Identification of Individual Differences in Responsivity to Prenatal Adversity using the Biological Signature related to Neighborhood Disadvantage Exposure

Gisele Sanda

Integrated Program in Neuroscience

Faculty of Medicine

McGill University

August 2023

A thesis submitted to McGill University in partial fulfillment of the requirements of the degree of Master of Science in Neuroscience.

Abstract

Genes are fundamental units of heredity that encode genetic information and serve as the basis for the development and functioning of all living organisms. Variations within the genetic code, as well as their interactions with environmental factors, underpin the diverse array of individual differences that ultimately dictate our susceptibility to various diseases.

Genome-wide association studies (GWAS) have increasingly generated informative genetic risk variants for mental health-related traits (Wray et al. 2012). These correlation studies highlight the complex and intricate polygenic nature of many psychiatric disorders. A standard GWAS, however, falls short of properly capturing the mechanisms that can control gene expression. In particular, the influence of epigenetic factors, which have a significant impact on gene regulation, is disregarded. Consequently, employing a multi-omics approach that integrates data from various sources, such as genomics, transcriptomics, and epigenomics, can build more accurate and informative risk assessment models.

Using a Neighbourhood Disadvantage (ND) EWAS (Reuben et al. 2020), we constructed an expression-based polygenic risk score (ePRS) weighted by Nacc (Nucleus Accumbens) tissue expression (Silveira et al. 2017a) and a methylation score for children using 3 different cohorts (GUSTO, ALSPAC, BIBO). We also calculated a prenatal adversity score by summing various sources of hardship experienced during the prenatal period to inform on the impact of early life environmental stressors. Multiple linear regression models were constructed to investigate the influence of the ePRS, methylation scores (M) and prenatal adversity (A) on socioemotional outcomes. We observed that variability was best explained by a multi-omics model (M + ePRS x A + ePRS +A) in the GUSTO cohort at 7 years. Using simple slope analysis, we showed that children at age 7 years old who have higher ePRS and who are exposed to high prenatal adversity have heightened emotional and behavioural problems in comparison to children who have low exposure to adversity. Meanwhile, we

observed that children with a higher. ePRS score in the ALSPAC cohort at 11 years has increased externalizing behavioural problems when exposed. To high levels of prenatal adversity. We also observed that children in the BIBO cohort who have high ePRS and who are not exposed to adversity have lower internalizing scores than children with lower ePRS.

We also investigated the biological background of the ePRS exploring its gene network, biological pathways and tissue expression. Our enrichment analysis revealed that the Neighbourhood Disadvantage (ND) gene network is prominently expressed in fetal development and young adulthood. The genes in the ND network are involved in the regulation of the synaptic vesicle cycle, synapse organization and nervous system development. These results highlight an important gene network involved in individual differences in neurodevelopmental processes, as well as vulnerability to behavioural problems in children.

Overall, this thesis aimed to explore the complex interplay between genes and the environment and their impact on childhood socioemotional problems, focusing on the role of variability in genetic and environmental factors in influencing susceptibility to these socioemotional issues.

Résumé

Les gènes sont des unités fondamentales de l'hérédité qui codent l'information génétique et servent de base au développement et au fonctionnement de tous les organismes vivants. Les variations au sein du code génétique, ainsi que leurs interactions avec les facteurs environnementaux, sont à la base de toute une série de différences individuelles qui déterminent en fin de compte notre susceptibilité à diverses maladies. Les études d'association à l'échelle du génome (GWAS) ont de plus en plus généré des variantes de risque génétique informatives pour les traits liés à la santé mentale (1). Ces études de corrélation mettent en évidence la nature polygénique complexe de nombreux troubles psychiatriques. Toutefois, une étude d'association pangénomique standard ne parvient pas à saisir correctement les mécanismes qui peuvent contrôler l'expression des gènes. En particulier, l'influence des facteurs épigénétiques, qui ont un impact significatif sur la régulation des gènes, n'est pas prise en compte. Par conséquent, l'utilisation d'une approche multi-omique qui intègre des données provenant de différentes sources, telles que la génomique, la transcriptomique et l'épigénomique, peut permettre d'élaborer des modèles d'évaluation des risques plus précis et plus informatifs.

À l'aide de l'EWAS (Neighbourhood Disadvantage) (2), nous avons construit un score de risque polygénique basé sur l'expression (ePRS) pondéré par l'expression du tissu Nacc (Nucleus Accumbens) (3) et un score de méthylation pour les enfants en utilisant 3 cohortes différentes (GUSTO, ALSPAC, BIBO). Nous avons également calculé un score d'adversité prénatale en additionnant diverses sources de difficultés rencontrées pendant la période prénatale afin d'obtenir des informations sur l'impact des facteurs de stress environnementaux au début de la vie. Des modèles de régression linéaire multiple ont été élaborés pour étudier l'influence du ePRS, des scores de méthylation (M) et de l'adversité prénatale (A) sur les résultats socio-émotionnels dans trois cohortes indépendantes à différents âges (GUSTO,

ALSPAC et BIBO). Nous avons observé que la variabilité était mieux expliquée par un modèle multi-omique (M + ePRS x A + ePRS +A) dans la cohorte GUSTO à 7 ans. En utilisant une analyse de pente simple, nous avons montré que les enfants âgés de 7 ans qui ont un ePRS plus élevé et qui sont exposés à une adversité prénatale élevée ont des problèmes émotionnels et comportementaux accrus par rapport aux enfants qui sont peu exposés à l'adversité. Parallèlement, nous avons observé que les enfants de la cohorte ALSPAC âgés de 11 ans et 8 mois ont des problèmes comportementaux extériorisés. Nous avons également observé que les enfants de la cohorte BIBO qui ont un ePRS élevé et qui ne sont pas exposés à l'adversité ont des scores d'intériorisation inférieurs à ceux des enfants qui ont un ePRS plus faible.

Nous avons également étudié le contexte biologique de l'ePRS en explorant son réseau de gènes, ses voies biologiques et l'expression des tissus. Notre analyse d'enrichissement a révélé que le réseau de gènes ND (Neighbourhood Disadvantage) est exprimé de manière proéminente dans le développement fœtal et chez le jeune adulte. Les gènes du réseau ND sont impliqués dans la régulation du cycle des vésicules synaptiques, l'organisation des synapses et le développement du système. Ces résultats mettent en évidence un important réseau de gènes impliqué dans les différences individuelles dans les processus de développement neurologique, ainsi que dans la vulnérabilité aux problèmes de comportement chez les enfants.

Dans l'ensemble, cette thèse visait à explorer l'interaction complexe entre les gènes et l'environnement et leur impact sur les problèmes de l'enfance, en se concentrant sur le rôle de la variabilité des facteurs génétiques et environnementaux dans l'influence de la susceptibilité à ces problèmes comportementaux.

Acknowledgment

I express my sincere gratitude to my supervisor, Dr. Patricia Silveira, for her consistent support and confidence in my ability to overcome challenges. Thank you for believing in me and being there for me throughout every step. There are no words to express my gratitude towards how amazing of a mentor you are. Obrigada por todo carinho e por sempre me ajudar a alcançar meus sonhos!

I would also like to thank the members of my advisory committee, Dr. Xiangfei Meng and Dr. Massimiliano Orri for tracking my progress and providing guidance with helpful feedback.

I extend a special note of appreciation to Irina Pokhvisneva and the dedicated Bioinformatics team whose expert guidance has equipped me with so much knowledge and essential skills. Thank you, Guillaume Elgbeili, for your incredible kindness in patiently guiding me through the intricacies of R, even when I was unfamiliar with the basics. Your teaching approach, grounded in the understanding that every journey begins with a first step, has been truly invaluable.

To my lab mates, your friendship and assistance in acclimating to a new environment have been greatly appreciated. Your support and kindness have truly made me feel at home. I will forever be grateful for all the kind hearts of the Meaney Lab, especially room E4101.

I am deeply thankful for the unwavering love and encouragement from my boyfriend, who has been my rock throughout this journey.

Finally, I would like to dedicate this thesis to my parents. Thank you for all your love and support throughout these years, without you, I would've never made it this far. I am truly fortunate to have you as my role model in life.

Contribution of authors

I wrote this thesis and the manuscript in Chapter 2 with editing from Dr. Patricia Silveira. Bioinformatics analyses were conducted by me with help from Irina Pokhvisneva and Guillaume Elgbeili; both also edited the manuscript. The ePRS scores were supervised by Dr. Patricia Silveira and conducted by Sachin Patel and Irina Pokhvisneva. The entire project was planned and supported by Dr. Patricia Silveira.

TABLE OF CONTENTS

CHAPTER 1. INTRODUCTION

1.1 Framing the questions

Psychiatric disorders are the leading contributor to global disability, affecting 1 in every 8 people or 970 million individuals around the world ("Global Burden of Disease Collaborative Network. Global Burden of Disease Study 2019 (GBD 2019) Population Estimates 1950-2019." 2020). Recent data from the Global Burden of Disease (GBD) 2020 report highlights the negative impact of the COVID-19 pandemic, showing an increase of 25.6% and 27.6% in anxiety and major depressive disorder in just one year, respectively (Santomauro et al. 2021). In 2019, 58 million children and adolescents were living with an anxiety disorder and 23 million were living with depression ("Global Burden of Disease Collaborative Network. Global Burden of Disease Study 2019 (GBD 2019) Population Estimates 1950-2019." 2020). Certain adverse conditions, such as poverty, violence or early life trauma, can increase the risk of developing psychopathology, whereas protective factors can aid in preventing the onset of these disorders. Genetic and epigenetic factors produce unique spectra of phenotypes and play a crucial role in the complexity of mental health-related traits. Identifying causal genetic variants and epigenetic markers that are sensitive to stressors would allow a better understanding of the underlying pathobiology of disorders.

The identification of reliable and informative genetic markers for mental health disorders has been hindered by the challenge of accessing human brain tissue. Collaborative efforts such as the Human Genome Project and the HapMap have contributed to the unravelling of the genetic basis of complex psychiatric diseases since recent advances in sequencing technologies through genome-wide association analyses (GWAS). More than 600 GWASs have been published and over 128,550 associations were made linking single nucleotide

polymorphism (SNP) to a disease or trait (Buniello et al. 2019; MacArthur et al. 2017). DNA microarrays enable cost-efficient testing techniques that provide a long-term repository biobank that will better represent a population in its entirety. Mapping gene loci associated with psychiatric disorder traits is of fundamental biological interest to make clinically relevant interferences as well as to early, more accurate diagnosis and personalized therapy.

Despite the success of GWAS, various limitations restrict the application of gene association studies in a clinical context. Firstly, the majority of trait or disease-associated SNPs (~93%) lie in non-coding regions of the genome and thus do not interfere directly with gene expression but may disrupt binding sites for transcription factors regulating expression levels (Maurano et al. 2012). Secondly, most gene-mapping studies are focused on single gene candidacy aiming to explain a complex disease trait with a reductionist approach ignoring the cellular concept that genes operate together and interact with each other and with environmental factors (e.g. gene x gene and gene x environment interactions) (Williams and Auwerx 2015). Lastly, GWAS is limited by the burden to correct for multiple comparison to reach statistical significance (Martin et al. 2019). This limitation can also be influenced by phenotypic variance which is determined by how strongly two allelic variants differ in their effect sizes and their frequency in the sample. These calculations are problematic for a GWAS regarding rare variants or SNPs with small effect sizes (Asimit and Zeggini 2010). Furthermore, identifying causal variants is complex due to a variety of factors including the incapability to standardize environmental experiences, as well as the inaccessibility to brain tissue through biological sample collection that is currently and mostly done by buccal cells or blood (Tarantino, Sullivan, and Meltzer-Brody 2011). Therefore, GWAS results heavily rely on follow-up studies gathering more information regarding environmental factors, epigenetics and/or tissue specificity to narrow disease-related loci.

The Polygenic Risk Score (PRS) is a useful tool for estimating an individual's genetic propensity to a trait or disease by summing up the effects of single nucleotide polymorphisms (SNPs) weighted by their corresponding effect sizes (Wray et al. 2014). However, PRS alone captures only a fraction of the genetic contribution to risk and integrating it with other risk metrics and accounting for tissue specificity can improve accuracy. To address this, our lab has developed the expression-based polygenic risk scores (ePRS), which considers co-expression gene networks in different tissues to contribute to disease risk or traits additively and synergistically (Silveira et al. 2017b; Hari Dass et al. 2019; Miguel et al. 2019; de Mendonça Filho et al. 2021; de Lima et al. 2022; Silveira and Meaney 2023). A critical question that will be addressed in this thesis is whether ePRS derived from methylation data can be used in combination with environmental data to predict variability in childhood problems.

Prenatal adversity, such as exposure to maternal stress or infection during gestation, has been identified as a major environmental stressor that can increase the risk of infants developing mental disorders later in life (Schlotz and Phillips 2009). Studies have shown that prenatal adversity can impact gene expression and induce epigenetic modifications, leading to alterations in brain development and function that increase the likelihood of developing disorders such as anxiety, depression, and schizophrenia (Monk, Spicer, and Champagne 2012). Moreover, individuals with a genetic predisposition to mental disorders may be particularly vulnerable to the effects of prenatal adversity, suggesting the existence of gene-environment interactions in the development of such disorders. Understanding the mechanisms by which prenatal adversity influences gene expression and brain development can inform the creation of interventions to prevent or mitigate the negative impact of environmental stressors on mental health.

The present thesis builds upon the findings of epigenetic risk factors from human population studies on behavioural phenotypes in children. Specifically, we aimed to investigate

if the ePRS is suitable for translating the findings from EWAS's back to the genetic variants associated with the epigenetic markers. To describe the functional relevance of these genetic variants, we addressed our hypothesis on three levels of analysis. We first (1) built scores representing the genetic information (ePRS), epigenetic information (methylation score) and environmental information (prenatal adversity score) for each individual from each cohort. We then proceeded to (2) explore interactions between multi-omics models and childhood problems to observe if the ePRS moderated the influences of environmental exposure, with or without the addition of the methylation score into the models. Finally, we (3) did a gene enrichment analysis to gain mechanistic insights into the gene network associated with the methylation markers from the EWAS and represented in the ePRS. The rationale for each of these studies follows:

(1) Given the significance of epigenetic processes in underlying biological responsivity to the environment, we rely heavily on the epigenome to gain insights into the transcription regulation to further embrace the complexity of individual traits. Current DNA methylation populational approaches use epigenome-wide association studies (EWAS) or methylation risk scores (MRS) to predict disease risk, which capture selected CpGs from high penetrant genes and do not reflect the true effects of complex traits where both genetic variants and CpG sites play a role in an intricate expression network. Therefore, we investigated the suitability of EWAS data (Reuben et al. 2020) to inform and complement the development of a genetic risk prediction model by creating an ePRS and a methylation score.

(2) Early life stressors such as prenatal adversities can exert profound influences on the neurodevelopment and cognitive-emotional phenotype of the offspring. Elucidating how genetic and epigenetic variants interact with prenatal adversity may provide insights in identifying biological markers that are susceptible to the environment.

Therefore, we explored whether biological signatures in a multi-omics model interact with prenatal adversity scores and how such interactions influence childhood problems.

(3) The conventional approach of single candidate genes has been found to be ineffective in explaining complex, non-Mendelian disorders. Psychopathologies are a prime example of such intricacy. By examining how genes interact with one another in a network, we can gain insights into how several variants, each with small effects, accumulate to influence the risk of multifactorial mental health disorders. To understand the functional and biological mechanisms underlying the ePRS gene network, we performed a gene enrichment analysis.

Currently, there is a gap in the genetic risk models to accurately capture the entirety of gene translation into phenotype and disease traits. Through this approach, we sought to contribute to the elucidation of complex interactions between gene variants, gene expression and environmental factors that contribute to the development and progression of various disorders. The subsequent section presents a comprehensive overview of our efforts to understand the complex nature of mental health risk and the current identification of genetic and epigenetic risk factors associated with childhood problems in response to early life adversity. It also provides an update on the status of multi-omics technologies and the potential for integrating multiple omics levels in functional genomics. We will also address genomics, prenatal adversity and neighborhood disadvantage as early-life environmental challenges.

1.2 Unravelling the complexity of mental disorders

The etiology of mental illnesses exhibits significant complexity in genetic architecture, as evidenced by the fact that the aggregate contribution of genetic risk factors only accounts for a fraction of the heritability observed across most mental health disorders. The recent and

rapid progress in psychiatric genomics has simultaneously created a set of opportunities and challenges. By combining large-scale human datasets with robust biostatistical approaches, we now have powerful tools that enable us to seek important clues regarding the puzzling nature of psychopathologies.

But why is our current understanding of psychiatric diseases limited? The limitations in our current comprehension of psychiatric disorders arise from the impact of genetic variations on biological mechanisms within molecular pathways, including neurotransmitter transmission. These variations can affect intermediate processes such as attention and working memory, which, in turn, can influence macro-level processes such as stress sensitivity and behavioral patterns. The interplay between these processes and their interactions with one another contribute to an individual's overall phenotype. Furthermore, susceptibility or resistance-conferring genetic variants interacting with environmental factors can lead to different phenotypes, ultimately affecting disease severity and progression.

Despite the growing recognition and research in mental health, our current understanding, diagnosis, and treatment remain rooted in a monocausal framework. The etiology of these disorders exhibits both multifinality meaning that the same causal factor can result in various mental health outcomes, and equifinality, meaning that different causal factors can lead to the same mental disorder (Cicchetti and Rogosch 1996). McLaughlin and colleagues, in their review of transdiagnostic risk and resilience to childhood trauma and psychopathology, argue that understanding how mechanisms and processes unfold over time, especially during childhood and adolescence, is crucial in preventing the development of psychopathology. This emphasizes the need for a developmental approach in the study of mental disorders, which considers the dynamic interplay between environmental stressors, genetic vulnerability, and neural processes (McLaughlin et al. 2020).

Furthermore, the challenges in understanding and treating mental disorders are not only limited to their pathobiology but also stem from societal stigmas and stereotypes surrounding mental illness. However, recent efforts in recognizing the importance of mental health in the job market, such as implementing laws that provide support and days off, signify a positive shift towards reducing the negative impact of societal attitudes on mental health. To fully understand the complex pathophysiology of mental disorders, it is necessary to combine scientific efforts with the recognition and support of the population. By acknowledging and addressing early symptoms, and seeking appropriate treatment, we can interrupt the progression of these disorders and prevent the development of later psychopathology.

In the following chapter, we will discuss current techniques in populational genomics and how they can contribute to our understanding of the multifaceted nature of mental health disorders, including the role of genetic and environmental factors, as well as the importance of multi-omics models in accurate risk assessment and effective prevention and treatment strategies.

1.3 Current functional genomics risk approaches for psychopathologies

The Polygenic Risk Score (PRS) is a powerful tool to infer heritability and explore the shared etiology of complex traits or diseases. While Genome-wide Complex Trait Analysis software (GCTA) (Yang et al. 2011) and LD score regression (LDSC) (Yang et al. 2010) can also be used for the same strategy, the PRS is the only approach that provides an estimate of genetic propensity to a trait or disease at the individual level. The PRS is calculated by the sum of the SNPs weighted by the corresponding genotype effect size estimates (Z- scores) identified in an independent discovery sample, creating a single value estimate for an individual's genetic liability to a specific trait or disease. Targeting high-risk patients can aid a subsequent stratified

medicine approach and exploit the pleiotropy of mental health diseases. However, PRS only captures part of the genetic contribution to risk, and genetic factors do not play the whole role in mental diseases. According to a review from Murray et al. 2020, the variance in mental health disorder liability described by PRS is 11% for schizophrenia, 4% for bipolar disorder, 4% for attention-deficit/hyperactivity disorder, 4% for depression, 2% for autism spectrum disorder, and 2% for anorexia nervosa (Murray et al. 2021). Therefore, the accuracy of risk assessment may be improved by integrating PRS with other risk metrics that not only account for genetic variance but include refined measurements that consider gene expression and tissue specificity.

In hindsight of this matter, our lab has created an expression-based polygenic risk score (ePRS) (Hari Dass et al. 2019), a novel technique for genomic risk profiling that creates a new perspective for enhanced functional genomics. This expertise has led to the publication of many articles focusing on individual risk differences in responsivity to environmental adversity in childhood (Batra et al. 2021; de Mendonça Filho et al. 2022; De Lima et al. 2020; Dalmaz et al. 2021; Barth et al. 2022; Miguel et al. 2019). This technique differs from traditional PRS as it considers co-expression gene networks in different tissues, where the combined effect of tissue-specific gene expression can additively and synergistically contribute to a disease risk or trait. While the original PRS focuses primarily on candidate associations between scattered loci and a trait/condition, the novel ePRS incorporates detailed levels of information on co-expression and tissue specificity. To incorporate the gene network, the ePRS uses co-expression RNA sequencing databases from human or animal models, owing to the dynamic nature of various polygenic diseases in the mental health field. To address tissue specificity, the ePRS uses variant-gene expression association slope given by GTEx, a post-mortem tissue bank that provides genetic effects on the transcription from a range of human tissues and organs ('Human genomics. The Genotype-Tissue Expression (GTEx) pilot analysis: multitissue gene

regulation in humans' 2015). With these attributes, the ePRS was for example able to better predict children with impulsivity, as well as individuals with high risk for addiction and Alzheimer's disease, in comparison to conventional PRS for ADHD, addiction, and dementia (Hari Dass et al. 2019).

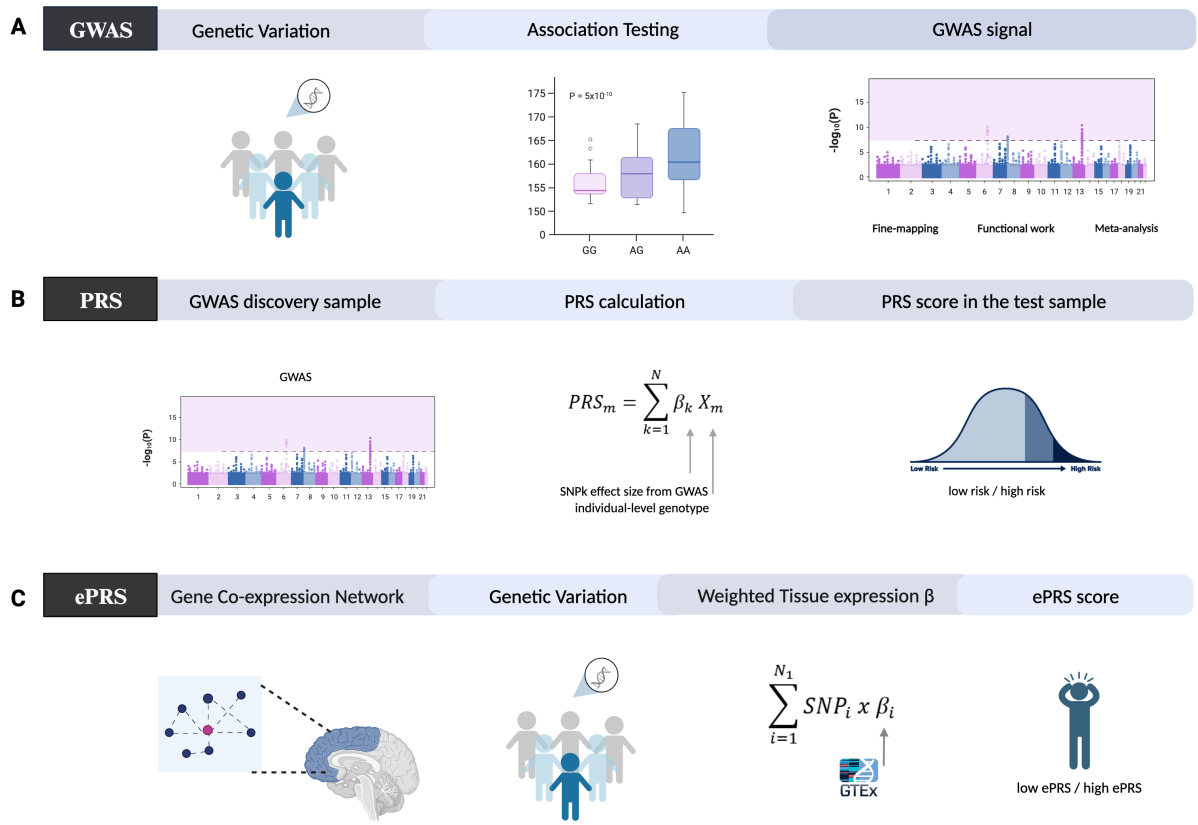


Figure 1: Flowchart for functional genomics risk approaches. Created in Biorender.com

(A) A GWAS requires genotype information from cases and controls or from a population sample. After quality control such as adjusting for population ancestry structure, a statistical analysis is performed to investigate whether the observed allele proportions are significantly more represented in one of the groups at each SNP. If a certain allele is statistically significantly more frequent in the group of individuals carrying the disease or trait (below the GWAS level of significance), it will be significant in the GWAS analysis. GWAS also estimates the effect size of this association, which quantifies the increased likelihood of developing the trait per risk allele count. These results are plotted in a Manhattan plot, which can serve as a platform for fine-mapping the genomic regions to find a true causal variant, identifying genes affected by the variant or identifying pathways, tissues and cell types implicated in this trait.

(B) The estimated PRS is informed by an external or discovery GWAS. The PRS is calculated in the target/test sample by the sum of the individual-level genotype weighted by the SNP effect sizes stated in a discovery GWAS. The final score is seen as a normally distributed score in the sample where the higher the score, the higher the genetic risk of trait or disease compared to the lower genetic risk score.

(C) The ePRS model is informed by a gene co-expression network identified using RNA sequencing data to characterize disease-relevant biological processes that are coregulated in certain tissues. Following the discovery of a gene network, the genetic variation within the co-expression network is mapped in an independent target sample to weight the SNPs based on tissue specificity, often utilizing the association between alleles and gene expression provided by GTEx. The final ePRS score is tissue-specific, mapping the relationship between functioning biological processes and the target sample genotype.

1.4 Beyond traditional genetic data: the role of epigenetics

Genotype risk prediction models mentioned above are of importance for the study of psychiatric phenotypes but are limited by not including epigenetic and/or environmental data. The field of epigenetics studies the changes in gene expression profiles that are not caused by the nucleotide sequence of the DNA. The four main components of epigenetics are DNA methylation, histone modification and chromatin remodelling, as well as non-coding RNA-mediated modifications with small non-coding RNAs, such as microRNAs (miRNAs).

