were specific to the euthymic phase, and four were implicated in mania. The intersection between BD phases led to the identification of 31 genes in Supplementary Table 4. A two-tailed GSEA was then performed to infer the activity (activation or repression) state of the MRs in each dataset. A total of 982 regulons had their activation state identified, with 167 in the manic phase, 437 in euthymia, and 378 in bipolar depression (Supplementary Table 5).

### 3.4 Drug Repurposing

To identify potential drug repurposing candidates in BD, we utilized the LINCS and cMap databases. These projects are related to pharmacology and employ systems biology approaches to study the large-scale interaction between drugs and cells [15]. In this study, we utilized the convergence of MRA, one-tailed GSEA, and two-tailed GSEA results to provide the input to unveil bioactive compounds and their mechanisms of action (MOAs). Thus, we obtained two regulons for mania, 16 related to the euthymic phase and 11 related to depression. The association of these regulons with their corresponding MOAs is depicted in Figure 2, while the comprehensive dataset containing information on drugs, MOAs, and regulons is provided in Supplementary Table 6. The initial list of compounds was filtered, retaining only the top 20 most prevalent drugs for each regulon in the different disease phases. The analyses were conducted for three phases of BD, suggesting a singular treatment for mania, euthymia, and depression and showing common drugs for the three phases (Figure 3).


Figure 2: Connectivity Map of regulons with their corresponding mechanisms of action in the different BD phases. A) Depression; B) Euthymia; C) Mania.

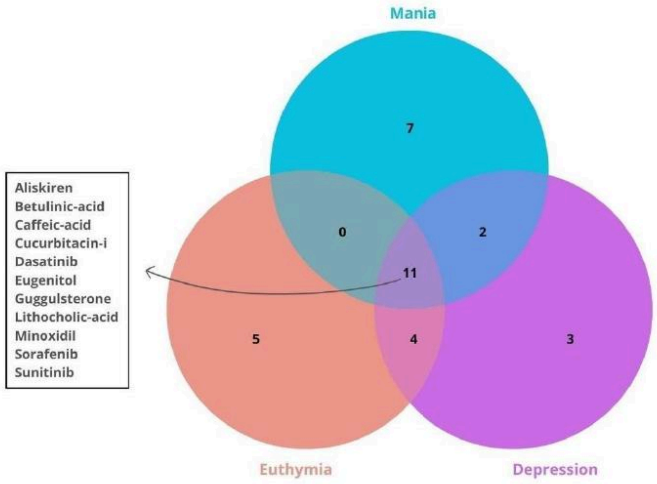


Figure 3: Venn Diagram representing the top 20 most prevalent drugs for each regulon in the different disease phases (depression, euthymia, and mania). Eleven compounds were common across all three phases of BD.



## 4. Discussion

When applied to Pharmacology, systems biology, and bioinformatics can assist the discovery of novel compounds or even new indications for FDA-approved drugs, a method known as drug repositioning. In this exploratory study, five microarray transcriptomics datasets representing different mood states of BD (euthymia, mania, depression) were selected from the GEO repository, and differential expression analyses were performed to identify DEGs and generate enriched gene sets. Then, we used the MRA to identify MRs responsible for changes in gene expression between subjects with BD and controls. Finally, using the LINCS and cMap databases, we explored the drug-gene interaction to identify the top drugs

or compounds whose mechanism of action was related to the gene expression signature of BD. Interestingly, some of these compounds were specific for mania, depression, or euthymia, while others were common to the three mood states of BD. They are FDA-approved drugs such as antineoplastic agents (dasatinib, sorafenib, and sunitinib) or antihypertensive agents (minoxidil and aliskiren) and bioactive compounds that are being tested in animal models such as guggulsterone, betulinic acid, caffeic acid, and others.

The repositioning of antihypertensive agents in psychiatry disorders is an underdeveloped area with promising results, as reported in an elegant systematic review [18]. In this regard, an observational study encompassing a cohort of 60,045 Finnish individuals revealed a significant correlation between using calcium channel blockers and dihydropyridines and decreased hospitalizations related to mood symptoms in BD patients [19]. Similarly, eight individuals with treatment-resistant BD experienced a substantial decrease in the frequency and severity of both manic and depressive episodes after undergoing diltiazem treatment - a calcium channel blocker agent compared to the period before diltiazem treatment, suggesting an effective adjunctive treatment in managing BD [20]. Calcium channel blockers agents play an interesting role as mood stabilizers, given their shared essential mechanism of action with lithium and carbamazepine, considered the primary treatment for BD[21,22]. Another class of antihypertensives with potential applicability in Psychiatry are the renin-angiotensin system antagonists [23]. For example, telmisartan as an adjunctive treatment for schizophrenia was tested in a randomized controlled trial with a notable reduction in the total score on the Positive and Negative Total Syndrome scale (PANSS) in the group of patients who received telmisartan

compared to placebo [24]. Furthermore, a significant association between angiotensin-converting enzyme inhibitors (ACE-I) and post-traumatic stress disorder (PTSD) was shown in an observational study involving more than 800 individuals. These results were also confirmed in subsequent analyses conducted using a large biorepository database (Partners Healthcare Biobank, N = 116,389) [25]. In accordance with previous studies, we also identified a renin-angiotensin system antagonist as a promising compound to be tested in BD.

Peculiarly, minoxidil was another repositioning drug identified in our findings. Although minoxidil is extensively used to treat alopecia, it was initially developed as a potent peripheral vasodilator agent for treating severe refractory hypertension. It exerts antihypertensive effects by opening adenosine triphosphate (ATP)-sensitive potassium channels. In this sense, a decreased expression of astrocytic ATP-sensitive potassium channel (Kir6.1/ATP) was found in the hippocampus of mice exposed to the chronic stress model, suggesting that the Kir6.1/K-ATP channel could hold promise as a potential target for the management of depression [26]. On the other hand, a randomized clinical trial to investigate the antidepressant effects of a potassium channel activator (e.g., Diazoxide) was interrupted due to severe adverse effects of Diazoxide [27]. Taken together, our results indicate that some of the targets involved in the mechanisms of action of certain antihypertensive agents appear promising candidates for Psychiatry, although this preliminary data needs further experimental validation studies. Our study also identified the potential applicability of ATP-competitive protein tyrosine kinase (PTKs) inhibitors (e.g., antineoplastic drugs) in psychopharmacology [28]. The Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway comprises a family of non-receptor PTKs that

modulate various cytokines, growth factors, and PTKs. Once activated, JAKs phosphorylate downstream targets; the primary effector in this category is the STAT family. Following phosphorylation by JAK, STATs dimerize and translocate to the nucleus, regulating the expression of several genes [29]. The JAK/STAT pathway has also stood out as a key mechanism in regulating synaptic plasticity, playing a role in neuronal and glial responses to injuries in the central nervous system (CNS). Specifically, the JAK2/STAT3 pathway is involved in long-term depression, which is a process through which synapses become less effective at transmitting signals over an extended period. JAK3 is also part of the mechanism of action of tricyclic antidepressants (such as amitriptyline), as demonstrated in a preclinical study of depression [30]. Additionally, the procognitive effects of ceramide are mediated by the JAK/STAT pathway. In summary, several lines of evidence support the role of JAK/STAT signaling in the pathogenesis of psychiatric disorders and psychopharmacology.

Furthermore, our study identified bioactive compounds with promising therapeutic properties for treating psychiatric disorders. Guggulsterone is a bioactive compound of Commiphora mukul that demonstrates antidepressant-like effects similar to fluoxetine in an animal model of chronic unpredictable stress-induced. These effects seem to be mediated by activating the brain-derived neurotrophic factor (BDNF) signaling pathway [31]. BDNF is a protein crucial in promoting brain growth, survival, and maintenance of neurons and is involved in various processes, such as neurogenesis, synaptic plasticity, and overall brain health. Previous studies have demonstrated alterations in plasma BDNF levels in BD patients [32] as well as the lithium‘s effects may be mediated by BDNF [33,] supporting guggulsterone as a

promising candidate for the treatment of BD. Betulinic acid is a triterpene tetracyclic found in several plants and exhibits a wide spectrum of pharmacological properties [34,35]. Betulinic acid has mainly demonstrated potent anticancer activities in multiple cancers by regulating JAK/STAT, VEGF, EGF/EGFR, TRAIL/TRAIL-R, AKT/mTOR, and ubiquitination pathways [34]. One important effect of this compound is its anti-inflammatory activity, which is most related to the inhibition of NF-kB and MAPK pathways [36]. Despite the lack of evidence in psychiatry, the effects of Betulinic acid were associated with cerebral blood flow and memory improvement in the vascular dementia model [37].

In addition to these compounds, caffeic acid seems to have some benefits for central nervous diseases. For instance, caffeic acid showed antidepressant-like effects in chronic stress models via epigenetic mechanisms. Mechanistically, it was observed that mRNA levels of hydroxymethylation [Ten-eleven translocation (TET)1-3] (associated with global DNA hydroxymethylation) was decreased in the stress group; an effect that was restored to normal levels following treatment with caffeic acid combined with paroxetine compared to control group [38]. Moreover, caffeic acid mitigates the decreased cortical BDNF mRNA expression induced by swim stress in wild-type mice [39]. Animal models of neurodegeneration, induced by amyloid beta, also demonstrated that caffeic acid reduced reactive oxygen species and lipid peroxidation and the expression of astrocytes-activated marker and microglia-activated marker; these effects were correlated with cognitive improvement as well [40].

To sum up, our preliminary data identified novel drug candidates using computational drug repurposing based on the transcriptome signature of individuals

with BD with subsequent analysis of drug-generated expression profiles. In the bioinformatics and Precision Medicine era, the use of innovative advancements, including system biology models and multi-omics approaches, are welcome to address important challenges involved in psychiatric disorders due to a) limited understanding of neurobiology, b) disease heterogeneity, c) lack of validated animal models. Although most of the drug candidates identified here are supported by the literature, our analysis was based on hypothesis generation, and experimental validation is required before clinical translation.

