ISTANBUL MEDENIYET UNIVERSITY
INSTITUTE OF GRADUATE STUDIES
DEPARTMENT OF BIOLOGICAL DATA SCIENCE

MULTIOMIC DATA ANALYSIS OF SCHIZOPHRENIA AND BIPOLAR DISORDER

Master's Thesis

Burak Cardak

March 2024

ISTANBUL MEDENIYET UNIVERSITY
INSTITUTE OF GRADUATE STUDIES
DEPARTMENT OF BIOLOGICAL DATA SCIENCE

MULTIOMIC DATA ANALYSIS OF SCHIZOPHRENIA AND BIPOLAR DISORDER

Master's Thesis

Burak Cardak

Supervisor

Assist. Prof. Dr. Muhammed Erkan Karabekmez

March 2024

THESIS JURY APPROVAL

This Master's Thesis titled "MULTIOMIC DATA ANALYSIS OF SCHIZOPHRENIA AND BIPOLAR DISORDER" written by Burak Cardak at the Department of Biological Data Science was accepted by our jury.

JURY MEMBERS **SIGNATURE**

Supervisor:

Assist. Prof. Dr. M. Erkan KARABEKMEZ

Istanbul Medeniyet University

Other Jury Members:

Prof. Dr. Hasan Güçlü

Istanbul Medeniyet University

Dr. Öğr. Üyesi Seyfullah Enes Kotil

Boğaziçi University

Date of Defense: 29/03/2024

STATEMENTS

Style and Reference Manual Statement

Having reviewed this thesis written under my supervision, I confirm that it has been written in accordance with APA Manual of Style and used its text reference format consistently throughout the entire text.

Assist. Prof. Dr. M. Erkan KARABEKMEZ

Declaration of Originality

I hereby declare that all information in this dissertation has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conducts, I have fully cited and referenced all material and results that are not original to this work.

Burak Cardak

PREFACE

This thesis represents a comprehensive exploration into the genetic and molecular aspects of bipolar disorder (BD) and schizophrenia (SZ). Utilizing a multi-omics approach, this study aims to unravel the complexities of these psychiatric conditions and contribute to their diagnosis, pathophysiology, and treatment.

Despite advancements in technology and data availability, the clinical application of multi-omics research in mental health poses challenges. The intricate interplay between genetic and environmental factors adds complexity to understanding BD and SZ. This thesis acknowledges these limitations while striving to provide valuable insights into these conditions.

I am grateful for the support, guidance, and contributions of Assist. Prof. Dr. M. Erkan Karabekmez who supported me during this journey.

No matter how much I express my gratitude to my dear mother, Çiğdem, and my dear father, Yılmaz, for raising me up to this day, it will never be enough.

I would like to extend my heartfelt thanks to my beloved wife, Nazlı Kılıç Çardak, for her unwavering support throughout, and to my precious daughter, Miss Leyla Inci Çardak, who was born just two weeks before the submission of this thesis.

I am also deeply indebted to my dear grandmother, Maviş, and my dear grandfather, Niyazi, who have taken care of me with all their hearts since my childhood.

And finally, I dedicate this to the esteemed martyrs who sacrificed their lives day and night to protect us in our beautiful country, and to the Turkish Armed Forces, of which my beloved father is also a member.

ÖZET

ŞİZOFRENİ VE BİPOLAR BOZUKLUĞA DAİR ÇOKLU OMİK VERİLERİN ANALİZİ

Çardak, Burak

Yüksek Lisans Tezi, Biyolojik Veri Bilimi Anabilim Dalı

Danışman: Dr. Öğr. Üyesi M. Erkan Karabekmez

Mart 2024

Giriş: Günümüzde, psikiyatrik bozuklukların anlaşılmasında biyopsikososyal model, tıp alanında uzun süre biyomedikal modele karşı çıkarak önemli bir alternatif sunmaktadır. Özellikle Ruhsal Bozuklukların Tanısal ve İstatistiksel El Kitabı 5. Edisyonu (DSM-5) ve Uluslararası Hastalık Sınıflaması On Birinci Revizyonu (ICD-11) gibi son dönem tanı ve sınıflandırma sistemlerinin kullanımı, ruh sağlığı bozukluklarını teşhis etmede psikolojik ve sosyal faktörleri göz önünde bulundurmasına rağmen, genellikle biyolojik bileşeni ihmal etmektedir. Bu durum, semptomlar, bulgular, süre ve rahatsızlık düzeyine dayalı olarak sınıflandırma yapmayı içeren DSM-5'in sınırlılıklarını ortaya koymaktadır. Ancak, bu tanı sistemleri, örtüşen semptomlar, kişisel klinik değerlendirmeler ve potansiyel kültürel farklılıklar gibi zorluklarla karşı karşıyadır. Sonuç olarak, genetik araştırmalar, geleneksel tanı sistemlerinin sınırlılıklarını açığa çıkararak, psikiyatrik bozuklukların ortak bir genetik temeli olduğunu ve kesin tanılar yerine daha karmaşık bir görüntü sunduğunu göstermektedir.

Biyolojik bileşenin entegrasyonu, özellikle de genetik ve epigenetik faktörlerin göz önüne alınması, psikiyatrik durumların anlaşılmasına önemli katkılar sağlar. Aile, ikiz ve evlatlık çalışmaları, psikiyatrik bozuklukların genetik temellerini açığa çıkararak, ruh sağlığı bozukluklarında kalıtımın %40 ila %80'ini açıklar. Ancak, psikiyatrik koşulları tek bir ana genin tanımlanmasını amaçlayan önceki Mendel çalışmaları yüksek yanlış pozitif oranlarından dolayı önemli zorluklarla karşılaşmıştır. Benzer şekilde, aday gen döneminde, nörotransmitterlere bağlı genler başlangıçta güçlü adaylar olarak kabul edilmiş, ancak sonunda yüksek sayıda yanlış pozitif sonuçlara yol açmıştır. Ancak, genom çapında ilişkilendirme çalışmalarının (GWAS) ortaya çıkışı,

alanda önemli bir sıçrama yapmış ve çeşitli psikiyatrik koşullarla ilişkilendirilen birden fazla genin keşfini sağlamıştır.

Epigenetik mekanizmalar, gen regülasyonunda kritik bir rol oynayarak gen ifadesini etkiler. Bu mekanizmalar arasında, deoksiribonükleik asit (DNA) metilasyonu, histon modifikasyonları ve ribonükleik asit (RNA) aracılı düzenlemeler bulunur. DNA metilasyonu, özellikle CpG adalarını hedefleyen metilasyon süreci yoluyla DNA'nın yapısını etkiler. Histon modifikasyonu ise DNA'yı saran histonlarda post-translasyonel modifikasyonlara neden olarak gen ifadesini etkileyebilir; bu modifikasyonlardan biri olan asetilasyon en yaygın olanıdır. Üçüncü olarak, mikroRNA'lar (miRNA), haberci RNA'ların (mRNA) proteinlere çevirisini baskılayarak gen ifadesini etkiler. Biyolojik süreçlerde kritik bir rol oynayan DNA metilasyonu, birçok araştırmada önemli bir rol oynamaktadır.

Genetik ve epigenetik araştırmalar arasındaki etkileşim ve karşılıklı ilişki oldukça önemlidir. Çevresel değişiklikler, DNA dizisini doğrudan değiştirmese de gen ifadesini etkileyen epigenetik mekanizmaları önemli ölçüde etkileyebilir. Bu durum, çevresel faktörlerin gen ifadesini epigenetik yollarla şekillendirebileceğinin potansiyelini vurgular. Bu, çevresel etkilerin genleri epigenetik mekanizmalar aracılığıyla etkileyerek gen ifadesini modüle edebileceği önemli bir konuyu ortaya koyar, bu da araştırılması ve anlaşılması gereken bir alanı oluşturur. Örneğin, Perroud ve arkadaşlarının (2016) çalışması, serotonin reseptörü 3A'nın metilasyon düzeyinin artmış hastalık şiddetiyle ilişkilendirildiğini ve çocukluk döneminde kötü muameleye maruz kalan bireylerde bipolar bozuklukla (BD) ilişkili olduğunu göstermektedir. Dolayısıyla, epigenetik mekanizmalar üzerine yapılan araştırmalar, çevresel faktörler ile genetik materyal arasındaki etkileşimi aydınlatır. Bu anlayış, ruhsal bozuklukların tanısında ve temel mekanizmaların anlaşılmasında yardımcı olur; bu da, bu epigenetik mekanizmaları hedefleyen yeni terapötik stratejilerin geliştirilmesine öncülük edebilir.

Genetik ve epigenetik faktörlerin yanı sıra, biyokimyasal ve moleküler araştırmaların psikiyatrik bozuklukların anlaşılmasına nasıl katkıda bulunduğu da incelenmelidir. Örneğin, dopamin, norepinefrin ve serotonin gibi nörotransmitterlerin rolü, çeşitli ruh sağlığı bozukluklarıyla ilişkilendirilmiştir. Ayrıca, beyin yapılarındaki anormallikler ve sinaptik işlevdeki değişiklikler de psikiyatrik bozuklukların anlaşılmasında önemli

bir rol oynar. Bu moleküler düzeydeki anlayış, psikotropik ilaçların geliştirilmesinde ve mevcut tedavilerin iyileştirilmesinde önemli bir rol oynar.

Sonuç olarak, psikiyatrik bozuklukların biyolojik temellerinin anlaşılması, tanısal kriterlerin geliştirilmesinde ve tedavilerin kişiselleştirilmesinde büyük bir potansiyele sahiptir. Genetik, epigenetik ve moleküler araştırmaların entegrasyonu, karmaşık mental sağlık sorunlarının nedenlerini daha iyi anlamamıza yardımcı olabilir ve böylece daha etkili tedavilerin geliştirilmesine olanak tanır. Bu nedenle, biyolojik araştırmaların psikiyatrik bozuklukların anlaşılmasındaki rolü, ruh sağlığı alanında önemli bir odak noktası olmaya devam etmektedir.

Metod: Çalışmada, insan beyinciklerinden elde edilen gen ifadesi ve DNA metilasyon verileri, Gene Expression Omnibus (GEO) veritabanından alındı. Toplamda 118 hasta verisi bulunmakta olup, bunların 36'sı BD, 39'u şizofreni (SZ) ve 43'ü kontrol grubundan oluşmaktadır.

Gen ifadesi veri setinde bazı hastalarda hasta başına üç ifade değeri bulunmaktadır. Bu değerler, diğer bireylerle karşılaştırıldığında belirgin şekilde daha yüksek korelasyonlar göstermektedir. Bu nedenle, her hastada yalnızca bir ifade değeri kullanılmış ve ilgili metilasyon verisiyle eşleştirilmiştir. Ayrıca, metilasyon verisi, çeşitli replikasyon türlerinin ne olduğunu belirtmeksizin 13 rastgele tekrar içermektedir. Bu nedenle, rastgele bir seçim yapılmış ve replikasyonlardan yalnızca biri gen ifadesi ile eşleştirilmiştir.

GSE35974'ten dışa aktarılan RNA-seq ifadesi verileri DESeq2 kullanılarak analiz edildi. DESeq2, RNA-seq verilerinin karşılaştırmalı analizi için sağlam bir nicel analiz yöntemi sağlar. DESeq2, RNA-seq verilerini analiz etmek için istatistiksel bir çerçeve olarak negatif binom dağılımına dayanır. Diferansiyel gen ekspresyonu analizi, Bonferroni düzeltilmiş p-değerlerine dayanarak filtrelenen farklı ekprese olmuş genlerin (DEG) belirlenmesi üzerine gerçekleştirildi.

RNA-seq verilerinin karakteristikleri genellikle normal dağılımı takip etmez. Sayım verileri, genellikle poisson ve binom dağılımı ile modellenebilir. DESeq2'nin kullanımında, negatif binom dağılımı, veri seti içinde ortalama ve varyans arasındaki ilişkiye dayandığı için uygun bir modeldir.

DESeq2, başlangıçta R istatistiksel hesaplama ortamı için Bioconductor projesi içinde bir paket olarak geliştirilmiştir. PyDESeq2, RNA-sekans verilerinin farklı ifade analizine odaklanan DESeq2'nin bir uygulaması olarak hizmet eder ve Python'un avantajlarını kullanır.

Diferansiyel metilasyon analizi, Fisher-exact testi kullanarak yapılmıştır. Çünkü literatürdeki hiç bir yöntemin diğerine üstünlüğü gösterilmemiştir. Bonferroni düzeltilmiş p-değerlerine dayanarak filtrelenen farklı metillenmiş genlerin (DMG) belirlenmesi üzerine gerçekleştirildi.

Daha sonra DEG ve DMGlerin kesişim kümesi bulunarak BD ve SZ gruplarında yukarı regule olup hipometillenen ve aşağı regule olup hipermetillenen genler ortaya çıkarılmıştır.

Son olarak da bulunan DEG ve DMGlerin gen ontolojisi (GO) analizleri g:Profiler kullanılarak yapılmıştır.

Sonuçlar: Çalışmada, BD ve SZ gibi psikiyatrik bozukluklar arasındaki gen ifadesi ve DNA metilasyonu farklılıklarının incelenmesi amaçlanmıştır. İnsan beyinciklerinden elde edilen gen ifadesi verileri ve DNA metilasyon verileri analiz edilmiştir.

Analiz sonuçlarına göre, BD ile kontrol grubu arasında 103 DEG tespit edilmiş olup bunların 74'ü upregüle ve 29'u ise downregüle olmuştur. Benzer şekilde, SZ ile kontrol grupları arasında 903 DEG belirlenmiştir ve bunların 331'i upregüle olmuşken 599'u downregüle olmuştur. Ayrıca, BD ile SZ grupları arasında yapılan karşılaştırmada 251 DEG saptanmıştır ve bunların 36'sı upregüle olmuşken 215'i downregüle olmuştur.

DNA metilasyon desenlerinin analizi ise, incelenen gruplar arasında belirgin farklılıklar olduğunu ortaya koymuştur. Örneğin, SZ ile kontrol grubu arasında 7352 DMG tespit edilmiştir ve bunların 3368'i hipermetillasyona uğrarken 3984'ü hipometillasyona uğramıştır. Benzer şekilde, BD ile kontrol grupları arasında 7552 DMG belirlenmiş olup bunların 3568'i hipermetillasyona uğrarken ve 3984'ü hipometillasyona uğramıştır. BD ile SZ grupları arasındaki karşılaştırmada ise 8077 DMG bulunmuştur ve bunların 4264'ü hipermetillasyona uğrarken 3813'ü hipometillasyona uğramıştır.

Sonuç olarak, bu çalışma, BD ve SZ gibi psikiyatrik bozuklukların moleküler mekanizmalarını anlamaya yönelik önemli bilgiler sunmuştur. Bu bulgular, bu bozuklukların patogenezini anlamak ve yeni tedavi stratejileri geliştirmek için temel oluşturabilir.

Tartışma: SZ ve BD gibi karmaşık nöropsikiyatrik hastalıkların moleküler patofizyolojisini anlamak için yaptığımız araştırma, bu hastalıklarda rol alan epigenetik ve genetik faktörleri incelemektedir. Özellikle, hipometilasyon ve yukarı regülasyonla ilişkilendirilen belirgin gen setlerinin tanımlanması, özellikle nörogelişimsel bozukluklarla potansiyel bağlantıları olanlar, anlayışımıza önemli bir katkı sağlıyor. Ayrıca, başlangıçta otizm spektrum bozukluğu (ASD) ve diğer nöropsikiyatrik durumlarla ilişkilendirilen bazı genler, daha yakından incelenmeyi hak eden yeni adaylar olarak ortaya çıkmaktadır. Epigenetik modifikasyonların, nörogelişim ve bağışıklık yanıtları arasındaki etkileşimlerin yanı sıra çeşitli psikiyatrik fenotipler arasında paylaşılan genetik temellerin keşfine yol açarak yeni bir bakış açısı sunmaktadır. Bu tartışmada, tanımlanan genleri derinlemesine inceleyerek, onların sinaptik iletim, bağışıklık düzenlemesi ve duyusal işleme üzerindeki rollerini keşfetmeyi amaçlıyoruz. Böylece, SZ ve BD'yi şekillendiren moleküler mekanizmalarla beraber bu hastalıkları anlamaya kapsamlı bir anlayış getirmeye çalıştık.

BD ve kontrol grupları arasında karşılaştırıldığında 'Bakır iyonuna hücresel yanıt' kategorisinde MT4, MT2A, MT1M, MT1E, MT1F, MT1H, MT1X ve MT1G genlerinin dikkate değer bir şekilde upregüle olduğu görülmektedir. Bu genler, metallothionein (MT) protein grubu ile ilişkilidir ve bulgularımız diğer çalışmalardan gelen destekleyici kanıtlarla uyumludur. Ayrıca, SZ'de MT1X'in upregüle olduğu gözlemlenmiştir, bu gözlem, Chen ve ark. (2013) tarafından yapılan araştırmada belirlenen benzer gen grupları ile paylaşılan temel patofizyolojiyi işaret edebilir.

İnsan metallothionein genleri, kognitif fonksiyonlar için esastır ve bakır ve çinko gibi metaller, oksidatif ajanlar ve inflamasyona yanıt vermede çeşitli rolleri vardır. Daha önceki çalışmalarda, nörotoksisiteye karşı koruyucu işlevleri belgelenmiştir. Serum MT-1'in düşük seviyeleri belirli bir çalışmada tanımlanmıştır, bu da SZ'de bu genlerin upregülasyonunu açıklayabilir. Bu fenomenin BD için de geçerli olabileceği, çünkü

çalışmamız benzer şekilde bu genlerin upregüle olduğunu ortaya koymuştur. Ayrıca, beyin inflamasyonunun hem SZ hem de BD'de doğrulandığına dikkat çekmek gerekir. Dolayısıyla, MT genlerinin upregülasyonu, bu durumların patogenezine karmaşık bir şekilde bağlı olabilir. Serum MT-1, SZ'yi öngörmek için değerli bir biyobelirteç olarak hizmet edebilir ve BD'deki gözlemlenen bu gen grubunun upregülasyonu, BD'li bireylerde SZ'den farklı gen ifadesi profili ortaya koyabilir (Yılmaz et al., 2023). Sonuç olarak, MT, SZ ve BD arasındaki ilişki henüz tam olarak keşfedilmemiştir ve daha fazla araştırma gerekmektedir.

BD, çeşitli patofizyolojik yorumlamalara rağmen, karmaşık bir psikiyatrik durumdur. Bağışıklık mekanizmalarındaki bozukluklar, BD'ye potansiyel bir açıklama getirebilir. Otoimmün koşullar ile ruhsal bozukluklar arasındaki bağlantı, bağışıklık sistemi ile BD arasında bir ilişki olduğunu göstermektedir. İnsan Lökosit Antijen (HLA) sistemi, hastalıklarla ilişkilendirilen bir gen grubudur. Bazı HLA genlerinin BD hastalarında düşük olduğu belirlenmiştir. Lityum, BD tedavisinde kullanılan bir ilaçtır ve HLA genlerinin tedaviye yanıt üzerinde etkili olabileceği düşünülmektedir. Özellikle, HLA-DRB1 aracılığıyla daha düşük inflamasyona sahip hastaların lityuma olumlu yanıt verdiği görülmüştür. HLA genleri, psikotropik tedavi yanıtı ve yan etkiler açısından da çeşitlilik gösterir. Bu genlerin farmakogenomik testlerde kullanılması, bireyselleştirilmiş tedavi için potansiyel sunabilir. Bu kapsamlı yaklaşım, psikiyatrik bozuklukların karmaşık mekanizmasını anlama ve daha etkili terapötik müdahaleler geliştirme yolunda önemli bir adım olabilir.

SNORD115, BD ve SZ'de ekspresyonu artan bir gen olarak öne çıkmaktadır. Bu bulgu araştırmamızla doğrulanmıştır. SNORD115, CRHR1, PBRM1, TAF1, DPM2 ve RALGPS1 olmak üzere beş diğer genin kontrolünü sağlar. Araştırmamızda SNORD115'in artışı ve PBRM1'in azalışı gözlemlendi ve her iki genin de BD, SZ ve katatoni ile ilişkisi literatürde belirtilmiştir. Özellikle, SNORD115'in CRHR1 ile ilişkisi ilgi çekicidir. SNORD115, kortikotropin hormon salınımı ile ilişkilendirilen CRHR1'in ekspresyonunu düzenler. BD hastalarında hipotalamus-hipofiz-böbrek üstü bezi (HPA) ekseni anormallikleri tanımlanmıştır. SNORD115'in CRHR1 üzerindeki düzenleyici etkisi ve CRHR1'in HPA ekseni üzerindeki rolü göz önüne alındığında, SNORD115'in BD'de artışı, bu hastalarda gözlenen HPA ekseni anormalliklerine katkıda bulunabilir. Bu bulgular, psikiyatrik bozukluklarda yer alan moleküler

etkileşimlerin karmaşıklığını vurgular ve SNORD115'in hedef genleriyle ve daha geniş moleküler peyzajla ilişkili karmaşık ilişkilerin daha fazla incelenmesi, yeni terapötik yöntemlerin ortaya çıkmasına ve temel patofizyolojinin anlaşılmasının geliştirilmesine ışık tutabilir.

Downregüle edilmiş PCLO, SYP, CAPZB ve KCNMB1 genlerinin GO analizi, duyusal algılamanın moleküler mekanizmalarına ilişkin potansiyel içgörüler sunmuştur. Özellikle, sinaptik işlevde önemli bir rol oynayan Piccolo Presynaptic Cytomatrix Protein'i kodlayan PCLO geni öne çıkar. Bulgularımız, bu genin BD ve majör depresyon hastalarında downregüle olduğunu gösteren önceki çalışmalarla uyumludur. PCLO'nun önemi, presinaptik aktif bölgedeki, sinaptik veziküllerin sinapslara bağlandığı yerdedir. Bu fonksiyon, monoaminlerin sinapslara salınımı için hayati önem taşır. PCLO'nun rolü sadece geleneksel sinapslarda değil, aynı zamanda duyusal iç saç hücrelerinde (IHC) de gözlemlenir, burada ribbon sinapsları, postsinaptik spiral ganglion nöronlarına bağlanarak ses iletilmesinde katkıda bulunur. Bu sinapslardaki Piccolo proteininin dahil olması özellikle dikkat çekicidir ve gözdeki rod hücrelerinde bulunması, görsel kodlamadaki rolünü de vurgular.