DNA methylation, considered to be the most extensively studied epigenetic modification, can change the accessibility of genes to the transcriptional machinery, thus, directly modulating the mRNA expression levels. DNAm involves the transfer of a methyl group by the DNA methyltransferase (DNMT) enzymes to the cytosine bases present in eukaryotic DNA, resulting in the addition of a methyl group to the C5 position of the cytosine residues (5-methylcytosine). This process is separated by two regulatory layers: *de novo* and maintenance methylation. *De novo* methylation results in establishing new methylation patterns to previously unmethylated DNA regions. This process is primarily done by DNMT3a and DNMT3b enzymes. Maintenance methylation, on the other hand, refers to the preservation of existing methylated patterns during replication. This is done by DNMT1 which recognizes hemimethylated CpG sites (where only one DNA strand is methylated) and adds a methyl group the newly synthesized strand to restore full methylation of the CpG site. Both regulations ensure the proper establishment and maintenance of DNAm patterns.

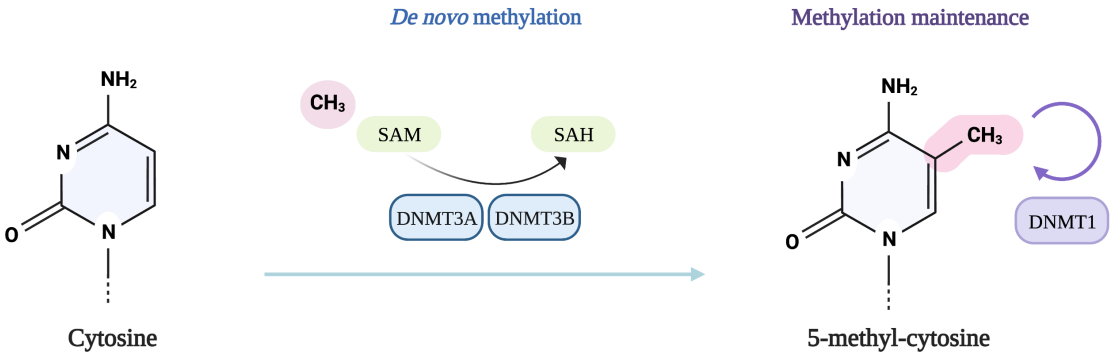


Figure 2: The mechanism of DNA methylation. Created in Biorender.com

The process of DNA methylation involves DNMT1, DNMT3A, and DNMT3B enzymes, which catalyze the addition of a methyl group to the fifth position of the pyrimidine ring of cytosine. These DNA methyltransferases (DNMTs) utilize S-adenosylmethionine (SAM) as a donor of the methyl group. As a result of this process, SAM gets converted to S-adenosylhomocysteine (SAH).

Recent scientific advancements have led to a paradigm shift from the early belief that DNAm has the same effects on transcription across all locations within the genome. It is now well known that the effects of DNAm on transcription are different and location-dependent. DNAm at gene promoters located within CPG islands is linked to the formation of a condensed chromatin structure, resulting in the suppression of gene expression, including X chromosome inactivation (Jaenisch and Bird 2003) and genomic imprinting (Li, Beard, and Jaenisch 1993). On the other hand, DNAm occurring at enhancers can promote transcriptional activation by recruiting transcriptional factors known to be crucial for early development (Ziller et al. 2013). Furthermore, other effects of DNAm have been associated with critical functions in a variety of biological processes suggesting roles in repression, promotion or modulation of the gene expression (Moore, Le, and Fan 2013).

DNAm can be highly sensitive to environmental factors and can be induced by stress, diet, smoking, medication and physical activity. Developmental factors also play a role in mitigating the DNAm profile. Prenatal adversities have been reported to have an effect on a widespread of DNAm changes at birth. Tobi et al. reported that DNAm at an enhancer linked to PIM3 expression mediated the association between prenatal famine exposure and BMI (Tobi et al. 2018). Suggesting that there is a connection between prenatal exposure to adversity and later-life metabolic health. Kundakovic et al. has also investigated how early-life adversity can increase the risk of psychopathology in later life due to epigenetic variation(Kundakovic et al. 2015). They showed that prenatal bisphenol A (BPA) exposure induces lasting DNA methylation changes in the transcriptionally relevant region of the BDNF gene in the hippocampus and blood of BALB/c mice, which is consistent with BDNF changes in the cord blood of humans exposed to high maternal BPA levels in utero. Additionally, studies of “Epigenetic clocks”, that are biological markers for aging, have associated epigenetic age acceleration with the onset of stress-related mental health problems (McGill et al. 2022).

Epigenetics is an exciting and growing field of research that has already made a significant impact on our understanding of the role of genes in the development of mental health disorders. Unlike genetics, which focuses on changes in the DNA sequence itself, epigenetics looks at the modifications above nucleotide sequences that can be influenced by environmental factors. The brain is particularly relevant to these influences, as it is the tissue that contains the highest levels of methylation (Ehrlich et al. 1982). This makes it an important area of study for understanding the molecular mechanisms underlying mental health disorders. In the subsequent section, we will dive into some of the latest research on epigenetics in mental health, highlighting the importance of this emerging field for improving our knowledge and treatment of these disorders.

1.5 Exploring DNAm effects in psychiatric disorders

The in-depth study of the molecular bases of mental illnesses can now be done through a new lens thanks to the area of epigenetics. DNA methylation (DNAm), one of the many epigenetic pathways, has become a major field of study for psychiatric diseases. The function of DNAm in a variety of psychiatric illnesses, including major depressive disorder (MDD), schizophrenia, bipolar disorder, and anxiety disorders, among others, has been the subject of numerous research. These analyses have uncovered fascinating relationships between particular genes or genomic areas engaged in crucial biological processes important to mental health.

Determining the age of onset for mental health disorders can be challenging, and understanding the time-dependent modifications of epigenetics by the environment adds another layer of complexity. Unravelling whether epigenetics acts as a mediator or a causal factor in this context is a complex task. Additionally, the concept of biological aging, which is

associated with various health risks, introduces another dimension. The way the epigenome is tied to this concept is called epigenetic clocks and it has been used to predict one's biological age. Epigenetic clock acceleration can be influenced by many aspects, such as smoking, alcohol use, and even Social Economic Status (SES) (Luo et al. 2020; McCrory et al. 2022; Oblak et al. 2021). This highlights the significance of projects that integrate multiple cohorts with longitudinal data, contributing to our understanding of the dynamic nature of DNA methylation (DNAm) patterns in mental health.

One of the key interests has been identifying methylation of specific genes and their potential as predictors in different psychiatric disorders. For example, Zhou et al demonstrated the role of DNAm in drug response in different psychopathologies including major depressive disorder (MDD) which is a prevalent mood disorder that is distinguished by periods of low mood, reduced motivation, and loss of interest in enjoyable activities (Zhou et al. 2021). With blood samples taken from patients diagnosed with schizophrenia, bipolar disorder and major depressive disorder, as well as healthy controls, researchers found significant differences in DNA methylation patterns in specific genes that are associated with drug response and neurotransmitter pathways in patients with psychiatric disorders compared to controls. Specifically, patients with schizophrenia and bipolar disorder showed methylation changes in genes that are involved in the dopaminergic and glutamatergic neurotransmitter systems, such as the promoter region of the DRD2 and GRIN1 genes. In patients with major depressive disorder, methylation changes were found in genes involved in the serotoninergic and neurotrophic pathways, including the promoter region of the SLC6A4 and BDNF genes. These findings suggest that DNA methylation changes in specific genes may contribute to the differences in treatment response observed in these disorders and allow a clinical approach to these targeted genes.

Overall, the emerging field of DNA methylation research in psychiatric disorders provides valuable insights into the underlying mechanisms and potential targets for therapeutic interventions.

1.6 EWAS and MRS

Genetics and epigenetics alone are insufficient to serve as the sole determinants of mental health. The development and manifestation of mental disorders are influenced by intricate interactions between gene expression networks and environmental factors. Acknowledging this complex nature, scientists have embarked on exploring the integration of epigenetic risk analysis, similar to current gene risk analysis, to gain deeper insights into the etiology of mental disorders. This chapter delves into the notion that the multifaceted nature of mental disorders arises not from single nucleotide polymorphisms (SNPs) or isolated CpG sites but rather from the intricate interplay and cumulative effects of multiple genetic and epigenetic factors.

Significant advancements in computational approaches and technology have accelerated epigenome research. The analysis of DNA methylation patterns on a genome-wide scale is made possible by high-throughput sequencing methods like next-generation sequencing. The identification and characterization of CpG sites and areas that display diverse methylation patterns in response to numerous biological activities and disease states have been made easier by these large epigenomic databases. To uncover these complex interactions, researchers have been integrating epigenetic data into epigenome-wide association studies (EWAS) or even by creating methylation risk scores (MRS).

For instance, a study in 2020 by Provençal et al. created a polyepigenetic score assessing differentially methylated CpG sites when human hippocampal progenitor cell line

and blood cells were exposed to glucocorticoids (Provençal et al. 2020). The weighted polyepigenetic score was applied to new-borns' cord blood DNA (n=817) and the score was significantly associated with maternal anxiety and depression. This suggests that early-life stress can induce epigenetic changes and modify vulnerability to later stress exposure.

Moreover, articles on methylation risk scores (MRS) have been of recent interest. Similar to the PRS calculation, MRS is defined by a linear combination of *n* CpG sites beta values *C* and weights *W*. Using certain methylation patterns at numerous CpG sites, this method enables the estimation of a person's epigenetic risk profile. MRS provides a valuable tool for assessing the cumulative impact of epigenetic modifications on disease susceptibility and other phenotypic outcomes.

$$MRS = \sum_{i=1}^{n} W_i C_i$$

A recent publication from Thompson et al. demonstrated the outstanding predictive performance of MRS on a variety of outcomes. Unexpectedly, the MRS-based imputations were more informative compared to PRS in 84 (92%) medication usage, 32 (94%) lab panel values, and 123 (82%) diagnoses, and in more than half of the cases, the imputation accuracy was more than doubled (Thompson et al. 2022). This article highlights the importance of including other measurements such as methylation, RNA, microbiome, metabolomics, or proteomic data when accessing individual risk. Tangentially, while polygenic risk scores provide unchangeable risk for a patient, methylation risk scores may provide a current risk state for the patient over the last months, and other information may provide risk within a timeline of days or even hours (e.g., RNA or metabolomics) (Thompson et al. 2022).

Another notable contribution to the field of psychiatric epigenetics comes from the recent study conducted by Charlie et al. published in 2022 (van den Oord et al. 2022). This

study specifically focused on the power of methylation risk scores in predicting psychiatric disorders. Notably, by developing an MRS specific to trauma-related outcomes, the score exhibited superior predictive capabilities compared to merely counting the occurrences of traumatic events. The trauma-related MRS captured the individualized long-term effects of trauma, enabling the prediction of various outcomes including depression, nicotine dependence, and alcohol use disorder.

Hence, the integration of epigenetics is promising for trait genetics studies since genes alone provide insufficient information to understand the full scope of non-monogenic phenotypes. By incorporating epigenetic information through methods like MRS, researchers can enhance their understanding of the underlying mechanisms and predictive models, paving the way for advancements in personalized medicine and the comprehensive assessment of individual risk profiles.

1.7 Impact of Early Environmental Stressors

The onset of mental health disorders is heavily influenced by the complex interplay between genes and environmental factors. The role of the environment in mental health disorders has been firmly assessed by twin studies (Manolio et al. 2009). Additionally, research has shown that early-life adversities, especially child abuse and/or neglect, have negative neurodevelopmental impacts that are associated with an increased susceptibility to develop psychiatric disorders later in life (Teicher and Samson 2016). One interesting data cites that by age 16 nearly 2 of 3 children have experienced some sort of traumatic experiences including parental death or family violence (Copeland et al. 2007).

Plausible mechanisms of such associations include blunted effects on the reward system (Mehta et al. 2010), amygdala hyperactivity and reduced hippocampal volume (Teicher and

Samson 2013). Nevertheless, prenatal stresses have also been linked to increased mental health risk (Coussons-Read 2013). Extensive studies have shown that maternal stress can lead to long-lasting cognitive and emotional consequences for their offspring (Lautarescu, Craig, and Glover 2020). Interestingly, Grundwald et al showed that pregnant female rats that were exposed to repeated social stress produced second-generation male offspring that displayed heightened anxious behavior compared to controls (Grundwald and Brunton 2015). This demonstrates that there is not only a direct but also a transgenerational transmission of prenatal stress that surpasses the first filial generation in rodents. In human studies, prenatal and postnatal stressor measurements, such as smoking during pregnancy or child neglect, can be important quantitative variables for genetic risk assessment models (Ekblad et al. 2010). In contrast, positive early life experiences such as high-quality of maternal care can buffer against stress (Jaffee, Takizawa, and Arseneault 2017). Therefore, environmental data such as pre/postnatal adversity are relevant to psychiatric symptomatology and are promising contributions to risk prediction models.

While there is a large variation in hardships children may experience, there is also variability in children's responses to adversity. To date, studies of gene and environment interactions follow two main theories that explain this variability. The first one of these theories, the diathesis-stress framework, states that some individuals possess an inherent vulnerability that can be triggered by environmental stress (Monroe and Simons 1991). This model suggests that the intensity of stress threshold to impact the development of a disorder varies according to one's degree of inherent vulnerability. The second theory is the differential susceptibility hypothesis, which suggests that an individual's biological context varies in their responsivity to their environment, some individuals respond more to environmental changes – be they negative or positive, with increased risk for pathology when exposed to negative environments but above average performance when faced with enriched positive environments

(Belsky 1997a). Some other individuals are not as sensitive and thus are not affected by either negative or positive experiences. Therefore, the idea of "vulnerability" has been steadily moved to "plasticity/responsivity" (Dalle Molle et al. 2017). The first evidence for the differential susceptibility model was proposed simultaneously and independently by Belsky (Belsky 1997b) and by Boyce (called biological sensitivity to context) (Boyce and Ellis 2005). An example of the genetic evidence for differential susceptibility was described by Pluess et al, showing that children with the DRD4 7-repeat allele were not only more likely to be diagnosed with ADHD when exposed to prenatal smoking, but they were also less likely to exhibit ADHD symptoms when exposed to a healthier intrauterine environment (Pluess, Belsky, and Neuman 2009). This indicates that genetic variants convey differential responsivity to environmental factors rather than vulnerability. Nevertheless, better evidence on what promotes resilient or susceptible profiles in children exposed to trauma or adversity is necessary for effective interventions to avoid or mitigate the immediate and long-term psychological repercussions.

1.8 The impact of Neighborhood Disadvantage on children's neurodevelopment

The present thesis aims to uncover plausible mechanisms of biological epigenetic and genetic markers related to neighborhood disadvantage exposure that is linked to childhood problems in response to prenatal adversity. The nature of most mental disorders is complex, and thus identifying GxE interaction effects on neurodevelopmental outcomes is fundamental. As previously described, these interactions provide the biological basis during the plastic period of childhood and the understanding of these relationships can contribute to the prevention of severe psychiatric consequences in later adulthood.

Considering the environment, the impact of neighborhood disadvantage on mental health and child upbringing is profound and cannot be overstated and can have far-reaching

consequences throughout adulthood (Christie-Mizell 2022). Growing up in disadvantaged neighborhoods exposes children to a range of adverse environmental factors, including poverty, violence, substance abuse, and limited access to resources and opportunities. The constant exposure to these unfavorable conditions contributes to chronic stress and disrupts the normal development of children's cognitive, emotional, and social capacities (Brooks-Gunn 1997; Leventhal and Brooks-Gunn 2003; McCoy et al. 2015).

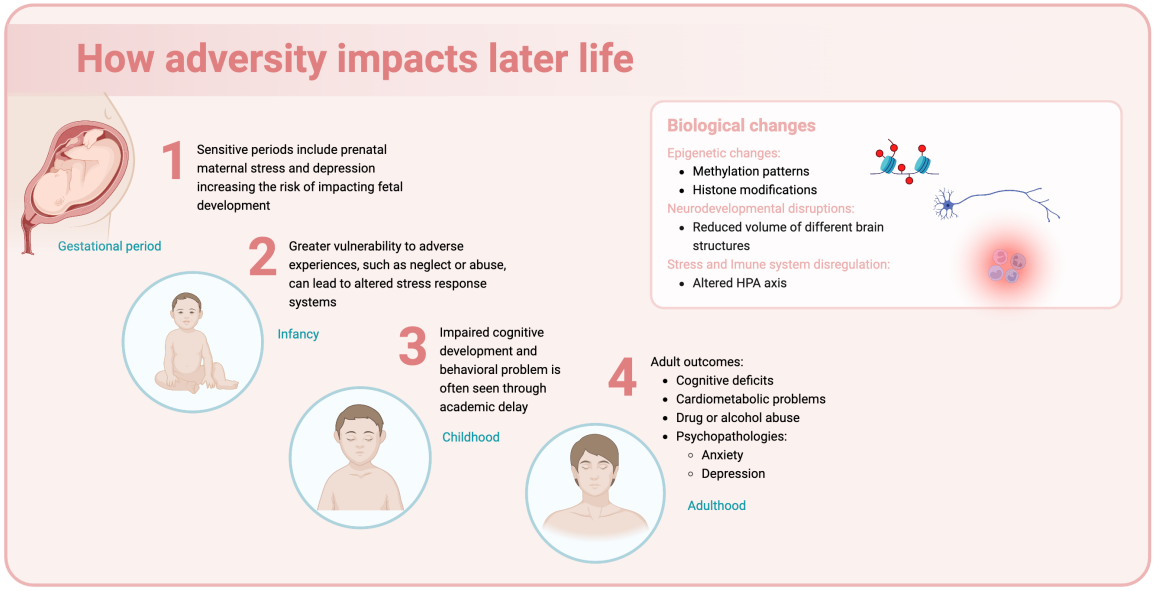


Figure 3: How adversity in early stages is linked to mental and physical health throughout adulthood. Created in Biorender.com

Exposure to adversity interacts with a child's genetic endowment, including variations in genetic polymorphisms, leading to a wide range of biological changes across various levels. These changes manifest as altered gene expression profiles, particularly in genes involved in stress response, neural plasticity, and inflammation. Moreover, adversity triggers epigenetic modifications, such as DNA methylation and histone modifications, which further modulate gene expression patterns and contribute to long-lasting neurodevelopmental disruptions. These disruptions affect crucial processes related to stress regulation, reward processing, and cognitive function. Collectively, these pathobiological changes in response to early life adversity have profound implications for mental and physical health outcomes in adulthood. They contribute to an increased vulnerability to mental health disorders and may worsen overall well-being.

Children residing in disadvantaged neighborhoods are more likely to experience higher levels of stress and trauma, which can have detrimental effects on their mental well-being (Santiago, Wadsworth, and Stump 2011). Chronic exposure to stressors may lead to an increased risk of developing mental health disorders, such as anxiety and depression. Moreover, neighborhood disadvantage can also contribute to behavioral problems, aggression, and delinquency among children and adolescents (Burt et al. 2016).

These responses have extensive systemic implications that are thought to eventually link adversity to physical and mental health (Figure 3). The higher levels of diurnal cortisol patterns are an example of an indicator of chronic physiological stress that has been linked to neighborhood disadvantage (Karb et al. 2012). Although the precise impacts may differ based on each individual, neighborhood conditions have also been connected to cortisol and blood pressure reactivity in response to stressful situations (Hackman et al. 2012). Neighborhood surroundings have an impact that goes beyond short-term stress and emotion since they can also have an impact on long-term stress and emotional functioning because of the cumulative effects of acute stress reactions and the long-term costs of adapting to unfavorable neighborhood environments. This process of adaptation might appear as either habituation or sensitization (increased reactivity with repeated exposures), each having distinct implications for the relationship between neighborhood stressors and health outcomes.

Children's socioemotional problems are made worse by the scarcity of high-quality healthcare and education options in underprivileged areas. Poor quality in educational options can impede intellectual growth and academic success, perpetuating the cycle of disadvantage. Moreover, lack of access to high-quality medical care could lead to misdiagnosed or untreated mental health disorders, which would have a longer-term negative effect on children’s development. Interestingly another factor that contributes to physical and mental health in low-income populations is the lack of quality nutritious food. The surroundings of fast foods can

contribute to malnutrition, affecting the potential healthy growth and becoming an increasingly important contributor to adult obesity, diabetes and cardiometabolic diseases (Black et al. 2013).

Additionally, the social environment of disadvantaged neighborhoods may lack supportive social networks and positive role models, leaving children with limited opportunities for positive socialization and mentorship. Social isolation and a higher prevalence of negative peer influences can contribute to maladaptive behaviors and increase the risk of engagement in risky activities, such as substance abuse. Notably, neurodevelopmental consequences may persist throughout adulthood. To circumvent this problem several community-based interventions and policies can buffer and modify future mental health consequences. This thesis offers multiple layers of analyses, where genetic risk profiling with additional epigenetic information can vastly improve our understanding of mental health by examining biological susceptibility and its interaction with the environment.

1.9 The Nucleus Accumbens

In the previous chapter, we explored how children who live in underprivileged areas are more likely to experience stress, trauma, and mental health problems because of their surroundings and how this can significantly impact their neurodevelopment. To successfully understand the biological underpinnings of these effects, it is imperative to acknowledge the profound influence that neighborhood disadvantage can exert on brain functioning and development.

Recent studies have shed light on living in disadvantaged neighborhoods and distinctive patterns of brain reactivity. For instance, consider a groundbreaking 2022 study involving a sample of twins aged 7–19 years (N = 354 families, 708 twins). In this research, these young

participants engaged in a socioemotional face processing fMRI task. Furthermore, a group of unrelated individuals from the same neighborhoods as the twins were enlisted to serve as informants on neighborhood social processes. What emerged from this study was truly noteworthy: neighborhood disadvantage was found to be associated with heightened reactivity in the right amygdala, particularly in response to threat (Suarez et al. 2022).

Interestingly, the nucleus accumbens (NAcc) and its associated neural systems are often implicated in various neurological and psychiatric disorders and have yet to be explored under a major cause which is the heightened cases of mental illnesses in disadvantaged populations.

The nucleus accumbens (NAcc), located in the ventral striatum, plays a crucial role in goal- and reward-based behavior. This region integrates information between the limbic and extrapyramidal motor systems by receiving neural projections from various midbrain regions, such as the ventral tegmental area (VTA), as well as regions involved in emotion (amygdala and cortex), motor functions (dorsal caudate and globus pallidus), and memory processes (hippocampus) (Jiang et al. 2023). Simultaneously, the NAcc sends signals to several interconnected regions including the cortex, amygdala, and hypothalamus, among others. Importantly, dysregulation of these neural pathways has been implicated in the development of major depressive disorder (MDD) and dysregulation of motivation, highlighting the significance of NAcc dysfunction in mental health (Salgado and Kaplitt 2015; Shiflett and Balleine 2010).

Both anxiety and depression, despite having distinct etiologies, share an intriguing similarity: the presence of reward-related abnormalities. This observation raises the possibility of developing refined treatments targeting dysfunction in the nucleus accumbens (NAcc), a key brain region involved in reward processing. Supporting this notion, a recent study revealed that adolescents with depression or anxiety exhibited reduced nucleus accumbens volume and

activation following reward receipt compared to healthy individuals (remaining significant even after excluding medicated individuals) (Auerbach et al. 2022). Additionally, multimodal modelling indicated that structural alterations in the NAcc were the sole predictors of depressive symptoms over a 6-month follow-up period. This suggests that alterations in the structure and function of the NAcc play a crucial role in characterizing depressed-anxious adolescents, with reduced volume specifically associated with depressive symptoms.

During the transition from childhood to adolescence, the NAcc undergoes significant changes. These functional changes may explain the age-related increase in depression in adolescents following exposure to early life stress (ELS). By exploring this idea, B.Goff conducted a study comparing 38 youths that have been previously institutionalized and 31 control individuals with no history of ELS (Goff et al. 2013). The findings revealed higher depression rates in adolescents with ELS, along with atypical NAcc development. Unlike the typical increase in NAcc reactivity seen in adolescence, the ELS group exhibited hypoactivation of the NAcc. Moreover, the lower reactivity in the ELS group was linked to higher depression scores. Understanding that the NAcc primarily consists of medium spiny neurons, which can be influenced by environmental factors, it is noteworthy that animal studies have also shown that exposure to maternal separation or mild prenatal stress can lead to alterations in the complexity of dendritic morphology in the accumbens (Monroy, Hernández-Torres, and Flores 2010; McClure, Ishtoyan, and Lyon 2004b). These results emphasize the importance of this age-related limbic structure suggesting a potential neural mechanism underlying the increased risk of later depression.

Abnormalities in the structure and function of the NAcc are thought to contribute to the development of these disorders. As such, understanding the role of the NAcc throughout different stages of life and its susceptibility to the effects of ELS is crucial for developing targeted interventions.

1.10 Multi-Omics Models Challenges and Future perspective

In our previous chapter, we examined how early life stress (ELS) impacts the Nucleus Accumbens (NAcc) and its potential association with increased depression risk during adolescence. The atypical development and hypoactivation of the NAcc in response to ELS underscore the intricate interplay between environmental factors and neural structure and function. Recognizing the NAcc's significance across various life stages and its vulnerability to ELS is pivotal for the development of targeted interventions. In this chapter, we will be discussing how we can use cutting-edge data integration and analytical techniques to advance our exploration.

Over the past few years, there have been significant advances in precision medicine, where medical treatment can be tailored to an individual's specific needs. These advances have been facilitated by the increasing availability of large-scale clinical datasets, including data from patient records, 24-hour monitoring devices, and smartphones. However, it is worth noting that only a small proportion of this data is currently being utilized for research purposes, highlighting the need for better integration and analysis of these complex datasets.

One promising approach for integrating different data types from multi-omics datasets is the use of Machine Learning and Systems Genomics approaches, which make use of data mining and predictive algorithms. Recent high-impact research studies have demonstrated the power of this approach in predicting mental health outcomes and identifying new therapeutic targets. Compared to studying a single data type in isolation, the integration of many data types through Machine Learning and Systems Genomics offers a more comprehensive understanding of phenotype-genotype interactions. This can pave the way for a more customized approach to healthcare, with individually tailored medical practices and treatments based on an individual's

unique genetic and clinical profile. However, there are still challenges to be addressed in the development and application of multi-omics models, including data quality control, data standardization, and effective integration of data from different sources.

