Genetic and epigenetic associations with child development and mental health in a South African birth cohort

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"Let he who waters, be watered also."

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Abbreviations

ADHD	Attention-deficit hyperactivity disorder
Bayley-III	Bayley Scales of Infant and Toddler Development, 3rd edition
CBCL	Child Behaviour Checklist
CpGs	Cytosine linked by a phosphate bond to Guanine
DCHS	Drakenstein Child Health Study
DNA	Deoxyribonucleic acid
DNAm	DNA methylation
EA	Epigenetic age
EAA	EA acceleration
EAGLE	Early Genetics and Lifecourse Epidemiology
EWAS	Epigenome-wide association studies
GA	Gestational age
GWAS	Genome-wide association Studies
HIV	Human immunodeficiency virus
HWE	Hardy-Weinberg Equilibrium
IBD	Identity by descent
LD	Linkage disequilibrium
LMICs	Low- and middle-income countries
MAF	Minor allele frequency
MeSH	Medical Subject Headings
MRS	Methylation risk scores
NDD	Neurodevelopmental disorder
OR	Odds ratio
PACE	Pregnancy and Childhood Epigenetics
PCA	Principal component analysis
PGC	Psychiatric Genomics Consortium
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-analysis
PRS	Polygenic risk scores
QC	Quality control
SES	Socioeconomic status
SNP	Single nucleotide polymorphism
UKBB	United Kingdom Biobank
WHO	World Health Organization
MDD	Major depressive disorder
CD	Conduct disorder

ASD	Autism spectrum disorder
SCZ	Schizophrenia
ALSPAC	Avon Longitudinal Study of Parents and Children
BD	Bipolar Disorder
DEP	Depression
ANX	Anxiety
DSM-IV	Diagnostic and statistical manual of mental disorders 4th edition
SDQ	Strengths and difficulties questionnaire
SASH	South African stress and health study
ASSIST	Alcohol, smoking and substance involvement screening test
EPDS	Edinburgh postnatal depression scale
PTSD	Post-traumatic stress disorder
iPSYCH	Integrative psychiatric research
HIC	High-income country
SR	Systematic review
LIMS	Laboratory Information Management System
K-SADS-PL	Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime version
BIBO	Longitudinal Basale Invloeden Op De Baby Ontwikkeling study
GLAKU	Glycyrrhizin in Licorice study
PedBE	Pediatric Buccal Epigenetic clock
SE	Standard error
ODD	Oppositional defiant disorder
NTR	Netherlands twin registry
NEU	Neuroticism
COGA	Collaborative Study on the Genetics of Alcoholism
NESCOG	Netherlands Study of Cognition, Enviroment and Genes
NCDS	National Child Development Study
MoBa	Norwegian mother, father and child cohort
BREATH	BRain dEvelopment and Air polluTion ultrafine particles in scHool children
SMFQ	Short mood and feelings questionnaire
WISC-III	Weschler Intelligence scale 3rd edition
TEACh	Test of Everyday Attention for Children
DANVA	Diagnostic Analysis of Nonverbal Accuracy
CI-BPD	Childhood Interview for DSM-IV Borderline Personality Disorder
SCDC	Social and communication disorders checklist

CCC	Children's communication checklist
SCARED	Screen for Child Anxiety Related Disorders
RS-DBD	Rating Scale for Disruptive Behaviour Disorders
HRTSE	Hit reaction time
ANT	Attention Network Test
CTNWR	Children's Test of Nonword Repetition
UCT	University of Cape Town
FHS	Faculty of Health Sciences
HREC	Human Research Ethics Committee

Abstract

Childhood developmental and mental disorders – including internalising and externalising symptoms – are prevalent in low- and middle-income countries (LMICs) such as South Africa. There is growing interest in the associations between polygenic risk scores (PRS), epigenetic age (EA) deviation, DNA methylation risk scores (MRS), and key neurodevelopmental disorders such as attention-deficit hyperactivity disorder (ADHD). However, most work has thus far been undertaken in high-income countries (HICs) with participants of European ancestry; and populations of African ancestry have been notably under-represented. The Drakenstein Child Health Study (DCHS), an ongoing South African birth cohort study, provides an opportunity to investigate the associations of PRS, EA deviation, and MRS, with childhood developmental and mental health outcomes, in an ancestrally diverse study population.

This doctoral project aimed to investigate potential genetic and epigenetic associations with adverse developmental and mental outcomes in children. This aim was addressed via five objectives. First, a systematic review was undertaken to collate existing work (both in HICs and LMICs) on associations between PRS (the weighted sum of risk alleles) and developmental and mental health disorders in childhood and adolescence. A second systematic review focused on associations between EA deviation (relative to chronological age) and the outcomes of interest. Third, empirical analyses of DCHS data investigated the relationship between a PRS for ADHD, and child developmental outcomes, as well as internalising and externalising symptoms. Fourth, the relationship between gestational EA deviation at birth, and child developmental and mental health outcomes in the DCHS, was explored. Finally, the association between MRS (the weighted sum of methylation markers' beta values) at birth, and the outcomes of interest, was investigated.

The systematic reviews adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines, and standard methods were used to collate and analyse the data. In the DCHS, a PRS for ADHD was generated (target n=958) using summary statistics from the Early Genetics and Lifecourse Epidemiology (EAGLE) consortium (discovery n=17,666). Gestational EA deviation at birth and MRS were calculated using DCHS umbilical cord blood samples (n=275) and summary statistics from the Pregnancy and Childhood Epigenetics (PACE) consortium (n=2,477 for the MRS analyses). Child developmental outcomes (i.e. cognitive, language and motor development) were derived from the Bayley Scales of Infant and Toddler Development, 3rd edition (Bayley-III), and child mental health outcomes (i.e. internalising and externalising symptoms) from the Child Behaviour Checklist (CBCL). Associations of interest were investigated using bivariate and multivariable linear and logistic regression models, controlling for relevant covariates (including sociodemographic characteristics, psychosocial risk factors, child anthropometric measures and genomic principal components).

In the first systematic review (of 14 studies, with ~50,000 participants), significant associations between PRS for several mental health disorders and adverse developmental/mental health outcomes were found. For example, a high ADHD PRS was found to be associated with adverse outcomes in childhood and adolescence in 5 of the 14 included studies. Additionally, 4 studies described associations between PRS for bipolar disorder and impaired cognitive function, and poor executive functioning, in children and adolescents; and 2 studies highlighted associations between schizophrenia PRS and ADHD, as well as internalising and externalising symptoms in children. In the second systematic review (of 4 studies with N~700 participants), gestational EA acceleration was found to be significantly associated with internalising symptoms in children.

The empirical analyses yielded no significant genetic or epigenetic associations with the developmental or mental health outcomes of interest in the DCHS children. However, trend-level associations were observed - in both the unadjusted and the adjusted models - between gestational EA deviation at birth and child externalising symptoms (at 42 months) in the DCHS (unadjusted $\beta = -0.19$, $p = 0.072$; adjusted $\beta = -0.17$, $p = 0.10$).

While limited by sample size and lack of ancestry-matched summary statistics, this work nonetheless represents a novel exploration of the potential genetic and epigenetic underpinnings of developmental and mental disorders in South African children. In future, further studies – ideally with larger sample sizes, ancestry-matched summary statistics and longitudinal developmental phenotype data – would be warranted to expand on this preliminary work. Ultimately, such research may provide insight into the genetic and epigenetic risk factors of developmental and mental health outcomes in children; and may inform targeted early interventions for at-risk children – particularly in resource-limited settings such as South Africa.

Chapter 1: Introduction

This chapter will provide a comprehensive background of previous literature, exploring first the prevalence of adverse developmental and mental health outcomes in children, followed by the genomic and epigenomic underpinnings of these outcomes. Thereafter, this chapter will address the need for ancestral diversity in global genomic and epigenomic research – which, to date, has been largely conducted in individuals of European ancestry. Finally, the aims and objectives of this doctoral project will be outlined.

1.1. Prevalence of adverse developmental and mental health outcomes in childhood

Child development is a complex and multifaceted process, spanning domains such as physical, cognitive, language, and emotional development (McCoy et al., 2016). Developmental disorders in childhood may manifest as dysfunction in attention, memory, perception, language, problem-solving, or social interaction (Lester et al., 2012). Globally, developmental delay affects 1-3% of children below the age of 5 years of age (Mithyantha et al., 2017). Perturbations during child development may lead to adverse sequelae persisting into adulthood (Kieling et al., 2011). For example, children may be at greater risk of incomplete schooling, poor academic performance, strained interpersonal relationships (e.g., family breakup and divorce), and long-term unemployment (Bagner et al., 2012, Scott et al., 2016).

Most mental health disorders – encompassing psychiatric and neurodevelopmental disorders - in children also present as emotional or behavioural outcomes which deviate from expected "norms" for their age groups (Kieling et al., 2011), as discussed further in *Chapter 2*. Attention-deficit hyperactivity disorder (ADHD), a neurodevelopmental disorder that emerges in childhood, is characterised by impaired attention, motor hyperactivity and impulsivity, with these difficulties often persisting into adulthood (Willner et al., 2016, Kessler et al., 2007). Mental health disorders in children collectively comprise the majority of health burden, accounting for approximately 3.4% of the global burden of disease (Cicchetti, 1984, Whiteford et al., 2013), with internalising and externalising symptoms among the most common paediatric psychiatric symptoms worldwide (Willner et al., 2016, Kessler et al., 2007). It is estimated that approximately one-third of individuals experience the onset of their first mental health disorder before the age of 14, and nearly half by the age of 18 (Solmi et al., 2022). The WHO estimates that mental health disorders affect 14% of 10- to 19-year-olds (WHO, 2021). In South Africa, although nationally representative data are not currently available (Fieggen et al., 2019), one study in rural SA – i.e. in eight villages in the Bushbuckridge district, Northern Province - reported a prevalence of intellectual disability – which reflects deficits in cognitive function along with a broad range of adaptive behaviours - in 3.56% of children aged 2 to 9 (N=6692) (Christianson et al., 2002). Further, large-scale data from other low- and middle-income countries (LMICs) estimated that 219 to 279 million children below the age of 5 years were at risk of not reaching their developmental potential (Grantham-McGregor et al., 2007) in academic, behavioural, and socioemotional domains (Black et al., 2017).

Given that the majority of the global child population resides in LMICs (Grantham-McGregor et al., 2007); and that the onset of most mental health disorders is often during childhood and adolescence, developmental and mental health during childhood should be prioritized - particularly in resource-limited countries. In such settings, child development and mental health are key, in order to optimise long-term trajectories, mitigate the risk for mental health outcomes later in life, and reduce the potential strain on health care systems (Scott et al., 2016).

1.2. Genomic underpinnings of adverse developmental and mental health outcomes

Child development and mental health may be influenced by a complex interplay of individual, familial and external factors (Lester et al., 2012, Bagner et al., 2012). Genetic mechanisms have been suggested as potential risk factors for adverse developmental and mental health outcomes in children (Lester et al., 2012). For example, a family study of 894 ADHD-diagnosed participants and 1135 control siblings (all aged 5 to17 years old) found that the risk for ADHD was nine-fold higher in participants who had a relative with ADHD diagnosis, compared to the control siblings (i.e. those who did not have a sibling with ADHD) (Chen et al., 2008).

Genome-wide association studies (GWAS) - a statistical genomics approach used to identify genomic variants that are associated with particular traits or disorders across the genome (Purcell et al., 2009) - have identified a number of genetic variants related to mental health disorders and behavioural traits in adults (Demontis et al., 2023, Demontis et al., 2019, Bulik-Sullivan et al., 2015). One such study, an ADHD GWAS meta-analysis of 12 European and Asian cohorts comprising 20,183 ADHD cases and 35,191 controls, identified 12 loci of genome-wide significance (Demontis et al., 2019). One of the genes identified (i.e. the *forkhead box P2*, FOXP*2* gene*)* has previously been implicated in speech and language development and has been hypothesized as a potential genetic risk factor for ADHD (Lai et al., 2003).

GWAS have also highlighted the polygenic architecture of complex disorders, where each identified variant has a small effect size on disease risk (Wray et al., 2013). Given that most mental health disorders are polygenic, a single variant is thus not informative for assessing disease risk (Purcell et al., 2009, Wray et al., 2013). Genetic loading through the aggregation of a set of risk variants may be needed to obtain a measure of risk liability for disorders (Bulik-Sullivan et al., 2015, Purcell et al., 2009, Wray et al., 2013). To this end, polygenic risk scores (PRS) are key (Purcell et al., 2009).

PRS enable the quantification of common risk alleles an individual carries for a given trait or disorder; and can provide an estimate of genetic liability to the phenotype of interest at the individual level (Purcell et al., 2009), PRS is discussed further in the *Chapter's 2 and 4*. Currently, several methods maybe used to calculate PRS. For example, in the "clumping/pruning and thresholding" approach, a reduced set of genetic variants is identified through pruning on linkage disequilibrium (LD) and clumping based on the evidence of association with phenotype of interest (Purcell et al.,2009). Thereafter, PRS are calculated by summing all the SNPs which meet the set p-value threshold(s), as implemented in tools such as PRSice or PLINK (Euesden et al., 2015, Chang et al., 2015). Alternative methods - such as the Bayesian LDpred approach - assess the best prediction genome-wide by modelling the correlation structure between variants, without attempting to identify a minimal subset of SNPs for prediction (Ge et al.,2019, Vilhjalmsson et al.,2015).

Clinically, PRS may hold promise in the early detection of disease; and may enable targeted preventative and/or therapeutic interventions (Folkersen, Pain et al. 2020). For example, a PRS for prostate cancer has been instrumental in identifying men at significantly higher risk for prostate cancer, thus guiding the decision for prostate-specific antigen (PSA) screening (Pashayan et al., 2015, Seibert et al., 2018). Similarly, PRS for Alzheimer's disease has been shown to stratify individuals based on the average age of disease onset (Desikan et al., 2017). Additionally, PRS for coronary artery disease have proven valuable in identifying high-risk individuals who would benefit most from statin therapy (Mega et al., 2015, Tada et al., 2016, Natarajan et al., 2017). However, the use of PRS across multiple phenotypes in a primary care setting is still limited (Hao, Kraft et al. 2022). For example, ensuring equal access and applicability across diverse populations would be key to avoiding exacerbating health disparities (Lewis and Vassos 2017). As current PRS methods rely on ancestry-matching between individual study participants and genotype-level summary statistics, these methods are generally applicable to only a small portion of the global population (Lewis and Vassos 2017) – as discussed further in Chapter 1, Section 1.4 – "There is a need for African genomic studies".

1.3. Epigenomic underpinnings of developmental and mental health outcomes

There has also been a growing interest in epigenetics as a way of understanding the interplay between genetics and the environment and their contribution to complex traits and diseases. Epigenetic variation often reflects both genetic and environmental exposures and holds promise to identify novel disease-associated genes and pathways that might not be discovered through genetic studies alone (Shah, Bonder et al. 2015).

Epigenetic mechanisms mediate mitotically heritable but reversible changes in gene expression through alterations in DNA methylation (DNAm) and chromatin structure, without altering the underlying genomic DNA sequence. Such mechanisms have emerged as potential biomarkers for understanding risk for mental disorders (Hannon et al., 2016). DNAm - the process of attaching methyl molecules to specific CpG (cytosine-phosphate-guanine) dinucleotide sites within DNA (Jaenisch and Bird, 2003) - is the most characterized epigenetic mechanism (Jaenisch and Bird, 2003). DNAm is primarily detected through the conversion of unmethylated cytosines with sodium bisulphate to uracil, which allows the methylated and unmethylated cytosines to be distinguished using array-based or sequencing-based technologies (Jaenisch and Bird, 2003). Unlike the relatively stable genome, the epigenome may be altered dynamically (and sometimes reversibly) across the lifespan by exposure to intrinsic or extrinsic factors (Cecil and Nigg, 2022). Notably, DNAm patterns are influenced by both genetic and environmental factors (Czamara et al., 2019). Although the DNA sequence remains largely unchanged throughout the lifespan, DNAm patterns can differ between tissue types within an individual and between individuals (Shah, Bonder et al. 2015). DNAm variation has been proposed as risk mechanisms for adverse developmental and mental health outcomes in children and adolescents, such as the risk for internalising problems in children (Szyf and Bick, 2013, Barker, 2018, Parade et al.,2016).

Epigenome-wide association studies (EWAS) investigate epigenetic variations associated with specific traits or disorders across the epigenome. Emerging evidence suggests that epigenetic (i.e. DNAm) perturbations in response to environmental exposures (e.g. maternal prenatal smoking) are strongly associated with ADHD risk (Kim et al., 2020). For example, one meta-analysis of 5 studies (N ~2500) reported associations between ADHD and DNAm (in cord blood) at 5 CpG sites, as well as associations between 3 CpG sites and social communication trajectories in children (Neumann et al., 2020). Further, an ADHD EWAS conducted as part of the Avon Longitudinal Study of Parents and Children (ALSPAC) in over 800 children and adolescents (aged 7 to 15 years old) reported that DNAm patterns at birth (in cord blood) differed between children who later obtained ADHD diagnosis at ages 7 and 15 years (Walton et al., 2017). Interestingly, this ADHD EWAS identified a significant DNAm hit in a CpG site annotated to the *ST3GAL3* gene (Walton et al., 2017), which was previously significantly associated with ADHD in a GWAS (comprising 20,183 ADHD cases and 35,191 controls) (Demontis et al., 2019). *ST3GAL3* has been linked to developmental delays and perturbed cognitive and motor functions (Hu et al., 2011, Edvardson et al., 2013).

Methylation risk scores (MRS), which are analogues of PRS (as detailed in *Chapter 6, Section 6.1*), have been instrumental in identifying risk related to lifestyle factors such as body mass index (BMI) (Shah et al., 2015). Further, MRS for smoking, alcohol consumption and educational attainment were found to be associated with risk for major depressive disorder (MDD) in individuals of European ancestry (Barbu et al., 2022). MRS have also been found to be useful in epidemiological analyses in which key phenotypes may not be accessible or were inaccurately recorded. For example, an MRS for smoking was found to be more accurate in quantifying both direct and indirect smoking exposures than self-report data (Zhang et al., 2019a).

Additionally, work on DNAm has led to important advances in our understanding of epigenetic ageing (EA), i.e. the estimation of an individual's age based on changes in their DNAm patterns (Horvath et al., 2015) (detailed in *Chapter 5, Section 5.1*). Of note, accelerated EA (relative to chronological age) has been found to be associated with increased risk for neurological diseases such as Parkinson's disease and Alzheimer's disease (Bressler et al., 2020, Marioni et al., 2015, Christiansen et al., 2016); as well as all-cause mortality in adults (Bressler et al., 2020, Marioni et al., 2015). Conversely, EA deceleration may be protective in adults, as evidenced by a recent finding that offspring of semi-supercentenarians (i.e. people who live until the ages 105 to109) have lower EA than their age-matched controls (Horvath et al., 2015, Chen et al., 2016). In children, however, accelerated gestational EA (relative to chronological gestational age) has been found to be

associated with increased birth weight and length, and resultant improved medical and neuropsychological outcomes in childhood (Khouja et al., 2018, Baron et al., 2012). Further, decelerated gestational EA has been found to be associated with adverse birth outcomes such as low birth weight (Knight et al., 2016, Simpkin et al., 2016, Simpkin et al., 2017), which may, in turn, be associated with cognitive delays, attention deficits and internalising symptoms (Mathewson et al., 2017). Thus, the functional relevance of gestational EA deviation (acceleration or deceleration) has not been fully elucidated. This underscores the need for further investigations in this area.

1.4. There is a need for African genomic studies

To date, the majority of genomic and epigenomic studies have been conducted in high-income countries (HICs); and have primarily included participants of European ancestry (Popejoy and Fullerton, 2016, Peterson et al., 2019b). For example, up to 78% of all included participants in GWAS are of European ancestry (Sirugo et al 2019; Duncan et al., 2019), **Figure 1.1.** This lack of diversity in studied populations limits the generalisability of trait associations and effect sizes (Duncan et al., 2019, Popejoy and Fullerton, 2016). Specifically, variants found to be associated with complex traits and diseases in European ancestral populations have not been widely replicated in other population groups (Duncan et al., 2019, Popejoy and Fullerton, 2016). Thus, there is a need to improve the ancestral diversity of such studies (Martin et al., 2018, Nielsen et al., 2017).

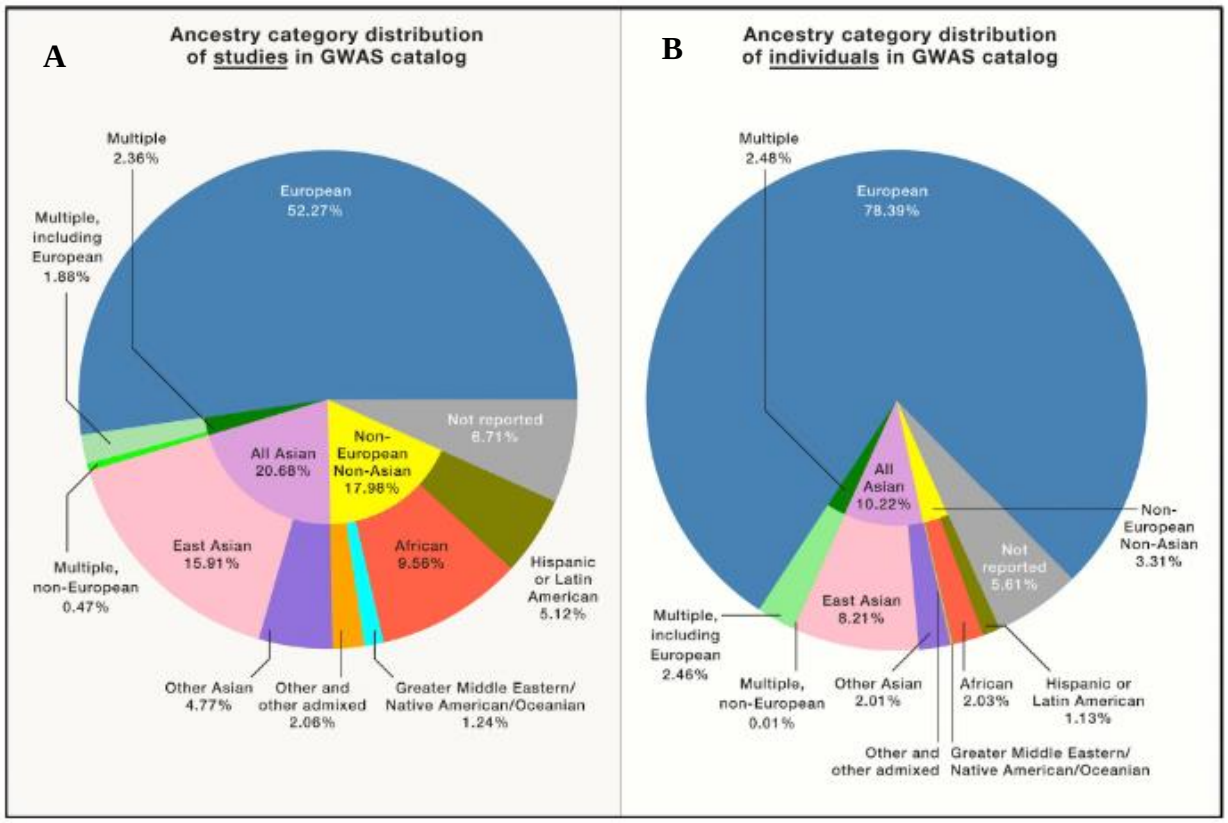

***Figure 1. 1 Distribution of ancestry categories in the GWAS catalogue based on (A) the study and (B) the total number of individuals* (Sirugo et al.,2019).**

From a scientific perspective, African genomes are characterised by significant diversity and unique variants, resulting from historical patterns of the origins of *homo sapiens* in Africa, and subsequent migration and admixture (Martin et al., 2018b, Sirugo et al., 2019). African ancestry populations also exhibit shorter blocks of linkage disequilibrium (LD, i.e., the non-random association of alleles of different loci) which allows for improved resolution in fine-mapping causal variants (Genovese et al., 2010). Therefore, increased inclusion of populations of African ancestry in global genomics research may provide valuable insights into human evolution (Martin et al., 2018, Tishkoff et al., 2009); and may deepen our understanding of the genetic basis of complex diseases such as mental health disorders (Martin et al., 2018, Sirugo et al., 2019, Nielsen et al., 2017). The inclusion of African populations may also lead to improved generalisability and transferability of GWAS summary statistics and PRS (Nielsen et al., 2017, Tishkoff et al., 2009). Currently, PRS prediction accuracy (using European ancestry summary statistics) is lowest within African populations (**Figure 1.2**) (Martin et al., 2018, Sirugo et al., 2019). Notably, the prediction accuracy and trans-ancestral transferability of PRS may be improved if more diverse populations are included in large-scale genomic studies (Majara et al., 2023, Bigdeli et al., 2019).

Similarly, ancestry-specific differences have also been observed in epigenetic research (Zhang et al., 2019b, Breeze et al., 2022). For example, current chromatin mapping resources, which are key in identifying regulatory elements associated with EWAS loci in tissue and cell types relevant to disease etiology, are European-centric (Breeze et al., 2016, Breeze et al., 2019). As a result, the extent to which the lack of diversity influences the interpretation of EWAS loci in non-European populations remains unknown (Breeze et al., 2022). In addition, a recent EWAS of kidney function reported that, although a similar number of epigenome-wide loci associated with estimated glomerular filtration rate was found in European Americans and African Americans; there were observable trans-ethnic differences in the differentially methylated positions (Breeze et al., 2021). Therefore, building more epigenetic datasets from diverse ancestries is key to draw more robust and informed conclusions regarding the transferability of MRS across ancestries (particularly as environmental and cultural differences may directly impact DNAm) (Breeze et al., 2022, Cecil and Nigg, 2022).

From a translational perspective, improved ancestral diversity in genomics and epigenomics research and increased inclusion of populations of African ancestry may also address current inequities in healthcare delivery between the developed and developing worlds (Whiteford et al., 2013, Bigdeli et al., 2019, Tekola-Ayele and Rotimi, 2015). Such inequities may be exacerbated by competing healthcare priorities, with public mental health care being marginalized (Whiteford et al., 2013). Thus, improved inclusivity and diversity may also help to build PRS and MRS that are useful for underserved populations (Majara et al., 2023).

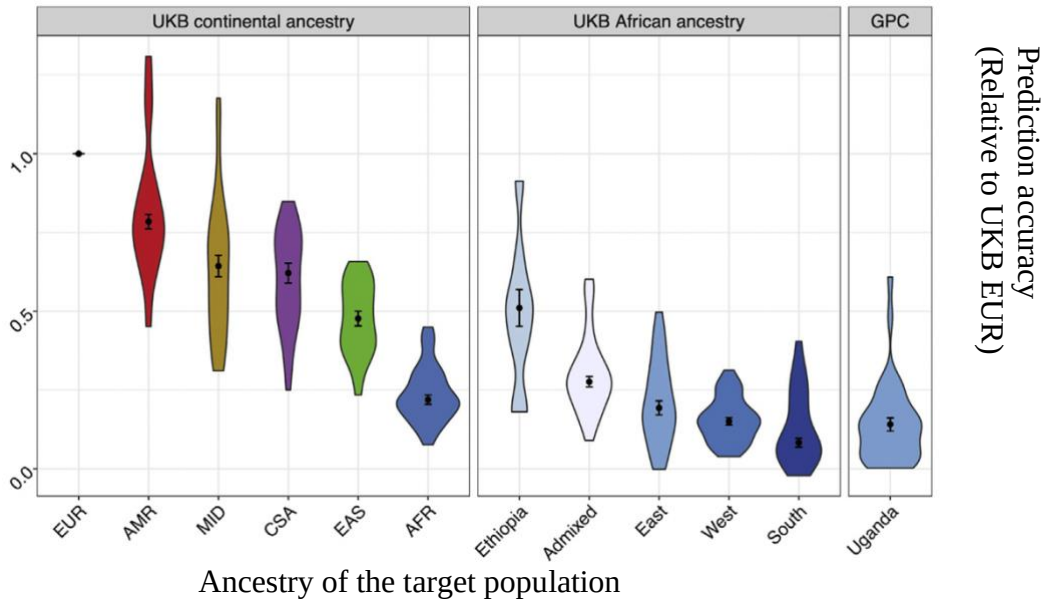

Figure 1. 2. PRS prediction accuracy decreases relative to European (EUR) ancestry individuals in diverse target datasets from the UK Biobank (UKB), (Majara et al., 2023). The target datasets were of globally diverse ancestries including regional African ancestry participants from UKB.

1.5. Study Rationale

Given that genomic and epigenomic mechanisms have emerged as potential risk factors for adverse developmental and mental health outcomes in children (Lester et al., 2012, Bulik-Sullivan et al., 2015), and that such outcomes may increase the risk for mental health disorders in later life (Colman et al., 2007, Copeland et al., 2009, Wachs, 2004), there is a strong rationale for further work in this field. Moreover, the predominant focus thus far on (adult and child) populations of European ancestry (Sirugo et al., 2019, Duncan et al., 2019) underscores the critical need for studies involving participants of other ancestral groups. Considering the complex nature of mental disorders - and that they develop over time, involving both environmental and genetic risk factors - full mechanistic insight is beneficial. It thus requires coordinated sets of several omics data, at multiple time points (Hasin et al., 2017).

A multi-omics approach - integrating genomic and epigenomic data - offers a more in-depth understanding of the biological mechanisms underlying complex and multifactorial disorders such as ADHD (Hasin et al., 2017, Bulik-Sullivan et al., 2015). By examining the interplay between different molecular layers, this approach may reveal new insights into disease aetiology and identify novel biomarkers for early diagnosis and targeted therapy (Hasin et al., 2017). Thus, the integration of genetic (PRS), epigenetic (EAA and MRS), and environmental data may provide a more holistic view of how these factors interact to influence child development and mental health outcomes (Lester et al., 2012, Bulik-Sullivan et al., 2015).

Specifically, PRS may help to identify individuals at higher genetic risk for certain diseases, potentially informing early interventions and personalised treatment plans. EA, which measures the biological age of tissues based on DNA methylation patterns, may provide insights into the effects of environmental risk factors on ageing and disease risk (Hasin et al., 2017). MRS, which captures methylation changes associated with environmental exposures, may enhance our understanding of the epigenetic influences on health (Zhang et al., 2019a).

In this regard, this doctoral project -employing a multi-omics approach - aimed thus to address these key research gaps by investigating the genetic and epigenetic risk factors for developmental and mental health outcomes in a study population of black African and admixed children in South Africa. To this end, two systematic reviews of the existing literature were undertaken - the first on polygenic risk for adverse developmental and mental health outcomes in childhood and adolescence (*Chapter* 2), and the second on associations between EA deviation and these adverse outcomes (*Chapter 3*). Thereafter, empirical analyses of data collected as part of the ongoing Drakenstein Child Health Study (DCHS) in South Africa, were undertaken (*Chapters 4 to 6*). The fourth chapter explores potential genetic associations, herein PRS for ADHD is generated, thereafter investigates the relationship between ADHD PRS and developmental as well as mental health outcomes in the DCHS children. Potential epigenetic associations are explored via analyses of EA deviation at birth (*Chapter 5*), generating ADHD MRS (*Chapter 6*), and investigating potential associations with the outcomes of interest in the DCHS children. The final chapter of this thesis (*Chapter 7*) provides an overall discussion - summarising key findings, outlining noteworthy limitations, and presenting considerations for future research in this field. Collectively, the findings of this thesis may thus ultimately contribute new knowledge to the understanding of the genetic and epigenetic underpinnings of child development and mental health, particularly in under-represented population groups.

1.6. Aims, Objectives and Hypotheses

Aim: To investigate genetic and epigenetic associations with development and mental health in childhood

Objectives

1. To systematically review the existing evidence on the contribution of polygenic risk to development and mental health in childhood and adolescence - *Chapter 2*

2. To systematically review the existing evidence on the association between epigenetic age (EA) deviation and development and mental health in childhood and adolescence - *Chapter 3*

3. To examine whether polygenic risk for attention-deficit/hyperactivity disorder (ADHD) is associated with development and mental health in childhood in the Drakenstein Child Health Study (DCHS) - *Chapter 4*

   Hypothesis: ADHD PRS will be associated with adverse child development and mental health outcomes in the DCHS.

4. To investigate the association between gestational EA deviation and development and mental health outcomes in childhood in the study population - *Chapter 5*

   Hypothesis: Gestational EA deviation will be associated with adverse child developmental and mental health outcomes in the DCHS.

5. To examine the association between DNA methylation risk for ADHD (based on CpG sites) and development and mental health in childhood in the study population - *Chapter 6*

   Hypothesis: ADHD MRS will be associated with adverse child developmental and mental health outcomes in the DCHS.

1.7. References

Bagner, d. M., rodríguez, g. M., blake, c. A., linares, d. & carter, a. S. 2012. Assessment of behavioral and emotional problems in infancy: a systematic review. *Clin child fam psychol rev,* 15**,** 113-28.

Barbu, m. C., amador, c., kwong, a. S. F., shen, x., adams, m. J., howard, d. M., walker, r. M., morris, s. W., min, j. L., liu, c., van dongen, j., ghanbari, m., relton, c., porteous, d. J., campbell, a., evans, k. L., whalley, h. C. & mcintosh, a. M. 2022. Complex trait methylation scores in the prediction of major depressive disorder. *Ebiomedicine,* 79**,** 104000.

Barker, e. D. 2018. Epigenetics, early adversity and child and adolescent mental health. *Psychopathology,* 51**,** 71-75.

Bigdeli, t. B., genovese, g., georgakopoulos, p., meyers, j. L., peterson, r. E., iyegbe, c. O., medeiros, h., valderrama, j., achtyes, e. D., kotov, r., stahl, e. A., abbott, c., azevedo, m. H., belliveau, r. A., bevilacqua, e., bromet, e. J., byerley, w., carvalho, c. B., chapman, s. B., delisi, l. E., dumont, a. L., o'dushlaine, c., evgrafov, o. V., fochtmann, l. J., gage, d., kennedy, j. L., kinkead, b., macedo, a., moran, j. L., morley, c. P., dewan, m. J., nemesh, j., perkins, d. O., purcell, s. M., rakofsky, j. J., scolnick, e. M., sklar, b. M., sklar, p., smoller, j. W., sullivan, p. F., macciardi, f., marder, s. R., gur, r. C., gur, r. E., braff, d. L., calkins, m. E., freedman, r. R., green, m. F., greenwood, t. A., lazzeroni, l. C., light, g. A., nuechterlein, k. H., radant, a. D., seidman, l. J., siever, l. J., silverman, j. M., stone, w. S., sugar, c. A., swerdlow, n. R., tsuang, d. W., tsuang, m. T., turetsky, b. I., nicolini, h., escamilla, m. A., vawter, m. P., sobell, j. L., malaspina, d., lehrer, d. S., buckley, p. F., rapaport, m. H., knowles, j. A., fanous, a. H., pato, m. T., mccarroll, s. A. & pato, c. N. 2019. Contributions of common genetic variants to risk of schizophrenia among individuals of african and latino ancestry. *Molecular psychiatry***,** 1-13.

Black, m. M., walker, s. P., fernald, l. C. H., andersen, c. T., digirolamo, a. M., lu, c., mccoy, d. C., fink, g., shawar, y. R., shiffman, j., devercelli, a. E., wodon, q. T., vargas-barón, e. & grantham-mcgregor, s. 2017. Early childhood development coming of age: science through the life course. *Lancet,* 389**,** 77-90.

Breeze, c. E., batorsky, a., lee, m. K., szeto, m. D., xu, x., mccartney, d. L., jiang, r., patki, a., kramer, h. J., eales, j. M., raffield, l., lange, l., lange, e., durda, p., liu, y., tracy, r. P., van den berg, d., evans, k. L., kraus, w. E., shah, s., tiwari, h. K., hou, l., whitsel, e. A., jiang, x., charchar, f. J., baccarelli, a. A., rich, s. S., morris, a. P., irvin, m. R., arnett, d. K., hauser, e. R., rotter, j. I., correa, a., hayward, c., horvath, s., marioni, r. E., tomaszewski, m., beck, s., berndt, s. I., london, s. J., mychaleckyj, j. C. & franceschini, n. 2021. Epigenome-wide association study of kidney function identifies trans-ethnic and ethnic-specific loci. *Genome med,* 13**,** 74.

Breeze, c. E., paul, d. S., van dongen, j., butcher, l. M., ambrose, j. C., barrett, j. E., lowe, r., rakyan, v. K., iotchkova, v., frontini, m., downes, k., ouwehand, w. H., laperle, j., jacques, p., bourque, g., bergmann, a. K., siebert, r., vellenga, e., saeed, s., matarese, f., martens, j. H. A., stunnenberg, h. G., teschendorff, a. E., herrero, j., birney, e., dunham, i. & beck, s. 2016. Eforge: a tool for identifying cell type-specific signal in epigenomic data. *Cell rep,* 17**,** 2137-2150.

Breeze, c. E., reynolds, a. P., van dongen, j., dunham, i., lazar, j., neph, s., vierstra, j., bourque, g., teschendorff, a. E., stamatoyannopoulos, j. A. & beck, s. 2019. Eforge v2.0: updated analysis of cell type-specific signal in epigenomic data. *Bioinformatics,* 35**,** 4767-4769.

Breeze, c. E., wong, j. Y. Y., beck, s., berndt, s. I. & franceschini, n. 2022. Diversity in ewas: current state, challenges, and solutions. *Genome med,* 14**,** 71.

Bulik-sullivan, b., finucane, h. K., anttila, v., gusev, a., day, f. R., loh, p. R., duncan, l., perry, j. R., patterson, n., robinson, e. B., daly, m. J., price, a. L. & neale, b. M. 2015. An atlas of genetic correlations across human diseases and traits. *Nat genet,* 47**,** 1236-41.

Caramaschi, d., neumann, a., cardenas, a., tindula, g., alemany, s., zillich, l., pesce, g., lahti, j. M. T., havdahl, a., mulder, r., felix, j. F., tiemeier, h., sirignano, l., frank, j., witt, s. H., rietschel, m., deuschle, m., huen, k., eskenazi, b., send, t. S., ferrer, m., gilles, m., de agostini, m., baïz, n., rifas-shiman, s. L., kvist, t., czamara, d., tuominen, s. T., relton, c. L., rai, d., london, s. J., räikkönen, k., holland, n., annesi-maesano, i., streit, f., hivert, m. F., oken, e., sunyer, j., cecil, c. A. M. & sharp, g. 2022. Meta-analysis

of epigenome-wide associations between dna methylation at birth and childhood cognitive skills. *Molecular psychiatry 2022 27:4,* 27**,** 2126-2135.

Cecil, c. A. M. & nigg, j. T. 2022. Epigenetics and adhd: reflections on current knowledge, research priorities and translational potential. *Mol diagn ther,* 26**,** 581-606.

Chen, w., zhou, k., sham, p., franke, b., kuntsi, j., campbell, d., fleischman, k., knight, j., andreou, p., arnold, r., altink, m., boer, f., boholst, m. J., buschgens, c., butler, l., christiansen, h., fliers, e., howe-forbes, r., gabriëls, i., heise, a., korn-lubetzki, i., marco, r., medad, s., minderaa, r., müller, u. C., mulligan, a., psychogiou, l., rommelse, n., sethna, v., uebel, h., mcguffin, p., plomin, r., banaschewski, t., buitelaar, j., ebstein, r., eisenberg, j., gill, m., manor, i., miranda, a., mulas, f., oades, r. D., roeyers, h., rothenberger, a., sergeant, j., sonuga-barke, e., steinhausen, h. C., taylor, e., thompson, m., faraone, s. V. & asherson, p. 2008. Dsm-iv combined type adhd shows familial association with sibling trait scores: a sampling strategy for qtl linkage. *Am j med genet b neuropsychiatr genet,* 147b**,** 1450-60.

Christianson, a. L., zwane, m. E., manga, p., rosen, e., venter, a., downs, d. & kromberg, j. G. 2002. Children with intellectual disability in rural south africa: prevalence and associated disability. *J intellect disabil res,* 46**,** 179-86.

Czamara, d., eraslan, g., page, c. M., lahti, j., lahti-pulkkinen, m., hämäläinen, e., kajantie, e., laivuori, h., villa, p. M., reynolds, r. M., nystad, w., håberg, s. E., london, s. J., o'donnell, k. J., garg, e., meaney, m. J., entringer, s., wadhwa, p. D., buss, c., jones, m. J., lin, d. T. S., macisaac, j. L., kobor, m. S., koen, n., zar, h. J., koenen, k. C., dalvie, s., stein, d. J., kondofersky, i., müller, n. S., theis, f. J., räikkönen, k. & binder, e. B. 2019. Integrated analysis of environmental and genetic influences on cord blood dna methylation in new-borns. *Nat commun,* 10**,** 2548.

Demontis, d., walters, g. B., athanasiadis, g., walters, r., therrien, k., nielsen, t. T., farajzadeh, l., voloudakis, g., bendl, j., zeng, b., zhang, w., grove, j., als, t. D., duan, j., satterstrom, f. K., bybjerg-grauholm, j., bækved-hansen, m., gudmundsson, o. O., magnusson, s. H., baldursson, g., davidsdottir, k., haraldsdottir, g. S., agerbo, e., hoffman, g. E., dalsgaard, s., martin, j., ribasés, m., boomsma, d. I., soler artigas, m., roth mota, n., howrigan, d., medland, s. E., zayats, t., rajagopal, v. M., havdahl, a., doyle, a., reif, a., thapar, a., cormand, b., liao, c., burton, c., bau, c. H. D., rovaris, d. L., sonuga-barke, e., corfield, e., grevet, e. H., larsson, h., gizer, i. R., waldman, i., brikell, i., haavik, j., crosbie, j., mcgough, j., kuntsi, j., glessner, j., langley, k., lesch, k.-p., rohde, l. A., hutz, m. H., klein, m., bellgrove, m., tesli, m., o'donovan, m. C., andreassen, o. A., leung, p. W. L., pan, p. M., joober, r., schachar, r., loo, s., witt, s. H., reichborn-kjennerud, t., banaschewski, t., hawi, z., daly, m. J., mors, o., nordentoft, m., mors, o., hougaard, d. M., mortensen, p. B., daly, m. J., faraone, s. V., stefansson, h., roussos, p., franke, b., werge, t., neale, b. M., stefansson, k., børglum, a. D., consortium, a. W. G. O. T. P. G. & i, p.-b. C. 2023. Genome-wide analyses of adhd identify 27 risk loci, refine the genetic architecture and implicate several cognitive domains. *Nature genetics,* 55**,** 198-208.

Demontis, d., walters, r. K., martin, j., mattheisen, m., als, t. D., agerbo, e., baldursson, g., belliveau, r., bybjerg-grauholm, j., bækvad-hansen, m., cerrato, f., chambert, k., churchhouse, c., dumont, a., eriksson, n., gandal, m., goldstein, j. I., grasby, k. L., grove, j., gudmundsson, o. O., hansen, c. S., hauberg, m. E., hollegaard, m. V., howrigan, d. P., huang, h., maller, j. B., martin, a. R., martin, n. G., moran, j., pallesen, j., palmer, d. S., pedersen, c. B., pedersen, m. G., poterba, t., poulsen, j. B., ripke, s., robinson, e. B., satterstrom, f. K., stefansson, h., stevens, c., turley, p., walters, g. B., won, h., wright, m. J., albayrak, ö., anney, r. J. L., arranz, m. J., banaschewski, t. J., bau, c., biederman, j., buitelaar, j. K., casas, m., charach, a., crosbie, j., dempfle, a., doyle, a. E., ebstein, r. P., elia, j., freitag, c., föcker, m., gill, m., grevet, e., hawi, z., hebebrand, j., herpertz-dahlmann, b., hervas, a., hinney, a., hohmann, s., holmans, p., hutz, m., ickowitz, a., johansson, s., kent, l., kittel-schneider, s., lambregts-rommelse, n., lehmkuhl, g., loo, s. K., mcgough, j. J., meyer, j., mick, e., middletion, f., miranda, a., mota, n. R., mulas, f., mulligan, a., nelson, f., nguyen, t. T., oades, r. D., o'donovan, m. C., owen, m. J., palmason, h., ramos-quiroga, j. A., renner, t. J., ribasés, m., rietschel, m., rivero, o., romanos, j., romanos, m., rothenberger, a., royers, h., et al. 2019. Discovery of the first genome-wide significant risk loci for attention deficit/hyperactivity disorder. *Nature genetics,* 51**,** 63-75.

Edvardson, s., baumann, a. M., mühlenhoff, m., stephan, o., kuss, a. W., shaag, a., he, l., zenvirt, s., tanzi, r., gerardy-schahn, r. & elpeleg, o. 2013. West syndrome caused by st3gal-iii deficiency. *Epilepsia,* 54**,** e24-7.

Fieggen, k. J., lambie, l. A. & donald, k. A. 2019. *Investigating developmental delay in south africa: a pragmatic approach*.

Genovese, g., friedman, d. J., ross, m. D., lecordier, l., uzureau, p., freedman, b. I., bowden, d. W., langefeld, c. D., oleksyk, t. K., uscinski knob, a. L., bernhardy, a. J., hicks, p. J., nelson, g. W., vanhollebeke, b., winkler, c. A., kopp, j. B., pays, e. & pollak, m. R. 2010. Association of trypanolytic apol1 variants with kidney disease in african americans. *Science,* 329**,** 841-5.

Hannon, e., dempster, e., viana, j., burrage, j., smith, a. R., macdonald, r., st clair, d., mustard, c., breen, g., therman, s., kaprio, j., toulopoulou, t., hulshoff pol, h. E., bohlken, m. M., kahn, r. S., nenadic, i., hultman, c. M., murray, r. M., collier, d. A., bass, n., gurling, h., mcquillin, a., schalkwyk, l. & mill, j. 2016. An integrated genetic-epigenetic analysis of schizophrenia: evidence for co-localization of genetic associations and differential dna methylation. *Genome biol,* 17**,** 176.

Horvath, s., pirazzini, c., bacalini, m. G., gentilini, d., di blasio, a. M., delledonne, m., mari, d., arosio, b., monti, d., passarino, g., de rango, f., d'aquila, p., giuliani, c., marasco, e., collino, s., descombes, p., garagnani, p. & franceschi, c. 2015. Decreased epigenetic age of pbmcs from italian semi-supercentenarians and their offspring. *Aging (albany ny),* 7**,** 1159-70.

Hu, h., eggers, k., chen, w., garshasbi, m., motazacker, m. M., wrogemann, k., kahrizi, k., tzschach, a., hosseini, m., bahman, i., hucho, t., mühlenhoff, m., gerardy-schahn, r., najmabadi, h., ropers, h. H. & kuss, a. W. 2011. St3gal3 mutations impair the development of higher cognitive functions. *Am j hum genet,* 89**,** 407-14.

Jaenisch, r. & bird, a. 2003. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. *Nat genet,* 33 suppl**,** 245-54.

Kieling, c., baker-henningham, h., belfer, m., conti, g., ertem, i., omigbodun, o., rohde, l. A., srinath, s., ulkuer, n. & rahman, a. 2011. Child and adolescent mental health worldwide: evidence for action. *Lancet,* 378**,** 1515-25.

Kim, j. H., kim, j. Y., lee, j., jeong, g. H., lee, e., lee, s., lee, k. H., kronbichler, a., stubbs, b., solmi, m., koyanagi, a., hong, s. H., dragioti, e., jacob, l., brunoni, a. R., carvalho, a. F., radua, j., thompson, t., smith, l., oh, h., yang, l., grabovac, i., schuch, f., fornaro, m., stickley, a., rais, t. B., salazar de pablo, g., shin, j. I. & fusar-poli, p. 2020. Environmental risk factors, protective factors, and peripheral biomarkers for adhd: an umbrella review. *Lancet psychiatry,* 7**,** 955-970.

Lai, c. S., gerrelli, d., monaco, a. P., fisher, s. E. & copp, a. J. 2003. Foxp2 expression during brain development coincides with adult sites of pathology in a severe speech and language disorder. *Brain,* 126**,** 2455-62.

Lester, b. M., marsit, c. J., conradt, e., bromer, c. & padbury, j. F. 2012. Behavioral epigenetics and the developmental origins of child mental health disorders. *J dev orig health dis,* 3**,** 395-408.

Majara, l., kalungi, a., koen, n., tsuo, k., wang, y., gupta, r., nkambule, l. L., zar, h., stein, d. J., kinyanda, e., atkinson, e. G. & martin, a. R. 2023. Low and differential polygenic score generalisability among african populations due largely to genetic diversity. *Hgg adv,* 4**,** 100184.

Mccoy, d. C., peet, e. D., ezzati, m., danaei, g., black, m. M., sudfeld, c. R., fawzi, w. & fink, g. 2016. Early childhood developmental status in low- and middle-income countries: national, regional, and global prevalence estimates using predictive modeling. *Plos med,* 13**,** e1002034.

Mithyantha, r., kneen, r., mccann, e. & gladstone, m. 2017. Current evidence-based recommendations on investigating children with global developmental delay. *Arch dis child,* 102**,** 1071-1076.

Neumann, a., walton, e., alemany, s., cecil, c., gonzález, j. R., jima, d. D., lahti, j., tuominen, s. T., barker, e. D., binder, e., caramaschi, d., carracedo, á., czamara, d., evandt, j., felix, j. F., fuemmeler, b. F., gutzkow, k. B., hoyo, c., julvez, j., kajantie, e., laivuori, h., maguire, r., maitre, l., murphy, s. K., murcia, m., villa, p. M., sharp, g., sunyer, j., raikkönen, k., bakermans-kranenburg, m., ijzendoorn, m. V., guxens, m., relton, c. L. & tiemeier, h. 2020. Association between dna methylation and adhd symptoms from birth to school age: a prospective meta-analysis. *Translational psychiatry,* 10**,** 30-30.

Peterson, r. E., kuchenbaecker, k., walters, r. K., chen, c. Y., popejoy, a. B., periyasamy, s., lam, m., iyegbe, c., strawbridge, r. J., brick, l., carey, c. E., martin, a. R., meyers, j. L., su, j., chen, j., edwards, a. C., kalungi, a., koen, n., majara, l., schwarz, e., smoller, j. W., stahl, e. A., sullivan, p. F., vassos, e., mowry, b., prieto, m. L., cuellar-barboza, a., bigdeli, t. B., edenberg, h. J., huang, h. & duncan, l. E. 2019. Genome-wide association studies in ancestrally diverse populations: opportunities, methods, pitfalls, and recommendations. *Cell,* 179**,** 589-603.