Araştırmamızda, SYP geninde dikkate değer bir downregülasyon gözlemlendi ve bu, sinaptik fonksiyon ve nöropsikiyatrik durumlar için potansiyel sonuçları olan önemli bir bulgu olarak belirlendi. Synapsin gen ailesinin bir üyesi olan SYP geni, sinaptofizin gibi proteinlerin kodlanmasında kritik bir rol oynar ve nörotransmitter salınımı ile sinaptogenezis de rol oynar. SYP'nin downregülasyonu, bu gen ile çeşitli nöropsikiyatrik durumlar arasında ilişkilendiren önceki araştırmalarla uyumlu bir şekilde, araştırmamızla örtüşmektedir. Bu bulgu, SYP'nin davranışı ve ruh hali modülasyonunda potansiyel rolünü vurgulamakta ve sinaptik protein seviyeleri ile belirli psikiyatrik semptomların ortaya çıkması arasında bir bağlantıyı önermektedir.

miRNA'lar, gen ifadesinin ve işlevsel yolların düzenlenmesinde önemli roller oynayarak, özellikle insanlarda SZ gibi çeşitli beyin bozukluklarında etkilidir. Özellikle, MIR-137 hakkında en büyük çalışmalardan birinde SZ ile ilişkili olduğu bulunmuştur. Araştırmamızda, SZ'nin GO analizinde MIR30E, MIR186, MIR29B2, MIR148B, MIR15A, MIR128-1, MIRLET7G, MIR124-1, MIRLET7F1 ve MIR181A2 genlerinin upregüle olduğu tespit edilmiştir. Örneğin, MIR30E'de yapılan

bir çalışmada, bu miRNA'nın SZ hastalarında kontrol gruplarına göre anlamlı derecede yüksek olduğu belirlenmiştir. MIR29B2 varyantlarının da SZ riskini artırdığı bulunmuştur. MIR-186'nın ise majör depresif bozukluk gibi belli ruh sağlığı bozukluklarıyla ilişkilendirildiği gözlemlenmiştir. MIR-148B'nin upregülasyonu hayvan modellerinde NMDAR'ın downregülasyonuna yol açabilir, bu da psikotik belirtilerle sonuçlanabilir. MIR128-1'in upregülasyonu normal beyin gelişimini etkileyebilir ve dolayısıyla SZ'nin patogenezinde rol oynayabilir. MIR-124 ise nöronal plastisiteyi düzenleyen beyine özgül miRNA'dır ve agresifliğe yol açabilen BDNF ve DRD4 genlerini hedef alarak pozitif belirtilerle ilişkilendirilmiştir. Bu bulgular, miRNA'ların SZ'nin patogenezinde kritik bir rol oynayabileceğini ve müdahale için bir hedef olarak tanımlanabileceğini göstermektedir.

BD'de hipermetile olan ve downregüle olan genlerin GO analizi, AP2M1, RPS6KA4, DPYSL2, DNM1, SH3GL2 ve CLTC gibi farklı metillenmiş ve düzenlenmiş genlerin "L1'in geri dönüşüm yolu" ile ilişkili olduğunu gösteriyor. L1, nöronal gelişimin bazı yönlerinde, özellikle aksonların uzaması ve nöronların göçü gibi, önemli bir rol oynamaktadır. L1'in aktin sitoskeleton ile koordinasyonu bu işlevler için hayati öneme sahiptir. Özellikle, DPYSL2 geninin hipermetilasyonu onun downregülasyonuna katkıda bulunabilir. Bu gen, nöronların gelişiminde yer alır ve akson oluşumunda hayati bir rol oynar. DPYSL2'nin düzensizliği nörogelişimde anormalliklere yol açabilir. Ayrıca, bu genin anormal metilasyonunun pediatrik BD hastalarında intihar riskinin artmasıyla ilişkilendirildiği gözlemlenmiştir. Bu bulgular, bu genin epigenetik düzenlemesinin çevresel risk faktörlerinden etkilenebileceğini göstermektedir. SH3GL2, endofilin A1'i kodlayan bir gen olup presinaptik terminallerde sinaptik vezikül geri dönüşümünde ve postsinaptik bölgede sinaptik plastisiteye katkı sağlar. Benzer şekilde, CLTC geni, L1 yolunun geri dönüşümünde AP2M1 ile birlikte işlev görerek klathrin aracılı endositozda kritik bir rol oynar. Bu genin hipermetilasyonu, BD ve SZ'nin patofizyolojisinde yatan mekanizmaların bir adayı olarak kabul edilmektedir. Bu araştırmalar, bu genlerin epigenetik düzenlemesinin psikiyatrik bozuklukların gelişimindeki rolünü anlamak için ilginç bir alan oluşturabilir.

BD'de histon proteinlerini kodlayan genlerin (HIST1H1D, HIST1H2BH, and HIST2H2AC) upregülasyonunu ve hipometilasyonunu vurgulayan araştırmamız, bu durumun altında yatan epigenetik mekanizmaların anlaşılmasına ışık tutmaktadır ve

bu genlerin epigenetik düzenlemedeki rollerinin daha fazla araştırılması için "meta-epigenom" terimini önermektedir. Ek olarak, valproik asidin histon deasetilaz inhibitörü olduğu da bilinmektedir. Sonuç olarak bu gen gruplarının farklılaşmış metilasyonu ve ekspresyonu BD'nin patofizyolojik mekanizmalarını açıklayabilir.

Sonuç olarak BD ve SZ hastalıklarının patofizyolojilerini açıklanması ve biyolojik belirteç bulunabilmesi için daha fazla çalışmaya ihtiyaç duyulmaktadır. Ayrıca çevresel faktörlerin bu hastalıkların oluşumundaki rolü de araştırmalıdır.

Anahtar Kelimeler: Bipolar Bozukluk, Şizofreni, Multiomik, Genetik, Epigenetik

ABSTRACT

MULTIOMIC DATA ANALYSIS OF SCHIZOPHRENIA AND BIPOLAR DISORDER

Cardak, Burak

MASTER'S THESIS, BIOLOGICAL DATA SCIENCE

Supervisor: Assist. Prof. Dr. M. Erkan Karabekmez

March 2024

This thesis investigates the genetic and molecular underpinnings of bipolar disorder (BD) and schizophrenia (SZ) through a multi-omics approach. The study aims to provide a comprehensive understanding of these psychiatric conditions by integrating gene expression and DNA methylation data obtained from human cerebellum samples. Differential expression analysis using DESeq2 and PyDESeq2 identified significant gene expression changes across psychiatric groups. Similarly, differential methylation analysis using the Fisher Exact test and CpGtools unveiled substantial DNA methylation differences among the studied groups. The analysis reveals significant differences in gene expression and DNA methylation patterns between patients with BD, SZ, and control individuals. Specifically, the comparison between SZ and control groups identifies 103 differentially expressed genes (DEGs) and 7352 differentially methylated genes (DMGs), while the comparison between BD and control groups reveals 903 DEGs and 7552 DMGs. Furthermore, the comparison between BD and SZ groups unveils 251 DEGs and 8077 DMGs. Functional enrichment analysis highlights the involvement of genes associated with neurodevelopmental disorders, synaptic transmission, and immune responses in the pathogenesis of BD and SZ. The analysis revealed significant upregulation of metallothionein genes in BD, suggesting their relevance to neuropsychiatric conditions, including SZ, and indicating their potential as biomarkers. Furthermore, differential expression patterns of human leukocyte antigen genes in BD patients underscore potential immune dysregulation, emphasizing personalized therapeutic approaches. Involvement of small nuclear ribonucleoproteins in splicing processes associated with BD suggests shared mechanisms with neurodegenerative disorders. Additionally, dysregulation of SNORD115 and its target

genes in both BD and SZ implicates molecular interactions in dysregulating the hypothalamic-pituitary-adrenal axis. Analysis of downregulated genes such as PCLO, SYP, CAPZB, and KCNMB1 revealed insights into common molecular mechanisms underlying sensory perception which might be related to auditory hallucinations in BD and SZ. Several upregulated miRNAs in SZ, including MIR30E, MIR186, MIR29B2, MIR148B, MIR15A, MIR128-1, MIRLET7G, MIR124-1, MIRLET7F1, and MIR181A2, were identified, suggesting their roles in SZ pathology. Altered levels of miR-186 and upregulation of miR-148B suggest involvement in drug-induced psychosis. Hypermethylation and downregulation of genes involved in the recycling pathway of L1 -cell adhesion molecule- in BD and upregulation and hypomethylation of genes encoding histone proteins shed light on potential epigenetic mechanisms underlying BD. These findings offer insights into the complex pathophysiology of BD and SZ, highlighting potential biomarkers and therapeutic targets for psychiatric disorders.

Key Words: Bipolar Disorder, Sczhizophrenia, Multiomics, Genetic, Epigenetic

I dedicate this thesis

to my beloved wife and daughter,

to all individuals suffering from psychiatric illnesses,

and to the Turkish Armed Forces.

TABLE OF CONTENTS

TABLE OF CONTENTS

LIST OF FIGURES

ABBREVIATIONS

ADRs	Adverse Drug Reactions
ASD	Autism Spectrum Sisorder
BA9	Brodmann Area 9
BD	Bipolar Disorder
CME	Clathrin Mediated Endocytosis
CNS	Central Nervous System
DEG	Differentially Expressed Gene
DMG	Differentially Methylated Gene
DNA	Deoxyribonucleic Acid
DSM-5	Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition
GEO	Gene Expression Omnibus
GO	Gene Ontology
GO-BP	Gene Ontology Biological Process
GWAS	Genome-Wide Association Studies
HLA	Human Leukocyte Antigen
HPA	Hypothalamic-Pituitary-Adrenal
ICD-11	International Classification of Diseases, 11th Revision
IHCs	Inner Hair Cells
KEGG	Kyoto Encyclopedia of Genes and Genomes
MDD	Major Depressive Disorder
MHC	Major Histocompatibility Complex
miRNAs	microRNAs

mRNA	messenger RNA
MT	Metallothionein
ncRNA	non-coding RNAs
NGS	Next-Generation Sequencing
NMDAR	NMDA Receptor
PCP	Phencyclidine
RISC	RNA-Induced Silencing Complex
RNA	Ribonucleic Acid
SZ	Schizophrenia
SNP	Single Nucleotide Polymorphism
snRNAs	small nuclear RNAs

1. INTRODUCTION

The biopsychosocial model is a scientific model designed to emphasize the limitations of the biomedical model, which held dominance in medicine over half a century ago (Engel, 1980). Despite the emergence of recent diagnostic and classification systems such as the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5), and the International Classification of Diseases, 11th Revision (ICD-11) in psychiatric conditions, these systems primarily incorporate the "psycho" and "social" aspects of the scientific model for diagnosis, often neglecting the "bio" component. The DSM-5 categorizes mental health disorders based on symptoms, signs, duration, and the level of distress they induce (American Psychiatric Association, 2013). While valuable, this system has limitations, including overlapping symptoms, subjective clinician assessments, and potential cultural differences. Subsequently, genetic studies have emerged as a challenge to traditional diagnostic systems (Smoller et al., 2019). There is a growing need for extensive genetic research in this field, highlighting the significance of incorporating biological aspects into the diagnostic model. Genetic research challenges current hierarchical diagnostic systems, revealing a 0.70 correlation of genetic factors among different mental health disorders. This suggests that psychiatric disorders share a common genetic foundation rather than being distinctly defined entities according to the DSM-5. Although there have been recent advancements in blood-based biomarkers and genetics of schizophrenia (SZ) and bipolar disorder (BD) for categorizing the diseases, gaining understanding, and diagnosing, these methods are not yet part of current medical practice (Lai et al., 2016; Frey et al., 2013; Gordovez & McMahon, 2020; Zamanpoor, 2020). Therefore, further research is needed in the "bio" part of the model to enhance our insight into these conditions and improve diagnostic systems.

Genetics constitutes the pivotal "bio" component of this model, significantly contributing to our understanding of psychiatric conditions. Substantial progress in this domain has significantly enhanced our comprehension of the genetic mechanisms underlying these conditions. Family, twin, and adoption studies have uncovered the

genetic underpinnings of psychiatric disorders, highlighting the profound influence of genetic factors for which heritability is around 40% to 80% in mental health disorders (Hoehe & Morris-Rosendahl, 2018). Given the high heritability of psychiatric conditions, these factors have been under investigation for over 60 years. The prior Mendelian studies aimed at identifying a single major gene underlying psychiatric conditions like SZ and BD led to significant challenges, notably a high false-positive rate. Similarly, during the candidate gene era, genes linked to neurotransmitters were initially considered strong candidates but eventually resulted in an elevated number of false-positive outcomes. However, the advent of genome-wide association studies (GWAS) marked a significant leap forward in the field, leading to numerous replicated advancements and the discovery of multiple genes associated with various psychiatric conditions (Kendler, 2022; Peedicayil, 2023). To sum up, the wealth of research suggests that psychiatric conditions involve multiple small-effect variants, forming a polygenic nature. Yet, the specific impact of each variant on the phenotype remains inadequately comprehended (Hoehe & Morris-Rosendahl, 2018).

Epigenetic mechanisms also play a significant role in gene regulation, leading to these mechanisms may contribute to the pathogenesis of psychiatric conditions by influencing gene expression. There are three major epigenetic mechanisms: deoxyribonucleic acid (DNA) methylation, histone modifications, and ribonucleic acid (RNA) mediated regulations. DNA methylation affects the DNA which undergoes covalent modifications through the methylation process primarily targeting CpG islands. Another epigenetic mechanism involves histone modification, wherein the histones enclosing DNA undergo post-translational modifications, with acetylation being one of the most prevalent modifications that can influence gene expression. Thirdly, microRNAs (miRNAs) play a role as an epigenetic mechanism by suppressing the translation of messenger RNA's (mRNA) into proteins, thereby influencing gene expression (Peedicayil, 2014). Among the extensively studied epigenetic alterations, DNA methylation plays a crucial part in various biological processes (Skvortsova et al., 2019; Li & Tollefsbol, 2021).

Genetics and epigenetics exhibit a mutual interplay or reciprocal relationship. While environmental changes might not readily modify the DNA sequence, they can significantly impact gene expression by influencing the epigenetic mechanisms that

govern gene expression. This phenomenon underscores the potential for environmental factors to shape genetic expression without altering the DNA sequence itself, a crucial aspect that warrants exploration and understanding. Despite the array of epigenetic mechanisms mentioned earlier, methylation stands out as the most extensively explored and prominent mechanism (Peedicayil, 2023). All of the psychiatric conditions including SZ and BD are determined by multiple genes, suggesting a polygenic nature (Peedicayil, 2023). When considering the polygenic nature of psychiatric conditions, the rationale often lies in the changes of underlying environmental factors, which includes early life adversity, maternal prenatal stress, poverty, social factors, etc., affecting genes via epigenetic mechanisms. For example, according to Perroud et al. (2016), the methylation of the serotonin receptor 3A (5HT3A4) is associated with increased disease severity and linked to childhood maltreatment with BD (Perroud et al., 2016). Therefore, research on epigenetic mechanisms can illuminate the reciprocal relationship between environmental factors and genetic material. This understanding aids in diagnosing mental disorders and provides insight into underlying mechanisms, facilitating the development of medications that target these epigenetic mechanisms.

The discovery of the double helical strand of DNA by Francis Crick and James D. Watson in 1953 marked a pivotal moment in scientific history. This breakthrough paved the way for the formulation of the central dogma, suggesting that genomic DNA not only defines species but also unravels the mysteries of life itself. In 1977, Frederick Sanger played a crucial role in the development of DNA sequencing technology. Over time, technological advancements have significantly enhanced sequencing, improving both accuracy and speed. As the 21st century unfolded, the Sanger sequencing method became a cornerstone in the monumental Human Genome Project. The advent of next-generation sequencing (NGS) further revolutionized genome sequencing, simplifying the process. Simultaneously, the exponential growth in data spurred the creation of innovative tools and methods for seamless data integration (Liu et al., 2012).

In addition to genomics, which involves the comprehensive study of the entire genome using NGS and microarrays, various 'omic' data types have emerged. Transcriptomics focuses on reading RNA sequences through the use of microarrays and RNA-Seq. Proteomics delves into the study of proteins, exploring their functions and structures

using mass spectrometry and gel electrophoresis. Metabolomics involves the analysis of metabolites in an organism, employing techniques such as nuclear magnetic resonance and liquid chromatography-mass spectrometry. Epigenomics, investigating epigenomic mechanisms, relies on methods like bisulfite sequencing and ChIP-Seq. The integration of different 'omic' data sets through advanced methods is referred to as multi-omic data analysis. This approach harnesses the power of diverse molecular information, opening new avenues for comprehensive biological insights (Subramanian et al., 2020).

The goal of multi-omics is to merge multiple omics datasets such as genomics, transcriptomics, and proteomics to assist in the analysis, visualization, and interpretation of biological processes with the ultimate aim of uncovering the intricacies thereof. Multi-omics initiatives have assumed a prominent role in the field of biomedical research, resulting in the emergence of fresh perspectives on biological phenomena and processes (Krassowski et al., 2020). Traditionally, researchers have examined mental health by studying individual subsystems such as genomics, transcriptomics, proteomics, and clinical data independently. It is now clear that these subsystems are interconnected in influencing mental health. Consequently, researchers are now exploring these subsystems collectively to gain a comprehensive understanding of the complex biology underlying psychiatric conditions, exploration of blood-based biomarkers, and markers for diagnosis. Despite the growing availability of data, the clinical application of this to research remains a significant challenge in mental health (Sathyanarayanan et al., 2023). On the other hand, integration of multi-omic analysis is increasingly becoming a crucial component of cancer treatment protocols. This entails a thorough examination of patient data to guide treatment approaches, identify subtypes, and uncover pathways (Liu et al., 2023; Fernandez-Rozadilla et al., 2023; Lindskrog et al., 2021).

BD is a persistent mood disorder distinguished by the presence of one or more episodes of mania intermingled with phases of depression. Manic episodes manifest as elevated mood, irritability, heightened energy, reduced sleep, rapid speech, racing thoughts, inflated self-confidence, and impulsivity (American Psychiatric Association, 2013; World Health Organization, 2019). Regardless of one's background, BD impacts over 1% of the global population. Moreover, BD is a chronic disorder resulting in cognitive

and functional limitations as well as an increased risk of mortality, notably due to suicide (Grande et al., 2016). While a diagnostic test is currently unavailable for this condition, clinical features are essential for its diagnosis. Furthermore, various subtypes of this condition have been identified and are distinguished based on the clinical features such as timing, symptom severity, and the presence of other psychiatric symptoms (Craddock & Sklar, 2013). While the intricate relationship between genotype and the manifestation of phenotypic traits in psychiatric disorders is undeniably convoluted, uncovering its genetic underpinnings remains a formidable challenge. The interaction of several genes with advanced genetic mechanisms, alongside the effects of environmental and unpredictable elements, constitutes the fundamental points (Craddock & Sklar, 2013; Gordovez & McMahon, 2020). BD is one of the most inheritable psychiatric conditions; therefore, its underlying genetic elements have been explored in the last decades with the rising of recent genetic advancements (Gordovez & McMahon, 2020; Harrison et al., 2018; Kato, 2019; McIntyre et al., 2020). High-throughput technologies including NGS tools alongside the advancements in bioinformatic methods have provided insight into the underlying mechanisms in BD in the last two decades (Niemsiri et al., 2023; Mokhtari et al., 2022).

There are several multi-omics research applied to BD. The research conducted by Niemsiri et al. (2023) utilized a combination of transcriptomic and genomic data, employing network analysis to discover genes linked to lithium response in individuals with BD. Abdolmaleky et al. (2019) conducted research analyzing transcriptomic and methylomic data to investigate pathways associated with the loss of lateralization in SZ and BD. Chen et al. (2014) conducted analyses in postmortem cerebellum that correlated methylation and expression data in subjects diagnosed with SZ and BD, identifying relations to PIK3R1, BTN3A3, NHLH1, and SLC16A7. Chen et al. (2018) identified that the transcription factor POU3F2 oversees a gene coexpression network involving both the transcriptomic data and microRNAs in brain tissue obtained from individuals diagnosed with BD and SZ. Fries et al. (2017) investigated DNA methylation patterns and mitochondrial DNA copy numbers in individuals with BD from their peripheral bloods to uncover mechanisms related to accelerated aging. Gandal et al. (2018) combined transcriptomic and genomic data obtained from brain

tissues, revealing pathological alterations in splicing and expression associated with both BD and SZ. The study conducted by Pisanu et al. (2018) highlighted the involvement of zinc finger proteins in the response to lithium treatment specifically observed in peripheral lymphoblasts. This insight was derived through the utilization of genome-wide expression and genotype data, shedding light on the role of these proteins in the context of lithium response. Pai et al. (2019) correlated altered IGF2 methylation patterns with dopamine synthesis, employing a comprehensive analysis of RNA expression, DNA methylation, and ChIP sequencing data. Brain tissues from Brodmann Area 9 (BA9) were examined by Zhao et al. (2015), demonstrating methylation changes that impacted RNA expression in both BD and SZ. Multi-omics research in BD unveils diverse molecular pathways and alterations through transcriptomic, genomic, and epigenomic analyses conducted on both brain tissue and peripheral blood samples, thereby identifying relevant genes and pathways.

The comprehension of the underlying pathology behind BD remains insufficiently elucidated. BD is among the most heritable mental illnesses. Related to this, previous research demonstrates a 40-70% risk of BD among monozygotic twins and a 5-10% risk among first-degree relatives (Craddock & Jones, 1999). While environmental factors, encompassing psychological and social mechanisms, contribute to the underlying pathophysiology of BD, studies -such as family, twin, and adoption studies- play a crucial role in providing evidence (Craddock & Sklar, 2013; Craddock & Jones, 1999). Nevertheless, uncovering its underlying genetic fundamentals poses a significant challenge. BD is not a monogenetic condition; instead, its genetic basis is complex, involving numerous genes, each with varying effect sizes. GWAS has enhanced our understanding of the genetic factors contributing to BD (Gordovez & McMahon, 2020). The most important genes associated with BD derived from GWAS studies can be summarized as ANK3, CACNA1C, TRANK1, DCLK3, DGKH, SCN2A, SLC4A1, GRIN2A, RIMS1, NCAN, TENM4, GLT8D1 (Gordovez & McMahon, 2020; Fabbri, 2021). Moreover, additional genetic markers have been identified in other research studies (Gordovez & McMahon, 2020; Fabbri, 2021).