This thesis delves into the multi-omics analysis of genetic variants across different layers of biological information, including genotype, transcriptomics and epigenomics. It investigates how these variants can be employed to examine alterations in biological networks and provide valuable knowledge regarding the unique ways individuals respond to stress. Specifically, we investigate how prenatal adversity interacts with genetic variants and epigenetic factors to influence the expression of socio-emotional childhood problems.

CHAPTER 2: Integrated model of neighborhood disadvantage-associated methylation and polygenic score associates with risk to psychopathology in children exposed to adversity.

Gisele Sanda[1], Barbara Barth [1,2, 3,] Danusa Mar Arcego [2,] Marielle Fortier[3], Birit Broekman[3], Yap Seng Chong[3], Mary Daniel[3], Zhen Ming Ngoh[3], Shayne Yeo[3], Ai Peng Tan[3], Evelyn Law[3], Michelle Kee[3], Mary Chong[3], Fabian Yap[3], Roseriet Beijers [7,8,] , Carolina de Weerth [8] , Marieke S.Tollenaar [9,10,] , Guillaume Elgbeili[2], Irina Pokhvisneva[2], Kieran O'Donnell[4], Michael J. Meaney[2,5, 6,] Patrícia Pelufo Silveira[2,3,6]

[1] Integrated Program in Neuroscience, McGill University; [2] Douglas Mental Health University Institute, Department of Psychiatry, Faculty of Medicine, McGill University, Montreal, QC, Canada; [3] Ludmer Centre for Neuroinformatics and Mental Health, Douglas Research Centre, McGill University, Montreal, QC, Canada. [4] Yale Child Study Center & Department of Obstetrics, Gynecology & Reproductive Sciences, Yale School of Medicine, Yale University; [5] Translational Neuroscience Programme, Singapore Institute for Clinical Sciences, Agency for Science, Technology and Research (A*STAR); [6] Department of Paediatrics, Yong Lin Loon School of Medicine, National University of Singapore, Singapore; [7,] Behavioural Science Institute, Radboud University, Nijmegen, the Netherlands; [8] Department of Cognitive Neuroscience, Donders Institute for Brain, Cognition & Behaviour, Radboud University Medical Center, Nijmegen, the Netherlands; [9]Clinical Psychology Unit, Institute of Psychology, Leiden University, Leiden, the Netherlands; [10]Leiden Institute for Brain and Cognition, Leiden University, Leiden, the Netherlands.

Keywords: gene networks, neighborhood disadvantage, early life adversity, expression-based polygenic score, methylation, multi-omics model

---

Abstract

Exposure to neighborhood disadvantage represents a chronic environmental stressor that has detrimental effects on youth development. Notably, individuals exposed to adversities early during development are prone to increased risk for a range of health disorders, including worsened mental health outcomes in comparison to their peers. However, not all children exposed to adversity will develop chronic diseases in the long term. This is due to important individual genetic and epigenetic differences accounting for mechanisms of vulnerability and susceptibility to adversity that are still to be discovered. In this study, we investigated whether interactions between methylation signals associated with Neighborhood Disadvantage and related expression-based polygenic score predict risk socioemotional behavior in children exposed to different levels of prenatal adversity severity. We constructed a methylation score considering the CpG sites discovered in the Neighborhood Disadvantage EWAS. We also

mapped these CpGs to the nearest genes and calculated an expression-based polygenic risk score (ePRS) in the same individuals. We then investigated the influence of ePRS, methylation score (M) and prenatal adversity (A) on socioemotional development outcomes in three independent cohorts with participants' ages ranging from childhood to early adolescence (GUSTO, ALSPAC and BIBO).

In the GUSTO cohort at 11 years, a multi-omics model (M + ePRS x A + ePRS + A) demonstrated the strongest explanatory power for the variability in socioemotional outcomes. High ePRS was associated with worsened behavioral problems in childhood when the individuals were exposed to high prenatal adversity but were linked to reduced risk for problems in the absence of prenatal adversity. In the ALSPAC cohort at 11 years and 8 months, the simpler models exhibited lower explanatory power, while the multi-omics interaction model showed promising results, particularly for Externalizing problems. The BIBO cohort yielded different results, with all models displaying lower explanatory power, with the multi-omics interaction model having a stronger association with Internalizing problems.

Such findings are critical for demonstrating how high exposure to adversity interacts with genomic features and neural circuits that together mediate risk and resilience in adulthood. Notably, these developmental consequences may persist throughout adulthood and highlight the importance of community-based interventions and policies that can buffer and modify future health consequences.

Introduction

It is well known that neighborhood disadvantage has consequential effects on children's mental health problems (Kosidou et al. 2011). Children from disadvantaged backgrounds are more likely to experience anxiety and depression and have poorer adult health including

physical disability and premature death (Kessler et al. 2010; Luby et al. 2017). Regarding fetal development, neighborhood disadvantage can also partake in intrauterine growth restriction through the poor nutritional status of the mother which leaves an inbuilt vulnerability to a range of problems in later life (Lahti et al. 2015; Hales and Barker 1992; Barker et al. 1993; Silveira et al. 2018). Similarly, indirect effects of adversity such as prenatal stress are a major risk factor for a range of adverse developmental problems, starting from birth with poor fetal growth and premature birth (Hedegaard et al. 1996; Bolten et al. 2011), childhood cognitive problems and impulsivity or hyperactivity (O'Connor et al. 2003; Laplante et al. 2004) to higher symptoms of depression and anxiety in young adulthood (Murphy et al. 2017; Davis and Sandman 2012).

Exposure to prenatal stress is not only associated with disease risk, but also with enduring effects on lasting biological changes including alterations in brain structure, function, and connectivity. In animal models, pregnant rats exposed to mild chronic stress produce offspring with reduced volumes and cell numbers in the nucleus accumbens (Nacc), a brain area involved in reward and emotional processes (McClure, Ishtoyan, and Lyon 2004a). Similar associations between early adversity and altered Nacc functional connectivity have been described (Fareri et al. 2017; Hanson et al. 2018; Marshall et al. 2018), which is significant for several clinical symptoms (Salgado and Kaplitt 2015), especially mood disorders (Sequeira et al. 2012). The Nacc is a major component of the mesocorticolimbic dopaminergic pathway (Yamaguchi et al. 2011). Exposure to prenatal stress may exert important effects on Nacc function, and this may be a key brain region for understanding susceptibility to adversity. Interestingly, while most individuals who emerge from such adverse conditions have a higher risk of later psychopathology, not all children exposed to distress will develop psychopathology in the long term(Baldwin and Degli Esposti 2021). The main reasons explaining these individual differences are due to predisposing genetic and epigenetic factors that can influence vulnerability and susceptibility. Genetics plays an important part in the development and onset

of mental diseases, as well as aiding protective factors. Finding stress-sensitive causative genetic variations and epigenetic modifications would enable a better understanding of the underlying time-dependent pathobiology of mental disorders (Parikshak, Gandal, and Geschwind 2015; Shonkoff et al. 2022). Traditional single-candidate genes do not inform the complexity of highly polygenic psychiatric disorders (Gandal et al. 2018). This is due to intrinsic expression networks that engage in several linked neurobiological pathways and processes (Gandal et al. 2016). Therefore, systems biology approaches and multi-omics modelling seem promising for understanding both genetic and epigenetic influences on neurobiological networks that may contribute to risk assessment models.

Here, we aim to investigate whether epigenetic changes associated with the exposure to neighborhood disadvantage described in a previous epigenetic-wide association study (Reuben et al. 2020) can predict the risk for childhood problems in response to prenatal adversity in different cohorts of children. We considered a multi-omics approach including the epigenetic changes associated with an adversity exposure (CpGs associated with neighborhood disadvantage described in Reuben (Reuben et al. 2020), combined in a methylation score), as well as genetic individual variability in the genes where these epigenetic changes were identified. For that, we used our novel method of genome profiling, informed by biological function, and based on the association between genotype and gene expression in a specific tissue (expression-based polygenic scores or ePRS). We focused on the nucleus accumbens as our brain region of interest given its strong associations with emotional outcomes (Sequeira et al. 2012; McClure, Ishtoyan, and Lyon 2004a), as a brain region important for the childhood adversity effects on the risk for psychopathologies. We also investigated whether different levels of exposure to prenatal adversity would influence the severity of childhood emotional problems.

Material and Methods

Samples

Three independent cohorts were included in our analysis: Growing Up in Singapore Towards Healthy Outcomes (GUSTO), Avon Longitudinal Study on Parents and Children (ALSPAC) and Basal Influences on Baby Development (BIBO). Informed consent was obtained from all participants (or their legal guardians).

The GUSTO cohort consisted of pregnant women 18 years old and above who were recruited at the National University Hospital and KK Women's and Children's Hospital in Singapore (Soh et al. 2014). The eligibility criterion for mothers was being of Chinese, Malay or Indian ethnicity with a homogenous parental ethnic background. The exclusion criteria were significant medical conditions for mothers, taking certain medications, and mixed marriages. The project was approved by The National Healthcare Group Domain Specific Review Board and the Sing Health Centralized Institutional Review Board for GUSTO. A total of 102 children had complete data (birth records, genotype, methylation data and complete behavioral questionnaire) and were included in the study.

The ALSPAC cohort included pregnant women from the former county of Avon, UK with expected delivery dates between April 1991 and December 1992 (Fraser et al. 2013). Additional recruitment was done later during Phases II, III and IV respectively, bringing the total sample size of prospective mother-child dyads to 15,658 mother-child dyads (Boyd et al. 2013). This study was approved by the ALSPAC Law and Ethics Committee, and by Local Research and Ethics Committees (a full list of the ethics committees that approved different aspects of the ALSPAC studies is available at http://www.bristol.ac.uk/alspac/researchers/research-ethics/). Consent for biological samples has been collected in accordance with the Human Tissue Act (2004). Informed consent for the

use of data collected via questionnaires and clinics was obtained from participants following the recommendations of the ALSPAC Ethics and Law Committee at the time. Please note that the study website contains details of all the data that is available through a fully searchable data dictionary and variable search tool reference the following webpage: http://www.bristol.ac.uk/alspac/researchers/our-data/ and the full ALSPAC data dictionary accessible at http://www.bris.ac.uk/alspac/researchers/data-access/data-dictionary/). A total of 656 children had all provided data (birth records, genotype, methylation data and complete behavioral questionnaire) and were included in the current analyses. Demographic characteristics for the ALSPAC cohort are provided in Table 3.

The BIBO cohort consisted of a community-based cohort from the Netherlands. This study is part of a project that examines how early caregiving affects children's growth. Mothers were enlisted while pregnant through flyers given out in Nijmegen, Arnhem, and nearby towns. The research adhered to the Helsinki Declaration. Before the study began, mothers agreed to write for themselves and their infants. Inclusion rules were a simple pregnancy with one baby, no drug use, and no ongoing health issues, physical or mental. Ethical approval for the study was approved by the Ethical Committee of the Faculty of Social Sciences, Radboud University, Nijmegen. A total of 119 children had all complete information (birth records, genotype, methylation data and complete behavioral questionnaire) and were included in the current study.

DNA Methylation data

In GUSTO, Infinium MethylationEPIC array (Illumina) was used to describe genome-wide DNA methylation from buccal cell–derived genomic DNA. Signal extraction from raw image files, quality control, and preprocessing steps were performed using R’s Minfi package

(Aryee et al. 2014). Standard Minfi quality control (QC; QC threshold < 10.5) was performed to have a call rate >99% and we removed all poorly performing samples whose probes had a call rate of <75%. DNA methylation of the sex chromosomes and biological sex was predicted and matched the reported sex in all cases. Samples with a buccal cell content of less than 55% were disqualified and for the measures of buccal cell heterogeneity, we used a well-established deconvolution method.

In ALSPAC, the methylation of DNA in white blood cells in ARIES kids was examined three times: at birth (cord blood), at 7 and 15-17 years of age (peripheral blood). DNA methylation was processed on maternal buffy coat samples using the Infinium HumanMethylation450 BeadChip ("Infinium 450K") and bisulphite converted with the Zymo Research's EZ DNA methylation Kit (Cat #: D5002). For this study, we used only methylation data collected at 7 years of age from peripheral blood.

In BIBO, buccal epithelial cells were collected from children at 5-6 years using the Infinium MethylationEPIC array (Illumina). DNA methylation quality control was performed using the meffil package in R (Min et al. 2018). All samples had a call rate above 99%, and poorly performed probes with call rates lower than 75% were removed. Buccal cell heterogeneity was estimated using a deconvolution approach (Smith et al. 2015) and samples with less than 55% buccal cell content were excluded.

Genotyping

For the extraction of genomic DNA from buccal samples provided by GUSTO participants we used the Isohelix DDK-50 kit (Isohelix, UK) and DNA Clean & Concentrate Kit (Zymo Research, USA). Genotyping was conducted utilizing the Infinium OmniExpressExome array and split by ethnicity for quality checks. Non-autosomal SNPs were

excluded, as were SNPs with < 95% call rates, 5% minor allele frequencies, 5% and a Hardy-Weinberg equilibrium p-value of 10-6. Variants in the 1000G reference panel that were discordant with their respective subpopulations were deleted. More precisely, variants with different allele codings than 1000G, as well as SNPs with frequencies that differ more than the threshold specified for the reference population (Chinese: EAS with a threshold of 0.20; Malay: EAS with a threshold of 0.30; Indian: SAS with a threshold of 0.20), were excluded. Samples with a call rate of less than 99%, cryptic relatedness, or sex/ethnic discrepancies were removed. The generated data were pre-phased using SHAPEIT v2.837 with family trio information. The Sanger Imputation Service was then used for imputation, with 1000G Phase 3 as the reference panel and the Positional Burrows-Wheeler Transform (PBWT) algorithm ("with PBWT, no pre-phasing" as the pipeline)(Rubinacci, Delaneau, and Marchini 2020). Imputed data that had biallelic SNPs and an INFO score > 0.80 were retained. Imputed genotyping data that were common in all three ethnicities (5,771,259 SNPs) were used for further analyses.

In the ALSPAC cohort, children were genotyped using the Illumina HumanHap550 quad chip genotyping platform by the Welcome Trust Sanger Institute, Cambridge, United Kingdom and the Laboratory Corporation of America, Burlington, NC, United States (Richmond et al. 2017). DNA was extracted from blood, cell lines, and mouthwash samples. A standard quality control (QC) process was applied. We excluded participants with inconsistent self-reported and genotyped sex, minimal or excessive heterozygosity, high levels of individual missingness (>3%), and insufficient sample replication (IBD < 0.8). SNPs with a call rate < 95%, a MAF < 1%, or those that were not in Hardy-Weinberg Equilibrium (HWE; p<5*10-7) were removed. Cryptic relatedness was measured as the proportion of identity by descent (IBD > 0.1). Related subjects that passed all other quality control thresholds were retained during subsequent phasing and imputation. For all the subjects that were retained (N = 9115), a total of 500,527 SNPs passed these quality control filters. Following the quality

control, the genotyping data was imputed using Impute v3 and the Haplotype Reference Consortium (HRC) imputation reference panel (release 1.1), total genotyping data resulted in 38,898,739 SNPs.

For BIBO, the QIAamp DNA Mini Kit (Qiagen, Netherlands) and DNA Clean & Concentrator columns (Zymo Research, USA) were used to isolate genomic DNA from buccal samples. After genotyping arrays, SNPs that had a call rate less than 95%, a minor allele frequency (MAF) less than 5% or did not follow the Hardy-Weinberg equilibrium ($p < 1e\text{-}20$) were excluded. Non-autosomal SNPs from our analysis and removed samples with call rates less than 95% were also excluded from the analysis. To perform genome-wide imputation, we utilized the Sanger Imputation Service (McCarthy et al. 2016).

To evaluate population structure, we conducted principal component analysis (PCA) (Patterson, Price, and Reich 2006a; Price et al. 2006) using PLINK 1.9 (Purcell et al. 2007) for each cohort on all genotyped SNPs that passed the quality control with MAF > 5% and the following pruning parameters: not in high linkage disequilibrium $r^2<0.20$ across 50kb region and an increment of 5 SNPs (GUSTO and BIBO) or a threshold of 1.01 for variance inflation factor, 100 SNPs region, a 5 SNPs step size (ALSPAC). Based on the screen plot, the first three principal components were the most informative of population structure in the three cohorts and were included in all analyses.

Methylation score calculation

In the Neighborhood Disadvantage EWAS by Reuben et al. (Reuben et al. 2020), estimates were identified to provide an ecological risk assessment based on 4 independent sources including local government data, criminal justice data, systematic social observation (using Google Street View), and surveys of neighborhood residents (conducted by the E-Risk

Study team) (Reuben et al. 2020). This EWAS ultimately provided an association effect between exposure to neighborhood disadvantage and their influence on affected CpG's methylation. For the methylation score calculation, in all cohorts we selected only the CpGs available in the Neighborhood Disadvantage EWAS (Reuben et al. 2020) with the FDR adjusted p-value < 0.15, resulting in 219 CpGs selected for the methylation ND score. Then, we kept only the CpGs available in the respective cohorts and weighted the methylation levels at each of the CpGs by the estimated beta value derived from the EWAS (association with Childhood Neighborhood Disadvantage corrected for smoking). Summation over all CpGs provided the Neighborhood Disadvantage (ND) methylation score. Variations in the methylation ND score represent individual methylation variation associated with the epigenetic responsivity to neighborhood disadvantage. The calculated ND methylation scores in three cohorts contained the following number of CpGs: GUSTO - 190, ALSPAC – 218, and BIBO – 198.

ePRS calculation

The ePRS score was calculated based on the SNPs located on the 177 genes related to the CpGs described in the Neighborhood Disadvantage EWAS reported from Reuben et al. (Reuben et al. 2020). The genetic score was created according to the protocol previously described by Silveira et al. (Silveira et al. 2017b; Hari Dass et al. 2019). First, we mapped the CpGs included in the ND methylation score in each cohort into the closest genes and selected all the SNPs from these genes using the *biomaRt* package (Durinck et al. 2005; Durinck et al. 2009). From this list of SNPs, we identified all the SNPs available in each cohort and applied linkage disequilibrium clumping ($r^2 < 0.2$ within 500kb region) to eliminate highly correlated SNPs. To link the score to a specific brain region, we weighted each SNP by the estimated

effect of the number of alleles at each SNP on gene expression in the nucleus accumbens (Nacc) provided by the Genotype-Tissue Expression (GTEx) ('Human genomics. The Genotype-Tissue Expression (GTEx) pilot analysis: multitissue gene regulation in humans' 2015). We used the sign of the estimated effect of the association with Childhood Neighborhood Disadvantage corrected for smoking from the Neighborhood Disadvantage EWAS (26). The sum of all SNPs provided the ND ePRS score (Figure 1). Variations in this score represent individual gene expression variation of the set of genes associated with epigenetic responsivity to Neighborhood disadvantage. The calculated ND ePRS scores in three cohorts contained the following number of SNPs (genes): GUSTO – 1272 SNPs (125 genes), ALSPAC – 7527 SNPs (146 genes), and BIBO –5651 SNPs (133 genes).

Environmental adversity - Cumulative Prenatal Adversity Score

To describe and quantify prenatal adversity conditions we used a cumulative prenatal adversity score as a measurement. To create this score, we utilized various indicators pre-established from the literature, such as maternal health and social economic status, as being related to worsening behavioral and health outcomes in children **(Silveira et al. 2017b)**. The Cumulative Prenatal Adversity Score predicts childhood problems to a larger extent than any single indicator in isolation **(Silveira et al. 2017b)**. One point was given for **each criterion that was met and all points were summed to obtain the adversity score.** Details of the adversity score can be seen in the reference (Silveira et al. 2017b).

Outcomes

To assess socio-emotional development and possible risk for the development of later psychopathology, we used the questionnaires CBCL (for GUSTO and BIBO) and the SDQ (for

ALSPAC). The Child Behavior Checklist (CBCL) is a questionnaire that includes 100 items that are used to evaluate emotional, behavioral, and social difficulties in preschool children (Achenbach 1991). The Strength and Difficulties Questionnaire (SDQ) is a questionnaire that consists of 25 questions representing 5 subscales (Conduct Problems, Inattention Hyperactivity, Emotional Symptoms, Peer Problems, and Prosocial Behavior) and a Total Difficulties score that is the sum of all subscales (Goodman 1997; Goodman, Meltzer, and Bailey 1998). Over the last two decades, the CBCL and SDQ have become the most used instruments for assessing mental health in children and adolescents. Both questionnaires are validated and demonstrate adequate reliability as a psychiatric assessment tool for the detection of a child mental health problems (Rescorla et al. 2007). CBCL Internalizing problems reflect anxious and depressive symptoms, social withdrawal, and somatic complaints, whereas CBCL Externalizing problems are characterized by hyperactivity, impulsivity, noncompliance, and aggression. The total difficulties score is derived by the summation of 100 items for an overall view of children's behavior. To match the SDQ scores to CBCL scores we computed externalizing and internalizing scales by summing the scores from the SDQ related to conduct problems, hyperactivity and prosocial scores into Externalizing problems, and emotional symptoms and peer problems into Internalizing problems. Externalizing, Internalizing and Total problems were considered as outcomes in the current study.

Gene-set enrichment analysis

Based on the list of ND ePRS genes we used co-expression values from GeneMania to query the possible biological relationship between the genes (Mostafavi et al. 2008). We explored the topological properties of this group of genes using the Cytoscape application (Shannon et al. 2003) and Cytoscape's NetworkAnalyzer (Assenov et al. 2008) plugin was used

to determine measures of centrality (degree and betweenness). The co-expression network's topological properties were examined by calculating centrality measures, including degree and betweenness. Bottlenecks were defined as genes with higher betweenness (measures how often a node occurs on all shortest paths between two nodes) and hub genes were defined as genes with high degree (high interactions or nodes highly connected) (Figure 6B). To explore the complex network of reactions important to biological processes we also analyzed protein-protein interactions mined from String (Szklarczyk et al. 2019).

Cell-type Specific Expression Analysis (CSEA) and Tissue-Specific Expression Analysis (TSEA) were also performed to investigate if these genes are differentially expressed in diverse tissues, and at which developmental time points (Dougherty et al. 2010; Xu et al. 2014). We used FUMA Gene2Function (Watanabe et al. 2017) to investigate the level of expression of these genes in 54 different tissue types from GTEX ('Human genomics. The Genotype-Tissue Expression (GTEx) pilot analysis: multitissue gene regulation in humans' 2015). We also investigated different psychopathological diseases associated with the ND network using Metacore® and Clarivate Analytics®.

Statistical Analysis

To determine the most predictive model for explaining childhood problems, we employed a step-by-step multiple linear regression analysis, sequentially adding variables to the model based on their statistical significance. We compared the adjusted R^2 values to assess the improvement in model fit with the addition of each variable. The adjusted R^2 is particularly suited to determining the best-fit model since it accounts for the number of predictors in the model and only increases if added predictors improve model fit. This approach allowed us to identify the key factors that contribute to child health outcomes.

To assess the distinctiveness of the variables, we conducted tests for multicollinearity and examined the variance inflation factor (VIF) for each variable in the models. The results revealed that all VIF values were below 1.3, indicating a lack of substantial intercorrelation among the variables and suggesting the absence of multicollinearity in our models.

Initial Model

The initial model included the dependent variables and Internalizing, Externalizing, and Total Problems as outcomes. We then performed step-by-step regression by iteratively adding variables.

Model type 1: simple models

(1) Methylation model: Y ~ M + covariates

(2) Genotype model: Y ~ ePRS + covariates

(3) Multi-omics model: Y ~ M + ePRS + covariates.

(4) Adversity model: Y ~ A + covariates

(5) Multi-omics + adversity model: Y ~ M + ePRS + A + covariates

Model type 2: ePRS, methylation and Prenatal Adversity Score interaction models

(6) Methylation interaction model: Y~ M x A + covariates

(7) Genotype interaction model: Y~ ePRS x A + covariates

(8) Methylation interaction + ePRS model: Y~ M x A + ePRS+ covariates

(9) Multi-omics interaction model: Y ~ M + A + ePRS x A + covariates.

(10) Two interactions model: Y ~ M x A + ePRS x A + covariates.

Baseline cohorts' characteristics description and statistical analyses were performed using R (R Core Team 2022). Multiple linear regression analysis was used to investigate the impacts of the polygenic score, methylation score, and adversity score on childhood socioemotional outcomes while controlling for sex, buccal cell counts or cell types and genetic principal components to adjust for population stratification (Patterson, Price, and Reich 2006b). For the analyses with a significant effect of the interaction term, a simple slope analysis was conducted to describe the differences in the ePRS effects between lower/ higher adversity groups. To adjust the regression analyses for the variation in buccal cell counts or blood cell types, in the respective models, we included principal components (PCs) from the buccal cell counts or from the blood cell types depending on how samples were collected in each cohort (see Table 1). The significance level for all tests was set at $p < 0.05$. The assumptions of the linear regression analysis in all models were checked and were not violated. In all models, the variance inflation factor (VIF) was below 1.3. Sensitivity analyses were also run with the influential cases removed.

Results

Descriptive analysis

Three independent cohorts of different ancestries were included in our analysis: Growing Up in Singapore Towards Healthy Outcomes (GUSTO), composed of participants of Chinese, Malay, and Indian backgrounds, the Avon Longitudinal Study on Parents and Children (ALSPAC), mostly composed of Caucasian participants from the UK and the Basal Influences on Baby Development (BIBO) included mostly Caucasian participants from

Netherlands (Table 1). No significant differences were found in the main confounding variables in GUSTO, ALSPAC and BIBO (Tables 2, 3 and 4 respectively).

Main findings

GUSTO

Initially, we examined the individual contributions of methylation (M), genotype (ePRS), and multi-omics (M + ePRS) models as separate predictors (Figure 1). However, the simple models (1-3) exhibited lower adjusted R-squared values, suggesting a relatively low model fit for predicting mental health in children when considering these variables in isolation. Additionally, we investigated the role of prenatal adversity (A) as an independent variable in model 4, which resulted in a noteworthy increase in the adjusted R-squared value compared to the simple models (1-3). This increase resulted in a meaningful and statistically significant ($p<0.001$) contribution of prenatal adversity to the prediction of childhood problems. Furthermore, we compared model 4 (including only adversity) to model 5 (including main effects from all predictors) and observed an increase in the adjusted R-squared value, indicating an improved predictive capability of model 5.