It is essential to acknowledge the limitations of our study. Firstly, there is restricted metadata availability within the dataset. This limitation diminishes our findings' overall efficacy compared to studies utilizing electronic health records. Our dataset lacks certain essential metadata, potentially constraining the depth and precision of our analyses. This fact can potentially impact the generalizability and robustness of our findings. Secondly, data was exclusively obtained from blood transcriptomics, which may limit sample representativeness. Also, we cannot rule out the influence of variables such as age, sex, race, and medications on findings. Thirdly, gene expression data came solely from blood samples, potentially not fully capturing the complex molecular changes associated with BD throughout the brain. Fourth, while our methodology provides a robust approach to gene expression analysis, it depends on computational models that are influenced by data quality and underlying assumptions. The selection of parameters can affect how the network is constructed, potentially resulting in different interpretations. Fifth, drug discovery is a multifaceted process beyond gene expression, involving critical aspects like pharmacokinetics and toxicology, which are also essential for developing new drugs.

Finally, this is an exploratory study that provides preliminary data regarding the mechanisms involved in the etiology of bipolar disorder. We aimed to give insights related to the molecular signature associated with BD rather than confirming or testing specific hypotheses. Therefore, our findings need to be validated through molecular biology methods in further studies.

In summary, our study underscored the promise of employing advanced gene expression analysis methods to identify new medications or repurpose existing ones to treat psychiatric disorders. This strategy offers a route to advance drug discovery and personalized medicine by tapping into genetic information. While our exploratory study furnishes preliminary data on the molecular signature of BD and intriguing compounds for its treatment, experimental validation of such findings in further studies is essential before clinical translation.

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**Authors' contributions**

ARR, MAB and PRZ conceived the presented idea. PRZ and MAB performed the analytical methods. MAB performed the statistical analysis. PRZ, ES, PHR, TS, GM, and QC wrote the manuscript. ARR and FK supervised the findings of this work. All authors discussed the results and contributed to the final manuscript.

**Availability of data and materials**

All data generated or analyzed during this study are included in this published article and supplementary files.

**Consent for publication**

All authors gave consent for publication in this Journal.

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## 6. ARTIGO CIENTÍFICO 3

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# Potential Candidates for Biomarkers in Bipolar Disorder: A Proteomic Approach through Systems Biology

**Paola Rampelotto Ziani[1,2], Jacson Gabriel Feiten[1,3], Jéferson Ferraz Goularte[1,3], Rafael Colombo[1,4], Bárbara Antqueviezc[1], Luiza Paul Géa[5], Adriane Ribeiro Rosa[1,2,3]**

[1]Laboratory of Molecular Psychiatry, Hospital Clinic of Porto Alegre, [2]Postgraduate Program in Biological Sciences: Pharmacology and Therapeutics - Institute of Basic Health Sciences, Federal University of Rio Grande do Sul, [3]Postgraduate Program in Psychiatry and Behavioral Sciences, Faculty of Medicine, Federal University of Rio Grande do Sul, Porto Alegre, [4]University of Caxias do Sul, Caxias do Sul, Brasil, [5]Department of Psychiatry and Behavioural Neurosciences, McMaster University, Hamilton, ON, Canada

Bipolar disorder (BD) is one of the most disabling diseases characterized by severe humor fluctuation. It is accompanied by cognitive and functional impairment in addiction to high suicide rates. BD is often underdiagnosed and treated incorrectly because many of the reported symptoms are not exclusive to the disorder. Once the diagnosis is exclusively clinical, it is not possible to state precisely. From that, proteomic approaches were used to identify, in a large scale, all proteins involved in cellular or tissue processes. This review aggregate data from blood proteomes, by using protein association network, of subjects with BD and healthy controls to suggest dysfunctional molecular pathways involved in disease. Original articles containing proteomic analysis were searched in PubMed. Seven studies were selected and data were extracted for posterior analysis. A protein-protein interaction network was created by STRING database. A final set of proteins in this network were employed as input in ClueGO and, the main biological process was visualized using R package pathview. The analysis revealed proteins associated with many biological processes, including growth and endocrine regulation, iron transportation, protease inhibition, protection against pathogens and cholesterol transport. Moreover, pathway analysis indicated the association of uncovered proteins with two main metabolic pathways: complement system and coagulation cascade. Thus, a better understanding on the pathophysiology of psychiatric disorders and the identification of potential biomarker candidates are essential to improve diagnostic, prognostic and design pharmacological strategies.

**KEY WORDS:** Blood; Biomarkers; Bipolar disorder; Proteomics; Systems biology.

## INTRODUCTION

Bipolar disorder (BD) is a chronic psychiatric illness characterized by recurrent and alternating episodes of mood that are often separated by periods of remission, also called "euthymia" (the Diagnostic and Statistical Manual of Mental Disorders 5th edition, DSM-V) [1]. BD affects almost 3% of the North American population and is associated with long-term cognitive and psychosocial impairment [2,3]. It is also associated with high rates of mortality by both natural causes and suicide [4].

BD is often underdiagnosed and untreated because many of the reported symptoms, including irritability, sleep disturbance, impulsive behavior, alcohol and substance abuse, are not exclusive to the disorder. Indeed, the mood fluctuations as well as the chronic and heterogeneous course of BD make it one of the most challenging illnesses to diagnose and treat [5,6]. The diagnosis of mood disorders is made by assessing symptoms through clinical interviews and based on the criteria established in the DSM-V or the International Statistical Classification of Diseases and Health-Related Problems version 10 (10th revision, ICD-10) [7]. However, the establishment of well-defined boundaries between distinct diagnostic catego-

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**Address for correspondence:** Adriane Ribeiro Rosa
Department of Pharmacology - Federal University of Rio Grande do Sul, Laboratory of Molecular Psychiatry - Hospital Clinic of Porto Alegre, Ramiro Barcelos 2350, Santa Cecília, Porto Alegre - RS, 90035-903, Brazil
E-mail: adrianerrosa@gmail.com
ORCID: https://orcid.org/0000-0001-8629-4625

ries can be challenging, especially among psychiatric disorder that share some biological aspects or have overlapping of symptoms such as BD and major depressive disorder (MDD) [8-11]. According to the literature, about 40% of BD patients are initially misdiagnosed as MDD, as most cases present with depressive episode at onset and seek medical assistance when depressed and not hypomanic. Consequently, BD patients might receive inadequate treatment which can aggravate the course of the illness and worsen the outcome. Therefore, the development of valid biomarkers for BD is critical to improve the diagnostic accuracy as well as to prognosis and treatment response in psychiatry [12].

The advances in omics approaches have created novel opportunities for identifying molecular signatures and/or biomarkers in various medical specialties, including psychiatry. For instance, proteomics is the science that allows the identification, in a large scale, of all proteins involved in cellular or tissue processes (i.e., proteomes) and seems to be essential for understanding the biological processes underlying health and disease [13,14]. As proteomics represents the translated and transcribed genetic information after epigenetic changes, it has been suggested that this analysis more reliably reflects the pathophysiology of a disease and the current state of the patient than genomic and transcriptomic analysis [15].

Therefore, a number of researchers throughout the world have preferred proteomics-based technologies in order to identify potential biomarker candidates for disease diagnosis, prognosis, and treatment response prediction. Within this rationale, some studies were also performed in BD [10,13,16-20]. The current review aimed to compare the plasma and serum proteomes of subjects with BD and health individuals using protein-protein interaction, in order to identify and propose associated dysfunctional biological pathways involved in BD.

## METHODS

### Studies Eligibility Criteria

For this review, we selected original articles reporting protein identification in the blood of individuals diagnosed with BD according to the following inclusion criteria:

- Studies including subjects with BD type I or II as confirmed by ICD-10, DSM-IV or DSM-V criteria;
- Comparative studies evaluating levels of protein in the peripheral blood (plasma or serum) of BD patients and healthy controls;
- Studies assessing protein levels in treated or drug-free patients with BD;
- Studies assessing protein levels in BD patients during euthymia or mood episodes;
- Studies using liquid chromatography and two-dimensional electrophoresis methods to separate proteins in peripheral blood of BD patients that differentiate or not the mood states and compared to healthy subjects;
- Studies that performed mass spectrometry or immunoassay multiplex analysis of proteins to identify the expression of proteins.

### Search Strategy and Study Selection

Publications were searched on PubMed in July, 2020 using the following search strategy: ("Proteomic" OR "Proteomic biomarker" OR "proteome") AND ("Serum" OR "plasma" OR "Blood") AND ("Bipolar disorder" OR "bipolar" OR "psychiatric illness"). The search yielded a total of 35 original studies on proteomic analysis in patients with BD. Four authors (PRZ, JGF, JFG, and ARR) revised the abstracts and methodologies to identify studies that match the inclusion criteria. The studies reporting major depressive disorder, schizophrenia, psychotic episodes and dementia were excluded (n = 31) as shown in Figure 1. Three relevant studies found in the reference list of selected articles were also included. Finally, seven articles were included, and the extracted data included information on proteins accession numbers (Uniprot Consortium)

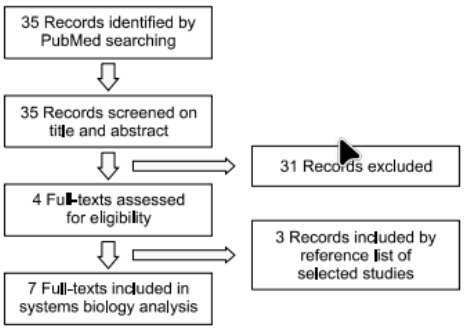


**Fig. 1.** Flowchart of eligibility criteria and research information.

and fold change (when available), BD diagnosis, and treatment (if applicable).