Epigenetic mechanisms, particularly DNA methylation, can impact gene expression in BD, representing the influence of environmental factors on DNA, and this has been investigated extensively for the last couple of decades. Certain environmental

elements, such as childhood trauma and chronic stress, interact via epigenetic mechanisms with DNA and are linked to an elevated occurrence of BD. As a result, these studies have identified numerous genes exhibiting differential methylation patterns associated with BD (Fries et al., 2016). Some of the genes are differentially methylated in BD, such as hypomethylation of HCG9 in brain tissues taken from prefrontal, parietal, and occipital cortices and corpus callosum (Kaminsky et al., 2012); hypomethylation of MB-COMT taken from the frontal lobe (Abdolmaleky et al., 2006); several differentially methylated genes were identified in samples taken from the frontal lobe (Mill et al., 2008); GABRB2 dysregulated samples taken from postmortem brain (Zhao et al., 2012); hypermethylation of TET1 samples taken from the parietal lobe; hypomethylation of HLA complex group 9 gene samples taken from the prefrontal, parietal, and occipital cortex; hypomethylation of COX-2 and hypermethylation of BDNF and DBNL samples taken from the frontal cortex (Rao et al., 2012; Gürel et al., 2020); hypomethylation of KCNQ3 samples taken from the prefrontal cortex (Kaminsky et al., 2015); hypomethylation of IGF2 samples taken from the frontal cortex (Pai et al., 2019); hypermethylation of Reelin (Ludwig & Dwivedi, 2016); hypermethylation of SLC6A4 sample taken from postmortem brain (Sugawara et al., 2011).

SZ is a multifaceted psychiatric condition characterized primarily by psychotic symptoms, encompassing disruptions in thought, perception, behavior, speech, cognition, and mood, and it holds a global prevalence of 1%, placing it among the top 10 conditions worldwide. Core symptoms involve thought disturbances, such as delusions, perceptual anomalies, such as hallucinations, and cognitive impairments, affecting attention and memory. Diagnostic guidelines outlined in the DSM-5 and ICD-11 necessitate symptoms persisting for at least one month for an accurate diagnosis of SZ (American Psychiatric Association, 2013; World Health Organization, 2019). Notable morphological alterations observed in SZ include lateral ventricular enlargement and reductions in overall brain volume. Functional changes in regions like the frontal cortex are important as well as other brain regions. Moreover, the dysregulation of dopamine has been extensively documented as a key chemical imbalance in the disorder. The primary treatment for this disorder involves antipsychotic medications that act by blocking dopamine receptors across various

brain regions. On the other hand, the estimations propose that approximately 80% of the susceptibility to SZ within a population can be attributed to heritable factors through assessments derived from twin and family studies (Jauhar et al., 2022; Marder & Cannon, 2019). SZ represents a complex and polygenic condition, wherein not only rare mutations but also common single nucleotide variants can contribute to its development, albeit with relatively minor individual effects (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014; Purcell et al., 2014). Furthermore, environmental factors such as adverse childhood events have been identified as potential factors to the susceptibility to SZ (Jauhar et al., 2022; Marder & Cannon, 2019).

Multiple multi-omics studies have been conducted in the context of SZ (Guo et al., 2023; Zhang et al., 2023; Mao et al., 2023; Zhou et al., 2022; Fan et al., 2022). Guo et al. (2023) analyzed the response to antipsychotic treatment using polygenic risk scores (PRS) and differential methylation-based polymethylation scores. They discovered associations between treatment response and specific genes such as LINC01795, DDHD2, SBNO1, KCNG2, SEMA7A, and RUFY1, indicating potential connections in gene-environment interactions. Zhang et al. (2023) integrated transcriptome-wide association studies, gene coexpression, and differentially expressed gene (DEG) analysis, identifying PCCB as a potential risk factor for SZ. Mao et al. (2023) conducted a review on spatial multiomic research, revealing GRM3 and CACNA1C as risk loci within the hippocampus of SZ patients. In their research, Zhou et al. (2022) conducted extensive genome-wide and epigenome-wide association studies within the context of SZ, utilizing peripheral blood samples to delineate the pertinent genetic factors linked to the onset of metabolic syndrome induced by antipsychotic medication. Fan et al. (2022) merged data from the intestinal microbiota and serum metabolome to investigate the interaction between gut microbiota and the immune system in SZ. There are various types of multi-omics analyses conducted on SZ patients, using samples from either brain tissues or peripheral tissues. These studies employ different combinations of methylation, gene expression, and metabolomics to identify potential genetic markers for SZ.

Despite its heritability and familial transmission, SZ poses a persistent challenge in pinpointing precise genetic determinants, despite substantial evidence supporting a genetic influence from family and twin studies (van de Leemput et al., 2016). Due to its polygenic nature and complexity, understanding the underlying neuropathology and molecular mechanisms of this psychiatric disorder remains complex, for example far beyond the understanding of single-gene disorders (Farsi & Sheng, 2023). Leveraging genetic advancements has allowed the classification of these genetic associations into three main categories: common variants, copy number variants, and rare coding variants, each of which has a different effect size on the risk of developing SZ. Recently, a multitude of rare coding variants with a significant impact on the development of the condition have been unearthed (Farsi & Sheng, 2023). Trubetskoy et al. (2022) identified around 120 genes associated with SZ through GWAS (Trubetskoy et al., 2022). The most important genes related to SC can be summarized as SLC39A8, C4, 22q11.2 microdeletion, 2p16.3 CNV, GRIN2A, GRIA3, SV2A, DNM3, CACNAQG, HCN4, SP4, SETD1A, ASH1L, NR3C2, HIST1H1E, ZMYM2, KDM6B, ANKRD12, STAG1, CUL1, HERC1, DAM120A, AKAP11 (Farsi & Sheng, 2023; Singh et al., 2022).

DNA methylation, among other epigenetic mechanisms, holds the potential to influence gene expression in SZ, signifying how environmental factors can affect DNA. Previous epigenome-wide studies from either from brain or peripheral tissues detected some differentially methylated genes CACNB2, PRKN, THAP1, KCNQ4, C17orf53, HTR2A, GAD1, RELN, GNA13, CAPNS1, GABPB2 (Chen et al., 2021).

Various studies have been conducted associated with BD, utilizing samples from postmortem brain tissues and peripheral tissues like saliva or blood cells. Considering the importance of investigating brain tissues from different regions in understanding pathophysiology of BD, the current feasibility allows for the data to be solely used for retrospective analysis. On the one hand, peripheral tissues have been extensively studied. Collecting blood samples is more convenient, and these tissues offer greater potential for prospective analysis (Mokhtari et al., 2022). Differences in gene expression across different tissues in mental health disorders like major depressive disorder (MDD) may vary. However, when we analyze enrichments, we consistently find similarities, especially in genes related to the immune system. Despite this, blood

analysis tends to provide less comprehensive insights compared to brain tissue, which is the primary site of occurrence for mental health disorders (Mokhtari et al., 2022).

The cerebellum is implicated in SZ, challenging the conventional notion that it is solely associated with movement disorders. Recent neuroimaging studies have revealed diminished activity in the cerebellum in individuals with SZ, potentially linked to blunted affect (Mothersill, Knee-Zaska, & Donohoe, 2016). Furthermore, investigations indicate that the connectivity between the prefrontal cortex and the cerebellum plays a role in contributing to negative symptoms observed in SZ (Brady et al., 2019).

In summary, the biopsychosocial model provides a comprehensive framework that extends beyond the limitations of the biomedical model, offering a holistic understanding of psychiatric conditions. While contemporary diagnostic systems primarily emphasize the 'psycho' and 'social' aspects, the integration of the 'bio' component, especially genetics and epigenetics, is crucial for a more nuanced comprehension of mental health disorders. Advances in genetic research challenge traditional diagnostic hierarchies, emphasizing the polygenic nature of psychiatric conditions. The burgeoning field of multi-omics, incorporating genomics, transcriptomics, proteomics, and epigenomics, promises to unravel intricate molecular pathways. As we delve into the complexities of BD and SZ, the convergence of genetic, epigenetic, and multi-omic approaches holds the key to deciphering the underlying mechanisms, ultimately paving the way for improved diagnostics and targeted interventions in the realm of mental health. By analyzing expression and methylation data, the aim is to unearth differentially expressed and differentially methylated genes, unraveling the intricate molecular landscapes that contribute to the pathogenesis of these disorders. This integrative investigation, encompassing genetic, epigenetic, and multi-omic dimensions, seeks to advance our knowledge, refine diagnostic paradigms, and open avenues for targeted therapeutic interventions in the complex realm of mental health.

2. METHODS

The gene expression data from human cerebellum were retrieved from GSE35974, and the DNA methylation data were retrieved from GSE38873 of the Gene Expression Omnibus (GEO) database (Chen et al., 2013; Chen et al., 2014). Gene expression data and DNA methylation data are available for 118 patients, comprising 36 with BD and 39 with SZ, and 43 control individuals.

Some patients within the gene expression dataset exhibited three expression values per patient. These values showed notably higher correlations compared to other individuals. Consequently, only one expression value was utilized and aligned with the corresponding methylation data. Additionally, the methylation data included 13 random replicates without specification regarding the type of replication. Hence, a random selection was made, and only one set of replicated data was chosen and paired with the gene expression profile.

The RNA-seq expression data exported from GSE35974 underwent analysis using DESeq2, a widely recognized statistical method for detecting DEGs (Love et al., 2014). DESeq2 incorporates methodological advancements alongside novel features, enabling a robust quantitative analysis of comparative RNA-seq data through the application of shrinkage estimators for both dispersion and fold change, thereby enhancing the reliability of differential gene expression detection. DESeq2 relies on the negative binomial distribution as part of its statistical framework for analyzing RNA-seq data.

The characteristics of RNA-seq count data do not typically follow a normal distribution. Count data can be modelled with poisson distribution and binomial distribution both of which are similar based on discrete events and shows the probability of an event in a population. In RNA-Seq data, numerous RNA transcripts are available, and the likelihood of selecting a specific transcript is exceedingly low. On the other hand, this also relies on the association between the mean and variance within dataset. If they are equal, poisson distribution is more appropriate.

In an analysis of RNA-Seq data, we observe different patterns. Genes with high expression levels show more variation between individuals, highlighting differences in their expression. On the other hand, genes with low expression levels display scattered and variable data, indicating less predictability. Unlike a typical distribution, where the average and spread are connected, RNA-Seq data doesn't follow this pattern. The variance in gene expression doesn't correspond proportionally to the mean expression level. This deviation from the norm makes the usual Poisson distribution, used for count data, unsuitable for accurately representing RNA-Seq characteristics. Variability between individuals is anticipated. In cases where the variance deviates from the mean, the Binomial distribution is an appropriate model. Particularly, when the mean is less than the variance, as seen in RNA-seq data, it aligns with the characteristics of the negative Binomial distribution.

DESeq2 was originally developed as a package within the Bioconductor project specifically designed for the R statistical computing environment (Love et al., 2014). PyDESeq2 serves as an implementation of DESeq2, focusing on the differential expression analysis of RNA-Seq data (Muzellec et al., 2023). This tool leverages the advantages of Python, utilizing libraries such as NumPy and SciPy for efficient computing. It also provides the capability to work seamlessly with machine learning and data science frameworks, benefitting from Python's widespread usage in programming. According to the findings in the study by Muzellec et al. (2023), the comparison between R and Python ultimately concludes that PyDESeq2 stands out as a rapid and dependable package for RNA-seq data's differential gene expression analysis.

Differential expression analysis was performed for comparisons between BD and Control groups, SZ and Control groups, and BD and SZ groups. DEGs were filtered based on Bonferroni-adjusted p-values below 0.05. Furthermore, for each comparison group, DEGs were categorized into three groups: total DEGs, upregulated DEGs, and downregulated DEGs. Each set of DEG groups underwent gene ontology (GO) analysis to elucidate their functional significance by using g:Profiler, which is a web

service designed to perform functional enrichment analysis and map gene identifiers (Kolberg et al., 2023).

Despite the existence of numerous diverse analysis methods for differential methylation analysis, none of them consistently secures the top rank across all benchmarking assessments (Piao et al., 2021). Hence, the DNA methylation data was analyzed using the Fisher Exact test, employing the CpGtools package that offers various modules designed for differential methylation analysis (Wei et al., 2021).

Differential methylation analysis was conducted for comparisons between BD and Control groups, SZ and Control groups, as well as BD and SZ groups. Differentially methylated genes (DMGs) were filtered based on adjusted p-values (Bonferroni correction) below 0.05. Additionally, for each comparison group, DMGs were classified into three groups: overall DMGs, hypermethylated DMGs, and hypomethylated DMGs.

Figure 1 in page 14 shows that the methodological workflow for the analysis of DEGs involved several key steps. Initially, data collection was performed from the GEO Database, comprising RNA-seq expression data essential for subsequent analyses. Subsequently, the obtained expression values were formatted into a suitable dataframe format to facilitate analysis using DESeq2, a widely recognized statistical method for detecting differential gene expression. Following this, the DESeq2 analysis was conducted, allowing for the identification of genes exhibiting significant expression changes across the studied conditions. The resulting DEGs were then scanned based on adjusted p-values (Bonferroni correction) below 0.05, indicating statistical significance. Finally, gene enrichment analysis was carried out using the g:Profiler tool, enabling the elucidation of the functional significance and biological pathways associated with the identified DEGs. This systematic workflow ensured a comprehensive and rigorous approach to uncovering key genes and pathways underlying the studied biological conditions.

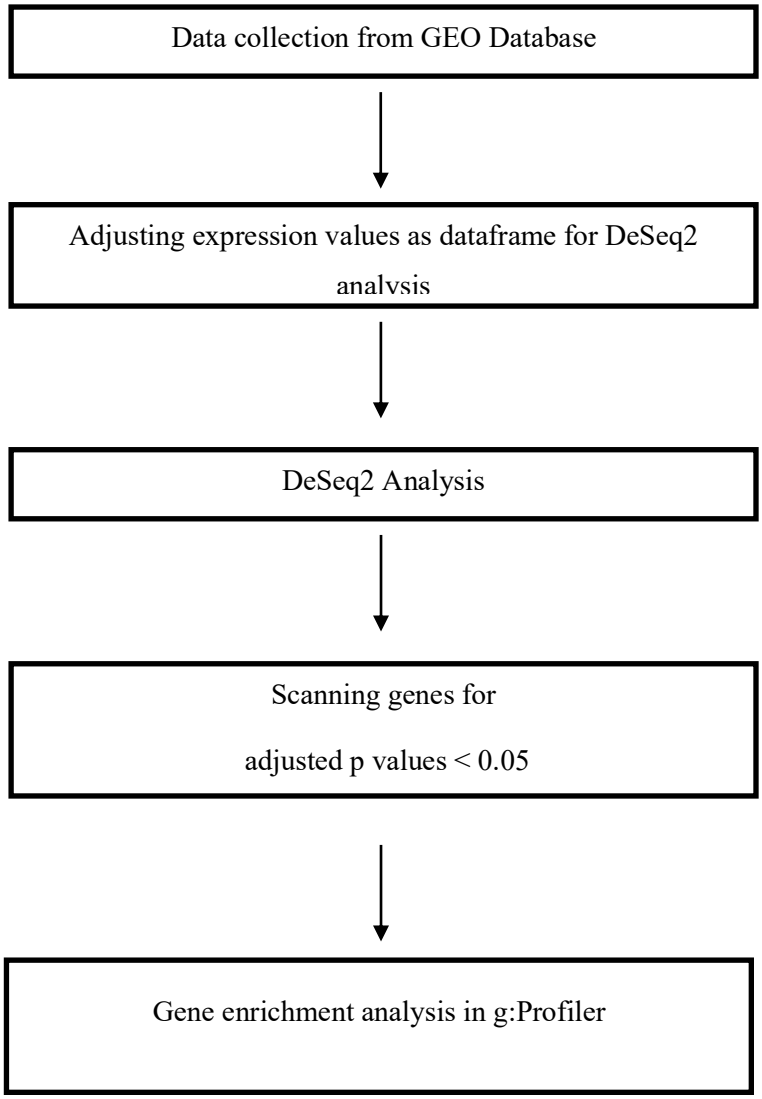

Figure 1. Workflow For DEG Analysis

3. RESULTS

The differential expression analysis highlights significant gene expression changes across distinct psychiatric groups as shown in Table 1. The SZ vs. control comparison revealed 103 DEGs, with 74 upregulated and 29 downregulated genes. Similarly, the BD vs. control groups exhibited a total of 903 DEGs, comprising 331 upregulated and 599 downregulated genes. Furthermore, the comparison between BD and SZ groups identified 251 DEGs, including 36 upregulated and 215 downregulated genes.

Table 1. Number of DEGs in Our Comparision Groups

	Overall DEGs	Upregulated DEGs	Downregulated DEGs
BD vs Control	103	74	29
SZ vs Control	903	331	599
BD vs SZ	251	36	215

Table 2. Number of DMGs in Our Comparision Groups

	Overall DMGs	Hypermethylated DMGs	Hypomethylated DMGs
BD vs Control	7552	3568	3984
SZ vs Control	7352	3368	3984
BD vs SZ	8077	4264	3813

Our analysis of DNA methylation patterns revealed substantial differences among the studied groups as shown in Table 2. Specifically, the SZ vs. control comparison identified a total of 7352 DMGs, with 3368 hypermethylated and 3984 hypomethylated genes. Similarly, the BD vs. control comparison showed 7552 DMGs, comprising 3568 hypermethylated and 3984 hypomethylated genes. Further, the BD

vs. SZ comparison unveiled 8077 DMGs, including 4264 hypermethylated and 3813 hypomethylated genes.

Supplementary Tables 1-9 presents statistically significant GO, gene ontology biological process (GO-BP) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways that DEGs in our comparison groups enriched.

The GO analysis of DEGs in BD revealed significant results, as detailed in Supplementary Table 1-3. Notably, 'cellular response to copper ion' and 'cellular response to zinc ion' in Supplementary Table 1 is associated with genes MT4, MT2A, MT1M, MT1E, MT1F, MT1H, MT1X, and MT1G, which exhibited significant upregulation in the comparison of BD and control groups. These genes are also involved in ‘response to metal ion’ and ‘response to inorganic substance’ processes. The genes HLA-DRA, HLA-DPA1, HLA-DRB1, HLA-DPB1, HLA-DQA1, and HLA-DRB3 are implicated in most of the GO-BPs in BD.

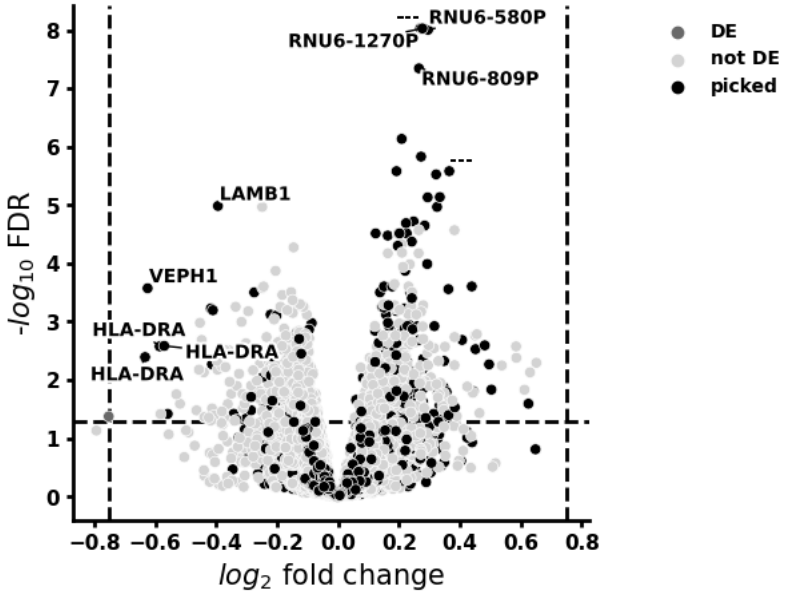

Figure 2. DEGs of BD Compared to Control Group

Figure 2 illustrates the differential expression of genes in BD compared to the control group, with the x-axis representing the log2 fold change for both upregulated and

downregulated genes. Notably, several genes stand out in this comparison, including HLA-DRA, VEPH1, the RNU6-1 gene family, and LAMB1. These genes exhibit significant changes in expression levels.

When specifically assessing upregulated genes in the GO analysis, detailed in Supplementary Table 2, distinctive outcomes emerged. Commonly involved in all these GO-BP were certain upregulated genes, such as the RNU6-1 gene family, aside from those associated with metal ion processes. RNU6-1 family is related to the GO-BP of the formation of quadruple SL/U4/U5/U6 small nuclear ribonucleoproteins (snRNP), which is notably implicated in this pathway. Additionally, upregulated gene families such as SNORD115 and ZNF also contribute significantly to various GO-BP, as indicated in Supplementary Table 2.

In Supplementary Table 3, the GO analysis of downregulated genes in BD is presented. While specific GO-BP are not identified, certain genes are more consistently associated with these processes, including FCER1G, FCRG2B, ATP13A2, KAT5, NCKAP1L, SYT1, RASGRP1, CORO1A, CHMP2A, PRNP, ITGB3, CD74, ATG5, CDK5, PTK2B, SYK, NR4A3, HLA-DRB1. Notably, downregulated genes, which analysed by using Reactome, PCLO, SYP, CAPZB, KCNMB1 are implicated in the sensory perception of sound.