Next, we added interaction terms with prenatal adversity score to the model. The multi-omics interaction model (9) emerged as the most informative among the various models for all three outcomes at 7 years old (Internalizing, Externalizing and Total Problems), displaying a higher adjusted R-squared value, which suggests that it explained a greater proportion of the variance in predicting childhood problems, adjusted for the number of predictors in the model (Figure 1). Moreover, we found a significant effect of multi-omics x adversity interaction term for all three outcomes: Internalizing ($p = 0.000168$), Externalizing ($p = 0.0001932$), and Total Problems ($p = 0.0004256$) at 7 years. The interaction term in each of the models was significantly associated with Internalizing ($p = 0.02557$) and Externalizing ($p = 0.03150$), but not Total Problems ($p=0.05461$).

Effects of Prenatal Adversity Severity

To examine the specific levels of severity of the prenatal adversity score at which the multi-omics x adversity interaction term effects were significant, we conducted a simple slope analysis (Figure 2). The results indicated that, for participants with higher exposure to prenatal adversity, an increase in ePRS was associated with higher Internalizing problems ($p = 0.04760$ at prenatal score 4, $p = 0.07006$ at prenatal score 3) and Externalizing problems ($p = 0.08871$ at prenatal score 4). Conversely, in the absence of prenatal adversity, higher ePRS was associated with lower Internalizing problems ($p = 0.05671$), Externalizing problems ($p = 0.02052$), and Total problems ($p = 0.06007$). This finding suggests that the combination of high ePRS and higher prenatal adversity exposure leads to amplified levels of childhood mental health and functioning, emphasizing the complex interplay between prenatal adversity, genetic susceptibility (ePRS), and their impact on child psychosocial development.

To explore different interactions, we incorporated the methylation interaction model (M x A) and genotype interaction model (ePRS x A) into our analysis. Within these interaction models, all of them (6-10) demonstrated either comparable or slightly improved adjusted R-squared values when compared to the adversity model alone (4) and similar to or less than the multi-omics + adversity model (5). Among the various models, the multi-omics interaction model (9) and the model including two interactions (10) exhibited the highest adjusted R-squared value, suggesting their superior predictive capacity for childhood mental health outcomes.

However, during the analysis, we encountered two strong influential cases that had a substantial effect on the fit of the methylation x adversity models (6,8,10). To address this issue, we removed the influential cases from the analysis, leading to a drastic reduction in the adjusted R-squared values for all three models that included this interaction (6,8,10). After

doing so, the multi-omics interaction model (9) emerged as the best predictor across all three outcomes: Internalizing, Externalizing, and Total Problems at 7 years.

ALSPAC

In the ALSPAC cohort at 11 years and 8 months of age, we observed similar patterns to those seen in the GUSTO cohort. The simple models (1-3) exhibited lower adjusted R-squared values, indicating modest model fit when considering these variables in isolation (Figure 3). Moreover, similar to GUSTO, we found a significant increase in the adjusted R-squared value for the adversity model (Model 4) compared to the simple models (1-3), demonstrating the meaningful and statistically significant ($p<0.001$) contribution of prenatal adversity to the prediction of childhood problems. However, when comparing model 4 to model 5 (including all main effects), the adjusted R-squared values were very close or slightly smaller for all dependent variables.

Subsequently, we introduced interactions with the prenatal adversity score as independent variables to the model, focusing on the multi-omics interaction model (Model 9) as it had shown the best fit in the GUSTO cohort. The multi-omics interaction model (Model 9) in the ALSPAC cohort demonstrated a high adjusted R-squared value, indicating that it explained a substantial proportion of the variance in predicting all three childhood problems, accounting for the number of predictors in the model.

Moreover, we found significant multi-omics interaction models for all three outcomes: Internalizing ($p = 0.01482$), Externalizing ($p = 0.01042$), and Total Problems ($p = 0.002304$) at 11 years and 8 months. The interaction term was significant for Externalizing ($p = 0.064519$), but not for Internalizing ($p = 0.2543$) and Total Problems ($p=0.016039$).

Effects of Prenatal Adversity Severity

To explore the specific levels of prenatal adversity score at which the multi-omics x adversity interaction term effects were significant, we conducted a simple slope analysis (Figure 4). The results indicated that, for participants with higher exposure to prenatal adversity, an increase in ePRS was associated with higher Externalizing problems at prenatal scores 3, 4, and 5 ($p = 0.046823644$, $p = 0.045372116$, and $p = 0.047257506$, respectively). This finding suggests that the combination of high ePRS and higher prenatal adversity exposure leads to amplified levels of childhood mental health problems, highlighting the complex interplay between prenatal adversity, genetic susceptibility (ePRS), and their impact on child psychosocial development.

BIBO

We extended our investigation to examine the predictive capability of the multi-omics model for childhood problems at 6 years using a sample group from the BIBO cohort. Surprisingly, the findings in the BIBO cohort diverged from those observed in the GUSTO and ALSPAC cohorts. Unlike the other cohorts, all models, including both simple and interaction models, exhibited lower adjusted R-squared values (adjusted R-squared < 0.04) (Figure 5). Moreover, contrary to the previous cohorts, the adversity model with prenatal adversity alone did not show a significant increase in adjusted R-squared compared to the simpler models.

Nonetheless, similar to GUSTO, the multi-omics interaction model (Model 9) in the BIBO cohort demonstrated a higher adjusted R-squared for Internalizing problems, suggesting that it explained a greater proportion of the variance in predicting Internalizing problems, accounting for the number of predictors in the model.

However, in contrast to GUSTO, we did not find a significant multi-omics interaction model for all three outcomes: Internalizing ($p = 0.1666$), Externalizing ($p = 0.2272$), and Total Problems ($p = 0.4409$) at 6 years. The interaction term in each of the models was significant for Internalizing ($p = 0.0984$), but not for Externalizing ($p = 0.2214$) and Total Problems ($p=0.1635$).

Effects of Prenatal Adversity Severity

To examine the specific levels of severity of the prenatal adversity score at which the effects were significant, we conducted a simple slope analysis (Figure 6). The results indicated that, for participants with higher exposure to prenatal adversity, an increase in ePRS was associated with higher Internalizing problems at prenatal score 0 ($p = 0.03721$). This finding suggests that the combination of low ePRS and no prenatal adversity exposure leads to lower levels of internalizing child problems.

Despite incorporating the methylation interaction model (M x A) and genotype interaction model (ePRS x A) into our analysis, the addition of these interactions did not substantially affect the predictive capability in our various models.

Biological and functional characterization of ND gene network

Early psychological adversity can have an impact on subsequent health via direct biological mechanisms, and this has important consequences for adult chronic physical and mental conditions (Scott et al. 2011). To explore the biological mechanisms of the associations seen in human cohorts, we performed enrichment analysis from the genes that were near the methylation CpG sites described in the original EWAS (Figure 7A). We began by exploring the possibility these genes might be working together in a network, rather than in isolation, following the principle of gene regulatory networks. Genes that are co-expressed are thought to be working together. Figure 7A depicts the ND co-expression gene network and shows that the genes are well connected. In fact, according to physical interactions (protein-protein interactions) mined from String (Szklarczyk et al. 2019), the ND gene network has significantly more interactions than expected by chance (p=0.000459). This strengthens the idea that these genes are working as a network. Amongst hubs-bottlenecks genes (considering betweenness and degree higher than +1SD above mean), represented by high degree and betweenness, which most likely have a key role in regulating the network, we found *BSN* (Bassoon Presynaptic Cytomatrix Protein gene), Fc mu receptor gene (*FAIM3*) and Dynamin 1 gene (*DNM1*) as the ones with the highest degree (Figure 7B). *BSN* is primarily expressed in neurons, and it encodes a scaffolding protein that has a role in cytoskeleton organization and recruitment of proteins relevant to presynaptic plasticity and regulated release of neurotransmitters (Montenegro-Venegas et al. 2020). *FAIM* plays a role in the immune responses by encoding the Fc receptor for IgM (Kubagawa et al. 2009). *DNM1* has a role in synaptic vesicle recycling by encoding dynamin-1 a GTPase that is particularly critical during postnatal development (Boumil et al. 2010).

To identify cell-type and specific tissues in the human brain associated with the ND gene network we performed an enrichment analysis using Cell-type Specific Expression

Analysis (CSEA) (Figure 8A) and Tissue Specific Expression Analysis (TSEA) tools (Figure 8B and 8C). The results reveal that the ND ePRS gene network is highly enriched in the brain during early development in the thalamus and striatum, as well as in the cortex during young adulthood (Dougherty et al. 2010; Xu et al. 2014). The network is also predominantly expressed in brain and pituitary tissues which might be related to the hypothalamic-pituitary axis, a pathway that has a key role in regulation of the endocrine system in response to stress.

The genes within the ND network were significantly enriched for biological processes associated with synaptic signalling and organization and nervous system development in Metacore® (Figure 9A). This finding is aligned with the hubs-bottlenecks from ND network, such as DNM1 and BSN, which have important roles in plasticity and neurotransmission. To investigate in which tissues these genes are expressed we used FUMA Gene2Function (Watanabe et al. 2017) and obtained their level of expression in 54 different tissue types from GTEX ('Human genomics. The Genotype-Tissue Expression (GTEx) pilot analysis: multitissue gene regulation in humans' 2015). The encoded genes that were highly expressed in the brain (right upper corner of Figure 9B) were significantly associated with different psychopathological diseases (Metacore®) such as anxiety, panic disorder, schizophrenia, and autism (Figure 9C). These results suggest that there are important genes in our ND network expressed in early fetal and young adulthood that are related to neurodevelopment and associated with psychopathology.

Discussion

As previously described, stressors occurring early in life have the most influential contribution to childhood psychosocial adversities (Scott et al. 2011). Growing up in disadvantaged neighborhoods is a major risk factor for acute and chronic physical and mental diseases (Taylor et al. 2020; Reuben et al. 2020; Chase-Lansdale 1997; Glymour et al. 2010). Our objective was to map the molecular/genetic architecture of responsivity to ND and using a multi-omics approach, including a polyepigenetic expression-based polygenic score, identify individuals who are more vulnerable to the effects of prenatal adversity. Our findings showed that some children will be at greater risk for emotional development problems than others after exposure to prenatal adversity, and risk can be identified by addressing genomics/epigenetic markers related to responsivity to neighborhood disadvantage.

Going beyond the conventional PRS that uses single nucleotide polymorphisms in G × E studies, our model managed to capture in a more accurate manner the complexity of biomolecular processes and thus inform the intrinsic mechanisms that can lead to the onset of susceptibility to psychiatric disorders. We used epigenome-wide DNAm array data from a neighborhood disadvantage study to construct a methylation score and a novel EWAS-based ePRS. While capturing the polygenicity of genes that respond to the environmental influence with changes in DNAm, we also took into consideration the different methylation profiles from the EWAS CpGs. The integration of EWAS data into a PRS has come to light in recent years enabling a broader view for populational studies. The recent publication of Kresovich et al. showed that the addition of a methylation-based risk score to existing genetic and questionnaire-based cohorts can significantly improve prediction for breast cancer (Kresovich et al. 2022). Tangentially, while polygenic risk scores provide unchangeable risk for a patient, methylation-based risk scores may provide a current risk state for the patient over the last months.

We demonstrated here that a linear regression adjusted by sex and buccal cell count (or blood cell type) that includes methylation scores and the ePRS with interaction with prenatal adversity exposure is statistically associated with internalizing, externalizing and total problems in GUSTO at 7 years. Additionally, ePRS originated from tissue expression of the Nacc, suggesting a strong relationship of this brain region to childhood problems at 7 years of age. The Nacc is a major dopaminergic projection area that collectively with the VTA, anterior hippocampus (aHipp), and medial prefrontal cortex (mPFC) form the mesocorticolimbic pathway that mediates reward processing. During development, proper establishment of neuronal circuits is crucial and involves complex tasks of cell proliferation, cell fate determination, cell migration, and cell and synapse formation that allow for the refined buildout of cognitive processing and mental health (Shohat, Amelan, and Shifman 2021). B.Goff showed that children exposed to early life adversity had NAcc hypoactivation during adolescence and lower Nacc reactivity was correlated with higher depressive scores (Goff et al. 2013). Similarly, Forbes et. al. have shown that DA-related functional polymorphisms increase ventral striatum reactivity and is associated with higher impulsivity (Forbes et al. 2009) and the same has been demonstrated in lesioned Nacc rat models (Cardinal et al. 2001). This suggests that disrupted or altered Nacc signalling can partake in pathological reward-related behaviors. This is reflected in our results, where models with the constructed ePRS using Nacc tissue expression predict externalizing problems (characterized by aggressive, oppositional, and oppositional behavior that can be provoked by hyperactive impulsive symptoms) and internalizing problems (characterized by social withdrawal, anxious and depressive symptoms) in children.). Therefore, Nacc is an important stress-related behavioral structure, and aligned with our findings, we show that the Nacc ND-related gene network is associated with neurological development and pathological diseases. Our enrichment analysis results show that the ND gene network is highly expressed in fetal and young adulthood tissues.

The genes within the network are seen to be involved in numerous aspects of neurodevelopmental processes. The ND gene network interestingly plays a role in the regulation of the synaptic vesicle cycle, synapse organization and system development, according to the gene ontology processes and hubs-bottlenecks associated with the network. While capturing the polygenicity of genes that respond to environmental factors with variations in DNAm, we took into consideration the various methylation patterns from the EWAS CpGs. One of the most inciting results is how the epigenome-wide DNAm data that was collected from adolescence with neighborhood disadvantage associates with genes that are expressed during early fetal developmental stages and young adulthood. This suggests that this network is highly preserved, and its expression is sensitive to critical developmental periods of firstly the arrangement of neural circuitry and secondly the development of childhood behavior.

GUSTO model 9 demonstrated the presence of a gradient linear pattern, indicating that the ePRS and prenatal adversity score (A) interact together to predict the severity of childhood internalizing and externalizing problems. Specifically, individuals with higher expression of the gene network associated with Neighborhood disadvantage (indicated by high ePRS) exhibited heightened susceptibility to childhood problems with increasing prenatal adversity exposure. These findings underscore the importance of considering these variables collectively to gain a deeper understanding of the gene-environment situation and its impact on the intricate mechanisms of child health outcomes.

Another intriguing finding was that significant interactions were evident with internalizing and externalizing problems, but not with the combined total problems score. As we had previously mentioned, the total problems score encompasses a broader spectrum of issues. As we previously discussed, the total problems score encompasses a wide array of issues, including not only internalizing and externalizing problems but also other factors such as sleeping difficulties. The amalgamation of these diverse scores into the total problems score

may lead to a dilution of specific effects and interactions that are associated with internalizing and externalizing problems when analyzed in combination.

Similar to the GUSTO cohort, the ALSPAC cohort also exhibited lower adjusted R-squared values for the simple models, with prenatal adversity playing a significant role in predicting childhood problems. The multi-omics interaction model demonstrated the second highest predictive capacity across all three outcomes, supporting the importance of considering gene-environment interactions in understanding childhood mental health outcomes.

An intriguing observation within the ALSPAC cohort pertains to the specificity of the interactions with externalizing scores. This holds intriguing implications, especially given that adolescence is often characterized by an increase in defiant behaviors. We hypothesize that this convergence on externalizing problems could be reflective of the defiance often observed during adolescence.

Furthermore, it's important to acknowledge the ongoing developmental processes of brain structures responsible for stress response (prefrontal cortex, hippocampus, amygdala) during childhood and adolescence. These structures follow diverse developmental trajectories, each with distinct windows of stress sensitivity. Bosch et al.'s findings (2012) highlighted the impact of adversities on the HPA-axis during puberty, emphasizing the temporal importance of stress exposure (Bosch et al. 2012). Notably, brain regions sensitive to stress hormones also respond to gonadal hormones, which surge during puberty. The transition from childhood to adolescence involves considerable biological and social adjustments, potentially leading to stress-induced changes in the post-pubertal brain, thereby contributing to an individual's vulnerability to psychopathologies (Goddings et al. 2019). Additionally, our findings underscore the critical importance of validating the findings in other cohorts to ensure the robustness and validity of gene-environment interaction analyses.

An interesting observation in the BIBO cohort was the presence of only two prenatal adversity levels (0 and 1), which resulted in a dichotomous distribution. Given that we have previously seen the significant role of prenatal adversity in the model, the limited variety of prenatal adversity levels in this cohort might contribute to the differences observed in comparison to the other cohorts. Notably, we found that the model performed well in predicting childhood problems for higher adversity scores, suggesting that the impact of prenatal adversity may be more pronounced in such cases. This underscores the potential influence of the range and severity of prenatal adversity exposure on the predictive capacity of our multi-omics model in this cohort.

In addition to constructing the linear models, we also observed that including information from blood or buccal epigenome, prenatal adversity and ePRS can provide a better prediction of childhood socio-emotional problems. We observed an increase in the adjusted R-squared value when the linear model has both epigenetic and genetic scores plus the interaction with prenatal adversity in comparison to when the scores are alone in the model in both GUSTO and ALSPAC. This suggests that the effect of genetic and epigenetic profiles is conditional to exposure to adversity and can improve the prediction rate.

In sum, we demonstrated a multi-omics approach to explore the risk to childhood socio-emotional behavior. Our study provides a methodology that captures the integration of epigenetic and polygenic scores for individual differences in susceptibility associated with early adversity exposure. This shows how complex the molecular processes of the neural circuit are and how adding more levels of information about gene tissue expression and epigenetics may better inform the identification of individuals who are susceptible to the long-term effects of prenatal adversity on psychiatric conditions.

Limitations

We note that our coverage of DNA modifications is currently incomplete as there are other epigenetic modifications, such as histone modification, acetylation, and micro-RNA that are not currently accessible. Another limitation is that our coverage of ethnicity in different cohorts lacks an African American-based cohort since Sharkey et. al estimated that black youth are 10 times more likely to live in a poor neighborhood than their white peers (Sharkey 2013).

Conclusion

Undoubtedly, there are many ways to improve the environment to protect newborns and children from disadvantaged locations with policies and initiatives on education, social welfare, employment, training, criminal policy, and youth unemployment. Our study can be a resource to support the development of policies that can help buffer the damaging and enduring effects of early adversity on mental and physical health.

Data Availability

For GUSTO, visit https://www.gusto.sg/. For ALSPAC, the study website contains details of all the data that is available through a fully searchable data dictionary and variable search tool at http://www.bristol.ac.uk/alspac/researchers/our-data/. For BIBO, visit https://dpblab.org/projects/bibo/.

Funding

This research was supported by The JPB Foundation through a grant to the JPB Research Network on Toxic Stress: A Project of the Center on the Developing Child at Harvard

University, the Canadian Institutes of Health Research (CIHR, PJT - 173237, PI Silveira PP) and the Ludmer Centre for Neuroinformatics & Mental Health.

The ALSPAC project receives foundational support from the UK Medical Research Council, Wellcome (Grant ref: 217065/Z/19/Z), and the University of Bristol. Detailed information about the funding grants can be accessed on the official ALSPAC website: (http://www.bristol.ac.uk/alspac/external/documents/grant-acknowledgements.pdf). The GWAS data was produced through collaboration with Sample Logistics and Genotyping Facilities at Wellcome Sanger Institute and LabCorp (Laboratory Corporation of America), with additional assistance from 23andMe. This publication is the collective effort of the authors, and Gisele Sanda and Patricia P. Silveira will assume responsibility as guarantors for the paper's content.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Acknowledgments

We are grateful for all participants and families that took part in these studies for (GUSTO, ALSPAC and BIBO). We are also thankful for all the team members of each cohort, which includes recruiters, interviewers, laboratory technicians, clinical and research scientists, volunteers, managers, and nurses.

Table 1: Overview of the three cohorts

Cohort	Sample size (male)	Ethnicity	Country	Methylation	Questionnaire
GUSTO	102 (56)	Chinese, Malay & Indian	Singapore	3m	CBCL 7y
ALSPAC	656 (325)	Mostly Caucasian	UK	7y	SDQ 11y
BIBO	119 (62)	Mostly Caucasian	Netherlands	5-6y	CBCL 6y

Table 2: Description of baseline characteristics in GUSTO sample for high and low ND ePRS score groups defined by median split of the ePRS score.

GUSTO				
Sample descriptive	**Total (n = 102)**	**Low ePRS (n = 51)**	**High ePRS (n = 51)**	***p***
Sex - Male	54.9% (56)	50.98% (26)	58.82% (30)	0.551
Birth weight (grams)	3116 (427)	3109 (387)	3123 (468)	0.870
Still breastfed at 3 months	56.9% (58)	54.9% (28)	58.8% (30)	0.842
Gestational Age (Weeks)	38.87 (1.27)	39.08 (1.25)	38.65 (1.28)	0.088
Maternal age at birth	30.35 (4.94)	30.89 (4.86)	29.8 (5)	0.270
Household income < SG$2000	19.6% (20)	23.5% (12)	15.7% (8)	0.454
Smoking during pregnancy	3.9% (4)	1.96% (1)	5.88% (3)	0.610
Self-reported ethnicity				0.183

Chinese	59.8% (61)	68.63% (35)	50.98% (26)	
Indian	13.7% (14)	9.8% (5)	17.65% (9)	
Malay	26.5% (27)	21.57% (11)	31.37% (16)	

Table 3: Description of baseline characteristics in ALSPAC sample for high and low ND ePRS score groups defined by median split of the ePRS score.

ALSPAC				
Sample descriptive	**Total (n = 656)**	**Low ePRS (n = 328)**	**High ePRS (n = 328)**	***p***
Sex - Male	49.5% (325)	50.3% (165)	48.78% (160)	0.755
Birth weight (grams)	3518 (460)	3513 (442)	3523 (478)	0.778
Still breastfed at 3 months	60.3% (395)	60.6% (198)	60.1% (197)	0.961
Gestational Age (Weeks)	39.70 (1.30)	39.66 (1.27)	39.73 (1.32)	0.527
Maternal age at birth	29.94 (4.33)	30.03 (4.26)	29.86 (4.40)	0.620
SES (Crowding index* above 1)	2.59% (17)	3.1% (10)	2.1% (7)	0.623
Smoking during pregnancy	13.6% (89)	11.6% (38)	15.6% (51)	0.171

*Low socioeconomic status (SES) in ALSPAC: crowding index higher than 0.75 at 2-year-and-9-month time point was considered as low SES. Crowding index was calculated by dividing the number of individuals living in the family dwelling by the number of rooms in the family dwelling and was used as a proxy measure of socioeconomic status.

Table 4: Description of baseline characteristics in BIBO sample for high and low ND ePRS score groups, defined by median split of the ePRS score.

BIBO				
Sample descriptive	**Total (n = 119)**	**Low ePRS (n = 57)**	**High ePRS (n = 62)**	**p**
Sex - Male	52.1% (62)	57.89% (33)	46.77% (29)	0.303
Birth weight (grams)	3589.84 (449.9)	3593.7 (450.15)	3586.29 (453.3)	0.929
Still breastfed at 3 months	64.41% (76)	57.89% (33)	70.49% (43)	0.217
Gestational Age (Weeks)	40.09 (1.22)	40.20 (1.30)	39.98 (1.14)	0.349
Maternal age at birth	32.93 (3.79)	32.18 (3.73)	33.61 (3.74)	0.038
Maternal education level (HBO or higher)	78.99% (94)	77.19% (44)	80.65% (50)	0.813

Figure 1: Comparison of simple and complex models by adjusted R-squared in GUSTO.