Popejoy, a. B. & fullerton, s. M. 2016. Genomics is failing on diversity. *Nature,* 538**,** 161-164.

Purcell, s. M., wray, n. R., stone, j. L., visscher, p. M., o'donovan, m. C., sullivan, p. F. & sklar, p. 2009. Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. *Nature,* 460**,** 748-52.

Scott, j. G., mihalopoulos, c., erskine, h. E., roberts, j. & rahman, a. 2016. Childhood mental and developmental disorders. *Disease control priorities, third edition (volume 4): mental, neurological, and substance use disorders***,** 145-161.

Szyf, m. & bick, j. 2013. Dna methylation: a mechanism for embedding early life experiences in the genome. *Child dev,* 84**,** 49-57.

Tekola-ayele, f. & rotimi, c. N. 2015. Translational genomics in low- and middle-income countries: opportunities and challenges. *Public health genomics,* 18**,** 242-7.

Van dongen, j., nivard, m. G., willemsen, g., hottenga, j. J., helmer, q., dolan, c. V., ehli, e. A., davies, g. E., van iterson, m., breeze, c. E., beck, s., suchiman, h. E., jansen, r., van meurs, j. B., heijmans, b. T., slagboom, p. E. & boomsma, d. I. 2016. Genetic and environmental influences interact with age and sex in shaping the human methylome. *Nat commun,* 7**,** 11115.

Walton, e., pingault, j. B., cecil, c. A., gaunt, t. R., relton, c. L., mill, j. & barker, e. D. 2017. Epigenetic profiling of adhd symptoms trajectories: a prospective, methylome-wide study. *Mol psychiatry,* 22**,** 250-256.

Whiteford, h. A., degenhardt, l., rehm, j., baxter, a. J., ferrari, a. J., erskine, h. E., charlson, f. J., norman, r. E., flaxman, a. D., johns, n., burstein, r., murray, c. J. & vos, t. 2013. Global burden of disease attributable to mental and substance use disorders: findings from the global burden of disease study 2010. *Lancet,* 382**,** 1575-86.

Wray, n. R., yang, j., hayes, b. J., price, a. L., goddard, m. E. & visscher, p. M. 2013. Pitfalls of predicting complex traits from snps. *Nat rev genet,* 14**,** 507-15.

Zhang, f., chen, w., zhu, z., zhang, q., nabais, m. F., qi, t., deary, i. J., wray, n. R., visscher, p. M., mcrae, a. F. & yang, j. 2019a. Osca: a tool for omic-data-based complex trait analysis. *Genome biol,* 20**,** 107.

Zhang, q., vallerga, c. L., walker, r. M., lin, t., henders, a. K., montgomery, g. W., he, j., fan, d., fowdar, j., kennedy, m., pitcher, t., pearson, j., halliday, g., kwok, j. B., hickie, i., lewis, s., anderson, t., silburn, p. A., mellick, g. D., harris, s. E., redmond, p., murray, a. D., porteous, d. J., haley, c. S., evans, k. L., mcintosh, a. M., yang, j., gratten, j., marioni, r. E., wray, n. R., deary, i. J., mcrae, a. F. & visscher, p. M. 2019b. Improved precision of epigenetic clock estimates across tissues and its implication for biological ageing. *Genome med,* 11**,** 54.

World health organization (2021). *Mental health of adolescents*. [online] world Health organization. Available at: https://www.who.int/news-room/fact-sheets/detail/adolescent-mental-health.

Pashayan, N., Duffy, S.W., Neal, D.E., Hamdy, F.C., Donovan, J.L., Martin, R.M., Harrington, P., Benlloch, S., Amin Al Olama, A., Shah, M., Kote-Jarai, Z., Easton, D.F., Eeles, R. and Pharoah, P.D. (2015). Implications of polygenic risk-stratified screening for prostate cancer on overdiagnosis. Genetics in Medicine, 17(10), pp.789–795. doi:https://doi.org/10.1038/gim.2014.192.

Seibert, T.M., Fan, C.C., Wang, Y., Zuber, V., Karunamuni, R., Parsons, J.K., Eeles, R.A., Easton, D.F., Kote-Jarai, Zs., Olama, A.A.A., Garcia, S.B., Muir, K., Grönberg, H., Wiklund, F., Aly, M., Schleutker, J., Sipeky, C., Tammela, T.L., Nordestgaard, B.G. and Nielsen, S.F. (2018). Polygenic hazard score to guide screening for aggressive prostate cancer: development and validation in large scale cohorts. BMJ, [online] 360, p.j5757. doi:https://doi.org/10.1136/bmj.j5757.

Mega, J.L., Stitziel, N.O., Smith, J.G., Chasman, D.I., Caulfield, M.J., Devlin, J.J., Nordio, F., Hyde, C.L., Cannon, C.P., Sacks, F.M., Poulter, N.R., Sever, P.S., Ridker, P.M., Braunwald, E., Melander, O., Kathiresan, S. and Sabatine, M.S. (2015). Genetic risk, coronary heart disease events, and the

clinical benefit of statin therapy: an analysis of primary and secondary prevention trials. The Lancet, [online] 385(9984), pp.2264–2271. doi:https://doi.org/10.1016/s0140-6736(14)61730-x.

Tada, H., Melander, O., Louie, J.Z., Catanese, J.J., Rowland, C.M., Devlin, J.J., Kathiresan, S. and Shiffman, D. (2016). Risk prediction by genetic risk scores for coronary heart disease is independent of self-reported family history. European Heart Journal, [online] 37(6), pp.561–567. doi:https://doi.org/10.1093/eurheartj/ehv462.

Natarajan, P., Young, R., Stitziel, N.O., Padmanabhan, S., Baber, U., Mehran, R., Sartori, S., Fuster, V., Reilly, D.F., Butterworth, A., Rader, D.J., Ford, I., Sattar, N. and Kathiresan, S. (2017). Polygenic Risk Score Identifies Subgroup With Higher Burden of Atherosclerosis and Greater Relative Benefit From Statin Therapy in the Primary Prevention Setting. Circulation, 135(22), pp.2091–2101. doi:https://doi.org/10.1161/circulationaha.116.024436.

Desikan, R.S., Fan, C.C., Wang, Y., Schork, A.J., Cabral, H.J., Cupples, L.A., Thompson, W.K., Besser, L., Kukull, W.A., Holland, D., Chen, C.-H., Brewer, J.B., Karow, D.S., Kauppi, K., Witoelar, A., Karch, C.M., Bonham, L.W., Yokoyama, J.S., Rosen, H.J. and Miller, B.L. (2017). Genetic assessment of age-associated Alzheimer disease risk: Development and validation of a polygenic hazard score. PLOS Medicine, 14(3), p.e1002258. doi:https://doi.org/10.1371/journal.pmed.1002258.

Hasin, Y., Seldin, M. and Lusis, A. (2017). Multi-omics approaches to disease. Genome Biology, 18(1). doi:https://doi.org/10.1186/s13059-017-1215-1.

Chapter 2: Polygenic risk associations with developmental and mental health outcomes in childhood and adolescence: A systematic review

This chapter is currently available in pre-print format (non-peer-reviewed) ahead of submission for publication: https://www.medrxiv.org/content/10.1101/2023.03.31.23287877v1**.**

2.1. Abstract

Background:

Neurodevelopmental and mental health disorders in childhood constitute an emerging global concern, with adverse sequelae which span children's physical, psychological and social well-being. The aetiology of these disorders is likely complex, multifactorial and polygenic. Polygenic risk scores (PRS), an estimate of an individual's genetic liability toward a disorder, have been increasingly used in psychiatric research to explore genetic associations with disorders of interest. However, limited work delineates polygenic associations with development and mental health in childhood populations.
We aimed to systematically review existing literature on associations between genetic risk (as measured by PRS) and neurodevelopmental and mental health outcomes in childhood and adolescence.

Methods:
Following the recommended Preferred Reporting Items for Meta-Analyses (PRISMA) guidelines, databases were searched using key search terms. The search commenced in March 2021. The studies eligible for inclusion were full-text articles investigating polygenic risk associations with neurodevelopmental and/or mental health outcomes in childhood or adolescence.

Results and conclusion:
Fourteen studies were eligible for inclusion in this systematic review. The association between higher PRS for attention-deficit/hyperactivity disorder (ADHD) and adverse developmental/mental health outcomes in childhood and adolescence was reported by five studies. Additionally, associations between PRS for bipolar disorder or major depressive disorder and adverse outcomes of interest were also described by two studies; and two studies highlighted associations between schizophrenia PRS and mental health disorders in childhood. The remaining studies highlighted shared polygenic contributions between and within neurodevelopmental and mental health disorders in children.

The findings of this systematic review suggest that PRS for neurodevelopmental and mental health disorders may be associated with adverse neurodevelopmental and mental health outcomes from early childhood to adolescence. In addition, these associations seemed not to be phenotype-specific, suggesting potential shared genetic variation across the phenotypes of interest.

2.2. Introduction

As discussed in *Chapter 1*, childhood developmental and mental health disorders – encompassing neurodevelopmental, behavioural and emotional disorders – are an emerging global concern, with adverse effects on children's physical, psychological and social well-being (Scott et al., 2016). Children with neurodevelopmental disorders (NDDs) often exhibit high rates of co-morbid mental health disorders – including internalising symptoms or disorders (e.g., anxiety, fear, sadness, depression, social withdrawal and somatic complaints); and/or externalising symptoms or disorders (e.g., aggression, ADHD and conduct disorder (CD)) (Carragher et al., 2015). NDDs and co-morbid mental health disorders in childhood are often also associated with cognitive and emotional regulation impairments (Tajik-Parvinchi et al., 2021) and difficulties in social communication and interactions (Barkley et al., 2006, Feder and Majnemer, 2007). Thus, childhood-onset NDDs and mental health disorders may impede educational and occupational achievement later in life (Masten et al., 2010, Sallis et al., 2019); and have been found to precede a range of adverse mental health sequelae across the lifespan (Cannon et al., 2002).

As discussed previously, the aetiology of NDDs and mental health disorders is complex and multifactorial (Sullivan and Geschwind, 2019); evidence suggests that shared, polygenic contributions may exist (Sullivan and Geschwind, 2019). GWAS, while powerful, have limited predictive power and results thereof are often non-specific - i.e. GWAS of mental health disorders are often not unique to a specific disorder (Wray et al., 2021). Thus, PRS have been increasingly used in psychiatric research to operationalise genetic risk associations with phenotypes of interest (Wray et al., 2021, Murray et al., 2021). These scores are calculated by summing the risk alleles an individual (from a target group) possesses, weighted by the effect size estimates (obtained from GWAS summary statistics from a discovery cohort) for a particular set of SNPs (Purcell et al., 2009, Choi et al., 2020).

Emerging population-based studies have suggested that PRS for NDDs and mental health disorders may be associated with behavioural problems in early childhood (Jansen et al., 2020, Krapohl et al., 2016). For example, in their recent study investigating polygenic risk associations for ADHD and autism spectrum disorder (ASD) in an ADHD and ASD clinical population (in 688 children and adolescents versus 943 adult controls), Jansen and colleagues found that a PRS for adult ADHD was significantly associated with ADHD diagnostic status in children and adolescents (Jansen et al., 2020). Similarly, a PRS for ADHD (in adults) has also been found to associate with attention and/or hyperactivity traits in children (Groen-Blokhuis et al., 2014; Martin et al., 2014); and with general genetic liability toward "broad psychopathology" in childhood (Brikell et al., 2020). There has also been novel work on associations between a PRS for schizophrenia (SCZ) and emotional and behavioural problems in children (Jansen et al., 2018). Further, SCZ PRS have also been associated with general psychopathology (including emotional and behavioural problems) in children (Neumann et al., 2016).

Despite the growing interest in delineating associations between PRS and NDDs/mental health disorders in childhood and adolescence; there is a paucity of such data, particularly when compared to available data in adults (Krapohl et al., 2016, Jansen et al., 2020). Further, to the best of our knowledge, no prior systematic reviews of these studies have been undertaken. Thus, an opportunity exists to delineate the number of studies in this field and the extent of potential associations. To this end, the aim of this analysis was to systematically review the existing literature on associations between genetic risk (measured by PRS) for NDDs and mental health disorders in childhood and adolescence.

2.3. Materials and Methods

The review was conducted per the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines (Moher et al., 2009); and subsequently registered on the International Prospective Register of Systematic Reviews (PROSPERO CRD42021274965). An electronic literature search was conducted using PubMed, Scopus, PsycINFO and ISI Web of Science. The primary reviewer (L.B Moyakhe) developed the search strategy in consultation with a second reviewer and a librarian in the Faculty of Health Sciences (FHS), University of Cape Town (UCT), South Africa. The search terms included "neurodevelopmental disorder," "polygenic score"," polygenic risk," "genetic risk score," "adolescent"," child," "childhood," "youth," and "developmental psychopathology." In addition, the MESH term "Neurodevelopment*" was used in various combinations (and modified as needed for each database). No search restrictions were added. The search commenced on 1 March 2021; full details of the search strategy are provided in the ***Appendix 1*** (Table 2).

2.3.1. Study selection criteria

Full-text published studies evaluating associations between PRS and neurodevelopment (neurodevelopmental outcomes/disorders); and between PRS and mental health (internalising and/or externalising behaviours) in childhood and adolescence were eligible for inclusion. Eligible studies included participants between the ages of two and eighteen years old. Studies of adult populations (older than eighteen); those focusing on neurological disorders or symptoms; animal models or GWAS without a PRS component; and all studies not published in English, were excluded.

2.3.2. Quality assessment

Quality assessment of the studies was conducted by two independent reviewers (L.B. Moyakhe and M. Mufford) using Q-Genie, a quality assessment tool for genetic studies (Sohani et al., 2016). Q-Genie comprises 11 items assessing studies for bias in the development of the research question, in the ascertainment of comparison groups, and in the classification of the outcome. Potential sources of bias and appropriateness of sample sizes were also assessed. Each item was marked on a 7-point Likert scale – studies were rated as moderate-quality if scoring a total of >32 but ≤40 on Q-Genie; and good-quality if scoring > 40. Complete details of the quality assessment ratings for each included study are provided in the ***Appendix 1*** (Table 3).

2.3.3. Data extraction

A data extraction form (adapted from the Cochrane Library's data collection form) (Lefebvre *et al*., 2011) was developed for this review, (***Appendix 1,*** Table 4**)**. The form was pilot-tested using two randomly selected studies that met inclusion criteria; and was further adapted when additional studies presented new information or data. Extracted information included sample demographics (e.g., age, ancestry); outcome phenotype (e.g., ADHD/ ASD, executive functioning, internalising disorders); outcome measure (e.g., the Child Behaviour Checklist); and the reported measures of association (e.g., means and standard deviations or odds ratios). The elimination process involved the removal of duplicates and the appraisal of study titles and abstracts to exclude non-relevant studies.

2.4. Results

2.4.1. Search results

Study selection was undertaken by the PhD candidate (L.B. Moyakhe). The initial electronic literature search yielded a total of 740 papers, of which 94 were duplicates, **Figure 2.1**. Thereafter, 646 papers were screened; and 629 were removed based on title and abstract. The remaining 17 were reviewed as full texts, and 9 were subsequently excluded - they did not meet the inclusion criteria. A total of 8 articles then underwent further assessment, including quality assessment of the studies (i.e., critical appraisal). This process was repeated by the second reviewer (M. Mufford), and any/all discrepancies were discussed and resolved by the two reviewers. An additional 7 papers were incorporated during this process, thus yielding a total of 15 papers that were reviewed and critically appraised. Thereafter, 1 study was removed after failing to meet quality assessment standards). Thus, a final total of 14 studies remained to review.

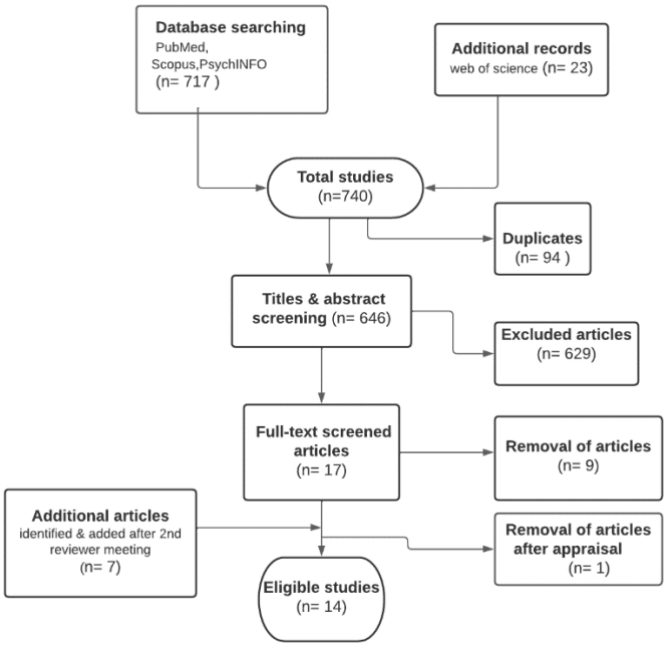

Figure 2. 1 PRISMA flow diagram of study selection

2.4.2. Study and sample characteristics

A detailed description of included studies (n=14) is presented in **Tables 2.1 and 2.2.** Per inclusion criteria for this review, all study participants were ≤18 years of age. Study participants were primarily of European ancestry; and from well-established cohorts – most commonly ALSPAC (Fraser et al., 2013). Included studies could broadly be categorised as either those investigating (i) PRS and neurodevelopmental outcomes (i.e., language, cognition); or (ii) PRS and mental health outcomes.

Table 2. 1Characteristics of included studies – PRS and neurodevelopmental outcomes

Reference	Discovery Cohort(s)	Target Cohort	Age (years)	Outcome Phenotype(s) (measures)	Sample Size	Polygenic Risk Score(s)	Relevant Findings
Aguilar-Lacasaña et al., 2020	• ADHD GWAS N= 55 374 • ASD GWAS N= 46 351	Population-based cohort (BREATHE project)	7 – 11	o Working memory: n-back task o Attention performance: n-back task, ANT (computer version) o ADHD symptoms: DSM-IV	N= 1667	- ADHD PRS - ASD PRS	ADHD PRS assoc with working memory performance
Jansen et al., 2020	• Discovery sample ADHD cases N= 20 183, controls N= 35 191 • Discovery sample ASD cases N= 18 381, controls N= 27 969 • Discovery sample SCZ cases N= 40 675, controls N=64 643 • NESCOG	"Inside out" outpatient sample	6 – 18	o ADHD symptoms (CBCL 1.5- 5 years) o ASD symptoms	• ADHD/ASD N= 688 • ADHD N= 280 • ASD N= 295	- ADHD PRS - ASD PRS - SCZ PRS	ADHD PRS assoc with: - ADHD symptoms - Combined ADHD /ASD symptoms
Martin et al., 2015	Population based GWAS (British & Irish children) with ADHD diagnosis cases N=727 and controls N= 5081	ALSPAC	7 – 10	o IQ (WISC-III) o Verbal working memory: WISC-III-digit span task o Cognitive inhibitory control: Diagnostic Analysis of the Faces subtest, counting span task (TEACh), Opposite worlds task o Facial emotional recognition o ADHD inattentive & impulsive traits: DAWBA o Social communication: SCDC o Pragmatic language scales: CCC	N= 6832	- ADHD PRS (in children)	ADHD PRS assoc with: - IQ - working memory performance

(Mistry et al., 2019b)	PGC working groups: • BD • SCZ • SCZ vs BD GWAS BD cases N= 20 129 SCZ cases N= 33 426	ALSPAC	8	○ Cognitive domains: (WISC-III) ○ Verbal IQ: derived from the information, similarities, arithmetic, vocabulary, comprehension, and forward and backward digit span subtests ○ Performance IQ: derived from the picture completion, picture arrangement, block design, coding and object assembly subtests ○ Total IQ: sum of PIQ and VIQ ○ Processing speed: WISC-III ○ Working memory: Freedom from distractibility score ○ Problem-solving: WISC-III ○ Executive function: TEACh ○ Attention: TEACh ○ Verbal Learning: CTNWR ○ Emotion recognition: DANVA	N= 8230	- BD PRS	BD PRS assoc with: - executive functioning - processing speed - performance IQ

Table 2. 2 Characteristics of included studies – PRS and mental health outcomes

Reference	Discovery Cohort(s)	Target Cohort	Age (years)	Outcome Phenotype(s) (measures)	Sample Size	Polygenic Risk Score(s)	Relevant Findings
Akingbuwa et al., 2020	GWAS data from 7 cohorts: • ALSPAC • Child and adolescent twin study (Sweden) • Generation R • MoBa • Northern Finland Birth Cohort of 1986 • Twins Early Development Study	Meta-analysis of the GWAS cohorts listed under discovery cohort(s) column	6 – 17	○ ADHD symptoms ○ Internalising symptoms ○ Social problems Scales used: ○ SDQ ○ CBCL ○ CBCL (Youth Self Report) ○ SDQ (Conners' Parent Rating Scale) ○ Autism-Tics, ADHD and other Comorbidities Inventory ○ Screen for Child Anxiety, emotional Disorders ○ Short Mood and Feelings Questionnaire ○ Screen for Child Anxiety Related Emotional Disorders ○ Short Mood and Feelings Questionnaire ○ Rating Scale for Disruptive Behavior Disorders	N= 42 998	- MDD PRS - NEU PRS	MD PRS assoc with: - Childhood ADHD symptoms - Internalising problems
Kwong et al., 2021	PGC working groups: • DEP • MDD • ANX • NEU • SCZ	ALSPAC	10 - 24	○ Depressive symptoms (self-reported) (SMFQ)	N= 6302	- DEP PRS - MDD PRS - ANX PRS - NEU PRS - SCZ PRS	- PRS for DEP, MDD, NEU assoc with adverse DEP symptoms

Mistry et al., 2019a	PGC-BD	ALSPAC	7 - 11	○ Emotional and Behavioural difficulties: SDQ ○ Assessment of childhood ADHD: DAWBA and DSM-IV ○ Assessment of borderline personality trait: CI-BPD	N= 8230	- BD PRS	- BD PRS assoc with ADHD symptoms
Nivard et al., 2017	PGC-SCZ2	• NTR • ALSPAC	7 - 15	NTR: ○ Psychopathology: DSM-IV ○ Anxiety: CBCL (maternal rating) ○ Depression: CBCL ○ OCD/ODD: CBCL (maternal rating) ALSPAC: ○ Psychopathology: (DAWBA)	• NTR: N=2588 • ALSPAC: N= 6127	- SCZ PRS	- SCZ PRS assoc with childhood psychopathology
Rice et al., 2019	• PGC-MDD • PGC-ADHD • PGC-SCZ	ALSPAC	10 - 18	○ Depressive symptoms trajectories: SMFQ	N= 7543	- MDD PRS - SCZ PRS - ADHD PRS	- MDD, ADHD and SCZ PRS assoc with DEP symptoms (age 12)
Salvatore et al., 2015	COGA adult GWAS	COGA	12 - 17	○ Subclinical externalising behaviour externalising disorders psychiatric interview ○ Impulsivity-related traits: externalising disorders psychiatric interview	N= 248	- Externalising disorders PRS	- Externalising disorders PRS assoc with subclinical externalising behaviour

Riglin et al., 2018	• PGC-SCZ • PGC-MDD	NCDS	7 - 16	o Emotional problems o Parent report of two depression/anxiety items: Rutter A scale for children (abbreviated version)	N= 5257	- ADHD PRS - MDD PRS	- SCZ PRS assoc with emotional problems (age 7)
Hannigan et al., 2021	PGC-SCZ	MoBa	18 months - 8 years	o Emotional and behavioural psychopathology: (BCL o Anxiety: SCARED o Depression: (MFQ o Disruptive Behaviour Disorders: RS-DBD o Conduct problems: RS-DBD o Hyperactivity and inattention: RS-DBD	N= 15 105	- SCZ PRS	SCZ PRS assoc with: - behavioural and emotional problems - symptoms of conduct disorder, ODD, ADHD in middle childhood

- **Phenotypes**: DEP=depression, MDD= major depressive disorder, ANX= anxiety, NEU= neuroticism and SCZ= schizophrenia SLI= specific language impairment, ASD= Autism spectrum disorder
- **Measures**: SMFQ= short mood and feelings questionnaire; WISC-III= Wechsler Intelligence Scale - III; TEACh= Test of Everyday Attention for Children; DANVA= Diagnostic Analysis of Nonverbal Accuracy; CI-BPD= the Childhood Interview for DSM-IV Borderline Personality Disorder; SCDC= Social & communication Disorders Checklist; CCC= Children's Communication Checklist, K-SADS Danish version of Schedule for Affective Disorders and Schizophrenia for School-Age Children; TROG-2= Test for Reception of Grammar (Danish version); RIST= Reynolds Intellectual Screening Test. SCARED= Screen for Child Anxiety Related Disorders; RS-DBD= Rating Scale for Disruptive Behaviour Disorders; HRTSE= Hit reaction time; ANT= Attention Network Test; CTNWR= Children's Test of Nonword Repetition
- **Cohorts**: PGC= Psychiatric Genomics Consortium, ALSPAC= Avon Longitudinal Study of Parents and Children; COGA = Collaborative Study on the Genetics of Alcoholism, NCDS= National Child Development Study, SAGE= Study of ADHD, Genes and Environment, MoBa= Norwegian Mother, Father, and Child Cohort, NTR= Netherlands Twin Registry, NESCOG= Netherlands Study of Cognition, Environment and Genes, BREATHE = BRain dEvelopment and Air polluTion ultrafine particles in scHool childrEn
- N's shown only for summary statistics provided for each study
- □□denotes an increased or higher variable □
- □□denotes a decreased variable

2.4.3. Quality assessment

Quality assessment within and between studies was validated using the Q-genie checklist (Sohani et al., 2016). Studies included in this review were those rated as good quality (i.e., with scores >40). One study was rated as poor quality and subsequently excluded from further analyses; two were rated as moderate quality, and twelve were rated as good quality (***Appendix 1,*** Table 3). Study limitations based on the Q-genie checklist centred mainly on methodological bias, limited sample size and low power. Due to the heterogeneity in the reported outcome measures and methodologies across the studies, a meta-analysis was not deemed to be feasible in this instance.

2.4.4. Studies investigating PRS and neurodevelopmental outcomes

Six of the included studies investigated associations between PRS for either neurodevelopmental or mental health disorders, and adverse neurodevelopmental outcomes (i.e., language, cognition, and behaviour) in childhood and adolescence, **Table 2.1**. The discovery cohorts included the GWAS of British and Irish children with ADHD (Martin et al., 2015); the Psychiatric Genomics Consortium Bipolar Disorder (PGC-BD) working group (Coleman et al., 2020); and the Psychiatric Genomics Consortium Schizophrenia (PGC-SCZ) working group (Ripke et al., 2014). Predictor phenotypes of interest in these cohorts were ASD, ADHD, bipolar disorder (BD), SCZ, and depression (DEP). Target cohorts included ALSPAC and the BRain dEvelopment and Air polluTion ultrafine particles in scHool childrEn (BREATH) project (Mar et al., 2013). The outcomes of interest included working memory, attention performance, cognitive domains, several aspects of IQ (e.g., performance IQ, verbal IQ etc.) and social communication (e.g., pragmatic language).

Most studies reported associations between ADHD PRS and adverse outcomes in childhood and adolescence was reported – which persisted with ADHD symptom trajectories (Sudre et al., 2020, Jansen et al., 2020, Riglin et al., 2016, Aguilar-Lacasaña et al., 2020, Martin et al., 2015, Nivard et al., 2017). For example, in their study of ADHD symptoms over time (assessed at seven longitudinal time points in participants aged4 to 17 years, Riglin and colleagues (2016) reported symptom persistence in participants with higher ADHD PRS. In line with these findings, Sudre and colleagues (2020) found worsening ADHD symptoms in participants (aged seven to sixteen years) with higher ADHD PRS (Sudre et al., 2020). Similarly, significant associations between ADHD PRS and ADHD clinical diagnosis, and between ADHD PRS and the combined ADHD/ASD status have been reported in children ages six to eighteen (Jansen et al., 2020). Higher ADHD PRS has also been found to be associated with poorer working memory performance and IQ in childhood (Aguilar-Lacasaña et al., 2020, Martin et al., 2015); and with the development of other neurodevelopmental disorders (i.e., social communication problems; impairment of pragmatic language; conduct problems) in children aged seven to nine years old (Riglin et al., 2016). Additionally, SCZ PRS was reported to be associated with ADHD symptoms in children and adolescents ages seven to sixteen years old (Nivard et al., 2017)

Associations between BD PRS and adverse outcomes in childhood were also reported. Specifically, an association was found between higher BD PRS and impaired cognitive function (i.e., poor performance IQ, and processing speed) and poor executive functioning in children aged eight years (Mistry et al., 2019b). A PRS for BD was also found to associate with childhood ADHD (Mistry *et al.*,2019a).

2.4.5. Studies investigating PRS and mental health outcomes

Eight studies investigated associations between PRS for mental health disorders and mental health outcomes in childhood and adolescence, **Table 2.2**. The discovery cohorts included the Major Depressive Disorder working group of the (PGC-MDD) (Wray et al., 2018), as well as PGC-SCZ,PGC-BD,PGC-NEU,PGC-ADHD; the Collaborative Study on the Genetics of Alcoholism (COGA) adult GWAS consortium (Edenberg et al., 2005); and the Netherlands Study of Cognition, Environment and Genes (NESCOG) consortium (Dick et al., 2018). Predictor phenotypes of interest included DEP, MDD, neuroticism (NEU), anxiety (ANX), SCZ and BD (Nivard et al., 2017, Kwong et al., 2021). The relevant target cohorts included ALSPAC (Fraser et al., 2013); the COGA adolescent sample (Edenberg et al., 2005); the Netherlands Twin Registry (NTR) (Ligthart et al., 2019); the "Inside Out" outpatient sample (Jansen et al., 2020); the National Child Development Study (NCDS) (Power and Elliott, 2006); and the Norwegian Mother, Father and Child Cohort (MoBa) (Magnus et al., 2016). Outcomes of interest included ADHD symptoms, internalising symptoms, social problems, depressive symptoms, emotional and behavioural difficulties, and externalising behaviour.

Most studies, higher PRS for mental disorders were associated with poorer mental health outcomes in children and adolescents (Kwong et al., 2021, Akingbuwa et al., 2020, Rice et al., 2019). For example, higher PRS for DEP, MDD and NEU were found to be associated with more severe depressive symptoms in adolescence (Kwong et al., 2021). Further, associations between genetic risk for MDD (indexed by PRS for adults), and psychopathology in children and adolescents (i.e., all ADHD symptoms, internalising problems and social problems) were described (Akingbuwa et al., 2020). A PRS for MDD was also found to be associated with depressive symptom trajectories in early adolescence (Rice et al., 2019).

Associations between SCZ PRS and adverse mental health outcomes were also described (Nivard et al. 2017, Hannigan et al., 2021, Salvatore et al., 2015, Riglin et al., 2018). For example, Nivard and colleagues (2017) reported that SCZ PRS was associated with psychiatric disorders in childhood (i.e., ANX, DEP, oppositional defiant disorder (ODD)/CD); and that the strength of these associations increased with participant age (predominantly for ADHD and ODD/CD) (Nivard et al. 2017). Riglin and colleagues (2018) also found that SCZ PRS associated with social and behavioural problems at the age of four years; as well as reporting associations between SCZ PRS and lower performance IQ, poor outcomes in social understanding and impaired language fluency (at age seven to nine years old). Associations have also been reported between a PRS for SCZ and emotional/behavioural problems in early childhood; as well as with CD, hyperactivity, inattention and ODD in middle childhood (Hannigan et al., 2021). Finally, Salvatore and colleagues (2015) reported that a PRS for externalising behaviour in adults associated with externalising and impulsivity-related behaviour in adolescents (aged twelve to seventeen years).

2.5. Discussion

This systematic review of fourteen eligible studies found a number of associations between PRS for NDDs/mental disorders and neurodevelopmental and mental health outcomes in childhood and adolescence. Five studies showed significant associations between a higher PRS for ADHD, and poorer neurodevelopmental and behavioural outcomes in children (Martin et al., 2015, Aguilar-Lacasaña et al., 2020, Sudre et al., 2020, Jansen et al., 2020, Riglin et al., 2016). These findings are in line with previous work describing associations between higher ADHD PRS and increased risk for neurodevelopmental, externalising and depressive symptoms in participants aged nine to twelve years (Brikell et al., 2020); and between ADHD PRS and pragmatic language difficulties at ages five and eight (Askeland et al., 2019).

Two studies found associations between a higher BD PRS and poorer executive functioning and processing speed in childhood (Mistry et al., 2019b, Mistry et al., 2019a), which is consistent with prior evidence of associations between BD PRS and deficits in executive functioning (Biederman et al., 2021). Similarly, two studies reported that genetic risk for SCZ (indexed by PRS) associated with adverse mental health outcomes in childhood and adolescence, and this association was found to be significant (Riglin et al., 2017, Nivard et al., 2017). Additionally, one study found that a higher SCZ PRS associated with an increased risk of behavioural symptoms, hyperactivity and inattention in early childhood (Nivard et al., 2017). This is in keeping with previously reported findings of associations between SCZ PRS and emotional recognition (i.e. reduced speed of emotion identification) (Germine et al., 2016).

The remaining four studies reported shared genetic variation (i.e., shared polygenic contributions) within and between NDDs and mental health disorders (Mistry et al., 2019a, Kwong et al., 2021, Akingbuwa et al., 2020, (Rice et al., 2019). For example, associations between BD PRS and childhood ADHD were also described (Mistry et al., 2019a). These cross- and within-trait associations are in line with prior work demonstrating associations between a PRS for several psychiatric disorders and depressive symptoms in adolescents (Kwong et al., 2021); as well as associations between an MDD PRS (in adults) and psychopathology in children and adolescents (Akingbuwa et al., 2020). Similarly, associations between SCZ PRS and depressive symptoms have been described previously (Rice et al., 2019). Further, prior cross-sectional and longitudinal studies have reported a higher prevalence of depression in children with ADHD versus controls (Demontis et al., 2019, Copeland et al., 2013, Lee et al., 2013). The genetic overlap observed in the studies included in this systematic review may thus suggest shared biological pathways underlying the neurodevelopmental and mental health outcomes of interest (Solovieff et al., 2013).

There are some noteworthy limitations of the studies included in this systematic review. For example, limited sample sizes of the target cohorts, which reduce the prediction power of PRS, thus impacting the ability to detect significant effects within the phenotypes of interest (Chatterjee et al., 2013). Further, the use of parent-rated assessments of neurodevelopmental and mental health outcomes could have led to over-or under-estimation of these phenotypes (Neumann et al., 2016). Several studies included in this review also used summary statistics from adult GWAS (as discovery datasets). In future, age-matched summary statistics could be informative to explore the age-specific effects of NDDs and mental health disorders (Raffington et al., 2020). Finally – though not an inclusion criterion – studies included in this review were of European ancestry populations, with no representation of other ancestral groups (including African ancestries). This is likely due to the limited representation of ancestrally diverse discovery and target datasets in the existing literature (Peterson et al., 2019a, Cavazos and Witte, 2020). Thus, diversity and inclusivity in such work is needed and would benefit to improve generalisability, trans-ancestral transferability and translational potential of PRS in future (Cavazos and Witte, 2020, Tishkoff et al., 2009, Peterson et al., 2019a).

Limitations specific to this review should also be acknowledged. First – though not formally assessed here – publication bias (e.g., skewing towards studies with positive findings) may have indirectly contributed to the studies included. Second, and due to the search strategy employed, it is possible that not all relevant studies were captured and analysed (potentially due to human error during the elimination of non-relevant studies).

Third, though considered *a priori*, a meta-analysis was not undertaken – due to heterogeneity in study design and outcomes measured across studies.

These limitations notwithstanding, this review – which demonstrates associations between ADHD, BD and SCZ PRS, and adverse neurodevelopmental and mental health outcomes in childhood and adolescence – highlights the emerging interest in the genomic underpinnings of NDDs and mental health disorders (Lester et al., 2012). Further, the genetic overlap observed between these disorders may be suggestive of shared biological pathways. In future, further work incorporating more ancestrally diverse cohorts and discovery datasets (ideally originating from child/adolescent GWAS), may contribute to an improved understanding of the genetic architecture of development and mental health in childhood and adolescence.

2.6. References

Aguilar-lacasaña, s., vilor-tejedor, n., jansen, p. R., lópez-vicente, m., bustamante, m., burgaleta, m., sunyer, j. & alemany, s. 2020. Polygenic risk for adhd and asd and their relation with cognitive measures in school children. *Psychol med***,** 1-9.

Akingbuwa, w. A., hammerschlag, a. R., jami, e. S., allegrini, a. G., karhunen, v., sallis, h., ask, h., askeland, r. B., baselmans, b., diemer, e., hagenbeek, f. A., havdahl, a., hottenga, j. J., mbarek, h., rivadeneira, f., tesli, m., van beijsterveldt, c., breen, g., lewis, c. M., thapar, a., boomsma, d. I., kuja-halkola, r., reichborn-kjennerud, t., magnus, p., rimfeld, k., ystrom, e., jarvelin, m. R., lichtenstein, p., lundstrom, s., munafò, m. R., plomin, r., tiemeier, h., nivard, m. G., bartels, m. & middeldorp, c. M. 2020. Genetic associations between childhood psychopathology and adult depression and associated traits in 42998 individuals: a meta-analysis. *Jama psychiatry,* 77**,** 715-728.

Askeland, r., hannigan, l., ask, h., tesli, m., corfield, e., andreassen, o. A., smith, g. D., reichborn-kjennerud, t. & havdahl, a. 2019. Early manifestations of genetic risk for neurodevelopmental disorders. *European neuropsychopharmacology,* 29**,** s252-s253.

Barkley, r. A., o'connell, t., murphy, k. R., anderson, d. & connor, d. F. 2006. Effects of two doses of alcohol on simulator driving performance in adults with attention-deficit/hyperactivity disorder. *Neuropsychology,* 20**,** 77-87.

Biederman, j., green, a., disalvo, m. & faraone, s. V. 2021. Can polygenic risk scores help identify pediatric bipolar spectrum and related disorders?: a systematic review. *Psychiatry res,* 299**,** 113843.

Brikell, i., larsson, h., lu, y., pettersson, e., chen, q., kuja-halkola, r., karlsson, r., lahey, b. B., lichtenstein, p. & martin, j. 2020. The contribution of common genetic risk variants for adhd to a general factor of childhood psychopathology. *Molecular psychiatry,* 25**,** 1809-1821.

Cannon, m., caspi, a., moffitt, t. E., harrington, h., taylor, a., murray, r. M. & poulton, r. 2002. Evidence for early-childhood, pan-developmental impairment specific to schizophreniform disorder: results from a longitudinal birth cohort. *Arch gen psychiatry,* 59**,** 449-56.

Cavazos, t. B. & witte, j. S. 2020. Inclusion of variants discovered from diverse populations improves polygenic risk score transferability. Biorxiv.

Chatterjee, n., wheeler, b., sampson, j., hartge, p., chanock, s. J. & park, j. H. 2013. Projecting the performance of risk prediction based on polygenic analyses of genome-wide association studies. *Nat genet,* 45**,** 400-5, 405e1-3.

Choi, s. W., mak, t. S. H. & o'reilly, p. F. 2020. Tutorial: a guide to performing polygenic risk score analyses. *Nature protocols.* Nature research.

Coleman, j. R. I., gaspar, h. A., bryois, j. & breen, g. 2020. The genetics of the mood disorder spectrum: genome-wide association analyses of more than 185,000 cases and 439,000 controls. *Biol psychiatry,* 88**,** 169-184.

Copeland, w. E., shanahan, l., erkanli, a., costello, e. J. & angold, a. 2013. Indirect comorbidity in childhood and adolescence. *Front psychiatry,* 4**,** 144.

Demontis, d., walters, r. K., martin, j., mattheisen, m., als, t. D., agerbo, e., baldursson, g., belliveau, r., bybjerg-grauholm, j., bækvad-hansen, m., cerrato, f., chambert, k., churchhouse, c., dumont, a., eriksson, n., gandal, m., goldstein, j. I., grasby, k. L., grove, j., gudmundsson, o. O., hansen, c. S., hauberg, m. E., hollegaard, m. V., howrigan, d. P., huang, h., maller, j. B., martin, a. R., martin, n. G., moran, j., pallesen, j., palmer, d. S., pedersen, c. B., pedersen, m. G., poterba, t., poulsen, j. B., ripke, s., robinson, e. B., satterstrom, f. K., stefansson, h., stevens, c., turley, p., walters, g. B., won, h., wright, m. J., albayrak, ö., anney, r. J. L., arranz, m. J., banaschewski, t. J., bau, c., biederman, j., buitelaar, j. K., casas, m., charach, a., crosbie, j., dempfle, a., doyle, a. E., ebstein, r. P., elia, j., freitag, c., föcker, m., gill, m., grevet, e., hawi, z., hebebrand, j., herpertz-dahlmann, b., hervas, a., hinney, a., hohmann, s., holmans, p., hutz, m., ickowitz, a., johansson, s., kent, l., kittel-schneider, s., lambregts-rommelse, n., lehmkuhl, g., loo, s. K., mcgough, j. J., meyer, j., mick, e., middletion, f., miranda, a., mota, n. R., mulas, f., mulligan, a., nelson, f., nguyen, t. T., oades, r. D., o'donovan, m.

C., owen, m. J., palmason, h., ramos-quiroga, j. A., renner, t. J., ribasés, m., rietschel, m., rivero, o., romanos, j., romanos, m., rothenberger, a., royers, h., et al. 2019. Discovery of the first genome-wide significant risk loci for attention deficit/hyperactivity disorder. *Nature genetics,* 51**,** 63-75.

Dick, d. M., barr, p. B., cho, s. B., cooke, m. E., kuo, s. I. C., lewis, t. J., neale, z., salvatore, j. E., savage, j. & su, j. 2018. Post-gwas in psychiatric genetics: a developmental perspective on the "other" next steps. *Genes, brain and behavior,* 17.

Edenberg, h. J., bierut, l. J., boyce, p., cao, m., cawley, s., chiles, r., doheny, k. F., hansen, m., hinrichs, t., jones, k., kelleher, m., kennedy, g. C., liu, g., marcus, g., mcbride, c., murray, s. S., oliphant, a., pettengill, j., porjesz, b., pugh, e. W., rice, j. P., rubano, t., shannon, s., steeke, r., tischfield, j. A., tsai, y. Y., zhang, c. & begleiter, h. 2005. Description of the data from the collaborative study on the genetics of alcoholism (coga) and single-nucleotidepolymorphism genotyping for genetic analysis workshop 14. *Bmc genetics,* 6**,** 1-8.

Feder, k. P. & majnemer, a. 2007. Handwriting development, competency, and intervention. *Developmental medicine & child neurology,* 49**,** 312-317.

Fraser, a., macdonald-wallis, c., tilling, k., boyd, a., golding, j., davey smith, g., henderson, j., macleod, j., molloy, l., ness, a., ring, s., nelson, s. M. & lawlor, d. A. 2013. Cohort profile: the avon longitudinal study of parents and children: alspac mothers cohort. *Int j epidemiol,* 42**,** 97-110.

Germine, l., robinson, e. B., smoller, j. W., calkins, m. E., moore, t. M., hakonarson, h., daly, m. J., lee, p. H., holmes, a. J., buckner, r. L., gur, r. C. & gur, r. E. 2016. Association between polygenic risk for schizophrenia, neurocognition and social cognition across development. *Translational psychiatry,* 6**,** e924-e924.

Jansen, a. G., dieleman, g. C., jansen, p. R., verhulst, f. C., posthuma, d. & polderman, t. J. C. 2020. Psychiatric polygenic risk scores as predictor for attention deficit/hyperactivity disorder and autism spectrum disorder in a clinical child and adolescent sample. *Behav genet,* 50**,** 203-212.

Krapohl, e., euesden, j., zabaneh, d., pingault, j. B., rimfeld, k., von stumm, s., dale, p. S., breen, g., o'reilly, p. F. & plomin, r. 2016. Phenome-wide analysis of genome-wide polygenic scores. *Mol psychiatry,* 21**,** 1188-93.

Kwong, a. S. F., morris, t. T., pearson, r. M., timpson, n. J., rice, f., stergiakouli, e. & tilling, k. 2021. Polygenic risk for depression, anxiety and neuroticism are associated with the severity and rate of change in depressive symptoms across adolescence. *Journal of child psychology and psychiatry and allied disciplines*.

Lee, s. H., ripke, s., neale, b. M., faraone, s. V., purcell, s. M., perlis, r. H., mowry, b. J., thapar, a., goddard, m. E., witte, j. S., absher, d., agartz, i., akil, h., amin, f., andreassen, o. A., anjorin, a., anney, r., anttila, v., arking, d. E., asherson, p., azevedo, m. H., backlund, l., badner, j. A., bailey, a. J., banaschewski, t., barchas, j. D., barnes, m. R., barrett, t. B., bass, n., battaglia, a., bauer, m., bayés, m., bellivier, f., bergen, s. E., berrettini, w., betancur, c., bettecken, t., biederman, j., binder, e. B., black, d. W., blackwood, d. H., bloss, c. S., boehnke, m., boomsma, d. I., breen, g., breuer, r., bruggeman, r., cormican, p., buccola, n. G., buitelaar, j. K., bunney, w. E., buxbaum, j. D., byerley, w. F., byrne, e. M., caesar, s., cahn, w., cantor, r. M., casas, m., chakravarti, a., chambert, k., choudhury, k., cichon, s., cloninger, c. R., collier, d. A., cook, e. H., coon, h., cormand, b., corvin, a., coryell, w. H., craig, d. W., craig, i. W., crosbie, j., cuccaro, m. L., curtis, d., czamara, d., datta, s., dawson, g., day, r., de geus, e. J., degenhardt, f., djurovic, s., donohoe, g. J., doyle, a. E., duan, j., dudbridge, f., duketis, e., ebstein, r. P., edenberg, h. J., elia, j., ennis, s., etain, b., fanous, a., farmer, a. E., ferrier, i. N., flickinger, m., fombonne, e., foroud, t., frank, j., franke, b., fraser, c., et al. 2013. Genetic relationship between five psychiatric disorders estimated from genome-wide snps. *Nat genet,* 45**,** 984-94.

Ligthart, l., van beijsterveldt, c. E. M., kevenaar, s. T., de zeeuw, e., van bergen, e., bruins, s., pool, r., helmer, q., van dongen, j., hottenga, j. J., van't ent, d., dolan, c. V., davies, g. E., ehli, e. A., bartels, m., willemsen, g., de geus, e. J. C. & boomsma, d. I. 2019. The netherlands twin register: longitudinal research based on twin and twin-family designs. *Twin res hum genet,* 22**,** 623-636.

Magnus, p., birke, c., vejrup, k., haugan, a., alsaker, e., daltveit, a. K., handal, m., haugen, m., høiseth, g., knudsen, g. P., paltiel, l., schreuder, p., tambs, k., vold, l. & stoltenberg, c. 2016. Cohort profile update: the norwegian mother and child cohort study (moba). *Int j epidemiol,* 45**,** 382-8.

Mar, á. P., joan, f., jesús, p., xavier, q. & jordi, s. 2013. Breathe project (brain development and air pollution ultrafine particles in school children): research protocol and first results. *Isee conference abstracts,* 2013**,** 3617-3617.

Martin, j., hamshere, m. L., stergiakouli, e., o'donovan, m. C. & thapar, a. 2015. Neurocognitive abilities in the general population and composite genetic risk scores for attention-deficit hyperactivity disorder. *Journal of child psychology and psychiatry,* 56**,** 648-656.

Masten, a. S., desjardins, c. D., mccormick, c. M., kuo, s. I. & long, j. D. 2010. The significance of childhood competence and problems for adult success in work: a developmental cascade analysis. *Dev psychopathol,* 22**,** 679-94.

Mistry, s., escott-price, v., florio, a. D., smith, d. J. & zammit, s. 2019a. Genetic risk for bipolar disorder and psychopathology from childhood to early adulthood. *Journal of affective disorders,* 246**,** 633-639.

Mistry, s., escott-price, v., florio, a. D., smith, d. J. & zammit, s. 2019b. Investigating associations between genetic risk for bipolar disorder and cognitive functioning in childhood. *Journal of affective disorders,* 259**,** 112-120.

Moher, d., liberati, a., tetzlaff, j. & altman, d. G. 2009. Preferred reporting items for systematic reviews and meta-analyses: the prisma statement. British medical journal publishing group.

Murray, g. K., lin, t., austin, j., mcgrath, j. J., hickie, i. B. & wray, n. R. 2021. Could polygenic risk scores be useful in psychiatry?: a review. *Jama psychiatry,* 78**,** 210-219.

Neumann, a., pappa, i., lahey, b. B., verhulst, f. C., medina-gomez, c., jaddoe, v. W., bakermans-kranenburg, m. J., moffitt, t. E., van, i. M. H. & tiemeier, h. 2016. Single nucleotide polymorphism heritability of a general psychopathology factor in children. *J am acad child adolesc psychiatry,* 55**,** 1038-1045.e4.

Nivard, m. G., gage, s. H., hottenga, j. J., van beijsterveldt, c. E. M., abdellaoui, a., bartels, m., baselmans, b. M. L., ligthart, l., pourcain, b. S., boomsma, d. I., munafò, m. R. & middeldorp, c. M. 2017. Genetic overlap between schizophrenia and developmental psychopathology: longitudinal and multivariate polygenic risk prediction of common psychiatric traits during development. *Schizophr bull,* 43**,** 1197-1207.

Peterson, r. E., kuchenbaecker, k., walters, r. K., chen, c.-y., popejoy, a. B., periyasamy, s., lam, m., iyegbe, c., strawbridge, r. J., brick, l., carey, c. E., martin, a. R., meyers, j. L., su, j., chen, j., edwards, a. C., kalungi, a., koen, n., majara, l., schwarz, e., smoller, j. W., stahl, e. A., sullivan, p. F., vassos, e., mowry, b., prieto, m. L., cuellar-barboza, a., bigdeli, t. B., edenberg, h. J., huang, h. & duncan, l. E. 2019. Genome-wide association studies in ancestrally diverse populations: opportunities, methods, pitfalls, and recommendations. *Cell,* 179**,** 589-603.

Power, c. & elliott, j. 2006. Cohort profiles. *International epidemiological association international journal of epidemiology,* 35**,** 34-41.

Purcell, s. M., wray, n. R., stone, j. L., visscher, p. M., o'donovan, m. C., sullivan, p. F. & sklar, p. 2009. Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. *Nature,* 460**,** 748-52.