The GO analysis of DEGs in SZ revealed significant results, outlined in Supplementary Table 4-6. Notably, certain MIR genes, including MIR30E, MIR186, MIR29B2, MIR148B, MIR15A, MIR128-1, MIRLET7G, MIR124-1, MIRLET7F1, MIR181A2, are implicated in the cellular function of the RNA-induced silencing complex (RISC), which is a multiprotein complex and emerge as an important feature in the GO analysis.

The GO analysis of exclusively upregulated genes reveals distinct GO-BP involved in SZ patients. Particularly, GO-BP such as 'regulation of gene expression,' 'miRNA-mediated post-transcriptional gene silencing,' 'regulatory Non-coding RNAs (ncRNA) mediated post-transcriptional gene silencing,' and 'post-transcriptional gene silencing' are common in this condition, as indicated in Supplementary Table 5.

Supplementary Table 6 presents the GO analysis of downregulated genes, revealing significant GO-BP in SZ patients compared to the control group. These processes include 'enzyme-linked receptor protein signaling pathway,' 'transmembrane receptor protein serine/threonine kinase signaling pathway,' 'integrin-mediated signaling pathway,' and 'basement membrane organization.

The GO analysis of DEGs in BD, compared to SZ, revealed significant results detailed in Supplementary Tables 7-9.

Key GO-BPs that are more pronounced in BD groups than in those with SZ are emphasized in Supplementary Table 7. Notably, genes such as NSUN4, ERCC6, METTL15, METTL16, THADA, SMARCB1, METTL6, and MEPCE are consistently implicated in the majority of these GO-BPs, underscoring their significant relevance in BD compared to SC.

In the analysis of upregulated genes, specific GO-BP emerge, including 'SRP-dependent cotranslational protein targeting to membrane, signal sequence recognition,' and 'protein targeting to ER'. Notably, the RN7SL gene family stands out as a key player in these pathways.

Table 3. Number of Genes After Multiomic Analysis of RNA Expression and Methylation Data

	Hypermethylated and Downregulated	Hypomethylated and Upregulated
BD vs Control Group	113	21
SZ vs Control Group	5	7
BD vs SZ	42	1

Table 3 presents a multiomic analysis of hypermethylated downregulated genes and hypomethylated upregulated genes in our comparison groups. In the BD vs control group, 113 genes are hypomethylated and downregulated, and 21 genes are hypomethylated and upregulated. The SC vs control group exhibits 5 hypermethylated downregulated genes and 7 hypomethylated upregulated genes. Comparing BD and

SC reveals 42 hypermethylated downregulated genes and 1 hypomethylated upregulated gene.

In Supplementary Table 10, the results of the GO analysis for hypermethylated and downregulated genes in BD compared to the control group are presented. GO-BP and Reactome underwent analysis. Notably, pathways such as the recycling pathway of L1, Retrograde neurotrophin signaling, Signaling by NTRK1 (TRKA), L1CAM interactions, DARPP-32 events, and signaling by NTRKs emerged as significantly important processes based on this analysis.

In Supplementary Table 11, the results highlight several significant GO-BP terms and KEGG pathways influenced within this gene group. Noteworthy pathways include 'regulation of transcription by RNA polymerase II,' 'transcription by RNA polymerase II,' and 'Epstein-Barr virus infection.' Particularly, the following genes play a role in these GO-BPs: PWAR, SIN3A, NTN1, ZNF451, PIK3R1, TRAF6, WNT8A, SALL3, and ATAD2.

The genes DHDDS, CD4, ITGA8, SCG2, and AKR1C2 exhibited hypermethylation and downregulation in the SC group when compared to normal samples. The results of the GO analysis are presented in Supplementary Table 12, revealing a significant association with molecular functions related to IL-16. Additionally, the transcription factors FKHL14 and Freac-7 were identified as significant in this analysis.

Seven genes (CDK8, TMEM56, STXBP5, SMOX, DIP2C, BARHL2, SMARCD2) exhibited significant concurrent hypomethylation and downregulation. GO analysis identified two molecular functions: norspermine:oxygen oxidoreductase activity and N1-acetylspermine:oxygen oxidoreductase (N1-acetylspermidine-forming) activity.

The multiomics analysis of both conditions yielded no discernible outcome in terms of significant GO.

4. DISCUSSION

In unraveling the intricate molecular landscape of SZ and BD, our investigation delved into the epigenetic and genetic factors governing these complex neuropsychiatric conditions. The identification of distinct gene sets associated with hypomethylation and upregulation in SZ and BD, particularly those with potential cross-links to neurodevelopmental disorders, adds a layer of complexity to our understanding. Notably, some genes initially linked to autism spectrum disorder (ASD) and other neuropsychiatric condition emerged as novel candidates deserving of closer scrutiny. The interplay between epigenetic modifications, neurodevelopment, and immune responses introduces a nuanced perspective, prompting exploration into shared genetic underpinnings across diverse psychiatric phenotypes. As we navigate through the molecular intricacies, the potential implications for biomarker discovery, targeted therapeutics, and personalized treatment strategies become increasingly apparent. In this discussion, we delve into the nuances of the identified genes, exploring their roles in synaptic transmission, immune regulation, and sensory processing, fostering a comprehensive understanding of the molecular landscape shaping SZ and BD.

The GO category 'Cellular response to copper ion' as depicted in Supplementary Table 1 is connected to the noteworthy upregulation of genes MT4, MT2A, MT1M, MT1E, MT1F, MT1H, MT1X, and MT1G when comparing BD and control groups. These genes are associated with the metallothionein (MT) protein group, and our findings are in line with supporting evidence from other studies (Seifuddin et al., 2013; Choi et al., 2008; Chen et al., 2013). Seifuddin et al. (2013) showed that an elevation in the expression of metallothionein genes was observed across all brain regions, with a specific emphasis on the prefrontal cortex. Our study, focusing on cerebellum samples, is also congruent with their research.

In our DEG analysis, MT1X is observed to be upregulated in SZ. This observation may indicate a shared underlying pathophysiology, consistent with the similar gene groups identified in the research by Chen et al. (2013).

The human metallothionein genes are localized in a cluster on chromosome 16 (Karin et al., 1984). This gene family is essential for neurocognitive functions, exhibiting diverse roles in responding to metals such as copper and zinc, oxidative agents, and inflammation. Their influence on cognition and protective functions against neurotoxicity has been documented in previous studies (Chen et al., 2013; Karin et al., 1984).

Low levels of serum MT-1 were identified in a particular study, potentially elucidating the upregulation of these genes in SZ. This phenomenon may also hold true for BD, as our study similarly reveals an upregulation of these genes. Moreover, brain inflammation has been substantiated in both SZ and BD. Thus, the upregulation of MT genes could be intricately linked to the pathogenesis of these conditions. Serum MT-1 could serve as a valuable biomarker for anticipating SZ, and its predictive utility may extend to BD given the observed upregulation of this gene group in individuals with BD in our DEG analysis of BD compared to control group (Yılmaz et al., 2023). To sum up, relation between MT, SZ and BD still remans undiscovered.

In the face of diverse pathophysiological interpretations for BD, it persists as an intricate and varied psychiatric phenomenon. One potential elucidation revolves around perturbed immune mechanisms in individuals with BD. In the context of our discussion, it is noteworthy that there is well-established evidence supporting a connection between autoimmune conditions and infections with mood disorders. This association involves various aspects, including alterations in different immune cell profiles, changes in cytokine release, elevated levels of immune markers in the circulation, and modifications in the inflammatory patterns within the central nervous system (CNS). All of these show that there is a relation between the immune system and BD (Tamouza et al., 2018; Barbosa et al., 2014).

The link between the Human Leukocyte Antigen (HLA) system and diseases was established half a century ago. HLA is a comprehensive and intricate system of genes located on chromosome 6, encoding proteins on the cell surface that play a regulatory role in the immune system. This system is commonly referred to as the major histocompatibility complex (MHC) in humans, comprising two major classes known as MHC-I and MHC-II. MHC-I includes three principal subtypes: HLA-A, HLA-B,

and HLA-C. These complexes primarily present antigens within cells to cytotoxic T cells, triggering the activation of relevant immune system cascades. On the other hand, MHC-II is situated on certain professional antigen-presenting cells, where they present extracellular antigens to immune cells, particularly CD4 T helper cells. Major subtypes of MHC-II are HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DQA2, HLA-DQB1, HLA-DQB2, HLA-DRA, HLA-DRB1, HLA-DRB2, HLA-DRB3, HLA-DRB4 and HLA-DRB5. This complex interaction underscores the intricate role of the HLA system in regulating immune responses and its potential implications for diseases.

Moreover, HLA genes exhibit the highest variance among genes and are associated with numerous diseases. Elevated expression levels of HLA peptides result in increased antigen presentation, thereby contributing to the development of autoimmune diseases and various other conditions. This emphasizes the significant role of HLA genes in the complex landscape of disease susceptibility and immune-related disorders. Some HLA genes are differentially downregulated in BD compared to control groups. These genes are HLA-DRA, HLA-DPA1, HLA-DRB1, HLA-DPB1, HLA-DQA1, and HLA-DRB3 are implicated in most of the GO-BPs in BD. In a study conducted on BD patients, HLA-DPA1 was identified as downregulated, particularly in samples obtained from the anterior cingulate cortex (Morgan et al., 2016). The reduced expression of this gene results in decreased MHC-II density on the cell surface, subsequently leading to diminished antigen presentation. This potential explanation aligns with findings that suggest an increased susceptibility to infections in individuals with BD (Morgan et al., 2016).

In one study, HLA-DRA and HLA-DRB3 were identified as differentially expressed in BD patients, with samples obtained from Brodmann Area 46 (Nakatani et al., 2006). Another research revealed that HLA-DRA exhibited a higher number of single nucleotide polymorphisms (SNPs) in individuals with BD compared to the general population (Calabrò et al., 2015). The distinct expression patterns of HLA-DRA in BD raise questions about the underlying mechanisms contributing to its differential regulation in this psychiatric condition. Further investigation into the specific genetic variations and functional implications of these findings may provide insights into the role of HLA-DRA in the context of BD pathology.

Lithium stands as the gold standard for both acute and maintenance treatment of BD, offering anti-manic and anti-depressant effects and proving to be the most effective medication in preventing relapses. Despite its gold standard status, responses to lithium vary among patients. A study has indicated a correlation between the response to lithium and the cluster of HLA genes. Our research aligns with this, demonstrating that the downregulation of these genes is associated with reduced antigen presentation and inflammation in BD patients. Notably, patients with HLA-DRB1-mediated lower inflammation show a favorable response to lithium, suggesting that lithium's anti-inflammatory effects may be particularly effective in this subgroup. On the contrary, non-responder patients to lithium may harbor other inflammatory processes that lithium cannot adequately address. Further exploration of the interplay between HLA genes, inflammation, and lithium response could enhance our understanding of treatment variability in BD (Le Clerc et al., 2021).

The diversity in HLA genetics extends to psychotropic treatment response and adverse drug reactions (ADRs). Class I and II HLA alleles partially mediate ADRs, with significant associations with Severe Cutaneous Adverse Drug Reactions such as Stevens-Johnson syndrome and toxic epidermal necrolysis. Implementing pre-emptive testing of HLA genotyping in clinical practice is crucial to prevent these side effects (Alchakee et al., 2022).

Our study and others highlight the potential of pharmacogenomic panels testing HLA variants in psychiatry patients for individualized therapy (Alchakee et al., 2022). Furthermore, understanding the intricate interplay between HLA genes, inflammation, and lithium response in BD sheds light on treatment variability. This comprehensive approach could significantly enhance our understanding of the complex landscape of psychiatric disorders and guide more effective and tailored therapeutic interventions.

ncRNA constitute a class of RNAs that do not undergo translation into proteins. These RNAs are categorized into two groups based on their nucleotide lengths. If their length exceeds 200 nucleotides, they are classified as long ncRNA, while those with shorter lengths fall under the category of small ncRNA. The latter includes miRNA, small interfering RNAs, small nucleolar RNAs, and small nuclear RNAs (snRNAs). Notably, snRNAs play a pivotal role by forming complexes with snRNPs to create

splicesomes, thereby facilitating alternative splicing mechanisms(Yoshino et al., 2022).

The genes responsible for coding snRNPs were identified to influence specific GO-BP, as evidenced by GO analysis of upregulated genes in BD. These GO-BPs encompass the formation of quadruple SL/U4/U5/U6 snRNP, spliceosomal tri-snRNP complex assembly, and spliceosomal snRNP assembly, detailed in Supplementary Table 2. This finding suggests a potential involvement of snRNPs in the dysregulation of splicing processes associated with BD.

Moreover, the impact of snRNPs extends beyond BD, as research has linked them to the production of abnormal proteins in Alzheimer's Disease(Yoshino et al., 2022). Understanding the role of snRNPs in both psychiatric and neurodegenerative disorders provides valuable insights into shared molecular mechanisms. Further exploration of the intricate connections between ncRNAs, particularly snRNPs, and their contribution to aberrant splicing in the context of BD is crucial for advancing our comprehension of these complex disorders.

SNORD115 emerges as a noteworthy gene, exhibiting upregulation in both BD and SZ, a finding corroborated by our research. This gene exerts control over five other genes, namely CRHR1, PBRM1, TAF1, DPM2, and RALGPS1. In our study, we observed an upregulation of SNORD115, coupled with the downregulation of PBRM1, aligning with previous evidence linking both genes to BD, SZ, and catatonia (Peter-Ross et al., 2018).

Of particular interest is the association between SNORD115 and CRHR1. SNORD115 regulates the expression of CRHR1, a gene implicated in corticotropin hormone release. Abnormalities in the hypothalamic-pituitary-adrenal (HPA) axis have been identified in BD patients. Given the regulatory influence of SNORD115 on CRHR1 and the established role of CRHR1 in the HPA axis, the upregulation of SNORD115 in BD may contribute to abnormalities in the HPA axis observed in these patients (Peter-Ross et al., 2018).

These findings underscore the complexity of the molecular interactions involved in psychiatric disorders, shedding light on potential mechanisms that extend beyond individual gene dysregulation. Further exploration of the intricate relationships

between SNORD115, its target genes, and the broader molecular landscape in BD and SZ may unravel novel therapeutic avenues and enhance our understanding of the underlying pathophysiology.

Reactome analysis of downregulated genes, namely PCLO, SYP, CAPZB, and KCNMB1, has unveiled potential insights into the molecular mechanisms underlying sensory perception. Notably, PCLO, a gene encoding Piccolo Presynaptic Cytomatrix Protein, stands out as a key player in synaptic function. Our findings align with previous studies demonstrating the downregulation of PCLO in individuals with BD and major depression patients (Choi et al., 2011).

PCLO's significance extends to its role in the presynaptic active zone, where it facilitates the attachment of synaptic vesicles into synapses. This function is crucial for the monoaminergic release into synapses, emphasizing its importance in neurotransmission. Beyond its role in conventional synapses, PCLO's influence is also observed in sensory inner hair cells (IHCs), where ribbon synapses connect to postsynaptic spiral ganglion neurons, contributing to sound encoding. The involvement of Piccolo protein in these synapses is particularly noteworthy, and its presence in rod cells in the eye further emphasizes its role in visual encoding (Voorn et al., 2021).

In our study, a noteworthy downregulation in the SYP gene was observed, marking a significant finding with potential implications for synaptic function and neuropsychiatric conditions. The SYP gene, a member of the synapsin gene family, plays a crucial role in encoding proteins such as synaptophysin, exerting influence on neurotransmitter release and synaptogenesis. The downregulation of SYP in our investigation aligns with previous research associating mutations in this gene with various neuropsychiatric conditions. Animal studies have provided additional insights into the impact of altered SYP expression on behavior. Specifically, studies on depressed subjects have demonstrated substantial decreases in synaptophysin, linking low levels of synaptophysin to manic-type behavior (Han et al., 2018; Gilabert-Juan et al., 2012). This finding underscores the potential role of SYP in modulating behavior and mood, suggesting a connection between synaptic protein levels and the manifestation of specific psychiatric symptoms.

The downregulation of CAPZB observed in BD patients in our study holds significance in the context of actin dynamics and cellular processes. CAPZB, a member of the F-actin capping protein family, serves a crucial role in regulating the growth of actin filaments. Studies have demonstrated that the deletion of CAPZB hinders the elongation of actin filaments, particularly those positioned on the cell surface of IHCs involved in sensing vibrations and auditory stimuli. The observed downregulation of CAPZB in BD patients suggests a potential link to the disruption of actin filament elongation, which could have implications for sensory perception (Avenarius et al., 2017).

Understanding the intricate role of CAPZB in actin dynamics provides insights into its broader impact on cellular functions. Actin filaments are fundamental components involved in various cellular processes, and their dysregulation can affect critical functions such as cellular morphology, migration, and mechanosensory responses. The specific association between CAPZB downregulation and impaired actin filament elongation in inner hair cells raises intriguing questions about the potential contributions of this molecular alteration to sensory dysfunction in individuals with BD.

Auditory hallucinations, a prevalent and distressing symptom in both SZ and BD, prompt discussions on their origin, whether rooted in primary sensory processing or higher brain functions (Doucet et al., 2019). While prior research has linked auditory hallucinations in patients to the auditory cortex, our study introduces a nuanced perspective. The downregulation of four genes (PCLO, SYP, CAPZB, and KCNMB1) identified in our investigation suggests a potential association with both high cognitive functions and primary sensory processes. Notably, these genes exhibit functionality in both IHCs and the brain.

The dual involvement of these genes in both peripheral sensory cells and central brain functions implies a comprehensive role in auditory perception. This duality suggests a potential connection between these genes and the occurrence of auditory hallucinations in individuals with SZ and BD. Moreover, the shared genetic underpinnings identified in our study provide a bridge between sensory processing abnormalities and higher

cognitive functions, supporting the hypothesis that dysregulated gene expression may contribute to the manifestation of auditory hallucinations.

This finding adds depth to our understanding of the complex interplay between genetic factors, sensory processing, and higher cognitive functions in the context of auditory hallucinations. Further investigations into the specific mechanisms through which these genes exert their influence on auditory perception could shed light on novel therapeutic targets for managing auditory hallucinations in psychiatric disorders. Additionally, exploring the potential link between these genes and the monoamine hypothesis in mood disorders may unveil shared molecular pathways contributing to the broader spectrum of psychiatric symptoms.

Many acknowledge that the majority of miRNAs discovered to date are found in the mammalian brain, playing essential roles in the regulation of neuronal growth, synaptic plasticity, and network interactivity (Zhang et al., 2023). miRNAs play a role in diverse brain disorders, particularly SZ, in humans and shape gene expression and functional pathways. Especially MIR-137 was found to be related to SZ in one of the largest studies. These genes are associated with miRNAs, a class of short, non-coding RNAs composed of 20–24 nucleotides involved in the post-transcriptional regulation of gene expression. Figure 3 shows that how miRNAs integrate into the RISC, which targets mRNAs, leading to translational inhibition of the target mRNA and subsequently silencing gene expression (Beveridge & Cairns, 2012).

In our research, we identified upregulated genes of MIR30E, MIR186, MIR29B2, MIR148B, MIR15A, MIR128-1, MIRLET7G, MIR124-1, MIRLET7F1, and MIR181A2 in GO analysis of SZ.

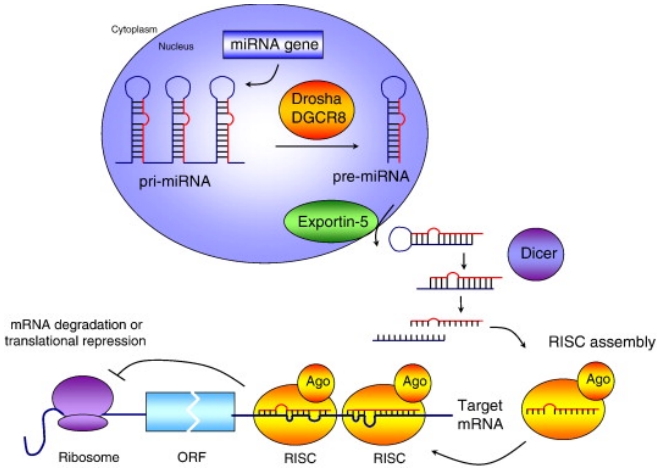

Figure 3. Overview of miRNA function

The expression of miR-30e was significantly higher in patients with SZ compared to control groups in a study, conducted in peripheral leukocytes. A rare SNP in MIR30E in the Han Chinese population is associated with an increased risk of SZ. This study's findings were also replicated in the Japanese population (Xu et al., 2010; Watanabe et al., 2013). Furthermore, miR-30e was differentially regulated in samples taken from the dorsolateral prefrontal cortex and peripheral blood mononuclear cells of SZ patients (Beveridge & Cairns, 2012; Grosu et al., 2023). A total of 502 genes were identified as potential targets of miR-30e. Additionally, a social defeat stress mouse model showed downregulation of miR-30e, associated with depression-like behavior and hippocampal neurogenesis. This miRNA was also found to be related to high levels in MDD cases involving suicide compared to those without. The intricate relationship between miR-30e and the pathophysiology of SZ warrants further investigation (Stapel et al., 2022).

Variants in MIR29B2 were also found to be related to an increased risk of SZ in our study. miR-29-2B exhibited varying expression patterns across specific brain regions, particularly during the shift from infancy to early childhood. Neurodevelopmental models also explain the emergence of SZ; therefore, miR29-2B might play a role in this, as it differentially expresses in various brain regions during early childhood (Reckziegel et al., 2022). This could also be associated with the noted differences in

the expression of miR-29b in the postmortem prefrontal cortex of individuals with SZ in comparison to those without psychiatric conditions (Brum et al., 2021).

miR-186 is commonly associated with cancer pathogenesis, impeding the migration and invasion of cancer cells by targeting multiple molecules (Wang et al., 2019). Conversely, altered levels of miR-186 have been found to be associated with certain mental health disorders, such as MDD, both in mouse models (Jiang et al., 2021) and human populations (Beveridge & Cairns, 2012); however, detailed information from human studies is currently limited. Moreover, miR-186, which targets numerous genes, shows increased levels in the prefrontal cortex of methamphetamine self-administering rats. Methamphetamine misuse is a well-known risk factor for SZ. Since the MIR186 gene is also upregulated in our study, this miRNA might be related to the pathophysiology of amphetamine-induced psychosis and SZ, making it a potential target in these conditions (Du et al., 2016).