GUSTO	Meth.	Gen.	Multi-omics	Adv.	Multi-omics + Adv.	Meth. x Adv	Gen. x Adv	Meth. x Adv + ePRS	Multi-omics interaction	Both interactions
Internalizing problems 7y	-0.00051	0.00293	0.00077	0.18312	0.18374	0.18296	0.20232	0.17583	0.21862	0.21545
Externalizing problems 7y	-0.02699	-0.02858	-0.00803	0.14542	0.18394	0.17856	0.16579	0.17945	0.21574	0.21882
Total problems 7y	-0.03602	-0.03559	-0.02789	0.15798	0.17508	0.17723	0.16799	0.17209	0.19911	0.20368

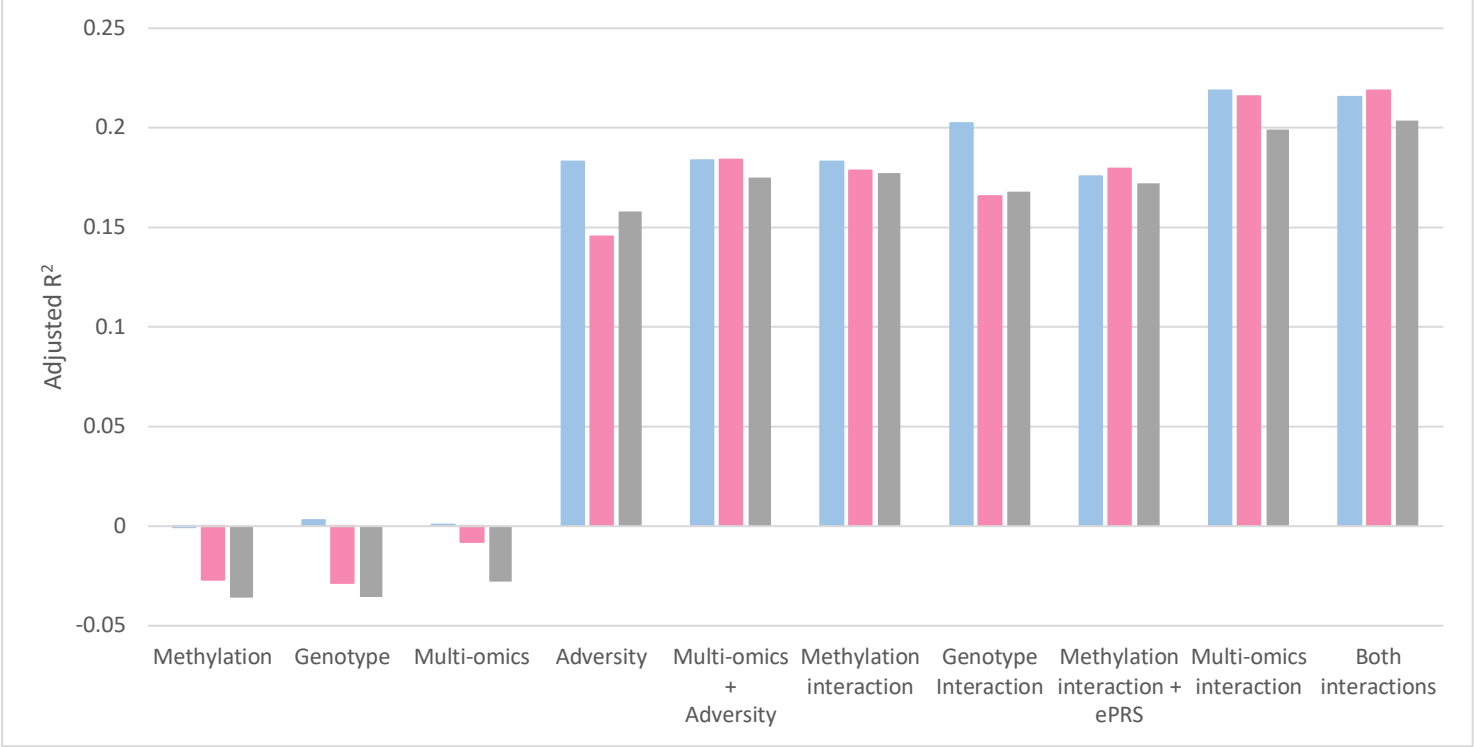
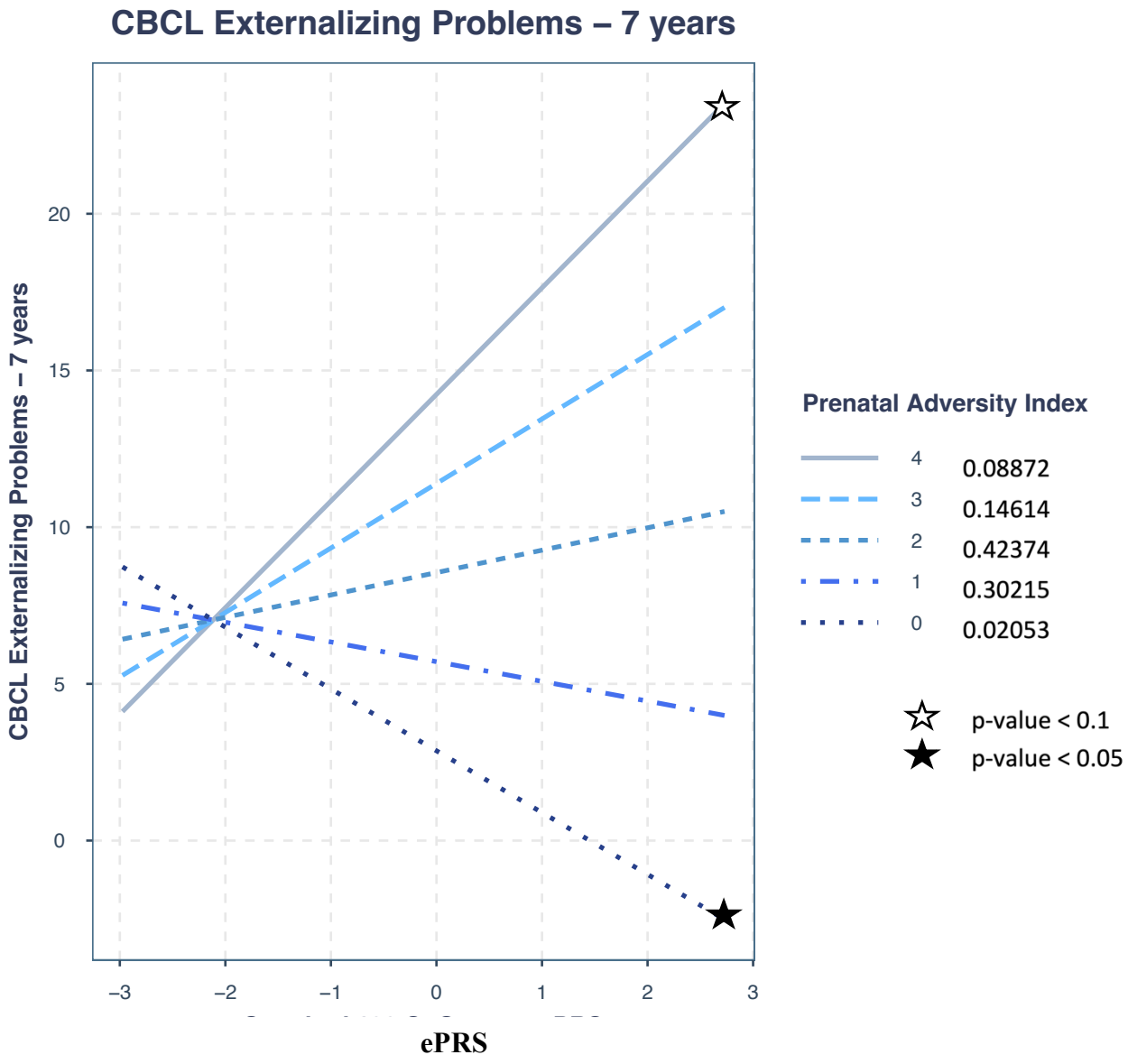
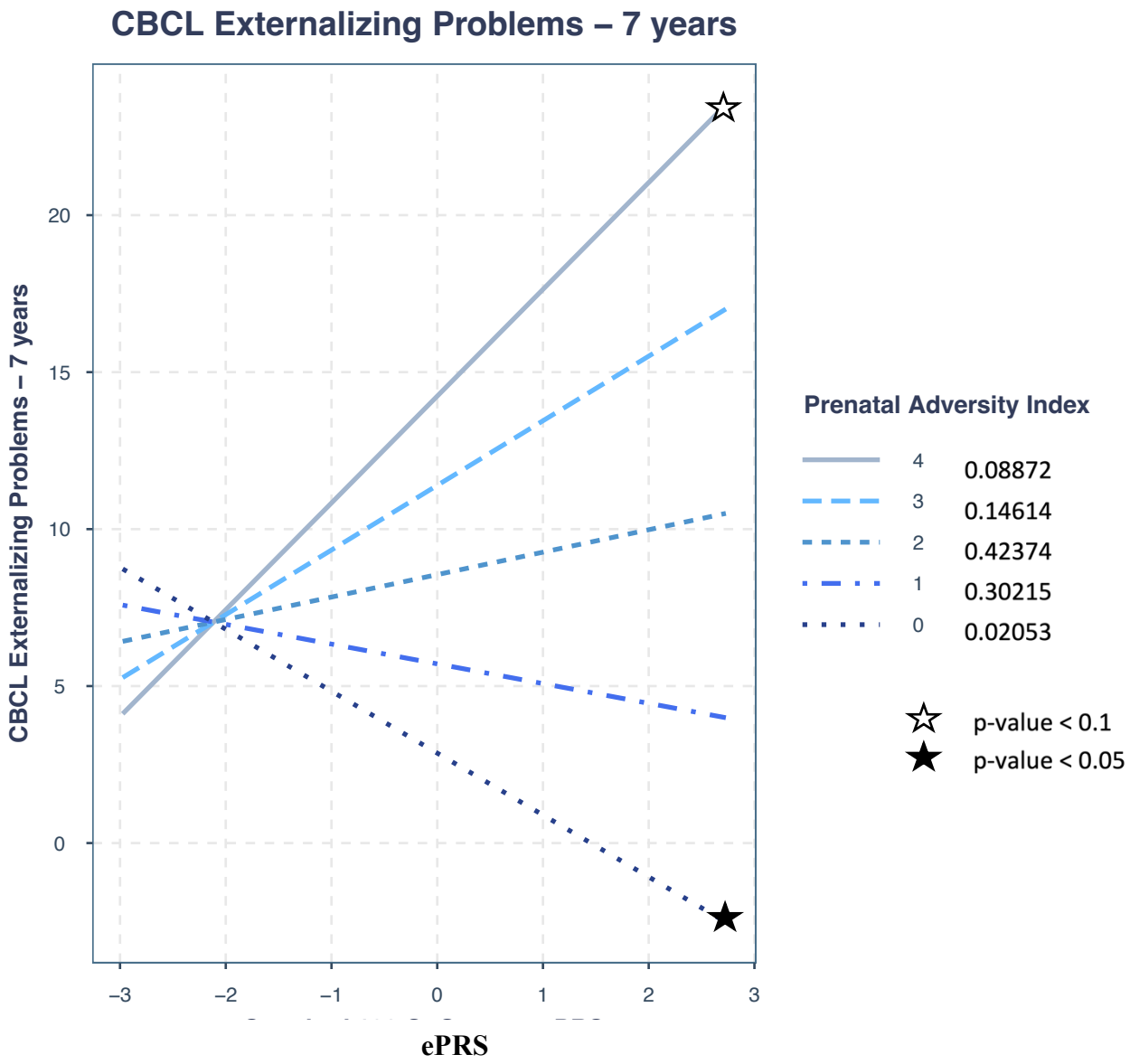


Figure 1: Adjusted R-squared estimates in GUSTO models. The model fit is determined by the adjusted R-squared represented by the y axis. The x axis represents the 10 different linear models, and the different colored columns represent Internalizing problems (blue), externalizing problems (pink) and total problems (gray).

Figure 2: Multiple linear regression and simple slope analysis demonstrating the effects of prenatal adversity x ePRS on socioemotional problems in 7-year-old children in GUSTO.




CBCL Externalizing Problems – 7 years
CBCL Externalizing Problems – 7 years
20
15
10
5
0
−3
−2
−1
0
1
2
3
ePRS
Prenatal Adversity Index
4
0.08872
3
0.14614
2
0.42374
1
0.30215
0
0.02053
p-value < 0.1
p-value < 0.05

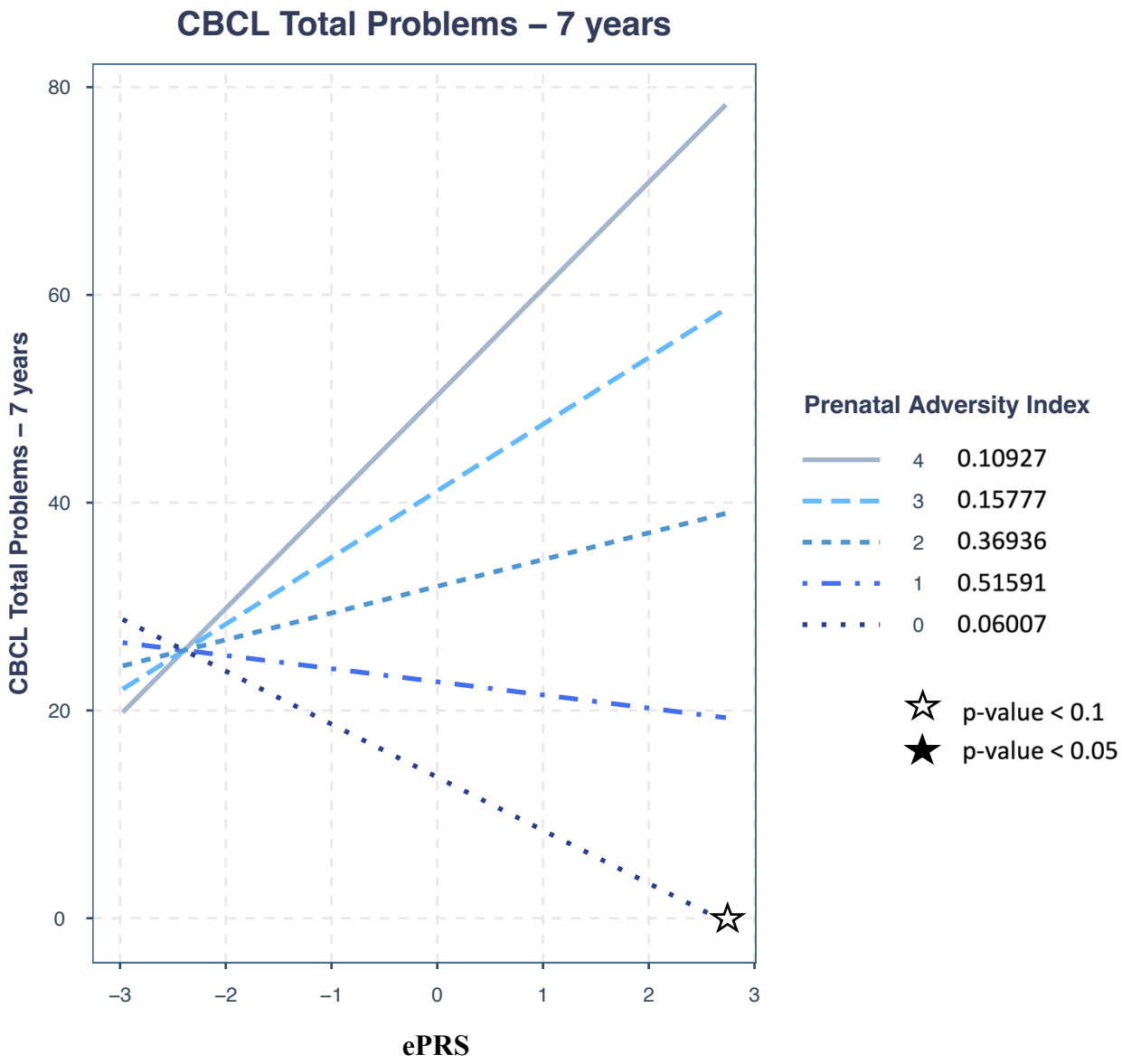


Figure 2: Simple slope analysis of different severity levels of early adversity in GUSTO at 7 years of age. White stars indicate significant associations ($p < 0.05$) and black stars indicate marginally significant associations ($p < 0.10$). At the higher values of ePRS, significant differences in predicted behavioural problems are seen contrasting high and low adversity groups, indicating that high ePRS individuals are susceptible to adversity and contribute differently to developmental outcomes.

Figure 3 Comparison of simple and complex models by adjusted R-squared in ALSPAC.

ALSPAC	Meth.	Gen.	Multi-omics	Adv.	Multi-omics + Adv.	Meth. x Adv	Gen. x Adv	Meth. x Adv + ePRS	Multi-omics interaction	Both interactions
Internalizing problems 11y8m	0.0064	0.00583	0.00497	0.01939	0.0188	0.0189	0.01845	0.01755	0.01926	0.01801
Externalizing problems 11y8m	-0.00106	-0.0068	-0.00153	0.01644	0.01709	0.01821	0.01922	0.01759	0.02078	0.0213
Total problems 11y8m	0.01161	0.01098	0.01017	0.02883	0.02587	0.0259	0.02884	0.02444	0.02734	0.02591

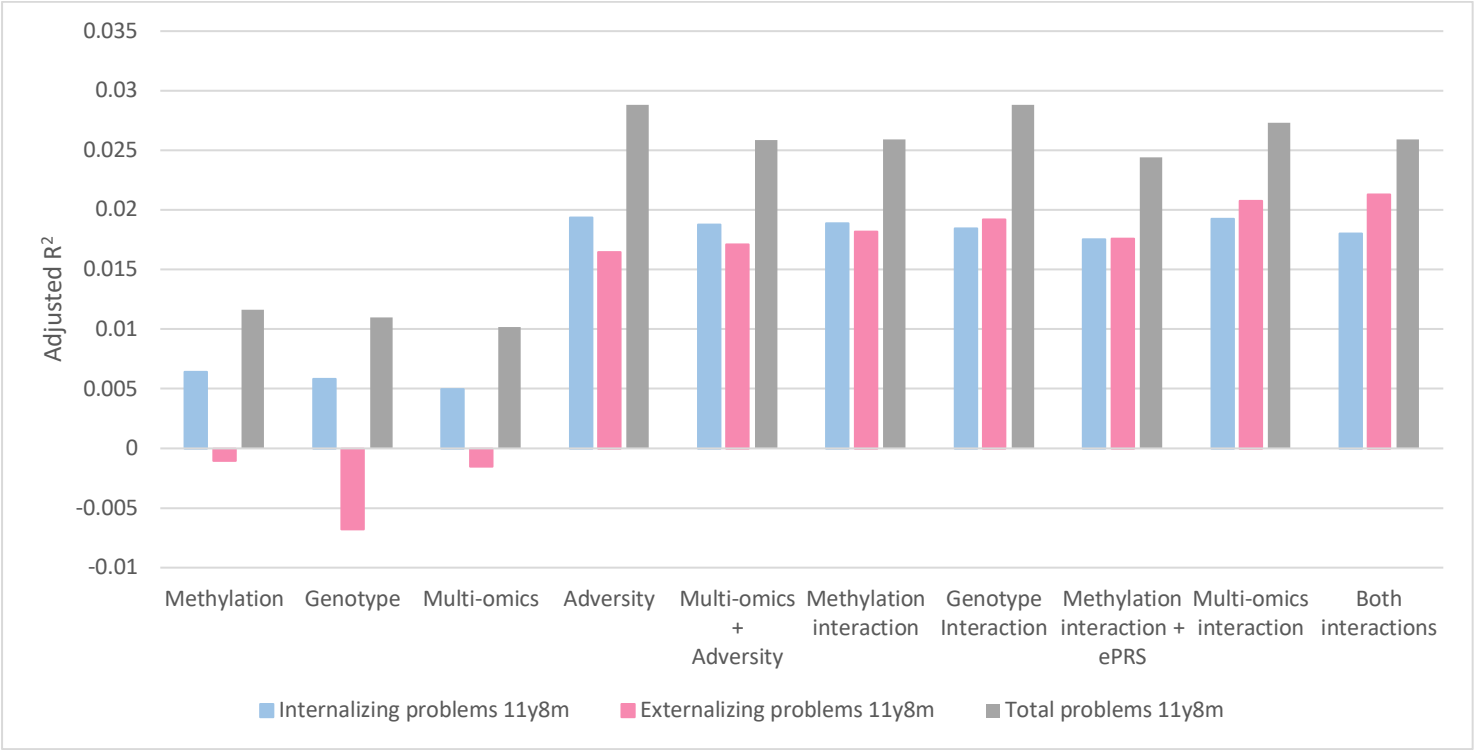


Figure 3: Adjusted R-squared estimates in ALSPAC models. The model fit is determined by the adjusted R-squared represented by the y-axis. The x-axis represents the 10 different linear models, and the different colored columns represent Internalizing problems (blue), externalizing problems (pink) and total problems (gray).

Figure 4 Simple slope analysis demonstrating the effects of prenatal adversity x ePRS on socioemotional problems at 11-year-old children in ALSPAC.

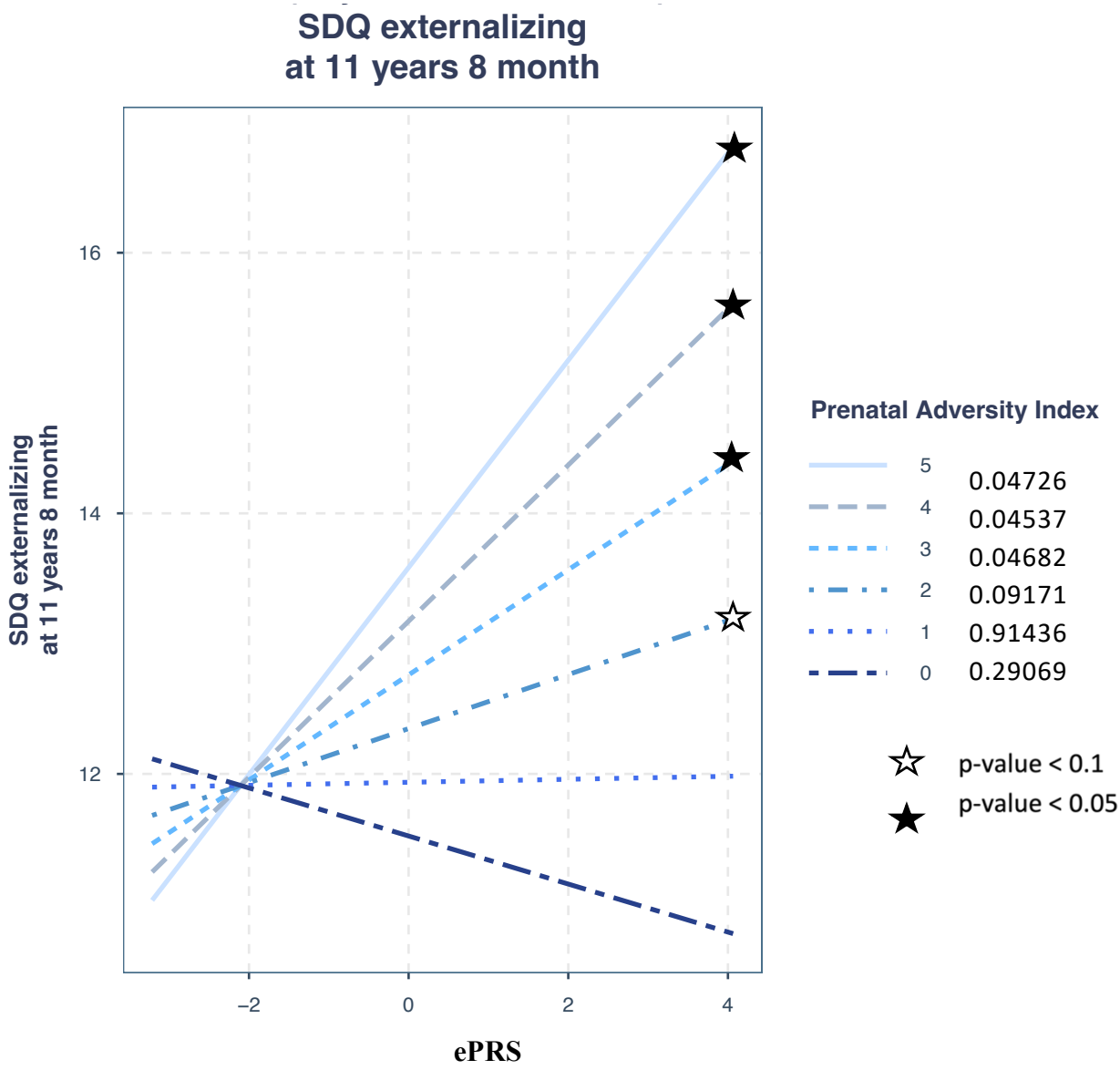


Figure 4: Simple slope analysis of different levels of early adversity severity in ALSPAC at 11 years and 8 months of age. White stars indicate p <0.05 and black stars indicate p <0.10. Significant differences in children with high ePRS scores are seen contrasting high ePRS individuals are susceptible to adversity and contribute to developmental outcomes.

Figure 5 Comparison of simple and complex models by adjusted R-squared in BIBO.

BIBO	Meth.	Gen.	Multi-omics	Adv.	Multi-omics + Adv.	Meth. x Adv	Gen. x Adv	Meth. x Adv + ePRS	Multi-omics interaction	Both interactions
Internalizing problems 6y	0.01112	0.00024	0.01935	-0.00708	0.01929	0.00523	0.02101	0.01309	0.03489	0.02677
Externalizing problems 6y	0.03363	0.01739	0.02576	0.02287	0.02039	0.03109	0.01940	0.02291	0.02493	0.02408
Total problems 6y	0.00302	-0.01363	-0.00525	-0.00612	-0.00845	-0.00640	-0.00453	-0.01496	0.00035	-0.00791

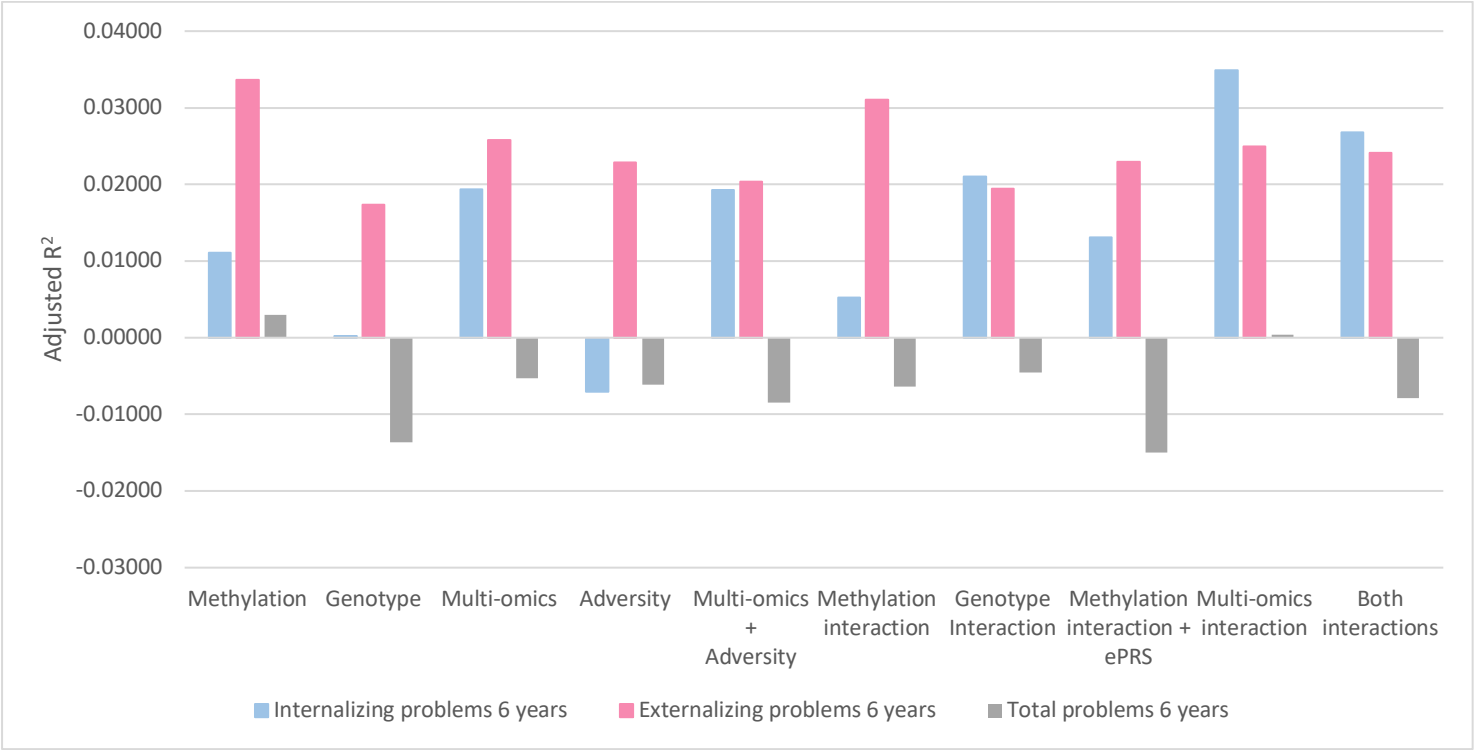


Figure 5: Adjusted R-squared estimates in BIBO models. The model fit is determined by the adjusted R-squared represented by the y-axis. The x-axis represents the 10 different linear models, and the different colored columns represent Internalizing problems (blue), externalizing problems (pink) and total problems (gray).