Raffington, l., mallard, t. & harden, k. P. 2020. Polygenic scores in developmental psychology: invite genetics in, leave biodeterminism behind. *Https://doi.org/10.1146/annurev-devpsych-051820-123945,* 2**,** 389-411.

Rice, f., riglin, l., thapar, a. K., heron, j., anney, r., o'donovan, m. C. & thapar, a. 2019. Characterizing developmental trajectories and the role of neuropsychiatric genetic risk variants in early-onset depression. *Jama psychiatry,* 76**,** 306-313.

Riglin, l., collishaw, s., richards, a., thapar, a. K., maughan, b., o'donovan, m. C. & thapar, a. 2017. Schizophrenia risk alleles and neurodevelopmental outcomes in childhood: a population-based cohort study. *The lancet psychiatry,* 4**,** 57-62.

Riglin, l., collishaw, s., thapar, a. K., dalsgaard, s., langley, k., smith, g. D., stergiakouli, e., maughan, b., o'donovan, m. C. & thapar, a. 2016. Association of genetic risk variants with attention-deficit/hyperactivity disorder trajectories in the general population. *Jama psychiatry,* 73**,** 1285-1292.

Ripke, s., neale, b. M., corvin, a., walters, j. T. R., farh, k. H., holmans, p. A., lee, p., bulik-sullivan, b., collier, d. A., huang, h., pers, t. H., agartz, i., agerbo, e., albus, m., alexander, m., amin, f., bacanu, s. A., begemann, m., belliveau, r. A., bene, j., bergen, s. E., bevilacqua, e., bigdeli, t. B., black, d. W., bruggeman, r., buccola, n. G., buckner, r. L., byerley, w., cahn, w., cai, g., campion, d., cantor, r. M., carr, v. J., carrera, n., catts, s. V., chambert, k. D., chan, r. C. K., chen, r. Y. L., chen, e. Y. H., cheng, w., cheung, e. F. C., chong, s. A., cloninger, c. R., cohen, d., cohen, n., cormican, p., craddock, n., crowley, j. J., curtis, d., davidson, m., davis, k. L., degenhardt, f., del favero, j., demontis, d., dikeos, d., dinan, t., djurovic, s., donohoe, g., drapeau, e., duan, j., dudbridge, f., durmishi, n., eichhammer, p., eriksson, j., escott-price, v., essioux, l., fanous, a. H., farrell, m. S., frank, j., franke, l., freedman, r., freimer, n. B., friedl, m., friedman, j. I., fromer, m., genovese, g., georgieva, l., giegling, i., giusti-rodríguez, p., godard, s., goldstein, j. I., golimbet, v., gopal, s., gratten, j., de haan, l., hammer, c., hamshere, m. L., hansen, m., hansen, t., haroutunian, v., hartmann, a. M., henskens, f. A., herms, s., hirschhorn, j. N., hoffmann, p., hofman, a., hollegaard, m. V., hougaard, d. M., ikeda, m., joa, i., et al. 2014. Biological insights from 108 schizophrenia-associated genetic loci. *Nature,* 511**,** 421-427.

Sallis, h., szekely, e., neumann, a., jolicoeur-martineau, a., van, i. M., hillegers, m., greenwood, c. M. T., meaney, m. J., steiner, m., tiemeier, h., wazana, a., pearson, r. M. & evans, j. 2019. General psychopathology, internalising and externalising in children and functional outcomes in late adolescence. *J child psychol psychiatry,* 60**,** 1183-1190.

Scott, j. G., mihalopoulos, c., erskine, h. E., roberts, j. & rahman, a. 2016. Childhood mental and developmental disorders. *Disease control priorities, third edition (volume 4): mental, neurological, and substance use disorders***,** 145-161.

Sohani, z. N., sarma, s., alyass, a., de souza, r. J., robiou-du-pont, s., li, a., mayhew, a., yazdi, f., reddon, h., lamri, a., stryjecki, c., ishola, a., lee, y. K., vashi, n., anand, s. S. & meyre, d. 2016. Empirical evaluation of the q-genie tool: a protocol for assessment of effectiveness. *Bmj open,* 6.

Solovieff, n., cotsapas, c., lee, p. H., purcell, s. M. & smoller, j. W. 2013. Pleiotropy in complex traits: challenges and strategies. *Nat rev genet,* 14**,** 483-95.

Sullivan, p. F. & geschwind, d. H. 2019. Defining the genetic, genomic, cellular, and diagnostic architectures of psychiatric disorders. *Cell,* 177**,** 162-183.

Tishkoff, s. A., reed, f. A., friedlaender, f. R., ehret, c., ranciaro, a., froment, a., hirbo, j. B., awomoyi, a. A., bodo, j. M., doumbo, o., ibrahim, m., juma, a. T., kotze, m. J., lema, g., moore, j. H., mortensen, h., nyambo, t. B., omar, s. A., powell, k., pretorius, g. S., smith, m. W., thera, m. A., wambebe, c., weber, j. L. & williams, s. M. 2009. The genetic structure and history of africans and african americans. *Science,* 324**,** 1035-44.

Wray, n. R., lin, t., austin, j., mcgrath, j. J., hickie, i. B., murray, g. K. & visscher, p. M. 2021. From basic science to clinical application of polygenic risk scores: a primer. *Jama psychiatry,* 78**,** 101-109.

Wray, n. R., ripke, s., mattheisen, m., trzaskowski, m., byrne, e. M., abdellaoui, a., adams, m. J., agerbo, e., air, t. M., andlauer, t. M. F., bacanu, s. A., bækvad-hansen, m., beekman, a. F. T., bigdeli, t. B., binder, e. B., blackwood, d. R. H., bryois, j., buttenschøn, h. N., bybjerg-grauholm, j., cai, n., castelao, e., christensen, j. H., clarke, t. K., coleman, j. I. R., colodro-conde, l., couvy-duchesne, b., craddock, n., crawford, g. E., crowley, c. A., dashti, h. S., davies, g., deary, i. J., degenhardt, f., derks, e. M., direk, n., dolan, c. V., dunn, e. C., eley, t. C., eriksson, n., escott-price, v., kiadeh, f. H. F., finucane, h. K., forstner, a. J., frank, j., gaspar, h. A., gill, m., giusti-rodríguez, p., goes, f. S., gordon, s. D., grove, j., hall, l. S., hannon, e., hansen, c. S., hansen, t. F., herms, s., hickie, i. B., hoffmann, p.,

homuth, g., horn, c., hottenga, j. J., hougaard, d. M., hu, m., hyde, c. L., ising, m., jansen, r., jin, f., jorgenson, e., knowles, j. A., kohane, i. S., kraft, j., kretzschmar, w. W., krogh, j., kutalik, z., lane, j. M., li, y., li, y., lind, p. A., liu, x., lu, l., macintyre, d. J., mackinnon, d. F., maier, r. M., maier, w., marchini, j., mbarek, h., mcgrath, p., mcguffin, p., medland, s. E., mehta, d., middeldorp, c. M., mihailov, e., milaneschi, y., milani, l., mill, j., mondimore, f. M., montgomery, g. W., mostafavi, s., mullins, n., nauck, m., ng, b., et al. 2018. Genome-wide association analyses identify 44 risk variants and refine the genetic architecture of major depression. *Nature genetics 2018 50:5,* 50**,** 668-681.

Chapter 3: Epigenetic age deviation and mental health in childhood and adolescence: A systematic review and meta-analysis

3.1. Abstract

Background:
Epigenetic age acceleration (EAA, i.e., higher EA relative to chronological age) may be linked to adverse mental health outcomes in children. Previously, EAA has been associated with advanced physical maturation and early pubertal development in adolescents. However, research on epigenomic changes and mental health outcomes in children remains limited. This systematic review aimed to investigate the associations between epigenetic age deviation (greater or lower EA age relative to chronological age) and mental health outcomes in childhood.

Methods:
This systematic review adhered to the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guidelines. Relevant terms were used to search the PubMed, Scopus, and PsychINFO (via Ebsco host) online databases. The search commenced in February 2022; only full-text studies published in English, involving participants under 18 years of age, and examining associations between epigenetic age deviation and child mental health outcomes were eligible for inclusion.

Results and conclusion:

Among the 4 studies that met the inclusion criteria for this review, 3 studies independently reported significant associations between EAA and internalising behaviour in children and adolescents (aged 4 to 17 years). However, a meta-analysis (OR=1.14, [95% CI, 0.86-1.49]) incorporating a subset of these studies (n=2) did not confirm this finding, though heterogeneity between studies was observed (I^2 =81%, p=0.022).

While the data are not consistent, the findings of this systematic review suggest that EAA holds promise as a potential biomarker for identifying children and adolescents at risk of internalising problems. Given the limited number of studies and the heterogeneity in effect size measures, further work is warranted to explore these preliminary findings.

3.2. Introduction

As discussed in *Chapter 1*, mental health disorders – including internalising behaviours (such as anxiety and depression) and externalising behaviours (such as disruptive behaviours and ADHD) - often emerge during childhood and adolescence (Kessler et al., 2007, Shaw et al., 2012). These disorders may lead to disrupted developmental trajectories and may precede adverse educational, social, and occupational outcomes that persist into adulthood (Shaw et al., 2012, Kieling et al., 2011, Sallis et al., 2019). Prior studies have also shown that risk for mental disorders during childhood and adolescence may be associated with advanced physical ageing (e.g. early pubertal timing) (Copeland et al., 2019). Epigenetic mechanisms, i.e. DNAm have been implicated as potential risk factors for mental health outcomes. For example, Barker and colleagues showed that a methylation index for inflammation risk correlated with internalising behaviours during childhood (Barker et al., 2018). Moreover, indicators of advanced biological ageing based on genomic DNAm have become of interest and have emerged as cross-tissue index of biological ageing (Horvath and Raj, 2018).

Epigenetic clocks utilise DNAm values at selected CpG sites (determined through machine learning approaches) to provide estimates of age across various tissues at different stages of life (Horvath, 2013, Jung et al., 2017). These estimates, referred to as epigenetic age (EA, or DNAmAge), have been found to predict age-related phenotypes (Horvath and Raj, 2018). Increased epigenetic age relative to chronological age (i.e., EA acceleration (EAA)), has been associated with adverse health outcomes (discussed further in *Chapter 5*), including cardiovascular disease and mortality in adults (Fransquet et al., 2019).

Emerging evidence also suggests potential associations between EAA and mental health disorders in adults, such as depression and bipolar disorder (Han et al., 2018, Fries et al., 2017). Similarly, EAA was found to be associated with early stress exposure in children, which in turn was positively linked to depressive behaviours in adolescence (Sumner et al.,2019). EA deceleration has also been associated with lower developmental maturity in children (Girchenko et al., 2017, Knight et al., 2018). However, studies evaluating early biomarkers that may link environmental factors to mental health outcomes in children are currently limited (Cerveira de Baumont et al., 2021). Moreover, there has been relatively minimal work investigating EA deviation (i.e. higher or lower EA) during childhood (Dieckmann et al., 2021). Therefore, this analysis's aim was to systematically review the existing evidence on the association between EA deviation and adverse developmental and mental health outcomes in childhood and adolescence. To the best of our knowledge, this was the first such review undertaken.

3.3. Methods

This review was conducted per the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines for protocol, search strategy, and risk of bias assessment (Moher et al., 2009); and subsequently registered on the International Prospective Register of Systematic Reviews (PROSPERO CRD42022348873). A comprehensive electronic database search was conducted in PubMed, Scopus and PsychINFO via Ebscohost, between February and September 2022. The search terms included "neurodevelopment disorders," "developmental psychopathology," "internalizing disorder," "externalizing disorder," "behaviour disorder," "youth," "epigenetic age" and "biological ageing". Full details of the search strategy and search terms are provided in the ***Appendix 2*** (Table 1).

3.3.1. Study selection criteria

Following the comprehensive search, title and abstract screening were conducted according to the inclusion criteria: full-text studies published in English and evaluating epigenetic age deviation were eligible for inclusion. Additionally, only studies of participants younger than 18 years old were eligible for inclusion (with no further demographic restrictions). Epigenome-wide association studies without an epigenetic age deviation component and/or not assessing developmental or mental health outcomes in childhood/adolescence were excluded.

3.3.2. Quality assessment

Two independent reviewers (L.B Moyakhe and T.C Chalumbila) conducted a quality assessment of the studies using Q-Genie (Sohani et al., 2016), as described in *Chapter 2, Section 2.3.* Potential sources of bias and appropriateness of sample sizes were also assessed. Complete details of the quality assessment ratings for each included study are provided in ***Appendix 2*** (Table 2).

3.3.3. Data extraction

Data were extracted independently by the two reviewers (L.B Moyakhe and T.C Chalumbila) using a data extraction form developed for this review, ***Appendi*x 2** (Table 2). Information extracted included study design, location, and sample characteristics; biological sampling; the measure (epigenetic clock) used to determine epigenetic age; the outcome phenotype (e.g., internalising disorders); the outcome measure (e.g., Child Behaviour Checklist); and the main study findings. The elimination process involved the removal of duplicates and the appraisal of study titles and abstracts to exclude non-relevant studies.

3.3.4. Meta-analysis

A Random-effects meta-analysis was performed using the *metafor* package in R (Viechtbauer, 2010). Specifying random-effects accounts for heterogeneity in the true associations which are attributable to factors that may lead to sample variation across cohorts (e.g. differences in measurements or sample characteristics). This meta-analysis was undertaken using odds ratios (OR) and confidence intervals (CI). A forest plot was also generated.

3.4. Results

3.4.1. Search results

Study selection was undertaken by the PhD candidate (L.B. Moyakhe). A total of 1200 studies were retrieved; 52 were duplicates and subsequently removed, **Figure 3.1**. Title and abstract screening were then performed on 1148 studies, after which only 8 studies were found to be eligible for full-text screening. Full-text screening and quality assessment then yielded 4 eligible studies, from which data were extracted and included for further analysis. Excluded studies were those with adult participants; those with non-epigenetic age measures (e.g., telomere length); and those not assessing the outcomes of interest.

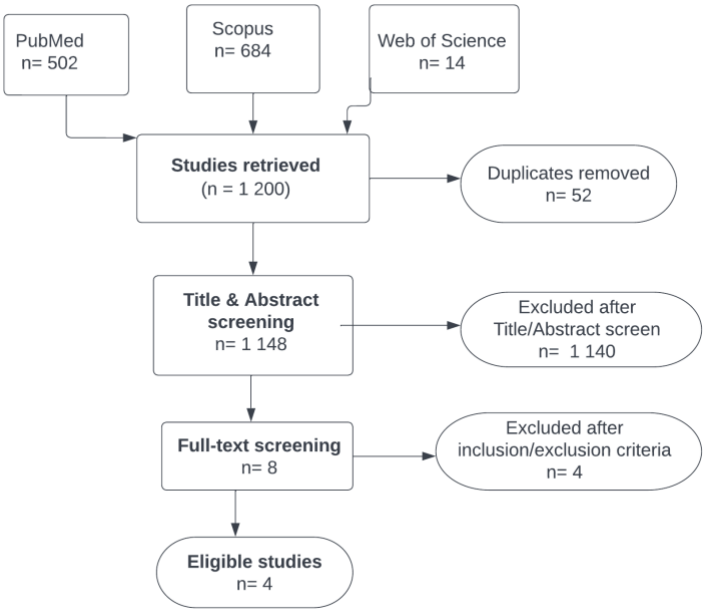

Figure 3. 1PRISMA flow diagram of study selection

3.4.2. Study and sample characteristics

A detailed description of the included studies (n=4) is presented in **Table 3.1**. Per the inclusion criteria for this review, all study participants were ≤18 years of age (ranging from 4 to 17 years old). Three of the studies utilised the Horvath epigenetic clock (Suarez et al., 2018a, Tollenaar et al., 2021, Cerveira de Baumont et al., 2021), a widely recognised measure of epigenetic ageing (Horvath, 2013); while the fourth study employed the Pediatric Buccal Epigenetic clock (PedBE) (Cerveira de Baumont et al., 2021), specifically designed for use with children (McEwen et al., 2020).

The prospective cohorts included in each of these four studies were diverse, spanning a number of geographical locations and community settings: The Glycyrrhizin in Licorice (GLAKU), a community-based cohort in Finland (Strandberg et al., 2002); the Longitudinal Basale Invloeden Op De Baby Ontwikkeling (BIBO) study in the Netherlands (Beijers et al., 2010); children recruited from public schools in Brazil (Salum et al., 2011); and the Berlin Longitudinal Children Study (Heim, 2017). The total sample size across all four studies was approximately 770 participants. The majority of these studies included participants of European ancestry (Tollenaar et al., 2021; Dammering et al., 2021b; Suarez et al., 2018), except for one study, which also had participants of diverse ancestry (e.g. mixed and African Brazilian participants) (Cerveira de Baumont et al., 2021). In terms of outcome measures, three of the included studies assessed internalising behaviour (Tollenaar et al., 2021; Dammering et al., 2021b; Suarez et al., 2018a); and of these, two studies also examined

externalising behaviour (Tollenaar et al., 2021; Suarez et al., 2018a). The fourth included study assessed anxiety disorders (Cerveira de Baumont et al., 2021).

Table 3. 1 Characteristics of included studies – epigenetic ageing and developmental and mental health outcomes in children

Reference	Target cohort	Age (years)	Sample Size	Ancestry	Relevant Findings	Outcome Phenotype	Outcome Measure	Epigenetic clock	Biological Sample
#Suarez et al., 2018	Glycyrrhizin in Licorice (GLAKU)	12.3	N=239	EUR	- EAA assoc with 18 to 34% higher odds for internalizing and thought problems - EAA was not significantly associated with cognition.	Internalising problems Externalising problems	Maternal report CBCL (6-18) The short form of the WISC-III for cognitive function	Horvath	Blood
#Tollenaar et al.,2021	Longitudinal Basale Invloeden Op De Baby Ontwikkeling (BIBO) study	6 - 10 (longitudinal)	N=148	EUR	- EAA assoc with internalising symptoms at 6 to 10 - Internalising symptoms at 2.5 and 4 years predicted EAA at age 6.	Internalising symptoms Externalising symptoms EAA at age 6	Maternal report CBCL 1.5-5 (at ages 2.5 and 4) CBCL 4-28 (at ages 6, 7 and 10) Follow-up: SDQ (ages 8 10)	Horvath	Buccal epithelial cells
CerveiradeBaumont et al., 2021	Children and adolescents were recruited from public schools in Brazil	Baseline 13.4 Follow-up 17.2	N=234, (N=76 after 5- year follow up)	EUR 63%, *African Brazilian 14%, *Mixed 21%	- No difference in EA between adolescents with/without an anxiety disorder.	Anxiety	Initial diagnoses: psychiatric evaluation Follow-up: K-SADS-PL <18	Horvath	Saliva
Dammering et al.,2021	Berlin Longitudinal Children Study	4.25	N=158	White: 144 (91 %) *White-black: 11 (7%) *White-Asian: 3 (2 %)	- Children with internalising disorder exhibited significant EAA compared to children without internalising disorder.	Internalising disorders	DSM-IV criteria using the electronic Preschool Age Psychiatric Assessment.	Pediatric buccal epigenetic (PedBE) clock	Saliva

- CBCL=Child Behaviour Checklist (versions, versions 1.5-5; 4-28; 6-18); K-SADS= Schedule for Affective Disorders and Schizophrenia for School-Age Children Present and Lifetime WISC-III= Wechsler-Intelligence-Scale-for-Children-III Version;SDQ= Strengths and Difficulties Questionnaire; DSM-IV= Diagnostic and Statistical Manual of Mental Disorders fourth edition. EUR = European

*Ancestry described as in the original study

*Ancestry % not reported by study but reported for consistency

Note: With the exception of Tollenaar et al (2021), ages are reported from studies as mean ages

Study included in meta-analysis

3.4.3. Quality assessment

The quality assessment of the included studies was conducted using the Q-Genie checklist (Sohani et al., 2016). All the studies included in this review were rated as good quality (i.e., with scores >40). The main limitations identified based on the Q-genie checklist were limited sample size and reduced statistical power across the studies. Due to heterogeneity in the effect size estimates reported by the individual studies, conducting a meta-analysis was only feasible for two of the four included studies.

3.4.4. Associations between epigenetic age (EA) deviation and internalising behaviour

Associations between epigenetic age deviation and internalising behaviour were examined in three studies involving children aged 4 to 13 years (Suarez et al., 2018a, Tollenaar et al., 2021, Dammering et al., 2021). Internalising behaviour was assessed using the Child Behavior Checklist (CBCL, versions 1.5-5; 4-28; 6-18) (Suarez et al., 2018a; Tollenaar et al., 2021), and the electronic Preschool Age Psychiatric Assessment (Bell, 1994) (Dammering et al., 2021), based on the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) (Guze et al.,1995). The individual study sample sizes were relatively small, ranging from 150 to 240 participants per study. While most participants were of European ancestry, two studies also included participants of diverse ancestry – such as mixed ancestry and African Brazilian (Cerveira de Baumont et al., 2021, Dammering et al., 2021). Overall, all three studies reported associations between EAA and internalising behaviour (Suarez et al., 2018a; Tollenaar et al., 2021; Dammering et al., 2021a). Notably, only one study utilised the PedBE clock (Dammering et al., 2021), and the remaining two studies utilised the Horvath clock as the measure of epigenetic ageing (Suarez et al., 2018a; Tollenaar et al., 2021). Thus, these two studies utilising the same epigenetic clock and comparable effect size estimates- reporting that EAA was associated with increased odds for internalising behaviour in children (OR=1.01, CI (0.96-1.07)) (Tollenaar et al., 2021); (OR= 1.34, CI (1.06-1.70)) (Suarez et al., 2018a) - were therefore eligible for meta-analysis.

3.4.5. Associations between EA deviation and anxiety disorders

One study examined the association between EAA and anxiety disorder (Cerveira de Baumont et al., 2021). In this study, the baseline assessment was conducted at about 13 years of age, with a follow-up assessment at 17 years old. Initial diagnoses were obtained from psychiatric evaluations and the Schedule for Affective Disorders and Schizophrenia for School-Age Children Present and Lifetime Version (K-SADS-PL) (Kaufman et al., 1997) for participants under 18 years old. This study's findings reported no significant difference in EAA between adolescents with and without anxiety disorders (Cerveira de Baumont et al., 2021).

3.4.6. Associations between EA deviation and externalising behaviour

Two studies examined associations between EA and externalising behaviour in children aged 2 to 13 years (Suarez et al., 2018a; Tollenaar et al., 2021). Both studies used the CBCL as the assessment measure; and neither study reported any significant associations between EA and externalising behaviour (Suarez et al., 2018a; Tollenaar et al., 2021).

3.4.7. Meta-analysis of EA and internalising behaviour

A random-effects meta-analysis was conducted on two of the four included studies, both of which investigated the associations between EAA and internalising behaviour (Suarez et al., 2018; Tollenaar et al., 2021). In contrast to the findings reported by the individual studies, the meta-analysis yielded no significant difference between EAA and internalising behaviour (OR=1.14, [95% CI, 0.86-1.49]), **Figure 3.2**. However, there was evidence of significant heterogeneity between the studies (I^2 = 81 %, p-value = 0.022). A meta-analysis of the remaining two studies was not performed due to heterogeneity in the effect-size measures. To ensure consistency in the analysis, the beta coefficients and standard errors reported by Tollenaar et.al (2021) were converted to OR and CI thus facilitating a direct comparison and synthesis of the results across the studies.

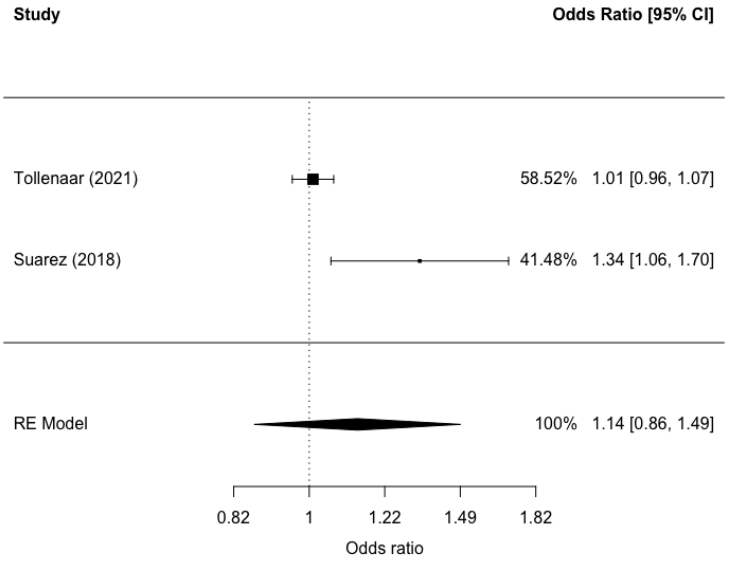

Figure 3. 2 Random-effects (RE) meta-analysis forest plot of the associations between epigenetic age acceleration (EAA) and internalising behaviour in children. The Bars represent confidence intervals corresponding to α=.05. The % represent the weights of the study. RE model p-value= 0.36, OR=1.14, 95% CI (0.86-1.49). Test for heterogeneity I^2= 81 %, p-value = 0.022. Data derived from Tollenaar et.al (2021) were converted from beta coefficients and standard errors to OR and CI for consistent comparison with the OR and CI reported by Suarez et.al (2018).

3.5. Discussion

This systematic review collated findings from four studies investigating the associations between EA deviation and mental health outcomes in childhood and adolescence. EAA was significantly associated with internalising behaviour in children in three of the four studies (Suarez et al., 2018a, Tollenaar et al., 2021, Dammering et al., 2021). For example, one study found EAA associated with an increased risk for internalising behaviour (Suarez et al., 2018a). Similarly, children with an internalising disorder diagnosis had higher EAA when compared to those without such a diagnosis (Dammering et al., 2021). In this study, children at risk for internalising behaviour also showed associations with EAA and physical growth (Dammering et al., 2021). These findings are consistent with previous literature linking EA with physical maturation in children (Copeland et al., 2019; Ellis et al., 2019). Further, prior studies reported associations between EAA and adverse outcomes such as internalising symptoms (e.g. depressive symptoms) in childhood and in late adolescence (ages 15 to 17) (Suarez et al., 2018b, Caro et al., 2023).

However, the meta-analysis conducted on a subset of the studies (n=2) in this systematic review did not confirm the findings observed in the individual studies. Substantial heterogeneity (e.g. in study settings or individual participant characteristics) was observed in the random-effects meta-analysis. Thus, considerable variation across the included studies may have contributed to the non-significant findings. This would align with prior evidence that the association between EAA and the development of internalising behaviours is dynamic and heterogenous within any given population (Caro et al., 2023, Barker and Maughan, 2009). For example, one study reported that EAA at birth is associated with lower internalising behaviours in early childhood for some children, while potentially higher internalising behaviours at the start of adolescence for others (Caro et al., 2023). Notably, one study in this review also reported no significant associations (i.e. between EAA and anxiety disorders in adolescence) (Cerveira de Baumont et al., 2021), thus aligning with the findings of the meta-analysis. This is consistent with previous literature in adults reporting no observable differences in EAA between patients with generalised anxiety disorder and healthy controls (Wolf et al., 2019).

This review has several noteworthy limitations. First, the search strategy and study selection process may have resulted in the omission of relevant studies. In addition, methodological heterogeneity (e.g. in the reported effect sizes and the use of different covariates) in the individual studies complicated direct inter-study comparisons and hindered meta-analysis. This resulted in a relatively small number of studies (n=2) being included in the meta-analysis.

The individual studies included in this review also had inherent limitations. These studies included relatively small sample sizes, which may have limited statistical power to detect subtle but meaningful significant effects. The prevalence of mental disorders (e.g. externalising problem behavior) in the included studies was also relatively low, which may have limited the power to detect significant associations (Suarez et al., 2018a). Thus, caution is needed when extrapolating these findings to larger study populations. Additionally, variations in participant recruitment methods, such as recruiting from schools (Cerveira de Baumont et al., 2021) compared to cohort studies (Tollenaar et al., 2021), may have introduced inconsistencies in diagnoses or phenotype classifications.

Finally, most of the studies in this review (n=3) made use of the Horvath epigenetic clock to measure EA deviation (Suarez et al., 2018a, Tollenaar et al., 2021, Cerveira de Baumont et al., 2021). This clock was previously reported to have reduced efficacy for estimating EA in children (Alisch et al., 2012, Higgins-Chen et al., 2020). Further, the Horvath clock (which was trained to predict chronological age) has been found to be less effective in detecting significant positive correlations between EAA and mental health outcomes (such as depression and schizophrenia), when compared to other epigenetic clocks trained for broader applications (such as predicting all-cause mortality) (Higgins-Chen et al., 2020). Thus, the performance of alternative epigenetic clocks (e.g. Pace of Aging methylation, PoA) in children and adolescents would warrant further exploration. Future studies could also benefit from comparing different epigenetic clocks for childhood-onset phenotypes in order to determine which clock is best suited in children.

In conclusion - notwithstanding these limitations - this systematic review highlights potential associations between EAA and adverse mental health outcomes in children and adolescents. While significant associations between EAA and internalising behaviour were described by individual studies, the meta-analysis did not confirm this finding. This underscores the need for larger and more well-powered studies, to enable further exploration of the utility of EA as early indicators of mental disorders in at-risk children.

3.6. References

Bagner, d. M., rodríguez, g. M., blake, c. A., linares, d. & carter, a. S. 2012. Assessment of behavioral and emotional problems in infancy: a systematic review. *Clin child fam psychol rev,* 15**,** 113-28.

Beijers, r., jansen, j., riksen-walraven, m. & de weerth, c. 2010. Maternal prenatal anxiety and stress predict infant illnesses and health complaints. *Pediatrics,* 126**,** e401-9.

Caramaschi, d., neumann, a., cardenas, a., tindula, g., alemany, s., zillich, l., pesce, g., lahti, j. M. T., havdahl, a., mulder, r., felix, j. F., tiemeier, h., sirignano, l., frank, j., witt, s. H., rietschel, m., deuschle, m., huen, k., eskenazi, b., send, t. S., ferrer, m., gilles, m., de agostini, m., baïz, n., rifas-shiman, s. L., kvist, t., czamara, d., tuominen, s. T., relton, c. L., rai, d., london, s. J., räikkönen, k., holland, n., annesi-maesano, i., streit, f., hivert, m. F., oken, e., sunyer, j., cecil, c. A. M. & sharp, g. 2022. Meta-analysis of epigenome-wide associations between dna methylation at birth and childhood cognitive skills. *Molecular psychiatry 2022 27:4,* 27**,** 2126-2135.

Cerveira de baumont, a., hoffmann, m. S., bortoluzzi, a., fries, g. R., lavandoski, p., grun, l. K., guimarães, l. S. P., guma, f., salum, g. A., barbé-tuana, f. M. & manfro, g. G. 2021. Telomere length and epigenetic age acceleration in adolescents with anxiety disorders. *Sci rep,* 11**,** 7716.

Copeland, w. E., worthman, c., shanahan, l., costello, e. J. & angold, a. 2019. Early pubertal timing and testosterone associated with higher levels of adolescent depression in girls. *J am acad child adolesc psychiatry,* 58**,** 1197-1206.

Dammering, f., martins, j., dittrich, k., czamara, d., rex-haffner, m., overfeld, j., de punder, k., buss, c., entringer, s., winter, s. M., binder, e. B. & heim, c. 2021. The pediatric buccal epigenetic clock identifies significant ageing acceleration in children with internalizing disorder and maltreatment exposure. *Neurobiology of stress,* 15.

Dieckmann, l., lahti-pulkkinen, m., kvist, t., lahti, j., dewitt, p. E., cruceanu, c., laivuori, h., sammallahti, s., villa, p. M., suomalainen-könig, s., eriksson, j. G., kajantie, e., raikkönen, k., binder, e. B. & czamara, d. 2021. Characteristics of epigenetic aging across gestational and perinatal tissues. *Clinical epigenetics,* 13**,** 97.

Fraga, m. F., ballestar, e., paz, m. F., ropero, s., setien, f., ballestar, m. L., heine-suñer, d., cigudosa, j. C., urioste, m., benitez, j., boix-chornet, m., sanchez-aguilera, a., ling, c., carlsson, e., poulsen, p., vaag, a., stephan, z., spector, t. D., wu, y. Z., plass, c. & esteller, m. 2005. Epigenetic differences arise during the lifetime of monozygotic twins. *Proc natl acad sci u s a,* 102**,** 10604-9.

Fransquet, p. D., wrigglesworth, j., woods, r. L., ernst, m. E. & ryan, j. 2019. The epigenetic clock as a predictor of disease and mortality risk: a systematic review and meta-analysis. Biomed central ltd.

Fries, g. R., bauer, i. E., scaini, g., wu, m. J., kazimi, i. F., valvassori, s. S., zunta-soares, g., walss-bass, c., soares, j. C. & quevedo, j. 2017. Accelerated epigenetic aging and mitochondrial dna copy number in bipolar disorder. *Transl psychiatry,* 7**,** 1283.

Girchenko, p., lahti, j., czamara, d., knight, a. K., jones, m. J., suarez, a., hämäläinen, e., kajantie, e., laivuori, h., villa, p. M., reynolds, r. M., kobor, m. S., smith, a. K., binder, e. B. & räikkönen, k. 2017. Associations between maternal risk factors of adverse pregnancy and birth outcomes and the offspring epigenetic clock of gestational age at birth. *Clin epigenetics,* 9**,** 49.

Han, l. K. M., aghajani, m., clark, s. L., chan, r. F., hattab, m. W., shabalin, a. A., zhao, m., kumar, g., xie, l. Y., jansen, r., milaneschi, y., dean, b., aberg, k. A., van den oord, e. & penninx, b. 2018. Epigenetic aging in major depressive disorder. *Am j psychiatry,* 175**,** 774-782.

Hannum, g., guinney, j., zhao, l., zhang, l., hughes, g., sadda, s. V., klotzle, b., bibikova, m., fan, j. B., gao, y., deconde, r., chen, m., rajapakse, i., friend, s., ideker, t. & zhang, k. 2013. Genome-wide methylation profiles reveal quantitative views of human aging rates. *Molecular cell,* 49**,** 359-367.

Heim, c. 2017. Immediate biological embedding of maltreatment in children: berlin longitudinal children study (berlinlcs). *Psychoneuroendocrinology,* 83**,** 7.

Higgins-chen, a. T., boks, m. P., vinkers, c. H., kahn, r. S. & levine, m. E. 2020. Schizophrenia and epigenetic aging biomarkers: increased mortality, reduced cancer risk, and unique clozapine effects. *Biol psychiatry,* 88**,** 224-235.

Horvath, s. 2013. Dna methylation age of human tissues and cell types. *Genome biology,* 14.

Horvath, s. & raj, k. 2018. Dna methylation-based biomarkers and the epigenetic clock theory of ageing. *Nat rev genet,* 19**,** 371-384.

Jung, s. E., shin, k. J. & lee, h. Y. 2017. Dna methylation-based age prediction from various tissues and body fluids. *Bmb rep,* 50**,** 546-553.

Kaufman, j., birmaher, b., brent, d., rao, u., flynn, c., moreci, p., williamson, d. & ryan, n. 1997. Schedule for affective disorders and schizophrenia for school-age children-present and lifetime version (k-sads-pl): initial reliability and validity data. *J am acad child adolesc psychiatry,* 36**,** 980-8.

Kessler, r. C., angermeyer, m., anthony, j. C., r, d. E. G., demyttenaere, k., gasquet, i., g, d. E. G., gluzman, s., gureje, o., haro, j. M., kawakami, n., karam, a., levinson, d., medina mora, m. E., oakley browne, m. A., posada-villa, j., stein, d. J., adley tsang, c. H., aguilar-gaxiola, s., alonso, j., lee, s., heeringa, s., pennell, b. E., berglund, p., gruber, m. J., petukhova, m., chatterji, s. & ustün, t. B. 2007. Lifetime prevalence and age-of-onset distributions of mental disorders in the world health organization's world mental health survey initiative. *World psychiatry,* 6**,** 168-76.

Kieling, c., baker-henningham, h., belfer, m., conti, g., ertem, i., omigbodun, o., rohde, l. A., srinath, s., ulkuer, n. & rahman, a. 2011. Child and adolescent mental health worldwide: evidence for action. *Lancet,* 378**,** 1515-25.

Knight, a. K., smith, a. K., conneely, k. N., dalach, p., loke, y. J., cheong, j. L., davis, p. G., craig, j. M., doyle, l. W. & theda, c. 2018. Relationship between epigenetic maturity and respiratory morbidity in preterm infants. *J pediatr,* 198**,** 168-173.e2.

Meijer, m., franke, b., sandi, c. & klein, m. 2023. Epigenome-wide dna methylation in externalizing behaviours: a review and combined analysis. *Neuroscience & biobehavioral reviews,* 145**,** 104997.

Moher, d., liberati, a., tetzlaff, j. & altman, d. G. 2009. Preferred reporting items for systematic reviews and meta-analyses: the prisma statement. British medical journal publishing group.

Quach, a., levine, m. E., tanaka, t., lu, a. T., chen, b. H., ferrucci, l., ritz, b., bandinelli, s., neuhouser, m. L., beasley, j. M., snetselaar, l., wallace, r. B., tsao, p. S., absher, d., assimes, t. L., stewart, j. D., li, y., hou, l., baccarelli, a. A., whitsel, e. A. & horvath, s. 2017. Epigenetic clock analysis of diet, exercise, education, and lifestyle factors. *Aging (albany ny),* 9**,** 419-446.

Sallis, h., szekely, e., neumann, a., jolicoeur-martineau, a., van, i. M., hillegers, m., greenwood, c. M. T., meaney, m. J., steiner, m., tiemeier, h., wazana, a., pearson, r. M. & evans, j. 2019. General psychopathology, internalising and externalising in children and functional outcomes in late adolescence. *J child psychol psychiatry,* 60**,** 1183-1190.

Salum, g. A., isolan, l. R., bosa, v. L., tocchetto, a. G., teche, s. P., schuch, i., costa, j. R., costa mde, a., jarros, r. B., mansur, m. A., knijnik, d., silva, e. A., kieling, c., oliveira, m. H., medeiros, e., bortoluzzi, a., toazza, r., blaya, c., leistner-segal, s., salles, j. F., silveira, p. P., goldani, m. Z., heldt, e. & manfro, g. G. 2011. The multidimensional evaluation and treatment of anxiety in children and adolescents: rationale, design, methods and preliminary findings. *Braz j psychiatry,* 33**,** 181-95.

Shaw, m., hodgkins, p., caci, h., young, s., kahle, j., woods, a. G. & arnold, l. E. 2012. A systematic review and analysis of long-term outcomes in attention deficit hyperactivity disorder: effects of treatment and non-treatment. *Bmc med,* 10**,** 99.

Sohani, z. N., sarma, s., alyass, a., de souza, r. J., robiou-du-pont, s., li, a., mayhew, a., yazdi, f., reddon, h., lamri, a., stryjecki, c., ishola, a., lee, y. K., vashi, n., anand, s. S. & meyre, d. 2016. Empirical evaluation of the q-genie tool: a protocol for assessment of effectiveness. *Bmj open,* 6.

Solmi, m., radua, j., olivola, m., croce, e., soardo, l., salazar de pablo, g., il shin, j., kirkbride, j. B., jones, p., kim, j. H., kim, j. Y., carvalho, a. F., seeman, m. V., correll, c. U. & fusar-poli, p. 2022. Age at onset of mental disorders worldwide: large-scale meta-analysis of 192 epidemiological studies. *Molecular psychiatry,* 27**,** 281-295.

Strandberg, t. E., andersson, s., järvenpää, a. L. & mckeigue, p. M. 2002. Preterm birth and licorice consumption during pregnancy. *Am j epidemiol,* 156**,** 803-5.

Suarez, a., lahti, j., czamara, d., lahti-pulkkinen, m., girchenko, p., andersson, s., strandberg, t. E., reynolds, r. M., kajantie, e., binder, e. B. & raikkonen, k. 2018a. The epigenetic clock and pubertal, neuroendocrine, psychiatric, and cognitive outcomes in adolescents. *Clin epigenetics,* 10**,** 96.

Suarez, a., lahti, j., czamara, d., lahti-pulkkinen, m., knight, a. K., girchenko, p., hämäläinen, e., kajantie, e., lipsanen, j., laivuori, h., villa, p. M., reynolds, r. M., smith, a. K., binder, e. B. & räikkönen, k. 2018b. The epigenetic clock at birth: associations with maternal antenatal depression and child psychiatric problems. *J am acad child adolesc psychiatry,* 57**,** 321-328.e2.

Sumner, j. A., colich, n. L., uddin, m., armstrong, d. & mclaughlin, k. A. 2019. Early experiences of threat, but not deprivation, are associated with accelerated biological aging in children and adolescents. *Biol psychiatry,* 85**,** 268-278.

Tollenaar, m. S., beijers, r., garg, e., nguyen, t. T. T., lin, d. T. S., macisaac, j. L., shalev, i., kobor, m. S., meaney, m. J., o'donnell, k. J. & de weerth, c. 2021. Internalizing symptoms associate with the pace of epigenetic aging in childhood. *Biological psychology,* 159**,** 108021.

Viechtbauer, w. 2010. Conducting meta-analyses in r with the metafor. *Journal of statistical software,* 36**,** 1-48.

Wolf, e. J., logue, m. W., morrison, f. G., wilcox, e. S., stone, a., schichman, s. A., mcglinchey, r. E., milberg, w. P. & miller, m. W. 2019. Posttraumatic psychopathology and the pace of the epigenetic clock: a longitudinal investigation. *Psychol med,* 49**,** 791-800.

Guze, s.b. (1995). Diagnostic and statistical manual of mental disorders, 4th ed. (dsm-iv). American journal of psychiatry, 152(8), pp.1228–1228. Doi:https://doi.org/10.1176/ajp.152.8.1228.

Barker, e.D. And maughan, b. (2009). Differentiating early-onset persistent versus childhood-limited Conduct problem youth. American journal of psychiatry, 166(8), pp.900–908. Doi:https://doi.Org/10.1176/appi.Ajp.2009.08121770.

Copeland, W.E., Worthman, C., Shanahan, L., Costello, E.J. and Angold, A. (2019). Early Pubertal Timing and Testosterone Associated With Higher Levels of Adolescent Depression in Girls. Journal of the American Academy of Child & Adolescent Psychiatry, 58(12), pp.1197–1206. doi:https://doi.org/10.1016/j.jaac.2019.02.007. Barker, E.D., Cecil, C.A.M., Walton, E., Houtepen, L.C.,

O'Connor, T.G., Danese, A.,

Jaffee, S.R., Jensen, S.K.G., Pariante, C., McArdle, W., Gaunt, T.R., Relton, C.L. and Roberts, S. (2018). Inflammation-related epigenetic risk and child and adolescent mental health: A prospective study from pregnancy to middle adolescence. Development and Psychopathology, 30(3), pp.1145–1156. doi:https://doi.org/10.1017/s0954579418000330.

Chapter 4: Associations of polygenic risk for ADHD with child development and mental health in a South African birth cohort study

4.1. Abstract

Background:

Childhood mental health disorders may have significant adverse implications for long-term psychological and social well-being. These disorders encompass both internalising and externalising conditions, such as attention-deficit hyperactivity disorder (ADHD). To date, there remains a paucity of data on genetic risk for ADHD in young children, particularly from LMICs, where a substantial proportion of the global childhood population resides. This analysis aimed to explore the association between polygenic risk scores (PRS) - the weighted sum of risk alleles - for ADHD, and adverse developmental and mental health outcomes in children in the Drakenstein Child Health Study (DCHS), a South African birth cohort.

Methods:

Child developmental and mental health outcomes were assessed with the Bayley Scales of Infant and Toddler Development 3rd edition (Bayley-III) and Child Behaviour Checklist (CBCL), respectively. Genome-wide association study summary statistics from the Early Genetics and Lifecourse Epidemiology (EAGLE) consortium (N=17, 666) were used to generate PRS in the target dataset, the DCHS (N=958). Associations between genetic risk (as indexed by PRS) for ADHD, and adverse developmental and mental health outcomes in the DCHS, were investigated using logistic regression models, controlling for covariates such as socioeconomic status, maternal substance use (alcohol consumption and tobacco use), child anthropometry (sex of the child, birthweight), and the first 10 genomic principal components to adjust for population stratification.

Results and conclusion:

In this analysis of DCHS children, no associations were found between genetic risk for ADHD (indexed by PRS) and adverse developmental or mental health outcomes. To the best of our knowledge, this study represents the first attempt to investigate genetic risk for ADHD in children from LMICs. Future work, incorporating ancestry-matched discovery datasets and larger target sample sizes (with increasing representation of African genomes), and incorporating other PRS, is warranted to enhance the accuracy, power, and trans-ancestral transferability of polygenic findings.

4.2. Introduction

As discussed in *Chapter 1,* childhood mental health disorders are prevalent - it is estimated that between 7% and 26% of children exhibit emotional or behavioural problems within the first five years of life (Egger and Angold, 2006, Wichstrøm et al., 2012). These problems often encompass several types of emotional and/or behavioural issues such as internalising symptoms (e.g., anxiety, depression) or externalising conditions (e.g., ADHD, conduct disorder) (Barican et al., 2022, Ogundele, 2018).

ADHD is of particular interest – this is a prevalent developmental disorder, affecting a substantial proportion of children globally (4-5% of children worldwide); with around 1 in 20 children in South Africa being affected (Schoeman and Liebenberg, 2017; Kessler et al., 2007). Moreover, ADHD has been associated with a range of adverse outcomes, including poor educational attainment, substance misuse, and adverse social, behavioural, and emotional outcomes later in life (Agnew-Blais et al., 2018; Stern et al., 2020).

The role of genetic risk for ADHD has been well-established, with twin and family studies reporting heritability estimates of 70-80% (Brikell et al., 2015; Larsson et al., 2014; Demontis et al., 2019). More recently, genome-wide association studies (GWAS) have been employed to explore the genetic basis of ADHD further (Demontis et al., 2019). However, the polygenic architecture of ADHD, as well as the limited predictive capacity of GWAS have necessitated alternative methods, including SNPs of non-genome-wide significance, to capture the genetic liability of complex disorders (Purcell et al., 2009).

As detailed in *Chapter 2,* PRS aggregate variant effects across the genome to estimate heritability; and infer genetic overlap between traits, providing an estimate of genetic liability to a particular trait at the individual level (Choi et al., 2020, Martin et al., 2019, Purcell et al., 2009). These scores are calculated by summing an individual's risk alleles, weighted by effect size estimates obtained from GWAS summary statistics, for a set of SNPs (Purcell et al., 2009, Choi et al., 2020). One such PRS calculation method is PRS-CS, a polygenic prediction method based on a Bayesian framework which includes all SNPs whilst adjusting the effect sizes for LD (Ge et al., 2019, Vilhjalmsson et al. 2015, Price et al. 2020). PRS-CS has been shown to outperform older polygenic methods in inferring continuous shrinkage priors-on SNP effect sizes. The method infers posterior effect sizes of SNPs using GWAS summary statistics and an external LD panel (Ge et al., 2019). The continuous shrinkage enables the amount of shrinkage applied to each genetic marker to be adaptive to the strength of its association signal in a GWAS, thus accommodating diverse underlying genetic architectures (Ge et al., 2019). Moreover, continuous shrinkage priors enable effect sizes for SNPs in each LD block to be updated jointly, in a multivariate manner rather than updating the effect size for each marker separately and sequentially; this allows for more accurate modelling of local LD patterns which provides substantial computational improvements (Ge et al., 2019).

To date, PRS for ADHD have been associated with attention and hyperactivity traits during childhood (Martin et al., 2014); with ADHD diagnostic status in children and adolescents (Jansen et al., 2020); and with genetic liability towards broad psychopathology in children (Brikell et al., 2020). ADHD PRS have also been reported to be associated with poor cognitive outcomes (i.e. poor working memory) in childhood (Aguilar-Lacasaña et al., 2020, Martin et al., 2015). Given that ADHD is a part of continuum of neurodevelopmental disorders encompassing cognitive, behavioural and emotional difficulties (Thapar and Cooper,2016), genetic factors contributing to ADHD may also impact various domains of child development (Tillman et al.,2011). Relatively little work has explored the use of PRS for childhood developmental and mental health disorders in non-European children. Moreover, most GWAS thus far have been conducted in high-income countries,

primarily with individuals of European ancestry – resulting in a notable underrepresentation of populations of African ancestry (Duncan et al., 2019, Tishkoff et al., 2009). This analysis aimed to investigate the association between genetic liability for ADHD (as indexed by PRS for ADHD), and adverse childhood developmental and mental health outcomes in the DCHS, an ongoing South African-based birth cohort study of black African and mixed ancestry participants (Zar et al., 2015, Stein et al., 2015).

4.3. Methods

4.3.1. Study participants

The DCHS is an ongoing, population-based South African birth cohort study investigating the early determinants of child development and health (Zar et al., 2015; Stein et al., 2015; Zar et al., 2019). Between March 2012 and March 2015, pregnant women attending routine antenatal care at two primary care clinics (Mbekweni or TC Newman clinic) - in the Drakenstein sub-district, Paarl, Western Cape - were recruited into the study. Participants were between 20- and 28-weeks' gestation at the time of recruitment and have been followed prospectively through childbirth and early childhood (Zar et al., 2015).

Consenting mothers completed self-report and clinician-administered assessment measures antenatally and at several postnatal time points (Donald et al., 2018), in their preferred language (English, Afrikaans or isiXhosa). The study population comprises 1143 infants (including 4 sets of twins and 1 set of triplets) born to 1137 mothers. This cohort includes both black African isiXhosa-speaking, and mixed ancestry Afrikaans-speaking participants (Zar et al., 2015; Stein et al., 2015); with little immigration or emigration (Stein et al., 2015).

The DCHS cohort is characterized by low socioeconomic status, single-parent households, and low levels of employment (Stein et al., 2015; Zar et al., 2015). This study population is thus representative of many other communities in South Africa and other LMICs (Donald et al., 2018). Previous sub-studies conducted within the DCHS have also reported high levels of maternal depression, substance abuse, and HIV exposure (Stein et al., 2015; Zar et al., 2019).

4.3.2. Maternal sociodemographic, biomedical and psychosocial characteristics

To assess sociodemographic characteristics of interest, a questionnaire adapted from the South African Stress and Health Study (SASH) (Myer et al., 2008) was utilised. Socioeconomic status (SES) was operationalised as the sum of standardised components such as education, income, assets, and employment, as previously described (Stein et al., 2015). Maternal biomedical information, including HIV status determined through rapid testing and self-report (Stein et al., 2015). Maternal anaemia was tested for during pregnancy (Donald et al., 2018), haemoglobin levels below 10g/dL were indicative of moderate to severe iron deficiency anaemia per WHO guidelines (WHO, 2011).