One theory underlying SZ is the glutamate and NMDA receptor (NMDAR) hypothesis, which emerged after observing psychotomimetic agents like phencyclidine (PCP) and ketamine, both NMDAR antagonists, leading to psychotic symptoms (Gunasekaran et al., 2022). Furthermore, NMDAR hypofunction induced by PCP and ketamine may result in dopaminergic dysregulation in the striatum and prefrontal cortex, contributing to the pathogenesis of SZ. miRNAs play a crucial role in this process, particularly the upregulation of miR-148B in animal models, which may lead to the downregulation of NMDAR and subsequent psychotic manifestations. Our study also identified upregulation of MIR148B in SZ patients compared to the control group. Similar to MIR186 gene, MIR148B might underlie the mechanisms of substance misuse and the manifestation of SZ (Du et al., 2016; Gunasekaran et al., 2022).

MIR128-1 was found to be upregulated in SZ patients compared to the control group. This brain-specific miRNA, especially neuron-specific, has been recently discovered and is associated with normal brain development. The upregulation of this miRNA in SZ suggests its potential role in the pathogenesis by influencing other genes. Therefore, further studies on miR128-1 may illuminate the pathogenesis of SZ, and its significance is particularly crucial as it is a neuron-specific miRNA (Smirnova et al., 2005; Shan et al., 2016). Similar to miR-128-1, miR-124 is also a brain-specific

miRNA that regulates neuronal plasticity. Polymorphism in MIR124-1 is associated with aggressiveness by targeting BDNF and DRD4 genes, both of which are related to positive symptoms of SZ. Therefore, the upregulation of miR-124-1 might be related to positive symptoms of SZ. Moreover, miR-124 may be involved in the etiology of MDD by regulating RGS4, which is also a vulnerability gene for SZ. Furthermore, promoter hypomethylation of the miR-124 gene is associated with MDD. Hence, miR-124 might also be related to negative symptoms of SZ (González-Giraldo et al., 2015; Zeng et al., 2018, 2021). Further research might enhance our understanding of SZ and could potentially identify it as a target for intervention.

GO analysis of hypermethylated and downregulated genes in BD compared to control groups shows “recycling pathway of L1” which is related to differentially methylated and regulated genes of AP2M1, RPS6KA4, DPYSL2, DNM1, SH3GL2, and CLTC. L1, a membrane-spanning protein, plays a role in certain aspects of neuronal development, including the extension of axons and the migration of neurons. Coordination of L1 and the actin cytoskeleton is paramount for these functions. F-actin moves from peripheral domain of the growth cone to central domain. L1 attaches to the actin intracelluler cytoskeleton via some other proteins, thereby linking extracellular ligans to F-actin flow. Internalization of L1 molecules as they are moved to the rear of the growth cone coupled to retrograde F-actin flow, recycling them to the leading edge plasma membrane. This is called recyling pathway of L1(Kamiguchi H., 2003).

Hypermethylation of the DPYSL2 gene may contribute to its downregulation. This gene is involved in the development of neurons, playing a vital role in the formation of axons. Dysregulation of DPYSL2 could lead to irregularities in neurodevelopment. Additionally, aberrant methylation of this gene has been associated with an increased risk of suicidality in pediatric BD patients (Kari et al., 2023). Lower levels of DPYSL2 protein in the frontal cortex were observed in individuals with SZ, BD, and MDD, consistent with the results obtained in our study (Johnston-Wilson et al., 2000). The reduced expression of DPYSL2 protein was specifically associated with SZ, with the expression level of DPYSL2 being particularly linked to positive symptoms (Izumi et al., 2022). On one hand, Lee et al. demonstrated decreased levels of DPYSL2 in the prefrontal cortex and hippocampus of rats exposed to prenatal stress (Lee et al., 2015).

Additionally, Garcia-Gonzalez et al. reported similar findings in C. elegans, a species with a DPYSL2 homolog called UNC-33. They showed that environmental stressors regulate the UNC-33 promoter. In summary, our study indicates that hypermethylation of this gene may lead to downregulation, and environmental stressors, which are risk factors for the development of SZ and BD, may regulate this gene. Further research on the effects of environmental risks, such as adverse childhood events, on both SZ and BD may provide insights into the epigenetic regulation of this gene (Garcia-Gonzalez et al., 2022).

SH3GL2 encodes endophilin A1, functioning in synaptic vesicle recycling at the presynaptic terminal and synaptic plasticity at the postsynaptic area (Yang et al., 2018). Carponi et al. demonstrated that the expression of SH3GL2 is implicated in suicidal ideation in both SZ and BD, particularly in relation to the glutamate system. Notably, substances like ketamine, an NMDA antagonist, have been associated with sudden suicidal ideation (Corponi et al., 2019). Therefore, similar to DPYSL2, we hypothesize that SH3GL2 may be influenced by environmental factors. Traglia et al. found in their study that levels of environmental pollutants may contribute to SNP-based heritability in ASD, and these loci are located on the SH3GL2 gene. Our research also suggests that hypermethylation may downregulate this gene, potentially serving as an epigenetic mechanism to mitigate the effects of environmental factors (Traglia et al., 2017).

CLTC, a gene encoding clathrin heavy chain 1, was identified in the GO analysis of hypermethylated and downregulated genes in BD. It plays a crucial role in the recycling of the L1 pathway, functioning alongside AP2M1 in clathrin-mediated endocytosis (CME), a process integral to L1 pathway recycling (Zhang et al., 2022; Kaksonen & Roux, 2018). CME is also considered a candidate for an underlying mechanism in the pathophysiology of both BD and SZ. Some antipsychotic drugs, such as chlorpromazine, strongly inhibit CME (Prichard et al., 2022). Alkelai et al. also found that single nucleotide variants and indels of CLTC are predominantly inherited in childhood-onset SZ, emphasizing its significance in both BD and SZ (Alkelai et al., 2023). Additionally, the CLTC gene is hypermethylated in obese patients with hyperlipidemia, influencing the endocytosis of low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) receptors. Psychiatric patients are

known to have a risk of metabolic syndrome, obesity, and hyperlipidemia (Miola et al., 2022). Therefore, epigenetic modification of common genes playing roles in similar pathways like CME in different tissues might be related to both neuropsychiatric and metabolic presentations of psychiatric conditions such as BD and SZ. Further research on this topic could shed light on this area. On the other hand, Filipovic et al. demonstrated in an animal model of depression in rats, comparing phenotypes of resistant and susceptible rats to chronic social isolation, that depression-resistant rats have upregulated CLTC gene expression. In contrast, our study suggests that hypermethylation of this gene might lead to its downregulation. As shown in the aforementioned study, this gene might be affected by epigenetic mechanisms leading to differential regulation. Therefore, further studies on the CLTC gene in humans might shed light on the effects of environmental factors on psychiatric disorders (Filipović et al., 2024).

DNM1 exhibited differential expression in BD as per a study (Föcking et al., 2016), although detailed information from this study was not provided.

In our study, 21 genes were identified as hypomethylated and upregulated in BD compared to the control group. However, the GO analysis did not reveal any specific GO-BP associated with these genes. The identified genes include HIST1H1D, PAWR, HIST1H2BH, RGS9, BTG3, SIN3A, MYL3, HIST2H2AC, NTN1, ZNF451, PIK3R1, TRIM51, TRAF6, FAH, WNT8A, SALL3, CDR1, MT1E, ATAD2, CDK2, and KCNN3.

Some genes stand out in this list, notably HIST1H1D, HIST1H2BH, and HIST2H2AC, which encode different histone proteins. DNA is wrapped around chromatin, the basic unit of which is called a nucleosome, consisting of 146-147 base pairs and a histone octamer with one H2A-H2B tetramer and two H3-H4 dimers. Post-translational modification of histone proteins may alter gene regulation (Zhang et al., 2021). Epigenetic gene regulation is a well-known mechanism for BD (Ludwig & Dwivedi, 2016). However, to our knowledge, there is limited research on genes coding for histone proteins in BD. Nevertheless, studies on histone expression have been conducted. Although expression dysregulation of histones in SZ has been found in other studies, we also provide evidence that some genes encoding histone proteins are

upregulated and hypomethylated. Interestingly, one of the medications used for BD, the mood stabilizer valproic acid, might act at an epigenetic level as a histone deacetylase inhibitor. This medication may partially correct underlying histone abnormalities (Sanders et al., 2013). On the other hand, research on the epigenetics of genes encoding histone proteins is ongoing. Typically, studies concentrate on histone modifications that affect gene expression. However, directing attention to the epigenetic modification of histone genes, similar to what we found in our study, might illuminate this topic. Additionally, emphasizing the necessity for studies that integrate histone acetylation, methylation, and transcriptome analysis could further advance our understanding of epigenetic regulation. So, we suggest the term 'meta-epigenome' to describe the study of genes of proteins involved in epigenetic mechanisms, such as histones.

ITGA8 is a gene that codes for the integrin subunit alpha 8 protein, a cell adhesion molecule. Supriyanto et al. (2013) demonstrated a missense mutation in this gene associated with SZ in Japanese female patients. While the alpha 8 subunit has been identified in neurons in rats, its investigation in humans is yet to be explored to our knowledge. Changes affecting the binding affinity of integrin in the ITGA8 gene may contribute to the development of SZ or act as a protective factor against it. A study related to Parkinson's disease revealed that the pathogenic ITGA8 influences basal dopamine levels, a crucial pathophysiological mechanism in SZ (Cacabelos et al., 2021). Conversely, another study demonstrated that the lack of ITGA8 leads to neuronal death and synaptic loss in the anterior cingulate cortex in rats (Kwak et al., 2021). Therefore, further research on this gene and its relationship to environmental factors might contribute invaluable knowledge to the field.

SCG2 is a gene encoding secretogranin II, expressed in the CNS and secreted by endocrine tissues. While the role of the SCG2 gene in the CNS is not fully understood, normal expression is associated with typical neurodevelopment, and aberrant expression may contribute to neurodevelopmental disorders. Secretogranin II plays a crucial role in packing neuropeptides into secretory vesicles (Filipović et al., 2024). In our research, we observed downregulation of this gene, potentially mediated by hypermethylation. Secretogranin II may transport orexin, MCH, NPY, and POMC by binding secretogranin III and releasing these peptides. Therefore, our study suggests a

possible link between hypermethylation and downregulation of SCG2 and dietary issues in SZ. Additionally, neuropeptides have been shown to modulate behavior in animal models. Dysregulation of neuropeptides in SZ might impact dopamine circuits in mesolimbic and mesocortical pathways, both of which are key pathophysiological mechanisms of SZ. In summary, uncovering the interaction between neuropeptides and neurotransmitters could provide a new understanding of SZ (Rodríguez et al., 2020).

Seven genes were found to be hypomethylated and upregulated in the gene analysis of SZ compared to the control group. None of them were previously associated with SZ to our knowledge; however, some are linked to other neuropsychiatric conditions. For instance, STXBP5, encoding syntaxin-binding protein 5, has been reported to be associated with ASD, intellectual disabilities, and seizures. This protein plays a role in synaptic transmission. Cukier et al. (2014) identified this gene running in multiple families with ASD, while the mother of one proband had BD. This suggests a potential association and overlap of genes between neurodevelopmental and neuropsychiatric conditions. On the other hand, the demethylation of CpGs in the STXBP5 gene, along with the rate of change, was correlated with improvement in cognition in a normal adolescent population (Jensen et al., 2023). Therefore, this gene and syntaxin-binding protein 5 play crucial roles in cognitive functions and synaptic transmission. Further research on this gene may provide insights into SZ pathophysiology.

5. CONCLUSION

The important results have been summarized in bullet points in sequence:

- Our analysis indicates a significant upregulation of MT genes in BD, suggesting their potential relevance to neuropsychiatric conditions, including SZ. The observed association between MT gene expression and brain inflammation underscores their potential as biomarkers for predicting and understanding these disorders
- This research delves into the complex relationship between BD and immune mechanisms, particularly focusing on the involvement of the HLA system. It reveals differential expression patterns of HLA genes in BD patients, suggesting potential implications for immune dysregulation and treatment response variability, emphasizing the importance of personalized therapeutic approaches in psychiatry.
- This research highlights the involvement of snRNPs in the dysregulation of splicing processes associated with BD, suggesting shared molecular mechanisms with neurodegenerative disorders like Alzheimer's Disease. Understanding these connections could provide valuable insights into the pathophysiology of BD and guide future research into ncRNA-mediated mechanisms in psychiatric disorders.
- The upregulation of SNORD115, alongside the downregulation of its target genes such as PBRM1, in both BD and SZ, suggests a potential role for SNORD115 in dysregulating the HPA axis and highlights the intricate molecular interactions contributing to these psychiatric disorders.
- The Reactome analysis of downregulated genes, including PCLO, SYP, CAPZB, and KCNMB1, reveals insights into the molecular mechanisms underlying sensory perception in BD and SZ. PCLO's involvement in synaptic function, particularly in monoaminergic release and sensory inner hair cells, underscores its significance in neurotransmission and sound encoding. Similarly, the downregulation of SYP suggests a role in modulating behavior

and mood through its influence on neurotransmitter release and synaptogenesis. Furthermore, the downregulation of CAPZB highlights potential disruptions in actin dynamics, affecting cellular functions and possibly contributing to sensory dysfunction. These findings suggest a dual involvement of these genes in both peripheral sensory cells and central brain functions, offering insights into auditory hallucinations in psychiatric disorders and potential therapeutic targets.

- Our study identified several upregulated miRNAs in SZ, including MIR30E, MIR186, MIR29B2, MIR148B, MIR15A, MIR128-1, MIRLET7G, MIR124-1, MIRLET7F1, and MIR181A2, indicating their potential roles in SZ pathology. Notably, miR-30e, miR-29-2B, and miR-186 showed differential expression patterns, suggesting involvement in neurodevelopment and SZ pathogenesis. These findings highlight the complex role of miRNAs in SZ and their potential as therapeutic targets for psychiatric disorders.
- Altered levels of miR-186, associated with mental health disorders like MDD, and upregulated in methamphetamine self-administering rats, suggest its involvement in amphetamine-induced psychosis and SZ. The NMDAR hypothesis of SZ implicates miRNAs, notably the upregulation of miR-148B, which may contribute to NMDAR downregulation and subsequent psychotic manifestations, highlighting the potential influence of miRNA dysregulation on drug-induced psychiatric disorders.
- Our study identified hypermethylation and downregulation of genes involved in the recycling pathway of L1 in BD, including DPYSL2, SH3GL2, and CLTC. These findings suggest potential epigenetic mechanisms influencing neurodevelopmental pathways and synaptic plasticity, with implications for environmental factors' role in BD and SZ. Further research into the interaction between genetic regulation, environmental stressors, and psychiatric disorders could elucidate novel therapeutic avenues and deepen our understanding of their pathophysiology.
- Our study highlights the upregulation and hypomethylation of genes encoding histone proteins in BD, shedding light on potential epigenetic mechanisms underlying the condition and suggesting the term "meta-epigenome" for further

exploration of these genes' roles in epigenetic regulation. Additionally, the mood stabilizer valproic acid's action as a histone deacetylase inhibitor suggests a potential therapeutic avenue targeting histone abnormalities in BD.

REFERENCES

Abdolmaleky, H. M., Cheng, K. H., Faraone, S. V., Wilcox, M., Glatt, S. J., Gao, F., Smith, C. L., Shafa, R., Aeali, B., Carnevale, J., Pan, H., Papageorgis, P., Ponte, J. F., Sivaraman, V., Tsuang, M. T., & Thiagalingam, S. (2006). Hypomethylation of MB-COMT promoter is a major risk factor for schizophrenia and bipolar disorder. *Human molecular genetics*, *15*(21), 3132–3145. https://doi.org/10.1093/hmg/ddl253

Abdolmaleky, H. M., Gower, A. C., Wong, C. K., Cox, J. W., Zhang, X., Thiagalingam, A., Shafa, R., Sivaraman, V., Zhou, J. R., & Thiagalingam, S. (2019). Aberrant transcriptomes and DNA methylomes define pathways that drive pathogenesis and loss of brain laterality/asymmetry in schizophrenia and bipolar disorder. *American journal of medical genetics. Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics*, *180*(2), 138–149. https://doi.org/10.1002/ajmg.b.32691

Alchakee, A., Ahmed, M., Eldohaji, L., Alhaj, H., & Saber-Ayad, M. (2022). Pharmacogenomics in Psychiatry Practice: The Value and the Challenges. *International journal of molecular sciences*, *23*(21), 13485. https://doi.org/10.3390/ijms232113485

Alkelai, A., Greenbaum, L., Shohat, S., Povysil, G., Malakar, A., Ren, Z., Motelow, J. E., Schechter, T., Draiman, B., Chitrit-Raveh, E., Hughes, D., Jobanputra, V., Shifman, S., Goldstein, D. B., & Kohn, Y. (2023). Genetic insights into childhood-onset schizophrenia: The yield of clinical exome sequencing. *Schizophrenia research*, *252*, 138–145. https://doi.org/10.1016/j.schres.2022.12.033

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders* (5th ed.). https://doi.org/10.1176/appi.books.9780890425596

Avenarius, M. R., Krey, J. F., Dumont, R. A., Morgan, C. P., Benson, C. B., Vijayakumar, S., Cunningham, C. L., Scheffer, D. I., Corey, D. P., Müller, U., Jones, S. M., & Barr-Gillespie, P. G. (2017). Heterodimeric capping protein is required for stereocilia length and width regulation. *The Journal of cell biology*, *216*(11), 3861–3881. https://doi.org/10.1083/jcb.201704171

Barbosa, I. G., Machado-Vieira, R., Soares, J. C., & Teixeira, A. L. (2014). The immunology of bipolar disorder. *Neuroimmunomodulation*, *21*(2-3), 117–122. https://doi.org/10.1159/000356539

Beveridge, N. J., & Cairns, M. J. (2012). MicroRNA dysregulation in schizophrenia. *Neurobiology of disease*, *46*(2), 263–271. https://doi.org/10.1016/j.nbd.2011.12.029

Brady, R. O., Jr, Gonsalvez, I., Lee, I., Öngür, D., Seidman, L. J., Schmahmann, J. D., Eack, S. M., Keshavan, M. S., Pascual-Leone, A., & Halko, M. A. (2019). Cerebellar-Prefrontal Network Connectivity and Negative Symptoms in Schizophrenia. *The American journal of psychiatry*, *176*(7), 512–520. https://doi.org/10.1176/appi.ajp.2018.18040429

Brum, C. B., Paixão-Côrtes, V. R., Carvalho, A. M., Martins-Silva, T., Carpena, M. X., Ulguim, K. F., Luquez, K. Y. S., Salatino-Oliveira, A., & Tovo-Rodrigues, L. (2021). Genetic variants in miRNAs differentially expressed during brain development and their relevance to psychiatric disorders susceptibility. *The world journal of biological psychiatry : the official journal of the World Federation of Societies of Biological Psychiatry*, *22*(6), 456–467. https://doi.org/10.1080/15622975.2020.1834618

Cacabelos, R., Carrera, I., Martínez, O., Alejo, R., Fernández-Novoa, L., Cacabelos, P., Corzo, L., Rodríguez, S., Alcaraz, M., Nebril, L., Tellado, I., Cacabelos, N., Pego, R., Naidoo, V., & Carril, J. C. (2021). Atremorine in Parkinson's disease: From dopaminergic neuroprotection to pharmacogenomics. *Medicinal research reviews*, *41*(5), 2841–2886. https://doi.org/10.1002/med.21838

Calabrò, M., Drago, A., Sidoti, A., Serretti, A., & Crisafulli, C. (2015). Genes involved in pruning and inflammation are enriched in a large mega-sample of patients affected by Schizophrenia and Bipolar Disorder and

controls. *Psychiatry research*, *228*(3), 945–949. https://doi.org/10.1016/j.psychres.2015.06.013

Chen, C., Cheng, L., Grennan, K., Pibiri, F., Zhang, C., Badner, J. A., Members of the Bipolar Disorder Genome Study (BiGS) Consortium, Gershon, E. S., & Liu, C. (2013). Two gene co-expression modules differentiate psychotics and controls. *Molecular psychiatry*, *18*(12), 1308–1314. https://doi.org/10.1038/mp.2012.146

Chen, C., Meng, Q., Xia, Y., Ding, C., Wang, L., Dai, R., Cheng, L., Gunaratne, P., Gibbs, R. A., Min, S., Coarfa, C., Reid, J. G., Zhang, C., Jiao, C., Jiang, Y., Giase, G., Thomas, A., Fitzgerald, D., Brunetti, T., Shieh, A., … Liu, C. (2018). The transcription factor POU3F2 regulates a gene coexpression network in brain tissue from patients with psychiatric disorders. *Science translational medicine*, *10*(472), eaat8178. https://doi.org/10.1126/scitranslmed.aat8178

Chen, C., Zhang, C., Cheng, L., Reilly, J. L., Bishop, J. R., Sweeney, J. A., Chen, H. Y., Gershon, E. S., & Liu, C. (2014). Correlation between DNA methylation and gene expression in the brains of patients with bipolar disorder and schizophrenia. *Bipolar disorders*, *16*(8), 790–799. https://doi.org/10.1111/bdi.12255

Chen, C., Zhang, C., Cheng, L., Reilly, J. L., Bishop, J. R., Sweeney, J. A., Chen, H. Y., Gershon, E. S., & Liu, C. (2014). Correlation between DNA methylation and gene expression in the brains of patients with bipolar disorder and schizophrenia. *Bipolar disorders*, *16*(8), 790–799. https://doi.org/10.1111/bdi.12255

Chen, Q., Li, D., Jin, W., Shi, Y., Li, Z., Ma, P., Sun, J., Chen, S., Li, P., & Lin, P. (2021). Research Progress on the Correlation Between Epigenetics and Schizophrenia. *Frontiers in neuroscience*, *15*, 688727. https://doi.org/10.3389/fnins.2021.688727

Choi, K. H., Elashoff, M., Higgs, B. W., Song, J., Kim, S., Sabunciyan, S., Diglisic, S., Yolken, R. H., Knable, M. B., Torrey, E. F., & Webster, M. J. (2008). Putative psychosis genes in the prefrontal cortex: combined analysis of gene expression microarrays. *BMC psychiatry*, *8*, 87. https://doi.org/10.1186/1471-244X-8-87