### Proteins Selection and Pathway Analysis

The protein-protein interaction data was downloaded from STRING database [21]. The data was imported to R 3.6 and the interactions with combined score equal to or less than 0.7 were removed. This procedure keeps only interaction with high confidence. Based on the list of differentially expressed proteins, a network was generated. Then, proteins that were found to be differentially expressed in three or more studies were selected in the

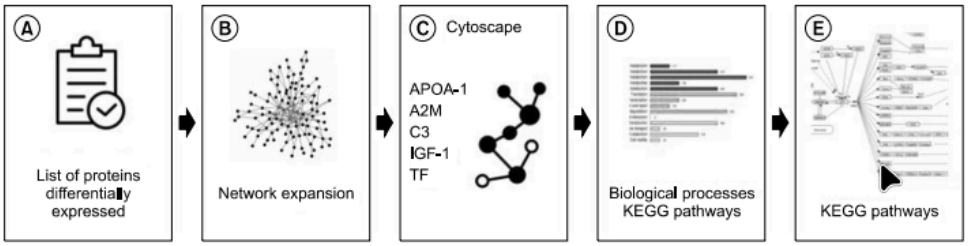


**Fig. 2.** Systems biology protocol. (A) A list of proteins differentially expressed was organized. (B) The list was imported to R 3.6.1 and the networks were created. (C) Based on the networks generated, the neighbors of the proteins differentially expressed found by three authors or more were selected. (D) This set of proteins was used as input to ClueGO. (E) The main biological process was visualized using the pathview R package as well as differentially expressed proteins.
APOA-1, apolipoprotein A-1; A2M, alpha-2-macroglobulin; C3, third component of complement; IGF-1, insulin growth factor-1; TF, transferrin; KEGG, Kyoto Encyclopedia of Genes and Genomes.

**Table 1.** Proteomic studies identifying BD peripheral biomarkers

| Author | Blood fraction | Subjects | Sample size | Proteomic technique | Results |
|---|---|---|---|---|---|
| Herberth *et al.* [19] (2011) | Serum | BD | 32 | LC-MS | 22 differentially expressed analytes compared HC |
| | | HC | 32 | | |
| | | BD | 16 | Multiplex | |
| | | HC | 15 | | |
| Alsaif *et al.* [16] (2012) | Serum | BD | 24 | Multiplex | 6 (serum) and 10 (plasma) differentially expressed proteins in BD compared to HC |
| | | HC | 21 | | |
| | Plasma | BD | 24 | | |
| | | HC | 21 | | 2 changed proteins in both fluids |
| Haenisch *et al.* [18] (2014) | Plasma | BD | 17 | Multiplex | 26 differentially expressed analytes compared HC |
| | | HC | 46 | | |
| Chen *et al.* [10] (2015) | Plasma | BD | 20 | 2-DE/MS | 3 differentially expressed proteins in BD compared to HC |
| | | HC | 30 | Multiplex | |
| Song *et al.* [13] (2015) | Plasma | Euthymic | 10 | 2-DE/MS | 32 differentially expressed proteins in BD compared to HC; |
| | | HC | 20 | | |
| | | Depressed HC | 20 | | 16 differentially expressed proteins in BD independently of mood; |
| | | Manic | 20 | | |
| | | HC | 15 | | 16 proteins associated with particular mood. |
| | | | 20 | | |
| de Jesus *et al.* [17] (2017) | Serum | BD | 14 | LC-MS/MS | 6 differentially expressed proteins in BD compared to HC |
| | | HCFN | 9 | | |
| | | BD | 14 | | |
| | | HCF | 3 | | |
| Ren *et al.* [20] (2017) | Plasma | BD | 30 | LC-MS/MS | 54 differentially expressed proteins in BD compared to HC |
| | | HC | 30 | | |

BD, bipolar disorder; HC, healthy control; HCFN, non familiar healthy control; HCF, familiar healthy control; LC, liquid chromatography; MS, mass spectrometry; 2-DE, two-dimensional electrophoresis.

**Table 2.** Summary of diagnostic biomarkers in BD

| Author | Groups | Up-regulated | Down-regulated |
|---|---|---|---|
| Herberth *et al.* [19] (2011) | BD x HC | C-C motif chemokine 16; tumor necrosis factor receptor superfamily member 5; CD40 ligand; connective tissue growth factor; endothelin-1; pro-epidermal growth factor; tumor necrosis factor ligand superfamily member 6; macrophage migration inhibitory factor; lymphotactin; luteinizing hormone; progesterone; testosterone; glutathione S-transferase A1; insulin-like growth factor-binding protein 2 | Apolipoprotein A-I; C-C motif chemokine 26; immunoglobulin A; immunoglobulin M; interleukin-13; kit ligand; tumor necrosis factor; apolipoprotein- C-III |
| Alsaif *et al.* [16] (2012) | BD x HC (plasma) | Lipoprotein-A; adrenocorticotropic hormone | Alpha-2-macroglobulin; macrophage migration inhibitory factor; macrophage inflammatory protein-3a; sex hormone-binding globulin; tenascin-C; apolipoprotein A; insulin-like growth factor I; monocyte chemotactic protein-4; platelet-derived growth factor subunit B; stem cell factor; superoxide dismutase; transferrin |
| Alsaif *et al.* [16] (2012) | BD x HC (serum) | Lipoprotein-A; macrophage migration inhibitory factor; insulin-like growth factor I; stem cell factor; superoxide dismutase | Alpha-2-macroglobulin; macrophage inflammatory protein-3a; sex hormone-binding globulin; tenascin-C; apolipoprotein A; monocyte chemotactic protein-4; platelet-derived growth factor subunit B; transferrin |
| Haenisch *et al.* [18] (2014) | BD x HC | S100 calcium binding protein B; interferon gamma induced protein 10; clusterin; complement C3; granulocyte colony stimulating factor; osteopontin; prostatic acid phosphatase; TIMP-1; C-peptide; hepatocyte growth factor; insulin; insulin like growth factor I; intact proinsulin; total proinsulin; vascular endothelial growth factor; peptide YY; chromogranin A; alpha 1 microglobulin; beta 2 microglobulin; matrix metalloproteinase 7; vitamin K dependent protein S; cystatin-C; apolipoprotein H | Apolipoprotein AI; myoglobin; sex hormone binding globulin |
| Chen *et al.* [10] (2015) | BD x HC | - | Complement component 3 isoform CRA_a; C4b-binding protein alpha chain; complement factor 1 |
| Song *et al.* [13] (2015) | BD x HC | Haptoglobin; apolipoprotein L1; afamin; pigment epithelium-derived factor; complement C4-B; vitamin D-binding protein; complement C4A3; carboxypeptidase B2; serotransferrin; fibrinogen beta chain; fibrinogen gamma chain; complement C3; hemopexin; keratin; complement subcomponent subunit C; complement factor I heavy chain; mannose-binding protein C; complement C4 gamma chain | Apolipoprotein A-I; carboxypeptidase N catalytic chain; N-acetylmuramoyl-L-alanine amidase; inter-alpha-trypsin inhibitor heavy chain H1; serum amyloid P-component; inter-alpha-trypsin inhibitor heavy chain H4; CD5 antigen-like; C4b-binding protein alpha chain; carbonic anhydrase 1; alpha-2-macroglobulin; complement factor H-related protein 1; complement C1r subcomponent; fibrinogen alpha chain |
| de Jesus *et al.* [17] (2017) | BD x HC | Albumin; apolipoprotein A-I | Complement C4-A; alpha-1-antitrypsin; apolipoprotein E; transferrin |
| Ren *et al.* [20] (2017) | BD x HC | Immunoblobulin light chain; full-length cDNA clone; immunoglobulin J chain; C4b-binding protein beta chain; hemoglobin beta subunit variant; myosin-reactive immunoglobulin heavy chain variable region; catalase; mutant hemoglobin alpha 2 globin chain; peroxiredoxin-2; carbonic anhydrase 1; superoxide dismutase; cDNA FLJ57106; haptoglobin; hemoglobin beta; alpha-2-macroglobulin; flavin reductase; Ig heavy chain variable region; beta globin; Ig kappa chain V-IV region Len; cDNA FLJ35079; insulin growth factor 1; IGL@; coagulation factor V; hemoglobin beta chain; protein S100; peroxiredoxin-1; alpha-1-acid glycoprotein 1; apolipoprotein A-I; anti-(ED-B) scFV; alpha-hemoglobin-stabilizing protein; delta-aminolevulinic acid dehydratase; immunglobulin heavy chain variable region; ATP-binding cassette sub-family B member 9; epididymis secretory protein; selenium-binding protein 1 | cDNA FLJ60397; brain acid soluble protein 1; Rab GDP dissociation inhibitor alpha; secreted phosphoprotein 24; amyloid beta A4 protein; endoglin; cDNA, FLJ95014; cathepsin S; thyroid peroxidase; ATP synthase subunit alpha; eukaryotic translation elongation factor 1 alpha; suprabasin; protein crumbs homolog 1; platelet endothelial cell adhesion molecule; sulfhydryl oxidase; trans-Golgi network integral membrane protein 2; multimerin-1; platelet basic protein; keratin-associated protein |

BD, bipolar disorder; HC, healthy control.

network. From this set, neighboring proteins of up to two degrees were selected, since proteins that are close tend to take part in similar biological processes. Each identified protein was converted and mapped onto its corresponding gene object. The final set of proteins was employed as input in ClueGO v2.5.7 (a Cytoscape v3.8 plug-in) [22,23] with the following parameters: two-sided hypergeometric (statistical test for the enrichment), Bonferroni step down correction, and kappa score of 0.4. In order to visualize the main biological process, the R package pathview version 1.24 [24] was used. Systems biology protocol was illustrated in Figure 2.

## RESULTS

The characteristics of all included studies are shown in Table 1. The set of proteins found to be differentially expressed between BD and healthy subjects in each study is listed in Table 2.