Maternal substance use, such as alcohol consumption and smoking, was assessed using the Alcohol, Smoking and Substance Involvement Screening Test (ASSIST) (WHO ASSIST Working Group, 2002). This tool assesses substance use and substance-related risk across 10 categories: tobacco, alcohol, cannabis, cocaine, inhalants, sedatives/sleeping pills, hallucinogens, opioids, amphetamine-type stimulants as well as a history of intravenous drug use. Total scores from each substance are obtained by summing individual item responses. Scores of 0-10 for alcohol and 0-3 for illicit drugs are indicative of low risk for substance-related health problems; 11-26 for alcohol and 4-26 for illicit drugs is indicative of moderate risk; and scores >26 indicate that an individual is at a high risk of experiencing severe problems as a result of their current pattern of use, and is likely to be dependent on the substance of interest (Stein et al., 2015, WHO ASSIST Working Group, 2002).

Objective measures of substance use were also utilised in the DCHS. For example, maternal urine cotinine, a biomarker for smoking, was measured antenatally and at birth, as well as for infants 6-10 weeks.

Additionally, maternal urine samples were tested antenatally via rapid urine dipstick for recent use of common illicit substances, as previously described (Stein et al., 2015).

4.3.3. Child anthropometric data

Child anthropometric data were obtained by trained DCHS staff (Zar et al., 2015, Donald et al., 2018, Donald et al., 2019). This included data on the sex of the child, birth weight as well as gestational age (Donald et al., 2018, Donald et al., 2019). Gestational age at delivery was based on ultrasound results (as available) or otherwise based on fundal height measurements and maternal reports of the last menstrual period (Donald et al., 2019). Birth weight was abstracted by trained study staff from hospital records at birth and was taken as a continuous measure at each time point thereafter (Donald et al., 2019).

4.3.4. Child developmental outcomes

Child development in the DCHS was assessed using the *Bayley Scales of Infant and Toddler Development, 3rd edition (Bayley-III)* (Bayley, 2006). This is a standardised tool to evaluate developmental functioning in children between 1 and 42 months of age, across five domains of development: cognition, language (expressive and receptive), motor (fine and gross), socio-emotional, and adaptive behaviour functioning. The Bayley-III has been used globally, including in LMIC settings such as South Africa (Malcolm-Smith et al., 2015, Ballot et al., 2017). Within the DCHS, the Bayley-III was administered to the entire cohort at 24 months, and fine motor development was again assessed in a subset of the study sample was assessed again under the fine motor domain at age 42 months (Zar et al., 2015, Donald et al., 2018). Direct observation was used for the cognitive, language, and motor domains, while caregiver report was used for the adaptive behaviour and socio-emotional domains. Scores were derived according to the Bayley-III manual (Bayley, 2006) using specialized software (the Bayley-III Scoring Assistant with Bayley-III PDA conduit). Composite scores were calculated for the cognitive, language, and motor domains, and scaled scores for each domain were calculated using age-specific reference norms, with a mean of 10 and a standard deviation of 3 (Donald et al., 2019). Infants with poor developmental outcomes were identified using a cut-off of 1 or more standard deviations below the mean scaled score in any domain, in line with prior studies within the DCHS (Stein et al., 2015, Donald et al., 2019). A cut-off composite score less than 84 was defined as "delayed", as reported in prior studies in similar age groups (Çelik et al., 2020).

4.3.5. Child mental health outcomes

Child mental health outcomes in the DCHS were assessed using the *Child Behaviour Checklist (CBCL)*, a parent-reported questionnaire used to evaluate behavioural and emotional problems in children aged between 1.5 and 5 years old (Achenbach, 2011). The questionnaire consists of 113 items, each scored on a three-point scale (0 = absent, 1 = occurs sometimes, 2 = occurs often) (Mazefsky et al., 2011), and measuring eight syndrome domains, i.e. anxious/depressed; depressed; somatic complaints; social problems; thought problems; attention problems; rule-breaking behaviour; and aggressive behaviour. The CBCL items may be further categorised into two higher-order factors – internalising and externalising symptoms. The CBCL is widely used internationally and has been shown to be reliable and applicable in the South African context (Malcolm-Smith et al., 2015, Barbarin et al., 2001). Norm-referenced t-scores were used to analyse higher-order internalising and externalising factors (Donald et al., 2018b). Children with scores of 64 or higher were classified as having externalising or internalising problems, in line with previous studies (Donald et al., 2018a).

4.3.6. Tissue sample collection and DNA extraction

Cord blood samples were collected at birth from newborns, and peripheral blood was collected from children postnatally at annual timepoints (Zar et al., 2015). Following the delivery of the newborn, blood from the umbilical cord was collected by trained medical staff to ensure minimal contamination with maternal blood. This procedure entailed first clamping and then cutting the umbilical cord, after which the clamp was released allowing the cord blood to drain by gravity into a kidney dish (Morin et al., 2017). The blood was then collected using a syringe for processing and stored at -80°C.

DNA was extracted from blood samples using the QIASymphony DSP DNA midi kit (Qiagen, Germany) and following a protocol established by the UCT Medical Microbiology Laboratory. DNA concentration was measured using BioDrop (Whitehead Scientific, South Africa) and normalized to a concentration of 5-10ng/µl.

All biological samples in the DCHS are tracked using a Laboratory Information Management System (LIMS) (Freezerworks, USA).

4.3.7. Polygenic risk scores

Quality control of discovery dataset: EAGLE consortium

Summary statistics from the Early Genetics and Lifecourse Epidemiology (EAGLE) Consortium were used as the discovery dataset for this analysis (Middeldorp et al., 2016). This dataset comprises approximately 17,000 children (below the age of 13), spanning nine population-based birth cohorts, most of whom (>90%) are of European ancestry (Middeldorp et al., 2016). At the time of writing, the EAGLE dataset was the largest available paediatric GWAS, comparable (in age and phenotype) to the DCHS target dataset, for which GWAS summary statistics for the phenotype of interest (i.e., ADHD) were also available. Quality control (QC) of these summary statistics included the removal of duplicated and ambiguous SNPs and the inclusion of variants with a minor allele frequency (MAF) of > 0.01, per standard recommendations (Choi et al., 2020).

Target dataset: DCHS

Pre-imputation quality control and principal component analysis (PCA)

For the target dataset (DCHS), genotyping data were generated using two arrays: the Illumina Global Screening Array (GSA, N= 153 cord blood samples and 722 child samples) and the PsychArray (N= 120 cord blood samples) (Illumina, San Diego, USA). The GSA has a total of 654,027 markers with 100,000 custom bead types (Illumina, San Diego, USA). The Illumina PsychArray contains 271,000 tag single nucleotide polymorphisms (SNPs), 77,000 markers from the Illumina Infinium Exome-24 BeadChip, and 50,000 additional markers which have previously been associated with psychiatric disorders (Illumina, San Diego, USA). QC was performed on each set of array data separately and merged thereafter. Only autosomal data were included in downstream analyses. Pre-imputation QC comprised the inclusion of variants with a MAF > 0.005 and a genotyping rate of at least 90%, and the removal of variants with a HWE p-value of < 0.001. A KING-cutoff value of 0.0422 was used to check for relatedness among participants (Manichaikul et al., 2010). A KING-cutoff is useful for separating unrelated pairs from close relatives, reducing the likelihood of false positives (Manichaikul et al., 2010). A sex check was also performed to ensure that the reported sex corresponded with the genomic sex. Prior to Principal Component Analysis (PCA), the dataset was LD pruned using a window size of 1000 variants, a window shift of 50 variants, and a pairwise r^2 threshold of 0.05. All QC, LD pruning, and PCA were conducted using Plink (versions 1.07 and 2) (Purcell et al., 2007).

Imputation quality control

The DCHS data were prepared for imputation using a pre-imputation-check tool (version 4.2.9) (http://www.well.ox.ac.uk/~wrayner/tools/)), which aligned the dataset to the preferred reference panel (Auton et al., 2015). Imputation was performed on the Michigan Imputation Server (Das et al., 2016), a web-based tool which uses minimac 3 (Das et al., 2016). The server divides the genomic datasets into segments and runs parallel analyses across these segments. It also performs automatic checks for strand orientation, file integrity, missingness, and MAF distribution. The DCHS dataset was imputed using the Haplotype Reference Consortium r1.12016 reference panel, which comprises 64,976 haplotypes from 32,488 samples (McCarthy et al., 2016). The Rsq filter was set at 0.3, removing more than 70% of the poorly imputed SNPs (Das et al., 2016).

Post-imputation quality control

The VCF format imputed data were processed using Plink (version 1.9) [http://pngu.mgh.harvard.edu/~purcell/plink/] (Purcell et al., 2007). Duplicated SNPs, as well as SNPs with a MAF of less than 25%, were removed from the dataset. The resultant imputed genotyping dataset comprised 5,291,952 variants from 958 children.

PRS Calculation

GWAS summary statistics from the EAGLE consortium were used to generate PRS in the DCHS (N=958). The PRS scores were calculated using PRS-CS (Ge et al., 2019), the LD panel was constructed using the African reference panel from the 1000 Genomes Project phase 3 samples, and the amount of shrinkage applied to each genetic marker (the global parameter shrinkage phi) was estimated automatically using a fully Bayesian approach (Ge et al., 2019). To generate the PRS based on the effect sizes from PRS-CS, Plink version 1.9 was used. All computations were performed using the UCT centralised High-Performance computing resources provided by UCT ICTS High-Performance Computing (*hpc.uct.ac.za*).

4.3.8. Statistical analyses

For the purposes of these analyses, the outcome variables for child development were cognition, language (receptive and expressive), and motor (fine and gross motor) domains on the Bayley-III. For child mental health, the outcome variables were internalising and externalising symptoms on the CBCL. The prevalence of child developmental delay (derived from dichotomised data from the Bayley-III), as well as internalising/externalising problems (from dichotomised data from the CBCL) in the DCHS sample, were calculated using Microsoft Excel and confirmed in R (R Core Team, 2013). The mean and standard deviations for all outcome variables were also calculated. A power analysis was conducted using the "pwr" package in R (R Core Team, 2013).

Logistic regression models were utilised to examine potential associations between PRS for ADHD, and adverse child development and mental health outcomes in the study sample. All regression analyses were performed in R (R Core Team, 2013). The complete set of covariates included in the models were prenatal maternal alcohol consumption, prenatal maternal smoking, maternal HIV status, SES quartile, sex of the child, and the first 10 genomic principal components (PCs) to adjust for population stratification (Stein et al., 2015; Donald et al., 2018b). The selection of these covariates was based on prior work in the DCHS – for example, higher SES has been found to be protective of psychopathology during child development (Donald et al., 2018b; Donald et al., 2019). By contrast, maternal HIV and substance use (such as antenatal smoking and/or

alcohol consumption) have been found to be associated with poorer developmental and anthropometric outcomes in childhood (Zar et al., 2015; Donald et al., 2019). A variable selection tool was utilised for this analysis as well as subsequent analyses. Variable selection determines the set of variables – i.e. a subset of variables from the complete set of variables - which is best for the model to ensure accurate predictions are made – by selecting covariates in the model that explained more than 80% of the variance in the models (Chowdhury and Turin, 2020). In these analyses *(including chapters 5 and 6)* the forward selection of variable method was employed using the *ols* package in R (R Core Team, 2013).

4.4. Results

4.4.1. Sociodemographic, biomedical and psychosocial characteristics

The sociodemographic, biomedical and psychosocial characteristics of the DCHS children included in this analysis are presented in Table 4.1. A total of 971 children with available phenotyping data were included in the final association models. Approximately half (51%) were male in this subset, **Table 4.1.** More than a quarter of the mothers (26%) in this study sample were of low SES, and almost a quarter (23%) of the children were born to HIV-positive mothers. The majority (77%) of children had been exposed to prenatal maternal smoking, and 14% had been exposed to prenatal maternal alcohol consumption.

Table 4. 1 Sociodemographic, biomedical and psychosocial characteristics of children with available phenotype and genotyping data in the DCHS

Variable	**N (%)**
N	971(100)
Sex of child Male Female	 475(51) 496 (49)
Low SES quartile	272 (28)
Prenatal maternal smoking (exposed)	731(78)
Prenatal maternal alcohol consumption (exposed)	125 (13)
Maternal HIV status (positive)	219 (23)

4.4.2. Child developmental and mental health outcomes

Of the children with available Bayley-III data at 24 months (N~700), almost a quarter (23%) were classified as having receptive language delay, and 16% were categorised each as having cognitive delay and expressive language delay, **Table 4.2**. Additionally, a tenth (10%) were classified as having gross motor delay, and 8% as having fine motor delay. Overall, the male children performed more poorly than the female children in the cognitive domain (p= 0.016) and the language domains (e.g. expressive language, p= 0.012), **Table 4.2**. At the follow-up assessment of fine motor developmental outcomes at 42 months, 10% of children were classified as having fine motor delay, which is consistent with the findings at 24 months.

Table 4. 2 Child developmental outcomes at 24 months (Bayley-III scaled-scores)

	Cognitive	**Language (receptive)**	**Language (expressive)**	**Motor (fine)**	**Motor (gross)**
Sample Size (n)	709	702	682	708	674
Male, n (%)	361 (51)	357 (51)	348 (51)	362 (51)	341 (51)
Female, n (%)	348 (49)	345 (49)	333 (49)	346 (49)	333 (49)
Mean	7.06	7.04	7.40	9.26	8.26
Standard deviation (SD)	1.86	1.95	2.31	2.49	2.32
Developmental delay**: n (%)	116 (16)	161 (23)	106 (16)	56 (8)	64 (10)
Male: developmental delay n (%)	71 (20)	99 (28)	66 (19)	34 (9)	28 (8)
Female: developmental delay n (%)	45 (13)	62 (18)	40 (12)	22 (6)	36 (11)
P-value (sex-stratified)	0.016*	0.003**	0.012*	0.109	0.317

**Developmental delay was categorised as Bayley-III scaled scores < 1 SD below the mean

*p<0.05; **p<0.01; #p $0.05 \leq p \leq 0.1$

Table 4. 3 Child fine-motor developmental outcomes at 42 months (Bayley-III scaled-scores)

	Motor development (Fine)
Sample Size	688
Male, n (%)	360 (52)
Female, n (%)	328 (48)
Mean	9.68
SD	1.74
Developmental delay	69 (10)
Male: developmental delay** n (%)	42 (12)
Female: developmental delay n (%)	27 (8)
P-value (sex-stratified)	0.071

**Developmental delay was categorised as Bayley-III scaled scores <1 SD below the mean

Longitudinal data was available for the CBCL data (i.e. internalising and externalising domains, from 24 months to 60 months), **Table 4.4**. For the children with available longitudinal CBCL data, 9% exhibited internalising problems at 24 months, whereas 5% exhibited externalising problems. At 42 months, the prevalence of internalising and externalising problems decreased to 2% and 3%, respectively; but increased again at 60 months to 10% of children exhibited internalising problems, and 4% exhibited externalising problems. Males exhibited more externalising problems than females at 60 months (p= 0.041) **Table 4.4**.

Table 4. 4 Child mental health outcomes at ages 24 months to 60 months (CBCL total-scores)

	Internalising symptoms			**Externalising symptoms**		
Age	**24 months**	**42 months**	**60 months**	**24 months**	**42 months**	**60 months**
Sample Size (n)	620	866	819	620	866	819
Males, n (%)	316 (51)	438 (51)	419 (51)	316 (51)	438 (51)	419 (51)
Females, n (%)	304 (49)	428 (49)	400 (49)	304 (49)	428 (49)	400 (49)
Mean	43.00	40.25	43.73	44.00	41.63	41.35
SD	12.29	10.21	13.02	10.41	10.58	10.39
Behavioural problems* n (%)	54 (9)	17 (2)	84 (10)	29 (5)	27 (3)	29 (4)
Behavioural problems: Males n (%)	30 (9)	9 (2)	45 (11)	19 (6)	14 (3)	20 (5)
Behavioural problems: Females n (%)	24 (8)	8 (2)	39 (10)	10 (3)	13 (3)	9 (3)
Behavioural problems, p-value (sex-stratified)	0.19	0.62	0.51	0.87	0.43	0.041*

*Internalising/ externalising problems were defined as CBCL scores > 64
*p<0.05

4.4.3. Associations between PRS for ADHD and child development and mental health outcomes

There were no statistically significant associations in the logistic regression models (controlled for relevant covariates) between polygenic risk for ADHD and adverse developmental and mental health outcomes (at ages 24 to 60 months), **Table 4.5**. However, trend-level associations were observed for ADHD PRS with internalising symptoms at 42 months (OR=1.22, p = 0.094). A linear regression though considered was not utilised as the relationship between ADHD PRS and the outcomes of interest is non-linear (as depicted by the

correlation plots, ***Appendix 3***, Figures 1 to 5). Thus, violating the linearity assumption which forms the basis for linear regression models (Sinharay, 2010).

Table 4. 5 Associations between ADHD PRS and developmental and mental health outcomes in the DCHS

	Cognitive delay (Bayley-III)	**Language delay (Bayley-III)**	**Motor Delay (Bayley-III)**	**Internalising problems (CBCL)**			**Externalising problems (CBCL)**		
Age	**24 months**	**24 months**	**24 months**	**24 months**	**42 months**	**60 months**	**24 months**	**42 months**	**60 months**
OR	0.96	1.01	0.92	0.89	1.22	1.02	1.04	1.0	1.12
SE	0.060	0.057	0.076	0.083	0.13	0.070	0.095	0.10	0.11
P-value	0.48	0.80	0.30	0.16	0.094[#]	0.76	0.68	0.96	0.27
Confidence Interval	0.85; 1.08	0.91; 1.14	0.80; 1.07	0.76; 1.05	0.97; 1.53	0.89; 1.17	0.86; 1.25	0.82; 1.21	0.91; 1.39

#p $0.05 \leq p \leq 0.1$: Trend level association

4.5. Discussion

In this investigation of the potential associations of ADHD genetic risk (as indexed by PRS) with child development and mental health outcomes in the DCHS, no significant associations were observed. This contrasts with prior work that has reported significant associations between ADHD PRS and adverse developmental and mental health outcomes, respectively (Stergiakouli et al., 2017, Neumann et al., 2022, Brikell et al., 2020, Askeland et al., 2019). For example, a higher ADHD PRS was reported to be associated with poorer cognitive outcomes in children with ADHD (Stergiakouli et al., 2017). Similarly, ADHD PRS was associated with language difficulties as well as with increased hyperactivity and inattention in children (Askeland et al., 2019). Previous literature also reported significant associations between ADHD PRS and externalising behaviour in children (Franke et al., 2016, Brikell et al., 2021). For example, Brikell and colleagues (2021) found that a higher ADHD PRS was associated with externalising symptoms in children aged nine and twelve. Similarly, Neumann and colleagues (2021) found that a higher ADHD PRS was linked to both internalising and externalising symptoms in younger children aged four to eight. These authors also reported an association between ADHD PRS and emotional problems in seven-year-old children (Neumann et al., 2021).

In the current study, the absence of statistically significant findings may be attributed to the analyses being underpowered. The sample size of the DCHS target dataset was relatively modest (N~ 900 genotyped) and the missing data at the relevant time points may have limited the predictive ability of the PRS. An *a priori* power analysis, suggested that the current sample had 70% power to detect PRS significant effects. Prior work by Wray et al (2014) recommends a target dataset of N ~2000 to optimise PRS prediction power (Chatterjee et al., 2013). Similarly, other studies reporting significant associations had target sample sizes of more than 10,000 participants (Neumann et al., 2022, Brikell et al., 2020, Askeland et al., 2019, Jansen et al., 2018).

While the prevalence of developmental delay and of adverse mental health outcomes (i.e. internalising and externalising problem behaviour) was low in the DCHS; significant sex-specific differences were observed. For example, the males performed more poorly in the developmental assessments (e.g., cognitive development) and mental health measures (e.g., externalising behaviour). This aligns with previous work within DCHS, which also reported that male children performed worse than female children in the cognitive and language (expressive and receptive language) domains (Donald et al., 2019). Similarly, prior studies have found that male children exhibited poorer fine motor and language skills compared to female children (aged 2 to 4 years) (Peyre et al., 2019, Eriksson et al., 2011). While the underpinnings of these observed sex-specific differences are yet to be well understood (Peyre et al., 2019, Eriksson et al., 2011), one proposed hypothesis is that differential environmental exposures (e.g. gender-typed play styles and toys) may exert an effect (Borstein et al.,2004, Caldera et al.,1989). For example, boys are exposed to action-oriented toys which may not stimulate language development at the same level as care-oriented toys may stimulate language development and expression in girls (Borstein et al.,2004, Caldera et al.,1989, Eriksson et al.,2011).

Further, differences in the genetic architecture of ADHD in different populations, along with inconsistencies in defining adverse development and mental health in children, may have led to the discrepancies and variability between the findings herein and those of previous literature. For example, some hyperactivity and aggressive behaviours may be considered part of "normal" child development, depending on the social context (Posserud et al., 2006). It is also possible that the effects of ADHD PRS on child developmental and mental health outcomes may have been mediated by environmental factors (e.g. parenting styles) that were not fully

captured nor investigated in the current analyses (Thapar et al., 2016, Hamshere et al., 2013). Further, given that many African languages do not have established terms to describe specific mental illnesses, emotions, or personality traits (Atilola, 2015), measurement bias may have influenced our findings in the mental health outcomes assessed using the CBCL (Zieff et al., 2022). However, within the DCHS, forward and backwards-translation methods of this tool were used to mitigate the potential for bias (Donald et al., 2018b).

Emerging research has also suggested better utility and improved prediction capacity of PRS in exploring ADHD genetic risk as a generalised liability factor (encompassing internalising, externalising, inattention, and ADHD symptoms in children) – as opposed to as an isolated phenotype (Caspi and Moffitt, 2018). Alternatively, combining different PRS and/or investigating general psychopathology in children may improve predictive ability of these risk scores (Neumann et al., 2022). Future work using different mental health PRS to investigate potential associations with child developmental and mental health outcomes in the DCHS would also be warranted. For example, Bolhuis and colleagues (2022) showed that a schizophrenia PRS is associated with an increased risk for mental health problems in children (Bolhuis et al., 2022). Additionally, combining clinical and environmental data with PRS could improve risk prediction, particularly within diverse ancestry populations such as in the DCHS. For example, Hujeol et al (2022) demonstrated the utility of combining PRS and family history of disease, showing that this approach improved the prediction accuracy of PRS - particularly in African ancestry populations (Hujoel, Loh et al., 2022).

A number of additional methodological limitations may also have contributed to the lack of significant findings in these analyses. First, the ancestry of the discovery sample (EAGLE) was not matched to the ancestry of the target dataset (DCHS), thus limiting the prediction accuracy of the PRS (Martin et al., 2019). Moreover, PRS are only able to capture a small portion of heritability due to common genetic variants (Assay et al.,2018). Thus, these scores may underestimate environmental effects in complex traits such as those within mental health outcomes (Assay et al., 2018, Wahbeh et al.,2021). However, the use of PRS-CS – which has been shown to perform better in trans-ancestry analysis than traditional PRS methods (such as pruning and thresholding) – may have improved trans-ancestral transferability in this instance (Ge et al., 2019, Ruan et al.,2022). PRS-CS leverages Bayesian inference to adjust for linkage disequilibrium (LD) across the genome, allowing for a more accurate estimation of polygenic scores, particularly in ancestrally diverse populations. This method also negates the need for pre-selecting variants (based on arbitrary p-value thresholds), providing a more nuanced and powerful approach to PRS computation (Ge et al., 2019). Similarly, incorporating the African LD reference panel from the 1000 Genomes Project was key in mitigating the limitations of trans-ancestral transferability. This is important as PRS combines causal and non-causal variants into a single score (Wang et al.,2020). Thus, an LD reference panel with African ancestry participants is needed to account for population-specific patterns of LD (Wang et al.,2020).

Second, the discovery summary statistics did not include any SNPs of genome-wide significance (i.e. this dataset may have been underpowered), which may have impeded accurate estimation of the effect sizes of ADHD. However, it is noteworthy that the PGC-ADHD meta-analysis (N=5,631 cases and 13,589 controls) – in which genome-wide significant SNPs were identified- had only 8% more statistical power than EAGLE to detect genome-wide significant effects (Middeldorp et al., 2019). However, efforts within EAGLE are underway, thus future analyses with larger sample sizes can be expected which will improve the power to detect additional variants of ADHD-related traits within the EAGLE consortium (Middeldorp et al., 2019).

In conclusion, this study did not find significant associations between genetic risk for ADHD (as indexed by PRS) and adverse developmental and mental health outcomes in the DCHS. Further work – ideally

incorporating larger target data sample sizes and discovery datasets originating from ancestry-matched childhood GWAS – is needed to increase statistical power to detect significant effects, and to allow more robust conclusions to be drawn. Nonetheless, despite its relatively limited sample size, the DCHS is a well-characterized and well-established cohort, that addresses the long-standing lack of representation of African ancestry participants in global genomics research (Stein et al., 2015, Zar et al., 2015). Further work in under-represented regions and population groups would contribute to a better understanding of the genomic basis of complex phenotypes and improve the generalisability and transferability of polygenic findings. Ultimately, such work may improve the predictive utility and trans-ancestral transferability of PRS in populations of African ancestry; as well as provide novel insight into the neurobiological understanding of development and mental health in early childhood in South African (and other under-represented) study populations.

4.6. References

Askeland, r., hannigan, l., ask, h., tesli, m., corfield, e., andreassen, o. A., smith, g. D., reichborn-kjennerud, t. & havdahl, a. 2019. Early manifestations of genetic risk for neurodevelopmental disorders. *European neuropsychopharmacology,* 29**,** s252-s253.

Atilola, o. 2015. Level of community mental health literacy in sub-saharan africa: current studies are limited in number, scope, spread, and cognizance of cultural nuances. *Nord j psychiatry,* 69**,** 93-101.

Barican, j. L., yung, d., schwartz, c., zheng, y., georgiades, k. & waddell, c. 2022. Prevalence of childhood mental disorders in high-income countries: a systematic review and meta-analysis to inform policymaking. *Evid based ment health,* 25**,** 36-44.

Bolhuis, k., steenkamp, l. R., blanken, l. M. E., neumann, a., jansen, p. R., hillegers, m. H. J., cecil, c. A. M., tiemeier, h. & kushner, s. A. 2022. Schizophrenia polygenic risk is associated with child mental health problems through early childhood adversity: evidence for a gene–environment correlation. *European child & adolescent psychiatry,* 31**,** 529-539.

Brikell, i., burton, c., mota, n. R. & martin, j. 2021. Insights into attention-deficit/hyperactivity disorder from recent genetic studies. *Psychological medicine*.

Brikell, i., larsson, h., lu, y., pettersson, e., chen, q., kuja-halkola, r., karlsson, r., lahey, b. B., lichtenstein, p. & martin, j. 2020. The contribution of common genetic risk variants for adhd to a general factor of childhood psychopathology. *Molecular psychiatry,* 25**,** 1809-1821.

Çelik, p., ayranci sucakli, i. & yakut, h. I. 2020. Which bayley-iii cut-off values should be used in different developmental levels? *Turk j med sci,* 50**,** 764-770.

Chatterjee, n., wheeler, b., sampson, j., hartge, p., chanock, s. J. & park, j. H. 2013. Projecting the performance of risk prediction based on polygenic analyses of genome-wide association studies.

Ge, t., chen, c. Y., ni, y., feng, y. A. & smoller, j. W. 2019. Polygenic prediction via bayesian regression and continuous shrinkage priors. *Nat commun,* 10**,** 1776.

Demontis, d., walters, r. K., martin, j., mattheisen, m., als, t. D., agerbo, e., baldursson, g., belliveau, r., bybjerg-grauholm, j., bækvad-hansen, m., cerrato, f., chambert, k., churchhouse, c., dumont, a., eriksson, n., gandal, m., goldstein, j. I., grasby, k. L., grove, j., gudmundsson, o. O., hansen, c. S., hauberg, m. E., hollegaard, m. V., howrigan, d. P., huang, h., maller, j. B., martin, a. R., martin, n. G., moran, j., pallesen, j., palmer, d. S., pedersen, c. B., pedersen, m. G., poterba, t., poulsen, j. B., ripke, s., robinson, e. B., satterstrom, f. K., stefansson, h., stevens, c., turley, p., walters, g. B., won, h., wright, m. J., albayrak, ö., anney, r. J. L., arranz, m. J., banaschewski, t. J., bau, c., biederman, j., buitelaar, j. K., casas, m., charach, a., crosbie, j., dempfle, a., doyle, a. E., ebstein, r. P., elia, j., freitag, c., föcker, m., gill, m., grevet, e., hawi, z., hebebrand, j., herpertz-dahlmann, b., hervas, a., hinney, a., hohmann, s., holmans, p., hutz, m., ickowitz, a., johansson, s., kent, l., kittel-schneider, s., lambregts-rommelse, n., lehmkuhl, g., loo, s. K., mcgough, j. J., meyer, j., mick, e., middletion, f., miranda, a., mota, n. R., mulas, f., mulligan, a., nelson, f., nguyen, t. T., oades, r. D., o'donovan, m. C., owen, m. J., palmason, h., ramos-quiroga, j. A., renner, t. J., ribasés, m., rietschel, m., rivero, o., romanos, j., romanos, m., rothenberger, a., royers, h., et al. 2019. Discovery of the first genome-wide significant risk loci for attention deficit/hyperactivity disorder. *Nature genetics,* 51**,** 63-75.

Donald, k. A., hoogenhout, m., du plooy, c. P., wedderburn, c. J., nhapi, r. T., barnett, w., hoffman, n., malcolm-smith, s., zar, h. J. & stein, d. J. 2018. Drakenstein child health study (dchs): investigating determinants of early child development and cognition. *Bmj paediatrics open,* 2**,** e000282-e000282.

Duncan, l., shen, h., gelaye, b., meijsen, j., ressler, k., feldman, m., peterson, r. & domingue, b. 2019. Analysis of polygenic risk score usage and performance in diverse human populations. *Nature communications 2019 10:1,* 10**,** 1-9.

Egger, h. L. & angold, a. 2006. Common emotional and behavioral disorders in preschool children: presentation, nosology, and epidemiology. *Journal of child psychology and psychiatry,* 47**,** 313-337.

Middeldorp, c. M., hammerschlag, a. R., ouwens, k. G., groen-blokhuis, m. M., st. Pourcain, b.,

Greven, c. U., pappa, i., tiesler, c. M. T., ang, w., nolte, i. M., vilor-tejedor, n., bacelis, j., ebejer, j. L., zhao, h., davies, g. E., ehli, e. A., evans, d. M., fedko, i. O., guxens, m., hottenga, j. J., hudziak, j. J., jugessur, a., kemp, j. P., krapohl, e., martin, n. G., murcia, m., myhre, r., ormel, j., ring, s. M., standl, m., stergiakouli, e., stoltenberg, c., thiering, e., timpson, n. J., trzaskowski, m., van der most, p. J., wang, c., nyholt, d. R., medland, s. E., neale, b., jacobsson, b., sunyer, j., hartman, c. A., whitehouse, a. J. O., pennell, c. E., heinrich, j., plomin, r., davey smith, g., tiemeier, h., posthuma, d. & boomsma, d. I. 2016. A genome-wide association meta-analysis of attention-deficit/hyperactivity disorder symptoms in population-based pediatric cohorts. *Journal of the american academy of child and adolescent psychiatry,* 55**,** 896-905.e6.

Neumann, a., jolicoeur-martineau, a., szekely, e., sallis, h. M., o'donnel, k., greenwood, c. M. T., levitan, r., meaney, m. J., wazana, a., evans, j. & tiemeier, h. 2021. Combined polygenic risk scores of different psychiatric traits predict general and specific psychopathology in childhood. *J child psychol psychiatry*.

Ogundele, m. O. 2018. Behavioural and emotional disorders in childhood: a brief overview for paediatricians. *World j clin pediatr,* 7**,** 9-26.

Posserud, m. B., lundervold, a. J. & gillberg, c. 2006. Autistic features in a total population of 7-9-year-old children assessed by the assq (autism spectrum screening questionnaire). *J child psychol psychiatry,* 47**,** 167-75.

Stein, d. J., koen, n., donald, k. A., adnams, c. M., koopowitz, s., lund, c., marais, a., myers, b., roos, a., sorsdahl, k., stern, m., tomlinson, m., van der westhuizen, c., vythilingum, b., myer, l., barnett, w., brittain, k. & zar, h. J. 2015. Investigating the psychosocial determinants of child health in africa: the drakenstein child health study. *J neurosci methods,* 252**,** 27-35.

Stergiakouli, e., davey smith, g., martin, j., skuse, d. H., viechtbauer, w., ring, s. M., ronald, a., evans, d. E., fisher, s. E., thapar, a. & st pourcain, b. 2017. Shared genetic influences between dimensional asd and adhd symptoms during child and adolescent development. *Molecular autism,* 8**,** 1-13.

Thapar, a., martin, j., mick, e., vasquez, a. A., langley, k., scherer, s. W., schachar, r., crosbie, j., williams, n., franke, b., elia, j., glessner, j., hakonarson, h., owen, m. J., faraone, s. V., o'donovan, m. C., holmans, p. & consortium, i. 2016. Psychiatric gene discoveries shape evidence on adhd's biology. *Molecular psychiatry,* 21**,** 1202-1207.

Tishkoff, s. A., reed, f. A., friedlaender, f. R., ehret, c., ranciaro, a., froment, a., hirbo, j. B., awomoyi, a. A., bodo, j. M., doumbo, o., ibrahim, m., juma, a. T., kotze, m. J., lema, g., moore, j. H., mortensen, h., nyambo, t. B., omar, s. A., powell, k., pretorius, g. S., smith, m. W., thera, m. A., wambebe, c., weber, j. L. & williams, s. M. 2009. The genetic structure and history of africans and african americans. *Science,* 324**,** 1035-44.

Peyre, h., hoertel, n., bernard, j.y., rouffignac, c., forhan, a., taine, m., heude, b. And ramus, f. (2019). Sex differences in psychomotor development during the preschool period: a longitudinal study of the effects of environmental factors and of emotional, behavioral, and social functioning. Journal of experimental child psychology, [online] 178, pp.369–384. Doi:https://doi.org/10.1016/j.jecp.2018.09.002.

Eriksson, m., marschik, p.b., tulviste, t., almgren, m., pérez pereira, m., wehberg, s., marjanovič-umek, l., gayraud, f., kovacevic, m. And gallego, c. (2011). Differences between girls and boys in emerging language skills: evidence from 10 language communities. British journal of developmental psychology, 30(2), pp.326–343. Doi:https://doi.org/10.1111/j.2044-835x.2011.02042.x.

Sinharay, s. (2010). An overview of statistics in education. International encyclopedia of education, pp.1–11. Doi:https://doi.org/10.1016/b978-0-08-044894-7.01719-x.

Chowdhury, m.z.i. And turin, t.c. (2020). Variable selection strategies

And its importance in clinical prediction modelling. Family medicine and community health, 8(1), p.e000262. Doi:https://doi.org/10.1136/fmch-2019-000262.

Assary, e., vincent, j.p., keers, r. And pluess, m. (2018). Gene-environment interaction and psychiatric

Disorders: review and future directions. Seminars in cell & developmental biology, 77, pp.133–143. Doi:https://doi.org/10.1016/j.semcdb.2017.10.016.

Wahbeh, m.h. and avramopoulos, d. (2021). Gene-environment interactions in schizophrenia: a literature Review. Genes, [online] 12(12), p.1850. Doi:https://doi.org/10.3390/genes12121850.

Zar, h. J., barnett, w., myer, l., stein, d. J. & nicol, m. P. 2015. Investigating the early-life Determinants of illness in africa: the drakenstein child health study. *Thorax,* 70**,** 592-594.

Zieff, m. R., fourie, c., hoogenhout, m. & donald, k. A. 2022. Psychometric properties of the aseba child behaviour checklist and youth self-report in sub-saharan africa - a systematic review. *Acta neuropsychiatr***,** 1-24.

Chapter 5: Gestational epigenetic age deviation and childhood development and mental health in the DCHS

5.1. Abstract

Background:

Epigenetic age (EA) acceleration (i.e., higher EA relative to chronological age) has been associated with adverse health outcomes and mortality in adults, and with early pubertal development in adolescence. However, potential associations between gestational EA deviation (i.e., accelerated, or decelerated EA relative to chronological age) at birth, and childhood neurodevelopmental and mental health outcomes remain poorly understood. Thus, this analysis aimed to investigate potential associations between gestational EA deviation and adverse developmental and mental health outcomes in early childhood – a critical period of neurodevelopment.

Methods:

Data were analysed from the Drakenstein Child Health Study (DCHS). Using DNAm data from umbilical cord blood samples, gestational EA deviation at birth was calculated. Child developmental and mental health outcomes were assessed with Bayley-III and CBCL, respectively. Initially, bivariate linear regression was employed to explore unadjusted associations between gestational EA residuals at birth, and child developmental and mental health outcomes. Thereafter, multivariable linear regression models were used to determine adjusted associations controlled for the relevant covariates – i.e. maternal depression, maternal prenatal smoking, sex of the child and the first 10 genomic principal components to adjust for population stratification.

Results and conclusion:

A study sample of 275 children from the DCHS was included in this analysis. In the adjusted multivariable models, no significant associations were found between gestational EA deviation at birth and developmental or mental health outcomes in the index children, between ages 24 and 60 months. However, trend-level associations between gestational EA deviation at birth and externalising symptoms were observed at 24 months, for both the unadjusted model ($\beta = -0.19$, $p = 0.072$) and the adjusted model ($\beta = -0.17$, $p = 0.10$). These preliminary findings suggest a need for further, more well-powered studies to investigate the potential clinical significance of gestational EA deviation at birth.

5.2. Introduction

As discussed in *Chapter 1 (Section 1.1),* childhood-onset developmental and mental health disorders have been linked to long-term adverse consequences (Barican et al., 2022, Ogundele, 2018). For example, internalising problems have been associated with anxiety and depression while externalising problems have been linked to difficulties in social and academic domains (Vassallo et al., 2002, Deighton et al., 2018, Goodwin et al., 2004). These complex disorders likely arise from multifaceted interactions between genetic and environmental factors (Cavalli and Heard, 2019). In recent years, research has increasingly highlighted the role of epigenetic changes in the development and manifestation of these disorders (Cavalli and Heard, 2019, Malecki et al., 2022).

Early childhood presents a critical period during which epigenetic changes, including DNA methylation (DNAm), - i.e., the addition of a methyl group to cytosines at the 5' position in cytosine-guanine dinucleotides (CpGs) *(further detailed in Chapter 6)* – may influence developmental trajectories (Cavalli and Heard, 2019). DNAm, along with other epigenetic modifications, plays a crucial role in regulating gene expression patterns and has been implicated in various biological processes, such as development and ageing (Liu et al., 2018, Barker, 2018).

Epigenetic clocks – consisting of DNAm profiles at specific age-related CpG sites – have been developed to estimate epigenetic age (EA) at different life stages, including childhood, and birth (Bell et al., 2019). As discussed previously (*Chapter 3, Section 3.1),* these clocks – distinguished by target tissue and age-related traits – offer valuable tools for studying development and ageing and can detect deviations between chronological age and EA (i.e. EA acceleration (EAA)or deceleration) (Horvath, 2013). They have emerged as potential indicators, not only of chronological age, but also of biological health (Horvath and Raj, 2018). While early epigenetic clocks were developed for adults, specialized clocks for estimating gestational EA at birth were later devised (Knight et al., 2016; Bohlin et al., 2016).

A number of studies in adults have explored the relationship between EA deviation and mental health outcomes (Han et al., 2018, Fries et al., 2017). For example, EAA has been linked to conditions such as major depression, bipolar disorder, and posttraumatic stress disorder (Han et al., 2018; Fries et al., 2017; Wolf et al., 2019); while EA deceleration may confer protective effects (e.g. reduced risk of all-cause mortality) in adults (Horvath et al., 2015; Chen et al., 2016). However, the understanding of epigenetic clocks in childhood is currently limited. Contrary to findings in adults, accelerated gestational EA at birth has been associated with positive outcomes, such as increased birth weight and improved neuropsychological outcomes during childhood (Khouja et al., 2018; Baron et al., 2012). Conversely, decelerated gestational EA at birth has been predictive of adverse outcomes such as low birth weight, cognitive delays, attention deficits and internalising symptoms (Knight et al., 2016; Simpkin et al., 2016, 2017; Mathewson et al., 2017; Tollenaar et al.,2021; Copeland et al.,2019).

Importantly, empirical research on the association between gestational EA deviation at birth and childhood developmental and mental health outcomes, particularly in children under the age of six years old, is sparse (Szyf, 2015; Tollenaar et al., 2021). Further, the vast majority of studies to date have been in participants of European ancestry, with a limited representation of African populations (Adepoju, 2022). To address these gaps in the literature, the aim of this analysis was to investigate the associations between gestational EA deviation at birth and childhood developmental and mental health outcomes in the DCHS children, at ages 24 months to 60 months.

5.3. Methods

5.3.1. Sociodemographic, biomedical and psychosocial characteristics

A comprehensive description of the relevant maternal sociodemographic, biomedical, and psychosocial measures in the DCHS is included in *Chapter 3 (Section 3.3).* For this analysis, SES quartiles were again generated for this study sample (Stein et al., 2015, Myer et al., 2008). Biomedical risk factors of interest included maternal HIV status (determined through rapid testing and self-report), and maternal anaemia (determined through haemoglobin test) (Stein et al., 2015, Zar et al., 2015). Psychosocial risk factors were prenatal maternal substance use (tobacco use, alcohol consumption), assessed using the ASSIST and urine cotinine measures (Humeniuk et al., 2008); and maternal depression, assessed using the Edinburgh Postnatal Depression Scale (EPDS), scores of ≥ 13 were considered indicative of depression (Donald et al., 2018a, Zar et al., 2015).

5.3.2. Child anthropometry, development and mental health measures

Child anthropometric data including the sex of the child, birth weight, and gestational age were obtained by trained DCHS staff (Donald et al., 2018a). Child developmental outcomes included cognitive development, language, and motor development, were assessed using Bayley-III (Bayley, 2006), as has been described previously *(Chapter 4, Section 4.3)*. In this analysis composite scores were reported, typical of other studies within DCHS (le Roux et al., 2018). Child mental health outcomes were assessed using the parent-rated CBCL (Achenbach, 2011) – *see also Chapter 4, Section 4.3*.

5.3.3. DNA methylation

Initially, DNAm data were available for 275 children, derived from non-contaminated umbilical cord blood (Morin et al., 2017) *(Full details on umbilical cord blood collection and DNA extraction are provided in Chapter 4).* One batch (n= 120) was generated using the Illumina Infinium HumanMethylation450 bead array (Illumina, San Diego, USA), which has over 450,000 methylation sites; while the second batch (n= 155) was generated using the Infinium Human MethylationEPIC BeadChip (Illumina, San Diego, USA), which has over 850,000 methylation sites. Pre-processing and statistics were done in R 3.5.1 (R Core Team, 2018). Raw-iDat files were imported to Rstudio where intensity values were converted into beta values. The 450K and EPIC datasets were then combined using the *minfi* package (Aryee et al., 2014). Background subtraction, colour correction and normalization were performed using the "preprocessFunnorm" function (Fortin et al., 2014). Batch effects were controlled for using "ComBat" from the R package *sva* (Leek et al., 2012). Cord blood cell type composition was predicted using the most recent cord blood reference dataset (Gervin et al., 2019) and the Improving cell mixture deconvolution by identifying optimal DNA methylation libraries (IDOL) algorithm and probe selection (Koestler et al., 2016). Following sample and probe filtration, 273 samples and 409,033 probes remained for downstream analyses (Pidsley et al., 2016, Price et al., 2013). The QC steps undertaken are expanded upon below.

Sample filtering

Samples were classified as outliers if detected by two or more of the following methods: "detectOutlier" function from the *lumi* package (Du et al., 2008); Hannum et al. (2013) using the *locFDR* package (Efron et al., 2015); and both the "outlyx" and "pfilter" functions from the *watermelon* package (Pidsley et al., 2013). Following pre-processing, technical replicates and sex-mismatched samples (i.e. samples for which reported sex did not match sex chromosome methylation signatures) were also removed.

Probe filtering

Probes which did not meet the following criteria were removed:

- Probes with Nas in ≥ 1% of samples or had a detection p value ≥ 1x10-16 in ≥ 1% of samples (n = 10,868) were removed.
- Probes which bind to the sex chromosomes were removed due to the distribution differences observed (n = 9,896).
- Probes with a sequence containing a SNP either at the CpG site being measured, or at the site of the single base pair extension with a minor allele frequency ≥ 1% (Price et al., 2013, Pidsley et al., 2016), were removed (n = 13,598).
- Autosomal probes which were predicted (*in silico)* to non-specifically bind to sex chromosomes were also removed (n = 9,698).

5.3.4. Epigenetic age deviation measures

Child gestational EA estimates were calculated using an epigenetic clock developed for newborns, which has been found to accurately estimate gestational age (GA) at birth (Knight et al., 2016). This gestational epigenetic clock (i.e. the Knight clock) was based on cord blood and blood spot samples obtained from 1434 neonates from 15 independent cohorts (Knight et al., 2016). Data are publicly available in repositories downloaded from the Gene Expression Omnibus (GEO): GSE36642, GSE62924 (Rojas et al., 2015), GSE51180 (Cruickshank et al., 2013), and GSE30870 (Heyn et al., 2012). For cord blood samples, GA was defined at birth; for blood spot samples, GA at birth was added to the number of days between birth and sampling (Knight et al., 2016). For all included datasets, methylation data were generated on either the Illumina Infinium HumanMethylation27 BeadChip or Infinium HumanMethylation450 bead array (Knight et al., 2016).

Knight and colleagues derived gestational EA at birth by regressing chronological GA (from early obstetric ultrasound or last menstrual period (LMP)) on DNAm levels, using a penalized regression model (Knight et al., 2016). They then utilised elastic net regression to select 148 CpG sites predictive of GA from a set of 16, 838 CpG sites that were available across all the training datasets (Knight et al., 2016). Ultimately, a subset of 90 CpG sites with epigenome-wide significance ($p<0.05$) was selected for the Knight (2016) clock. The correlation between this clock and clinically estimated GA was 0.99, thus indicating a strong fit of the model (Knight et al., 2016). For the purposes of this analysis, gestational EA deviation at birth was computed as the residuals of the linear model between gestational EA at birth and chronological GA (Bressler et al., 2020).

5.3.5. Statistical analyses

All analyses described here were performed in R (R Core Team, 2013). The outcome variables for child development were derived from composite scores of the cognition, language, and motor domains of the Bayley-III; and child mental health outcomes were internalising and externalising symptoms of the CBCL. The means and standard deviations for all outcome variables were also calculated and were assessed as continuous variables.

Unadjusted bivariate models, followed by multivariable linear regression models (to control for potential covariates) were conducted to explore potential associations between gestational EA deviation at birth, and child developmental and mental health outcomes in the study sample. Potential covariates of interest included prenatal maternal alcohol consumption, prenatal maternal smoking, maternal HIV, maternal anaemia, SES quartile, sex of the child, birth weight and the first 10 genomic principal components to adjust for population stratification (Stein et al., 2015; Donald et al., 2018a). These covariates were selected based on prior evidence in the DCHS that older child age and higher SES may be protective factors for child development (Donald et al., 2018a, 2019). Conversely, maternal HIV and prenatal smoking and/or alcohol consumption have been found to be associated with poorer anthropometric and developmental outcomes in childhood in this cohort (Zar et al., 2015; Donald et al., 2019). Additionally, maternal HIV, anaemia, and low SES have been previously associated with EA deviation and poor developmental and mental health outcomes in childhood (Oblak et al., 2021; Ronkainen et al., 2022). To improve model performance (discussed previously in *Chapter 4, Section 4.3.8*), a forward selection of variables was employed using the *ols* package in R) (Smith, 2018) - the resultant model included maternal depression, maternal prenatal smoking, and the sex of the child as covariates.

5.4. Results

5.4.1. Sociodemographic, biomedical and psychosocial characteristics

This study sample comprised 275 children with DNAm phenotype data, **Table 5.1**. Almost half (44%) were female, and the prevalence of preterm birth was 12%. Almost a third (29%) of the children were exposed to maternal prenatal smoking, and 16% to prenatal alcohol consumption. A quarter of the children were born to HIV-positive mothers (25%), and more than a quarter (27%) of mothers from low SES. Almost a fifth (19%) of the mothers in this study sample were anaemic during their pregnancies and almost a third (30%) had depression.

Table 5. 1 Sociodemographic, biomedical and psychosocial characteristics of the study sample

Variable	**N (%)**
N	275 (100)
Sex of child (female)	122 (44)
Preterm birth (premature)	32 (12)
SES quartile (low SES)	75 (27)
Prenatal smoking (exposed)	82 (30)
Prenatal alcohol consumption (exposed)	46 (16)
Maternal HIV status (positive)	68 (25)
Maternal anaemia	52 (19)
Maternal depression	83 (30)

5.4.2. Child developmental and mental health outcomes

In this study sample, n=194 (71%) of the children had complete Bayley-III data at the 24-month timepoint, **Table 5.2**. Within this subset, approximately 15% were classified as having cognitive delay, 16% with language delay, and 14% with motor delay. Overall, there were sex-specific differences observed, with males having developmental delay in the language domains (p=0.016).

Table 5. 2 Child developmental outcomes at 24 months (Bayley-III composite scores) in subset of study sample

	Cognitive	**Language**	**Motor**
Sample Size (n)	194	183	183
Male, n (%)	109 (56)	104 (57)	100 (55)
Female, n (%)	85 (44)	79 (43)	83 (45)
Mean	85	84.23	93.22
Standard deviation	8.95	12.33	13.31
Developmental delay:** n (%)	30 (15)	29 (16)	26 (14)
Developmental delay: Males n (%)	20 (18)	21 (20)	16 (16)
Developmental delay: Females n (%)	10 (12)	8 (10)	10 (12)
Delay, P-value (sex-stratified)	0.068#	0.016*	0.24

**Developmental delay was defined as Bayley-III composite scores < 1 SD below the mean
*p<0.05; #p $0.05 \leq p \leq 0.1$

At 24 months, half (50%) of the study sample (n=138) had available CBCL data, with increased sample sizes at 42 months (n=216) and 60 months (n=218). Among these children, approximately one-tenth exhibited internalising problems (12%) or externalising problems (11%), **Table 5.3**. At 42 months, the prevalence of

internalising problems detected was 3%, while 4% exhibited externalising problems. At 60 months, the prevalence of internalising problems was then found to increase to 14%, while 7% exhibited externalising problems. Of note, 157 children (57% of this study sample) had missing CBCL data for at least one timepoint. Similarly, there were significant sex-specific differences observed for mental health outcomes in the indexed children. Overall, males exhibited externalising problem behaviour at 42 months.