Choi, K. H., Higgs, B. W., Wendland, J. R., Song, J., McMahon, F. J., & Webster, M. J. (2011). Gene expression and genetic variation data implicate PCLO in bipolar disorder. *Biological psychiatry*, *69*(4), 353–359. https://doi.org/10.1016/j.biopsych.2010.09.042

Ciuculete, D. M., Voisin, S., Kular, L., Welihinda, N., Jonsson, J., Jagodic, M., Mwinyi, J., & Schiöth, H. B. (2020). Longitudinal DNA methylation changes at MET may alter HGF/c-MET signalling in adolescents at risk for depression. *Epigenetics*, *15*(6-7), 646–663. https://doi.org/10.1080/15592294.2019.1700628

Comprehensive DNA Methylation Analysis on the Illumina® Infinium® Assay Platform

Contributed by Daniel J. Weisenberger, David Van Den Berg, Fei Pan, Benjamin P. Berman, and Peter W. Laird, University of Southern California, Keck School of Medicine, USC/Norris Comprehensive Cancer Center, Los Angeles, CA 90033

Corponi, F., Bonassi, S., Vieta, E., Albani, D., Frustaci, A., Ducci, G., Landi, S., Boccia, S., Serretti, A., & Fabbri, C. (2019). Genetic basis of psychopathological dimensions shared between schizophrenia and bipolar disorder. *Progress in neuro-psychopharmacology & biological psychiatry*, *89*, 23–29. https://doi.org/10.1016/j.pnpbp.2018.08.023

Craddock, N., & Jones, I. (1999). Genetics of bipolar disorder. *Journal of medical genetics*, *36*(8), 585–594. https://doi.org/10.1136/jmg.36.8.585

Craddock, N., & Sklar, P. (2013). Genetics of bipolar disorder. *Lancet (London, England)*, *381*(9878), 1654–1662. https://doi.org/10.1016/S0140-6736(13)60855-7

Cukier, H. N., Dueker, N. D., Slifer, S. H., Lee, J. M., Whitehead, P. L., Lalanne, E., Leyva, N., Konidari, I., Gentry, R. C., Hulme, W. F., Booven, D. V., Mayo, V., Hofmann, N. K., Schmidt, M. A., Martin, E. R., Haines, J. L., Cuccaro, M. L., Gilbert, J. R., & Pericak-Vance, M. A. (2014). Exome sequencing of extended families with autism reveals genes shared across

neurodevelopmental and neuropsychiatric disorders. *Molecular autism*, *5*(1), 1. https://doi.org/10.1186/2040-2392-5-1

Dendrou, C. A., Petersen, J., Rossjohn, J., & Fugger, L. (2018). HLA variation and disease. *Nature reviews. Immunology*, *18*(5), 325–339. https://doi.org/10.1038/nri.2017.143

Doucet, G. E., Luber, M. J., Balchandani, P., Sommer, I. E., & Frangou, S. (2019). Abnormal auditory tonotopy in patients with schizophrenia. *NPJ schizophrenia*, *5*(1), 16. https://doi.org/10.1038/s41537-019-0084-x

Du, H. Y., Cao, D. N., Chen, Y., Wang, L., Wu, N., & Li, J. (2016). Alterations of prefrontal cortical microRNAs in methamphetamine self-administering rats: From controlled drug intake to escalated drug intake. *Neuroscience letters*, *611*, 21–27. https://doi.org/10.1016/j.neulet.2015.11.016

Engel G. L. (1980). The clinical application of the biopsychosocial model. *The American journal of psychiatry*, *137*(5), 535–544. https://doi.org/10.1176/ajp.137.5.535

Fabbri C. (2021). The Role of Genetics in Bipolar Disorder. *Current topics in behavioral neurosciences*, *48*, 41–60. https://doi.org/10.1007/7854_2020_153

Fan, Y., Gao, Y., Ma, Q., Yang, Z., Zhao, B., He, X., Yang, J., Yan, B., Gao, F., Qian, L., Wang, W., Zhu, F., & Ma, X. (2022). Multi-Omics Analysis Reveals Aberrant Gut-Metabolome-Immune Network in Schizophrenia. *Frontiers in immunology*, *13*, 812293. https://doi.org/10.3389/fimmu.2022.812293

Farsi, Z., & Sheng, M. (2023). Molecular mechanisms of schizophrenia: Insights from human genetics. *Current opinion in neurobiology*, *81*, 102731. https://doi.org/10.1016/j.conb.2023.102731

Fernandez-Rozadilla, C., Timofeeva, M., Chen, Z., Law, P., Thomas, M., Schmit, S., Díez-Obrero, V., Hsu, L., Fernandez-Tajes, J., Palles, C., Sherwood, K., Briggs, S., Svinti, V., Donnelly, K., Farrington, S., Blackmur, J., Vaughan-Shaw, P., Shu, X. O., Long, J., Cai, Q., … Peters, U. (2023). Deciphering colorectal cancer genetics through multi-omic analysis of 100,204 cases and 154,587 controls of European and east Asian ancestries. *Nature genetics*, *55*(1), 89–99. https://doi.org/10.1038/s41588-022-01222-9

Filipović, D., Novak, B., Xiao, J., Tadić, P., & Turck, C. W. (2024). Prefrontal cortical synaptoproteome profile combined with machine learning predicts resilience towards chronic social isolation in rats. *Journal of psychiatric research*, *172*, 221–228. Advance online publication. https://doi.org/10.1016/j.jpsychires.2024.02.042

Föcking, M., Dicker, P., English, J. A., Schubert, K. O., Dunn, M. J., & Cotter, D. R. (2011). Common proteomic changes in the hippocampus in schizophrenia and bipolar disorder and particular evidence for involvement of cornu ammonis regions 2 and 3. *Archives of general psychiatry*, *68*(5), 477–488. https://doi.org/10.1001/archgenpsychiatry.2011.43

Föcking, M., Dicker, P., Lopez, L. M., Hryniewiecka, M., Wynne, K., English, J. A., Cagney, G., & Cotter, D. R. (2016). Proteomic analysis of the postsynaptic density implicates synaptic function and energy pathways in bipolar disorder. *Translational psychiatry*, *6*(11), e959. https://doi.org/10.1038/tp.2016.224

Frey, B. N., Andreazza, A. C., Houenou, J., Jamain, S., Goldstein, B. I., Frye, M. A., Leboyer, M., Berk, M., Malhi, G. S., Lopez-Jaramillo, C., Taylor, V. H., Dodd, S., Frangou, S., Hall, G. B., Fernandes, B. S., Kauer-Sant'Anna, M., Yatham, L. N., Kapczinski, F., & Young, L. T. (2013). Biomarkers in bipolar disorder: a positional paper from the International Society for Bipolar Disorders Biomarkers Task Force. *The Australian and New Zealand journal of psychiatry*, *47*(4), 321–332. https://doi.org/10.1177/0004867413478217

Fries, G. R., Bauer, I. E., Scaini, G., Wu, M. J., Kazimi, I. F., Valvassori, S. S., Zunta-Soares, G., Walss-Bass, C., Soares, J. C., & Quevedo, J. (2017). Accelerated epigenetic aging and mitochondrial DNA copy number in bipolar disorder. *Translational psychiatry*, *7*(12), 1283. https://doi.org/10.1038/s41398-017-0048-8

Fries, G. R., Li, Q., McAlpin, B., Rein, T., Walss-Bass, C., Soares, J. C., & Quevedo, J. (2016). The role of DNA methylation in the pathophysiology and treatment of bipolar disorder. *Neuroscience and biobehavioral reviews*, *68*, 474–488. https://doi.org/10.1016/j.neubiorev.2016.06.010

Gandal, M. J., Zhang, P., Hadjimichael, E., Walker, R. L., Chen, C., Liu, S., Won, H., van Bakel, H., Varghese, M., Wang, Y., Shieh, A. W., Haney, J., Parhami, S., Belmont, J., Kim, M., Moran Losada, P., Khan, Z., Mleczko, J., Xia, Y., Dai, R., … Geschwind, D. H. (2018). Transcriptome-wide isoform-level dysregulation in ASD, schizophrenia, and bipolar disorder. *Science (New York, N.Y.)*, *362*(6420), eaat8127. https://doi.org/10.1126/science.aat8127

Garcia-Gonzalez, B., Avant, S., Carassco-Pena, A., Miranda, M. C., Salazar, K., Torres, E., & Holgado, A. (2022). Investigating the regulation of the *unc-33*promoter by environmental stressors. *microPublication biology*, *2022*, 10.17912/micropub.biology.000651. https://doi.org/10.17912/micropub.biology.000651

Gilabert-Juan, J., Varea, E., Guirado, R., Blasco-Ibáñez, J. M., Crespo, C., & Nácher, J. (2012). Alterations in the expression of PSA-NCAM and synaptic proteins in the dorsolateral prefrontal cortex of psychiatric disorder patients. *Neuroscience letters*, *530*(1), 97–102. https://doi.org/10.1016/j.neulet.2012.09.032

González-Giraldo, Y., Camargo, A., López-León, S., Adan, A., & Forero, D. A. (2015). A functional SNP in MIR124-1, a brain expressed miRNA gene, is associated with aggressiveness in a Colombian sample. *European psychiatry : the journal of the Association of European Psychiatrists*, *30*(4), 499–503. https://doi.org/10.1016/j.eurpsy.2015.03.002

Gordovez, F. J. A., & McMahon, F. J. (2020). The genetics of bipolar disorder. *Molecular psychiatry*, *25*(3), 544–559. https://doi.org/10.1038/s41380-019-0634-7

Gordovez, F. J. A., & McMahon, F. J. (2020). The genetics of bipolar disorder. *Molecular psychiatry*, *25*(3), 544–559. https://doi.org/10.1038/s41380-019-0634-7

Grande, I., Berk, M., Birmaher, B., & Vieta, E. (2016). Bipolar disorder. *Lancet (London, England)*, *387*(10027), 1561–1572. https://doi.org/10.1016/S0140-6736(15)00241-X

Grosu, Ș. A., Dobre, M., Milanesi, E., & Hinescu, M. E. (2023). Blood-Based MicroRNAs in Psychotic Disorders-A Systematic

Review. *Biomedicines*, *11*(9), 2536. https://doi.org/10.3390/biomedicines11092536

Gunasekaran, S., Jacob, R. S., & Omkumar, R. V. (2022). Differential expression of miR-148b, miR-129-2 and miR-296 in animal models of schizophrenia-Relevance to NMDA receptor hypofunction. *Neuropharmacology*, *210*, 109024. https://doi.org/10.1016/j.neuropharm.2022.109024

Guo, L. K., Su, Y., Zhang, Y. Y., Yu, H., Lu, Z., Li, W. Q., Yang, Y. F., Xiao, X., Yan, H., Lu, T. L., Li, J., Liao, Y. D., Kang, Z. W., Wang, L. F., Li, Y., Li, M., Liu, B., Huang, H. L., Lv, L. X., Yao, Y., … Yue, W. H. (2023). Prediction of treatment response to antipsychotic drugs for precision medicine approach to schizophrenia: randomized trials and multiomics analysis. *Military Medical Research*, *10*(1), 24. https://doi.org/10.1186/s40779-023-00459-7

Gürel, Ç., Kuşçu, G. C., Yavaşoğlu, A., & Biray Avcı, Ç. (2020). The clues in solving the mystery of major psychosis: The epigenetic basis of schizophrenia and bipolar disorder. *Neuroscience and biobehavioral reviews*, *113*, 51–61. https://doi.org/10.1016/j.neubiorev.2020.03.005

Han, L., Wang, L., Tang, S., Yuan, L., Wu, S., Du, X., Xiang, Y., Qu, X., Liu, H., Luo, H., Qin, X., & Liu, C. (2018). ITGB4 deficiency in bronchial epithelial cells directs airway inflammation and bipolar disorder-related behavior. *Journal of neuroinflammation*, *15*(1), 246. https://doi.org/10.1186/s12974-018-1283-5

Harrison, P. J., Geddes, J. R., & Tunbridge, E. M. (2018). The Emerging Neurobiology of Bipolar Disorder. *Trends in neurosciences*, *41*(1), 18–30. https://doi.org/10.1016/j.tins.2017.10.006

Hoehe, M. R., & Morris-Rosendahl, D. J. (2018). The role of genetics and genomics in clinical psychiatry. *Dialogues in clinical neuroscience*, *20*(3), 169–177. https://doi.org/10.31887/DCNS.2018.20.3/mhoehe

Hotta, K., Hosaka, M., Tanabe, A., & Takeuchi, T. (2009). Secretogranin II binds to secretogranin III and forms secretory granules with orexin, neuropeptide Y, and POMC. *The Journal of endocrinology*, *202*(1), 111–121. https://doi.org/10.1677/JOE-08-0531

https://hbctraining.github.io/DGE_workshop_salmon_online/lessons/01c_RNAseq_count_distribution.html

https://www.bioconductor.org/packages/release/bioc/html/DESeq2.html

Izumi, R., Hino, M., Nagaoka, A., Shishido, R., Kakita, A., Hoshino, M., Kunii, Y., & Yabe, H. (2022). Dysregulation of DPYSL2 expression by mTOR signaling in schizophrenia: Multi-level study of postmortem brain. *Neuroscience research*, *175*, 73–81. https://doi.org/10.1016/j.neures.2021.09.004

Jauhar, S., Johnstone, M., & McKenna, P. J. (2022). Schizophrenia. *Lancet (London, England)*, *399*(10323), 473–486. https://doi.org/10.1016/S0140-6736(21)01730-X

Jensen, D., Chen, J., Turner, J. A., Stephen, J. M., Wang, Y. P., Wilson, T. W., Calhoun, V. D., & Liu, J. (2023). Epigenetic associations with adolescent grey matter maturation and cognitive development. *Frontiers in genetics*, *14*, 1222619. https://doi.org/10.3389/fgene.2023.1222619

Jiang, M., Gu, Y. F., Cai, J. F., Wang, A., He, Y., & Feng, Y. L. (2021). MiR-186-5p Dysregulation Leads to Depression-like Behavior by De-repressing SERPINF1 in Hippocampus. *Neuroscience*, *479*, 48–59. https://doi.org/10.1016/j.neuroscience.2021.10.005

Johnston-Wilson, N. L., Sims, C. D., Hofmann, J. P., Anderson, L., Shore, A. D., Torrey, E. F., & Yolken, R. H. (2000). Disease-specific alterations in frontal cortex brain proteins in schizophrenia, bipolar disorder, and major depressive disorder. The Stanley Neuropathology Consortium. *Molecular psychiatry*, *5*(2), 142–149. https://doi.org/10.1038/sj.mp.4000696

Kaksonen, M., & Roux, A. (2018). Mechanisms of clathrin-mediated endocytosis. *Nature reviews. Molecular cell biology*, *19*(5), 313–326. https://doi.org/10.1038/nrm.2017.132

Kamiguchi H. (2003). The mechanism of axon growth: what we have learned from the cell adhesion molecule L1. *Molecular neurobiology*, *28*(3), 219–228. https://doi.org/10.1385/MN:28:3:219

Kaminsky, Z., Jones, I., Verma, R., Saleh, L., Trivedi, H., Guintivano, J., Akman, R., Zandi, P., Lee, R. S., & Potash, J. B. (2015). DNA methylation and

expression of KCNQ3 in bipolar disorder. *Bipolar disorders*, *17*(2), 150–159. https://doi.org/10.1111/bdi.12230

Kaminsky, Z., Tochigi, M., Jia, P., Pal, M., Mill, J., Kwan, A., Ioshikhes, I., Vincent, J. B., Kennedy, J. L., Strauss, J., Pai, S., Wang, S. C., & Petronis, A. (2012). A multi-tissue analysis identifies HLA complex group 9 gene methylation differences in bipolar disorder. *Molecular psychiatry*, *17*(7), 728–740. https://doi.org/10.1038/mp.2011.64

Kari, D., Mijiti, P., Zou, S., & Zhang, P. (2023). Study on the correlation between suicidal ideation and ABI3BP gene、DPYSL2 gene methylation in pediatric bipolar disorder with depressive episode. *Heliyon*, *10*(1), e23680. https://doi.org/10.1016/j.heliyon.2023.e23680

Karin, M., Eddy, R. L., Henry, W. M., Haley, L. L., Byers, M. G., & Shows, T. B. (1984). Human metallothionein genes are clustered on chromosome 16. *Proceedings of the National Academy of Sciences of the United States of America*, *81*(17), 5494–5498. https://doi.org/10.1073/pnas.81.17.5494

Kato T. (2019). Current understanding of bipolar disorder: Toward integration of biological basis and treatment strategies. *Psychiatry and clinical neurosciences*, *73*(9), 526–540. https://doi.org/10.1111/pcn.12852

Kendler K. S. (2022). Incremental advances in psychiatric molecular genetics and nosology. *World psychiatry : official journal of the World Psychiatric Association (WPA)*, *21*(3), 415–416. https://doi.org/10.1002/wps.20999

Kolberg, L., Raudvere, U., Kuzmin, I., Adler, P., Vilo, J., & Peterson, H. (2023). g:Profiler-interoperable web service for functional enrichment analysis and gene identifier mapping (2023 update). *Nucleic acids research*, *51*(W1), W207–W212. https://doi.org/10.1093/nar/gkad347

Krassowski, M., Das, V., Sahu, S. K., & Misra, B. B. (2020). State of the Field in Multi-Omics Research: From Computational Needs to Data Mining and Sharing. Frontiers in genetics, 11, 610798. https://doi.org/10.3389/fgene.2020.610798

Kwak, J. H., Kim, S., Yu, N. K., Seo, H., Choi, J. E., Kim, J. I., Choi, D. I., Kim, M. W., Kwak, C., Lee, K., & Kaang, B. K. (2021). Loss of the neuronal

genome organizer and transcription factor CTCF induces neuronal death and reactive gliosis in the anterior cingulate cortex. *Genes, brain, and behavior*, *20*(2), e12701. https://doi.org/10.1111/gbb.12701

Lai, C. Y., Scarr, E., Udawela, M., Everall, I., Chen, W. J., & Dean, B. (2016). Biomarkers in schizophrenia: A focus on blood based diagnostics and theranostics. *World journal of psychiatry*, *6*(1), 102–117. https://doi.org/10.5498/wjp.v6.i1.102

Le Clerc, S., Lombardi, L., Baune, B. T., Amare, A. T., Schubert, K. O., Hou, L., Clark, S. R., Papiol, S., Cearns, M., Heilbronner, U., Degenhardt, F., Tekola-Ayele, F., Hsu, Y. H., Shekhtman, T., Adli, M., Akula, N., Akiyama, K., Ardau, R., Arias, B., Aubry, J. M., … Tamouza, R. (2021). HLA-DRB1 and HLA-DQB1 genetic diversity modulates response to lithium in bipolar affective disorders. *Scientific reports*, *11*(1), 17823. https://doi.org/10.1038/s41598-021-97140-7

Lee, H., Joo, J., Nah, S. S., Kim, J. W., Kim, H. K., Kwon, J. T., Lee, H. Y., Kim, Y. O., & Kim, H. J. (2015). Changes in Dpysl2 expression are associated with prenatally stressed rat offspring and susceptibility to schizophrenia in humans. *International journal of molecular medicine*, *35*(6), 1574–1586. https://doi.org/10.3892/ijmm.2015.2161

Li, S., & Tollefsbol, T. O. (2021). DNA methylation methods: Global DNA methylation and methylomic analyses. *Methods (San Diego, Calif.)*, *187*, 28–43. https://doi.org/10.1016/j.ymeth.2020.10.002

Lim, S. H., Sung, Y. J., Jo, N., Lee, N. Y., Kim, K. S., Lee, D. Y., Kim, N. S., Lee, J., Byun, J. Y., Shin, Y. B., & Lee, J. R. (2021). Nanoplasmonic immunosensor for the detection of SCG2, a candidate serum biomarker for the early diagnosis of neurodevelopmental disorder. *Scientific reports*, *11*(1), 22764. https://doi.org/10.1038/s41598-021-02262-7

Lindskrog, S. V., Prip, F., Lamy, P., Taber, A., Groeneveld, C. S., Birkenkamp-Demtröder, K., Jensen, J. B., Strandgaard, T., Nordentoft, I., Christensen, E., Sokac, M., Birkbak, N. J., Maretty, L., Hermann, G. G., Petersen, A. C., Weyerer, V., Grimm, M. O., Horstmann, M., Sjödahl, G., Höglund, M., … Dyrskjøt, L. (2021). An integrated multi-omics analysis identifies prognostic

molecular subtypes of non-muscle-invasive bladder cancer. *Nature communications*, *12*(1), 2301. https://doi.org/10.1038/s41467-021-22465-w

Liu, L., Li, Y., Li, S., Hu, N., He, Y., Pong, R., Lin, D., Lu, L., & Law, M. (2012). Comparison of next-generation sequencing systems. *Journal of biomedicine & biotechnology*, *2012*, 251364. https://doi.org/10.1155/2012/251364

Liu, Z., Li, X., Gao, Y., Liu, J., Feng, Y., Liu, Y., Wang, J., Wang, C., Wang, D., He, J., Han, W., Mei, Q., & Sun, Y. (2023). Epigenetic reprogramming of Runx3 reinforces CD8 + T-cell function and improves the clinical response to immunotherapy. *Molecular cancer*, *22*(1), 84. https://doi.org/10.1186/s12943-023-01768-0

Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome biology*, *15*(12), 550. https://doi.org/10.1186/s13059-014-0550-8

Ludwig, B., & Dwivedi, Y. (2016). Dissecting bipolar disorder complexity through epigenomic approach. *Molecular psychiatry*, *21*(11), 1490–1498. https://doi.org/10.1038/mp.2016.123

Ludwig, B., & Dwivedi, Y. (2016). Dissecting bipolar disorder complexity through epigenomic approach. *Molecular psychiatry*, *21*(11), 1490–1498. https://doi.org/10.1038/mp.2016.123

Mao, Q., Huang, S., Luo, X., Liu, P., Wang, X., Wang, K., Zhang, Y., Chen, B., & Luo, X. (2023). Spatial Multiomics Analysis in Psychiatric Disorders. *EC psychology and psychiatry*, *12*(6), 1–5.