### Study-protein Network

We generated two types of networks: study-protein (Figs. 3 and 4) and protein-protein (Fig. 5) interaction networks. Study-protein networks have two types of nodes, one that represents each study and another that represents the proteins identified as differentially expressed in the studies. In order to maintain the accuracy of the results obtained, our analysis was made through the presentation of each author and the type of sample (serum or plasma). Furthermore, it has a comparison made by mood state (depression, euthymia, or mania) which was described in one study [13]. Usually, most sets of differentially expressed proteins found by each study are exclusive, unique, and specific. It is possible to observe that protein expression can differ within and between studies. For instance, Alsaif *et al.* [16] found that insulin growth factor-1 (IGF-1), superoxide dismutase 1, KIT ligand, and macrophage migration inhibitory factor displayed an opposite expression profile in plasma (underexpressed) compared to the serum (overexpressed).

Overall, we observed heterogeneity among studies re-

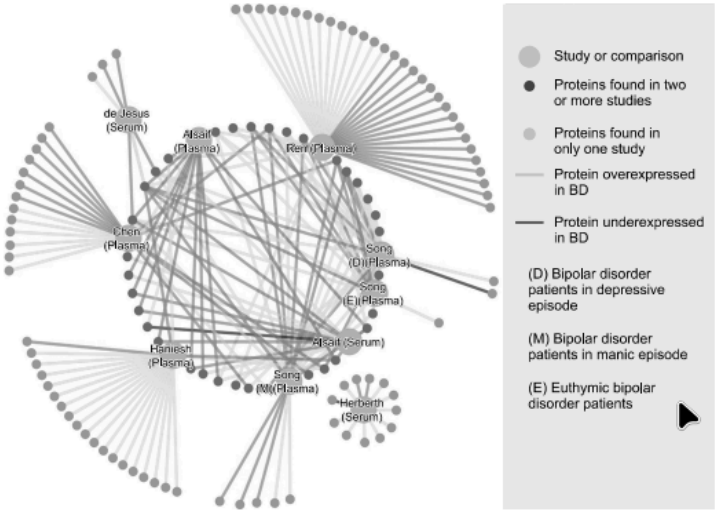


**Fig. 3.** Study-protein interaction network. The seven studies selected presented individual and shared proteins. Alsaif *et al.* [16] found a shared set of proteins between plasma and serum. The proteomics profile found by Song *et al.* [13] was very similar between different disease states and shared some proteins with the profile found by Ren *et al.* [20] and Chen *et al.* [10]. Alsaif presented results based on sample type (serum and plasma), meanwhile, Song stratifies studies based on different disease stages (depression, euthymia, and mania). The rest of the authors did not differentiate the disease stage. All studies, except Alsaif, found uniquely expressed proteins. One study (Herberth *et al.* [19]) showed 12 uniquely expressed proteins, therefore there were no proteins in common with other studies and it was not bound to the main network.
BD, bipolar disorder.

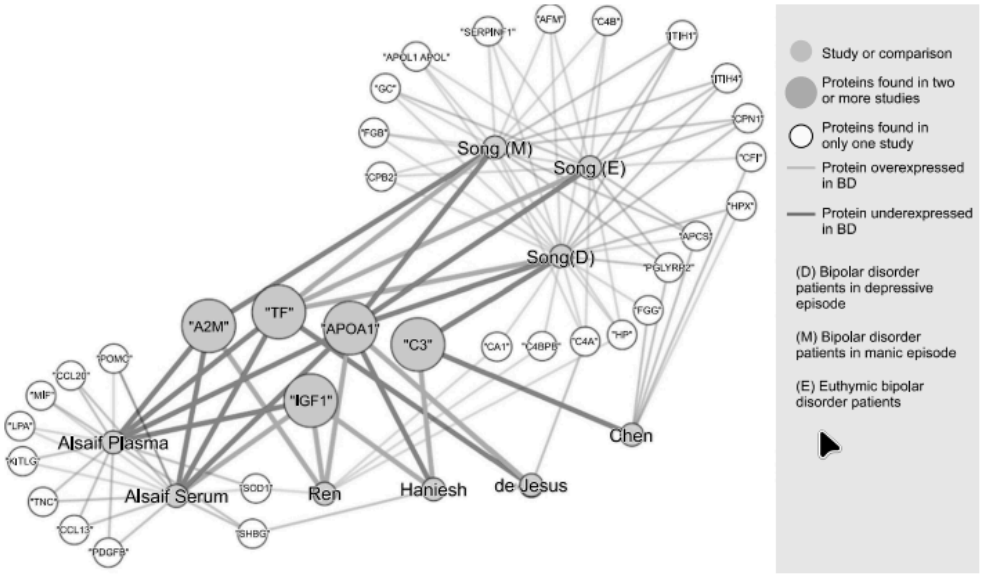


**Fig. 4.** Study-protein network analysis of differentially expressed proteins. Five proteins that presented significant change in expression between bipolar disorder and healthy control samples were included in the analysis. These network proteins are involved in growth regulation, endocrine system, iron transportation, protease inhibition, defense against pathogens and cholesterol transport.
Song, Song *et al.* [13]; Chen, Chen *et al.* [10]; de Jesus, de Jesus *et al.* [17]; Haenisch, Haenisch *et al.* [18]; Alsaif, Alsaif *et al.* [16]; Ren, Ren *et al.* [20]. APOA-1, apolipoprotein A-1; A2M, alpha-2-macroglobulin; C3, third component of complement; IGF-1, insulin growth factor-1; BD, bipolar disorder.

garding the number of proteins differentially expressed between individuals with BD and controls, which may be as a result of sample characteristic and methodological differences between studies. For instance, patients were on pharmacological treatment in five studies, while only one study included drug-free BD patients [19]. Also, most studies included chronic BD subjects, except for one that recruited patients in the first mood episode [10]. All experiments used plasma or serum as blood fraction. Furthermore, there were methodological differences regarding of protein depletion and quantification techniques, sample size, mass spectrometry database, and data and statistical analysis used in each study.

We identified 123 differentially expressed proteins from the seven articles included [10,13,16-20]. A total of 112 (91.1%) proteins were found differentially expressed by at least one study, 6 (4.9%) by two studies, and 3 (2.4%) by three studies. In particular, transferrin (TF) and apolipoprotein A-1 (APOA-1) were identified in four and five different studies, respectively (Fig. 4). Therefore, to identify potential biomarkers in BD, we decided to focus on the proteins identified in three or more studies. As a result, our analysis revealed the following five proteins as relevant in BD: TF, APOA1, alpha-2-macroglobulin (A2M), complement C3 (C3), and IGF-1 (Fig. 4). Based on the interactions from STRING database, we were able to found proteins directly or indirectly connected with them (PPBP, CXCL8, INS, CXCL10, PC, B2M, POMC, DCTN1, MMP10, CD1E, PAI1, PAI2, PDGFD, GNG5, RANBP2). This set of proteins was used as input in ClueGO, which showed that the proteins that interact closely are associated with the coagulation cascade.

### Main KEGG Pathway-related Selected Proteins and Neighbors

The most significant Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway was the complement and coagulation cascade (p-adj. Bonferroni = $1.0 \times 10^{-13}$). This

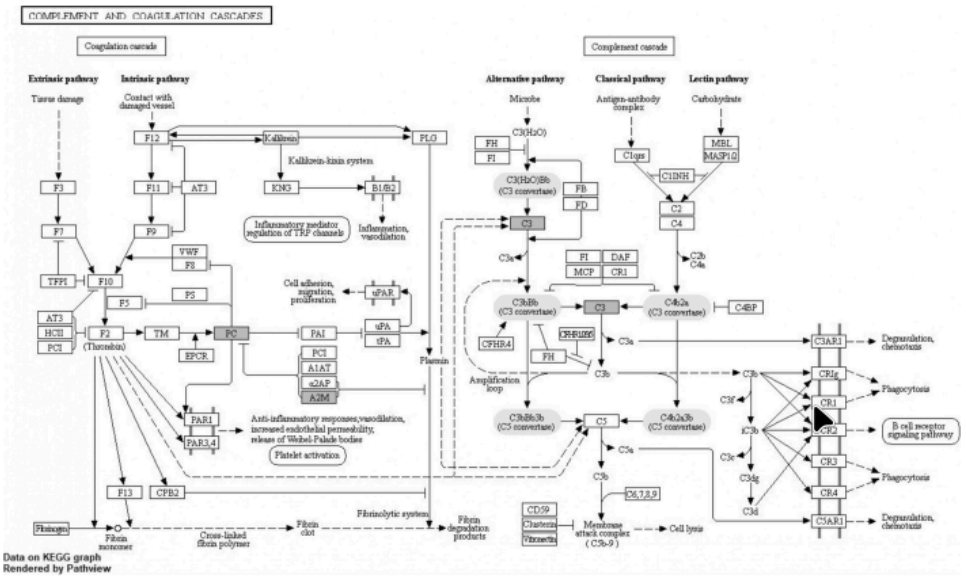


**Fig. 5.** Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis. Complement and coagulation cascade (p-adj. Bonferroni = $1.0 \times 10^{-13}$) were the two main signaling pathways involved in bipolar disorder biological processes. Grey squares represent differentially expressed proteins found by three or more authors. A2M forms a complex with PCI, alpha a1AT, alpha 2AP. This complex of proteins inhibits PC. Furthermore, alpha2AP and A2M complex inhibits plasmin activity. C3 is part of the alternative pathway and integrates this pathway with classical and lectin pathways. This figure can be visualized in more details here (https://www.kegg.jp/kegg-bin/highlight_pathway?scale=1.0&map=map04610&keyword=coagulation).