Table 5. 3 Child mental health outcomes at ages 24 months to 60 months (CBCL total -scores) in a subset of the study sample

	Internalising symptoms			**Externalising symptoms**		
Age	**24 months**	**42 months**	**60 months**	**24 months**	**42 months**	**60 months**
Sample Size (n)	138	216	218	138	216	218
Males n (%)	75 (54)	118 (55)	125 (57)	75 (54)	118 (55)	125 (57)
Females n (%)	63(46)	98 (45)	93 (43)	63 (46)	98 (45)	93 (43)
Mean	48.14	40.78	44.85	47.91	43.73	42.21
SD	12.29	10.30	13.68	11.60	11.77	10.91
Behavioural problems** n (%)	17 (12)	6 (3)	31 (14)	15 (11)	8 (4)	15 (7)
Behavioural problems: Males n (%)	10 (13)	2 (2)	20 (16)	11 (15)	5 (4)	11 (9)
Behavioural problems: Females n (%)	7 (11)	4 (4)	11 (12)	4 (6)	3 (3)	4 (4)
Behavioural problems, P-value (sex-stratified)	0.47	0.41	0.11	$0.071^{\#}$	0.041^{*}	$0.071^{\#}$

**Internalising/ externalising problems were defined as CBCL t-scores > 64

*$p<0.05$; # $0.05 \leq p \leq 0.1$

5.4.3. Associations between gestational EA deviation at birth, and child developmental and mental health outcomes

The unadjusted bivariate regression models and multivariable linear regressions revealed no statistically significant associations between gestational EA deviation at birth and the outcomes of interest, **Tables 5.4 to 5.7**. However, interesting trend-level associations were observed between EA deviation and externalising problems at 24 months, both in the unadjusted models ($\beta = -0.19$, $p = 0.072$) and the adjusted models ($\beta = -0.17$, $p = 0.10$), **Tables 5.6 and 5.7**.

Table 5. 4 Bivariate regression analysis of associations between gestational EA deviation at birth and child developmental outcomes at 24 months

	Cognitive development			**Language development**			**Motor development**		
	estimate	CI	*p*	estimate	CI	*p*	estimate	CI	*p*
Gestational EA deviation at birth	0.022	0.12; 0.17	0.76	0.099	-0.10; 0.30	0.33	-0.015	-0.23; 0.20	0.89

Table 5. 5 Multivariable regression analysis of associations between gestational EA deviation at birth and child developmental outcomes at 24 months

	Cognitive development			**Language development**			**Motor development**		
	estimate	CI	*p*	estimate	CI	*p*	estimate	CI	*p*
Gestational EA deviation at birth	-0.031	-0.18; 0.12	0.68	0.080	-0.12; 0.28	0.43	-0.0063	-0.24; 0.23	0.96
Sex of child (Female)	-1.56	-4.18; 1.06	0.24	-4.17	-7.76; -0.58	0.023*	0.46	-3.63; 4.59	0.83
Maternal anaemia	-0.86	-4.05; 2.33	0.60	0.72	-3.726; 5.18	0.75	-0.22	-5.363; 4.93	0.93
Maternal depression	-3.85	-6.65; -1.06	0.007**	-5.94	-9.80; -2.08	0.003*	-2.67	-7.10; 1.76	0.24
Prenatal maternal smoking	2.41	-0.58; 5.40	0.11	-1.05	-5.18; 3.08	0.62	-2.80	-7.44; 1.83	0.23

*p<0.05; **p<0.01

Table 5. 6 Bivariate regression analysis of associations between gestational EA deviation at birth and child mental health outcomes from 24 months to 60 months

	24 months						**42 months**						**60 months**					
	Internalising symptoms			**Externalising symptoms**			**Internalising symptoms**			**Externalising symptoms**			**Internalising symptoms**			**Externalising symptoms**		
	estimate	CI	*p*	estimate	CI	*p*	estimate	CI	*p*	estimate	CI	*p*	estimate	CI	*p*	estimate	CI	*p*
EA deviation	-0.063	-0.28;0.16	0.57	-0.19	-0.39; 0.017	0.072#	-0.044	-0.20; 0.11	0.57	-0.061	-0.23; 0.11	0.48	-0.089	-0.30; 0.12	0.41	-0.102	-0.36; 0.16	0.43

#p $0.05 \leq p \leq 0.1$

Table 5. 7 Multivariable regression analysis of associations between gestational EA deviation at birth and child mental health outcomes from 24 months to 60 months

	24 months						**42 months**						**60 months**					
	Internalising symptoms			**Externalising symptoms**			**Internalising symptoms**			**Externalising symptoms**			**Internalising symptoms**			**Externalising symptoms**		
	estimate	CI	*p*	estimate	CI	*p*	estimate	CI	*p*	estimate	CI	*p*	estimate	CI	*p*	estimate	CI	*p*
EA deviation	-0.034	-0.26; 0.19	0.8	-0.17	-0.37; 0.04	0.1	-0.023	-0.18; 0.13	0.78	0.006	-0.17; 0.18	0.95	-0.066	-0.28; 0.15	0.55	-0.042	-0.29; 0.21	0.74
Sex of child (Female)	1.92	-2.25; 6.08	0.36	2.46	-1.32; 6.23	0.2	0.72	-2.04; 3.47	0.61	4.82	1.83; 7.81	0.002**	2.89	-0.82; 6.60	0.13	1.6	-3.66; 6.85	0.55
Maternal anaemia	2.68	-2.12; 7.48	0.27	2.9	-1.45; 7.26	0.19	-0.65	-3.92; 2.61	0.69	0.66	-2.89; 4.20	0.72	4.51	-0.023; 9.05	0.051#	2.95	-3.04; 8.94	0.33
Maternal depression	2.73	-1.82; 7.27	0.24	2.92	-1.20; 7.04	0.16	-2.42	-5.34; 0.51	0.1	-2.07	-5.24; 1.10	0.2	2.61	-1.38; 6.60	0.2	2.21	-3.29; 7.79	0.42
Pre-natal smoking	6.77	1.77; 11.76	0.008**	5.25	0.72; 9.78	0.024*	0.47	-2.89; 3.84	0.78	2.57	-1.09; 6.22	0.17	0.89	-3.59; 5.37	0.7	1.47	-4.66; 7.61	0.63

*p<0.05; **p<0.01; #p $0.05 \leq p \leq 0.1$

5.5. Discussion

The primary findings of this analysis yielded no statistically significant associations between gestational EA deviation at birth, and developmental or mental health outcomes in the DCHS study sample. This is consistent with previous work that did not find associations between EA deviation and externalising symptoms or adverse cognitive outcomes in children (Suarez et al., 2018a, Tollenaar et al., 2021). Similarly, the meta-analysis of n=2 studies, conducted as part of this doctoral project (see *Chapter 3, Section 3.4.7)*, also yielded no significant associations between EA deviation and child internalising symptoms (OR =1.14, [95CI, 0.86-1.49]).

Of note, there were trend-level associations between EA deviation at birth and externalising symptoms at 24 months in the DCHS index infants (p=0.072). This is aligned with prior work by Tollenaar and colleagues (2021), who reported associations between EA deviation and externalising symptoms in children (aged 6 to 10 years old), which did not reach statistical significance (p= 0.083). On the other hand, a number of studies have reported significant associations between EA deviation and internalising symptoms in children (Tollenaar et al., 2021, Sumner et al., 2019, Suarez et al., 2018a; Dammering et al. 2021). For example, one study demonstrated that EAA accurately predicted internalising symptoms diagnosis in children (aged 4 years) (Dammering et al., 2021). Interestingly, the prevalence of internalising and externalising symptoms in the DCHS children increased at 42 months with a decrease again at 60 months. This spike at 42 months may be attributed to developmental milestones in the children. As children mature, they develop improved emotional regulation and coping mechanisms (Fanti and Henrich, 2010, Campbell,1994, Dupaul et al., 2001), moreover they tend to have improved language skills and as a result are better equipped to communicate their needs thus leading to reduced frustration – which often manifests as externalising problem behaviour such as aggression and disruptive behaviour (Fanti and Henrich, 2010).

The discrepancies between the current study findings and prior literature in this field may be attributed to several factors. First, differences in the biological samples and epigenetic clocks used in this study, compared to those reported in previous studies, may have contributed to these non-significant findings. Different tissues have different DNAm and EA profiles (Zhang et al., 2013) - for example, DNAm from buccal cells has been found to be hypomethylated when compared to blood (Lowe et al.,2014) - with DNAm in cord blood (as analyses in this study) reflecting the intrauterine environment, while saliva does not (Neumann et al., 2020). This study utilised the Knight epigenetic clock, which has been found to be accurate in measuring gestational EA at birth (Knight et al., 2016). Conversely, other studies have used the Horvath clock (Suarez et al., 2018a; Tollenaar et al., 2021), which may not be optimally suited for measuring gestational EA in newborns (Knight et al., 2016, Schroeder et al., 2011). Second, unmeasured mediating and moderating factors may have influenced the current study findings. For example, previous research suggests that the association between EAA at birth and internalising behaviour in children may be moderated by exposure to maltreatment (Dammering et al., 2021).

Noteworthy limitations of this study included potential biases (e.g., reduced accuracy) in the epigenetic clock used, particularly when applied to multi-ancestral cohorts such as the DCHS (Horvath et al., 2016). Nevertheless, the Knight clock (2016) has previously been used in ancestrally diverse populations (Wang and Zhou, 2021), thus was a suitable measure of gestational EA in this cohort. The performance of epigenetic clocks is influenced by the tissue type and data on which they were trained (Horvath and Raj 2018). Most current epigenetic clocks have been trained to predict chronological age using blood or saliva, thus limiting their ability to capture age- and disease-related DNAm variation across different tissues (Higgins-Chen, Boks et al. 2020). Thus, ascertaining the biological or clinical significance and implications of variation in EA is challenging and not yet well understood (Horvath and Raj 2018). Integrating both biological ageing and disease-related biomarkers may therefore advance the development of epigenetic biomarkers of development and mental health outcomes in children (Bozack, Rifas-Shiman et al. 2023). Given that chronological age is not always consistent with biological age, measures of ageing that track clinical parameters (e.g. Pace of Aging methylation, (PoA)), may be better suited in children and adolescents (Belsky, Caspi et al. 2020). Biomarkers of system integrity and biological ageing -e.g. lifespan or physical functioning (Levine et al., 2018) - may also

be suitable alternatives for measuring EA deviations in children/adolescents (Belsky, Caspi et al. 2020, Niccodemi, Menta et al. 2022).

Sex- and age-related differences may significantly influence the performance of epigenetic clocks (Bozack et al.,2023, Gopalan et al.,2017). For example, in a recent study of N=485 newborns, females were found to exhibit lower EAA compared to the male newborns, suggesting slower biological ageing in the early life stages (Bozack et al.,2023). Additionally, a comprehensive longitudinal examination of EA throughout childhood may provide valuable insights, as associations between gestational EA and developmental maturity in newborns have been shown to fluctuate during childhood (Bright et al., 2019). Finally, incorporating more participants of diverse ancestry in such work may improve cross-population transferability and translation of study findings – which would be key in mitigating existing health disparities.

The relatively small DCHS sample size (n~300) may also have limited the ability to detect statistically significant associations. Of note, though, this sample size is comparable to those of other similar cohorts in the literature (ranges from N=90 to N=240) (Suarez et al., 2018a, Tollenaar et al., 2021, Dammering et al., 2021). Given the relative dearth of longitudinal epigenetic research, a priori determination of effect sizes for power calculation is challenging (Neumann et al., 2020). In addition, the variance of DNAm between biological sample may influence epigenetic power calculations (Tsai and Bell, 2015, Neumann et al., 2020).

Given the existing evidence that EAA predicts adverse health outcomes and mortality in adults (Marioni et al., 2015), there is a need for further investigation into gestational EA for long-term developmental and mental health outcomes, particularly in children and other vulnerable population groups. Future research foci may thus include precursors and/or potential mediators or moderators of EAA in children (such as exposure to prenatal trauma and stress) (Koen et al., 2014; Stein et al., 2015). Such investigations could contribute to a deeper mechanistic understanding of how environmental stressors become biologically embedded in the epigenome (Aristizabal et al., 2020).

In conclusion, although not significant, trend-level associations were identified between EA deviation and externalising symptoms at 42 months in the DCHS study sample. This preliminary finding warrants further investigation in larger samples, and with longitudinal developmental data – in order to investigate the functional relevance of gestational EA deviation at birth and during development. Enhancing statistical power through larger sample sizes could also provide more robust data. Nevertheless, the study provides a novel exploration of the implications of EA deviation, in a well-characterized South African birth cohort, with both epigenomic data and phenotype data. In future, such studies may offer valuable insights into the biological impact of EA deviation in at-risk children - which may, in turn, ultimately inform efforts to improve early-life interventions and health outcomes.

5.6. References

Aizer, a., stroud, l. & buka, s. 2016. Maternal stress and child outcomes: evidence from siblings. *J hum resour,* 51**,** 523-555.

Akingbuwa, w. A., hammerschlag, a. R., jami, e. S., allegrini, a. G., karhunen, v., sallis, h., ask, h., askeland, r. B., baselmans, b., diemer, e., hagenbeek, f. A., havdahl, a., hottenga, j. J., mbarek, h., rivadeneira, f., tesli, m., van beijsterveldt, c., breen, g., lewis, c. M., thapar, a., boomsma, d. I., kuja-halkola, r., reichborn-kjennerud, t., magnus, p., rimfeld, k., ystrom, e., jarvelin, m. R., lichtenstein, p., lundstrom, s., munafò, m. R., plomin, r., tiemeier, h., nivard, m. G., bartels, m. & middeldorp, c. M. 2020. Genetic associations between childhood psychopathology and adult depression and associated traits in 42998 individuals: a meta-analysis. *Jama psychiatry,* 77**,** 715-728.

Alisch, r. S., barwick, b. G., chopra, p., myrick, l. K., satten, g. A., conneely, k. N. & warren, s. T. 2012. Age-associated dna methylation in pediatric populations. *Genome res,* 22**,** 623-32.

Aristizabal, m. J., anreiter, i., halldorsdottir, t., odgers, c. L., mcdade, t. W., goldenberg, a., mostafavi, s., kobor, m. S., binder, e. B., sokolowski, m. B. & o'donnell, k. J. 2020. Biological embedding of experience: a primer on epigenetics. *Proc natl acad sci u s a,* 117**,** 23261-23269.

Aryee, m. J., jaffe, a. E., corrada-bravo, h., ladd-acosta, c., feinberg, a. P., hansen, k. D. & irizarry, r. A. 2014. Minfi: a flexible and comprehensive bioconductor package for the analysis of infinium dna methylation microarrays. *Bioinformatics,* 30**,** 1363-9.

Barbarin, o. A., richter, l. & dewet, t. 2001. Exposure to violence, coping resources, and psychological adjustment of south african children. *American journal of orthopsychiatry,* 71**,** 16-25.

Barican, j. L., yung, d., schwartz, c., zheng, y., georgiades, k. & waddell, c. 2022. Prevalence of childhood mental disorders in high-income countries: a systematic review and meta-analysis to inform policymaking. *Evid based ment health,* 25**,** 36-44.

Barker, e. D., walton, e. & cecil, c. A. M. 2018. Annual research review: dna methylation as a mediator in the association between risk exposure and child and adolescent psychopathology. *J child psychol psychiatry,* 59**,** 303-322.

Barkley, r. A., o'connell, t., murphy, k. R., anderson, d. & connor, d. F. 2006. Effects of two doses of alcohol on simulator driving performance in adults with attention-deficit/hyperactivity disorder. *Neuropsychology,* 20**,** 77-87.

Bayley, n. 2006. *Bayley scales of infant and toddler development*, psychcorp, pearson.

Beijers, r., jansen, j., riksen-walraven, m. & de weerth, c. 2010. Maternal prenatal anxiety and stress predict infant illnesses and health complaints. *Pediatrics,* 126**,** e401-9.

Bell, c. G., lowe, r., adams, p. D., baccarelli, a. A., beck, s., bell, j. T., christensen, b. C., gladyshev, v. N., heijmans, b. T., horvath, s., ideker, t., issa, j. P. J., kelsey, k. T., marioni, r. E., reik, w., relton, c. L., schalkwyk, l. C., teschendorff, a. E., wagner, w., zhang, k. & rakyan, v. K. 2019. Dna methylation aging clocks: challenges and recommendations. Biomed central ltd.

Biederman, j., green, a., disalvo, m. & faraone, s. V. 2021. Can polygenic risk scores help identify pediatric bipolar spectrum and related disorders?: a systematic review. *Psychiatry res,* 299**,** 113843.

Cannon, m., caspi, a., moffitt, t. E., harrington, h., taylor, a., murray, r. M. & poulton, r. 2002. Evidence for early-childhood, pan-developmental impairment specific to schizophreniform disorder: results from a longitudinal birth cohort. *Arch gen psychiatry,* 59**,** 449-56.

Caramaschi, d., neumann, a., cardenas, a., tindula, g., alemany, s., zillich, l., pesce, g., lahti, j. M. T., havdahl, a., mulder, r., felix, j. F., tiemeier, h., sirignano, l., frank, j., witt, s. H., rietschel, m., deuschle, m., huen, k., eskenazi, b., send, t. S., ferrer, m., gilles, m., de agostini, m., baïz, n., rifas-shiman, s. L., kvist, t., czamara, d., tuominen, s. T., relton, c. L., rai, d., london, s. J., räikkönen, k., holland, n., annesi-maesano, i., streit, f., hivert, m. F., oken, e., sunyer, j., cecil, c. A. M. & sharp, g. 2022. Meta-analysis of epigenome-wide associations between dna methylation at birth and childhood cognitive skills. *Molecular psychiatry 2022 27:4,* 27**,** 2126-2135.

Cavalli, g. & heard, e. 2019. Advances in epigenetics link genetics to the environment and disease. *Nature,* 571**,** 489-499.

Cavazos, t. B. & witte, j. S. 2020. Inclusion of variants discovered from diverse populations improves polygenic risk score transferability. Biorxiv.

Cecil, c. A. M. & nigg, j. T. 2022. Epigenetics and adhd: reflections on current knowledge, research priorities and translational potential. *Mol diagn ther,* 26**,** 581-606.

Çelik, p., ayranci sucakli, i. & yakut, h. I. 2020. Which bayley-iii cut-off values should be used in different developmental levels? *Turk j med sci,* 50**,** 764-770.

Cerveira de baumont, a., hoffmann, m. S., bortoluzzi, a., fries, g. R., lavandoski, p., grun, l. K., guimarães, l. S. P., guma, f., salum, g. A., barbé-tuana, f. M. & manfro, g. G. 2021. Telomere length and epigenetic age acceleration in adolescents with anxiety disorders. *Sci rep,* 11**,** 7716.

Chen, j., gatev, e., everson, t., conneely, k. N., koen, n., epstein, m. P., kobor, m. S., zar, h. J., stein, d. J. & hüls, a. 2023. Pruning and thresholding approach for methylation risk scores in multi-ancestry populations. *Epigenetics,* 18**,** 2187172.

Cherkasova, m., sulla, e. M., dalena, k. L., pondé, m. P. & hechtman, l. 2013. Developmental course of attention deficit hyperactivity disorder and its predictors. *J can acad child adolesc psychiatry,* 22**,** 47-54.

Choi, s. W., mak, t. S. H. & o'reilly, p. F. 2020. Tutorial: a guide to performing polygenic risk score analyses. *Nature protocols.* Nature research.

Christensen, g. M., marcus, m., vanker, a., eick, s. M., malcolm-smith, s., smith, a., dunn, e. C., suglia, s. F., chang, h. H., zar, h. J., stein, d. J. & hüls, a. 2023. Sensitive periods for exposure to indoor air pollutants and psychosocial factors in association with symptoms of psychopathology at school-age in a south african birth cohort. *Medrxiv*.

Christensen, g. M., rowcliffe, c., chen, j., vanker, a., koen, n., jones, m. J., gladish, n., hoffman, n., donald, k. A., wedderburn, c. J., kobor, m. S., zar, h. J., stein, d. J. & hüls, a. 2022. In-utero exposure to indoor air pollution or tobacco smoke and cognitive development in a south african birth cohort study. *Sci total environ,* 834**,** 155394.

Coleman, j. R. I., gaspar, h. A., bryois, j. & breen, g. 2020. The genetics of the mood disorder spectrum: genome-wide association analyses of more than 185,000 cases and 439,000 controls. *Biol psychiatry,* 88**,** 169-184.

Copeland, w. E., shanahan, l., erkanli, a., costello, e. J. & angold, a. 2013. Indirect comorbidity in childhood and adolescence. *Front psychiatry,* 4**,** 144.

Copeland, w. E., worthman, c., shanahan, l., costello, e. J. & angold, a. 2019. Early pubertal timing and testosterone associated with higher levels of adolescent depression in girls. *J am acad child adolesc psychiatry,* 58**,** 1197-1206.

Cruickshank, m. N., oshlack, a., theda, c., davis, p. G., martino, d., sheehan, p., dai, y., saffery, r., doyle, l. W. & craig, j. M. 2013. Analysis of epigenetic changes in survivors of preterm birth reveals the effect of gestational age and evidence for a long term legacy. *Genome med,* 5**,** 96.

Dammering, f., martins, j., dittrich, k., czamara, d., rex-haffner, m., overfeld, j., de punder, k., buss, c., entringer, s., winter, s. M., binder, e. B. & heim, c. 2021. The pediatric buccal epigenetic clock identifies significant ageing acceleration in children with internalizing disorder and maltreatment exposure. *Neurobiology of stress,* 15.

Das, s., forer, l., schönherr, s., sidore, c., locke, a. E., kwong, a., vrieze, s. I., chew, e. Y., levy, s., mcgue, m., schlessinger, d., stambolian, d., loh, p. R., iacono, w. G., swaroop, a., scott, l. J., cucca, f., kronenberg, f., boehnke, m., abecasis, g. R. & fuchsberger, c. 2016. Next-generation genotype imputation service and methods. *Nat genet,* 48**,** 1284-1287.

Dick, d. M., barr, p. B., cho, s. B., cooke, m. E., kuo, s. I. C., lewis, t. J., neale, z., salvatore, j. E., savage, j. & su, j. 2018. Post-gwas in psychiatric genetics: a developmental perspective on the "other" next steps. *Genes, brain and behavior,* 17.

Dick, k. J., nelson, c. P., tsaprouni, l., sandling, j. K., aïssi, d., wahl, s., meduri, e., morange, p. E., gagnon, f., grallert, h., waldenberger, m., peters, a., erdmann, j., hengstenberg, c., cambien, f., goodall, a. H., ouwehand, w. H., schunkert, h., thompson, j. R., spector, t. D., gieger, c., trégouët, d. A., deloukas, p. & samani, n. J. 2014. Dna methylation and body-mass index: a genome-wide analysis. *Lancet,* 383**,** 1990-8.

Dieckmann, l., lahti-pulkkinen, m., kvist, t., lahti, j., dewitt, p. E., cruceanu, c., laivuori, h., sammallahti, s., villa, p. M., suomalainen-König, s., eriksson, j. G., kajantie, e., raikkönen, k., binder, e. B. & czamara, d. 2021. Characteristics of epigenetic aging across gestational and perinatal tissues. *Clinical epigenetics,* 13**,** 97.

Donald, k. A., hoogenhout, m., du plooy, c. P., wedderburn, c. J., nhapi, r. T., barnett, w., hoffman, n., malcolm-smith, s., zar, h. J. & stein, d. J. 2018b. Drakenstein child health study (dchs): investigating determinants of early child development and cognition. *Bmj paediatr open,* 2**,** e000282.

Donald, k. A., wedderburn, c. J., barnett, w., nhapi, r. T., rehman, a. M., stadler, j. A. M., hoffman, n., koen, n., zar, h. J. & stein, d. J. 2019. Risk and protective factors for child development: an observational south african birth cohort. *Plos med,* 16**,** e1002920.

Du, p., kibbe, w. A. & lin, s. M. 2008. Lumi: a pipeline for processing illumina microarray. *Bioinformatics,* 24**,** 1547-8.

Duncan, l., shen, h., gelaye, b., meijsen, j., ressler, k., feldman, m., peterson, r. & domingue, b. 2019. Analysis of polygenic risk score usage and performance in diverse human populations. *Nature communications 2019 10:1,* 10**,** 1-9.

Edenberg, h. J., bierut, l. J., boyce, p., cao, m., cawley, s., chiles, r., doheny, k. F., hansen, m., hinrichs, t., jones, k., kelleher, m., kennedy, g. C., liu, g., marcus, g., mcbride, c., murray, s. S., oliphant, a., pettengill, j., porjesz, b., pugh, e. W., rice, j. P., rubano, t., shannon, s., steeke, r., tischfield, j. A., tsai, y. Y., zhang, c. & begleiter, h. 2005. Description of the data from the collaborative study on the genetics of alcoholism (coga) and single-nucleotidepolymorphism genotyping for genetic analysis workshop 14. *Bmc genetics,* 6**,** 1-8.

Egger, h. L. & angold, a. 2006. Common emotional and behavioral disorders in preschool children: presentation, nosology, and epidemiology. *Journal of child psychology and psychiatry,* 47**,** 313-337.

Eze, i. C., imboden, m., kumar, a., von eckardstein, a., stolz, d., gerbase, m. W., künzli, n., pons, m., kronenberg, f., schindler, c. & probst-hensch, n. 2016. Air pollution and diabetes association: modification by type 2 diabetes genetic risk score. *Environ int,* 94**,** 263-271.

Faleschini, s., rifas-shiman, s. L., tiemeier, h., oken, e. & hivert, m. F. 2019. Associations of prenatal and postnatal maternal depressive symptoms with offspring cognition and behavior in mid-childhood: a prospective cohort study. *Int j environ res public health,* 16.

Feder, k. P. & majnemer, a. 2007. Handwriting development, competency, and intervention. *Developmental medicine & child neurology,* 49**,** 312-317.

Feil, d., abrishamcar, s., christensen, g. M., vanker, a., koen, n., kilanowski, a., hoffman, n., wedderburn, c. J., donald, k. A., kobor, m. S., zar, h. J., stein, d. J. & hüls, a. 2023. Dna methylation as a potential mediator of the association between indoor air pollution and neurodevelopmental delay in a south african birth cohort. *Clin epigenetics,* 15**,** 31.

Fortin, j. P., labbe, a., lemire, m., zanke, b. W., hudson, t. J., fertig, e. J., greenwood, c. M. & hansen, k. D. 2014. Functional normalization of 450k methylation array data improves replication in large cancer studies. *Genome biol,* 15**,** 503.

Fraga, m. F., ballestar, e., paz, m. F., ropero, s., setien, f., ballestar, m. L., heine-suñer, d., cigudosa, j. C., urioste, m., benitez, j., boix-chornet, m., sanchez-aguilera, a., ling, c., carlsson, e., poulsen, p., vaag, a., stephan, z., spector, t. D., wu, y. Z., plass, c. & esteller, m. 2005. Epigenetic differences arise during the lifetime of monozygotic twins. *Proc natl acad sci u s a,* 102**,** 10604-9.

Franke, b., van hulzen, k. J. E., arias-vasquez, a., bralten, j., hoogman, m., klein, m., van donkelaar, m. M. J., hakobjan, m. M. H., heister, a. J. G. A. M., makkinje, r. R. R., naber, m. A. M., van der marel, s. S. L., mostert, j. C., brunner, h. G., van bokhoven, h., zwiers, m. P., buitelaar, j. K., fernández, g., fisher, s.

E., francks, c., stein, j. L., hibar, d. P., thompson, p. M., ripke, s., anttila, v., neale, b. M., farh, k. H., bulik-sullivan, b., huang, h., fromer, m., goldstein, j. I., daly, m. J., walters, r. K., smoller, j. W., lee, p., belliveau, r. A., bergen, s. E., bevilacqua, e., chambert, k. D., genovese, g., o'dushlaine, c., scolnick, e. M., mccarroll, s. A., moran, j. L., palotie, a., petryshen, t. L., erk, s., heinz, a., mohnke, s., romanczuk-seiferth, n., walter, h., delisi, l. E., mccarley, r. W., mesholam-gately, r. I., seidman, l. J., nichols, t. E., neale, m. C., mcintosh, a. M., papmeyer, m., sprooten, e., lawrie, s. M., sussmann, j. E., mcmahon, f. J., yao, y., meyer-lindenberg, a., schwarz, e., grimm, o., mattheisen, m., agerbo, e., demontis, d., hansen, t., mors, o., olsen, l., rasmussen, h. B., børglum, a. D., mortensen, p. B., werge, t., andreassen, o. A., brown, a. A., athanasiu, l., hartberg, c. B., haukvik, u., melle, i., gruber, o., kraemer, b., keil, m., sachdev, p. S., mather, k. A., thalamuthu, a., armstrong, n. J., assareh, a. A., brodaty, h., reppermund, s., wen, w., roiz-santiañez, r., perez-iglesias, r., saykin, a. J., kim, s., nho, k., risacher, s. L., et al. 2016. Genetic influences on schizophrenia and subcortical brain volumes: large-scale proof of concept. *Nature neuroscience,* 19**,** 420-431.

Fransquet, p. D., wrigglesworth, j., woods, r. L., ernst, m. E. & ryan, j. 2019. The epigenetic clock as a predictor of disease and mortality risk: a systematic review and meta-analysis. Biomed central ltd.

Fraser, a., macdonald-wallis, c., tilling, k., boyd, a., golding, j., davey smith, g., henderson, j., macleod, j., molloy, l., ness, a., ring, s., nelson, s. M. & lawlor, d. A. 2013. Cohort profile: the avon longitudinal study of parents and children: alspac mothers cohort. *Int j epidemiol,* 42**,** 97-110.

Fries, g. R., bauer, i. E., scaini, g., wu, m. J., kazimi, i. F., valvassori, s. S., zunta-soares, g., walss-bass, c., soares, j. C. & quevedo, j. 2017. Accelerated epigenetic aging and mitochondrial dna copy number in bipolar disorder. *Transl psychiatry,* 7**,** 1283.

Germine, l., robinson, e. B., smoller, j. W., calkins, m. E., moore, t. M., hakonarson, h., daly, m. J., lee, p. H., holmes, a. J., buckner, r. L., gur, r. C. & gur, r. E. 2016. Association between polygenic risk for schizophrenia, neurocognition and social cognition across development. *Translational psychiatry,* 6**,** e924-e924.

Gervin, k., salas, l. A., bakulski, k. M., van zelm, m. C., koestler, d. C., wiencke, j. K., duijts, l., moll, h. A., kelsey, k. T., kobor, m. S., lyle, r., christensen, b. C., felix, j. F. & jones, m. J. 2019. Systematic evaluation and validation of reference and library selection methods for deconvolution of cord blood dna methylation data. *Clin epigenetics,* 11**,** 125.

Girchenko, p., lahti, j., czamara, d., knight, a. K., jones, m. J., suarez, a., hämäläinen, e., kajantie, e., laivuori, h., villa, p. M., reynolds, r. M., kobor, m. S., smith, a. K., binder, e. B. & räikkönen, k. 2017. Associations between maternal risk factors of adverse pregnancy and birth outcomes and the offspring epigenetic clock of gestational age at birth. *Clin epigenetics,* 9**,** 49.

Hamshere, m. L., stergiakouli, e., langley, k., martin, j., holmans, p., kent, l., owen, m. J., gill, m., thapar, a., o'donovan, m. & craddock, n. 2013. Shared polygenic contribution between childhood attention-deficit hyperactivity disorder and adult schizophrenia. *Br j psychiatry,* 203**,** 107-11.

Han, l. K. M., aghajani, m., clark, s. L., chan, r. F., hattab, m. W., shabalin, a. A., zhao, m., kumar, g., xie, l. Y., jansen, r., milaneschi, y., dean, b., aberg, k. A., van den oord, e. & penninx, b. 2018. Epigenetic aging in major depressive disorder. *Am j psychiatry,* 175**,** 774-782.

Hannon, e., dempster, e., viana, j., burrage, j., smith, a. R., macdonald, r., st clair, d., mustard, c., breen, g., therman, s., kaprio, j., toulopoulou, t., hulshoff pol, h. E., bohlken, m. M., kahn, r. S., nenadic, i., hultman, c. M., murray, r. M., collier, d. A., bass, n., gurling, h., mcquillin, a., schalkwyk, l. & mill, j. 2016. An integrated genetic-epigenetic analysis of schizophrenia: evidence for co-localization of genetic associations and differential dna methylation. *Genome biol,* 17**,** 176.

Hannon, e., schendel, d., ladd-acosta, c., grove, j., hansen, c. S., andrews, s. V., hougaard, d. M., bresnahan, m., mors, o., hollegaard, m. V., bækvad-hansen, m., hornig, m., mortensen, p. B., børglum, a. D., werge, t., pedersen, m. G., nordentoft, m., buxbaum, j., daniele fallin, m., bybjerg-grauholm, j., reichenberg, a. & mill, j. 2018. Elevated polygenic burden for autism is associated with differential dna methylation at birth. *Genome med,* 10**,** 19.

Hannum, g., guinney, j., zhao, l., zhang, l., hughes, g., sadda, s. V., klotzle, b., bibikova, m., fan, j. B., gao, y., deconde, r., chen, m., rajapakse, i., friend, s., ideker, t. & zhang, k. 2013. Genome-wide methylation profiles reveal quantitative views of human aging rates. *Molecular cell,* 49**,** 359-367.

Heim, c. 2017. Immediate biological embedding of maltreatment in children: berlin longitudinal children study (berlinlcs). *Psychoneuroendocrinology,* 83**,** 7.

Heyn, h., li, n., ferreira, h. J., moran, s., pisano, d. G., gomez, a., diez, j., sanchez-mut, j. V., setien, f., carmona, f. J., puca, a. A., sayols, s., pujana, m. A., serra-musach, j., iglesias-platas, i., formiga, f., fernandez, a. F., fraga, m. F., heath, s. C., valencia, a., gut, i. G., wang, j. & esteller, m. 2012. Distinct dna methylomes of newborns and centenarians. *Proc natl acad sci u s a,* 109**,** 10522-7.

Hibler, e., huang, l., andrade, j. & spring, b. 2019. Impact of a diet and activity health promotion intervention on regional patterns of dna methylation. *Clin epigenetics,* 11**,** 133.

Higgins-chen, a. T., boks, m. P., vinkers, c. H., kahn, r. S. & levine, m. E. 2020. Schizophrenia and epigenetic aging biomarkers: increased mortality, reduced cancer risk, and unique clozapine effects. *Biol psychiatry,* 88**,** 224-235.

Horvath, s. 2013. Dna methylation age of human tissues and cell types. *Genome biology,* 14.

Horvath, s. & raj, k. 2018. Dna methylation-based biomarkers and the epigenetic clock theory of ageing. *Nat rev genet,* 19**,** 371-384.

Hüls, a. & czamara, d. 2020. Methodological challenges in constructing dna methylation risk scores. Taylor and francis inc.

Humeniuk, r., ali, r., babor, t. F., farrell, m., formigoni, m. L., jittiwutikarn, j., de lacerda, r. B., ling, w., marsden, j., monteiro, m., nhiwatiwa, s., pal, h., poznyak, v. & simon, s. 2008. Validation of the alcohol, smoking and substance involvement screening test (assist). *Addiction,* 103**,** 1039-1047.

Joehanes, r., just, a. C., marioni, r. E., pilling, l. C., reynolds, l. M., mandaviya, p. R., guan, w., xu, t., elks, c. E., aslibekyan, s., moreno-macias, h., smith, j. A., brody, j. A., dhingra, r., yousefi, p., pankow, j. S., kunze, s., shah, s. H., mcrae, a. F., lohman, k., sha, j., absher, d. M., ferrucci, l., zhao, w., demerath, e. W., bressler, j., grove, m. L., huan, t., liu, c., mendelson, m. M., yao, c., kiel, d. P., peters, a., wang-sattler, r., visscher, p. M., wray, n. R., starr, j. M., ding, j., rodriguez, c. J., wareham, n. J., irvin, m. R., zhi, d., barrdahl, m., vineis, p., ambatipudi, s., uitterlinden, a. G., hofman, a., schwartz, j., colicino, e., hou, l., vokonas, p. S., hernandez, d. G., singleton, a. B., bandinelli, s., turner, s. T., ware, e. B., smith, a. K., klengel, t., binder, e. B., psaty, b. M., taylor, k. D., gharib, s. A., swenson, b. R., liang, l., demeo, d. L., o'connor, g. T., herceg, z., ressler, k. J., conneely, k. N., sotoodehnia, n., kardia, s. L., melzer, d., baccarelli, a. A., van meurs, j. B., romieu, i., arnett, d. K., ong, k. K., liu, y., waldenberger, m., deary, i. J., fornage, m., levy, d. & london, s. J. 2016. Epigenetic signatures of cigarette smoking. *Circ cardiovasc genet,* 9**,** 436-447.

Jung, s. E., shin, k. J. & lee, h. Y. 2017. Dna methylation-based age prediction from various tissues and body fluids. *Bmb rep,* 50**,** 546-553.

Kaufman, j., birmaher, b., brent, d., rao, u., flynn, c., moreci, p., williamson, d. & ryan, n. 1997. Schedule for affective disorders and schizophrenia for school-age children-present and lifetime version (k-sads-pl): initial reliability and validity data. *J am acad child adolesc psychiatry,* 36**,** 980-8.

Kieling, c., baker-henningham, h., belfer, m., conti, g., ertem, i., omigbodun, o., rohde, l. A., srinath, s., ulkuer, n. & rahman, a. 2011. Child and adolescent mental health worldwide: evidence for action. *Lancet,* 378**,** 1515-25.

Knight, a. K., craig, j. M., theda, c., bækvad-hansen, m., bybjerg-grauholm, j., hansen, c. S., hollegaard, m. V., hougaard, d. M., mortensen, p. B., weinsheimer, s. M., werge, t. M., brennan, p. A., cubells, j. F., newport, d. J., stowe, z. N., cheong, j. L. Y., dalach, p., doyle, l. W., loke, y. J., baccarelli, a. A., just, a. C., wright, r. O., téllez-rojo, m. M., svensson, k., trevisi, l., kennedy, e. M., binder, e. B., iurato, s., czamara, d., räikkönen, k., lahti, j. M. T., pesonen, a. K., kajantie, e., villa, p. M., laivuori, h., hämäläinen, e., park, h. J., bailey, l. B., parets, s. E., kilaru, v., menon, r., horvath, s., bush, n. R.,

lewinn, k. Z., tylavsky, f. A., conneely, k. N. & smith, a. K. 2016. An epigenetic clock for gestational age at birth based on blood methylation data. *Genome biology,* 17.

Knight, a. K., smith, a. K., conneely, k. N., dalach, p., loke, y. J., cheong, j. L., davis, p. G., craig, j. M., doyle, l. W. & theda, c. 2018. Relationship between epigenetic maturity and respiratory morbidity in preterm infants. *J pediatr,* 198**,** 168-173.e2.

Koen, n., wyatt, g. E., williams, j. K., zhang, m., myer, l., zar, h. J. & stein, d. J. 2014. Intimate partner violence: associations with low infant birthweight in a south african birth cohort. *Metabolic brain disease,* 29**,** 281-299.

Kwong, a. S. F., morris, t. T., pearson, r. M., timpson, n. J., rice, f., stergiakouli, e. & tilling, k. 2021. Polygenic risk for depression, anxiety and neuroticism are associated with the severity and rate of change in depressive symptoms across adolescence. *Journal of child psychology and psychiatry and allied disciplines*.

Laird, P.W. (2010). Principles and challenges of genome-wide DNA methylation analysis. Nature Reviews Genetics, 11(3), pp.191–203. doi:https://doi.org/10.1038/nrg2732.

Lee, s. H., ripke, s., neale, b. M., faraone, s. V., purcell, s. M., perlis, r. H., mowry, b. J., thapar, a., goddard, m. E., witte, j. S., absher, d., agartz, i., akil, h., amin, f., andreassen, o. A., anjorin, a., anney, r., anttila, v., arking, d. E., asherson, p., azevedo, m. H., backlund, l., badner, j. A., bailey, a. J., banaschewski, t., barchas, j. D., barnes, m. R., barrett, t. B., bass, n., battaglia, a., bauer, m., bayés, m., bellivier, f., bergen, s. E., berrettini, w., betancur, c., bettecken, t., biederman, j., binder, e. B., black, d. W., blackwood, d. H., bloss, c. S., boehnke, m., boomsma, d. I., breen, g., breuer, r., bruggeman, r., cormican, p., buccola, n. G., buitelaar, j. K., bunney, w. E., buxbaum, j. D., byerley, w. F., byrne, e. M., caesar, s., cahn, w., cantor, r. M., casas, m., chakravarti, a., chambert, k., choudhury, k., cichon, s., cloninger, c. R., collier, d. A., cook, e. H., coon, h., cormand, b., corvin, a., coryell, w. H., craig, d. W., craig, i. W., crosbie, j., cuccaro, m. L., curtis, d., czamara, d., datta, s., dawson, g., day, r., de geus, e. J., degenhardt, f., djurovic, s., donohoe, g. J., doyle, a. E., duan, j., dudbridge, f., duketis, e., ebstein, r. P., edenberg, h. J., elia, j., ennis, s., etain, b., fanous, a., farmer, a. E., ferrier, i. N., flickinger, m., fombonne, e., foroud, t., frank, j., franke, b., fraser, c., et al. 2013. Genetic relationship between five psychiatric disorders estimated from genome-wide snps. *Nat genet,* 45**,** 984-94.

Leek, j. T., johnson, w. E., parker, h. S., jaffe, a. E. & storey, j. D. 2012. The sva package for removing batch effects and other unwanted variation in high-throughput experiments. *Bioinformatics,* 28**,** 882-3.

Ligthart, l., van beijsterveldt, c. E. M., kevenaar, s. T., de zeeuw, e., van bergen, e., bruins, s., pool, r., helmer, q., van dongen, j., hottenga, j. J., van't ent, d., dolan, c. V., davies, g. E., ehli, e. A., bartels, m., willemsen, g., de geus, e. J. C. & boomsma, d. I. 2019. The netherlands twin register: longitudinal research based on twin and twin-family designs. *Twin res hum genet,* 22**,** 623-636.

Liu, C., Jiao, C., Wang, K. and Yuan, N. (2018). DNA Methylation and Psychiatric Disorders. Progress in Molecular Biology and Translational Science, [online] 157, pp.175–232. doi:https://doi.org/10.1016/bs.pmbts.2018.01.006.

Magnus, p., birke, c., vejrup, k., haugan, a., alsaker, e., daltveit, a. K., handal, m., haugen, m., høiseth, g., knudsen, g. P., paltiel, l., schreuder, p., tambs, k., vold, l. & stoltenberg, c. 2016. Cohort profile update: the norwegian mother and child cohort study (moba). *Int j epidemiol,* 45**,** 382-8.

Malcolm-smith, s., woolley, d. & ward, c. L. 2015. Examining empathy and its association with aggression in young western cape children. *Journal of child and adolescent mental health,* 27**,** 135-147.

Malecki, k. M. C., andersen, j. K., geller, a. M., harry, g. J., jackson, c. L., james, k. A., miller, g. W. & ottinger, m. A. 2022. Integrating environment and aging research: opportunities for synergy and acceleration. *Front aging neurosci,* 14**,** 824921.

Manichaikul, a., mychaleckyj, j. C., rich, s. S., daly, k., sale, m. & chen, w. M. 2010. Robust relationship inference in genome-wide association studies. *Bioinformatics,* 26**,** 2867-73.

Mar, á. P., joan, f., jesús, p., xavier, q. & jordi, s. 2013. Breathe project (brain development and air pollution ultrafine particles in school children): research protocol and first results. *Isee conference abstracts,* 2013**,** 3617-3617.

Masten, a. S., desjardins, c. D., mccormick, c. M., kuo, s. I. & long, j. D. 2010. The significance of childhood competence and problems for adult success in work: a developmental cascade analysis. *Dev psychopathol,* 22**,** 679-94.

Mazefsky, c. A., anderson, r., conner, c. M. & minshew, n. 2011. Child behavior checklist scores for school-aged children with autism: preliminary evidence of patterns suggesting the need for referral. *Journal of psychopathology and behavioral assessment,* 33**,** 31-37.

Mccarthy, s., das, s., kretzschmar, w., delaneau, o., wood, a. R., teumer, a., kang, h. M., fuchsberger, c., danecek, p., sharp, k., luo, y., sidore, c., kwong, a., timpson, n., koskinen, s., vrieze, s., scott, l. J., zhang, h., mahajan, a., veldink, j., peters, u., pato, c., van duijn, c. M., gillies, c. E., gandin, i., mezzavilla, m., gilly, a., cocca, m., traglia, m., angius, a., barrett, j. C., boomsma, d., branham, k., breen, g., brummett, c. M., busonero, f., campbell, h., chan, a., chen, s., chew, e., collins, f. S., corbin, l. J., smith, g. D., dedoussis, g., dorr, m., farmaki, a. E., ferrucci, l., forer, l., fraser, r. M., gabriel, s., levy, s., groop, l., harrison, t., hattersley, a., holmen, o. L., hveem, k., kretzler, m., lee, j. C., mcgue, m., meitinger, t., melzer, d., min, j. L., mohlke, k. L., vincent, j. B., nauck, m., nickerson, d., palotie, a., pato, m., pirastu, n., mcinnis, m., richards, j. B., sala, c., salomaa, v., schlessinger, d., schoenherr, s., slagboom, p. E., small, k., spector, t., stambolian, d., tuke, m., tuomilehto, j., van den berg, l. H., van rheenen, w., volker, u., wijmenga, c., toniolo, d., zeggini, e., gasparini, p., sampson, m. G., wilson, j. F., frayling, t., de bakker, p. I., swertz, m. A., mccarroll, s., kooperberg, c., dekker, a., altshuler, d., willer, c., iacono, w., ripatti, s., et al. 2016. A reference panel of 64,976 haplotypes for genotype imputation. *Nat genet,* 48**,** 1279-83.

Mcrae, a. F., powell, j. E., henders, a. K., bowdler, l., hemani, g., shah, s., painter, j. N., martin, n. G., visscher, p. M. & montgomery, g. W. 2014. Contribution of genetic variation to transgenerational inheritance of dna methylation. *Genome biol,* 15**,** r73.

Meijer, m., franke, b., sandi, c. & klein, m. 2023. Epigenome-wide dna methylation in externalizing behaviours: a review and combined analysis. *Neuroscience & biobehavioral reviews,* 145**,** 104997.

Mill, j. & petronis, a. 2008. Pre- and peri-natal environmental risks for attention-deficit hyperactivity disorder (adhd): the potential role of epigenetic processes in mediating susceptibility. *J child psychol psychiatry,* 49**,** 1020-30.

Montoro, e., lawson, d. J., burrows, k., suderman, m., bretherick, a. D., richardson, t. G., klughammer, j., iotchkova, v., sharp, g., al khleifat, a., shatunov, a., iacoangeli, a., mcardle, w. L., ho, k. M., kumar, a., söderhäll, c., soriano-tárraga, c., giralt-steinhauer, e., kazmi, n., mason, d., mcrae, a. F., corcoran, d. L., sugden, k., kasela, s., cardona, a., day, f. R., cugliari, g., viberti, c., guarrera, s., lerro, m., gupta, r., bollepalli, s., mandaviya, p., zeng, y., clarke, t. K., walker, r. M., schmoll, v., czamara, d., ruiz-arenas, c., rezwan, f. I., marioni, r. E., lin, t., awaloff, y., germain, m., aïssi, d., zwamborn, r., van eijk, k., dekker, a., van dongen, j., hottenga, j. J., willemsen, g., xu, c. J., barturen, g., català-moll, f., kerick, m., wang, c., melton, p., elliott, h. R., shin, j., bernard, m., yet, i., smart, m., gorrie-stone, t., shaw, c., al chalabi, a., ring, s. M., pershagen, g., melén, e., jiménez-conde, j., roquer, j., lawlor, d. A., wright, j., martin, n. G., montgomery, g. W., moffitt, t. E., poulton, r., esko, t., milani, l., metspalu, a., perry, j. R. B., ong, k. K., wareham, n. J., matullo, g., sacerdote, c., panico, s., caspi, a., arseneault, l., gagnon, f., ollikainen, m., kaprio, j., felix, j. F., rivadeneira, f., tiemeier, h., van, i. M. H., et al. 2021. Genomic and phenotypic insights from an atlas of genetic effects on dna methylation. *Nat genet,* 53**,** 1311-1321.

Moher, d., liberati, a., tetzlaff, j. & altman, d. G. 2009. Preferred reporting items for systematic reviews and meta-analyses: the prisma statement. British medical journal publishing group.

Moore, l. D., le, t. & fan, g. 2013. Dna methylation and its basic function. *Neuropsychopharmacology,* 38**,** 23-38.

Moreaux, j., bruyer, a., veyrune, j. L., goldschmidt, h., hose, d. & klein, b. 2014. Dna methylation score is predictive of myeloma cell sensitivity to 5-azacitidine. *Br j haematol,* 164**,** 613-6.

Morin, a. M., gatev, e., mcewen, l. M., macisaac, j. L., lin, d. T. S., koen, n., czamara, d., räikkönen, k., zar, h. J., koenen, k., stein, d. J., kobor, m. S. & jones, m. J. 2017. Maternal blood contamination of collected cord blood can be identified using dna methylation at three cpgs. *Clin epigenetics,* 9**,** 75.

Murphy, t. M. & mill, j. 2014. Epigenetics in health and disease: heralding the ewas era. *Lancet,* 383**,** 1952-4.

Murray, g. K., lin, t., austin, j., mcgrath, j. J., hickie, i. B. & wray, n. R. 2021. Could polygenic risk scores be useful in psychiatry?: a review. *Jama psychiatry,* 78**,** 210-219.

Myer, l., stein, d. J., grimsrud, a., seedat, s. & williams, d. R. 2008. Social determinants of psychological distress in a nationally-representative sample of south african adults. *Social science and medicine,* 66**,** 1828-1840.