Marder, S. R., & Cannon, T. D. (2019). Schizophrenia. *The New England journal of medicine*, *381*(18), 1753–1761. https://doi.org/10.1056/NEJMra1808803

McIntyre, R. S., Berk, M., Brietzke, E., Goldstein, B. I., López-Jaramillo, C., Kessing, L. V., Malhi, G. S., Nierenberg, A. A., Rosenblat, J. D., Majeed, A., Vieta, E., Vinberg, M., Young, A. H., & Mansur, R. B. (2020). Bipolar disorders. *Lancet (London, England)*, *396*(10265), 1841–1856. https://doi.org/10.1016/S0140-6736(20)31544-0

Mehta, D., Iwamoto, K., Ueda, J., Bundo, M., Adati, N., Kojima, T., & Kato, T. (2014). Comprehensive survey of CNVs influencing gene expression in the human brain and its implications for pathophysiology. *Neuroscience research*, *79*, 22–33. https://doi.org/10.1016/j.neures.2013.10.009

Mill, J., Tang, T., Kaminsky, Z., Khare, T., Yazdanpanah, S., Bouchard, L., Jia, P., Assadzadeh, A., Flanagan, J., Schumacher, A., Wang, S. C., & Petronis, A. (2008). Epigenomic profiling reveals DNA-methylation changes associated with major psychosis. *American journal of human genetics*, *82*(3), 696–711. https://doi.org/10.1016/j.ajhg.2008.01.008

Miola, A., De Filippis, E., Veldic, M., Ho, A. M., Winham, S. J., Mendoza, M., Romo-Nava, F., Nunez, N. A., Gardea Resendez, M., Prieto, M. L., McElroy, S. L., Biernacka, J. M., Frye, M. A., & Cuellar-Barboza, A. B. (2022). The genetics of bipolar disorder with obesity and type 2 diabetes. *Journal of affective disorders*, *313*, 222–231. https://doi.org/10.1016/j.jad.2022.06.084

Mokhtari, A., Porte, B., Belzeaux, R., Etain, B., Ibrahim, E. C., Marie-Claire, C., Lutz, P. E., & Delahaye-Duriez, A. (2022). The molecular pathophysiology of mood disorders: From the analysis of single molecular layers to multi-omic integration. *Progress in neuro-psychopharmacology & biological psychiatry*, *116*, 110520. https://doi.org/10.1016/j.pnpbp.2022.110520

Morgan, L. Z., Rollins, B., Sequeira, A., Byerley, W., DeLisi, L. E., Schatzberg, A. F., Barchas, J. D., Myers, R. M., Watson, S. J., Akil, H., Bunney, W. E., Jr, & Vawter, M. P. (2016). Quantitative Trait Locus and Brain Expression of HLA-DPA1 Offers Evidence of Shared Immune Alterations in Psychiatric Disorders. *Microarrays (Basel, Switzerland)*, *5*(1), 6. https://doi.org/10.3390/microarrays5010006

Mothersill, O., Knee-Zaska, C., & Donohoe, G. (2016). Emotion and Theory of Mind in Schizophrenia-Investigating the Role of the Cerebellum. *Cerebellum (London, England)*, *15*(3), 357–368. https://doi.org/10.1007/s12311-015-0696-2

Muzellec, B., Teleńczuk, M., Cabeli, V., & Andreux, M. (2023). PyDESeq2: a python package for bulk RNA-seq differential expression

analysis. *Bioinformatics (Oxford, England)*, *39*(9), btad547. https://doi.org/10.1093/bioinformatics/btad547

Nakatani, N., Hattori, E., Ohnishi, T., Dean, B., Iwayama, Y., Matsumoto, I., Kato, T., Osumi, N., Higuchi, T., Niwa, S., & Yoshikawa, T. (2006). Genome-wide expression analysis detects eight genes with robust alterations specific to bipolar I disorder: relevance to neuronal network perturbation. *Human molecular genetics*, *15*(12), 1949–1962. https://doi.org/10.1093/hmg/ddl118

Niemsiri, V., Rosenthal, S. B., Nievergelt, C. M., Maihofer, A. X., Marchetto, M. C., Santos, R., Shekhtman, T., Alliey-Rodriguez, N., Anand, A., Balaraman, Y., Berrettini, W. H., Bertram, H., Burdick, K. E., Calabrese, J. R., Calkin, C. V., Conroy, C., Coryell, W. H., DeModena, A., Eyler, L. T., Feeder, S., … Kelsoe, J. R. (2023). Focal adhesion is associated with lithium response in bipolar disorder: evidence from a network-based multi-omics analysis. *Molecular psychiatry*, 10.1038/s41380-022-01909-9. Advance online publication. https://doi.org/10.1038/s41380-022-01909-9

Pai, S., Li, P., Killinger, B., Marshall, L., Jia, P., Liao, J., Petronis, A., Szabó, P. E., & Labrie, V. (2019). Differential methylation of enhancer at IGF2 is associated with abnormal dopamine synthesis in major psychosis. *Nature communications*, *10*(1), 2046. https://doi.org/10.1038/s41467-019-09786-7

Peedicayil J. (2014). Epigenetic approaches for bipolar disorder drug discovery. *Expert opinion on drug discovery*, *9*(8), 917–930. https://doi.org/10.1517/17460441.2014.922537

Peedicayil J. (2023). Genome-Environment Interactions and Psychiatric Disorders. *Biomedicines*, *11*(4), 1209. https://doi.org/10.3390/biomedicines11041209

Perroud, N., Zewdie, S., Stenz, L., Adouan, W., Bavamian, S., Prada, P., Nicastro, R., Hasler, R., Nallet, A., Piguet, C., Paoloni-Giacobino, A., Aubry, J. M., & Dayer, A. (2016). METHYLATION OF SEROTONIN RECEPTOR 3A IN ADHD, BORDERLINE PERSONALITY, AND BIPOLAR DISORDERS: LINK WITH SEVERITY OF THE DISORDERS AND CHILDHOOD MALTREATMENT. *Depression and anxiety*, *33*(1), 45–55. https://doi.org/10.1002/da.22406

Peter-Ross E. M. (2018). Molecular hypotheses to explain the shared pathways and underlying pathobiological causes in catatonia and in catatonic presentations in neuropsychiatric disorders. *Medical hypotheses*, *113*, 54–64. https://doi.org/10.1016/j.mehy.2018.02.009

Piao, Y., Xu, W., Park, K. H., Ryu, K. H., & Xiang, R. (2021). Comprehensive Evaluation of Differential Methylation Analysis Methods for Bisulfite Sequencing Data. *International journal of environmental research and public health*, *18*(15), 7975. https://doi.org/10.3390/ijerph18157975

Pisanu, C., Congiu, D., Costa, M., Chillotti, C., Ardau, R., Severino, G., Angius, A., Heilbronner, U., Hou, L., McMahon, F. J., Schulze, T. G., Del Zompo, M., & Squassina, A. (2018). Convergent analysis of genome-wide genotyping and transcriptomic data suggests association of zinc finger genes with lithium response in bipolar disorder. *American journal of medical genetics. Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics*, *177*(7), 658–664. https://doi.org/10.1002/ajmg.b.32663

Prichard, K. L., O'Brien, N. S., Murcia, S. R., Baker, J. R., & McCluskey, A. (2022). Role of Clathrin and Dynamin in Clathrin Mediated Endocytosis/Synaptic Vesicle Recycling and Implications in Neurological Diseases. *Frontiers in cellular neuroscience*, *15*, 754110. https://doi.org/10.3389/fncel.2021.754110

Purcell, S. M., Moran, J. L., Fromer, M., Ruderfer, D., Solovieff, N., Roussos, P., O'Dushlaine, C., Chambert, K., Bergen, S. E., Kähler, A., Duncan, L., Stahl, E., Genovese, G., Fernández, E., Collins, M. O., Komiyama, N. H., Choudhary, J. S., Magnusson, P. K., Banks, E., Shakir, K., … Sklar, P. (2014). A polygenic burden of rare disruptive mutations in schizophrenia. *Nature*, *506*(7487), 185–190. https://doi.org/10.1038/nature12975

Rao, J. S., Keleshian, V. L., Klein, S., & Rapoport, S. I. (2012). Epigenetic modifications in frontal cortex from Alzheimer's disease and bipolar disorder patients. *Translational psychiatry*, *2*(7), e132. https://doi.org/10.1038/tp.2012.55

Reckziegel, R., Czepielewski, L. S., Hasse-Sousa, M., Martins, D. S., de Britto, M. J., Lapa, C. O., Schwartzhaupt, A. W., & Gama, C. S. (2022). Heterogeneous trajectories in schizophrenia: insights from neurodevelopment and neuroprogression models. *Revista brasileira de psiquiatria (Sao Paulo, Brazil : 1999)*, *44*(1), 74–80. https://doi.org/10.1590/1516-4446-2020-1670

Rodríguez, B., Nani, J. V., Almeida, P. G. C., Brietzke, E., Lee, R. S., & Hayashi, M. A. F. (2020). Neuropeptides and oligopeptidases in schizophrenia. *Neuroscience and biobehavioral reviews*, *108*, 679–693. https://doi.org/10.1016/j.neubiorev.2019.11.024

Sanders, A. R., Göring, H. H., Duan, J., Drigalenko, E. I., Moy, W., Freda, J., He, D., Shi, J., MGS, & Gejman, P. V. (2013). Transcriptome study of differential expression in schizophrenia. *Human molecular genetics*, *22*(24), 5001–5014. https://doi.org/10.1093/hmg/ddt350

Sathyanarayanan, A., Mueller, T. T., Ali Moni, M., Schueler, K., ECNP TWG Network members, Baune, B. T., Lio, P., Mehta, D., Baune, B. T., Dierssen, M., Ebert, B., Fabbri, C., Fusar-Poli, P., Gennarelli, M., Harmer, C., Howes, O. D., Janzing, J. G. E., Lio, P., Maron, E., Mehta, D., … Xicota, L. (2023). Multi-omics data integration methods and their applications in psychiatric disorders. *European neuropsychopharmacology : the journal of the European College of Neuropsychopharmacology*, *69*, 26–46. https://doi.org/10.1016/j.euroneuro.2023.01.001

Schizophrenia Working Group of the Psychiatric Genomics Consortium (2014). Biological insights from 108 schizophrenia-associated genetic loci. *Nature*, *511*(7510), 421–427. https://doi.org/10.1038/nature13595

Schubert, K. O., Föcking, M., Prehn, J. H., & Cotter, D. R. (2012). Hypothesis review: are clathrin-mediated endocytosis and clathrin-dependent membrane and protein trafficking core pathophysiological processes in schizophrenia and bipolar disorder?. *Molecular psychiatry*, *17*(7), 669–681. https://doi.org/10.1038/mp.2011.123

Seifuddin, F., Pirooznia, M., Judy, J. T., Goes, F. S., Potash, J. B., & Zandi, P. P. (2013). Systematic review of genome-wide gene expression

studies of bipolar disorder. *BMC psychiatry*, *13*, 213. https://doi.org/10.1186/1471-244X-13-213

Shan, Z. N., Tian, R., Zhang, M., Gui, Z. H., Wu, J., Ding, M., Zhou, X. F., & He, J. (2016). miR128-1 inhibits the growth of glioblastoma multiforme and glioma stem-like cells via targeting BMI1 and E2F3. *Oncotarget*, *7*(48), 78813–78826. https://doi.org/10.18632/oncotarget.12385

Singh, T., Poterba, T., Curtis, D., Akil, H., Al Eissa, M., Barchas, J. D., Bass, N., Bigdeli, T. B., Breen, G., Bromet, E. J., Buckley, P. F., Bunney, W. E., Bybjerg-Grauholm, J., Byerley, W. F., Chapman, S. B., Chen, W. J., Churchhouse, C., Craddock, N., Cusick, C. M., DeLisi, L., … Daly, M. J. (2022). Rare coding variants in ten genes confer substantial risk for schizophrenia. *Nature*, *604*(7906), 509–516. https://doi.org/10.1038/s41586-022-04556-w

Skvortsova, K., Stirzaker, C., & Taberlay, P. (2019). The DNA methylation landscape in cancer. *Essays in biochemistry*, *63*(6), 797–811. https://doi.org/10.1042/EBC20190037

Smirnova, L., Gräfe, A., Seiler, A., Schumacher, S., Nitsch, R., & Wulczyn, F. G. (2005). Regulation of miRNA expression during neural cell specification. *The European journal of neuroscience*, *21*(6), 1469–1477. https://doi.org/10.1111/j.1460-9568.2005.03978.x

Smoller, J. W., Andreassen, O. A., Edenberg, H. J., Faraone, S. V., Glatt, S. J., & Kendler, K. S. (2019). Psychiatric genetics and the structure of psychopathology. *Molecular psychiatry*, *24*(3), 409–420. https://doi.org/10.1038/s41380-017-0010-4

Stapel, B., Xiao, K., Gorinski, N., Schmidt, K., Pfanne, A., Fiedler, J., Richter, I., Vollbrecht, A. L., Thum, T., Kahl, K. G., & Ponimaskin, E. (2022). MicroRNAs as novel peripheral markers for suicidality in patients with major depressive disorder. *Frontiers in psychiatry*, *13*, 1020530. https://doi.org/10.3389/fpsyt.2022.1020530

Subramanian, I., Verma, S., Kumar, S., Jere, A., & Anamika, K. (2020). Multi-omics Data Integration, Interpretation, and Its Application. *Bioinformatics and*

biology insights, *14*, 1177932219899051. https://doi.org/10.1177/1177932219899051

Sugawara, H., Iwamoto, K., Bundo, M., Ueda, J., Miyauchi, T., Komori, A., Kazuno, A., Adati, N., Kusumi, I., Okazaki, Y., Ishigooka, J., Kojima, T., & Kato, T. (2011). Hypermethylation of serotonin transporter gene in bipolar disorder detected by epigenome analysis of discordant monozygotic twins. *Translational psychiatry*, *1*(7), e24. https://doi.org/10.1038/tp.2011.26

Supriyanto, I., Watanabe, Y., Mouri, K., Shiroiwa, K., Ratta-Apha, W., Yoshida, M., Tamiya, G., Sasada, T., Eguchi, N., Okazaki, K., Shirakawa, O., Someya, T., & Hishimoto, A. (2013). A missense mutation in the ITGA8 gene, a cell adhesion molecule gene, is associated with schizophrenia in Japanese female patients. *Progress in neuro-psychopharmacology & biological psychiatry*, *40*, 347–352. https://doi.org/10.1016/j.pnpbp.2012.11.002

Tamouza, R., Oliveira, J., Etain, B., Bengoufa, D., Hamdani, N., Manier, C., Mariaselvam, C., Sundaresh, A., Bellivier, F., Henry, C., Kahn, J. P., Krishnamoorthy, R., Charron, D., & Leboyer, M. (2018). HLA genetics in bipolar disorder. *Acta psychiatrica Scandinavica*, *138*(5), 464–471. https://doi.org/10.1111/acps.12912

Traglia, M., Croen, L. A., Lyall, K., Windham, G. C., Kharrazi, M., DeLorenze, G. N., Torres, A. R., & Weiss, L. A. (2017). Independent Maternal and Fetal Genetic Effects on Midgestational Circulating Levels of Environmental Pollutants. *G3 (Bethesda, Md.)*, *7*(4), 1287–1299. https://doi.org/10.1534/g3.117.039784

Trubetskoy, V., Pardiñas, A. F., Qi, T., Panagiotaropoulou, G., Awasthi, S., Bigdeli, T. B., Bryois, J., Chen, C. Y., Dennison, C. A., Hall, L. S., Lam, M., Watanabe, K., Frei, O., Ge, T., Harwood, J. C., Koopmans, F., Magnusson, S., Richards, A. L., Sidorenko, J., Wu, Y., … Schizophrenia Working Group of the Psychiatric Genomics Consortium (2022). Mapping genomic loci implicates genes and synaptic biology in schizophrenia. *Nature*, *604*(7906), 502–508. https://doi.org/10.1038/s41586-022-04434-5

van de Leemput, J., Hess, J. L., Glatt, S. J., & Tsuang, M. T. (2016). Genetics of Schizophrenia: Historical Insights and Prevailing Evidence. *Advances in genetics*, *96*, 99–141. https://doi.org/10.1016/bs.adgen.2016.08.001

Voorn, R. A., & Vogl, C. (2020). Molecular Assembly and Structural Plasticity of Sensory Ribbon Synapses-A Presynaptic Perspective. *International journal of molecular sciences*, *21*(22), 8758. https://doi.org/10.3390/ijms21228758

Wang, D., Liu, S., Warrell, J., Won, H., Shi, X., Navarro, F. C. P., Clarke, D., Gu, M., Emani, P., Yang, Y. T., Xu, M., Gandal, M. J., Lou, S., Zhang, J., Park, J. J., Yan, C., Rhie, S. K., Manakongtreecheep, K., Zhou, H., Nathan, A., … Gerstein, M. B. (2018). Comprehensive functional genomic resource and integrative model for the human brain. *Science (New York, N.Y.)*, *362*(6420), eaat8464. https://doi.org/10.1126/science.aat8464

Wang, S., Shi, X., Wu, M., & Ma, S. (2019). Horizontal and vertical integrative analysis methods for mental disorders omics data. *Scientific reports*, *9*(1), 13430. https://doi.org/10.1038/s41598-019-49718-5

Wang, Z., Sha, H. H., & Li, H. J. (2019). Functions and mechanisms of miR-186 in human cancer. *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie*, *119*, 109428. https://doi.org/10.1016/j.biopha.2019.109428

Watanabe, Y., Iijima, Y., Egawa, J., Nunokawa, A., Kaneko, N., Arinami, T., Ujike, H., Inada, T., Iwata, N., Kunugi, H., Itokawa, M., Sasaki, T., Ozaki, N., Hashimoto, R., Shibuya, M., Igeta, H., & Someya, T. (2013). Replication in a Japanese population that a MIR30E gene variation is associated with schizophrenia. *Schizophrenia research*, *150*(2-3), 596–597. https://doi.org/10.1016/j.schres.2013.08.028

Wei, T., Nie, J., Larson, N. B., Ye, Z., Eckel-Passow, J. E., Robertson, K. D., Kocher, J. A., & Wang, L. (2021). CpGtools: a python package for DNA methylation analysis. *Bioinformatics (Oxford, England)*, *37*(11), 1598–1599. https://doi.org/10.1093/bioinformatics/btz916

World Health Organization. (2019). *International statistical classification of diseases and related health problems* (11th ed.). https://icd.who.int/

Xie, C., Leung, Y. K., Chen, A., Long, D. X., Hoyo, C., & Ho, S. M. (2019). Differential methylation values in differential methylation analysis. *Bioinformatics (Oxford, England)*, *35*(7), 1094–1097. https://doi.org/10.1093/bioinformatics/bty778

Xu, Y., Li, F., Zhang, B., Zhang, K., Zhang, F., Huang, X., Sun, N., Ren, Y., Sui, M., & Liu, P. (2010). MicroRNAs and target site screening reveals a pre-microRNA-30e variant associated with schizophrenia. *Schizophrenia research*, *119*(1-3), 219–227. https://doi.org/10.1016/j.schres.2010.02.1070

Yang, Y., Chen, J., Guo, Z., Deng, S., Du, X., Zhu, S., Ye, C., Shi, Y. S., & Liu, J. J. (2018). Endophilin A1 Promotes Actin Polymerization in Dendritic Spines Required for Synaptic Potentiation. *Frontiers in molecular neuroscience*, *11*, 177. https://doi.org/10.3389/fnmol.2018.00177

Yılmaz, S., Kılıç, N., Kaya, Ş., & Taşcı, G. (2023). A Potential Biomarker for Predicting Schizophrenia: Metallothionein-1. *Biomedicines*, *11*(2), 590. https://doi.org/10.3390/biomedicines11020590

Yoshino, Y., & Dwivedi, Y. (2020). Non-Coding RNAs in Psychiatric Disorders and Suicidal Behavior. *Frontiers in psychiatry*, *11*, 543893. https://doi.org/10.3389/fpsyt.2020.543893

Zamanpoor M. (2020). Schizophrenia in a genomic era: a review from the pathogenesis, genetic and environmental etiology to diagnosis and treatment insights. *Psychiatric genetics*, *30*(1), 1–9. https://doi.org/10.1097/YPG.0000000000000245

Zeng, D., He, S., Yu, S., Li, G., Ma, C., Wen, Y., Shen, Y., Yu, Y., & Li, H. (2018). Analysis of the association of *MIR124-1* and its target gene *RGS4*polymorphisms with major depressive disorder and antidepressant response. *Neuropsychiatric disease and treatment*, *14*, 715–723. https://doi.org/10.2147/NDT.S155076

Zeng, D., He, S., Zhao, N., Hu, M., Gao, J., Yu, Y., Huang, J., Shen, Y., & Li, H. (2021). Promoter Hypomethylation of miR-124 Gene Is Associated With Major Depressive Disorder. *Frontiers in molecular neuroscience*, *14*, 771103. https://doi.org/10.3389/fnmol.2021.771103

Zhang, H. C., Du, Y., Chen, L., Yuan, Z. Q., & Cheng, Y. (2023). MicroRNA schizophrenia: Etiology, biomarkers and therapeutic targets. *Neuroscience and biobehavioral reviews*, *146*, 105064. https://doi.org/10.1016/j.neubiorev.2023.105064

Zhang, Q., Chen, B., Yang, P., Wu, J., Pang, X., & Pang, C. (2022). Bioinformatics-based study reveals that AP2M1 is regulated by the circRNA-miRNA-mRNA interaction network and affects Alzheimer's disease. *Frontiers in genetics*, *13*, 1049786. https://doi.org/10.3389/fgene.2022.1049786

Zhang, W., Zhang, M., Xu, Z., Yan, H., Wang, H., Jiang, J., Wan, J., Tang, B., Liu, C., Chen, C., & Meng, Q. (2023). Human forebrain organoid-based multi-omics analyses of PCCB as a schizophrenia associated gene linked to GABAergic pathways. *Nature communications*, *14*(1), 5176. https://doi.org/10.1038/s41467-023-40861-2