A2M, alpha-2-macroglobulin; PCI, protein C inhibitor; α1AT, alpha-1-antitrypsin; α2AP, alpha-2-antiplasmin; C3, third component of complement.

pathway has plasmin as the common molecule. A2M forms a complex with protein C inhibitor, alpha-1-antitrypsin and alpha-2-antiplasmin (α2AP). This complex of proteins inhibits PC. Furthermore, α2AP and A2M complex inhibits plasmin activity. C3 is part of the alternative pathway and integrates this pathway with classical and lectin pathways. Haenisch *et al.* [18] found PC (Protein C, Inactivator Of Coagulation Factors Va And VIIIa) was overexpressed in blood of BD patients.

## DISCUSSION

In the present study, we gathered data on differentially expressed proteins in the plasma and serum proteomes of subjects with BD compared to healthy controls. Hence, we sought to identify potential biomarkers for discriminating between patients and controls and dysfunctional molecular pathways underlying the pathophysiology of BD. Here, we identified five proteins: IGF-1, TF, A2M, C3, and APOA1. Overall, these proteins are involved in common biological process such as growth regulation, endocrine system, free iron transportation, protease inhibition, defense against pathogens and cholesterol transport. Specifically, to understand the high-level functions and utilities of the uncovered proteins, KEGG pathway analysis revealed that proteins were associated with two main metabolic pathways, the complement system and coagulation cascade. The relevance of each protein and potential interaction mechanisms in the context of BD are described below.

### C3

C3 is a protein of the human complement system involved in host defense against pathogens in the bloodstream. It acts stimulating both the innate and adaptive immune systems, eliminating apoptotic cells and cell de-

bris [25]. C3 is mainly produced in the liver [26], however, it is also expressed in adipose tissue of obese man [27] and in the brain of humans and mice [28]. The complement activation triggered by different stimuli converges to C3 activation and cleavage by proteases into effectors molecules such as C3b (mainly involved in the pathogen opsonization and further elimination), C3a and C5a (which are potent anaphylatoxins that promote inflammation), and C5b-9-membrane attack complex (MAC) (mainly involved in the lysis of target pathogens) [29]. Some studies have shown C3 alterations in serum from bipolar patients. For instance, C3 levels were lower in BD patients, independent of mood state, compared to healthy controls [30]. Similar results were found in patients with chronic BD treated with lithium [31]. However, Reginia *et al.* [32] demonstrated an increase in C3a, C5a, C5b-9 concentrations in blood of euthymic bipolar disorder patients who were not treated with lithium in the past 5 years. Our review showed that C3 was differentially expressed in three studies while underexpressed levels were found in two of them. Despite this evidence, the role of the complement system in the etiology of BD is still unclear. One possible explanation is that the complement system is involved in neuroinflammation process that it is also part of the etiology of BD [32]. The main suggested mechanism by which the peripheral immune system interacts with the central nervous system (CNS) is the increased permeability of the blood-brain barrier (BBB), which has been shown to be disrupted in patients with BD. Consequently, the complement components may penetrate the CNS [32]. Specific receptors of complement are present in neurons (such as C5aR C3aR) and in oligodendrocytes (C5b-9) and may trigger antiapoptotic signaling. Additionally, the over activation of the complement system, including C3, and microglia is involved in early synaptic pruning in the hippocampus of humans/mice [33], which may promote the secretion of proinflammatory cytokines by glial cells and induce neuronal damage and death. This mechanism explains, in part, the neurodegenerative process found in CNS diseases such as Alzheimer disease [34].

## APOA-1

The APOA-1 is a protein involved in the high-density lipoprotein (HDL) maturation, cholesterol efflux from artery wall cells, and reverse transport of cholesterol [35,36]. APOA-1 is mainly synthesized by hepatocytes and duodenojejunal mucosa cells [37,38], and released as lipid-free or lipid-poor APOA-1 and small HDL [38]. In individuals with schizophrenia (SZ), APOA-1 protein concentration seems to be reduced in the cerebrospinal fluid (CSF), red blood cells, post-mortem liver, dorsolateral prefrontal cortex, and serum [39]. Lower level of APOA-1 was also found in BD I patients compared to healthy controls [40], and a negative correlation between APOA-1 levels and patients treated with lithium has been demonstrated [41]. On the other hand, APOA-1 levels may not be altered in BD patients before the onset of symptoms [42], suggesting that changes occur later in the course of the disorder. In our review, APOA-1 was underexpressed in three studies while two others showed that this protein overexpressed. According to literature, APOA-1 is involved in HDL-accepting cholesterol process from macrophages, resulting in a smaller amount of cholesterol to be oxidized and consequent lower local inflammation [43]. Thus, there may be an interesting relationship between peripheral and central APOA-1 levels that could be mediated by inflammatory mechanisms. Furthermore, APOA-1 is able to act as an anti-inflammatory molecule, in part by limiting the macrophage cholesterol efflux or preventing macrophage chemotaxis towards chemokines coagulation cascade (CC) [44]. In addition to *in vitro* studies, there is evidence that BD is associated with inflammatory processes, suggesting the influence of blood protein alteration on the brain. A recent study showed an upregulation of pro-inflammatory cytokines decreased chemokines secretion in macrophages exposed to serum of patients during manic and depressive episodes compared to those in euthyma [45]. Moreover, dysfunction in macrophage activity occurs in the late stage of BD, due to low cytokine secretion by macrophages in response to inflammatory environment [46]. Many signaling proinflammatory molecules are upregulated during acute episodes of BD supporting the concept of a chronic low-grade inflammatory state in BD [47,48]. An interesting meta-analysis suggests that BD is accompanied by dysregulation of the immune response by demonstrating elevated levels of interleukin, its receptors, and tumor necrosis factor-alpha (IL-2R, sIL-6R, TNF-α, sTNFR1, IL-4) in patients compared with healthy controls [49].

**TF**

TF is an iron transporter glycoprotein that plays important roles in human physiology. Iron, in turn, has relevant functions in biological systems such as DNA metabolism, oxygen transport, and energy production [50]. However, free iron can be toxic inducing oxidative damage, thus TF acts on the safe transportation through the body [51]. Iron can be carried from blood to the brain through TF receptors located in the endothelial cells of the BBB, internalize the protein-iron complex releasing ferrous iron to the CNS [51,52]. Synthesized predominantly by hepatocytes, TF is expressed in several tissues including the brain [52]. A study using separation methods showed that TF in the CSF can be derived from blood [52]. There is evidence that altered TF is associated with pathologies including MDD [53] and schizophrenia [54]. A study involving antidepressant drug-naive patients with MDD showed a decrease in serotransferrin levels suggesting a relationship between the initial state of disease and immune response [53]. Tsai *et al.* [55] also found increased TF receptors in BD patients during acute mania and in subsequent remission. We also showed an overexpression of TF levels in three disease stages. Song *et al.* [13] author, in contrast to two authors, that presented TF down-regulated levels. Interestingly, another study found lower coagulation measures for fibrinogen and plasminogen activator inhibitor, and higher levels of plasmin-α2-antiplasmin complex in anxiety or depressed patients on serotonergic antidepressant treatment than in patients without these agents [56]. These findings indicate an activation of coagulation factors in the direction of a hypercoagulable state in patients with psychiatric disorders. This hypercoagulable state may explain, in part, the higher risk for cardiovascular diseases associated with anxiety and mood disorders. Depressed patients have been demonstrated increased baseline platelet activation, suggesting a mechanism by which depression is a key risk factor in vascular disease [57]. A recent study showed the ability of TF to potentiate thrombin and FXIIa activity, two important coagulation enzymes. Elevated levels of TF found in atherosclerotic plasma are related to the maintenance of coagulation balance [58]. Moreover, there is evidence showing a relationship between TF alterations and cardiovascular diseases [59,60]. It is possible to find a variety of studies relating to central nervous system diseases and coagulation cascade [61-63].

**IGF-1**

IGF-1 is a protein similar in molecular structure to insulin which plays an important role in growth regulation and endocrine system through increased glucose uptake and decreased hepatic glycogenolysis and gluconeogenesis, thus improving insulin sensitivity [64,65]. IGF-1 belongs to a group of polypeptides where most of the mRNA is detected in the liver, kidney, brain, and myocardium. IGF-1 gene expression is stimulated by growth hormone production, which in turn is suppressed by high levels of IGF-1 suggesting a feedback compensatory mechanism [66]. Many factors such as age and gender influence these protein levels. Higher levels of IGF-1 are produced in the initial phases of life while a decrement is common during aging [66,67]. As IGF-1 is present in critical brain regions such as olfactory bulb and hippocampus [68], this peptide exerts modulatory effects including synaptic plasticity [69], neuronal excitability [70], cognitive function, and behavior [71,72]. In BD, Kim and collaborators have suggested that IGF-1 can be a trait marker for BD due to its relevant roles in the pathophysiology of the condition [73]. An in vitro experiment demonstrated that IGF-1 increased lithium sensitivity in lymphoblastoid cell lines from non-responders BD patients [74]. Corroborating with previous data, we showed elevated peripheral levels of IGF-1 in euthymic BD patients compared to healthy controls [75]. Not only BD but also MDD patients presented high levels of IGF-1 when compared to healthy controls [76]. Our review found an overexpression of IGF-1 levels in serum from patients with BD while underexpression was found in plasma suggesting that these alterations may be tissue-specific. Several studies have associated IGF-1 levels alterations with inflammatory diseases such as obesity [77], and diabetes [78]. It has been proposed that obesity promotes chronic low-grade inflammation in periphery, and IGF-1 resistance. Inflammation, on the other hand, enhances IGF-1 resistance. Both factors play a relevant role in triggering CNS disorder [77,79]. Evidence with animal models showed that central administration of IGF-1 decreased the expression of inflammatory markers, suggesting a reduction in depressive-like behavior [80]. This evidence leads us to believe that IGF-1 collaborates positively in inflammatory changes that psychiatric illnesses can cause.