Nabais, m. F., gadd, d. A., hannon, e., mill, j., mcrae, a. F. & wray, n. R. 2023. An overview of dna methylation-derived trait score methods and applications. *Genome biology,* 24**,** 28.

Nabais, m. F., laws, s. M., lin, t., vallerga, c. L., armstrong, n. J., blair, i. P., kwok, j. B., mather, k. A., mellick, g. D., sachdev, p. S., wallace, l., henders, a. K., zwamborn, r. A. J., hop, p. J., lunnon, k., pishva, e., roubroeks, j. A. Y., soininen, h., tsolaki, m., mecocci, p., lovestone, s., kłoszewska, i., vellas, b., furlong, s., garton, f. C., henderson, r. D., mathers, s., mccombe, p. A., needham, m., ngo, s. T., nicholson, g., pamphlett, r., rowe, d. B., steyn, f. J., williams, k. L., anderson, t. J., bentley, s. R., dalrymple-alford, j., fowder, j., gratten, j., halliday, g., hickie, i. B., kennedy, m., lewis, s. J. G., montgomery, g. W., pearson, j., pitcher, t. L., silburn, p., zhang, f., visscher, p. M., yang, j., stevenson, a. J., hillary, r. F., marioni, r. E., harris, s. E., deary, i. J., jones, a. R., shatunov, a., iacoangeli, a., van rheenen, w., van den berg, l. H., shaw, p. J., shaw, c. E., morrison, k. E., al-chalabi, a., veldink, j. H., hannon, e., mill, j., wray, n. R. & mcrae, a. F. 2021. Meta-analysis of genome-wide dna methylation identifies shared associations across neurodegenerative disorders. *Genome biology,* 22.

Neumann, a., jolicoeur-martineau, a., szekely, e., sallis, h. M., o'donnel, k., greenwood, c. M. T., levitan, r., meaney, m. J., wazana, a., evans, j. & tiemeier, h. 2021. Combined polygenic risk scores of different psychiatric traits predict general and specific psychopathology in childhood. *J child psychol psychiatry*.

Neumann, a., jolicoeur-martineau, a., szekely, e., sallis, h. M., o'donnel, k., greenwood, c. M. T., levitan, r., meaney, m. J., wazana, a., evans, j. & tiemeier, h. 2022. Combined polygenic risk scores of different psychiatric traits predict general and specific psychopathology in childhood. *J child psychol psychiatry,* 63**,** 636-645.

Neumann, a., pappa, i., lahey, b. B., verhulst, f. C., medina-gomez, c., jaddoe, v. W., bakermans-kranenburg, m. J., moffitt, t. E., van, i. M. H. & tiemeier, h. 2016. Single nucleotide polymorphism heritability of a general psychopathology factor in children. *J am acad child adolesc psychiatry,* 55**,** 1038-1045.e4.

Neumann, a., walton, e., alemany, s., cecil, c., gonzález, j. R., jima, d. D., lahti, j., tuominen, s. T., barker, e. D., binder, e., caramaschi, d., carracedo, á., czamara, d., evandt, j., felix, j. F., fuemmeler, b. F., gutzkow, k. B., hoyo, c., julvez, j., kajantie, e., laivuori, h., maguire, r., maitre, l., murphy, s. K., murcia, m., villa, p. M., sharp, g., sunyer, j., raikkönen, k., bakermans-kranenburg, m., ijzendoorn, m. V., guxens, m., relton, c. L. & tiemeier, h. 2020. Association between dna methylation and adhd symptoms from birth to school age: a prospective meta-analysis. *Translational psychiatry,* 10**,** 30-30.

Nivard, m. G., gage, s. H., hottenga, j. J., van beijsterveldt, c. E. M., abdellaoui, a., bartels, m., baselmans, b. M. L., ligthart, l., pourcain, b. S., boomsma, d. I., munafò, m. R. & middeldorp, c. M. 2017. Genetic overlap between schizophrenia and developmental psychopathology: longitudinal and multivariate polygenic risk prediction of common psychiatric traits during development. *Schizophr bull,* 43**,** 1197-1207.

Oblak, l., van der zaag, j., higgins-chen, a. T., levine, m. E. & boks, m. P. 2021. A systematic review of biological, social and environmental factors associated with epigenetic clock acceleration. *Ageing research reviews,* 69**,** 101348-101348.

Ogundele, m. O. 2018. Behavioural and emotional disorders in childhood: a brief overview for paediatricians. *World j clin pediatr,* 7**,** 9-26.

Palladino, v. S., mcneill, r., reif, a. & kittel-schneider, s. 2019. Genetic risk factors and gene-environment interactions in adult and childhood attention-deficit/hyperactivity disorder. *Psychiatric genetics,* 29**,** 63-78.

Parade, s. H., ridout, k. K., seifer, r., armstrong, d. A., marsit, c. J., mcwilliams, m. A. & tyrka, a. R. 2016. Methylation of the glucocorticoid receptor gene promoter in preschoolers: links with internalizing behavior problems. *Child dev,* 87**,** 86-97.

Peterson, r. E., kuchenbaecker, k., walters, r. K., chen, c.-y., popejoy, a. B., periyasamy, s., lam, m., iyegbe, c., strawbridge, r. J., brick, l., carey, c. E., martin, a. R., meyers, j. L., su, j., chen, j., edwards, a. C., kalungi, a., koen, n., majara, l., schwarz, e., smoller, j. W., stahl, e. A., sullivan, p. F., vassos, e., mowry, b., prieto, m. L., cuellar-barboza, a., bigdeli, t. B., edenberg, h. J., huang, h. & duncan, l. E. 2019. Genome-wide association studies in ancestrally diverse populations: opportunities, methods, pitfalls, and recommendations. *Cell,* 179**,** 589-603.

Pidsley, r., cc, y. W., volta, m., lunnon, k., mill, j. & schalkwyk, l. C. 2013. A data-driven approach to preprocessing illumina 450k methylation array data. *Bmc genomics,* 14**,** 293.

Pidsley, r., zotenko, e., peters, t. J., lawrence, m. G., risbridger, g. P., molloy, p., van djik, s., muhlhausler, b., stirzaker, c. & clark, s. J. 2016. Critical evaluation of the illumina methylationepic beadchip microarray for whole-genome dna methylation profiling. *Genome biol,* 17**,** 208.

Pingault, j.-b., viding, e., galera, c., greven, c. U., zheng, y., plomin, r. & rijsdijk, f. 2015. Genetic and environmental influences on the developmental course of attention-deficit/hyperactivity disorder symptoms from childhood to adolescence. *Jama psychiatry,* 72**,** 651-658.

Posserud, m. B., lundervold, a. J. & gillberg, c. 2006. Autistic features in a total population of 7-9-year-old children assessed by the assq (autism spectrum screening questionnaire). *J child psychol psychiatry,* 47**,** 167-75.

Power, c. & elliott, j. 2006. Cohort profiles. *International epidemiological association international journal of epidemiology,* 35**,** 34-41.

Price, m. E., cotton, a. M., lam, l. L., farré, p., emberly, e., brown, c. J., robinson, w. P. & kobor, m. S. 2013. Additional annotation enhances potential for biologically-relevant analysis of the illumina infinium humanmethylation450 beadchip array. *Epigenetics chromatin,* 6**,** 4.

Quach, a., levine, m. E., tanaka, t., lu, a. T., chen, b. H., ferrucci, l., ritz, b., bandinelli, s., neuhouser, m. L., beasley, j. M., snetselaar, l., wallace, r. B., tsao, p. S., absher, d., assimes, t. L., stewart, j. D., li, y., hou, l., baccarelli, a. A., whitsel, e. A. & horvath, s. 2017. Epigenetic clock analysis of diet, exercise, education, and lifestyle factors. *Aging (albany ny),* 9**,** 419-446.

Raffington, l., mallard, t. & harden, k. P. 2020. Polygenic scores in developmental psychology: invite genetics in, leave biodeterminism behind. *Https://doi.org/10.1146/annurev-devpsych-051820-123945,* 2**,** 389-411.

Rakyan, v. K., down, t. A., balding, d. J. & beck, s. 2011. Epigenome-wide association studies for common human diseases. *Nat rev genet,* 12**,** 529-41.

Rice, f., riglin, l., thapar, a. K., heron, j., anney, r., o'donovan, m. C. & thapar, a. 2019. Characterizing developmental trajectories and the role of neuropsychiatric genetic risk variants in early-onset depression. *Jama psychiatry,* 76**,** 306-313.

Riglin, l., collishaw, s., richards, a., thapar, a. K., maughan, b., o'donovan, m. C. & thapar, a. 2017. Schizophrenia risk alleles and neurodevelopmental outcomes in childhood: a population-based cohort study. *The lancet psychiatry,* 4**,** 57-62.

Riglin, l., collishaw, s., thapar, a. K., dalsgaard, s., langley, k., smith, g. D., stergiakouli, e., maughan, b., o'donovan, m. C. & thapar, a. 2016. Association of genetic risk variants with attention-

deficit/hyperactivity disorder trajectories in the general population. *Jama psychiatry,* 73**,** 1285-1292.

Rijlaarsdam, j., cecil, c. A. M., relton, c. L. & barker, e. D. 2022. Epigenetic profiling of social communication trajectories and co-occurring mental health problems: a prospective, methylome-wide association study. *Dev psychopathol,* 34**,** 854-863.

Ripke, s., neale, b. M., corvin, a., walters, j. T. R., farh, k. H., holmans, p. A., lee, p., bulik-sullivan, b., collier, d. A., huang, h., pers, t. H., agartz, i., agerbo, e., albus, m., alexander, m., amin, f., bacanu, s. A., begemann, m., belliveau, r. A., bene, j., bergen, s. E., bevilacqua, e., bigdeli, t. B., black, d. W., bruggeman, r., buccola, n. G., buckner, r. L., byerley, w., cahn, w., cai, g., campion, d., cantor, r. M., carr, v. J., carrera, n., catts, s. V., chambert, k. D., chan, r. C. K., chen, r. Y. L., chen, e. Y. H., cheng, w., cheung, e. F. C., chong, s. A., cloninger, c. R., cohen, d., cohen, n., cormican, p., craddock, n., crowley, j. J., curtis, d., davidson, m., davis, k. L., degenhardt, f., del favero, j., demontis, d., dikeos, d., dinan, t., djurovic, s., donohoe, g., drapeau, e., duan, j., dudbridge, f., durmishi, n., eichhammer, p., eriksson, j., escott-price, v., essioux, l., fanous, a. H., farrell, m. S., frank, j., franke, l., freedman, r., freimer, n. B., friedl, m., friedman, j. I., fromer, m., genovese, g., georgieva, l., giegling, i., giusti-rodríguez, p., godard, s., goldstein, j. I., golimbet, v., gopal, s., gratten, j., de haan, l., hammer, c., hamshere, m. L., hansen, m., hansen, t., haroutunian, v., hartmann, a. M., henskens, f. A., herms, s., hirschhorn, j. N., hoffmann, p., hofman, a., hollegaard, m. V., hougaard, d. M., ikeda, m., joa, i., et al. 2014. Biological insights from 108 schizophrenia-associated genetic loci. *Nature,* 511**,** 421-427.

Rojas, d., rager, j. E., smeester, l., bailey, k. A., drobná, z., rubio-andrade, m., stýblo, m., garcía-vargas, g. & fry, r. C. 2015. Prenatal arsenic exposure and the epigenome: identifying sites of 5-methylcytosine alterations that predict functional changes in gene expression in newborn cord blood and subsequent birth outcomes. *Toxicol sci,* 143**,** 97-106.

Ronkainen, j., heiskala, a., vehmeijer, f. O. L., lowry, e., caramaschi, d., estrada gutierrez, g., heiss, j. A., hummel, n., keikkala, e., kvist, t., kupsco, a., melton, p. E., pesce, g., soomro, m. H., vives-usano, m., baiz, n., binder, e., czamara, d., guxens, m., mustaniemi, s., london, s. J., rauschert, s., vääräsmäki, m., vrijheid, m., ziegler, a.-g., annesi-maesano, i., bustamante, m., huang, r.-c., hummel, s., just, a. C., kajantie, e., lahti, j., lawlor, d., räikkönen, k., järvelin, m.-r., felix, j. F. & sebert, s. 2022. Maternal haemoglobin levels in pregnancy and child dna methylation: a study in the pregnancy and childhood epigenetics consortium. *Epigenetics,* 17**,** 19-31.

Sallis, h., szekely, e., neumann, a., jolicoeur-martineau, a., van, i. M., hillegers, m., greenwood, c. M. T., meaney, m. J., steiner, m., tiemeier, h., wazana, a., pearson, r. M. & evans, j. 2019. General psychopathology, internalising and externalising in children and functional outcomes in late adolescence. *J child psychol psychiatry,* 60**,** 1183-1190.

Salum, g. A., isolan, l. R., bosa, v. L., tocchetto, a. G., teche, s. P., schuch, i., costa, j. R., costa mde, a., jarros, r. B., mansur, m. A., knijnik, d., silva, e. A., kieling, c., oliveira, m. H., medeiros, e., bortoluzzi, a., toazza, r., blaya, c., leistner-segal, s., salles, j. F., silveira, p. P., goldani, m. Z., heldt, e. & manfro, g. G. 2011. The multidimensional evaluation and treatment of anxiety in children and adolescents: rationale, design, methods and preliminary findings. *Braz j psychiatry,* 33**,** 181-95.

Schoeman, r. & liebenberg, r. 2017. The south african society of psychiatrists/psychiatry management group management guidelines for adult attention-deficit/hyperactivity disorder. *South african journal of psychiatry,* 23**,** 14-14.

Scott, j. G., mihalopoulos, c., erskine, h. E., roberts, j. & rahman, a. 2016. Childhood mental and developmental disorders. *Disease control priorities, third edition (volume 4): mental, neurological, and substance use disorders*, 145-161.

Shaw, m., hodgkins, p., caci, h., young, s., kahle, j., woods, a. G. & arnold, l. E. 2012. A systematic review and analysis of long-term outcomes in attention deficit hyperactivity disorder: effects of treatment and non-treatment. *Bmc med,* 10**,** 99.

Sohani, z. N., sarma, s., alyass, a., de souza, r. J., robiou-du-pont, s., li, a., mayhew, a., yazdi, f., reddon, h., lamri, a., stryjecki, c., ishola, a., lee, y. K., vashi, n., anand, s. S. & meyre, d. 2016. Empirical evaluation of the q-genie tool: a protocol for assessment of effectiveness. *Bmj open,* 6.

Solmi, m., radua, j., olivola, m., croce, e., soardo, l., salazar de pablo, g., il shin, j., kirkbride, j. B., jones, p., kim, j. H., kim, j. Y., carvalho, a. F., seeman, m. V., correll, c. U. & fusar-poli, p. 2022. Age at onset of mental disorders worldwide: large-scale meta-analysis of 192 epidemiological studies. *Molecular psychiatry,* 27**,** 281-295.

Solovieff, n., cotsapas, c., lee, p. H., purcell, s. M. & smoller, j. W. 2013. Pleiotropy in complex traits: challenges and strategies. *Nat rev genet,* 14**,** 483-95.

Stein, d. J., koen, n., donald, k. A., adnams, c. M., koopowitz, s., lund, c., marais, a., myers, b., roos, a., sorsdahl, k., stern, m., tomlinson, m., van der westhuizen, c., vythilingum, b., myer, l., barnett, w., brittain, k. & zar, h. J. 2015. Investigating the psychosocial determinants of child health in africa: the drakenstein child health study. *J neurosci methods,* 252**,** 27-35.

Stergiakouli, e., davey smith, g., martin, j., skuse, d. H., viechtbauer, w., ring, s. M., ronald, a., evans, d. E., fisher, s. E., thapar, a. & st pourcain, b. 2017. Shared genetic influences between dimensional asd and adhd symptoms during child and adolescent development. *Molecular autism,* 8**,** 1-13.

Strandberg, t. E., andersson, s., järvenpää, a. L. & mckeigue, p. M. 2002. Preterm birth and licorice consumption during pregnancy. *Am j epidemiol,* 156**,** 803-5.

Suarez, a., lahti, j., czamara, d., lahti-pulkkinen, m., girchenko, p., andersson, s., strandberg, t. E., reynolds, r. M., kajantie, e., binder, e. B. & raikkonen, k. 2018a. The epigenetic clock and pubertal, neuroendocrine, psychiatric, and cognitive outcomes in adolescents. *Clin epigenetics,* 10**,** 96.

Suarez, a., lahti, j., czamara, d., lahti-pulkkinen, m., knight, a. K., girchenko, p., hämäläinen, e., kajantie, e., lipsanen, j., laivuori, h., villa, p. M., reynolds, r. M., smith, a. K., binder, e. B. & räikkönen, k. 2018b. The epigenetic clock at birth: associations with maternal antenatal depression and child psychiatric problems. *J am acad child adolesc psychiatry,* 57**,** 321-328.e2.

SUDRE, G., SHARP, W., KUNDZICZ, P., BOUYSSI-KOBAR, M., NORMAN, L., CHOUDHURY, S. & SHAW, P. 2020. Predicting the course of ADHD symptoms through the integration of childhood genomic, neural, and cognitive features. *Molecular Psychiatry*.

Sullivan, p. F. & geschwind, d. H. 2019. Defining the genetic, genomic, cellular, and diagnostic architectures of psychiatric disorders. *Cell,* 177**,** 162-183.

Sumner, j. A., colich, n. L., uddin, m., armstrong, d. & mclaughlin, k. A. 2019. Early experiences of threat, but not deprivation, are associated with accelerated biological aging in children and adolescents. *Biol psychiatry,* 85**,** 268-278.

Szyf, m. & bick, j. 2013. Dna methylation: a mechanism for embedding early life experiences in the genome. *Child dev,* 84**,** 49-57.

Thapar, a., martin, j., mick, e., vasquez, a. A., langley, k., scherer, s. W., schachar, r., crosbie, j., williams, n., franke, b., elia, j., glessner, j., hakonarson, h., owen, m. J., faraone, s. V., o'donovan, m. C., holmans, p. & consortium, i. 2016. Psychiatric gene discoveries shape evidence on adhd's biology. *Molecular psychiatry,* 21**,** 1202-1207.

Tishkoff, s. A., reed, f. A., friedlaender, f. R., ehret, c., ranciaro, a., froment, a., hirbo, j. B., awomoyi, a. A., bodo, j. M., doumbo, o., ibrahim, m., juma, a. T., kotze, m. J., lema, g., moore, j. H., mortensen, h., nyambo, t. B., omar, s. A., powell, k., pretorius, g. S., smith, m. W., thera, m. A., wambebe, c., weber, j. L. & williams, s. M. 2009. The genetic structure and history of africans and african americans. *Science,* 324**,** 1035-44.

Tollenaar, m. S., beijers, r., garg, e., nguyen, t. T. T., lin, d. T. S., macisaac, j. L., shalev, i., kobor, m. S., meaney, m. J., o'donnell, k. J. & de weerth, c. 2021. Internalizing symptoms associate with the pace of epigenetic aging in childhood. *Biological psychology,* 159**,** 108021.

Viechtbauer, w. 2010. Conducting meta-analyses in r with the metafor. *Journal of statistical software,* 36**,** 1-48.

Walton, e., pingault, j. B., cecil, c. A., gaunt, t. R., relton, c. L., mill, j. & barker, e. D. 2017. Epigenetic profiling of adhd symptoms trajectories: a prospective, methylome-wide study. *Mol psychiatry,* 22**,** 250-256.

Wichstrøm, l., berg-nielsen, t. S., angold, a., egger, h. L., solheim, e. & sveen, t. H. 2012. Prevalence of psychiatric disorders in preschoolers. *Journal of child psychology and psychiatry,* 53**,** 695-705.

Wolf, e. J., logue, m. W., morrison, f. G., wilcox, e. S., stone, a., schichman, s. A., mcglinchey, r. E., milberg, w. P. & miller, m. W. 2019. Posttraumatic psychopathology and the pace of the epigenetic clock: a longitudinal investigation. *Psychol med,* 49**,** 791-800.

Wray, n. R., lee, s. H., mehta, d., vinkhuyzen, a. A., dudbridge, f. & middeldorp, c. M. 2014. Research review: polygenic methods and their application to psychiatric traits. *J child psychol psychiatry,* 55**,** 1068-87.

Wray, n. R., lin, t., austin, j., mcgrath, j. J., hickie, i. B., murray, g. K. & visscher, p. M. 2021. From basic science to clinical application of polygenic risk scores: a primer. *Jama psychiatry,* 78**,** 101-109.

Wray, n. R., ripke, s., mattheisen, m., trzaskowski, m., byrne, e. M., abdellaoui, a., adams, m. J., agerbo, e., air, t. M., andlauer, t. M. F., bacanu, s. A., bækvad-hansen, m., beekman, a. F. T., bigdeli, t. B., binder, e. B., blackwood, d. R. H., bryois, j., buttenschøn, h. N., bybjerg-grauholm, j., cai, n., castelao, e., christensen, j. H., clarke, t. K., coleman, j. I. R., colodro-conde, l., couvy-duchesne, b., craddock, n., crawford, g. E., crowley, c. A., dashti, h. S., davies, g., deary, i. J., degenhardt, f., derks, e. M., direk, n., dolan, c. V., dunn, e. C., eley, t. C., eriksson, n., escott-price, v., kiadeh, f. H. F., finucane, h. K., forstner, a. J., frank, j., gaspar, h. A., gill, m., giusti-rodríguez, p., goes, f. S., gordon, s. D., grove, j., hall, l. S., hannon, e., hansen, c. S., hansen, t. F., herms, s., hickie, i. B., hoffmann, p., homuth, g., horn, c., hottenga, j. J., hougaard, d. M., hu, m., hyde, c. L., ising, m., jansen, r., jin, f., jorgenson, e., knowles, j. A., kohane, i. S., kraft, j., kretzschmar, w. W., krogh, j., kutalik, z., lane, j. M., li, y., li, y., lind, p. A., liu, x., lu, l., macintyre, d. J., mackinnon, d. F., maier, r. M., maier, w., marchini, j., mbarek, h., mcgrath, p., mcguffin, p., medland, s. E., mehta, d., middeldorp, c. M., mihailov, e., milaneschi, y., milani, l., mill, j., mondimore, f. M., montgomery, g. W., mostafavi, s., mullins, n., nauck, m., ng, b., et al. 2018. Genome-wide association analyses identify 44 risk variants and refine the genetic architecture of major depression. *Nature genetics 2018 50:5,* 50**,** 668-681.

Zar, h. J., barnett, w., myer, l., stein, d. J. & nicol, m. P. 2015. Investigating the early-life determinants of illness in africa: the drakenstein child health study. *Thorax,* 70**,** 592-594.

Zhang, b., zhou, y., lin, n., lowdon, r. F., hong, c., nagarajan, r. P., cheng, j. B., li, d., stevens, m., lee, h. J., xing, x., zhou, j., sundaram, v., elliott, g., gu, j., shi, t., gascard, p., sigaroudinia, m., tlsty, t. D., kadlecek, t., weiss, a., o'geen, h., farnham, p. J., maire, c. L., ligon, k. L., madden, p. A., tam, a., moore, r., hirst, m., marra, m. A., zhang, b., costello, j. F. & wang, t. 2013. Functional dna methylation differences between tissues, cell types, and across individuals discovered using the m&m algorithm. *Genome res,* 23**,** 1522-40.

Zhou, k., dempfle, a., arcos-burgos, m., bakker, s. C., banaschewski, t., biederman, j., buitelaar, j., castellanos, f. X., doyle, a., ebstein, r. P., ekholm, j., forabosco, p., franke, b., freitag, c., friedel, s., gill, m., hebebrand, j., hinney, a., jacob, c., lesch, k. P., loo, s. K., lopera, f., mccracken, j. T., mcgough, j. J., meyer, j., mick, e., miranda, a., muenke, m., mulas, f., nelson, s. F., nguyen, t. T., oades, r. D., ogdie, m. N., palacio, j. D., pineda, d., reif, a., renner, t. J., roeyers, h., romanos, m., rothenberger, a., schäfer, h., sergeant, j., sinke, r. J., smalley, s. L., sonuga-barke, e., steinhausen, h. C., van der meulen, e., walitza, s., warnke, a., lewis, c. M., faraone, s. V. & asherson, p. 2008. Meta-analysis of genome-wide linkage scans of attention deficit hyperactivity disorder. *Am j med genet b neuropsychiatr genet,* 147b**,** 1392-8.

Zieff, m. R., fourie, c., hoogenhout, m. & donald, k. A. 2022. Psychometric properties

of the aseba child behaviour checklist and youth self-report in sub-saharan africa - a systematic review. *Acta neuropsychiatr*, 1-24.efron b, brit turnbull b, narasimhan b. 2015. Locfdr: computes local false discovery rates. R package version 1.1-8. Available from: https://cran.r-project.org/package=locfdr

R Core Team. 2018. R: A language and environment for statistical computing. Available from: https://www.r-project.org/

Koestler, D.C., Jones, M.J., Usset, J., Christensen, B.C., Butler, R.A., Kobor, M.S., Wiencke, J.K. and Kelsey, K.T. (2016). Improving cell mixture deconvolution by identifying optimal DNA methylation libraries (IDOL). BMC Bioinformatics, 17(1). doi:https://doi.org/10.1186/s12859-016-0943-7.

le Roux, S.M., Donald, K.A., Brittain, K., Phillips, T.K., Zerbe, A., Nguyen, K.K., Strandvik, A., Kroon, M., Abrams, E.J. and Myer, L. (2018). Neurodevelopment of breastfed HIV-exposed uninfected and HIV-unexposed children in South Africa. AIDS, 32(13), pp.1781–1791. doi:https://doi.org/10.1097/qad.0000000000001872.

Fanti, K.A. and Henrich, C.C. (2010). Trajectories of pure and co-occurring internalizing and externalizing problems from age 2 to age 12: Findings from the National Institute of Child Health and Human Development Study of Early Child Care. Developmental Psychology, 46(5), pp.1159–1175. doi:https://doi.org/10.1037/a0020659.

Campbell, S.B. (1994). Hard-to-manage preschool boys: Externalizing behavior, social competence, and family context at two-year followup. Journal of Abnormal Child Psychology, 22(2), pp.147–166. doi:https://doi.org/10.1007/bf02167897.

Dupaul, g.J., Mcgoey, k.E., Eckert, t.L. And VANBRAKLE, J. (2001). Preschool children With attention-deficit/hyperactivity disorder: impairments in behavioral, social, and school functioning. Journal of the american academy of child & adolescent psychiatry, 40(5), pp.508–515. Doi:https://doi.Org/10.1097/00004583-200105000-00009.

Deighton, J., Humphrey, N., Belsky, J., Boehnke, J., Vostanis, P. and Patalay, P. (2017). Longitudinal pathways between mental health difficulties and academic performance during middle childhood and early adolescence. *British Journal of Developmental Psychology*, 36(1), pp.110–126. doi:https://doi.org/10.1111/bjdp.12218.

Vassallo, S., Australian Institute Of Family Studies, Crime Prevention Victoria and Australian Temperament Project (2002). Patterns and precursors of adolescent antisocial behaviour. Melbourne: Crime Prevention Victoria.

Goodwin, R.D., Fergusson, D.M. and Horwood, L.J. (2004). Early anxious/withdrawn behaviours predict later internalising disorders. Journal of Child Psychology and Psychiatry, 45(4), pp.874–883. doi:https://doi.org/10.1111/j.1469-7610.2004.00279.x.

Fanti, K.A. and Henrich, C.C. (2010). Trajectories of pure and co-occurring internalizing and externalizing problems from age 2 to age 12: Findings from the National Institute of Child Health and Human Development Study of Early Child Care. Developmental Psychology, 46(5), pp.1159–1175. doi:https://doi.org/10.1037/a0020659.

Chapter 6: DNA methylation risk for ADHD and childhood development and mental health in the DCHS

6.1. Abstract

Background:

Internalising and externalising behaviours in early childhood may be early indicators of children's development and risk for mental health disorders. DNA methylation (DNAm) is the most widely studied epigenetic marker in mental disorders, including attention-deficit and hyperactivity disorder (ADHD). Studies have described associations between DNAm and risk for internalising behaviours. Recently, polygenic risk score (PRS) techniques have been adapted for quantifying DNAm risk for adverse health outcomes. This analysis aimed to investigate whether DNAm risk for ADHD (as indexed by methylation risk scores) is associated with poor developmental and mental health outcomes in the Drakenstein Child Health Study (DCHS).

Methods:

Methylation risk scores (MRS) are defined as the weighted sum of an individual's DNAm beta values from pre-selected CpG sites. For this analysis, MRS were generated using external weights from the ADHD epigenome-wide summary statistics from the Pregnancy and Childhood Epigenetics (PACE) consortium. Associations between MRS for ADHD and adverse developmental and mental health outcomes (assessed with the Bayley III and CBCL, respectively) were investigated using unadjusted bivariate and multivariable linear regression models; controlling for sex of the child, socioeconomic status (SES), maternal substance use, maternal HIV status, and the first 10 genomic principal components (to adjust for population stratification).

Results and conclusion:

No significant associations were found between the MRS for ADHD and adverse developmental and mental health outcomes in this study sample. In future, more well-powered studies would be warranted to determine the potential utility of MRS in quantifying risk for developmental and mental health disorders; and to improve the trans-ancestral transferability of these risk scores. Nonetheless, this study represents the first attempt to utilise MRS to index risk for adverse development and mental health in children, and the first to do so in an LMIC (South African) setting.

6.2. Introduction

As discussed in *Chapter 1 (Section 1.1)*, DNAm - the most well-characterised epigenetic modification (Liu et al., 2018) – has been suggested to play a role in cognitive function, growth and development (Moore et al., 2013, Caramaschi et al., 2022,RiJlaarsdam et al.,2022). DNAm has also been associated with the timing of physical development which in turn associates with internalising behaviours during childhood (Barker et al.,2018). Further, changes in DNAm patterns have been implicated as a potential risk factor for a number of adverse mental health outcomes (McRae et al., 2014, Mill and Petronis, 2008). For example, perturbations in DNAm have emerged as a potential mechanism in the development of ADHD (Moore et al., 2013) - *as detailed in Chapter 4 (Section 4.1)*; and umbilical cord DNAm may hold promise as a biomarker for ADHD risk in children (Neumann et al., 2020).

EWAS have also identified a number of DNAm sites associated with childhood ADHD symptoms (Walton et al., 2017), and with internalising symptoms in children (Parade et al., 2016). However, these associations have generally been found to have small effect sizes (Barker et al., 2018, Walton et al., 2017). More recently, there has been growing interest in adapting polygenic risk score (PRS) techniques for use in DNAm data, in order to generate methylation risk scores (MRS) (Hüls and Czamara, 2020).

MRS represent the weighted sum of an individual's beta values (obtained from a target dataset) of a pre-selected number of CpG sites from a discovery dataset (Hüls and Czamara, 2020) - see also *Chapter 1 (Section 1.3)*. Here, beta-values represent the degree of methylation for each CpG site, ranging from 0 (unmethylated) to 1 (completely methylated). To date, MRS have been utilised as potential biomarkers of environmental exposures (e.g. smoking) (Elliot et al.,2014); in association analyses (primarily yielding non-significant findings) as a dimension reduction approach in interaction and mediation analyses (Das et al., 2016); and for the prediction of an individual's risk of disease or as a predictor of treatment success (Moreaux et al., 2014, Eze et al., 2016). For example, in their study of potential differences in smoking-related loci between South Asian and European individuals, Elliot and colleagues (2014) reported that an MRS for smoking was able to predict current smoking behaviour among the indexed individuals. Previous studies in the DCHS have shown DNAm-based associations (i.e. changes in gestational EA) with indoor air pollution and childhood psychopathology (Christensen et al., 2023). Similarly, there is evidence of the predictive utility of DNAm for psychiatric disorders such as schizophrenia (Hannon et al., 2016). These authors showed that epigenetic variation at multiple loci across the genome associated with schizophrenia; this suggests that DNAm may mediate the association between environmental stressors, and diseases and symptoms (Hannon et al., 2016).

While prior research has reported associations between DNA and internalising symptoms during childhood (Maughan et al., 2009, Barker et al., 2018), these studies did not account for developmental trajectories - which can impact psychopathology risk. Additionally, there is a paucity of studies investigating associations between MRS and mental health disorders - minimal work has investigated potential associations between MRS for mental health disorders (such as ADHD), and development and mental health in childhood (Szyf and Bick, 2013, Walton et al., 2017). Given that ADHD is a commonly occurring neurodevelopmental disorder -1 in 20 children in South Africa affected, ~5% children globally (Schoeman and Liebenberg, 2017, Kessler et al., 2007) – and its genetic and epigenetic risk remains unclear, DNAm may be a promising risk mechanism. Thus, this analysis aimed to investigate the associations between MRS for ADHD and childhood developmental and mental health outcomes in the DCHS. To the best of our knowledge, this represents the first such investigation (and the first in a LMIC context).

6.3. Methods

6.3.1. Sociodemographic, biomedical and psychosocial characteristics

As described fully in *Chapter 4, Section 4.3*, DCHS mothers in this study sample completed a battery of measures administered by trained study staff during the antenatal visits. These included measures of SES quartiles, maternal HIV status, prenatal substance use (alcohol and tobacco use) and maternal depression (Stein et al., 2015).

6.3.2. Child anthropometry, development and mental health measures

Child anthropometric measures were sex of the child and birth weight. Child developmental domains of interest were cognition, language and motor skills, assessed using the Bayley-III; and mental health outcomes were internalising and externalising symptoms using the CBCL (Donald et al.,2018, Zar et al., 2015). These outcomes were assessed using a combination of observational and parent/caregiver report measures, as described in detail in *Chapter 4, Section 4.3* and were operationalised as composite scores assessed as continuous variables (Donald et al., 2018a, Le Roux et al., 2018).

6.3.3. Methylation risk scores (MRS) for ADHD

Target data - DCHS:

As described previously *(Chapter 5, Section 5.3)*, DNAm measures were derived from non-contaminated cord blood samples from 273 children in the DCHS (Morin et al., 2017). The DNAm data were generated on the Infinium Human Methylation EPIC BeadChip (n=153) (Illumina, San Diego, USA) or the Illumina Infinium HumanMethylation450 bead array (n=120) (Illumina, San Diego, USA). Pre-processing and quality control procedures are detailed fully in *Chapter 5, Section 5.3*.

An *a priori* power analysis was undertaken to determine the sample size required for detecting a significant association between ADHD methylation risk and the outcomes of interest, **Figure 6.1**. The "G-power" tool was used, with an alpha level of 0.05, an effect size of 0.5 and power of 80% (Faul et al.,2009). This power analysis indicated - for an effect size of 0.5 - a minimum sample size of 128 participants would be required. Thus, given the current sample size (N=273), this analysis was sufficiently powered to detect significant effects in our target sample.

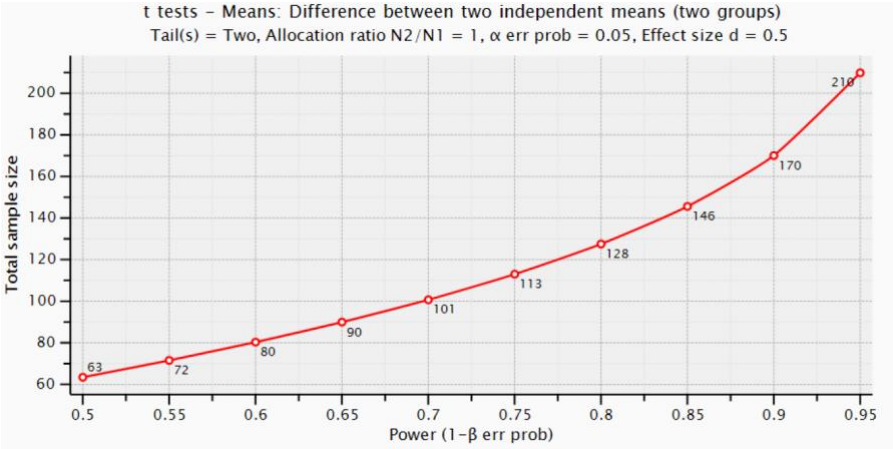

Figure 6. 1 A priori power analysis curve for associations between ADHD methylation risk and adverse developmental and mental health outcomes in the DCHS study sample

Discovery data - PACE:

The Pregnancy and Childhood Epigenetics (PACE) consortium has collated data from 39 studies, with epigenome-wide DNAm data for more than 29,000 samples from pregnant women, newborns and children (Felix et al., 2018). Included participants are of Hispanic, White, European, Latino, Black and "other" ancestry (Felix et al., 2018). For this analysis, summary statistics from an EWAS of ADHD symptoms - with cord blood DNAm data generated using the Illumina Infinium HumanMethylation450K BeadChip (Illumina, San Diego, USA) - were used as the discovery dataset (Neumann et al., 2020). This dataset comprised 2,477 children (ages 4 to 15 years), and included information on effect size, standard error, and p-values for each CpG site associated with ADHD (Neumann et al., 2020). Given the large sample size, combination of several cohorts, participants comparable in age to the DCHS children, cord-blood DNAm data, and phenotype of interest (ADHD) (Neumann et al., 2020); the PACE ADHD summary statistics were a suitable discovery dataset for this analysis.

MRS calculation

A MRS for ADHD was calculated as the weighted sum of methylation beta levels of CpG sites, using the PACE-ADHD summary statistics as external weights (Chen et al., 2023, Hüls and Czamara, 2020). To account for the correlation between CpG sites, the *CoMeBack* R package was used to estimate co-methylated regions (CMRs) (Gatev et al., 2020). This method "chains" adjacent array probes less than 2 kb apart if i) the reference human genome annotation shows a set of unmeasured genomic CpGs between them, and ii) the density of unmeasured genomic CpGs between them is at least one CpG every 400 bp (Gatev et al., 2020). This results in one unit in which multiple CpGs are "chained" together. Correlations between DNAm levels were then calculated for all array probes within each unit. A region is considered a CMR if all pairs of adjacent probes in a unit have a correlation square (R^2) greater than 0.3 (Hüls and Czamara, 2020).

The MRS was then generated using clumping and thresholding (C+T), a method similar to the pruning (P) and threshold (T) methods in traditional PRS calculations (Purcell et al., 2007). Clumping was undertaken by retaining only one CpG site per CMR in the dataset, i.e. the site with the lowest and most significant p-value in the PACE-ADHD summary statistics. Thereafter, p-value thresholding was performed for the pruned set of CpG sites by applying different p-value thresholds (i.e., 0.05, 0.005, 5 X 10^{-4}). These p-value thresholds are in line with those used in prior DCHS MRS analyses (Chen et al., 2023). Only CpGs which reached a p-value below these defined thresholds were included in the final MRS calculation. For each p-value threshold, the MRS was calculated as the weighted sum of beta-values, with the weights corresponding to effect sizes for

each CpG from the summary statistics. The R^2 between the phenotype of interest (i.e. ADHD) and the MRS obtained for each p-value threshold was then calculated as a measure of prediction accuracy. The p-value threshold with the highest R^2 was used in the downstream analyses (Chen et al., 2023).

6.3.4. Statistical analyses

Potential associations between the ADHD MRS and developmental and mental health outcomes were investigated using unadjusted bivariate and multivariable linear regression models in R (R Core Team, 2013). Covariates of interest were the sex of the child, SES quartile, prenatal maternal alcohol consumption, prenatal maternal smoking, maternal HIV status, and the first 10 genomic PCs (to adjust for population stratification). Covariate selection was informed by prior findings in the DCHS - for example, higher SES has been identified as a protective factor during child development (Donald et al., 2018a, 2019), while maternal HIV and substance use (smoking and alcohol consumption) have been linked to adverse developmental and anthropometric outcomes during childhood (Donald et al., 2018a, Donald et al., 2019, Zar et al., 2015). Similar to the approach described in *Chapter 4*, a forward selection of variables was employed using the *ols* package in R) (Smith, 2018) - the resultant model included maternal depression, maternal prenatal smoking, and the sex of the child as covariates. All analyses were stratified by array, due to probe differences during MRS calculation - per prior work in the DCHS (Chen et al., 2023).

6.4. Results

6.4.1. Sociodemographic, biomedical and psychosocial characteristics

Full sociodemographic, biomedical and psychosocial characteristics of this study sample are provided in *Chapter 4, Section 4.4*. In summary, of the N=275 children with phenotype data, almost half were female (44%); more than a quarter (27%) were from families with low SES; and almost a third (31%) had depression.

6.4.2. Child developmental and mental health outcomes

Complete details of the developmental and mental health outcomes of this study sample are provided in *Chapter 4, Section 4.4*. Of note, 15% of the children were found to have cognitive delay, 16% to have language delay and 14% to have motor delay on the Bayley III at age 24 months. Mental health outcomes were assessed longitudinally (via the CBCL at 24, 42 and 60 months). Approximately a tenth of children were found to have either internalising (12%) or externalising (11%) problems at 24 months. At 42 months, this prevalence was found to be reduced (i.e. 3% of children were found to have internalising problems, and 4% externalising problems). At 60 months, an increased prevalence was again observed, with 16% found to exhibit internalising problems, and 7% externalising problems.

6.4.3. Associations between methylation risk for ADHD and child developmental and mental health outcomes

The MRS at the p-value threshold of 0.05 (comprising 19,2023 CpG sites) obtained the highest R^2 value of 0.5% (***Appendix* 4**, Tables 1 and 2); and was thus used in the association testing. The highest R^2 reached was 0.5%. There were no significant associations between MRS for ADHD and the development or mental outcomes in the unadjusted bivariate and multivariable linear regression models **Tables 6.1-6.8**.

Table 6. 1 Bivariate linear regression analysis of potential associations between methylation risk for ADHD and child developmental outcomes at 24 months (450K samples)

	Cognitive development			**Language development**			**Motor development**		
	estimate	CI	*p*	estimate	CI	*p*	estimate	CI	*p*
MRS (0.05)	0.0023	-0.032; 0.037	*0.89*	0.026	-0.025;0.077	*0.31*	0.032	-0.022;0.086	0.24

Table 6. 2 Multivariable linear regression analysis of potential associations between methylation risk for ADHD and child developmental outcomes at 24 months (450K samples)

	Cognitive development			**Language development**			**Motor development**		
	estimate	CI	*p*	estimate	CI	*p*	estimate	CI	*p*
MRS (0.05)	0.0002	-0.034; 0.035	0.99	0.032	-0.020; 0.084	0.22	0.024	-0.032; 0.080	0.39
Child sex (female)	0.49	-3.75; 4.73	0.99	-3.49	-9.80; 2.82	0.27	2.37	-4.62; 9.36	0.5
Maternal depression	-5.58	-9.92; -1.23	0.013*	-4.71	-11.07; 1.65	0.14	-0.56	-7.992; 6.88	0.88
Prenatal smoking	1.82	-2.97; 6.62	0.45	-4.54	11.59; 2.51	0.2	-5.5	-13.401; 2.40	0.17

*p<0.05

Table 6. 3 Bivariate linear regression analysis of potential associations between methylation risk for ADHD and child mental health outcomes at 24 months to 60 months (450K samples)

	24 months						42 months						60 months					
	Internalising symptoms			Externalising symptoms			Internalising symptoms			Externalising symptoms			Internalising symptoms			Externalising symptoms		
	estimate	CI	*p*	estimate	CI	*p*	estimate	CI	*p*	estimate	CI	*p*	estimate	CI	*p*	estimate	CI	*p*
MRS (0.05)	0.002	-0.055;0.059	0.94	-0.01	-0.064; 0.045	0.73	-0.01	-0.05; 0.025	0.56	-0.012	-0.050; 0.027	0.56	-0.014	-0.070; 0.042	0.61	-0.012	-0.054; 0.030	0.57

Table 6. 4 Multivariable linear regression analyses of potential associations between methylation risk for ADHD and child mental health outcomes at 24 months to 60 months (450K samples)

	24 months						**42 months**						**60 months**					
	Internalising symptoms			**Externalising symptoms**			**Internalising symptoms**			**Externalising symptoms**			**Internalising symptoms**			**Externalising symptoms**		
	estimate	CI	*p*	estimate	CI	*p*	estimate	CI	*p*	estimate	CI	*p*	estimate	CI	*p*	estimate	CI	*p*
MRS (0.05)	0.02	-0.04; 0.07	0.63	0.01	-0.05; 0.06	0.8	-0.01	-0.04; 0.03	0.71	-0.01	-0.05; 0.03	0.5	-0.01	-0.07; 0.04	0.65	-0.01	-0.05; 0.03	0.69
Child sex (female)	-0.54	7.44; 6.36	0.88	-2.4	-8.64; 3.84	0.44	-1.5	5.89; 2.89	0.5	2.91	-1.88; 7.69	0.23	1.45	-5.28; 8.17	0.67	1.1	-3.78; 5.99	0.65
Maternal depression	7.01	-0.16; 14.17	0.06$^{\#}$	8.96	2.48; 15.44	0.01**	-2.22	-6.57; 2.14	0.32	-1.61	6.46; 3.14	0.5	6.33	-0.15; 12.82	0.06$^{\#}$	3.73	-0.98; 8.44	0.12
Prenatal smoking	4.7	-2.88; 12.27	0.22	6.15	-0.70; 13.00	0.077$^{\#}$	1.00	-4.27; 6.21	0.71	0.75	-4.96; 6.46	0.8	3.61	-3.89; 11.11	0.34	4.71	-0.74; 10.16	0.09$^{\#}$

*p<0.05; #p $0.05 \leq p \leq 0.1$

Table 6. 5 Bivariate linear regression analysis of potential associations between methylation risk for ADHD and child developmental outcomes at 24 months (EPIC samples)

	Cognitive development			**Language development**			**Motor development**		
	estimate	CI	*p*	estimate	CI	*p*	estimate	CI	*p*
MRS (0.05)	0.01	-0.03; 0.01	0.71	0.01	-0.03; 0.06	0.56	0.02	-0.03; 0.08	0.40

Table 6. 6 Multivariable linear regression analysis of potential associations between methylation risk for ADHD and child developmental outcomes at 24 months (EPIC samples)

	Cognitive development			**Language development**			**Motor development**		
	estimate	CI	*p*	estimate	CI	*p*	estimate	CI	*p*
MRS (0.05)	0.01	-0.03; 0.04	0.82	0.01	-0.04; 0.06	0.75	0.022	-0.04; 0.08	0.48
Child sex (Female)	-1.96	-5.87; 1.95	0.32	-3.10	-7.86; 1.66	0.20	-0.84	-6.90; 5.23	0.78
Maternal depression	-2.5	-6.90; 1.91	0.26	-4.34	-9.73; 1.04	0.11	-2.45	-9.32; 4.41	0.48
Prenatal smoking	1.86	-3.11; 6.80	0.46	-0.03	-6.19; 6.12	0.99	-1.99	-9.61; 5.62	0.60

Table 6. 7 Bivariate linear regression analysis of potential associations between methylation risk for ADHD and child mental health outcomes from 24 months to 60 months (EPIC samples)

	24 months						**42 months**						**60 months**					
	Internalising symptoms			**Externalising symptoms**			**Internalising symptoms**			**Externalising symptoms**			**Internalising symptoms**			**Externalising symptoms**		
	estimate	CI	*p*	estimate	CI	*p*	estimate	CI	*p*	estimate	CI	*p*	estimate	CI	*p*	estimate	CI	*p*
MRS (0.05)	0.03	-0.04; 0.10	0.38	-0.03	-0.08; 0.03	0.4	-0.02	-0.06; 0.02	0.3	0.004	-0.04; 0.05	0.86	-0.01	-0.06; 0.04	0.72	-0.01	-0.05; 0.03	0.67

Table 6. 8 Multivariable linear regression analysis of potential associations between methylation risk for ADHD and child developmental outcomes from 24 months to 60 months (EPIC samples)

	24 months						**42 months**						**60 months**					
	Internalising symptoms			**Externalising symptoms**			**Internalising symptoms**			**Externalising symptoms**			**Internalising symptoms**			**Externalising symptoms**		
	estimate	CI	*p*	estimate	CI	*p*	estimate	CI	*p*	estimate	CI	*p*	estimate	CI	*p*	estimate	CI	*p*
MRS (0.05)	0.04	-0.03; 0.11	0.28	-0.03	-0.08; 0.03	0.35	-0.019	-0.056; 0.017	0.3	0.01	-0.04; 0.05	0.78	-0.01	-0.05; 0.04	0.84	-0.01	-0.05; 0.03	0.71
Child sex (Female)	3.56	2.81; 9.93	0.27	7.61	2.53; 12.70	0.004**	2.98	0.58; 6.55	0.1	5.94	1.94; 9.94	0.004**	4.73	-0.25; 9.72	0.06#	3.67	-0.46; 7.81	0.08#
Maternal depression	0.84	-6.78; 8.46	0.83	-3.00	9.09;3.08	0.32	-3.55	7.59; 0.46	0.08#	-4.31	-8.83; 0.21	0.06#	2.4	-3.39; 8.20	0.41	0.35	-4.46; 5.16	0.89
Pre-natal smoking	8.84	-0.47; 18.16	0.06#	2.64	-4.79; 10.08	0.48	-3.22	-8.15; 1.72	0.2	0.76	-4.78; 6.30	0.77	-4.27	-11.34; 2.81	0.23	-2.89	-8.76; 2.98	0.33

*p<0.05; #p $0.05 \leq p \leq 0.1$; **p<0.01

6.5. Discussion

This analysis – which sought to investigate potential associations between MRS for ADHD, and adverse developmental and mental health outcomes in the DCHS index children aged 24 months to 60 months – yielded no significant findings with the outcomes of interest. This is in line with prior work by Hannon and colleagues (2018) quantifying methylomic variation in 1263 infants from the Integrative Psychiatric Research (iPSYCH) cohort (Pedersen et al., 2017). In this study, no significant associations were reported between MRS for autism spectrum disorder (ASD) at birth, and the development of ASD symptoms in childhood (Hannon et al., 2018). The complex aetiology and phenotypic heterogeneity of many mental disorders may have accounted for the lack of significant findings in this analysis (Palladino et al., 2019, Nabais et al.,2023). For example, several different ADHD subtypes exist, each with distinct environmental risk factors (Palladino et al., 2019). Similarly, phenotypic heterogeneity makes mental health disorders difficult to study within epigenetics – where DNAm is dynamic in nature and can thus change throughout an individual's lifetime (Cecil and Nigg, 2022, Nabais et al.,2023). Moreover, the potential effects of psychopathology risk can also be both cumulative and time-sensitive, as such, result in significant variation in a population (Barker et al., 2018).