Figure 6 Simple slope analysis demonstrating the effects of prenatal adversity x ePRS on socioemotional problems at 6y children in BIBO.

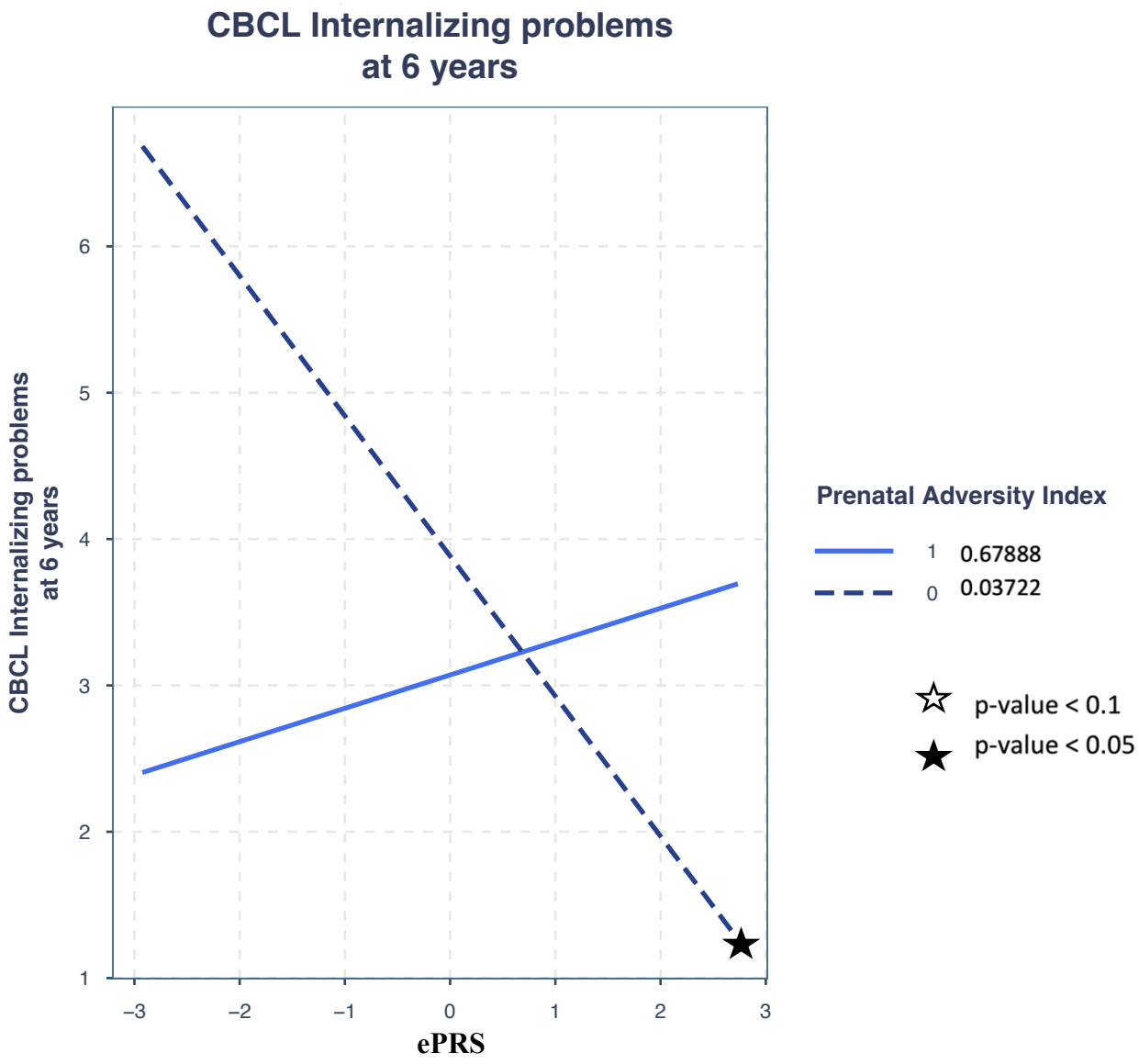
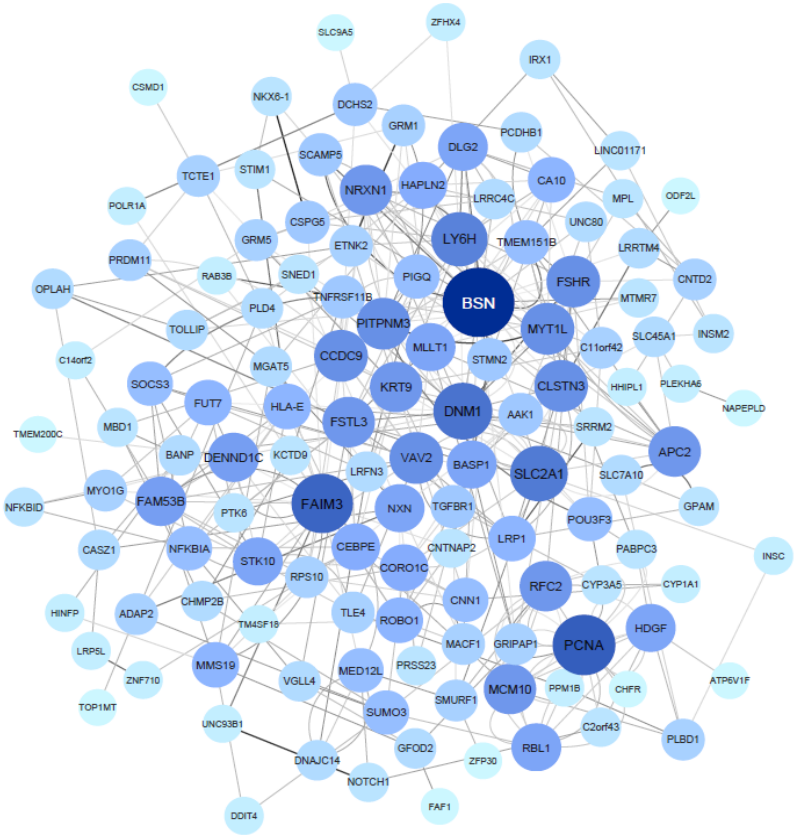


Figure 6: Simple slope analysis of different levels of early adversity severity in BIBO at 6 years of age. White stars indicate p <0.05 and black stars indicate p <0.10. Significant effects were seen at prenatal score 0 in Internalizing Problems.

Figure 7. Topological properties of the ND gene network

A)



B)

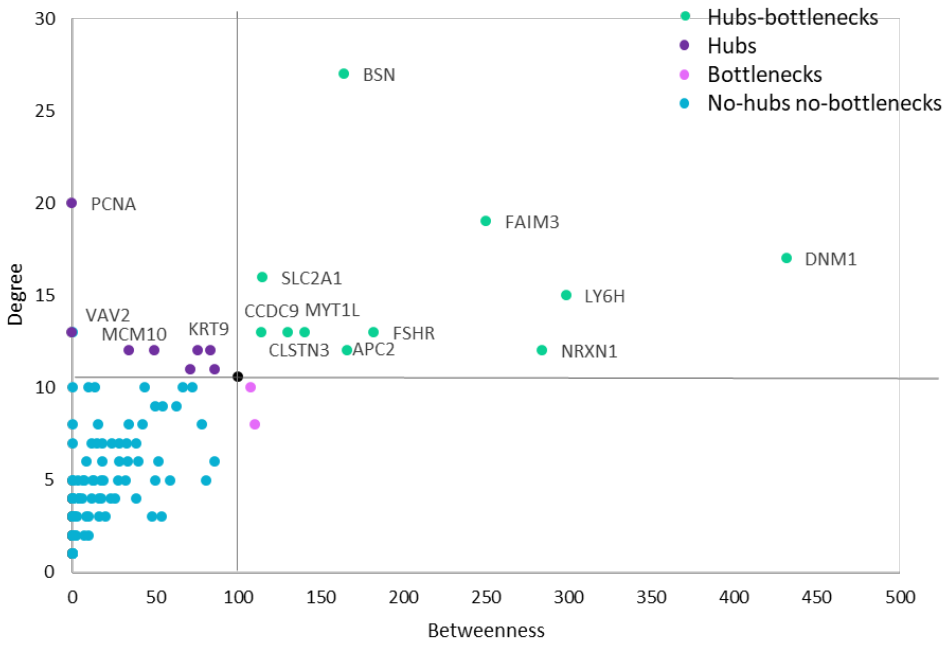
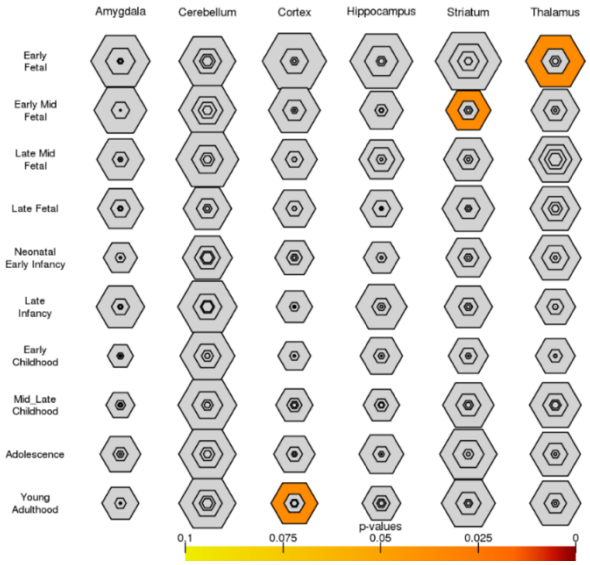


Figure 7A: NaCC ND co-expression gene network. The genes from the ND co-expression gene network are represented by nodes, by which the size of the node and the color intensity is proportionate to the degree of connectivity. Bigger and darker blue nodes represent genes with the highest degree (more connected). The edges of the nodes indicate co-expression, where the darkest lines represent a higher co-expression relationship.

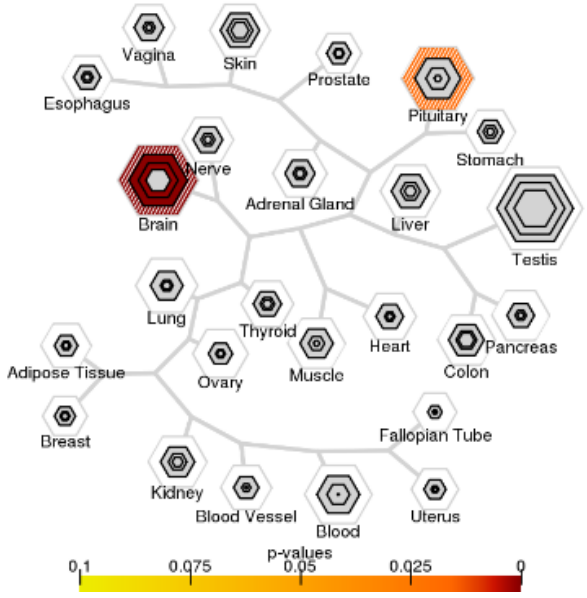
Figure 7B: Topological properties of the ND gene network, showing hubs (high connectivity genes with degrees higher than +1SD above the mean), bottlenecks (highly influential genes that serve as bridges in the network with betweenness higher than +1SD above the mean), and hubs-bottlenecks. Horizontal and vertical lines in black indicate mean +1 SD for betweenness and degree. We see that the ND co-expression gene network is well connected, representing a cohesive network and the genes most important to the overall network structure, according to degree and betweenness centrality measures are, Bassoon Presynaptic Cytomatrix Protein gene (*BSN*), Fc mu receptor gene (*FAIM3*) and Dynamin 1 gene (*DNM1*).

Figure 8. Cell-type and Tissue Specific Expression Analysis associated with ND network.

A)



B)



C)

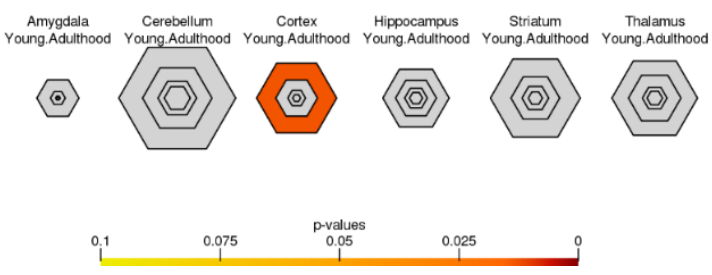
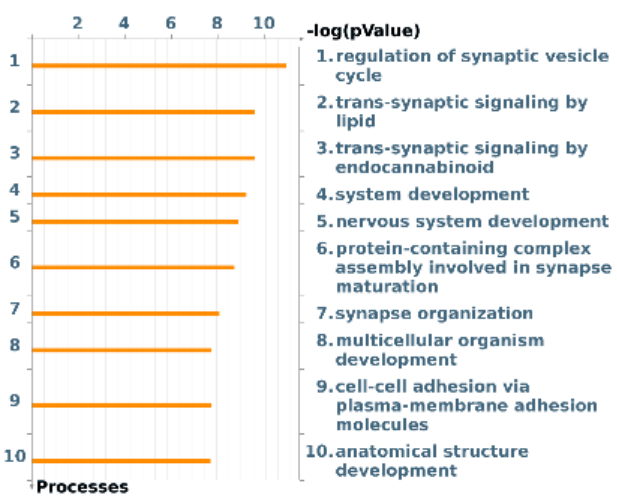


Figure 8A: Cell-type Specific Expression Analysis (CSEA)

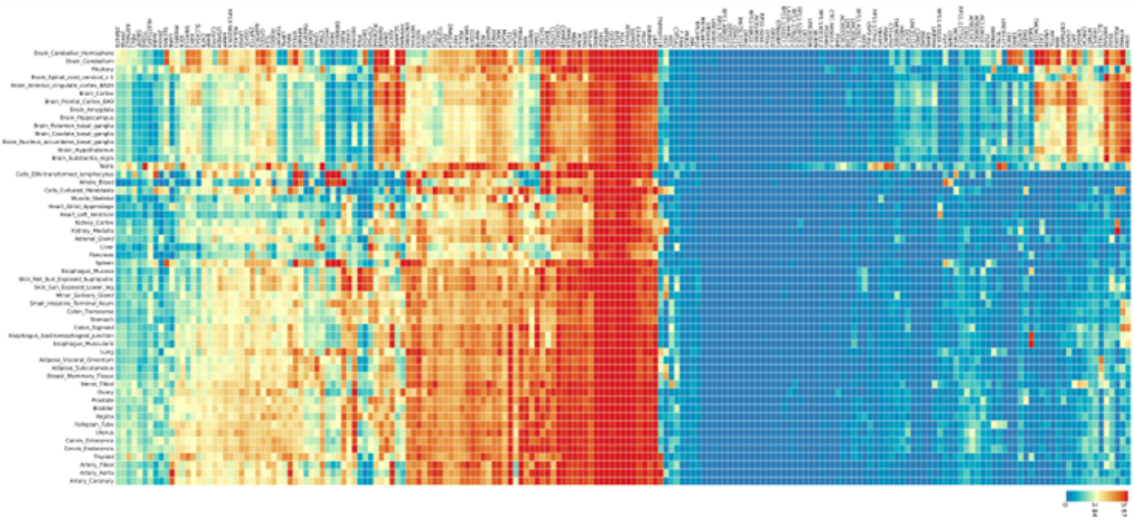
Figure 8B 8C: Tissue-Specific Expression Analysis (TSEA). TSEA confirms that the gene network The levels of the hexagons represent varying stringencies for enrichment going from least specific lists (outer hexagons) to most specific (center). The size of the hexagon is scaled to the size of the gene list, and the color represents the FDR-adjusted p-values of the overlap between the genes in the network and the list of enriched genes in the specific cell or tissue. The ND gene network is enriched during early and mid-fetal life in the striatum and thalamus, and cortex during early development. In general, the network is predominantly expressed in brain and pituitary tissues.

Figure 9: Combined enrichment analysis of the ND gene network

A)



B)



C)

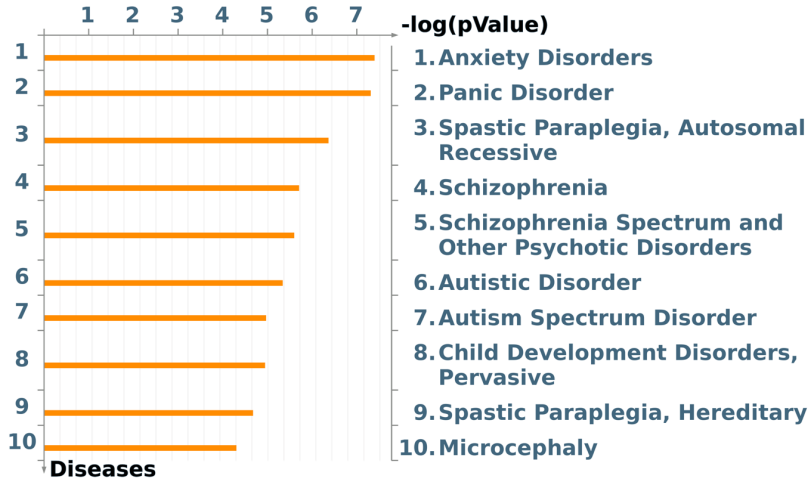


Figure 9A: Metacore pathway map, network, and processes. Combined enrichment analysis for the gene network shows enrichment for predominantly Notch signaling pathway suggesting that the ND gene network has a brain maturation role in neurodevelopment. (Metacore®, FDR<0.05)

Figure 9B: Expression levels of the ND network in 54 different tissue types from GTEX using theFUMA gene2function. Benjamin-Hochberg (FDR) p-value <0.05.

Figure 9C: Metacore disease analysis for the significant genes from brain tissues in FUMA gene2function.

CHAPTER 3. SUPPLEMENTAL MATERIALS

Supplemental Material

Supplementary Table 1: Results of linear regression analyses in GUSTO

	Internalizing Problems - 7 years				Externalizing Problems - 7 years				Total Problems - 7 years			
	B	**SE**	**t**	**p-value**	**B**	**SE**	**t**	**p-value**	**B**	**SE**	**t**	**p-value**
(Intercept)	-0.161	12.157	-0.013	0.989	1.836	14.487	0.127	0.899	-5.928	46.461	-0.128	0.899
Gender	-1.108	1.078	-1.028	0.307	-0.251	1.285	-0.195	0.846	-2.280	4.120	-0.553	0.581
Genetics PC1	39.336	19.649	2.002	**0.048**	-4.002	23.414	-0.171	0.865	26.545	75.092	0.353	0.725
Genetics PC2	-3.198	18.256	-0.175	0.861	-3.736	21.755	-0.172	0.864	-28.904	69.770	-0.414	0.680
Genetics PC3	9.961	16.748	0.595	0.554	-15.984	19.957	-0.801	0.425	-19.235	64.006	-0.301	0.765
Buccal cell count	0.047	0.170	0.278	0.781	0.013	0.203	0.062	0.951	0.271	0.651	0.417	0.678
Standardized ND score at 3 months	-1.004	0.586	-1.715	0.090	-1.837	0.698	-2.631	**0.010**	**-4.809**	2.239	-2.148	**0.034**
Standard NACC ePRS	-1.355	0.702	-1.930	0.057	-1.972	0.837	-2.357	**0.021**	*-5.108*	2.683	-1.904	*0.060*
Prenatal Adversity	2.345	0.459	5.105	**<0.001**	2.842	0.547	5.193	**<0.001**	**9.189**	1.756	5.234	**<0.001**
ePRS x Adversity interaction	1.172	0.516	2.270	**0.026**	1.343	0.615	2.184	**0.032**	*3.841*	1.973	1.947	*0.055*

*All p-values are calculated with two-tailed hypothesis

Supplementary Table 2: Results of linear regression analyses in ALSPAC

	Internalizing Problems - 7 years				Externalizing Problems - 7 years				Total Problems - 7 years			
	B	SE	t	p-value	B	SE	t	p-value	B	SE	t	p-value
(Intercept)	**1.930**	0.198	9.735	**<0.001**	**11.535**	0.205	56.291	**<0.001**	**4.920**	0.341	14.425	**<0.001**
Gender	0.098	0.207	0.471	0.638	0.223	0.214	1.043	0.297	**0.887**	0.356	2.495	**0.013**
Genetics PC1	5.544	8.939	0.620	0.535	-2.315	9.239	-0.251	0.802	5.713	15.378	0.371	0.710
Genetics PC2	9.458	9.305	1.016	0.310	8.982	9.585	0.937	0.349	18.230	15.954	1.143	0.254
Genetics PC3	1.497	9.243	0.162	0.871	-5.763	9.541	-0.604	0.546	-5.818	15.881	-0.366	0.714
Cell Count PC1	**2.899**	1.137	2.550	**0.011**	1.265	1.174	1.077	0.282	**5.088**	1.954	2.604	**0.009**
Cell Count PC2	0.131	2.128	0.062	0.951	1.149	2.193	0.524	0.600	0.113	3.650	0.031	0.975
Cell Count PC3	1.877	3.322	0.565	0.572	3.129	3.431	0.912	0.362	3.792	5.710	0.664	0.507
Standardized ND score	**-0.140**	0.113	-1.239	**<0.001**	0.167	0.117	1.425	0.155	0.012	0.195	0.063	0.950
Standard NACC ePRS	-0.191	0.169	-1.131	0.258	-0.184	0.174	-1.057	0.291	-0.301	0.290	-1.038	0.299
Prenatal Adversity score	**0.342**	0.105	3.271	**0.001**	**0.412**	0.108	3.811	**0.000**	**0.631**	0.180	3.503	**0.000**
ePRS x Adversity interaction	0.117	0.102	1.141	0.254	**0.196**	0.106	1.852	**0.065**	*0.247*	0.176	1.405	*0.160*

*All p-values are calculated with two-tailed hypothesis. However, as this is a replication cohort, interactions should be tested as a one-tailed hypothesis, i.e. significance threshold for interactions going in the same direction as GUSTO should be p=0.1 in this table.

Supplementary Table 3: Results of linear regression analyses in BIBO

	Internalizing Problems - 7 years				Externalizing Problems - 7 years				Total Problems - 7 years			
	B	SE	t	p-value	B	SE	t	p-value	B	SE	t	p-value
(Intercept)	**10.539**	4.954	2.127	**0.036**	6.557	6.581	0.996	0.321	**31.800**	14.686	2.165	**0.033**
Gender	0.793	0.731	1.085	0.280	0.543	0.971	0.559	0.578	1.248	2.168	0.576	0.566
Genetics PC1	-2.744	4.140	-0.663	0.509	-2.286	5.500	-0.416	0.679	-8.154	12.274	-0.664	0.508
Genetics PC2	-0.663	4.142	-0.160	0.873	-8.620	*5.503*	-1.566	*0.120*	-11.661	12.280	-0.950	0.344
Genetics PC3	-1.570	4.300	-0.365	0.716	-12.555	**5.713**	-2.198	**0.030**	-16.098	12.748	-1.263	0.209
Buccal Cell Count	*-0.088*	0.064	-1.367	*0.174*	-0.003	0.085	-0.035	0.972	-0.189	0.191	-0.991	0.324
Standardized ND score	*0.660*	0.411	1.607	*0.111*	0.695	0.546	1.274	0.205	1.509	1.217	1.240	0.218
Standard NACC ePRS	**-0.957**	0.454	-2.109	**0.037**	-0.315	0.603	-0.522	0.602	-1.455	1.346	-1.081	0.282
Prenatal Adversity score	-0.813	0.760	-1.069	0.288	-0.681	1.010	-0.674	0.502	-1.944	2.254	-0.862	0.390
ePRS x Adversity interaction	**1.185**	0.711	1.667	**0.098**	1.162	0.945	1.230	0.221	*2.958*	2.108	1.403	*0.164*

*All p-values are calculated with two-tailed hypothesis. However, as this is a replication cohort, interactions should be tested as a one-tailed hypothesis, i.e., significance threshold for interactions going in the same direction as GUSTO should be p=0.1 in this table.

CHAPTER 4. DISCUSSION

We found that a multiple linear regression model adjusted for sex and buccal cell count (or blood cell type) that combines methylation scores and the ePRS interaction with prenatal adversity exposure has effects on internalizing, externalizing, and total problems in GUSTO children at 7 years. Furthermore, the ePRS was derived from Nacc tissue expression, suggesting a strong link between this brain area and children's difficulties at 7 years of age. The Nacc is a dopaminergic projection region that, together with the VTA, anterior hippocampus (aHipp), and medial prefrontal cortex (mPFC), forms the mesocorticolimbic circuit, which mediates reward processing (Kleinridders and Pothos 2019).

The formation of neural circuits is critical throughout initial developmental stages and involves complicated processes such as cell proliferation, cell fate determination, cell migration and cell and synapse formation, which allow for the refined buildout of cognitive processing and mental wellness. B.Goff discovered that adolescents who experienced hardship early in life exhibited NAcc hypoactivation during adolescence and that reduced Nacc reactivity was associated with a greater depression score (Goff et al. 2013). Similarly, Forbes et al. revealed that DA-related functional polymorphisms enhance ventral striatum responsiveness, which is linked with increased impulsivity, and the same has been observed in Nacc rat models with lesioned striatum (Forbes et al. 2009). This shows that aberrant reward-related behaviors can be caused by interrupted or altered Nacc signalling. This is reflected in our findings, which show that models with the constructed ePRS using Nacc tissue expression predict externalizing problems (characterized by aggressive, oppositional, and oppositional behavior that can be triggered by hyperactive impulsive symptoms) and internalizing problems (characterized by social withdrawal, anxious, and depressive symptoms) in children.