**A2M**

As part of a glycoproteins group, A2M is present in vertebrates body fluids with diversified roles. One of the most important functions is the inhibition of proteases without directly blocking protease active site. It is widely involved in body protection against proteolytic activity [81]. Moreover, A2M has the ability to connect to several non-protease ligands such as cytokines, growth factors, and apolipoproteins [82]. Evidence shows that A2M is altered in a variety of illnesses including Alzheimer's disease [83], Parkinson's disease, and schizophrenia [84]. Also, there is data demonstrating three new genes predicting depression in response to stress including the A2M gene through "omics" approach [85]. Further, patients predisposed to develop depression have high levels of A2M [86], besides patients diagnosed with depression have elevated levels of A2M [87,88], in parallel with our data, that showed A2M up-regulated in only one study. Also, our analysis presented A2M levels down-regulated specifically in the mania group of Song study [13]. Interestingly, recent research showed altered levels of A2M in patients on the first episode psychosis, suggesting that acute phase proteins are involved with schizophrenic illness [89]. Acute-phase proteins are changed in response to inflammatory status and have presented a relationship with a mental disorder [89-91]. Although there is little evidence of A2M presence in BD patients blood, other mental illnesses have reported this change, like mentioned above.

**Coagulation Cascade and Complement System in BD**

From the analysis of proteins differentially expressed in BD, we identified the involvement of two main signalling pathways. Although the precise mechanisms underlying the interaction between the complement system (CS) and the CC are still not fully elucidated, current research has indicated a bidirectional modulation between these systems. The CS seems to be derived from the serine protease reaction cascade, which is encoded by the same ancestor genes as the coagulation factors [92,93]. Besides a common origin, these systems also share similar roles, including promoting the first defense line against infections and tissue repairing, while potentially contributing to either homeostasis or the development of pathological conditions [94]. Here, we observed that A2M, from the CC, and C3, a component of the CS, have been found differentially expressed in BD. Such finding corroborates previous evidence supporting the crosstalk between CC and CS and further implicates this interaction in the pathophysiology of the disorder.

Like the CS, the CC is characterized by a highly regulated and coordinated event that culminates in clot formation and, when combined with the fibrinolytic system and platelets, constitutes the hemostasis system (Fig. 5). The activation of the CC comprises primary and secondary hemostasis, and it is usually accompanied by the activation of inflammatory mechanisms [95]. The primary hemostasis is characterized by the activation and aggregation of platelets and culminates with the formation of fibrin by thrombin. This event is also accompanied by an acute inflammatory response to control tissue damage, stop loss of blood and prevent microbial infection. During the second hemostasis, plasmin dissolves fibrin along with reparative inflammatory cells in a combined effort to remodel and repair damaged tissue [96]. Hence, under physiological conditions, a strictly controlled hemostasis system confers minimal risk of complications or failed response.

On the other hand, the dysregulation of the acute phase response, as a result of a disproportionate CC activation of inflammatory signalling, can be detrimental to tissue repair and homeostasis. Therefore, the proper regulation of this pathway relies on modulatory anticoagulant mechanisms such as the protein C pathway (PC), the tissue factor pathway inhibitor and the antithrombin-heparin pathway [97]. Overall, these mechanisms inhibit most of the factors that become activated throughout the CC, being the PC considered the major one [98]. As a coordinated mechanism is also essential for the thrombus resolution and wound healing, circulating α2AP, and A2M represents the main modulators of the fibrinolytic system. Thus, the downregulation of A2M in BD, as observed by most of the studies included in this review, results in a lack of control of both PC pathway and plasmin activity; thus, upregulating the anticoagulation capacity and fibrinolytic system, respectively. Specifically, activated PC impairs the procoagulant effects of thrombin [95], while enhanced plasmin activity increases CS activation (Fletcher-Sandersjöö *et al.* [99], discussed below). The possible overregulation of coagulation by anticoagulation mechanisms can result in abnormal bleeding, which is not usually reported in psychiatric disorders. However, pharmacological treatment has been suggested to interfere in the balance of the

CC causing haematological side effects [100].

Throughout the CC, there are several steps involving the activation of the CS components. For instance, activated platelets present surface molecules, such as P-selectin and C1q receptor, that activate the alternative and classical pathways of the CS, respectively [93,94]. Fibrinogen is a potent acute phase reactant and inflammatory mediator [101]. Also, thrombin and plasmin can activate C3 and C5 in the coagulation site independently of C3 conversion (Fletcher-Sandersjöö *et al.* [99]; Fig. 5, dashed line). Besides chemo attractant properties, activated CS components C3a and C5a induce the activation, aggregation and degranulation of platelets, promote calcium influx and enhance procoagulant activity [102]. Also, the formation of C5a favours neutrophils and monocytes recruitment, while C5b contributes to MAC formation, which further augments platelet activation and aggregation [93]. Thus, the crosstalk between these systems is suggested to generate a self-strengthening cycle.

CS activity is directly related to an increased prothrombotic and antifibrinolytic state. For instance, mannan-binding lectin serine proteases initiate the lectin pathway of the CS and form activated thrombin, suggesting that the CS can generate the end product of the CC [99]. Also, the modulatory mechanisms of the CS, including the C1 inhibitor and C4-binding protein, play a dual role as regulatory proteins in both systems. In the CC, these proteins ultimately cleave activated factors V and VIII, reducing the activation of procoagulant mechanisms [93]. On the contrary, C1 inhibitor has also been shown to inhibit plasmin, which consequently reduces fibrinolytic activity and increases thrombogenesis [103]. Therefore, dysfunctional CS activation has been implicated in pathogenic mechanisms underlying hemolytic and thrombotic diseases, as hyperactivation of this pathway is associated with both systemic inflammation and thrombosis [94, 104,105]. Such events have been extensively reported and characterized in sepsis, whereas a chronic low-grade inflammatory state is commonly observed in BD [48].

Albeit the crosstalk between CS and CC has not been fully understood yet, the CS is considered the primary mediator, while C3 represents the common component of this interaction [94]. Accordingly, our results indicate an altered expression of C3 in BD, which seems to be downregulated in the disorder [32,106]. Reduced levels of C3 may be a result of higher consumption and activation of CS components, being compatible with a peripheral and central proinflammatory milieu. As previously discussed, inflammation can promote coagulation and, specifically, IL-6 has been shown to increase platelet activation and aggregation, which further augments the secretion of other inflammatory markers [107,108]. Peripheral proinflammatory cytokines are known to be increased in BD, especially during mood episodes [48,109], and to downregulate the PC pathway–the main anticoagulant system [98]. In sum, the dysregulation of the complement system triggers the activation of hemostatic factors, consequently leading to thrombosis and intravascular coagulation. On the other hand, thrombosis or tissue damage activates the CS amplifying the inflammatory response and promoting additional local tissue injury.

Due to this interplay, extant literature supports the implication of CC and its regulatory mechanism—including the CS—in BD. Interestingly, in sepsis, the levels of active CS components seem to be higher in the serum than in the plasma, and evidence shows a better correlation between CS and hemostasis parameters than with other inflammatory markers [110]. Then, increased activation of CS during coagulation might be mediated by the platelets, which are considered a non-specific first line inflammatory marker and suggested to play a role in psychiatric disorders [111]. Razouki *et al.* [112,113] have found that BD, among others, might be a predictive factor associated with more time below the target therapeutic range for treatment with warfarin, which points out to an impaired anticoagulation control among BD patients. Moreover, more recent evidence of the involvement of these systems in BD implicates other signalling pathways. For instance, C5a induces the upregulation of the plasminogen activator inhibitor-1 (PAI-1), a regulator of the fibrinolytic pathway, which inhibits the tissue plasmin activator (tPA) [94]. Besides promoting a procoagulant effect, the inhibition of tPA may contribute to impair its activity in converting proBDNF to BDNF [114]. The role of BDNF in BD and other psychiatric disorders has been extensively investigated [115,116]. For instance, the tPA-BDNF pathway has been implicated in MDD. Specifically, tPA, BDNF, and BDNF/proBDNF ratio were lower in MDD, while 8-week antidepressant treatment rescued those levels [117]. In BD, peripheral BDNF levels are commonly found to be reduced during mania and depression [118], as well as tPA, proBDNF, TrkB and p75NTR [119].

Hence, combined peripheral levels of these markers—but not each marker individually—have been proposed to present a good accuracy of diagnosis and differentiation among SZ, BD at different episodes, MDD and healthy controls. Interestingly, authors have found a good diagnostic efficacy for differentiating mania from depression in BD. As both CC and CS seem to be involved in BD, the investigation of these pathways may be informative of pathophysiological mechanisms involved in the disorder and potentially indicative of a marker of state (e.g., mood episode) [120].

### Limitations

This review presented a number of limitations. First, methodological differences such as protein levels assay (multiplex assay and chromatography) may have contributed to the heterogeneous results. Immunoassay is based in a specific interaction between antibody and target, whereas chromatography separates molecules according to their solubility, size, and mass. Considering that immunoassay has a high specificity it is likely that chromatography does not present all proteins detected by multiplex assay. On the other hand, multiplex is characterized by being selective, not covering all proteins separated in chromatographic analysis. This leads us to believe that different methods of analysis can generate heterogeneous results. Second, sample characteristics including demographic variables (such as ethnicity, gender and age), mood state, symptom severity, chronicity, and comorbidities among others are also factors that may influence the protein expression. Additionally, we can not rule out biomarkers differences according to patients with first episodes versus those with chronic course [121]. Third, it is possible to find a variety of physiological alterations in patients according to the drug therapy [122,123]. Since interferences related to weight gain, gastrointestinal disturbances, neural tube defects, up until cytochrome P450 enzymes induction [123], may be a precursor of proteomic modification.

It is possible to note a great heterogeneity among studies in both uniquely expressed proteins and a number of proteins. Those differences may be explained to some extent by biological sample type, subjects characteristics, and proteomic technique carried out.