Thus, MRS may be more suitable for predicting biomedical phenotypes such as smoking behaviour (Bollepalli et al.,2019), body mass index (Shah et al.,2015, Zhange et al.,2019), and risk for type 2 diabtetes mellitus (Cheng et al.,2023). Biomedical phenotypes are often less heterogeneous, with clearer biological pathways and more robust associations that are often reproducible across different studies. Additionally, environmental exposures related to smoking as well as the metabolic processes underlying BMI have well-established associations with DNAm, therefore are a more stable basis for developing MRS (Nabais et al.,2023). For example, in their work identifying allergy diseases, Kilanowski and colleagues (2022) found that MRS for allergy phenotypes predicted aeroallergy sensitization in children and adolescents (Kilanowski et al.,2022). An alternative approach may be to combine MRS with electronic health records (EHR) (Thompson et al., 2022). For example, Thompson (2022) reported that adding MRS to existing EHR-based imputation frameworks improved MRS performance in the clinical setting (Thompson et al., 2022)."

Notably, previous studies have reported significant associations between differential DNAm at birth and ADHD symptoms (Neumann et al., 2020), as well as developmental outcomes in childhood (Caramaschi et al., 2022). For example, a recent study of ~2,000 children found that differential DNAm at birth was associated with cognitive development in children (aged 4 to 9 years) (Caramaschi et al., 2022). However, this study undertook an EWAS, rather than an MRS-based analysis. Thus, the utility of MRS in developmental outcomes or in the context of mental disorders remains to be elucidated.

A number of noteworthy limitations may have influenced these study findings. First, most currently available EWAS summary statistics are substantially smaller in sample size than those from GWAS (Min et al., 2021). For example, the PACE-ADHD EWAS discovery dataset used in this study comprises approximately 2,000 participants (Neumann et al., 2020); while the PGC-ADHD GWAS has a sample size of more than 20,000 participants (Demontis et al., 2019). As larger sample sizes are key to increase statistical power to detect significant effects (Min et al., 2021), there are now ongoing efforts to collate existing EWAS datasets for meta-analyses and related analyses (e.g. within the PACE) (Min et al., 2021, Joehanes et al., 2016). Second, given dynamic age-related DNAm changes during childhood (Alisch et al., 2012, Neumann et al., 2020), DNAm risk for later neurodevelopmental and/or mental health disorders may not yet have been detectable in the DCHS umbilical cord samples (Thapar et al., 2016). Rather, umbilical cord DNAm may act as a marker for (or mediator of) prenatal risk factors (Neumann et al., 2020, Pingault et al., 2015).

Third, the effects of inter-population variability (e.g. HICs vs LMICs) and differing environmental exposures may impact the accuracy and generalisability of MRS (kader et al.,2016, Fraser et al., 2012, Elliot et al., 2014). For example, there is emerging evidence that DNAm may be a marker of untested environmental factors affecting mental health disorders via independent pathways (Neumann et al., 2020). In the context of the

DCHS, there is prior evidence that such environmental/psychosocial risk factors (e.g. Low SES, trauma exposure) may influence newborn DNAm profiles (Ronkainen et al., 2022, Oblak et al., 2021).

Fourth, a significant limitation of DNAm as a potential biomarker for mental health disorders (including ADHD) is its tissue specificity (Smith et al., 2014). Easily accessible tissues such as blood and saliva may not accurately reflect DNAm patterns in the central nervous system (Smith et al., 2014). However, while Smith et al. (2014) demonstrated distinct DNAm profiles in brain regions versus peripheral tissues (i.e. blood and saliva); these authors also reported a positive correlation between DNAm in the brain and DNAm in peripheral tissues (Smith et al., 2014). Moreover, saliva-derived DNAm has shown promise in identifying genetic risk factors for mental disorders such as BP, SCZ, and MDD in adults (Nohesara et al., 2011; Ghadirivasfi et al., 2011; Fuchikami et al., 2011). Thus, accessible tissues such as cord blood may be valuable for studying mental health phenotypes such as ADHD.

Finally, the paucity of available summary statistics from populations of African ancestry may have contributed to the current study findings. Most available EWAS summary statistics disproportionally represent participants of European ancestry (Wray et al., 2014, Majara et al., 2023). It is well-recognized that differing correlation structures between ancestries affect the predictive power and transferability of PRS. However, the extent to which ancestry influences MRS is less understood (Nabais et al., 2023). It is noteworthy, though, that European-derived summary statistics have previously been found to be suitable for use within African and admixed populations (Chen et al., 2023). Nevertheless, the analysis and interpretation of MRS findings across multi-ancestry (and ancestry-discordant) populations should be undertaken with caution; as environmental, cultural, and social/societal differences may impact the cross-ancestry applicability of MRS calculations (Chen et al., 2023, Hannon et al., 2016).

In conclusion, although analysis did not yield any significant associations between MRS for ADHD and poor development and mental health outcomes in the DCHS children; this is the first study (to our knowledge) to use MRS to investigate potential associations between ADHD risk and child development as well as mental health. Further work into the potential utility of MRS as early biomarkers for identifying at risk children – particularly in LMICs and in participants of African ancestry - is warranted. In addition, research integrating both genetic risk (captured by PRS) and MRS may improve the statistical power and prediction accuracy of these risk scores. Such an approach may further contribute to elucidating the epigenetic risk factors underlying poor development and risk for mental disorders in children.

6.6. References

Alisch, r. S., barwick, b. G., chopra, p., myrick, l. K., satten, g. A., conneely, k. N. & warren, s. T. 2012. Age-associated dna methylation in pediatric populations. *Genome res,* 22**,** 623-32.

Aristizabal, m. J., anreiter, i., halldorsdottir, t., odgers, c. L., mcdade, t. W., goldenberg, a., mostafavi, s., kobor, m. S., binder, e. B., sokolowski, m. B. & o'donnell, k. J. 2020. Biological embedding of experience: a primer on epigenetics. *Proc natl acad sci u s a,* 117**,** 23261-23269.

Barker, e. D., walton, e. & cecil, c. A. M. 2018. Annual research review: dna methylation as a mediator in the association between risk exposure and child and adolescent psychopathology. *J child psychol psychiatry,* 59**,** 303-322.

Brikell, i., kuja-halkola, r. & larsson, h. 2015. Heritability of attention-deficit hyperactivity disorder in adults. *Am j med genet b neuropsychiatr genet,* 168**,** 406-413.

Caramaschi, d., neumann, a., cardenas, a., tindula, g., alemany, s., zillich, l., pesce, g., lahti, j. M. T., havdahl, a., mulder, r., felix, j. F., tiemeier, h., sirignano, l., frank, j., witt, s. H., rietschel, m., deuschle, m., huen, k., eskenazi, b., send, t. S., ferrer, m., gilles, m., de agostini, m., baïz, n., rifas-shiman, s. L., kvist, t., czamara, d., tuominen, s. T., relton, c. L., rai, d., london, s. J., räikkönen, k., holland, n., annesi-maesano, i., streit, f., hivert, m. F., oken, e., sunyer, j., cecil, c. A. M. & sharp, g. 2022. Meta-analysis of epigenome-wide associations between dna methylation at birth and childhood cognitive skills. *Molecular psychiatry 2022 27:4,* 27**,** 2126-2135.

Cecil, c. A. M. & nigg, j. T. 2022. Epigenetics and adhd: reflections on current knowledge, research priorities and translational potential. *Mol diagn ther,* 26**,** 581-606.

Chen, j., gatev, e., everson, t., conneely, k. N., koen, n., epstein, m. P., kobor, m. S., zar, h. J., stein, d. J. & hüls, a. 2023. Pruning and thresholding approach for methylation risk scores in multi-ancestry populations. *Epigenetics,* 18**,** 2187172.

Cherkasova, m., sulla, e. M., dalena, k. L., pondé, m. P. & hechtman, l. 2013. Developmental course of attention deficit hyperactivity disorder and its predictors. *J can acad child adolesc psychiatry,* 22**,** 47-54.

Christensen, g. M., marcus, m., vanker, a., eick, s. M., malcolm-smith, s., smith, a., dunn, e. C., suglia, s. F., chang, h. H., zar, h. J., stein, d. J. & hüls, a. 2023. Sensitive periods for exposure to indoor air pollutants and psychosocial factors in association with symptoms of psychopathology at school-age in a south african birth cohort. *Medrxiv*.

Christensen, g. M., rowcliffe, c., chen, j., vanker, a., koen, n., jones, m. J., gladish, n., hoffman, n., donald, k. A., wedderburn, c. J., kobor, m. S., zar, h. J., stein, d. J. & hüls, a. 2022. In-utero exposure to indoor air pollution or tobacco smoke and cognitive development in a south african birth cohort study. *Sci total environ,* 834**,** 155394.

Das, s., forer, l., schönherr, s., sidore, c., locke, a. E., kwong, a., vrieze, s. I., chew, e. Y., levy, s., mcgue, m., schlessinger, d., stambolian, d., loh, p. R., iacono, w. G., swaroop, a., scott, l. J., cucca, f., kronenberg, f., boehnke, m., abecasis, g. R. & fuchsberger, c. 2016. Next-generation genotype imputation service and methods. *Nat genet,* 48**,** 1284-1287.

Dick, k. J., nelson, c. P., tsaprouni, l., sandling, j. K., aïssi, d., wahl, s., meduri, e., morange, p. E., gagnon, f., grallert, h., waldenberger, m., peters, a., erdmann, j., hengstenberg, c., cambien, f., goodall, a. H., ouwehand, w. H., schunkert, h., thompson, j. R., spector, t. D., gieger, c., trégouët, d. A., deloukas, p. & samani, n. J. 2014. Dna methylation and body-mass index: a genome-wide analysis. *Lancet,* 383**,** 1990-8.

Donald, k. A., hoogenhout, m., du plooy, c. P., wedderburn, c. J., nhapi, r. T., barnett, w., hoffman, n., malcolm-smith, s., zar, h. J. & stein, d. J. 2018. Drakenstein child health study (dchs): investigating determinants of early child development and cognition. *Bmj paediatr open,* 2**,** e000282.

Donald, k. A., wedderburn, c. J., barnett, w., nhapi, r. T., rehman, a. M., stadler, j. A. M., hoffman, n., koen, n., zar, h. J. & stein, d. J. 2019. Risk and protective factors for child development: an observational south african birth cohort. *Plos med,* 16**,** e1002920.

Eze, i. C., imboden, m., kumar, a., von eckardstein, a., stolz, d., gerbase, m. W., künzli, n., pons, m., kronenberg, f., schindler, c. & probst-hensch, n. 2016. Air pollution and diabetes association: modification by type 2 diabetes genetic risk score. *Environ int,* 94**,** 263-271.

Faleschini, s., rifas-shiman, s. L., tiemeier, h., oken, e. & hivert, m. F. 2019. Associations of prenatal and postnatal maternal depressive symptoms with offspring cognition and behavior in mid-childhood: a prospective cohort study. *Int j environ res public health,* 16.

Feil, d., abrishamcar, s., christensen, g. M., vanker, a., koen, n., kilanowski, a., hoffman, n., wedderburn, c. J., donald, k. A., kobor, m. S., zar, h. J., stein, d. J. & hüls, a. 2023. Dna methylation as a potential mediator of the association between indoor air pollution and neurodevelopmental delay in a south african birth cohort. *Clin epigenetics,* 15**,** 31.

Girchenko, p., lahti, j., czamara, d., knight, a. K., jones, m. J., suarez, a., hämäläinen, e., kajantie, e., laivuori, h., villa, p. M., reynolds, r. M., kobor, m. S., smith, a. K., binder, e. B. & räikkönen, k. 2017. Associations between maternal risk factors of adverse pregnancy and birth outcomes and the offspring epigenetic clock of gestational age at birth. *Clin epigenetics,* 9**,** 49.

Hannon, e., dempster, e., viana, j., burrage, j., smith, a. R., macdonald, r., st clair, d., mustard, c., breen, g., therman, s., kaprio, j., toulopoulou, t., hulshoff pol, h. E., bohlken, m. M., kahn, r. S., nenadic, i., hultman, c. M., murray, r. M., collier, d. A., bass, n., gurling, h., mcquillin, a., schalkwyk, l. & mill, j. 2016. An integrated genetic-epigenetic analysis of schizophrenia: evidence for co-localization of genetic associations and differential dna methylation. *Genome biol,* 17**,** 176.

Hannon, e., schendel, d., ladd-acosta, c., grove, j., hansen, c. S., andrews, s. V., hougaard, d. M., bresnahan, m., mors, o., hollegaard, m. V., bækvad-hansen, m., hornig, m., mortensen, p. B., børglum, a. D., werge, t., pedersen, m. G., nordentoft, m., buxbaum, j., daniele fallin, m., bybjerg-grauholm, j., reichenberg, a. & mill, j. 2018. Elevated polygenic burden for autism is associated with differential dna methylation at birth. *Genome med,* 10**,** 19.

Hibler, e., huang, l., andrade, j. & spring, b. 2019. Impact of a diet and activity health promotion intervention on regional patterns of dna methylation. *Clin epigenetics,* 11**,** 133.

Hüls, a. & czamara, d. 2020. Methodological challenges in constructing dna methylation risk scores. Taylor and francis inc.

Joehanes, r., just, a. C., marioni, r. E., pilling, l. C., reynolds, l. M., mandaviya, p. R., guan, w., xu, t., elks, c. E., aslibekyan, s., moreno-macias, h., smith, j. A., brody, j. A., dhingra, r., yousefi, p., pankow, j. S., kunze, s., shah, s. H., mcrae, a. F., lohman, k., sha, j., absher, d. M., ferrucci, l., zhao, w., demerath, e. W., bressler, j., grove, m. L., huan, t., liu, c., mendelson, m. M., yao, c., kiel, d. P., peters, a., wang-sattler, r., visscher, p. M., wray, n. R., starr, j. M., ding, j., rodriguez, c. J., wareham, n. J., irvin, m. R., zhi, d., barrdahl, m., vineis, p., ambatipudi, s., uitterlinden, a. G., hofman, a., schwartz, j., colicino, e., hou, l., vokonas, p. S., hernandez, d. G., singleton, a. B., bandinelli, s., turner, s. T., ware, e. B., smith, a. K., klengel, t., binder, e. B., psaty, b. M., taylor, k. D., gharib, s. A., swenson, b. R., liang, l., demeo, d. L., o'connor, g. T., herceg, z., ressler, k. J., conneely, k. N., sotoodehnia, n., kardia, s. L., melzer, d., baccarelli, a. A., van meurs, j. B., romieu, i., arnett, d. K., ong, k. K., liu, y., waldenberger, m., deary, i. J., fornage, m., levy, d. & london, s. J. 2016. Epigenetic signatures of cigarette smoking. *Circ cardiovasc genet,* 9**,** 436-447.

Koen, n., wyatt, g. E., williams, j. K., zhang, m., myer, l., zar, h. J. & stein, d. J. 2014. Intimate partner violence: associations with low infant birthweight in a south african birth cohort. *Metabolic brain disease,* 29**,** 281-299.

Lee, s. H., ripke, s., neale, b. M., faraone, s. V., purcell, s. M., perlis, r. H., mowry, b. J., thapar, a., goddard, m. E., witte, j. S., absher, d., agartz, i., akil, h., amin, f., andreassen, o. A., anjorin, a., anney, r., anttila, v., arking, d. E., asherson, p., azevedo, m. H., backlund, l., badner, j. A., bailey, a. J., banaschewski, t., barchas, j. D., barnes, m. R., barrett, t. B., bass, n., battaglia, a., bauer, m., bayés, m., bellivier, f., bergen, s. E., berrettini, w., betancur, c., bettecken, t., biederman, j., binder, e. B., black, d. W., blackwood, d. H., bloss, c. S., boehnke, m., boomsma, d. I., breen, g., breuer, r.,

bruggeman, r., cormican, p., buccola, n. G., buitelaar, j. K., bunney, w. E., buxbaum, j. D., byerley, w. F., byrne, e. M., caesar, s., cahn, w., cantor, r. M., casas, m., chakravarti, a., chambert, k., choudhury, k., cichon, s., cloninger, c. R., collier, d. A., cook, e. H., coon, h., cormand, b., corvin, a., coryell, w. H., craig, d. W., craig, i. W., crosbie, j., cuccaro, m. L., curtis, d., czamara, d., datta, s., dawson, g., day, r., de geus, e. J., degenhardt, f., djurovic, s., donohoe, g. J., doyle, a. E., duan, j., dudbridge, f., duketis, e., ebstein, r. P., edenberg, h. J., elia, j., ennis, s., etain, b., fanous, a., farmer, a. E., ferrier, i. N., flickinger, m., fombonne, e., foroud, t., frank, j., franke, b., fraser, c., et al. 2013. Genetic relationship between five psychiatric disorders estimated from genome-wide snps. *Nat genet,* 45**,** 984-94.

Mcrae, a. F., powell, j. E., henders, a. K., bowdler, l., hemani, g., shah, s., painter, j. N., martin, n. G., visscher, p. M. & montgomery, g. W. 2014. Contribution of genetic variation to transgenerational inheritance of dna methylation. *Genome biol,* 15**,** r73.

Mill, j. & petronis, a. 2008. Pre- and peri-natal environmental risks for attention-deficit hyperactivity disorder (adhd): the potential role of epigenetic processes in mediating susceptibility. *J child psychol psychiatry,* 49**,** 1020-30.

Min, j. L., hemani, g., hannon, e., dekkers, k. F., castillo-fernandez, j., luijk, r., carnero-montoro, e., lawson, d. J., burrows, k., suderman, m., bretherick, a. D., richardson, t. G., klughammer, j., iotchkova, v., sharp, g., al khleifat, a., shatunov, a., iacoangeli, a., mcardle, w. L., ho, k. M., kumar, a., söderhäll, c., soriano-tárraga, c., giralt-steinhauer, e., kazmi, n., mason, d., mcrae, a. F., corcoran, d. L., sugden, k., kasela, s., cardona, a., day, f. R., cugliari, g., viberti, c., guarrera, s., lerro, m., gupta, r., bollepalli, s., mandaviya, p., zeng, y., clarke, t. K., walker, r. M., schmoll, v., czamara, d., ruiz-arenas, c., rezwan, f. I., marioni, r. E., lin, t., awaloff, y., germain, m., aïssi, d., zwamborn, r., van eijk, k., dekker, a., van dongen, j., hottenga, j. J., willemsen, g., xu, c. J., barturen, g., català-moll, f., kerick, m., wang, c., melton, p., elliott, h. R., shin, j., bernard, m., yet, i., smart, m., gorrie-stone, t., shaw, c., al chalabi, a., ring, s. M., pershagen, g., melén, e., jiménez-conde, j., roquer, j., lawlor, d. A., wright, j., martin, n. G., montgomery, g. W., moffitt, t. E., poulton, r., esko, t., milani, l., metspalu, a., perry, j. R. B., ong, k. K., wareham, n. J., matullo, g., sacerdote, c., panico, s., caspi, a., arseneault, l., gagnon, f., ollikainen, m., kaprio, j., felix, j. F., rivadeneira, f., tiemeier, h., van, i. M. H., et al. 2021. Genomic and phenotypic insights from an atlas of genetic effects on dna methylation. *Nat genet,* 53**,** 1311-1321.

Moore, l. D., le, t. & fan, g. 2013. Dna methylation and its basic function. *Neuropsychopharmacology,* 38**,** 23-38.

Moreaux, j., bruyer, a., veyrune, j. L., goldschmidt, h., hose, d. & klein, b. 2014. Dna methylation score is predictive of myeloma cell sensitivity to 5-azacitidine. *Br j haematol,* 164**,** 613-6.

Murphy, t. M. & mill, j. 2014. Epigenetics in health and disease: heralding the ewas era. *Lancet,* 383**,** 1952-4.

Nabais, m. F., gadd, d. A., hannon, e., mill, j., mcrae, a. F. & wray, n. R. 2023. An overview of dna methylation-derived trait score methods and applications. *Genome biology,* 24**,** 28.

Neumann, a., walton, e., alemany, s., cecil, c., gonzález, j. R., jima, d. D., lahti, j., tuominen, s. T., barker, e. D., binder, e., caramaschi, d., carracedo, á., czamara, d., evandt, j., felix, j. F., fuemmeler, b. F., gutzkow, k. B., hoyo, c., julvez, j., kajantie, e., laivuori, h., maguire, r., maitre, l., murphy, s. K., murcia, m., villa, p. M., sharp, g., sunyer, j., raikkönen, k., bakermans-kranenburg, m., ijzendoorn, m. V., guxens, m., relton, c. L. & tiemeier, h. 2020. Association between dna methylation and adhd symptoms from birth to school age: a prospective meta-analysis. *Translational psychiatry,* 10**,** 30-30.

Oblak, l., van der zaag, j., higgins-chen, a. T., levine, m. E. & boks, m. P. 2021. A systematic review of biological, social and environmental factors associated with epigenetic clock acceleration. *Ageing research reviews,* 69**,** 101348-101348.

Palladino, v. S., mcneill, r., reif, a. & kittel-schneider, s. 2019. Genetic risk factors and gene-environment interactions in adult and childhood attention-deficit/hyperactivity disorder. *Psychiatric genetics,* 29**,** 63-78.

Parade, s. H., ridout, k. K., seifer, r., armstrong, d. A., marsit, c. J., mcwilliams, m. A. & tyrka, a. R. 2016. Methylation of the glucocorticoid receptor gene promoter in preschoolers: links with internalizing behavior problems. *Child dev,* 87**,** 86-97.

Pingault, j.-b., viding, e., galera, c., greven, c. U., zheng, y., plomin, r. & rijsdijk, f. 2015. Genetic and environmental influences on the developmental course of attention-deficit/hyperactivity disorder symptoms from childhood to adolescence. *Jama psychiatry,* 72**,** 651-658.

Rakyan, v. K., down, t. A., balding, d. J. & beck, s. 2011. Epigenome-wide association studies for common human diseases. *Nat rev genet,* 12**,** 529-41.

Rijlaarsdam, j., cecil, c. A. M., relton, c. L. & barker, e. D. 2022. Epigenetic profiling of social communication trajectories and co-occurring mental health problems: a prospective, methylome-wide association study. *Dev psychopathol,* 34**,** 854-863.

Ronkainen, j., heiskala, a., vehmeijer, f. O. L., lowry, e., caramaschi, d., estrada gutierrez, g., heiss, j. A., hummel, n., keikkala, e., kvist, t., kupsco, a., melton, p. E., pesce, g., soomro, m. H., vives-usano, m., baiz, n., binder, e., czamara, d., guxens, m., mustaniemi, s., london, s. J., rauschert, s., vääräsmäki, m., vrijheid, m., ziegler, a.-g., annesi-maesano, i., bustamante, m., huang, r.-c., hummel, s., just, a. C., kajantie, e., lahti, j., lawlor, d., räikkönen, k., järvelin, m.-r., felix, j. F. & sebert, s. 2022. Maternal haemoglobin levels in pregnancy and child dna methylation: a study in the pregnancy and childhood epigenetics consortium. *Epigenetics,* 17**,** 19-31.

Stein, d. J., koen, n., donald, k. A., adnams, c. M., koopowitz, s., lund, c., marais, a., myers, b., roos, a., sorsdahl, k., stern, m., tomlinson, m., van der westhuizen, c., vythilingum, b., myer, l., barnett, w., brittain, k. & zar, h. J. 2015. Investigating the psychosocial determinants of child health in africa: the drakenstein child health study. *J neurosci methods,* 252**,** 27-35.

Szyf, m. & bick, j. 2013. Dna methylation: a mechanism for embedding early life experiences in the genome. *Child dev,* 84**,** 49-57.

Thapar, a., martin, j., mick, e., vasquez, a. A., langley, k., scherer, s. W., schachar, r., crosbie, j., williams, n., franke, b., elia, j., glessner, j., hakonarson, h., owen, m. J., faraone, s. V., o'donovan, m. C., holmans, p. & consortium, i. 2016. Psychiatric gene discoveries shape evidence on adhd's biology. *Molecular psychiatry,* 21**,** 1202-1207.

Walton, e., pingault, j. B., cecil, c. A., gaunt, t. R., relton, c. L., mill, j. & barker, e. D. 2017. Epigenetic profiling of adhd symptoms trajectories: a prospective, methylome-wide study. *Mol psychiatry,* 22**,** 250-256.

Wray, n. R., lee, s. H., mehta, d., vinkhuyzen, a. A., dudbridge, f. & middeldorp, c. M. 2014. Research review: polygenic methods and their application to psychiatric traits. *J child psychol psychiatry,* 55**,** 1068-87.

Zar, h. J., barnett, w., myer, l., stein, d. J. & nicol, m. P. 2015. Investigating the early-life determinants of illness in africa: the drakenstein child health study. *Thorax,* 70**,** 592-594.

Zhou, k., dempfle, a., arcos-burgos, m., bakker, s. C., banaschewski, t., biederman, j., buitelaar, j., castellanos, f. X., doyle, a., ebstein, r. P., ekholm, j., forabosco, p., franke, b., freitag, c., friedel, s., gill, m., hebebrand, j., hinney, a., jacob, c., lesch, k. P., loo, s. K., lopera, f., mccracken, j. T., mcgough, j. J., meyer, j., mick, e., miranda, a., muenke, m., mulas, f., nelson, s. F., nguyen, t. T., oades, r. D., ogdie, m. N., palacio, j. D., pineda, d., reif, a., renner, t. J., roeyers, h., romanos, m., rothenberger, a., schäfer, h., sergeant, j., sinke, r. J., smalley, s. L., sonuga-barke, e., steinhausen, h. C., van der meulen, e., walitza, s., warnke, a., lewis, c. M., faraone, s. V. & asherson, p. 2008. Meta-analysis of genome-wide linkage scans of attention deficit hyperactivity disorder. *Am j med genet b neuropsychiatr genet,* 147b**,** 1392-8.

Aryee, M.J., Jaffe, A.E., Corrada-Bravo, H., Ladd-Acosta, C., Feinberg, A.P., Hansen, K.D.,

Irizarry, R.A., 2014. Minfi: A flexible and comprehensive Bioconductor package for the analysis of Infinium DNA methylation microarrays. Bioinformatics 30, 1363–1369. https://doi.org/10.1093/bioinformatics/btu049

Fortin, J.-P., Labbe, A., Lemire, M., Zanke, B.W., Hudson, T.J., Fertig, E.J., Greenwood, C.M., Hansen, K.D., 2014. Functional normalization of 450k methylation array data improves replication in large cancer studies. Genome Biol. 15, 503. https://doi.org/10.1186/s13059-014-0503-2

Gervin, K., Salas, L.A., Bakulski, K.M., Van Zelm, M.C., Koestler, D.C., Wiencke, J.K., Duijts, L., Moll, H.A., Kelsey, K.T., Kobor, M.S., Lyle, R., Christensen, B.C., Felix, J.F., Jones, M.J., 2019. Systematic evaluation and validation of reference and library selection methods for deconvolution of cord blood DNA methylation data. Clin. Epigenetics 11, 1–15. https://doi.org/10.1186/s13148-019-0717-y

Leek, J.T., Johnson, W.E., Parker, H.S., Jaffe, A.E., Storey, J.D., 2012. The sva package for removing batch effects and other unwanted variation in high-throughput experiments. Bioinformatics 28, 882–883. https://doi.org/10.1093/bioinformatics/bts034

Morin, A.M., Gatev, E., McEwen, L.M., Macisaac, J.L., Lin, D.T.S., Koen, N., Czamara, D., Räikkönen, K., Zar, H.J., Koenen, K., Stein, D.J., Kobor, M.S., Jones, M.J., 2017. Maternal blood contamination of collected cord blood can be identified using DNA methylation at three CpGs. Clin. Epigenetics 9, 1–9. https://doi.org/10.1186/s13148-017-0370-2

R Core Team, 2018. R: A language and environment for statistical computing.

Pedersen, C.B., Bybjerg-Grauholm, J., Pedersen, M.G., Grove, J., Agerbo, E., Bækvad-Hansen, M., Poulsen, J.B., Hansen, C.S., McGrath, J.J., Als, T.D., Goldstein, J.I., Neale, B.M., Daly, M.J., Hougaard, D.M., Mors, O., Nordentoft, M., Børglum, A.D., Werge, T. and Mortensen, P.B. (2017). The iPSYCH2012 case–cohort sample: new directions for unravelling genetic and environmental architectures of severe mental disorders. Molecular Psychiatry, 23(1), pp.6–14. doi:https://doi.org/10.1038/mp.2017.196.

Kilanowski, A., Thiering, E., Wang, G., Kumar, A., Kress, S., Flexeder, C., Bauer, C., Dietrich Berdel, Andrea von Berg, Bergström, A., Gappa, M., Heinrich, J., Gunda Herberth, Sibylle Koletzko, Kull, I., Melén, E., Schikowski, T., Peters, A. and Standl, M. (2022). Allergic disease trajectories up to adolescence: Characteristics, early-life, and genetic determinants. Allergy, 78(3), pp.836–850. doi:https://doi.org/10.1111/all.15511.

Kader, F. and Ghai, M. (2016). DNA methylation-based variation between human populations. Molecular Genetics and Genomics, [online] 292(1), pp.5–35. doi:https://doi.org/10.1007/s00438-016-1264-2.

Fraser, H.B., Lam, L.L., Neumann, S.M. and Kobor, M.S. (2012). Population-specificity of human DNA methylation. Genome Biology, 13(2), p.R8. doi:https://doi.org/10.1186/gb-2012-13-2-r8.

Garg, P., Joshi, R.S., Watson, C. and Sharp, A.J. (2018). A survey of inter-individual variation in DNA methylation identifies environmentally responsive co-regulated networks of epigenetic variation in the human genome. PLOS Genetics, 14(10), p.e1007707. doi:https://doi.org/10.1371/journal.pgen.1007707.

Nohesara, S., Ghadirivasfi, M., Mostafavi, S., Eskandari, M.-R., Ahmadkhaniha, H., Thiagalingam, S. and Abdolmaleky, H.M. (2011). DNA hypomethylation of MB-COMT promoter in the DNA derived from saliva in schizophrenia and bipolar disorder. Journal of Psychiatric Research, 45(11), pp.1432–1438. doi:https://doi.org/10.1016/j.jpsychires.2011.06.013.

Ghadirivasfi, M., Nohesara, S., Ahmadkhaniha, H.-R., Eskandari, M.-R., Mostafavi, S., Thiagalingam, S. and Abdolmaleky, H.M. (2011). Hypomethylation of the serotonin receptor type-2A Gene (HTR2A) at T102C polymorphic site in DNA derived from the saliva of patients with schizophrenia and bipolar disorder. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics, 156(5), pp.536–545. doi:https://doi.org/10.1002/ajmg.b.31192.

Fuchikami, M., Morinobu, S., Segawa, M., Okamoto, Y., Yamawaki, S., Ozaki, N., Inoue, T., Kusumi, I., Koyama, T., Tsuchiyama, K. and Terao, T. (2011). DNA Methylation Profiles of the Brain-Derived Neurotrophic Factor (BDNF) Gene as a Potent Diagnostic Biomarker in Major Depression. PLoS ONE, [online] 6(8), p.e23881. doi:https://doi.org/10.1371/journal.pone.0023881.

Chapter 7: Discussion and Conclusion

7.1. Summary of findings

This doctoral project aimed to investigate potential genetic and epigenetic associations with child development and mental health through five objectives. Two systematic reviews were conducted, adhering to PRISMA guidelines, which collated existing literature on genetic risk (assessed by PRS) *(Chapter 2)* and epigenetic risk factors (i.e. EA deviation) *(Chapter 3)* for poor developmental and mental health outcomes in children and adolescents. Thereafter, empirical analyses were undertaken focused on genetic risk (via ADHD PRS) *(Chapter 4)* and epigenetic risk (assessed through gestational EA deviation *(Chapter 5)* and ADHD MRS *(Chapter 6*) for adverse developmental and mental health outcomes in the DCHS children.

The key findings of this thesis are summarized below:

1. First, the systematic review on genetic (polygenic) risk for developmental and mental disorders in children and adolescents identified 14 eligible studies (with ~50,000 participants). The findings of this review suggested that PRS for neurodevelopmental and mental health disorders (such as ADHD) may be associated with adverse outcomes (such as impaired language development, externalising behaviour and ADHD diagnosis) from early childhood to adolescence (Sudre et al., 2020, Jansen et al., 2020, Riglin et al., 2016). These findings align with previous literature which described associations between ADHD PRS and increased risk for externalising behaviour, as well as language difficulties in children (Askeland et al., 2019, Brikell et al., 2020).

2. The second systematic review of epigenetic risk for developmental and mental disorders in children (assessed via EA deviation), identified 4 eligible studies (with ~700 participants). In this review, EA acceleration (EAA) was found to be significantly associated with internalising symptoms in children. However, the meta-analysis of a subset of these studies (n=2) did not replicate this finding. In line with systematic review, previous literature has reported that children with internalising symptoms were found to have EAA, when compared to children without this diagnosis (Dammering et al., 2021).

3. The first empirical analysis of data from the DCHS yielded no significant associations between a PRS for ADHD and adverse developmental or mental health outcomes in the index children. In line with this finding, one prior study also yielded no significant associations between ADHD PRS and internalising symptoms in children (Brikell et al., 2020). However, most literature has reported significant associations between PRS for ADHD and adverse developmental and mental health outcomes in children (Stergiakouli et al., 2017, Neumann et al., 2022, Brikell et al., 2020, Askeland et al., 2019). For instance, PRS for ADHD was found to be associated with poor cognitive outcomes in children with ADHD (Stergiakouli et al., 2017). Prior work has also reported that a higher ADHD PRS associated with both internalising and externalising symptoms in children (Neumann et al., 2021).

4. In the second empirical analysis, no significant associations were found between gestational EA deviation at birth and the outcomes of interest in the DCHS. However, noteworthy trend-level associations between gestational EA deviation at birth and externalising behaviour at 42 months were found. Aligned with the non-significant findings of this analysis, two recent studies reported only trend-level associations between EAA and externalising symptoms in children (Tollenaar et al., 2021, Meijer et al., 2023). However, most studies to date have yielded significant associations between EAA and internalising behavior (Tollenaar et al., 2021, Dammering et al., 2021, Sumner et al., 2019).

5. The final empirical analysis also yielded no significant associations between MRS for ADHD and adverse developmental and mental health outcomes in the DCHS. This suggests that MRS may have better utility in measuring prenatal exposures (e.g. to smoking), rather than in predicting development or mental health outcomes. While prior EWAS have reported significant associations between differential DNAm at birth and ADHD symptoms in children (Caramaschi et al., 2022, Neumann et al., 2020); there is also evidence of non-significant associations between DNAm and internalising disorders (i.e. ASD MRS) (Hannon et al., 2018), which aligns with the non-signifcant findings of my analyses. At the time of writing, this doctoral study was the first to utilise MRS as a biomarker for adverse development and mental health - much of the published literature has investigated the utility of MRS in biomedical indices such as smoking behaviour and the effects of air pollution (Hüls and Czamara, 2020, Chen et al., 2023, Hibler et al., 2019, Dick et al., 2014, Feil et al., 2023).

Overall, while the empirical findings of this thesis were not in line with the initial hypotheses *(Chapter 1, Section 1.2*), this work does represent a novel exploration of genetic and epigenetic risk factors for child development and mental health. Further, given the LMIC context of the DCHS, as well as its representation of African ancestry participants and its focus on early child development, this study is well poised in addressing this long-standing gap in the literature.

7.2. Noteworthy limitations

Limitations inherent to the systematic reviews

The process of study elimination (per the PRISMA guidelines, (Moher et al., 2009)) may have resulted in the omission of relevant studies. Further, the included studies had relatively small sample sizes - for example, none of the four studies included in the EA deviation systematic review had more than n=300 participants (Suarez et al., 2018a, Tollenaar et al., 2021, Cerveira de Baumont et al., 2021, Dammering et al., 2021). This may thus have affected the statistical power to detect significant effects within these studies. Both systematic reviews also had substantial study heterogeneity. Thus, a meta-analysis was not feasible for the first (PRS) review; and only two studies could be meta-analysed for the second (EA deviation) review. Thus, the robustness of the aforementioned associations remains to be confirmed.

Limitations inherent to the empirical analyses

First, as publicly available summary statistics are primarily of European ancestry (Martin et al., 2018, Sirugo et al., 2019, Duncan et al., 2019), the generalisability and transferability of findings – as well as the prediction power of PRS within the target ancestry (African and mixed ancestry participants) were limited (Majara et al., 2023).

Second, while the DCHS is a well-characterised birth cohort, the relatively modest sample size of this dataset (comprising genotype data for n=958 and epigenetic data for n=273 children) may have resulted in reduced statistical power to detect significant effects. Replication of this work with more well-powered target datasets will be key, particularly given the relatively small effect sizes of ADHD methylation differences (Neumann et al., 2020).

Third, missing DCHS data (e.g. for developmental outcomes on the Bayley III at 24 months) likely affected the sample size and statistical power of these analyses; as well as limiting potential longitudinal analyses of developmental trajectories. Additionally, the lack of a repeated-measures analysis for the internalising and externalising outcomes (despite the availability of select DCHS data at multiple time points) is a noteworthy limitation. While each time point was analysed separately using regression models, this approach may not have fully captured the longitudinal nature of these data. Thus, a repeated-measures framework could provide more comprehensive insights into the trajectory of these mental health outcomes over time.

Fourth, moderation analyses (to assess for potential moderating factors) were not undertaken, as this was beyond the scope of this doctoral project. The impact of environmental influences - such as maternal prenatal psychological distress and exposure to violence – on child development and mental health is well-documented (Aizer et al., 2016, Kamp et al., 2021, Ferguson et al., 2013). Incorporating moderating factors could provide a more nuanced understanding of the pathways through which these environmental stressors may impact developmental and mental health outcomes.

Finally, the utility of epigenetic clocks and MRS as potential risk biomarkers for adverse developmental and mental health outcomes in children is still not well understood (Dieckmann et al., 2021). Much of the available literature has been on adults, assessing biomedical outcomes such as risk for diabetes and BMI and allergy diseases (Hibler et al., 2019, Dick et al., 2014, Feil et al., 2023). Investigating epigenetic associations with development and mental health is also challenging, in part due to cross-tissue variability (Fraga et al., 2005, Laird, 2010, Alisch et al., 2012). For example, most prior work on EA has measured DNAm in saliva samples (Suarez et al., 2018a, Tollenaar et al., 2021, Cerveira de Baumont et al., 2021). In the DCHS, umbilical cord blood samples were used. Thus, Different tissues within the body have different DNAm and EA profiles (Zhang et al., 2013). DNAm profiles are also largely influenced by environmental exposures such as smoking) (Nabais et al., 2023); and are known to fluctuate over the lifespan (Alisch et al., 2012). Therefore, risk for

later neurodevelopmental and/or mental health disorders may not yet have been detectable in the DCHS umbilical cord samples and at the assessed timepoints (Thapar et al., 2016).

7.3. Future considerations

Future research efforts may benefit from addressing some of these limitations, as detailed below:

Inclusion and representation of ancestrally diverse study participants

Available and accessible summary statistics of participants of more diverse ancestry (including those of African ancestry) would enable researchers to capitalise on the LD structures from diverse population groups (which would be beneficial in fine-mapping causal variants) (Genovese et al., 2010). This could, in turn, enhance prediction capacity of PRS, epigenetic clocks as well as MRS; and improve the transferability of study findings across ancestral groups. Ultimately, improved inclusivity and diversity may also contribute to mitigating existing disparities in access to health care and delivery systems between HICs and LMICs (Whiteford et al., 2013, Bigdeli et al., 2019, Tekola-Ayele and Rotimi, 2015).

Increase in sample size

Future studies – with increased sample sizes (and increased power to detect significant effects) would be warranted. Additionally, combining multiple PRS would aid in improving predictive utility of these scores (Neumann et al.,2022). For example, work by Neumann and colleagues combining PRS of different mental health disorders (including ADHD) showed significant associations with internalising and externalising symptoms in children (Neumann et al.,2022). Moreover, it may be key to incorporate saliva derived DNAm data. Saliva is easily accessible and its suitability as a biomarker for mental health outcomes has previously been reported (Smith et al., 2014).

Assessment of longitudinal outcomes

In future, the use of longitudinal phenotype data could aid in providing a more accurate understanding of developmental trajectories and risk for mental health disorders. Prior research has shown that the developmental trajectories for internalising disorders in children is dynamic and heterogenous (Barker et al., 2019), moreover DNAm in response to environmental exposures influence developmental trajectories (including risk for psychopathology in children) (Barker et al., 2017). Therefore, longitudinal data with repeated measurements is key to understanding the dynamic and heterogeneity between DNAm and mental health outcomes in children.

Mediation and Moderating factors analyses

Future work, exploring mediation analyses in the context of DCHS is warranted. Prior research has reported that exposure to trauma (e.g. violence) associates with EAA in children, which in turn associates with depressive behaviours during adolescence (Sumner et al., 2019). Thus, including precursors and mediator/ or moderators may be beneficial in this context, given the high prenatal exposures to inter-partner violence reported by the mothers in the DCHS, exploring these effects on the children could be informative (Koen et al., 2014; Stein et al., 2015).

Systems biology approach

In future analyses in the DCHS, it may be worthwhile to combine both PRS and MRS. This approach has previously been used to investigate predictors of BMI and height in the Lothian Birth cohort participants (n=1366) (Shah, Bonder et al. 2015). This study reported that PRS and MRS explained 8% and 7%, respectively, of the variance in BMI, and 14% when fitted jointly. Almost no variation in height was observed (Shah, Bonder et al. 2015). These finding suggest that the contributions of PRS and MRS in this context were both independent and additive (Shah, Bonder et al. 2015). Additionally, incorporating additional biological measures such as transcriptomics and proteomics, may be beneficial in providing a more comprehensive and

nuanced understanding of the complex interplay between genetics, epigenetics, and environmental factors in shaping child development and mental health outcomes (Hasin et al.,2017).

7.4. Conclusion

To the best of my knowledge, this has been the first study to utilize a multi-omics approach to investigate associations between genetic and epigenetic risk factors for adverse developmental and mental health outcomes in African and admixed children. The integration of PRS, EA deviation measures from epigenetic clocks and MRS presents a potential avenue for early insights into children's susceptibility to developmental and mental health disorders. Future work in this field - with larger sample sizes and ancestry-matched summary statistics - is warranted to expand on this preliminary study, and to increase generalisability of findings. Such efforts may inform targeted early interventions for identifying children at-risk for poor outcomes, particularly in resource-limited settings such as South Africa - thus potentially improving trajectories and promoting long-term well-being.

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7.5. References

Alisch, r. S., barwick, b. G., chopra, p., myrick, l. K., satten, g. A., conneely, k. N. & warren, s. T. 2012. Age-associated dna methylation in pediatric populations. *Genome res,* 22**,** 623-32.

Aristizabal, m. J., anreiter, i., halldorsdottir, t., odgers, c. L., mcdade, t. W., goldenberg, a., mostafavi, s., kobor, m. S., binder, e. B., sokolowski, m. B. & o'donnell, k. J. 2020. Biological embedding of experience: a primer on epigenetics. *Proc natl acad sci u s a,* 117**,** 23261-23269.

Aryee, m. J., jaffe, a. E., corrada-bravo, h., ladd-acosta, c., feinberg, a. P., hansen, k. D. & irizarry, r. A. 2014. Minfi: a flexible and comprehensive bioconductor package for the analysis of infinium dna methylation microarrays. *Bioinformatics,* 30**,** 1363-9.

Askeland, r., hannigan, l., ask, h., tesli, m., corfield, e., andreassen, o. A., smith, g. D., reichborn-kjennerud, t. & havdahl, a. 2019. Early manifestations of genetic risk for neurodevelopmental disorders. *European neuropsychopharmacology,* 29**,** s252-s253.

Atilola, o. 2015. Level of community mental health literacy in sub-saharan africa: current studies are limited in number, scope, spread, and cognizance of cultural nuances. *Nord j psychiatry,* 69**,** 93-101.

Bagner, d. M., rodríguez, g. M., blake, c. A., linares, d. & carter, a. S. 2012. Assessment of behavioral and emotional problems in infancy: a systematic review. *Clin child fam psychol rev,* 15**,** 113-28.

Barbarin, o. A., richter, l. & dewet, t. 2001. Exposure to violence, coping resources, and psychological adjustment of south african children. *American journal of orthopsychiatry,* 71**,** 16-25.

Barbu, m. C., amador, c., kwong, a. S. F., shen, x., adams, m. J., howard, d. M., walker, r. M., morris, s. W., min, j. L., liu, c., van dongen, j., ghanbari, m., relton, c., porteous, d. J., campbell, a., evans, k. L., whalley, h. C. & mcintosh, a. M. 2022. Complex trait methylation scores in the prediction of major depressive disorder. *Ebiomedicine,* 79**,** 104000.

Barican, j. L., yung, d., schwartz, c., zheng, y., georgiades, k. & waddell, c. 2022. Prevalence of childhood mental disorders in high-income countries: a systematic review and meta-analysis to inform policymaking. *Evid based ment health,* 25**,** 36-44.

Barker, e. D. 2018. Epigenetics, early adversity and child and adolescent mental health. *Psychopathology,* 51**,** 71-75.

Barker, e. D., walton, e. & cecil, c. A. M. 2018. Annual research review: dna methylation as a mediator in the association between risk exposure and child and adolescent psychopathology. *J child psychol psychiatry,* 59**,** 303-322.

Barkley, r. A., o'connell, t., murphy, k. R., anderson, d. & connor, d. F. 2006. Effects of two doses of alcohol on simulator driving performance in adults with attention-deficit/hyperactivity disorder. *Neuropsychology,* 20**,** 77-87.

Beijers, r., jansen, j., riksen-walraven, m. & de weerth, c. 2010. Maternal prenatal anxiety and stress predict infant illnesses and health complaints. *Pediatrics,* 126**,** e401-9.

Bell, c. G., lowe, r., adams, p. D., baccarelli, a. A., beck, s., bell, j. T., christensen, b. C., gladyshev, v. N., heijmans, b. T., horvath, s., ideker, t., issa, j. P. J., kelsey, k. T., marioni, r. E., reik, w., relton, c. L., schalkwyk, l. C., teschendorff, a. E., wagner, w., zhang, k. & rakyan, v. K. 2019. Dna methylation aging clocks: challenges and recommendations. Biomed central ltd.

Biederman, j., green, a., disalvo, m. & faraone, s. V. 2021. Can polygenic risk scores help identify pediatric bipolar spectrum and related disorders?: a systematic review. *Psychiatry res,* 299**,** 113843.

Bigdeli, t. B., genovese, g., georgakopoulos, p., meyers, j. L., peterson, r. E., iyegbe, c. O., medeiros, h., valderrama, j., achtyes, e. D., kotov, r., stahl, e. A., abbott, c., azevedo, m. H., belliveau, r. A., bevilacqua, e., bromet, e. J., byerley, w., carvalho, c. B., chapman, s. B., delisi, l. E., dumont, a. L., o'dushlaine, c., evgrafov, o. V., fochtmann, l. J., gage, d., kennedy, j. L., kinkead, b., macedo, a., moran, j. L., morley, c. P., dewan, m. J., nemesh, j., perkins, d. O., purcell, s. M., rakofsky, j. J., scolnick, e. M., sklar, b. M., sklar, p., smoller, j. W., sullivan, p. F., macciardi, f., marder, s. R., gur, r. C., gur, r. E., braff, d. L., calkins, m. E., freedman, r. R., green, m. F., greenwood, t. A., lazzeroni, l. C.,

light, g. A., nuechterlein, k. H., radant, a. D., seidman, l. J., siever, l. J., silverman, j. M., stone, w. S., sugar, c. A., swerdlow, n. R., tsuang, d. W., tsuang, m. T., turetsky, b. I., nicolini, h., escamilla, m. A., vawter, m. P., sobell, j. L., malaspina, d., lehrer, d. S., buckley, p. F., rapaport, m. H., knowles, j. A., fanous, a. H., pato, m. T., mccarroll, s. A. & pato, c. N. 2019. Contributions of common genetic variants to risk of schizophrenia among individuals of african and latino ancestry. *Molecular psychiatry***,** 1-13.

Black, m. M., walker, s. P., fernald, l. C. H., andersen, c. T., digirolamo, a. M., lu, c., mccoy, d. C., fink, g., shawar, y. R., shiffman, j., devercelli, a. E., wodon, q. T., vargas-barón, e. & grantham-mcgregor, s. 2017. Early childhood development coming of age: science through the life course. *Lancet,* 389**,** 77-90.

Bolhuis, k., steenkamp, l. R., blanken, l. M. E., neumann, a., jansen, p. R., hillegers, m. H. J., cecil, c. A. M., tiemeier, h. & kushner, s. A. 2022. Schizophrenia polygenic risk is associated with child mental health problems through early childhood adversity: evidence for a gene–environment correlation. *European child & adolescent psychiatry,* 31**,** 529-539.

Breeze, c. E., batorsky, a., lee, m. K., szeto, m. D., xu, x., mccartney, d. L., jiang, r., patki, a., kramer, h. J., eales, j. M., raffield, l., lange, l., lange, e., durda, p., liu, y., tracy, r. P., van den berg, d., evans, k. L., kraus, w. E., shah, s., tiwari, h. K., hou, l., whitsel, e. A., jiang, x., charchar, f. J., baccarelli, a. A., rich, s. S., morris, a. P., irvin, m. R., arnett, d. K., hauser, e. R., rotter, j. I., correa, a., hayward, c., horvath, s., marioni, r. E., tomaszewski, m., beck, s., berndt, s. I., london, s. J., mychaleckyj, j. C. & franceschini, n. 2021. Epigenome-wide association study of kidney function identifies trans-ethnic and ethnic-specific loci. *Genome med,* 13**,** 74.

Breeze, c. E., paul, d. S., van dongen, j., butcher, l. M., ambrose, j. C., barrett, j. E., lowe, r., rakyan, v. K., iotchkova, v., frontini, m., downes, k., ouwehand, w. H., laperle, j., jacques, p., bourque, g., bergmann, a. K., siebert, r., vellenga, e., saeed, s., matarese, f., martens, j. H. A., stunnenberg, h. G., teschendorff, a. E., herrero, j., birney, e., dunham, i. & beck, s. 2016. Eforge: a tool for identifying cell type-specific signal in epigenomic data. *Cell rep,* 17**,** 2137-2150.

Breeze, c. E., reynolds, a. P., van dongen, j., dunham, i., lazar, j., neph, s., vierstra, j., bourque, g., teschendorff, a. E., stamatoyannopoulos, j. A. & beck, s. 2019. Eforge v2.0: updated analysis of cell type-specific signal in epigenomic data. *Bioinformatics,* 35**,** 4767-4769.