As a result, Nacc is a key stress-related behavioral structure, and our findings support the notion that the Nacc gene network associated with responsivity to ND is linked to brain development and pathological illnesses. The findings of our enrichment analysis suggest that the ND gene network is significantly expressed in fetal and young adulthood (Figure 10A). The genes in the network are thought to be engaged in a variety of neurodevelopmental processes (Figure 11A). NOTCH signalling is one of the pathways that appear often in the enrichment analysis. This route is vital in early neurodevelopment, learning, and memory and is critical for neuronal differentiation in early development (Lasky and Wu 2005). According to the gene ontology processes and hubs-bottlenecks connected with the network, the ND gene network also plays an intriguing function in the control of the synaptic vesicle cycle, synapse structure, and system development. One of the most intriguing findings is how epigenome-wide DNAm data gathered during adolescence in response to neighborhood deprivation interacts with genes expressed throughout early fetal developmental stages and young adulthood. This network appears to be well conserved, and its expression is sensitive to important developmental phases in both neural circuitry architecture and early behavior development.

In other cohorts, we found that the substantial interaction with prenatal adversity was lost at later ages (11y ALSPAC) and that the complete linear model had little significance at earlier stages. In ALSPAC, the complete multi-omics interaction model for each childhood issue is significant, however, there is no significant interaction between adversity and ePRS in the model. Notably, our intriguing observation within the ALSPAC cohort revolves around the specificity of interactions with externalizing scores, particularly pertinent given the behavioral changes often associated with adolescence. We hypothesize that the convergence on externalizing problems may reflect the defiance commonly observed during adolescence.

The interaction between adversity and the ePRS is seen to play a huge role in our model. In our 3rd cohort BIBO, we saw significant effects in the internalizing problem when children were exposed to lower levels of prenatal adversity. However, it is important to acknowledge that the range of adversity in this sample was relatively low. This limited range of adversity might have attenuated the predictive power of the interaction between polygenic risk and adversity. Future research with larger sample sizes and a wider range of adversity levels should be conducted to provide a more comprehensive understanding of the interplay between genetic vulnerability and environmental factors in forecasting childhood mental health problems.

In addition to building linear models, we discovered that incorporating information from the blood or buccal epigenome, prenatal adversity, and ePRS can help predict childhood socio-emotional problems. In both GUSTO and ALSPAC, we found that when the linear model included both epigenetic and genetic scores, as well as the interaction with prenatal adversity, the adjusted R-squared value increased. This implies that the influence of genetic and epigenetic profiles is conditional on adversity experience and can increase prediction rate.

Beyond the standard PRS, which employs single nucleotide polymorphisms in GxE studies, our model was able to capture the intricacy of biomolecular processes more precisely and illuminate some inherent mechanisms that might contribute to the start of vulnerability to psychiatric diseases. We created a methylation score and a unique EWAS-based ePRS using epigenome-wide DNAm array data from neighborhood disadvantage research. We considered the varied methylation patterns from the EWAS CpGs while capturing the polygenicity of genes that respond to environmental influences with variations in DNAm. In recent years, the integration of EWAS data into a PRS has emerged, allowing for a larger picture for populational investigations. Tangentially, while polygenic risk scores provide unchangeable

risk for a patient, methylation-based risk scores may provide a current risk state for the patient over the last months.

In conclusion, we presented a multi-omics strategy for investigating risk factors for childhood socioemotional behavior. Our research presents a mechanism for capturing individual variations in susceptibility linked with early adversity experience by integrating epigenetic and polygenic scores. This demonstrates how intricate the brain circuit's molecular processes are, and how adding more layers of knowledge regarding gene tissue expression and epigenetics may better illuminate the reality of genetic predisposition relevant to mental illnesses.

CHAPTER 5. BIBLIOGRAPHY

Achenbach, T. M. 1991. 'Manual for The Child Behavior Checklist/4-18 and 1991 Profile', *University of Vermont, Department of Psychiatry*.

Aryee, M. J., A. E. Jaffe, H. Corrada-Bravo, C. Ladd-Acosta, A. P. Feinberg, K. D. Hansen, and R. A. Irizarry. 2014. 'Minfi: a flexible and comprehensive Bioconductor package for the analysis of Infinium DNA methylation microarrays', *Bioinformatics*, 30: 1363-9.

Asimit, Jennifer, and Eleftheria Zeggini. 2010. 'Rare variant association analysis methods for complex traits', *Annual review of genetics*, 44: 293-308.

Assenov, Y., F. Ramírez, S. E. Schelhorn, T. Lengauer, and M. Albrecht. 2008. 'Computing topological parameters of biological networks', *Bioinformatics*, 24: 282-4.

Auerbach, Randy P., David Pagliaccio, Nicholas A. Hubbard, Isabelle Frosch, Rebecca Kremens, Elizabeth Cosby, Robert Jones, Viviana Siless, Nicole Lo, Aude Henin, Stefan G. Hofmann, John D. E. Gabrieli, Anastasia Yendiki, Susan Whitfield-Gabrieli, and Diego A. Pizzagalli. 2022. 'Reward-Related Neural Circuitry in Depressed and Anxious Adolescents: A Human Connectome Project', *Journal of the American Academy of Child & Adolescent Psychiatry*, 61: 308-20.

Baldwin, Jessie R., and Michelle Degli Esposti. 2021. 'Triangulating evidence on the role of perceived versus objective experiences of childhood adversity in psychopathology', *JCPP Advances*, 1: e12010.

Barker, D. J. P., C. N. Hales, C. H. D. Fall, C. Osmond, K. Phipps, and P. M. S. Clark. 1993. 'Type 2 (non-insulin-dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): relation to reduced fetal growth', *Diabetologia*, 36: 62-67.

Barth, Barbara, Danusa Mar Arcego, Euclides José de Mendonça Filho, Randriely Merscher de Lima, Sachin Patel, Omar Khedr, Zihan Wang, Carine Parent, Carla Dalmaz, and André Krumel Portella. 2022. 'Cardio-Metabolic and Psychiatric Comorbidities: Early Adversity-DAT1 Gene Network Interactions', *Biological Psychiatry*, 91: S79.

Batra, Aashita, Lawrence M. Chen, Zihan Wang, Carine Parent, Irina Pokhvisneva, Sachin Patel, Robert D. Levitan, Michael J. Meaney, and Patricia Pelufo Silveira. 2021. 'Early Life Adversity and Polygenic Risk for High Fasting Insulin Are Associated With Childhood Impulsivity', *Frontiers in Neuroscience*, 15.

Belsky, J. 1997a. 'Theory testing, effect-size evaluation, and differential susceptibility to rearing influence: the case of mothering and attachment', *Child Dev*, 68: 598-600.

Belsky, Jay. 1997b. 'Variation in Susceptibility to Environmental Influence: An Evolutionary Argument', *Psychological Inquiry*, 8: 182-86.

Black, R. E., C. G. Victora, S. P. Walker, Z. A. Bhutta, P. Christian, M. de Onis, M. Ezzati, S. Grantham-McGregor, J. Katz, R. Martorell, and R. Uauy. 2013. 'Maternal and child undernutrition and overweight in low-income and middle-income countries', *Lancet*, 382: 427-51.

Bolten, M. I., H. Wurmser, A. Buske-Kirschbaum, M. Papoušek, K. M. Pirke, and D. Hellhammer. 2011. 'Cortisol levels in pregnancy as a psychobiological predictor for birth weight', *Arch Womens Ment Health*, 14: 33-41.

Bosch, Nienke M., Harriëtte Riese, Sijmen A. Reijneveld, Martin P. Bakker, Frank C. Verhulst, Johan Ormel, and Albertine J. Oldehinkel. 2012. 'Timing matters: Long term effects of adversities from prenatal period up to adolescence on adolescents'

cortisol stress response. The TRAILS study', *Psychoneuroendocrinology*, 37: 1439-47.
Boumil, Rebecca M., Verity A. Letts, Monica C. Roberts, Christine Lenz, Connie L. Mahaffey, Zhong-wei Zhang, Tobias Moser, and Wayne N. Frankel. 2010. 'A Missense Mutation in a Highly Conserved Alternate Exon of Dynamin-1 Causes Epilepsy in Fitful Mice', *PLOS Genetics*, 6: e1001046.
Boyce, W. T., and B. J. Ellis. 2005. 'Biological sensitivity to context: I. An evolutionary-developmental theory of the origins and functions of stress reactivity', *Development and Psychopathology*, 17: 271-301.
Boyd, A., J. Golding, J. Macleod, D. A. Lawlor, A. Fraser, J. Henderson, L. Molloy, A. Ness, S. Ring, and G. Davey Smith. 2013. 'Cohort Profile: the 'children of the 90s'--the index offspring of the Avon Longitudinal Study of Parents and Children', *Int J Epidemiol*, 42: 111-27.
Brooks-Gunn, Jeanne. 1997. *Neighborhood poverty: Context and consequences for children* (Russell Sage Foundation).
Buniello, A., J. A. L. MacArthur, M. Cerezo, L. W. Harris, J. Hayhurst, C. Malangone, A. McMahon, J. Morales, E. Mountjoy, E. Sollis, D. Suveges, O. Vrousgou, P. L. Whetzel, R. Amode, J. A. Guillen, H. S. Riat, S. J. Trevanion, P. Hall, H. Junkins, P. Flicek, T. Burdett, L. A. Hindorff, F. Cunningham, and H. Parkinson. 2019. 'The NHGRI-EBI GWAS Catalog of published genome-wide association studies, targeted arrays and summary statistics 2019', *Nucleic Acids Res*, 47: D1005-d12.
Burt, S. A., K. L. Klump, D. Gorman-Smith, and J. M. Neiderhiser. 2016. 'Neighborhood Disadvantage Alters the Origins of Children's Nonaggressive Conduct Problems', *Clin Psychol Sci*, 4: 511-26.
Cardinal, R. N., D. R. Pennicott, C. L. Sugathapala, T. W. Robbins, and B. J. Everitt. 2001. 'Impulsive choice induced in rats by lesions of the nucleus accumbens core', *Science*, 292: 2499-501.
Chase-Lansdale, Lindsay. 1997. 'Neighborhood and family influences on the intellectual and behavioral competence of preschool and early school-age children: Context and consequences for children.' in, *Neighborhood poverty: Context and consequences for children* (Russell Sage Foundation).
Christie-Mizell, C. A. 2022. 'Neighborhood Disadvantage and Poor Health: The Consequences of Race, Gender, and Age among Young Adults', *Int J Environ Res Public Health*, 19.
Cicchetti, Dante, and Fred Rogosch. 1996. 'Equifinality and Mutifinality in Developmental Psychopathology', *Development and Psychopathology*, 8: 597-600.
Copeland, W. E., G. Keeler, A. Angold, and E. J. Costello. 2007. 'Traumatic events and posttraumatic stress in childhood', *Arch Gen Psychiatry*, 64: 577-84.
Coussons-Read, M. E. 2013. 'Effects of prenatal stress on pregnancy and human development: mechanisms and pathways', *Obstet Med*, 6: 52-57.
Dalle Molle, R., H. Fatemi, A. Dagher, R. D. Levitan, P. P. Silveira, and L. Dubé. 2017. 'Gene and environment interaction: Is the differential susceptibility hypothesis relevant for obesity?', *Neurosci Biobehav Rev*, 73: 326-39.
Dalmaz, Carla, Barbara Barth, Irina Pokhvisneva, Zihan Wang, Sachin Patel, Jorge A. Quillfeldt, Euclides J. Mendonça Filho, Randriely Merscher Sobreira de Lima, Danusa M. Arcego, and Roberto Britto Sassi. 2021. 'Prefrontal cortex VAMP1 gene network moderates the effect of the early environment on cognitive flexibility in children', *Neurobiology of Learning and Memory*, 185: 107509.
Davis, Elysia Poggi, and Curt A. Sandman. 2012. 'Prenatal psychobiological predictors of anxiety risk in preadolescent children', *Psychoneuroendocrinology*, 37: 1224-33.

de Lima, R. M. S., B. Barth, D. Mar Arcego, E. J. de Mendonça Filho, S. Patel, Z. Wang, I. Pokhvisneva, C. Parent, R. D. Levitan, M. S. Kobor, A. P. S. de Vasconcellos Bittencourt, M. J. Meaney, C. Dalmaz, and P. P. Silveira. 2022. 'Leptin receptor co-expression gene network moderates the effect of early life adversity on eating behavior in children', *Commun Biol*, 5: 1092.

De Lima, Randriely Merscher Sobreira, Barbara Barth, Danusa Mar Arcego, Euclides José de Mendonça Filho, Andrew Clappison, Sachin Patel, Zihan Wang, Irina Pokhvisneva, Roberto Britto Sassi, and Geoffrey B. C. Hall. 2020. 'Amygdala 5-HTT gene network moderates the effects of postnatal adversity on attention problems: anatomo-functional correlation and epigenetic changes', *Frontiers in Neuroscience*, 14: 198.

de Mendonça Filho, E. J., B. Barth, D. R. Bandeira, R. M. S. de Lima, D. M. Arcego, C. Dalmaz, I. Pokhvisneva, R. B. Sassi, G. B. C. Hall, M. J. Meaney, and P. P. Silveira. 2021. 'Cognitive Development and Brain Gray Matter Susceptibility to Prenatal Adversities: Moderation by the Prefrontal Cortex Brain-Derived Neurotrophic Factor Gene Co-expression Network', *Front Neurosci*, 15: 744743.

de Mendonça Filho, Euclides José, Barbara Barth, Denise Ruschel Bandeira, Randriely Merscher Sobreira de Lima, Danusa Mar Arcego, Carla Dalmaz, Irina Pokhvisneva, Roberto Britto Sassi, Geoffrey B. C. Hall, and Michael J. Meaney. 2022. 'Background: Previous studies focused on the relationship between prenatal conditions and neurodevelopmental outcomes later in life, but few have explored the interplay between gene co-expression networks and prenatal adversity conditions on cognitive development trajectories and gray matter density. Methods: We analyzed the moderation effects of an expression polygenic score (ePRS)', *Gene and Environment Interactions in Neurodevelopmental Disorders*.

Dougherty, J. D., E. F. Schmidt, M. Nakajima, and N. Heintz. 2010. 'Analytical approaches to RNA profiling data for the identification of genes enriched in specific cells', *Nucleic Acids Res*, 38: 4218-30.

Durinck, S., Y. Moreau, A. Kasprzyk, S. Davis, B. De Moor, A. Brazma, and W. Huber. 2005. 'BioMart and Bioconductor: a powerful link between biological databases and microarray data analysis', *Bioinformatics*, 21: 3439-40.

Durinck, S., P. T. Spellman, E. Birney, and W. Huber. 2009. 'Mapping identifiers for the integration of genomic datasets with the R/Bioconductor package biomaRt', *Nat Protoc*, 4: 1184-91.

Ehrlich, M., M. A. Gama-Sosa, L. H. Huang, R. M. Midgett, K. C. Kuo, R. A. McCune, and C. Gehrke. 1982. 'Amount and distribution of 5-methylcytosine in human DNA from different types of tissues of cells', *Nucleic Acids Res*, 10: 2709-21.

Ekblad, Mikael, Mika Gissler, Liisa Lehtonen, and Jyrki Korkeila. 2010. 'Prenatal Smoking Exposure and the Risk of Psychiatric Morbidity Into Young Adulthood', *Archives of General Psychiatry*, 67: 841-49.

Fareri, Dominic S., Laurel Gabard-Durnam, Bonnie Goff, Jessica Flannery, Dylan G. Gee, Daniel S. Lumian, Christina Caldera, and Nim Tottenham. 2017. 'Altered ventral striatal–medial prefrontal cortex resting-state connectivity mediates adolescent social problems after early institutional care', *Development and Psychopathology*, 29: 1865-76.

Forbes, E. E., S. M. Brown, M. Kimak, R. E. Ferrell, S. B. Manuck, and A. R. Hariri. 2009. 'Genetic variation in components of dopamine neurotransmission impacts ventral striatal reactivity associated with impulsivity', *Molecular Psychiatry*, 14: 60-70.

Fraser, A., C. Macdonald-Wallis, K. Tilling, A. Boyd, J. Golding, G. Davey Smith, J. Henderson, J. Macleod, L. Molloy, A. Ness, S. Ring, S. M. Nelson, and D. A. Lawlor.

2013. 'Cohort Profile: the Avon Longitudinal Study of Parents and Children: ALSPAC mothers cohort', *Int J Epidemiol*, 42: 97-110.
Gandal, M. J., J. R. Haney, N. N. Parikshak, V. Leppa, G. Ramaswami, C. Hartl, A. J. Schork, V. Appadurai, A. Buil, T. M. Werge, C. Liu, K. P. White, S. Horvath, and D. H. Geschwind. 2018. 'Shared molecular neuropathology across major psychiatric disorders parallels polygenic overlap', *Science*, 359: 693-97.
Gandal, M. J., V. Leppa, H. Won, N. N. Parikshak, and D. H. Geschwind. 2016. 'The road to precision psychiatry: translating genetics into disease mechanisms', *Nat Neurosci*, 19: 1397-407.
"Global Burden of Disease Collaborative Network. Global Burden of Disease Study 2019 (GBD 2019) Population Estimates 1950-2019." In. 2020. Seattle, United States of America: Institute for Health Metrics and Evaluation (IHME).
Glymour, M. M., M. Mujahid, Q. Wu, K. White, and E. J. Tchetgen Tchetgen. 2010. 'Neighborhood disadvantage and self-assessed health, disability, and depressive symptoms: longitudinal results from the health and retirement study', *Ann Epidemiol*, 20: 856-61.
Goddings, Anne-Lise, Adriene Beltz, Jiska S. Peper, Eveline A. Crone, and Barbara R. Braams. 2019. 'Understanding the Role of Puberty in Structural and Functional Development of the Adolescent Brain', *Journal of Research on Adolescence*, 29: 32-53.
Goff, B., D. G. Gee, E. H. Telzer, K. L. Humphreys, L. Gabard-Durnam, J. Flannery, and N. Tottenham. 2013. 'Reduced nucleus accumbens reactivity and adolescent depression following early-life stress', *Neuroscience*, 249: 129-38.
Goodman, R. 1997. 'The Strengths and Difficulties Questionnaire: a research note', *J Child Psychol Psychiatry*, 38: 581-6.
Goodman, R., H. Meltzer, and V. Bailey. 1998. 'The Strengths and Difficulties Questionnaire: a pilot study on the validity of the self-report version', *Eur Child Adolesc Psychiatry*, 7: 125-30.
Grundwald, N. J., and P. J. Brunton. 2015. 'Prenatal stress programs neuroendocrine stress responses and affective behaviors in second generation rats in a sex-dependent manner', *Psychoneuroendocrinology*, 62: 204-16.
Hackman, Daniel, Laura Betancourt, Nancy Brodsky, Hallam Hurt, and Martha Farah. 2012. 'Neighborhood disadvantage and adolescent stress reactivity', *Frontiers in Human Neuroscience*, 6.
Hales, C. N., and D. J. Barker. 1992. 'Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis', *Diabetologia*, 35: 595-601.
Hanson, Jamie L., Annchen R. Knodt, Bartholomew D. Brigidi, and Ahmad R. Hariri. 2018. 'Heightened connectivity between the ventral striatum and medial prefrontal cortex as a biomarker for stress-related psychopathology: understanding interactive effects of early and more recent stress', *Psychological Medicine*, 48: 1835-43.
Hari Dass, Shantala A., Kathryn McCracken, Irina Pokhvisneva, Lawrence M. Chen, Elika Garg, Thao T. T. Nguyen, Zihan Wang, Barbara Barth, Moein Yaqubi, Lisa M. McEwen, Julie L. MacIsaac, Josie Diorio, Michael S. Kobor, Kieran J. O'Donnell, Michael J. Meaney, and Patricia P. Silveira. 2019. 'A biologically-informed polygenic score identifies endophenotypes and clinical conditions associated with the insulin receptor function on specific brain regions', *EBioMedicine*, 42: 188-202.
Hedegaard, M., T. B. Henriksen, N. J. Secher, M. C. Hatch, and S. Sabroe. 1996. 'Do stressful life events affect duration of gestation and risk of preterm delivery?', *Epidemiology*, 7: 339-45.

'Human genomics. The Genotype-Tissue Expression (GTEx) pilot analysis: multitissue gene regulation in humans'. 2015. *Science*, 348: 648-60.
Jaenisch, R., and A. Bird. 2003. 'Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals', *Nat Genet*, 33 Suppl: 245-54.
Jaffee, S. R., R. Takizawa, and L. Arseneault. 2017. 'Buffering effects of safe, supportive, and nurturing relationships among women with childhood histories of maltreatment', *Psychol Med*, 47: 2628-39.
Jiang, Yajie, Manshu Zou, Yeqing Wang, and Yuhong Wang. 2023. 'Nucleus accumbens in the pathogenesis of major depressive disorder: A brief review', *Brain Research Bulletin*, 196: 68-75.
Karb, Rebecca A., Michael R. Elliott, Jennifer B. Dowd, and Jeffrey D. Morenoff. 2012. 'Neighborhood-level stressors, social support, and diurnal patterns of cortisol: The Chicago Community Adult Health Study', *Social Science & Medicine*, 75: 1038-47.
Kessler, R. C., K. A. McLaughlin, J. G. Green, M. J. Gruber, N. A. Sampson, A. M. Zaslavsky, S. Aguilar-Gaxiola, A. O. Alhamzawi, J. Alonso, M. Angermeyer, C. Benjet, E. Bromet, S. Chatterji, G. de Girolamo, K. Demyttenaere, J. Fayyad, S. Florescu, G. Gal, O. Gureje, J. M. Haro, C. Y. Hu, E. G. Karam, N. Kawakami, S. Lee, J. P. Lépine, J. Ormel, J. Posada-Villa, R. Sagar, A. Tsang, T. B. Ustün, S. Vassilev, M. C. Viana, and D. R. Williams. 2010. 'Childhood adversities and adult psychopathology in the WHO World Mental Health Surveys', *Br J Psychiatry*, 197: 378-85.
Kleinridders, André, and Emmanuel N. Pothos. 2019. 'Impact of Brain Insulin Signaling on Dopamine Function, Food Intake, Reward, and Emotional Behavior', *Current Nutrition Reports*, 8: 83-91.
Kosidou, K., C. Dalman, M. Lundberg, J. Hallqvist, G. Isacsson, and C. Magnusson. 2011. 'Socioeconomic status and risk of psychological distress and depression in the Stockholm Public Health Cohort: a population-based study', *J Affect Disord*, 134: 160-7.
Kresovich, Jacob K., Zongli Xu, Katie M. O'Brien, Min Shi, Clarice R. Weinberg, Dale P. Sandler, and Jack A. Taylor. 2022. 'Blood DNA methylation profiles improve breast cancer prediction', *Molecular Oncology*, 16: 42-53.
Kubagawa, H., S. Oka, Y. Kubagawa, I. Torii, E. Takayama, D. W. Kang, G. L. Gartland, L. F. Bertoli, H. Mori, H. Takatsu, T. Kitamura, H. Ohno, and J. Y. Wang. 2009. 'Identity of the elusive IgM Fc receptor (FcmuR) in humans', *J Exp Med*, 206: 2779-93.
Kundakovic, Marija, Kathryn Gudsnuk, Julie B. Herbstman, Deliang Tang, Frederica P. Perera, and Frances A. Champagne. 2015. 'DNA methylation of BDNF as a biomarker of early-life adversity', *Proceedings of the National Academy of Sciences*, 112: 6807-13.
Lahti, M., J. G. Eriksson, K. Heinonen, E. Kajantie, J. Lahti, K. Wahlbeck, S. Tuovinen, A. K. Pesonen, M. Mikkonen, C. Osmond, D. J. P. Barker, and K. Räikkönen. 2015. 'Late preterm birth, post-term birth, and abnormal fetal growth as risk factors for severe mental disorders from early to late adulthood', *Psychological Medicine*, 45: 985-99.
Laplante, D. P., R. G. Barr, A. Brunet, G. Galbaud du Fort, M. L. Meaney, J. F. Saucier, P. R. Zelazo, and S. King. 2004. 'Stress during pregnancy affects general intellectual and language functioning in human toddlers', *Pediatr Res*, 56: 400-10.
Lasky, Joseph L., and Hong Wu. 2005. 'Notch Signaling, Brain Development, and Human Disease', *Pediatric Research*, 57: 104-09.

Lautarescu, Alexandra, Michael C. Craig, and Vivette Glover. 2020. 'Chapter Two - Prenatal stress: Effects on fetal and child brain development.' in Angela Clow and Nina Smyth (eds.), *International Review of Neurobiology* (Academic Press).

Leventhal, T., and J. Brooks-Gunn. 2003. 'Moving to opportunity: an experimental study of neighborhood effects on mental health', *Am J Public Health*, 93: 1576-82.

Li, E., C. Beard, and R. Jaenisch. 1993. 'Role for DNA methylation in genomic imprinting', *Nature*, 366: 362-5.

Luby, Joan L., Deanna Barch, Diana Whalen, Rebecca Tillman, and Andy Belden. 2017. 'Association Between Early Life Adversity and Risk for Poor Emotional and Physical Health in Adolescence: A Putative Mechanistic Neurodevelopmental Pathway', *JAMA Pediatrics*, 171: 1168-75.

Luo, Audrey, Jeesun Jung, Martha Longley, Daniel B. Rosoff, Katrin Charlet, Christine Muench, Jisoo Lee, Colin A. Hodgkinson, David Goldman, Steve Horvath, Zachary A. Kaminsky, and Falk W. Lohoff. 2020. 'Epigenetic aging is accelerated in alcohol use disorder and regulated by genetic variation in APOL2', *Neuropsychopharmacology*, 45: 327-36.

MacArthur, J., E. Bowler, M. Cerezo, L. Gil, P. Hall, E. Hastings, H. Junkins, A. McMahon, A. Milano, J. Morales, Z. M. Pendlington, D. Welter, T. Burdett, L. Hindorff, P. Flicek, F. Cunningham, and H. Parkinson. 2017. 'The new NHGRI-EBI Catalog of published genome-wide association studies (GWAS Catalog)', *Nucleic Acids Res*, 45: D896-d901.

Manolio, Teri A., Francis S. Collins, Nancy J. Cox, David B. Goldstein, Lucia A. Hindorff, David J. Hunter, Mark I. McCarthy, Erin M. Ramos, Lon R. Cardon, Aravinda Chakravarti, Judy H. Cho, Alan E. Guttmacher, Augustine Kong, Leonid Kruglyak, Elaine Mardis, Charles N. Rotimi, Montgomery Slatkin, David Valle, Alice S. Whittemore, Michael Boehnke, Andrew G. Clark, Evan E. Eichler, Greg Gibson, Jonathan L. Haines, Trudy F. C. Mackay, Steven A. McCarroll, and Peter M. Visscher. 2009. 'Finding the missing heritability of complex diseases', *Nature*, 461: 747-53.