## CONCLUSION

In sum, this review demonstrates a potential biological signature in BD patients based on proteomic analysis. We compared blood proteomes, by using protein association network, of subjects with BD and healthy controls to suggest dysfunctional molecular pathways involved in disease. The results revealed proteins associated with several biological processes, including growth and endocrine regulation, iron transportation, protease inhibition, protection against pathogens and cholesterol transport. Moreover, pathway analysis indicated the association of uncovered proteins with two main metabolic pathways: complement system and coagulation cascade. Many of these physiological processes are related to psychiatric disorders. Therefore, it is important to identify possible biomarkers for mental illnesses differentiation. Since psychiatry still strongly relies on clinical judgment, there is a risk for misdiagnosis and, consequently, inadequate/erroneous treatment. Thus, it is essential to improve the current knowledge on the pathophysiology of psychiatric disorders and underlying molecular patterns.

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■ **Conflicts of Interest**

No potential conflict of interest relevant to this article was reported.

■ **Author Contributions**

Conceived the research study: Adriane Ribeiro Rosa, Rafael Colombo. Writing of the manuscript and collected data: Paola Rampelotto Ziani, Adriane Ribeiro Rosa, Jacson Gabriel Feiten, Jéferson Ferraz Goularte, Bárbara Antqueviezc. Data analysis and interpretation: Jacson Gabriel Feiten. Interpretation of the results and critically

reviewed the final draft of the manuscript: Adriane Ribeiro Rosa, Rafael Colombo, Paola Rampelotto Ziani, Jéferson Ferraz Goularte, Luiza Paul Géa. All authors contributed to the article and approved the submitted version.

## ■ ORCID

Paola Rampelotto Ziani
https://orcid.org/0000-0003-3070-3535
Jacson Gabriel Feiten
https://orcid.org/0000-0003-0414-6421
Jéferson Ferraz Goularte
https://orcid.org/0000-0002-1229-702X
Rafael Colombo https://orcid.org/0000-0002-2794-0671
Bárbara Antqueviezc
https://orcid.org/0000-0003-0170-0597
Luiza Paul Géa https://orcid.org/0000-0002-8465-0785
Adriane Ribeiro Rosa
https://orcid.org/0000-0001-8629-4625

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## 7. ARTIGO CIENTÍFICO 4

Artigo científico intitulado: Proteomic Insights into Biology of Bipolar Disorder: Implications for Health Complexity and Mortality

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**Proteomic Insights into Biology of Bipolar Disorder: Implications for Health Complexity and Mortality**

**Proteomic Profile in Bipolar Disorder**

Paola Rampelotto Ziani1,2, Marco Antônio de Bastiani2, Pietra Paiva Alves1, Pedro Henrique da Rosa1,2, Tainá Schons1, Giovana Mezzomo1,2, Ellen Scotton1,2, Flávio Kapczinski1,2,3, Adriane R Rosa1,2,3

1 Laboratory of Molecular Psychiatry, Hospital Clinic of Porto Alegre (HCPA), Porto Alegre, RS, Brazil

2 Department of Pharmacology and Graduate Program of Pharmacology and Therapeutics, Institute for Basic Medical Science, University Federal do Rio Grande do Sul, Brazil

3 Graduate Program in Psychiatry and Behavioral Sciences, School of Medicine, University Federal of Rio Grande do Sul

(UFRGS), Porto Alegre, RS, Brazil.

Corresponding author: arrosa@hcpa.edu.br

Rua Ramiro Barcelos, 2350, Santa Cecília, Hospital de Clínicas de Porto Alegre, Porto Alegre, RS, Brazil - Postal Code: 90410-000

ORCID: 0000-0001-8629-4625

Abstract

Bipolar disorder (BD) is a debilitating condition associated with a high prevalence of medical comorbidities and premature mortality. This is the first study to explore, through high-throughput-omics combined with bioinformatics, molecular signatures, pathways, and main medical diseases related to different stages of BD. Blood samples from BD patients (n=10) classified into high (BD+) or poor functioning (BD-), based on functional and cognitive status, and healthy controls (n=5) were analyzed using mass spectrometry-based proteomic analysis. Bioinformatics was performed to detect biological processes, pathways, and diseases related to BD. Eight proteins exclusively characterized the molecular profile of patients with BD+ compared to HC, while 26 altered proteins were observed in the BD- group. These altered proteins were mainly enriched in biological processes related to lipid metabolism, complement system and coagulation cascade, and cardiovascular diseases; all these changes were more prominent in the BD- group. These findings may represent systemic alterations that occur with the progression of the illness and a possible link between BD and medical comorbidities. Such comprehensive understanding provides valuable insights for targeted interventions, addressing mental and physical health aspects in subjects with BD.

Keywords: Mass spectrometry, molecular signatures, bioinformatics, biomarkers, complement system, coagulation cascade.

# CAPÍTULO III

Discussão e Conclusão

## 8. DISCUSSÃO

A fisiopatologia do TB se revela de maneira heterogênea, envolvendo fatores genéticos, comportamentais e ambientais. A complexidade das manifestações clínicas muitas vezes leva a diagnósticos equivocados ou subestimados, o que por sua compromete a implementação de abordagens terapêuticas efetivas. Nesse contexto desafiador, a compreensão aprofundada da doença, aliada à busca por marcadores específicos, emerge como um imperativo. Portanto, estratégias que integram variados níveis de informação, juntamente com a pesquisa de marcadores específicos, são essenciais para aprimorar a precisão diagnóstica e favorecer intervenções farmacológicas mais eficazes neste contexto clínico.

Na primeira etapa desta pesquisa, foram empregados dados de microarranjos, obtidos a partir do banco de dados GEO, que estavam disponíveis em amostras de sangue de pacientes com TB para conduzir análises *in silico*. Enquanto a Análise de Regulador Mestre permite a identificação de reguladores-chave que exercem controle sobre muitos genes em uma rede gênica (CAI et al., 2020), a Análise de Enriquecimento de Conjuntos de Genes ajuda a identificar conjuntos de genes coordenadamente envolvidos em processos biológicos específicos (SUBRAMANIAN et al., 2005). Essas duas ferramentas em conjunto possibilitaram a descoberta de padrões biológicos importantes envolvidos na doença. Foram identificados 59 candidatos a reguladores mestres no TB, sendo que o DMTF1 foi o único gene presente nos três estados de humor. Além disso, identificamos 134 genes enriquecidos, dos quais 2 (CLOCK e SETDB2) foram identificados no estado de depressão, 3 (ZNF358, ZNF653 e ZNF787) na eutimia e 4 (ELF4, ZNF467, ZNF512 e ZNF91) na mania. Essa descoberta sugere que os genes identificados podem indicar o estado da doença, ou seja, alterações que ocorrem durante os episódios de humor, contribuindo para uma compreensão mais abrangente e precisa do transtorno. No entanto, é importante destacar que a confirmação e validação dessas descobertas requerem a realização de estudos adicionais no futuro. Este

passo é essencial para garantir a robustez e a confiabilidade dos resultados obtidos até o momento.

Adicionalmente, o segundo artigo científico uniu os dados transcriptômicos previamente discutidos a abordagens computacionais, destacando o emprego dos bancos de dados cMap e LINCS. Essa integração teve como propósito identificar novos compostos bioativos ou medicamentos aprovados pela FDA, cujos mecanismos de ação estivessem relacionados à assinatura de expressão gênica associada ao TB. O cMap foi utilizado para explorar padrões de resposta transcriptômica a tratamentos específicos, enquanto o LINCS permitiu uma análise abrangente de perturbações celulares, contribuindo para a identificação e compreensão de agentes potencialmente relevantes no contexto do TB. Curiosamente, alguns desses compostos eram específicos para mania, depressão ou eutimia, enquanto outros eram comuns aos três estados de humor. Eles incluem medicamentos aprovados pela FDA, como agentes antineoplásicos (dasatinibe, sorafenibe e sunitinibe) ou agentes anti-hipertensivos (minoxidil e alisquireno), e compostos bioativos que estão sendo testados em modelos animais, como gugulsterona, ácido betulínico, ácido cafeico, entre outros. Boa parte destes compostos ou medicamentos aprovados já foram descritos em estudos prévios que estabeleceram uma provável conexão entre os mecanismos de ação dos compostos identificados com a neurobiologia dos transtornos psiquiátricos, em especial, o TB (CARNOVALE et al., 2023; GULBINS et al., 2016; HU et al., 2020; LI et al., 2022; LIU et al., 2017). Assim, nosso trabalho identificou novos candidatos a medicamentos por meio da reutilização computacional de medicamentos, com base na assinatura transcriptômica de indivíduos com TB, seguida da análise subsequente dos perfis de expressão gerados pelos medicamentos. Na era da bioinformática e Medicina de Precisão, o uso de avanços inovadores, incluindo modelos de biologia de sistemas e abordagens multiômicas, é bem-vindo para lidar com desafios importantes relacionados a transtornos psiquiátricos,

devido a) à compreensão limitada da neurobiologia, b) à heterogeneidade da doença e c) à falta de modelos animais validados. Embora a maioria dos candidatos a medicamentos identificados aqui tenha respaldo na literatura, nossa análise foi baseada na geração de hipóteses, e a validação experimental é necessária antes da tradução clínica.