Zhang, Y., Sun, Z., Jia, J., Du, T., Zhang, N., Tang, Y., Fang, Y., & Fang, D. (2021). Overview of Histone Modification. *Advances in experimental medicine and biology*, *1283*, 1–16. https://doi.org/10.1007/978-981-15-8104-5_1

Zhao, C., Wang, F., Pun, F. W., Mei, L., Ren, L., Yu, Z., Ng, S. K., Chen, J., Tsang, S. Y., & Xue, H. (2012). Epigenetic regulation on GABRB2 isoforms expression: developmental variations and disruptions in psychotic disorders. *Schizophrenia research*, *134*(2-3), 260–266. https://doi.org/10.1016/j.schres.2011.11.029

Zhao, H., Xu, J., Pang, L., Zhang, Y., Fan, H., Liu, L., Liu, T., Yu, F., Zhang, G., Lan, Y., Bai, J., Li, X., & Xiao, Y. (2015). Genome-wide DNA methylome reveals the dysfunction of intronic microRNAs in major psychosis. *BMC medical genomics*, *8*, 62. https://doi.org/10.1186/s12920-015-0139-4

Zhou, W., Sun, J., Huai, C., Liu, Y., Chen, L., Yi, Z., Lv, Q., Song, C., Zhu, W., Liu, C., Weng, S., Wu, H., Sun, Y., Zhang, R., Wu, L., Li, M., Zhu, J., Zhang, Y., Wei, M., Guo, Y., … Qin, S. (2022). Multi-omics analysis identifies rare variation in leptin/PPAR gene sets and hypermethylation of ABCG1 contribute to antipsychotics-induced metabolic syndromes. *Molecular psychiatry*, *27*(12), 5195–5205. https://doi.org/10.1038/s41380-022-01759-5

ATTACHMENTS

ATTACHMENT 1. Results of g:profiler Analysis of DEGs and DMGs of SZ and BD

Supplementary Table 1. GO and KEGG Analysis of Overall DEG of BD and Control

source	term_name	term_id	adjusted_p_value
GO:BP	cellular response to copper ion	GO:0071280	1E-05
GO:BP	cellular response to zinc ion	GO:0071294	4E-04
GO:BP	response to copper ion	GO:0046688	4E-04
GO:BP	antigen processing and presentation of exogenous peptide antigen via MHC class II	GO:0019886	6E-04
GO:BP	antigen processing and presentation of exogenous peptide antigen	GO:0002478	8E-04
GO:BP	protein-containing complex organization	GO:0043933	1E-03
GO:BP	antigen processing and presentation of peptide antigen via MHC class II	GO:0002495	2E-03
GO:BP	cell activation	GO:0001775	3E-03
GO:BP	leukocyte activation	GO:0045321	3E-03
GO:BP	antigen processing and presentation of peptide or polysaccharide antigen via MHC class II	GO:0002504	6E-03
GO:BP	antigen processing and presentation of peptide antigen	GO:0048002	6E-03
GO:BP	antigen processing and presentation of exogenous antigen	GO:0019884	7E-03
GO:BP	response to metal ion	GO:0010038	7E-03
GO:BP	lymphocyte activation	GO:0046649	9E-03
GO:BP	leukocyte cell-cell adhesion	GO:0007159	1E-02
GO:BP	response to inorganic substance	GO:0010035	2E-02
GO:BP	cellular component organization	GO:0016043	2E-02
GO:BP	response to cadmium ion	GO:0046686	2E-02
GO:BP	regulation of leukocyte cell-cell adhesion	GO:1903037	2E-02
GO:BP	regulation of cell adhesion	GO:0030155	3E-02
GO:BP	localization	GO:0051179	4E-02
GO:BP	regulation of cell-cell adhesion	GO:0022407	5E-02
GO:BP	synaptic vesicle cycle	GO:0099504	5E-02

KEGG	Phagosome	KEGG:04145	1E-02

Supplementary Table 2. GO and KEGG Analysis of Upregulated DEG of BD and Control

source	term_name	term_id	adjusted_p_value
GO:BP	mRNA trans splicing, via spliceosome	GO:0000365	3.01E-13
GO:BP	formation of quadruple SL/U4/U5/U6 snRNP	GO:0000353	3.01E-13
GO:BP	mRNA trans splicing, SL addition	GO:0045291	3.01E-13
GO:BP	spliceosomal tri-snRNP complex assembly	GO:0000244	8.68E-13
GO:BP	spliceosomal snRNP assembly	GO:0000387	2.91E-11
GO:BP	protein-RNA complex assembly	GO:0022618	2.58E-06
GO:BP	protein-RNA complex organization	GO:0071826	3.40E-06
GO:BP	mRNA splicing, via spliceosome	GO:0000398	7.37E-07
GO:BP	RNA splicing, via transesterification reactions with bulged adenosine as nucleophile	GO:0000377	7.37E-07
GO:BP	RNA splicing, via transesterification reactions	GO:0000375	8.38E-06
GO:BP	RNA splicing	GO:0008380	1.94E-05
GO:BP	ribonucleoprotein complex biogenesis	GO:0022613	2.34E-04
GO:BP	mRNA processing	GO:0006397	3.94E-03
GO:BP	mRNA metabolic process	GO:0016071	5.97E-02
GO:BP	protein-containing complex assembly	GO:0065003	1.19E-01
GO:BP	RNA processing	GO:0006396	1.28E-03
GO:BP	protein-containing complex organization	GO:0043933	2.28E-01
GO:BP	macromolecule biosynthetic process	GO:0009059	4.76E-02
GO:BP	gene expression	GO:0010467	1.12E-04
GO:BP	RNA metabolic process	GO:0016070	3.10E-04
GO:BP	cellular component assembly	GO:0022607	4.60E-03
GO:BP	cellular aromatic compound metabolic process	GO:0006725	1.54E-05
GO:BP	nucleic acid metabolic process	GO:0090304	1.60E-04
GO:BP	cellular component biogenesis	GO:0044085	1.83E-05
GO:BP	heterocycle metabolic process	GO:0046483	8.58E-04
GO:BP	nucleobase-containing compound metabolic process	GO:0006139	1.33E-06
GO:BP	organic cyclic compound metabolic process	GO:1901360	1.90E-06
GO:BP	cellular biosynthetic process	GO:0044249	8.80E-05

GO:BP	cellular response to copper ion	GO:0071280	1.28E-07
GO:BP	organic substance biosynthetic process	GO:1901576	2.01E-07
GO:BP	cellular nitrogen compound metabolic process	GO:0034641	3.16E-06
GO:BP	biosynthetic process	GO:0009058	3.18E-05
GO:BP	response to copper ion	GO:0046688	4.07E-07
GO:BP	cellular response to zinc ion	GO:0071294	6.43E-07
GO:BP	macromolecule metabolic process	GO:0043170	4.25E-05
GO:BP	cellular metabolic process	GO:0044237	1.87E-04
GO:BP	response to zinc ion	GO:0010043	4.24E-04
GO:BP	cellular response to inorganic substance	GO:0071241	2.92E-03
GO:BP	cellular response to cadmium ion	GO:0071276	1.38E-02
GO:BP	organic substance metabolic process	GO:0071704	2.50E-02
GO:BP	cellular component organization	GO:0016043	2.77E-02
GO:BP	cellular response to metal ion	GO:0071248	3.23E-02
KEGG	MicroRNAs in cancer	KEGG:05206	1.97E-06
KEGG	Mineral absorption	KEGG:04978	2.15E-06
KEGG	Herpes simplex virus 1 infection	KEGG:05168	1.76E-02

Supplementary Table 3. GO and KEGG Analysis of Downregulated DEG of BD and Control

source	term_name	term_id	adjusted_p_value
GO:BP	localization	GO:0051179	1.40E-08
GO:BP	transport	GO:0006810	3.82E-08
GO:BP	establishment of localization	GO:0051234	5.14E-08
GO:BP	positive regulation of cellular process	GO:0048522	9.00E-08
GO:BP	organonitrogen compound metabolic process	GO:1901564	1.66E-06
GO:BP	positive regulation of biological process	GO:0048518	5.11E-06
GO:BP	regulation of biological quality	GO:0065008	8.48E-06
GO:BP	vesicle-mediated transport	GO:0016192	1.35E-05
GO:BP	cell activation	GO:0001775	5.59E-05
GO:BP	regulation of localization	GO:0032879	1.02E-04
GO:BP	regulation of transport	GO:0051049	1.08E-04
GO:BP	exocytosis	GO:0006887	1.82E-04

GO:BP	small molecule metabolic process	GO:0044281	1.93E-04
GO:BP	leukocyte activation	GO:0045321	2.54E-04
GO:BP	regulation of vesicle-mediated transport	GO:0060627	4.60E-04
GO:BP	organelle organization	GO:0006996	4.75E-04
GO:BP	synaptic vesicle cycle	GO:0099504	4.94E-04
GO:BP	response to stimulus	GO:0050896	9.91E-04
GO:BP	regulation of exocytosis	GO:0017157	1.00E-03
GO:BP	cellular localization	GO:0051641	1.18E-03
GO:BP	regulation of cell adhesion	GO:0030155	1.20E-03
GO:BP	leukocyte cell-cell adhesion	GO:0007159	1.21E-03
GO:BP	lymphocyte activation	GO:0046649	1.39E-03
GO:BP	response to stress	GO:0006950	1.56E-03
GO:BP	protein metabolic process	GO:0019538	2.06E-03
GO:BP	macromolecule modification	GO:0043412	2.35E-03
GO:BP	vacuole organization	GO:0007033	2.64E-03
GO:BP	regulation of cytokine production	GO:0001817	2.71E-03
GO:BP	vesicle-mediated transport in synapse	GO:0099003	2.84E-03
GO:BP	cytokine production	GO:0001816	3.32E-03
GO:BP	regulation of cell-cell adhesion	GO:0022407	3.82E-03
GO:BP	membrane organization	GO:0061024	4.80E-03
GO:BP	regulation of leukocyte cell-cell adhesion	GO:1903037	5.76E-03
GO:BP	regulation of cellular component organization	GO:0051128	5.88E-03
GO:BP	catabolic process	GO:0009056	6.05E-03
GO:BP	cell death	GO:0008219	8.01E-03
GO:BP	antigen processing and presentation of exogenous peptide antigen	GO:0002478	8.63E-03
GO:BP	export from cell	GO:0140352	8.72E-03
GO:BP	positive regulation of immune system process	GO:0002684	1.02E-01
GO:BP	nitrogen compound transport	GO:0071705	1.10E-02
GO:BP	organonitrogen compound biosynthetic process	GO:1901566	1.11E-02
GO:BP	T cell activation	GO:0042110	1.14E-02
GO:BP	secretion	GO:0046903	1.20E-02
GO:BP	antigen processing and presentation of exogenous peptide antigen via MHC class II	GO:0019886	1.28E-02
GO:BP	secretion by cell	GO:0032940	1.45E-02

GO:BP	regulated exocytosis	GO:0045055	1.46E-02
GO:BP	organic substance transport	GO:0071702	1.55E-02
GO:BP	antigen processing and presentation of peptide antigen	GO:0048002	1.79E-02
GO:BP	apoptotic process	GO:0006915	2.04E-02
GO:BP	leukocyte activation involved in immune response	GO:0002366	2.31E-02
GO:BP	positive regulation of cellular metabolic process	GO:0031325	2.37E-02
GO:BP	programmed cell death	GO:0012501	2.39E-02
GO:BP	macroautophagy	GO:0016236	2.46E-02
GO:BP	cellular response to stimulus	GO:0051716	2.50E-02
GO:BP	regulation of organelle organization	GO:0033043	2.65E-02
GO:BP	cellular homeostasis	GO:0019725	2.68E-02
GO:BP	cell activation involved in immune response	GO:0002263	2.84E-02
GO:BP	organelle localization	GO:0051640	3.19E-02
GO:BP	positive regulation of cellular biosynthetic process	GO:0031328	3.21E-02
GO:BP	establishment of localization in cell	GO:0051649	3.28E-02
GO:BP	antigen processing and presentation of peptide antigen via MHC class II	GO:0002495	3.34E-02
GO:BP	carbohydrate derivative metabolic process	GO:1901135	3.76E-02
GO:BP	positive regulation of macromolecule biosynthetic process	GO:0010557	4.06E-02
GO:BP	positive regulation of biosynthetic process	GO:0009891	4.32E-02
GO:BP	positive regulation of metabolic process	GO:0009893	4.36E-02
GO:BP	antigen processing and presentation of exogenous antigen	GO:0019884	4.78E-02
GO:BP	homeostatic process	GO:0042592	4.95E-02
KEGG	Phagosome	KEGG:04145	6.30E-04
KEGG	Biosynthesis of cofactors	KEGG:01240	1.53E-02
KEGG	Synaptic vesicle cycle	KEGG:04721	2.28E-02
KEGG	Ferroptosis	KEGG:04216	3.44E-02
KEGG	Nucleotide metabolism	KEGG:01232	4.63E-02

Supplementary Table 4. GO and KEGG Analysis of Overall DEG of SZ and Control

source	term_name	term_id	adjusted_p_value
GO:MF	neurotrophin TRK receptor binding	GO:0005167	6.78E-04
GO:MF	neurotrophin TRKA receptor binding	GO:0005168	6.78E-04
GO:MF	neurotrophin receptor binding	GO:0005165	7.33E-03

GO:CC	RISC complex	GO:0016442	6.75E-03
GO:CC	RNAi effector complex	GO:0031332	6.75E-03
KEGG	MicroRNAs in cancer	KEGG:05206	2.55E-05
KEGG	Choline metabolism in cancer	KEGG:05231	2.84E-02

Supplementary Table 5. GO and KEGG Analysis of Upregulated DEG of SZ and Control

source	term_name	term_id	adjusted_p_value
GO:BP	regulation of gene expression	GO:0010468	1.00E-02
GO:BP	regulation of macromolecule biosynthetic process	GO:0010556	1.74E-02
GO:BP	miRNA-mediated post-transcriptional gene silencing	GO:0035195	2.19E-02
GO:BP	regulatory ncRNA-mediated post-transcriptional gene silencing	GO:0035194	2.42E-02
GO:BP	post-transcriptional gene silencing	GO:0016441	2.70E-02
GO:BP	regulation of cellular biosynthetic process	GO:0031326	2.89E-02
GO:BP	regulation of biosynthetic process	GO:0009889	3.27E-02
GO:BP	right ventricular cardiac muscle tissue morphogenesis	GO:0003221	4.93E-02
KEGG	MicroRNAs in cancer	KEGG:05206	2.03E-09
KEGG	Choline metabolism in cancer	KEGG:05231	4.01E-03

Supplementary Table 6. GO and KEGG Analysis of Downregulated DEG of SZ and Control

source	term_name	term_id	adjusted_p_value
GO:BP	enzyme-linked receptor protein signaling pathway	GO:0007167	6.54E-03
GO:BP	transmembrane receptor protein serine/threonine kinase signaling pathway	GO:0007178	1.75E-02
GO:BP	regulation of response to stimulus	GO:0048583	2.04E-02
GO:BP	cellular response to endogenous stimulus	GO:0071495	2.65E-02
GO:BP	positive regulation of response to stimulus	GO:0048584	3.00E-02
GO:BP	basement membrane organization	GO:0071711	3.18E-02
GO:BP	integrin-mediated signaling pathway	GO:0007229	3.36E-02
KEGG	Focal adhesion	KEGG:04510	3.22E-02

Supplementary Table 7. GO Analysis of Overall DEG of BD and SZ

source	term_name	term_id	adjusted_p_value

GO:BP	ncRNA metabolic process	GO:0034660	1.02E-04
GO:BP	regulation of cell cycle process	GO:0010564	5.20E-03
GO:BP	RNA methylation	GO:0001510	6.86E-03
GO:BP	tRNA metabolic process	GO:0006399	9.98E-03
GO:BP	rRNA metabolic process	GO:0016072	2.19E-02
GO:BP	RNA modification	GO:0009451	2.61E-02
GO:BP	cellular response to stress	GO:0033554	4.66E-02

Supplementary Table 8. GO Analysis of Upregulated DEG of BD and SZ

source	term_name	term_id	adjusted_p_value
GO:BP	SRP-dependent cotranslational protein targeting to membrane, signal sequence recognition	GO:0006617	2.44E-04
GO:BP	SRP-dependent cotranslational protein targeting to membrane	GO:0006614	3.20E-04
GO:BP	cotranslational protein targeting to membrane	GO:0006613	3.54E-04
GO:BP	protein targeting to ER	GO:0045047	4.83E-04
GO:BP	establishment of protein localization to endoplasmic reticulum	GO:0072599	5.20E-04
GO:BP	protein localization to endoplasmic reticulum	GO:0070972	8.65E-04
GO:BP	protein targeting to membrane	GO:0006612	2.00E-03
GO:BP	establishment of protein localization to membrane	GO:0090150	1.10E-02
GO:BP	protein targeting	GO:0006605	1.84E-02
GO:BP	establishment of protein localization to organelle	GO:0072594	4.67E-02
GO:CC	signal recognition particle, endoplasmic reticulum targeting	GO:0005786	4.40E-05
GO:CC	signal recognition particle	GO:0048500	4.40E-05
GO:CC	ribonucleoprotein complex	GO:1990904	2.79E-03
GO:CC	protein-containing complex	GO:0032991	2.38E-02

Supplementary Table 9. GO Analysis of Downregulated DEG of BD and SZ

source	term_name	term_id	adjusted_p_value
GO:BP	ncRNA metabolic process	GO:0034660	1.31E-04
GO:BP	regulation of cell cycle process	GO:0010564	1.67E-03
GO:BP	RNA methylation	GO:0001510	4.06E-03
GO:BP	cellular response to stress	GO:0033554	9.42E-03

GO:BP	rRNA metabolic process	GO:0016072	1.09E-02
GO:BP	RNA modification	GO:0009451	1.42E-02
GO:BP	cell cycle process	GO:0022402	2.99E-02
GO:BP	tRNA metabolic process	GO:0006399	3.38E-02
GO:BP	protein-DNA complex organization	GO:0071824	4.35E-02
GO:BP	DNA damage response	GO:0006974	4.73E-02
GO:BP	snRNA modification	GO:0040031	5.00E-02

Supplementary Table 10: GO Analysis of Hypermethylated and Downregulated Genes in BD

source	term_name	term_id	adjusted_p_value
GO:BP	regulation of localization	GO:0032879	2.01E-02
GO:BP	regulation of vesicle-mediated transport	GO:0060627	2.12E-02
GO:BP	regulation of cellular component organization	GO:0051128	2.41E-02
GO:BP	regulation of transport	GO:0051049	2.69E-02
GO:BP	ribonucleotide biosynthetic process	GO:0009260	3.94E-02
KEGG	Synaptic vesicle cycle	KEGG:04721	2.81E-02
REAC	Recycling pathway of L1	REAC:R-HSA-437239	4.62E-04
REAC	Retrograde neurotrophin signalling	REAC:R-HSA-177504	9.61E-04
REAC	Signaling by NTRK1 (TRKA)	REAC:R-HSA-187037	6.36E-03
REAC	L1CAM interactions	REAC:R-HSA-373760	9.44E-03
REAC	DARPP-32 events	REAC:R-HSA-180024	9.65E-03
REAC	Signaling by NTRKs	REAC:R-HSA-166520	2.01E-02
REAC	Formation of annular gap junctions	REAC:R-HSA-196025	2.12E-02
REAC	Gap junction degradation	REAC:R-HSA-190873	2.41E-02
REAC	Clathrin-mediated endocytosis	REAC:R-HSA-8856828	2.69E-02
REAC	Nucleotide biosynthesis	REAC:R-HSA-8956320	3.94E-02

Supplementary Table 11. GO Analysis of Hypomethylated and Upregulated Genes in BD

source	term_name	term_id	adjusted_p_value
GO:BP	regulation of transcription by RNA polymerase II	GO:0006357	5.12E-03
GO:BP	transcription by RNA polymerase II	GO:0006366	7.80E-03
KEGG	Epstein-Barr virus infection	KEGG:05169	8.00E-03
KEGG	Small cell lung cancer	KEGG:05222	1.59E-02

Supplementary Table 12. GO Analysis of Hypermethylated and Downregulated Genes in SZ

source	term_name	term_id	adjusted_p_value
GO:MF	interleukin-16 binding	GO:0042011	4.99E-02
GO:MF	interleukin-16 receptor activity	GO:0042012	4.99E-02
TF	Factor: FKHL14; motif: TMNAAACARKAASA	TF:M08882	2.44E-02
TF	Factor: Freac-7; motif: WNNANATAAAYANNNN; match class: 1	TF:M00293_1	2.53E-02

Supplementary Table 13. GO Analysis of Hypomethylated and Upregulated Genes in SZ

source	term_name	term_id	adjusted_p_value
GO:MF	norspermine:oxygen oxidoreductase activity	GO:0052894	5.00E-02
GO:MF	N1-acetylspermine:oxygen oxidoreductase (N1-acetylspermidine-forming) activity	GO:0052895	5.00E-02
GO:CC	extrinsic component of neuronal dense core vesicle membrane	GO:0098674	4.99E-02
GO:CC	extrinsic component of dense core granule membrane	GO:0098922	4.99E-02

CURRICULUM VITAE

PERSONAL INFORMATION

Name and Surname: Burak Cardak

Nationality: Turkish

Date of Birth and Place: 19 July 1995, Golcuk

E-mail: burak.cardak@yahoo.com

EDUCATION

Degree	University	Graduation Year
Doctor of Medicine (MD) Graduate	Istanbul Medeniyet University, Faculty of Medicine, Medicine	2021

WORK EXPERIENCE

Year	Hospital	Position
2021-2022	Ministry of Health, Istanbul, Turkey	Prehospital Emergency Doctor
2022-2023	Medway Maritime Hospital, Gillingham, Kent, United Kingdom	Junior Doctor
2023-2024	Kendray Hospital, Barnsley, South Yorkshire, United Kingdom	Core Psychiatry Trainee

LANGUAGES

Native Turkish speaker with advanced proficiency in English.