Marshall, Narcis A., Hilary A. Marusak, Kelsey J. Sala-Hamrick, Laura M. Crespo, Christine A. Rabinak, and Moriah E. Thomason. 2018. 'Socioeconomic disadvantage and altered corticostriatal circuitry in urban youth', *Human Brain Mapping*, 39: 1982-94.

Martin, A. R., M. J. Daly, E. B. Robinson, S. E. Hyman, and B. M. Neale. 2019. 'Predicting Polygenic Risk of Psychiatric Disorders', *Biol Psychiatry*, 86: 97-109.

Maurano, M. T., R. Humbert, E. Rynes, R. E. Thurman, E. Haugen, H. Wang, A. P. Reynolds, R. Sandstrom, H. Qu, J. Brody, A. Shafer, F. Neri, K. Lee, T. Kutyavin, S. Stehling-Sun, A. K. Johnson, T. K. Canfield, E. Giste, M. Diegel, D. Bates, R. S. Hansen, S. Neph, P. J. Sabo, S. Heimfeld, A. Raubitschek, S. Ziegler, C. Cotsapas, N. Sotoodehnia, I. Glass, S. R. Sunyaev, R. Kaul, and J. A. Stamatoyannopoulos. 2012. 'Systematic localization of common disease-associated variation in regulatory DNA', *Science*, 337: 1190-5.

McCarthy, S., S. Das, W. Kretzschmar, O. Delaneau, A. R. Wood, A. Teumer, H. M. Kang, C. Fuchsberger, P. Danecek, K. Sharp, Y. Luo, C. Sidore, A. Kwong, N. Timpson, S. Koskinen, S. Vrieze, L. J. Scott, H. Zhang, A. Mahajan, J. Veldink, U. Peters, C. Pato, C. M. van Duijn, C. E. Gillies, I. Gandin, M. Mezzavilla, A. Gilly, M. Cocca, M. Traglia, A. Angius, J. C. Barrett, D. Boomsma, K. Branham, G. Breen, C. M. Brummett, F. Busonero, H. Campbell, A. Chan, S. Chen, E. Chew, F. S. Collins, L. J. Corbin, G. D. Smith, G. Dedoussis, M. Dorr, A. E. Farmaki, L. Ferrucci, L. Forer, R. M. Fraser, S. Gabriel, S. Levy, L. Groop, T. Harrison, A. Hattersley, O. L. Holmen, K. Hveem, M. Kretzler, J. C. Lee, M. McGue, T. Meitinger, D. Melzer, J. L. Min, K.

L. Mohlke, J. B. Vincent, M. Nauck, D. Nickerson, A. Palotie, M. Pato, N. Pirastu, M. McInnis, J. B. Richards, C. Sala, V. Salomaa, D. Schlessinger, S. Schoenherr, P. E. Slagboom, K. Small, T. Spector, D. Stambolian, M. Tuke, J. Tuomilehto, L. H. Van den Berg, W. Van Rheenen, U. Volker, C. Wijmenga, D. Toniolo, E. Zeggini, P. Gasparini, M. G. Sampson, J. F. Wilson, T. Frayling, P. I. de Bakker, M. A. Swertz, S. McCarroll, C. Kooperberg, A. Dekker, D. Altshuler, C. Willer, W. Iacono, S. Ripatti, N. Soranzo, K. Walter, A. Swaroop, F. Cucca, C. A. Anderson, R. M. Myers, M. Boehnke, M. I. McCarthy, and R. Durbin. 2016. 'A reference panel of 64,976 haplotypes for genotype imputation', *Nat Genet*, 48: 1279-83.

McClure, W. O., A. Ishtoyan, and M. Lyon. 2004a. 'Very mild stress of pregnant rats reduces volume and cell number in nucleus accumbens of adult offspring: some parallels to schizophrenia', *Brain Res Dev Brain Res*, 149: 21-8.

McClure, William O., Armine Ishtoyan, and Melvin Lyon. 2004b. 'Very mild stress of pregnant rats reduces volume and cell number in nucleus accumbens of adult offspring: some parallels to schizophrenia', *Developmental Brain Research*, 149: 21-28.

McCoy, D. C., M. C. Connors, P. A. Morris, H. Yoshikawa, and A. H. Friedman-Krauss. 2015. 'Neighborhood Economic Disadvantage and Children's Cognitive and Social-Emotional Development: Exploring Head Start Classroom Quality as a Mediating Mechanism', *Early Child Res Q*, 32: 150-59.

McCrory, Cathal, Giovanni Fiorito, Aisling M. O'Halloran, Silvia Polidoro, Paolo Vineis, and Rose Anne Kenny. 2022. 'Early life adversity and age acceleration at mid-life and older ages indexed using the next-generation GrimAge and Pace of Aging epigenetic clocks', *Psychoneuroendocrinology*, 137: 105643.

McGill, M. G., I. Pokhvisneva, A. S. Clappison, L. M. McEwen, R. Beijers, M. S. Tollenaar, H. Pham, M. Z. L. Kee, E. Garg, E. J. de Mendonça Filho, N. Karnani, P. P. Silveira, M. S. Kobor, C. de Weerth, M. J. Meaney, and K. J. O'Donnell. 2022. 'Maternal Prenatal Anxiety and the Fetal Origins of Epigenetic Aging', *Biol Psychiatry*, 91: 303-12.

McLaughlin, Katie A., Natalie L. Colich, Alexandra M. Rodman, and David G. Weissman. 2020. 'Mechanisms linking childhood trauma exposure and psychopathology: a transdiagnostic model of risk and resilience', *BMC Medicine*, 18: 96.

Mehta, M. A., E. Gore-Langton, N. Golembo, E. Colvert, S. C. Williams, and E. Sonuga-Barke. 2010. 'Hyporesponsive reward anticipation in the basal ganglia following severe institutional deprivation early in life', *J Cogn Neurosci*, 22: 2316-25.

Miguel, Patrícia Maidana, Lenir Orlandi Pereira, Barbara Barth, Euclides José de Mendonça Filho, Irina Pokhvisneva, Thao T. T. Nguyen, Elika Garg, Bruna Regis Razzolini, Dawn Xin Ping Koh, and Heather Gallant. 2019. 'Prefrontal cortex dopamine transporter gene network moderates the effect of perinatal hypoxic-ischemic conditions on cognitive flexibility and brain gray matter density in children', *Biological Psychiatry*, 86: 621-30.

Min, J. L., G. Hemani, G. Davey Smith, C. Relton, and M. Suderman. 2018. 'Meffil: efficient normalization and analysis of very large DNA methylation datasets', *Bioinformatics*, 34: 3983-89.

Monk, C., J. Spicer, and F. A. Champagne. 2012. 'Linking prenatal maternal adversity to developmental outcomes in infants: the role of epigenetic pathways', *Dev Psychopathol*, 24: 1361-76.

Monroe, S. M., and A. D. Simons. 1991. 'Diathesis-stress theories in the context of life stress research: implications for the depressive disorders', *Psychol Bull*, 110: 406-25.

Monroy, Elibeth, Elizabeth Hernández-Torres, and Gonzalo Flores. 2010. 'Maternal separation disrupts dendritic morphology of neurons in prefrontal cortex, hippocampus, and nucleus accumbens in male rat offspring', *Journal of Chemical Neuroanatomy*, 40: 93-101.
Montenegro-Venegas, Carolina, Anil Annamneedi, Sheila Hoffmann-Conaway, Eckart D. Gundelfinger, and Craig C. Garner. 2020. 'BSN (bassoon) and PRKN/parkin in concert control presynaptic vesicle autophagy', *Autophagy*, 16: 1732-33.
Moore, Lisa D., Thuc Le, and Guoping Fan. 2013. 'DNA Methylation and Its Basic Function', *Neuropsychopharmacology*, 38: 23-38.
Mostafavi, Sara, Debajyoti Ray, David Warde-Farley, Chris Grouios, and Quaid Morris. 2008. 'GeneMANIA: a real-time multiple association network integration algorithm for predicting gene function', *Genome Biology*, 9: S4.
Murphy, Shannon K., Anna M. Fineberg, Seth D. Maxwell, Lauren B. Alloy, Lauren Zimmermann, Nickilou Y. Krigbaum, Barbara A. Cohn, Deborah A. G. Drabick, and Lauren M. Ellman. 2017. 'Maternal infection and stress during pregnancy and depressive symptoms in adolescent offspring', *Psychiatry Research*, 257: 102-10.
Murray, G. K., T. Lin, J. Austin, J. J. McGrath, I. B. Hickie, and N. R. Wray. 2021. 'Could Polygenic Risk Scores Be Useful in Psychiatry?: A Review', *JAMA Psychiatry*, 78: 210-19.
O'Connor, T. G., J. Heron, J. Golding, and V. Glover. 2003. 'Maternal antenatal anxiety and behavioural/emotional problems in children: a test of a programming hypothesis', *J Child Psychol Psychiatry*, 44: 1025-36.
Oblak, Lara, Jeroen van der Zaag, Albert T. Higgins-Chen, Morgan E. Levine, and Marco P. Boks. 2021. 'A systematic review of biological, social and environmental factors associated with epigenetic clock acceleration', *Ageing Research Reviews*, 69: 101348.
Parikshak, N. N., M. J. Gandal, and D. H. Geschwind. 2015. 'Systems biology and gene networks in neurodevelopmental and neurodegenerative disorders', *Nat Rev Genet*, 16: 441-58.
Patterson, N., A. L. Price, and D. Reich. 2006a. 'Population structure and eigenanalysis', *PLoS Genet*, 2: e190.
Patterson, Nick, Alkes L. Price, and David Reich. 2006b. 'Population Structure and Eigenanalysis', *PLOS Genetics*, 2: e190.
Pluess, Michael, Jay Belsky, and Rosalind J. Neuman. 2009. 'Prenatal Smoking and Attention-Deficit/Hyperactivity Disorder: DRD4-7R as a Plasticity Gene', *Biological Psychiatry*, 66: e5-e6.
Price, A. L., N. J. Patterson, R. M. Plenge, M. E. Weinblatt, N. A. Shadick, and D. Reich. 2006. 'Principal components analysis corrects for stratification in genome-wide association studies', *Nat Genet*, 38: 904-9.
Provençal, N., J. Arloth, A. Cattaneo, C. Anacker, N. Cattane, T. Wiechmann, S. Röh, M. Ködel, T. Klengel, D. Czamara, N. S. Müller, J. Lahti, K. Räikkönen, C. M. Pariante, and E. B. Binder. 2020. 'Glucocorticoid exposure during hippocampal neurogenesis primes future stress response by inducing changes in DNA methylation', *Proc Natl Acad Sci U S A*, 117: 23280-85.
Purcell, S., B. Neale, K. Todd-Brown, L. Thomas, M. A. Ferreira, D. Bender, J. Maller, P. Sklar, P. I. de Bakker, M. J. Daly, and P. C. Sham. 2007. 'PLINK: a tool set for whole-genome association and population-based linkage analyses', *Am J Hum Genet*, 81: 559-75.
R Core Team. 2022. "R: A language and environment for statistical computing." In. Vienna, Austria: R Foundation for Statistical Computing.

Rescorla, Leslie, Thomas Achenbach, Masha Y. Ivanova, Levent Dumenci, Fredrik Almqvist, Niels Bilenberg, Hector Bird, Chen Wei, Anca Dobrean, Manfred Döpfner, Nese Erol, Eric Fombonne, Antonio Fonseca, Alessandra Frigerio, Hans Grietens, Helga Hannesdottir, Yasuko Kanbayashi, Michael Lambert, B. O. Larsson, Patrick Leung, Liu Xianchen, Asghar Minaei, Mesfin S. Mulatu, Torunn S. Novik, Kyung-Ja Oh, Alexandra Roussos, Michael Sawyer, Zeynep Simsek, Hans-Christoph Steinhausen, Sheila Weintraub, John Weisz, Christa Winkler Metzke, Tomasz Wolanczyk, Hao-Jan Yang, Nelly Zilber, Rita Zukauskiene, and Frank Verhulst. 2007. 'Behavioral and Emotional Problems Reported by Parents of Children Ages 6 to 16 in 31 Societies', *Journal of Emotional and Behavioral Disorders*, 15: 130-42.

Reuben, Aaron, Karen Sugden, Louise Arseneault, David L. Corcoran, Andrea Danese, Helen L. Fisher, Terrie E. Moffitt, Joanne B. Newbury, Candice Odgers, Joey Prinz, Line J. H. Rasmussen, Ben Williams, Jonathan Mill, and Avshalom Caspi. 2020. 'Association of Neighborhood Disadvantage in Childhood With DNA Methylation in Young Adulthood', *JAMA Network Open*, 3: e206095.

Richmond, Rebecca C., Nicholas J. Timpson, Janine F. Felix, Tom Palmer, Romy Gaillard, George McMahon, George Davey Smith, Vincent W. Jaddoe, and Debbie A. Lawlor. 2017. 'Using Genetic Variation to Explore the Causal Effect of Maternal Pregnancy Adiposity on Future Offspring Adiposity: A Mendelian Randomisation Study', *PLOS Medicine*, 14: e1002221.

Rubinacci, Simone, Olivier Delaneau, and Jonathan Marchini. 2020. 'Genotype imputation using the Positional Burrows Wheeler Transform', *PLOS Genetics*, 16: e1009049.

Salgado, Sanjay, and Michael G. Kaplitt. 2015. 'The Nucleus Accumbens: A Comprehensive Review', *Stereotactic and Functional Neurosurgery*, 93: 75-93.

Santiago, Catherine DeCarlo, Martha E. Wadsworth, and Jessica Stump. 2011. 'Socioeconomic status, neighborhood disadvantage, and poverty-related stress: Prospective effects on psychological syndromes among diverse low-income families', *Journal of Economic Psychology*, 32: 218-30.

Santomauro, Damian F., Ana M. Mantilla Herrera, Jamileh Shadid, Peng Zheng, Charlie Ashbaugh, David M. Pigott, Cristiana Abbafati, Christopher Adolph, Joanne O. Amlag, Aleksandr Y. Aravkin, Bree L. Bang-Jensen, Gregory J. Bertolacci, Sabina S. Bloom, Rachel Castellano, Emma Castro, Suman Chakrabarti, Jhilik Chattopadhyay, Rebecca M. Cogen, James K. Collins, Xiaochen Dai, William James Dangel, Carolyn Dapper, Amanda Deen, Megan Erickson, Samuel B. Ewald, Abraham D. Flaxman, Joseph Jon Frostad, Nancy Fullman, John R. Giles, Ababi Zergaw Giref, Gaorui Guo, Jiawei He, Monika Helak, Erin N. Hulland, Bulat Idrisov, Akiaja Lindstrom, Emily Linebarger, Paulo A. Lotufo, Rafael Lozano, Beatrice Magistro, Deborah Carvalho Malta, Johan C. Månsson, Fatima Marinho, Ali H. Mokdad, Lorenzo Monasta, Paulami Naik, Shuhei Nomura, James Kevin O'Halloran, Samuel M. Ostroff, Maja Pasovic, Louise Penberthy, Robert C. Reiner Jr, Grace Reinke, Antonio Luiz P. Ribeiro, Aleksei Sholokhov, Reed J. D. Sorensen, Elena Varavikova, Anh Truc Vo, Rebecca Walcott, Stefanie Watson, Charles Shey Wiysonge, Bethany Zigler, Simon I. Hay, Theo Vos, Christopher J. L. Murray, Harvey A. Whiteford, and Alize J. Ferrari. 2021. 'Global prevalence and burden of depressive and anxiety disorders in 204 countries and territories in 2020 due to the COVID-19 pandemic', *The Lancet*, 398: 1700-12.

Schlotz, Wolff, and David I. W. Phillips. 2009. 'Fetal origins of mental health: Evidence and mechanisms', *Brain, Behavior, and Immunity*, 23: 905-16.

Scott, K. M., M. Von Korff, M. C. Angermeyer, C. Benjet, R. Bruffaerts, G. de Girolamo, J. M. Haro, J. P. Lépine, J. Ormel, J. Posada-Villa, H. Tachimori, and R. C. Kessler.

2011. 'Association of childhood adversities and early-onset mental disorders with adult-onset chronic physical conditions', *Arch Gen Psychiatry*, 68: 838-44.
Sequeira, Adolfo, Ling Morgan, David M. Walsh, Preston M. Cartagena, Prabhakara Choudary, Jun Li, Alan F. Schatzberg, Stanley J. Watson, Huda Akil, Richard M. Myers, Edward G. Jones, William E. Bunney, and Marquis P. Vawter. 2012. 'Gene Expression Changes in the Prefrontal Cortex, Anterior Cingulate Cortex and Nucleus Accumbens of Mood Disorders Subjects That Committed Suicide', *PloS one*, 7: e35367.
Shannon, P., A. Markiel, O. Ozier, N. S. Baliga, J. T. Wang, D. Ramage, N. Amin, B. Schwikowski, and T. Ideker. 2003. 'Cytoscape: a software environment for integrated models of biomolecular interaction networks', *Genome Res*, 13: 2498-504.
Sharkey, Patrick. 2013. *Stuck in place: Urban neighborhoods and the end of progress toward racial equality* (University of Chicago Press).
Shiflett, Michael W., and Bernard W. Balleine. 2010. 'At the limbic–motor interface: disconnection of basolateral amygdala from nucleus accumbens core and shell reveals dissociable components of incentive motivation', *European Journal of Neuroscience*, 32: 1735-43.
Shohat, S., A. Amelan, and S. Shifman. 2021. 'Convergence and Divergence in the Genetics of Psychiatric Disorders From Pathways to Developmental Stages', *Biol Psychiatry*, 89: 32-40.
Shonkoff, J. P., W. T. Boyce, N. R. Bush, M. R. Gunnar, T. K. Hensch, P. Levitt, M. J. Meaney, C. A. Nelson, N. Slopen, D. R. Williams, and P. P. Silveira. 2022. 'Translating the Biology of Adversity and Resilience Into New Measures for Pediatric Practice', *Pediatrics*, 149.
Silveira, P. P., and M. J. Meaney. 2023. 'Examining the biological mechanisms of human mental disorders resulting from gene-environment interdependence using novel functional genomic approaches', *Neurobiol Dis*, 178: 106008.
Silveira, P. P., I. Pokhvisneva, C. Parent, S. Cai, A. S. S. Rema, B. F. P. Broekman, A. Rifkin-Graboi, M. Pluess, K. J. O'Donnell, and M. J. Meaney. 2017a. 'Cumulative prenatal exposure to adversity reveals associations with a broad range of neurodevelopmental outcomes that are moderated by a novel, biologically informed polygenetic score based on the serotonin transporter solute carrier family C6, member 4 (SLC6A4) gene expression', *Dev Psychopathol*, 29: 1601-17.
Silveira, Patrícia P., Irina Pokhvisneva, Hélène Gaudreau, Anne Rifkin-Graboi, Birit F. P. Broekman, Meir Steiner, Robert Levitan, Carine Parent, Josie Diorio, and Michael J. Meaney. 2018. 'Birth weight and catch up growth are associated with childhood impulsivity in two independent cohorts', *Scientific Reports*, 8: 13705.
Silveira, Patrícia P., Irina Pokhvisneva, Carine Parent, Shirong Cai, Anu Sathyan Sathyapalan Rema, Birit F. P. Broekman, Anne Rifkin-Graboi, Michael Pluess, Kieran J. O'Donnell, and Michael J. Meaney. 2017b. 'Cumulative prenatal exposure to adversity reveals associations with a broad range of neurodevelopmental outcomes that are moderated by a novel, biologically informed polygenetic score based on the serotonin transporter solute carrier family C6, member 4', *Development and Psychopathology*, 29: 1601-17.
Smith, A. K., V. Kilaru, T. Klengel, K. B. Mercer, B. Bradley, K. N. Conneely, K. J. Ressler, and E. B. Binder. 2015. 'DNA extracted from saliva for methylation studies of psychiatric traits: evidence tissue specificity and relatedness to brain', *Am J Med Genet B Neuropsychiatr Genet*, 168b: 36-44.
Soh, S. E., M. T. Tint, P. D. Gluckman, K. M. Godfrey, A. Rifkin-Graboi, Y. H. Chan, W. Stünkel, J. D. Holbrook, K. Kwek, Y. S. Chong, and S. M. Saw. 2014. 'Cohort profile:

Growing Up in Singapore Towards healthy Outcomes (GUSTO) birth cohort study', *Int J Epidemiol*, 43: 1401-9.
Suarez, G. L., S. A. Burt, A. M. Gard, J. Burton, D. A. Clark, K. L. Klump, and L. W. Hyde. 2022. 'The impact of neighborhood disadvantage on amygdala reactivity: Pathways through neighborhood social processes', *Dev Cogn Neurosci*, 54: 101061.
Szklarczyk, Damian, Annika L. Gable, David Lyon, Alexander Junge, Stefan Wyder, Jaime Huerta-Cepas, Milan Simonovic, Nadezhda T. Doncheva, John H. Morris, Peer Bork, Lars J. Jensen, and Christian von Mering. 2019. 'STRING v11: protein–protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets', *Nucleic Acids Research*, 47: D607-D13.
Tarantino, L. M., P. F. Sullivan, and S. Meltzer-Brody. 2011. 'Using animal models to disentangle the role of genetic, epigenetic, and environmental influences on behavioral outcomes associated with maternal anxiety and depression', *Front Psychiatry*, 2: 44.
Taylor, R. L., S. R. Cooper, J. J. Jackson, and D. M. Barch. 2020. 'Assessment of Neighborhood Poverty, Cognitive Function, and Prefrontal and Hippocampal Volumes in Children', *JAMA Netw Open*, 3: e2023774.
Teicher, M. H., and J. A. Samson. 2013. 'Childhood maltreatment and psychopathology: A case for ecophenotypic variants as clinically and neurobiologically distinct subtypes', *Am J Psychiatry*, 170: 1114-33.
Teicher, Martin H., and Jacqueline A. Samson. 2016. 'Annual research review: enduring neurobiological effects of childhood abuse and neglect', *Journal of child psychology and psychiatry*, 57: 241-66.
Thompson, Mike, Brian L. Hill, Nadav Rakocz, Jeffrey N. Chiang, Daniel Geschwind, Sriram Sankararaman, Ira Hofer, Maxime Cannesson, Noah Zaitlen, and Eran Halperin. 2022. 'Methylation risk scores are associated with a collection of phenotypes within electronic health record systems', *npj Genomic Medicine*, 7: 50.
Tobi, E. W., R. C. Slieker, R. Luijk, K. F. Dekkers, A. D. Stein, K. M. Xu, P. E. Slagboom, E. W. van Zwet, L. H. Lumey, and B. T. Heijmans. 2018. 'DNA methylation as a mediator of the association between prenatal adversity and risk factors for metabolic disease in adulthood', *Sci Adv*, 4: eaao4364.
van den Oord, Charlie L. J. D., William E. Copeland, Min Zhao, Lin Ying Xie, Karolina A. Aberg, and Edwin J. C. G. van den Oord. 2022. 'DNA methylation signatures of childhood trauma predict psychiatric disorders and other adverse outcomes 17 years after exposure', *Molecular Psychiatry*, 27: 3367-73.
Watanabe, Kyoko, Erdogan Taskesen, Arjen van Bochoven, and Danielle Posthuma. 2017. 'Functional mapping and annotation of genetic associations with FUMA', *Nature Communications*, 8: 1826.
Williams, E. G., and J. Auwerx. 2015. 'The Convergence of Systems and Reductionist Approaches in Complex Trait Analysis', *Cell*, 162: 23-32.
Wray, N. R., S. H. Lee, D. Mehta, A. A. Vinkhuyzen, F. Dudbridge, and C. M. Middeldorp. 2014. 'Research review: Polygenic methods and their application to psychiatric traits', *J Child Psychol Psychiatry*, 55: 1068-87.
Wray, N. R., M. L. Pergadia, D. H. Blackwood, B. W. Penninx, S. D. Gordon, D. R. Nyholt, S. Ripke, D. J. MacIntyre, K. A. McGhee, A. W. Maclean, J. H. Smit, J. J. Hottenga, G. Willemsen, C. M. Middeldorp, E. J. de Geus, C. M. Lewis, P. McGuffin, I. B. Hickie, E. J. van den Oord, J. Z. Liu, S. Macgregor, B. P. McEvoy, E. M. Byrne, S. E. Medland, D. J. Statham, A. K. Henders, A. C. Heath, G. W. Montgomery, N. G. Martin, D. I. Boomsma, P. A. Madden, and P. F. Sullivan. 2012. 'Genome-wide

association study of major depressive disorder: new results, meta-analysis, and lessons learned', *Mol Psychiatry*, 17: 36-48.
Xu, X., A. B. Wells, D. R. O'Brien, A. Nehorai, and J. D. Dougherty. 2014. 'Cell type-specific expression analysis to identify putative cellular mechanisms for neurogenetic disorders', *J Neurosci*, 34: 1420-31.
Yamaguchi, Tsuyoshi, Hui-Ling Wang, Xueping Li, Tsz H. Ng, and Marisela Morales. 2011. 'Mesocorticolimbic Glutamatergic Pathway', *The Journal of Neuroscience*, 31: 8476.
Yang, J., B. Benyamin, B. P. McEvoy, S. Gordon, A. K. Henders, D. R. Nyholt, P. A. Madden, A. C. Heath, N. G. Martin, G. W. Montgomery, M. E. Goddard, and P. M. Visscher. 2010. 'Common SNPs explain a large proportion of the heritability for human height', *Nat Genet*, 42: 565-9.
Yang, J., S. H. Lee, M. E. Goddard, and P. M. Visscher. 2011. 'GCTA: a tool for genome-wide complex trait analysis', *Am J Hum Genet*, 88: 76-82.
Zhou, J., M. Li, X. Wang, Y. He, Y. Xia, J. A. Sweeney, R. F. Kopp, C. Liu, and C. Chen. 2021. 'Drug Response-Related DNA Methylation Changes in Schizophrenia, Bipolar Disorder, and Major Depressive Disorder', *Front Neurosci*, 15: 674273.
Ziller, M. J., H. Gu, F. Müller, J. Donaghey, L. T. Tsai, O. Kohlbacher, P. L. De Jager, E. D. Rosen, D. A. Bennett, B. E. Bernstein, A. Gnirke, and A. Meissner. 2013. 'Charting a dynamic DNA methylation landscape of the human genome', *Nature*, 500: 477-81.