No terceiro artigo desta tese foi conduzida uma revisão da literatura com o objetivo de identificar potenciais candidatos a biomarcadores no TB por meio de uma análise *in sílico* do proteoma sanguíneo. Neste estudo, foram coletados dados sobre proteínas diferencialmente expressas nos proteomas de plasma e soro de indivíduos com TB. Buscou-se identificar possíveis biomarcadores para distinguir entre pacientes e controles saudáveis, bem como compreender as vias moleculares disfuncionais subjacentes à fisiopatologia do TB. Nos estudos abordados, os resultados principais destacam a proeminência de cinco proteínas distintas: IGF-1, TF, A2M, C3 e APOA1. Em termos gerais, essas proteínas estão envolvidas em processos biológicos comuns como regulação do crescimento, sistema endócrino, transporte de ferro livre, inibição de proteases, defesa contra patógenos e transporte de colesterol. Através da análise de vias do KEGG, encontramos duas principais vias metabólicas: o sistema complemento e a cascata de coagulação. A interação entre os sistemas de coagulação e complemento no TB sugere uma comunicação bidirecional, influenciando a expressão diferencial de proteínas (KREM; CERA, 2002; ONCUL; AFSHAR-KHARGHAN, 2020). O sistema de complemento tem origem na cascata de reações da serina protease, compartilhando uma ancestralidade comum com os fatores de coagulação (KREM; CERA, 2002; ONCUL; AFSHAR-KHARGHAN, 2020). A ativação da cascata de coagulação compreende a hemostasia primária e secundária, e geralmente é acompanhada pela ativação de mecanismos inflamatórios (LUYENDYK; SCHOENECKER; FLICK, 2019). Por outro lado, a disfunção na ativação do sistema complemento pode contribuir para a patologia, resultando em um estado pró-trombótico e antifibrinolítico aumentado (FLETCHER-SANDERSJÖÖ;

MAEGELE; BELLANDER, 2020). Assim, a interação entre esses sistemas pode desempenhar um papel na resposta inflamatória, coagulação e lesões locais em TB. Portanto, compreender esses mecanismos, juntamente com a identificação das proteínas envolvidas, oferece informações cruciais sobre indicadores do estado do TB.

Reconhecer as limitações em nossa pesquisa é fundamental, englobando desde a heterogeneidade das amostras até as restrições algorítmicas. Alguns estudos não fornecem informações clínicas completas, como estado de humor, duração da doença, comorbidades ou farmacoterapia, fatores que podem impactar os resultados. A seleção de conjuntos de dados minimizou as disparidades amostrais, porém, ao utilizar exclusivamente dados de transcriptômica ou proteômica periféricos, a representatividade global das amostras e a complexidade cerebral podem estar limitadas. As técnicas bioinformáticas estão em constante evolução, tornando desafiadora a escolha da ferramenta adequada para análises específicas. Algumas ferramentas podem apresentar limitações de precisão ou capacidade de processamento. No artigo que propusemos fármacos e compostos bioativos, é importante ressaltar que a descoberta de medicamentos é um processo multifacetado que vai além da expressão gênica, envolvendo aspectos críticos como farmacocinética e toxicologia, que são igualmente essenciais para o desenvolvimento de novos fármacos. Por fim, estudos de validação envolvendo técnicas de biologia molecular são necessários para confirmar nossos achados.

Por fim, no quarto artigo da tese buscamos confirmar os achados do estudo acima em uma amostra brasileira. Portanto, usamos sangue periférico de pacientes com TB oriundos do Programa de Tratamento do Transtorno de Humor Bipolar (PROTHABI) do Hospital de Clínicas de Porto Alegre (HCPA), por meio de LC-MS/MS. Inicialmente, os pacientes foram divididos em dois grupos: bom funcionamento (BD+) ou mau funcionamento (BD-), considerando seu estado funcional e cognitivo conforme as escalas FAST e COBRA (LIMA et

al., 2018; ROSA et al., 2007, 2009, 2014). De maneira singular, as descobertas extraídas de nossos resultados destacaram um perfil molecular diferenciado entre pacientes com desempenho cognitivo e funcional classificado como bom e mau. Essa singularidade sugere que possíveis variações nas proteínas circulantes periféricas podem estar intrinsecamente associadas a cada grupo, identificando assim variações moleculares significativas provavelmente associadas com a progressão da doença. Os resultados mostraram, ainda, que as proteínas diferencialmente expressas estavam principalmente envolvidas no metabolismo lipídico, no sistema complemento e na cascata de coagulação. Assim, nossos achados corroboram estudos anteriores - inclusive o primeiro artigo desta tese - indicando que a interação entre o sistema de complemento e a cascata de coagulação está envolvida na etiologia e progressão do TB (REGINIA et al., 2018; RODRIGUES et al., 2022; ZIANI et al., 2022). Outro importante resultado foi a proeminência de doenças cardiovasculares associadas, especialmente, ao TB com baixo funcionamento. Em condições normais, um sistema de hemostasia estritamente regulado apresenta risco mínimo de complicações ou respostas inadequadas. No entanto, a desregulação pode resultar em respostas plaquetárias hiperativas, formação anormal de fibrina e fibrinólise prejudicada, criando um ambiente propício a eventos cardiovasculares. Além disso, a intercomunicação entre os sistemas de complemento e coagulação tem o potencial de amplificar processos inflamatórios no sistema vascular, aumentando o risco de eventos cardiovasculares (CHAUDHRY; USAMA; BABIKER, 2023; HU et al., 2020). É importante destacar que as doenças cardiovasculares são comorbidades comuns entre pacientes com TB, fato que pode explicar, parcialmente, a disparidade na mortalidade entre indivíduos com TB e a população em geral (SCHOEPF; HEUN, 2015). Essa compreensão abrangente oferece insights valiosos para intervenções direcionadas, abordando aspectos de saúde mental e mecanismos subjacentes a comorbidades no TB. Por fim, as proteínas identificadas revelaram uma correlação entre TB e a variável “número de

medicações". Boa parte dos pacientes, em especial, aqueles com mau funcionamento são polimedicados, o que deve representar quadros mais crônicos e de difícil tratamento. Pacientes com regimes mais complexos de tratamento estão também associados com as alterações mais proeminentes no sistema do complemento e coagulação e comorbidades médicas. Por outro lado, não podemos descartar o efeito das medicações (psicofármacos) nas alterações moleculares aqui identificadas.

Algumas limitações devem ser consideradas ao interpretar os resultados deste estudo. Em primeiro lugar, o número de amostras foi relativamente pequeno e todos os pacientes estavam sob tratamento farmacológico. A polimedicação introduz complexidades adicionais que exigem uma análise minuciosa, uma vez que os agentes farmacológicos podem influenciar diretamente as respostas biológicas investigadas e gerar efeitos sinérgicos ou antagonistas nos resultados observados. A variabilidade individual na resposta ao tratamento é um fator significativo, tornando desafiador tirar conclusões definitivas sobre as associações entre as proteínas identificadas e condições de saúde comórbidas. Em segundo lugar, algoritmos e métodos de análise podem influenciar significativamente os resultados. Diferentes algoritmos podem gerar interpretações distintas a partir dos mesmos conjuntos de dados, e a interpretação biológica de resultados obtidos por abordagens bioinformáticas pode ser desafiadora. Devido a isso, colaboramos com um bioinformático experiente para trabalhar com os dados. Em terceiro lugar, o perfil proteômico foi obtido a partir de uma amostra de sangue, potencialmente não capturando integralmente as complexas mudanças moleculares associadas ao TB em todo o cérebro. Essas limitações são cruciais para uma compreensão completa e crítica dos resultados obtidos.

## 9. CONCLUSÃO

De acordo com os resultados alcançados nesta pesquisa, é possível inferir que:

- A diferenciação entre indivíduos saudáveis e pacientes com TB por meio de biomarcadores séricos ainda é um grande desafio. Contudo, é possível obter respostas mais definitivas quando se utiliza biologia de sistemas e técnicas de bioinformática para correlacionar conjuntos de proteínas e transcritos já identificados nesse transtorno.
- A análise transcriptômica identificou reguladores mestre e genes específicos relacionados aos estados de humor no TB, contribuindo para uma compreensão mais precisa e abrangente do transtorno.
- A abordagem de fármaco-gene revelou compostos bioativos e medicamentos existentes, sugerindo potenciais opções terapêuticas (a ser adequadamente testadas) para diferentes fases do TB.
- A revisão do proteoma sanguíneo identificou cinco proteínas (IGF-1, TF, A2M, C3 e APOA1) associadas ao TB, destacando sua ligação com as vias do sistema complemento e coagulação.
- A análise exploratória de proteínas em pacientes evidenciou a associação do TB com o metabolismo lipídico, sistema complemento e cascata de coagulação, além de correlacionar comorbidades, em especial as cardiovasculares e a polifarmacoterapia, destacando a importância da gestão integrada da doença.

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# ANEXO

## Carta de Aprovação do Projeto pelo Comitê de Ética de Pesquisas com Seres Humanos

**HOSPITAL DE CLÍNICAS DE PORTO ALEGRE**

**Grupo de Pesquisa e Pós Graduação**

**Carta de Aprovação**

**Projeto**

2019/0640

**Pesquisadores:**

**ADRIANE RIBEIRO ROSA**

| | | |
|---|---|---|
| GIOVANA DALPIAZ | ELLEN SCOTTON | BARBARA ANTQUEVIEZC PINTO |
| IVES CAVALCANTE PASSOS | RAFAEL COLOMBO | JACSON GABRIEL FEITEN |
| PAOLA ZIANI | | |

**Número de Participantes:** 120

**Título:** PERFIL PROTEÔMICO EM PACIENTES COM TRANSTORNO BIPOLAR: FOCO NO DESENVOLVIMENTO DE BIOMARCADORES

Este projeto foi APROVADO em seus aspectos éticos, metodológicos, logísticos e financeiros para ser realizado no Hospital de Clínicas de Porto Alegre.
Esta aprovação está baseada nos pareceres dos respectivos Comitês de Ética e do Serviço de Gestão em Pesquisa.

- Os pesquisadores vinculados ao projeto não participaram de qualquer etapa do processo de avalição de seus projetos.

- O pesquisador deverá apresentar relatórios semestrais de acompanhamento e relatório final ao Grupo de Pesquisa e Pós-Graduação (GPPG).

11/12/2019

Assinado digitalmente por:
**PATRICIA ASHTON PROLLA**
Grupo de Pesquisa e Pós-graduação
03/01/2020 11:49:34

Impresso do sistema AGHUse-Pesquisa por CRISTIAN FIDALGO CABRAL em 11/12/2019 11:49:53

# ANEXO

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**Carta de Aprovação**

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2019/0640

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