Breeze, c. E., wong, j. Y. Y., beck, s., berndt, s. I. & franceschini, n. 2022. Diversity in ewas: current state, challenges, and solutions. *Genome med,* 14**,** 71.

Brikell, i., burton, c., mota, n. R. & martin, j. 2021. Insights into attention-deficit/hyperactivity disorder from recent genetic studies. *Psychological medicine*.

Brikell, i., kuja-halkola, r. & larsson, h. 2015. Heritability of attention-deficit hyperactivity disorder in adults. *Am j med genet b neuropsychiatr genet,* 168**,** 406-413.

Brikell, i., larsson, h., lu, y., pettersson, e., chen, q., kuja-halkola, r., karlsson, r., lahey, b. B., lichtenstein, p. & martin, j. 2020. The contribution of common genetic risk variants for adhd to a general factor of childhood psychopathology. *Molecular psychiatry,* 25**,** 1809-1821.

Bulik-sullivan, b., finucane, h. K., anttila, v., gusev, a., day, f. R., loh, p. R., duncan, l., perry, j. R., patterson, n., robinson, e. B., daly, m. J., price, a. L. & neale, b. M. 2015. An atlas of genetic correlations across human diseases and traits. *Nat genet,* 47**,** 1236-41.

Cannon, m., caspi, a., moffitt, t. E., harrington, h., taylor, a., murray, r. M. & poulton, r. 2002. Evidence for early-childhood, pan-developmental impairment specific to schizophreniform disorder: results from a longitudinal birth cohort. *Arch gen psychiatry,* 59**,** 449-56.

Caramaschi, d., neumann, a., cardenas, a., tindula, g., alemany, s., zillich, l., pesce, g., lahti, j. M. T., havdahl, a., mulder, r., felix, j. F., tiemeier, h., sirignano, l., frank, j., witt, s. H., rietschel, m., deuschle, m., huen, k., eskenazi, b., send, t. S., ferrer, m., gilles, m., de agostini, m., baïz, n., rifas-shiman, s. L., kvist, t., czamara, d., tuominen, s. T., relton, c. L., rai, d., london, s. J., räikkönen, k., holland, n., annesi-maesano, i., streit, f., hivert, m. F., oken, e., sunyer, j., cecil, c. A. M. & sharp, g.

2022. Meta-analysis of epigenome-wide associations between dna methylation at birth and childhood cognitive skills. *Molecular psychiatry 2022 27:4,* 27**,** 2126-2135.

Cavalli, g. & heard, e. 2019. Advances in epigenetics link genetics to the environment and disease. *Nature,* 571**,** 489-499.

Cavazos, t. B. & witte, j. S. 2020. Inclusion of variants discovered from diverse populations improves polygenic risk score transferability. Biorxiv.

Cecil, c. A. M. & nigg, j. T. 2022. Epigenetics and adhd: reflections on current knowledge, research priorities and translational potential. *Mol diagn ther,* 26**,** 581-606.

Cerveira de baumont, a., hoffmann, m. S., bortoluzzi, a., fries, g. R., lavandoski, p., grun, l. K., guimarães, l. S. P., guma, f., salum, g. A., barbé-tuana, f. M. & manfro, g. G. 2021. Telomere length and epigenetic age acceleration in adolescents with anxiety disorders. *Sci rep,* 11**,** 7716.

Chen, j., gatev, e., everson, t., conneely, k. N., koen, n., epstein, m. P., kobor, m. S., zar, h. J., stein, d. J. & hüls, a. 2023. Pruning and thresholding approach for methylation risk scores in multi-ancestry populations. *Epigenetics,* 18**,** 2187172.

Chen, w., zhou, k., sham, p., franke, b., kuntsi, j., campbell, d., fleischman, k., knight, j., andreou, p., arnold, r., altink, m., boer, f., boholst, m. J., buschgens, c., butler, l., christiansen, h., fliers, e., howe-forbes, r., gabriëls, i., heise, a., korn-lubetzki, i., marco, r., medad, s., minderaa, r., müller, u. C., mulligan, a., psychogiou, l., rommelse, n., sethna, v., uebel, h., mcguffin, p., plomin, r., banaschewski, t., buitelaar, j., ebstein, r., eisenberg, j., gill, m., manor, i., miranda, a., mulas, f., oades, r. D., roeyers, h., rothenberger, a., sergeant, j., sonuga-barke, e., steinhausen, h. C., taylor, e., thompson, m., faraone, s. V. & asherson, p. 2008. Dsm-iv combined type adhd shows familial association with sibling trait scores: a sampling strategy for qtl linkage. *Am j med genet b neuropsychiatr genet,* 147b**,** 1450-60.

Cherkasova, m., sulla, e. M., dalena, k. L., pondé, m. P. & hechtman, l. 2013. Developmental course of attention deficit hyperactivity disorder and its predictors. *J can acad child adolesc psychiatry,* 22**,** 47-54.

Choi, s. W., mak, t. S. H. & o'reilly, p. F. 2020. Tutorial: a guide to performing polygenic risk score analyses. *Nature protocols.* Nature research.

Christensen, g. M., marcus, m., vanker, a., eick, s. M., malcolm-smith, s., smith, a., dunn, e. C., suglia, s. F., chang, h. H., zar, h. J., stein, d. J. & hüls, a. 2023. Sensitive periods for exposure to indoor air pollutants and psychosocial factors in association with symptoms of psychopathology at school-age in a south african birth cohort. *Medrxiv*.

Christensen, g. M., rowcliffe, c., chen, j., vanker, a., koen, n., jones, m. J., gladish, n., hoffman, n., donald, k. A., wedderburn, c. J., kobor, m. S., zar, h. J., stein, d. J. & hüls, a. 2022. In-utero exposure to indoor air pollution or tobacco smoke and cognitive development in a south african birth cohort study. *Sci total environ,* 834**,** 155394.

Christianson, a. L., zwane, m. E., manga, p., rosen, e., venter, a., downs, d. & kromberg, j. G. 2002. Children with intellectual disability in rural south africa: prevalence and associated disability. *J intellect disabil res,* 46**,** 179-86.

Coleman, j. R. I., gaspar, h. A., bryois, j. & breen, g. 2020. The genetics of the mood disorder spectrum: genome-wide association analyses of more than 185,000 cases and 439,000 controls. *Biol psychiatry,* 88**,** 169-184.

Copeland, w. E., shanahan, l., erkanli, a., costello, e. J. & angold, a. 2013. Indirect comorbidity in childhood and adolescence. *Front psychiatry,* 4**,** 144.

Copeland, w. E., worthman, c., shanahan, l., costello, e. J. & angold, a. 2019. Early pubertal timing and testosterone associated with higher levels of adolescent depression in girls. *J am acad child adolesc psychiatry,* 58**,** 1197-1206.

Cruickshank, m. N., oshlack, a., theda, c., davis, p. G., martino, d., sheehan, p., dai, y., saffery, r., doyle, l. W. & craig, j. M. 2013. Analysis of epigenetic changes in survivors of preterm birth reveals the effect of gestational age and evidence for a long term legacy. *Genome med,* 5**,** 96.

Czamara, d., eraslan, g., page, c. M., lahti, j., lahti-pulkkinen, m., hämäläinen, e., kajantie, e., laivuori, h., villa, p. M., reynolds, r. M., nystad, w., håberg, s. E., london, s. J., o'donnell, k. J., garg, e., meaney, m. J., entringer, s., wadhwa, p. D., buss, c., jones, m. J., lin, d. T. S., macisaac, j. L., kobor, m. S., koen, n., zar, h. J., koenen, k. C., dalvie, s., stein, d. J., kondofersky, i., müller, n. S., theis, f. J., räikkönen, k. & binder, e. B. 2019. Integrated analysis of environmental and genetic influences on cord blood dna methylation in new-borns. *Nat commun,* 10**,** 2548.

Dammering, f., martins, j., dittrich, k., czamara, d., rex-haffner, m., overfeld, j., de punder, k., buss, c., entringer, s., winter, s. M., binder, e. B. & heim, c. 2021. The pediatric buccal epigenetic clock identifies significant ageing acceleration in children with internalizing disorder and maltreatment exposure. *Neurobiology of stress,* 15.

Das, s., forer, l., schönherr, s., sidore, c., locke, a. E., kwong, a., vrieze, s. I., chew, e. Y., levy, s., mcgue, m., schlessinger, d., stambolian, d., loh, p. R., iacono, w. G., swaroop, a., scott, l. J., cucca, f., kronenberg, f., boehnke, m., abecasis, g. R. & fuchsberger, c. 2016. Next-generation genotype imputation service and methods. *Nat genet,* 48**,** 1284-1287.

Demontis, d., walters, g. B., athanasiadis, g., walters, r., therrien, k., nielsen, t. T., farajzadeh, l., voloudakis, g., bendl, j., zeng, b., zhang, w., grove, j., als, t. D., duan, j., satterstrom, f. K., bybjerg-grauholm, j., bækved-hansen, m., gudmundsson, o. O., magnusson, s. H., baldursson, g., davidsdottir, k., haraldsdottir, g. S., agerbo, e., hoffman, g. E., dalsgaard, s., martin, j., ribasés, m., boomsma, d. I., soler artigas, m., roth mota, n., howrigan, d., medland, s. E., zayats, t., rajagopal, v. M., havdahl, a., doyle, a., reif, a., thapar, a., cormand, b., liao, c., burton, c., bau, c. H. D., rovaris, d. L., sonuga-barke, e., corfield, e., grevet, e. H., larsson, h., gizer, i. R., waldman, i., brikell, i., haavik, j., crosbie, j., mcgough, j., kuntsi, j., glessner, j., langley, k., lesch, k.-p., rohde, l. A., hutz, m. H., klein, m., bellgrove, m., tesli, m., o'donovan, m. C., andreassen, o. A., leung, p. W. L., pan, p. M., joober, r., schachar, r., loo, s., witt, s. H., reichborn-kjennerud, t., banaschewski, t., hawi, z., daly, m. J., mors, o., nordentoft, m., mors, o., hougaard, d. M., mortensen, p. B., daly, m. J., faraone, s. V., stefansson, h., roussos, p., franke, b., werge, t., neale, b. M., stefansson, k., børglum, a. D., consortium, a. W. G. O. T. P. G. & i, p.-b. C. 2023. Genome-wide analyses of adhd identify 27 risk loci, refine the genetic architecture and implicate several cognitive domains. *Nature genetics,* 55**,** 198-208.

Dick, d. M., barr, p. B., cho, s. B., cooke, m. E., kuo, s. I. C., lewis, t. J., neale, z., salvatore, j. E., savage, j. & su, j. 2018. Post-gwas in psychiatric genetics: a developmental perspective on the "other" next steps. *Genes, brain and behavior,* 17.

Dick, k. J., nelson, c. P., tsaprouni, l., sandling, j. K., aïssi, d., wahl, s., meduri, e., morange, p. E., gagnon, f., grallert, h., waldenberger, m., peters, a., erdmann, j., hengstenberg, c., cambien, f., goodall, a. H., ouwehand, w. H., schunkert, h., thompson, j. R., spector, t. D., gieger, c., trégouët, d. A., deloukas, p. & samani, n. J. 2014. Dna methylation and body-mass index: a genome-wide analysis. *Lancet,* 383**,** 1990-8.

Dieckmann, l., lahti-pulkkinen, m., kvist, t., lahti, j., dewitt, p. E., cruceanu, c., laivuori, h., sammallahti, s., villa, p. M., suomalainen-könig, s., eriksson, j. G., kajantie, e., raikkönen, k., binder, e. B. & czamara, d. 2021. Characteristics of epigenetic aging across gestational and perinatal tissues. *Clinical epigenetics,* 13**,** 97.

Donald, k. A., hoogenhout, m., du plooy, c. P., wedderburn, c. J., nhapi, r. T., barnett, w., hoffman, n., malcolm-smith, s., zar, h. J. & stein, d. J. 2018a. Drakenstein child health study (dchs): investigating determinants of early child development and cognition. *Bmj paediatr open,* 2**,** e000282.

Donald, k. A., wedderburn, c. J., barnett, w., nhapi, r. T., rehman, a. M., stadler, j. A. M., hoffman, n., koen, n., zar, h. J. & stein, d. J. 2019. Risk and protective factors for child development: an observational south african birth cohort. *Plos med,* 16**,** e1002920.

Du, p., kibbe, w. A. & lin, s. M. 2008. Lumi: a pipeline for processing illumina microarray. *Bioinformatics,* 24**,** 1547-8.

Duncan, l., shen, h., gelaye, b., meijsen, j., ressler, k., feldman, m., peterson, r. & domingue, b. 2019. Analysis of polygenic risk score usage and performance in diverse human populations. *Nature communications 2019 10:1,* 10**,** 1-9.

Edenberg, h. J., bierut, l. J., boyce, p., cao, m., cawley, s., chiles, r., doheny, k. F., hansen, m., hinrichs, t., jones, k., kelleher, m., kennedy, g. C., liu, g., marcus, g., mcbride, c., murray, s. S., oliphant, a., pettengill, j., porjesz, b., pugh, e. W., rice, j. P., rubano, t., shannon, s., steeke, r., tischfield, j. A., tsai, y. Y., zhang, c. & begleiter, h. 2005. Description of the data from the collaborative study on the genetics of alcoholism (coga) and single-nucleotidepolymorphism genotyping for genetic analysis workshop 14. *Bmc genetics,* 6**,** 1-8.

Edvardson, s., baumann, a. M., mühlenhoff, m., stephan, o., kuss, a. W., shaag, a., he, l., zenvirt, s., tanzi, r., gerardy-schahn, r. & elpeleg, o. 2013. West syndrome caused by st3gal-iii deficiency. *Epilepsia,* 54**,** e24-7.

Egger, h. L. & angold, a. 2006. Common emotional and behavioral disorders in preschool children: presentation, nosology, and epidemiology. *Journal of child psychology and psychiatry,* 47**,** 313-337.

Eze, i. C., imboden, m., kumar, a., von eckardstein, a., stolz, d., gerbase, m. W., künzli, n., pons, m., kronenberg, f., schindler, c. & probst-hensch, n. 2016. Air pollution and diabetes association: modification by type 2 diabetes genetic risk score. *Environ int,* 94**,** 263-271.

Faleschini, s., rifas-shiman, s. L., tiemeier, h., oken, e. & hivert, m. F. 2019. Associations of prenatal and postnatal maternal depressive symptoms with offspring cognition and behavior in mid-childhood: a prospective cohort study. *Int j environ res public health,* 16.

Feder, k. P. & majnemer, a. 2007. Handwriting development, competency, and intervention. *Developmental medicine & child neurology,* 49**,** 312-317.

Feil, d., abrishamcar, s., christensen, g. M., vanker, a., koen, n., kilanowski, a., hoffman, n., wedderburn, c. J., donald, k. A., kobor, m. S., zar, h. J., stein, d. J. & hüls, a. 2023. Dna methylation as a potential mediator of the association between indoor air pollution and neurodevelopmental delay in a south african birth cohort. *Clin epigenetics,* 15**,** 31.

Fieggen, k. J., lambie, l. A. & donald, k. A. 2019. *Investigating developmental delay in south africa: a pragmatic approach*.

Fortin, j. P., labbe, a., lemire, m., zanke, b. W., hudson, t. J., fertig, e. J., greenwood, c. M. & hansen, k. D. 2014. Functional normalization of 450k methylation array data improves replication in large cancer studies. *Genome biol,* 15**,** 503.

Fraga, m. F., ballestar, e., paz, m. F., ropero, s., setien, f., ballestar, m. L., heine-suñer, d., cigudosa, j. C., urioste, m., benitez, j., boix-chornet, m., sanchez-aguilera, a., ling, c., carlsson, e., poulsen, p., vaag, a., stephan, z., spector, t. D., wu, y. Z., plass, c. & esteller, m. 2005. Epigenetic differences arise during the lifetime of monozygotic twins. *Proc natl acad sci u s a,* 102**,** 10604-9.

Franke, b., van hulzen, k. J. E., arias-vasquez, a., bralten, j., hoogman, m., klein, m., van donkelaar, m. M. J., hakobjan, m. M. H., heister, a. J. G. A. M., makkinje, r. R. R., naber, m. A. M., van der marel, s. S. L., mostert, j. C., brunner, h. G., van bokhoven, h., zwiers, m. P., buitelaar, j. K., fernández, g., fisher, s. E., francks, c., stein, j. L., hibar, d. P., thompson, p. M., ripke, s., anttila, v., neale, b. M., farh, k. H., bulik-sullivan, b., huang, h., fromer, m., goldstein, j. I., daly, m. J., walters, r. K., smoller, j. W., lee, p., belliveau, r. A., bergen, s. E., bevilacqua, e., chambert, k. D., genovese, g., o'dushlaine, c., scolnick, e. M., mccarroll, s. A., moran, j. L., palotie, a., petryshen, t. L., erk, s., heinz, a., mohnke, s., romanczuk-seiferth, n., walter, h., delisi, l. E., mccarley, r. W., mesholam-gately, r. I., seidman, l. J., nichols, t. E., neale, m. C., mcintosh, a. M., papmeyer, m., sprooten, e., lawrie, s. M., sussmann, j. E., mcmahon, f. J., yao, y., meyer-lindenberg, a., schwarz, e., grimm, o., mattheisen, m., agerbo, e., demontis, d., hansen, t., mors, o., olsen, l., rasmussen, h. B., børglum, a. D., mortensen, p. B., werge, t., andreassen, o. A., brown, a. A., athanasiu, l., hartberg, c. B., haukvik, u., melle, i., gruber, o., kraemer, b., keil, m., sachdev, p. S., mather, k. A., thalamuthu, a., armstrong, n. J., assareh, a. A.,

brodaty, h., reppermund, s., wen, w., roiz-santiañez, r., perez-iglesias, r., saykin, a. J., kim, s., nho, k., risacher, s. L., et al. 2016. Genetic influences on schizophrenia and subcortical brain volumes: large-scale proof of concept. *Nature neuroscience,* 19**,** 420-431.

Fransquet, p. D., wrigglesworth, j., woods, r. L., ernst, m. E. & ryan, j. 2019. The epigenetic clock as a predictor of disease and mortality risk: a systematic review and meta-analysis. Biomed central ltd.

Fraser, a., macdonald-wallis, c., tilling, k., boyd, a., golding, j., davey smith, g., henderson, j., macleod, j., molloy, l., ness, a., ring, s., nelson, s. M. & lawlor, d. A. 2013. Cohort profile: the avon longitudinal study of parents and children: alspac mothers cohort. *Int j epidemiol,* 42**,** 97-110.

Fries, g. R., bauer, i. E., scaini, g., wu, m. J., kazimi, i. F., valvassori, s. S., zunta-soares, g., walss-bass, c., soares, j. C. & quevedo, j. 2017. Accelerated epigenetic aging and mitochondrial dna copy number in bipolar disorder. *Transl psychiatry,* 7**,** 1283.

Ge, t., chen, c. Y., ni, y., feng, y. A. & smoller, j. W. 2019. Polygenic prediction via bayesian regression and continuous shrinkage priors. *Nat commun,* 10**,** 1776.

Genovese, g., friedman, d. J., ross, m. D., lecordier, l., uzureau, p., freedman, b. I., bowden, d. W., langefeld, c. D., oleksyk, t. K., uscinski knob, a. L., bernhardy, a. J., hicks, p. J., nelson, g. W., vanhollebeke, b., winkler, c. A., kopp, j. B., pays, e. & pollak, m. R. 2010. Association of trypanolytic apol1 variants with kidney disease in african americans. *Science,* 329**,** 841-5.

Germine, l., robinson, e. B., smoller, j. W., calkins, m. E., moore, t. M., hakonarson, h., daly, m. J., lee, p. H., holmes, a. J., buckner, r. L., gur, r. C. & gur, r. E. 2016. Association between polygenic risk for schizophrenia, neurocognition and social cognition across development. *Translational psychiatry,* 6**,** e924-e924.

Gervin, k., salas, l. A., bakulski, k. M., van zelm, m. C., koestler, d. C., wiencke, j. K., duijts, l., moll, h. A., kelsey, k. T., kobor, m. S., lyle, r., christensen, b. C., felix, j. F. & jones, m. J. 2019. Systematic evaluation and validation of reference and library selection methods for deconvolution of cord blood dna methylation data. *Clin epigenetics,* 11**,** 125.

Girchenko, p., lahti, j., czamara, d., knight, a. K., jones, m. J., suarez, a., hämäläinen, e., kajantie, e., laivuori, h., villa, p. M., reynolds, r. M., kobor, m. S., smith, a. K., binder, e. B. & räikkönen, k. 2017. Associations between maternal risk factors of adverse pregnancy and birth outcomes and the offspring epigenetic clock of gestational age at birth. *Clin epigenetics,* 9**,** 49.

Hamshere, m. L., stergiakouli, e., langley, k., martin, j., holmans, p., kent, l., owen, m. J., gill, m., thapar, a., o'donovan, m. & craddock, n. 2013. Shared polygenic contribution between childhood attention-deficit hyperactivity disorder and adult schizophrenia. *Br j psychiatry,* 203**,** 107-11.

Han, l. K. M., aghajani, m., clark, s. L., chan, r. F., hattab, m. W., shabalin, a. A., zhao, m., kumar, g., xie, l. Y., jansen, r., milaneschi, y., dean, b., aberg, k. A., van den oord, e. & penninx, b. 2018. Epigenetic aging in major depressive disorder. *Am j psychiatry,* 175**,** 774-782.

Hannon, e., dempster, e., viana, j., burrage, j., smith, a. R., macdonald, r., st clair, d., mustard, c., breen, g., therman, s., kaprio, j., toulopoulou, t., hulshoff pol, h. E., bohlken, m. M., kahn, r. S., nenadic, i., hultman, c. M., murray, r. M., collier, d. A., bass, n., gurling, h., mcquillin, a., schalkwyk, l. & mill, j. 2016. An integrated genetic-epigenetic analysis of schizophrenia: evidence for co-localization of genetic associations and differential dna methylation. *Genome biol,* 17**,** 176.

Hannon, e., schendel, d., ladd-acosta, c., grove, j., hansen, c. S., andrews, s. V., hougaard, d. M., bresnahan, m., mors, o., hollegaard, m. V., bækvad-hansen, m., hornig, m., mortensen, p. B., børglum, a. D., werge, t., pedersen, m. G., nordentoft, m., buxbaum, j., daniele fallin, m., bybjerg-grauholm, j., reichenberg, a. & mill, j. 2018. Elevated polygenic burden for autism is associated with differential dna methylation at birth. *Genome med,* 10**,** 19.

Hannum, g., guinney, j., zhao, l., zhang, l., hughes, g., sadda, s. V., klotzle, b., bibikova, m., fan, j. B., gao, y., deconde, r., chen, m., rajapakse, i., friend, s., ideker, t. & zhang, k. 2013. Genome-wide methylation profiles reveal quantitative views of human aging rates. *Molecular cell,* 49**,** 359-367.

Heim, c. 2017. Immediate biological embedding of maltreatment in children: berlin longitudinal children study (berlinlcs). *Psychoneuroendocrinology,* 83**,** 7.

Heyn, h., li, n., ferreira, h. J., moran, s., pisano, d. G., gomez, a., diez, j., sanchez-mut, j. V., setien, f., carmona, f. J., puca, a. A., sayols, s., pujana, m. A., serra-musach, j., iglesias-platas, i., formiga, f., fernandez, a. F., fraga, m. F., heath, s. C., valencia, a., gut, i. G., wang, j. & esteller, m. 2012. Distinct dna methylomes of newborns and centenarians. *Proc natl acad sci u s a,* 109**,** 10522-7.

Hibler, e., huang, l., andrade, j. & spring, b. 2019. Impact of a diet and activity health promotion intervention on regional patterns of dna methylation. *Clin epigenetics,* 11**,** 133.

Higgins-chen, a. T., boks, m. P., vinkers, c. H., kahn, r. S. & levine, m. E. 2020. Schizophrenia and epigenetic aging biomarkers: increased mortality, reduced cancer risk, and unique clozapine effects. *Biol psychiatry,* 88**,** 224-235.

Horvath, s. 2013. Dna methylation age of human tissues and cell types. *Genome biology,* 14.

Horvath, s., pirazzini, c., bacalini, m. G., gentilini, d., di blasio, a. M., delledonne, m., mari, d., arosio, b., monti, d., passarino, g., de rango, f., d'aquila, p., giuliani, c., marasco, e., collino, s., descombes, p., garagnani, p. & franceschi, c. 2015. Decreased epigenetic age of pbmcs from italian semi-supercentenarians and their offspring. *Aging (albany ny),* 7**,** 1159-70.

Horvath, s. & raj, k. 2018. Dna methylation-based biomarkers and the epigenetic clock theory of ageing. *Nat rev genet,* 19**,** 371-384.

Hu, h., eggers, k., chen, w., garshasbi, m., motazacker, m. M., wrogemann, k., kahrizi, k., tzschach, a., hosseini, m., bahman, i., hucho, t., mühlenhoff, m., gerardy-schahn, r., najmabadi, h., ropers, h. H. & kuss, a. W. 2011. St3gal3 mutations impair the development of higher cognitive functions. *Am j hum genet,* 89**,** 407-14.

Hüls, a. & czamara, d. 2020. Methodological challenges in constructing dna methylation risk scores. Taylor and francis inc.

Humeniuk, r., ali, r., babor, t. F., farrell, m., formigoni, m. L., jittiwutikarn, j., de lacerda, r. B., ling, w., marsden, j., monteiro, m., nhiwatiwa, s., pal, h., poznyak, v. & simon, s. 2008. Validation of the alcohol, smoking and substance involvement screening test (assist). *Addiction,* 103**,** 1039-1047.

Jaenisch, r. & bird, a. 2003. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. *Nat genet,* 33 suppl**,** 245-54.

Jansen, a. G., dieleman, g. C., jansen, p. R., verhulst, f. C., posthuma, d. & polderman, t. J. C. 2020. Psychiatric polygenic risk scores as predictor for attention deficit/hyperactivity disorder and autism spectrum disorder in a clinical child and adolescent sample. *Behav genet,* 50**,** 203-212.

Jansen, a. G., jansen, p. R., posthuma, d., dieleman, g. C. & polderman, t. 2018. Associating psychiatric polygenic risk scores with neurodevelopmental disorders in a clinical child and adolescent sample. *Behavior genetics,* 48**,** 481-481.

Joehanes, r., just, a. C., marioni, r. E., pilling, l. C., reynolds, l. M., mandaviya, p. R., guan, w., xu, t., elks, c. E., aslibekyan, s., moreno-macias, h., smith, j. A., brody, j. A., dhingra, r., yousefi, p., pankow, j. S., kunze, s., shah, s. H., mcrae, a. F., lohman, k., sha, j., absher, d. M., ferrucci, l., zhao, w., demerath, e. W., bressler, j., grove, m. L., huan, t., liu, c., mendelson, m. M., yao, c., kiel, d. P., peters, a., wang-sattler, r., visscher, p. M., wray, n. R., starr, j. M., ding, j., rodriguez, c. J., wareham, n. J., irvin, m. R., zhi, d., barrdahl, m., vineis, p., ambatipudi, s., uitterlinden, a. G., hofman, a., schwartz, j., colicino, e., hou, l., vokonas, p. S., hernandez, d. G., singleton, a. B., bandinelli, s., turner, s. T., ware, e. B., smith, a. K., klengel, t., binder, e. B., psaty, b. M., taylor, k. D., gharib, s. A., swenson, b. R., liang, l., demeo, d. L., o'connor, g. T., herceg, z., ressler, k. J., conneely, k. N., sotoodehnia, n., kardia, s. L., melzer, d., baccarelli, a. A., van meurs, j. B., romieu, i., arnett, d. K., ong, k. K., liu, y., waldenberger, m., deary, i. J., fornage, m., levy, d. & london, s. J. 2016. Epigenetic signatures of cigarette smoking. *Circ cardiovasc genet,* 9**,** 436-447.

Jung, s. E., shin, k. J. & lee, h. Y. 2017. Dna methylation-based age prediction from various tissues and body fluids. *Bmb rep,* 50**,** 546-553.

Kaufman, j., birmaher, b., brent, d., rao, u., flynn, c., moreci, p., williamson, d. & ryan, n. 1997. Schedule for affective disorders and schizophrenia for school-age children-present and lifetime version (k-sads-pl): initial reliability and validity data. *J am acad child adolesc psychiatry,* 36**,** 980-8.

Kessler, r. C., angermeyer, m., anthony, j. C., r, d. E. G., demyttenaere, k., gasquet, i., g, d. E. G., gluzman, s., gureje, o., haro, j. M., kawakami, n., karam, a., levinson, d., medina mora, m. E., oakley browne, m. A., posada-villa, j., stein, d. J., adley tsang, c. H., aguilar-gaxiola, s., alonso, j., lee, s., heeringa, s., pennell, b. E., berglund, p., gruber, m. J., petukhova, m., chatterji, s. & ustün, t. B. 2007. Lifetime prevalence and age-of-onset distributions of mental disorders in the world health organization's world mental health survey initiative. *World psychiatry,* 6**,** 168-76.

Kieling, c., baker-henningham, h., belfer, m., conti, g., ertem, i., omigbodun, o., rohde, l. A., srinath, s., ulkuer, n. & rahman, a. 2011. Child and adolescent mental health worldwide: evidence for action. *Lancet,* 378**,** 1515-25.

Kim, j. H., kim, j. Y., lee, j., jeong, g. H., lee, e., lee, s., lee, k. H., kronbichler, a., stubbs, b., solmi, m., koyanagi, a., hong, s. H., dragioti, e., jacob, l., brunoni, a. R., carvalho, a. F., radua, j., thompson, t., smith, l., oh, h., yang, l., grabovac, i., schuch, f., fornaro, m., stickley, a., rais, t. B., salazar de pablo, g., shin, j. I. & fusar-poli, p. 2020. Environmental risk factors, protective factors, and peripheral biomarkers for adhd: an umbrella review. *Lancet psychiatry,* 7**,** 955-970.

Knight, a. K., craig, j. M., theda, c., bækvad-hansen, m., bybjerg-grauholm, j., hansen, c. S., hollegaard, m. V., hougaard, d. M., mortensen, p. B., weinsheimer, s. M., werge, t. M., brennan, p. A., cubells, j. F., newport, d. J., stowe, z. N., cheong, j. L. Y., dalach, p., doyle, l. W., loke, y. J., baccarelli, a. A., just, a. C., wright, r. O., téllez-rojo, m. M., svensson, k., trevisi, l., kennedy, e. M., binder, e. B., iurato, s., czamara, d., räikkönen, k., lahti, j. M. T., pesonen, a. K., kajantie, e., villa, p. M., laivuori, h., hämäläinen, e., park, h. J., bailey, l. B., parets, s. E., kilaru, v., menon, r., horvath, s., bush, n. R., lewinn, k. Z., tylavsky, f. A., conneely, k. N. & smith, a. K. 2016. An epigenetic clock for gestational age at birth based on blood methylation data. *Genome biology,* 17.

Knight, a. K., smith, a. K., conneely, k. N., dalach, p., loke, y. J., cheong, j. L., davis, p. G., craig, j. M., doyle, l. W. & theda, c. 2018. Relationship between epigenetic maturity and respiratory morbidity in preterm infants. *J pediatr,* 198**,** 168-173.e2.

Koen, n., wyatt, g. E., williams, j. K., zhang, m., myer, l., zar, h. J. & stein, d. J. 2014. Intimate partner violence: associations with low infant birthweight in a south african birth cohort. *Metabolic brain disease,* 29**,** 281-299.

Krapohl, e., euesden, j., zabaneh, d., pingault, j. B., rimfeld, k., von stumm, s., dale, p. S., breen, g., o'reilly, p. F. & plomin, r. 2016. Phenome-wide analysis of genome-wide polygenic scores. *Mol psychiatry,* 21**,** 1188-93.

Kwong, a. S. F., morris, t. T., pearson, r. M., timpson, n. J., rice, f., stergiakouli, e. & tilling, k. 2021. Polygenic risk for depression, anxiety and neuroticism are associated with the severity and rate of change in depressive symptoms across adolescence. *Journal of child psychology and psychiatry and allied disciplines*.

Lai, c. S., gerrelli, d., monaco, a. P., fisher, s. E. & copp, a. J. 2003. Foxp2 expression during brain development coincides with adult sites of pathology in a severe speech and language disorder. *Brain,* 126**,** 2455-62.

Laird, p. W. 2010. Principles and challenges of genomewide dna methylation analysis. *Nat rev genet,* 11**,** 191-203.

Lee, s. H., ripke, s., neale, b. M., faraone, s. V., purcell, s. M., perlis, r. H., mowry, b. J., thapar, a., goddard, m. E., witte, j. S., absher, d., agartz, i., akil, h., amin, f., andreassen, o. A., anjorin, a., anney, r., anttila, v., arking, d. E., asherson, p., azevedo, m. H., backlund, l., badner, j. A., bailey, a. J., banaschewski, t., barchas, j. D., barnes, m. R., barrett, t. B., bass, n., battaglia, a., bauer, m., bayés, m., bellivier, f., bergen, s. E., berrettini, w., betancur, c., bettecken, t., biederman, j., binder, e. B.,

black, d. W., blackwood, d. H., bloss, c. S., boehnke, m., boomsma, d. I., breen, g., breuer, r., bruggeman, r., cormican, p., buccola, n. G., buitelaar, j. K., bunney, w. E., buxbaum, j. D., byerley, w. F., byrne, e. M., caesar, s., cahn, w., cantor, r. M., casas, m., chakravarti, a., chambert, k., choudhury, k., cichon, s., cloninger, c. R., collier, d. A., cook, e. H., coon, h., cormand, b., corvin, a., coryell, w. H., craig, d. W., craig, i. W., crosbie, j., cuccaro, m. L., curtis, d., czamara, d., datta, s., dawson, g., day, r., de geus, e. J., degenhardt, f., djurovic, s., donohoe, g. J., doyle, a. E., duan, j., dudbridge, f., duketis, e., ebstein, r. P., edenberg, h. J., elia, j., ennis, s., etain, b., fanous, a., farmer, a. E., ferrier, i. N., flickinger, m., fombonne, e., foroud, t., frank, j., franke, b., fraser, c., et al. 2013. Genetic relationship between five psychiatric disorders estimated from genome-wide snps. *Nat genet,* 45**,** 984-94.

Leek, j. T., johnson, w. E., parker, h. S., jaffe, a. E. & storey, j. D. 2012. The sva package for removing batch effects and other unwanted variation in high-throughput experiments. *Bioinformatics,* 28**,** 882-3.

Lester, b. M., marsit, c. J., conradt, e., bromer, c. & padbury, j. F. 2012. Behavioral epigenetics and the developmental origins of child mental health disorders. *J dev orig health dis,* 3**,** 395-408.

Ligthart, l., van beijsterveldt, c. E. M., kevenaar, s. T., de zeeuw, e., van bergen, e., bruins, s., pool, r., helmer, q., van dongen, j., hottenga, j. J., van't ent, d., dolan, c. V., davies, g. E., ehli, e. A., bartels, m., willemsen, g., de geus, e. J. C. & boomsma, d. I. 2019. The netherlands twin register: longitudinal research based on twin and twin-family designs. *Twin res hum genet,* 22**,** 623-636.

Liu, c., jiao, c., wang, k. & yuan, n. 2018. Dna methylation and psychiatric disorders. Elsevier b.v.

Magnus, p., birke, c., vejrup, k., haugan, a., alsaker, e., daltveit, a. K., handal, m., haugen, m., høiseth, g., knudsen, g. P., paltiel, l., schreuder, p., tambs, k., vold, l. & stoltenberg, c. 2016. Cohort profile update: the norwegian mother and child cohort study (moba). *Int j epidemiol,* 45**,** 382-8.

Majara, l., kalungi, a., koen, n., tsuo, k., wang, y., gupta, r., nkambule, l. L., zar, h., stein, d. J., kinyanda, e., atkinson, e. G. & martin, a. R. 2023. Low and differential polygenic score generalisability among african populations due largely to genetic diversity. *Hgg adv,* 4**,** 100184.

Malcolm-smith, s., woolley, d. & ward, c. L. 2015. Examining empathy and its association with aggression in young western cape children. *Journal of child and adolescent mental health,* 27**,** 135-147.

Malecki, k. M. C., andersen, j. K., geller, a. M., harry, g. J., jackson, c. L., james, k. A., miller, g. W. & ottinger, m. A. 2022. Integrating environment and aging research: opportunities for synergy and acceleration. *Front aging neurosci,* 14**,** 824921.

Manichaikul, a., mychaleckyj, j. C., rich, s. S., daly, k., sale, m. & chen, w. M. 2010. Robust relationship inference in genome-wide association studies. *Bioinformatics,* 26**,** 2867-73.

Mar, á. P., joan, f., jesús, p., xavier, q. & jordi, s. 2013. Breathe project (brain development and air pollution ultrafine particles in school children): research protocol and first results. *Isee conference abstracts,* 2013**,** 3617-3617.

Martin, j., hamshere, m. L., stergiakouli, e., o'donovan, m. C. & thapar, a. 2014. Genetic risk for attention-deficit/hyperactivity disorder contributes to neurodevelopmental traits in the general population. *Biol psychiatry,* 76**,** 664-71.

Martin, j., hamshere, m. L., stergiakouli, e., o'donovan, m. C. & thapar, a. 2015. Neurocognitive abilities in the general population and composite genetic risk scores for attention-deficit hyperactivity disorder. *Journal of child psychology and psychiatry,* 56**,** 648-656.

Masten, a. S., desjardins, c. D., mccormick, c. M., kuo, s. I. & long, j. D. 2010. The significance of childhood competence and problems for adult success in work: a developmental cascade analysis. *Dev psychopathol,* 22**,** 679-94.

Mazefsky, c. A., anderson, r., conner, c. M. & minshew, n. 2011. Child behavior checklist scores for school-aged children with autism: preliminary evidence of patterns suggesting the need for referral. *Journal of psychopathology and behavioral assessment,* 33**,** 31-37.

Mccarthy, s., das, s., kretzschmar, w., delaneau, o., wood, a. R., teumer, a., kang, h. M., fuchsberger, c., danecek, p., sharp, k., luo, y., sidore, c., kwong, a., timpson, n., koskinen, s., vrieze, s., scott, l. J., zhang, h., mahajan, a., veldink, j., peters, u., pato, c., van duijn, c. M., gillies, c. E., gandin, i.,

mezzavilla, m., gilly, a., cocca, m., traglia, m., angius, a., barrett, j. C., boomsma, d., branham, k., breen, g., brummett, c. M., busonero, f., campbell, h., chan, a., chen, s., chew, e., collins, f. S., corbin, l. J., smith, g. D., dedoussis, g., dorr, m., farmaki, a. E., ferrucci, l., forer, l., fraser, r. M., gabriel, s., levy, s., groop, l., harrison, t., hattersley, a., holmen, o. L., hveem, k., kretzler, m., lee, j. C., mcgue, m., meitinger, t., melzer, d., min, j. L., mohlke, k. L., vincent, j. B., nauck, m., nickerson, d., palotie, a., pato, m., pirastu, n., mcinnis, m., richards, j. B., sala, c., salomaa, v., schlessinger, d., schoenherr, s., slagboom, p. E., small, k., spector, t., stambolian, d., tuke, m., tuomilehto, j., van den berg, l. H., van rheenen, w., volker, u., wijmenga, c., toniolo, d., zeggini, e., gasparini, p., sampson, m. G., wilson, j. F., frayling, t., de bakker, p. I., swertz, m. A., mccarroll, s., kooperberg, c., dekker, a., altshuler, d., willer, c., iacono, w., ripatti, s., et al. 2016. A reference panel of 64,976 haplotypes for genotype imputation. *Nat genet,* 48**,** 1279-83.

Mccoy, d. C., peet, e. D., ezzati, m., danaei, g., black, m. M., sudfeld, c. R., fawzi, w. & fink, g. 2016. Early childhood developmental status in low- and middle-income countries: national, regional, and global prevalence estimates using predictive modeling. *Plos med,* 13**,** e1002034.

Mcrae, a. F., powell, j. E., henders, a. K., bowdler, l., hemani, g., shah, s., painter, j. N., martin, n. G., visscher, p. M. & montgomery, g. W. 2014. Contribution of genetic variation to transgenerational inheritance of dna methylation. *Genome biol,* 15**,** r73.

Meijer, m., franke, b., sandi, c. & klein, m. 2023. Epigenome-wide dna methylation in externalizing behaviours: a review and combined analysis. *Neuroscience & biobehavioral reviews,* 145**,** 104997.

Middeldorp, c. M., hammerschlag, a. R., ouwens, k. G., groen-blokhuis, m. M., st. Pourcain, b., greven, c. U., pappa, i., tiesler, c. M. T., ang, w., nolte, i. M., vilor-tejedor, n., bacelis, j., ebejer, j. L., zhao, h., davies, g. E., ehli, e. A., evans, d. M., fedko, i. O., guxens, m., hottenga, j. J., hudziak, j. J., jugessur, a., kemp, j. P., krapohl, e., martin, n. G., murcia, m., myhre, r., ormel, j., ring, s. M., standl, m., stergiakouli, e., stoltenberg, c., thiering, e., timpson, n. J., trzaskowski, m., van der most, p. J., wang, c., nyholt, d. R., medland, s. E., neale, b., jacobsson, b., sunyer, j., hartman, c. A., whitehouse, a. J. O., pennell, c. E., heinrich, j., plomin, r., davey smith, g., tiemeier, h., posthuma, d. & boomsma, d. I. 2016. A genome-wide association meta-analysis of attention-deficit/hyperactivity disorder symptoms in population-based pediatric cohorts. *Journal of the american academy of child and adolescent psychiatry,* 55**,** 896-905.e6.

Mill, j. & petronis, a. 2008. Pre- and peri-natal environmental risks for attention-deficit hyperactivity disorder (adhd): the potential role of epigenetic processes in mediating susceptibility. *J child psychol psychiatry,* 49**,** 1020-30.

Min, j. L., hemani, g., hannon, e., dekkers, k. F., castillo-fernandez, j., luijk, r., carnero-montoro, e., lawson, d. J., burrows, k., suderman, m., bretherick, a. D., richardson, t. G., klughammer, j., iotchkova, v., sharp, g., al khleifat, a., shatunov, a., iacoangeli, a., mcardle, w. L., ho, k. M., kumar, a., söderhäll, c., soriano-tárraga, c., giralt-steinhauer, e., kazmi, n., mason, d., mcrae, a. F., corcoran, d. L., sugden, k., kasela, s., cardona, a., day, f. R., cugliari, g., viberti, c., guarrera, s., lerro, m., gupta, r., bollepalli, s., mandaviya, p., zeng, y., clarke, t. K., walker, r. M., schmoll, v., czamara, d., ruiz-arenas, c., rezwan, f. I., marioni, r. E., lin, t., awaloff, y., germain, m., aïssi, d., zwamborn, r., van eijk, k., dekker, a., van dongen, j., hottenga, j. J., willemsen, g., xu, c. J., barturen, g., català-moll, f., kerick, m., wang, c., melton, p., elliott, h. R., shin, j., bernard, m., yet, i., smart, m., gorrie-stone, t., shaw, c., al chalabi, a., ring, s. M., pershagen, g., melén, e., jiménez-conde, j., roquer, j., lawlor, d. A., wright, j., martin, n. G., montgomery, g. W., moffitt, t. E., poulton, r., esko, t., milani, l., metspalu, a., perry, j. R. B., ong, k. K., wareham, n. J., matullo, g., sacerdote, c., panico, s., caspi, a., arseneault, l., gagnon, f., ollikainen, m., kaprio, j., felix, j. F., rivadeneira, f., tiemeier, h., van, i. M. H., et al. 2021. Genomic and phenotypic insights from an atlas of genetic effects on dna methylation. *Nat genet,* 53**,** 1311-1321.

Mistry, s., escott-price, v., florio, a. D., smith, d. J. & zammit, s. 2019a. Genetic risk for bipolar disorder and psychopathology from childhood to early adulthood. *Journal of affective disorders,* 246**,** 633-639.

Mistry, s., escott-price, v., florio, a. D., smith, d. J. & zammit, s. 2019b. Investigating associations between genetic risk for bipolar disorder and cognitive functioning in childhood. *Journal of affective disorders,* 259**,** 112-120.
Mithyantha, r., kneen, r., mccann, e. & gladstone, m. 2017. Current evidence-based recommendations on investigating children with global developmental delay. *Arch dis child,* 102**,** 1071-1076.
Moher, d., liberati, a., tetzlaff, j. & altman, d. G. 2009. Preferred reporting items for systematic reviews and meta-analyses: the prisma statement. British medical journal publishing group.
Moore, l. D., le, t. & fan, g. 2013. Dna methylation and its basic function. *Neuropsychopharmacology,* 38**,** 23-38.
Moreaux, j., bruyer, a., veyrune, j. L., goldschmidt, h., hose, d. & klein, b. 2014. Dna methylation score is predictive of myeloma cell sensitivity to 5-azacitidine. *Br j haematol,* 164**,** 613-6.
Morin, a. M., gatev, e., mcewen, l. M., macisaac, j. L., lin, d. T. S., koen, n., czamara, d., räikkönen, k., zar, h. J., koenen, k., stein, d. J., kobor, m. S. & jones, m. J. 2017. Maternal blood contamination of collected cord blood can be identified using dna methylation at three cpgs. *Clin epigenetics,* 9**,** 75.
Murphy, t. M. & mill, j. 2014. Epigenetics in health and disease: heralding the ewas era. *Lancet,* 383**,** 1952-4.
Murray, g. K., lin, t., austin, j., mcgrath, j. J., hickie, i. B. & wray, n. R. 2021. Could polygenic risk scores be useful in psychiatry?: a review. *Jama psychiatry,* 78**,** 210-219.
Myer, l., stein, d. J., grimsrud, a., seedat, s. & williams, d. R. 2008. Social determinants of psychological distress in a nationally-representative sample of south african adults. *Social science and medicine,* 66**,** 1828-1840.
Nabais, m. F., gadd, d. A., hannon, e., mill, j., mcrae, a. F. & wray, n. R. 2023. An overview of dna methylation-derived trait score methods and applications. *Genome biology,* 24**,** 28.
Nabais, m. F., laws, s. M., lin, t., vallerga, c. L., armstrong, n. J., blair, i. P., kwok, j. B., mather, k. A., mellick, g. D., sachdev, p. S., wallace, l., henders, a. K., zwamborn, r. A. J., hop, p. J., lunnon, k., pishva, e., roubroeks, j. A. Y., soininen, h., tsolaki, m., mecocci, p., lovestone, s., kłoszewska, i., vellas, b., furlong, s., garton, f. C., henderson, r. D., mathers, s., mccombe, p. A., needham, m., ngo, s. T., nicholson, g., pamphlett, r., rowe, d. B., steyn, f. J., williams, k. L., anderson, t. J., bentley, s. R., dalrymple-alford, j., fowder, j., gratten, j., halliday, g., hickie, i. B., kennedy, m., lewis, s. J. G., montgomery, g. W., pearson, j., pitcher, t. L., silburn, p., zhang, f., visscher, p. M., yang, j., stevenson, a. J., hillary, r. F., marioni, r. E., harris, s. E., deary, i. J., jones, a. R., shatunov, a., iacoangeli, a., van rheenen, w., van den berg, l. H., shaw, p. J., shaw, c. E., morrison, k. E., al-chalabi, a., veldink, j. H., hannon, e., mill, j., wray, n. R. & mcrae, a. F. 2021. Meta-analysis of genome-wide dna methylation identifies shared associations across neurodegenerative disorders. *Genome biology,* 22.
Neumann, a., jolicoeur-martineau, a., szekely, e., sallis, h. M., o'donnel, k., greenwood, c. M. T., levitan, r., meaney, m. J., wazana, a., evans, j. & tiemeier, h. 2021. Combined polygenic risk scores of different psychiatric traits predict general and specific psychopathology in childhood. *J child psychol psychiatry*.
Neumann, a., jolicoeur-martineau, a., szekely, e., sallis, h. M., o'donnel, k., greenwood, c. M. T., levitan, r., meaney, m. J., wazana, a., evans, j. & tiemeier, h. 2022. Combined polygenic risk scores of different psychiatric traits predict general and specific psychopathology in childhood. *J child psychol psychiatry,* 63**,** 636-645.
Neumann, a., pappa, i., lahey, b. B., verhulst, f. C., medina-gomez, c., jaddoe, v. W., bakermans-kranenburg, m. J., moffitt, t. E., van, i. M. H. & tiemeier, h. 2016. Single nucleotide polymorphism heritability of a general psychopathology factor in children. *J am acad child adolesc psychiatry,* 55**,** 1038-1045.e4.
Neumann, a., walton, e., alemany, s., cecil, c., gonzález, j. R., jima, d. D., lahti, j., tuominen, s. T., barker, e. D., binder, e., caramaschi, d., carracedo, á., czamara, d., evandt, j., felix, j. F., fuemmeler, b. F., gutzkow, k. B., hoyo, c., julvez, j., kajantie, e., laivuori, h., maguire, r., maitre, l., murphy, s. K.,

murcia, m., villa, p. M., sharp, g., sunyer, j., raikkönen, k., bakermans-kranenburg, m., ijzendoorn, m. V., guxens, m., relton, c. L. & tiemeier, h. 2020. Association between dna methylation and adhd symptoms from birth to school age: a prospective meta-analysis. *Translational psychiatry,* 10**,** 30-30.

Nivard, m. G., gage, s. H., hottenga, j. J., van beijsterveldt, c. E. M., abdellaoui, a., bartels, m., baselmans, b. M. L., ligthart, l., pourcain, b. S., boomsma, d. I., munafò, m. R. & middeldorp, c. M. 2017. Genetic overlap between schizophrenia and developmental psychopathology: longitudinal and multivariate polygenic risk prediction of common psychiatric traits during development. *Schizophr bull,* 43**,** 1197-1207.

Oblak, l., van der zaag, j., higgins-chen, a. T., levine, m. E. & boks, m. P. 2021. A systematic review of biological, social and environmental factors associated with epigenetic clock acceleration. *Ageing research reviews,* 69**,** 101348-101348.

Ogundele, m. O. 2018. Behavioural and emotional disorders in childhood: a brief overview for paediatricians. *World j clin pediatr,* 7**,** 9-26.

Palladino, v. S., mcneill, r., reif, a. & kittel-schneider, s. 2019. Genetic risk factors and gene-environment interactions in adult and childhood attention-deficit/hyperactivity disorder. *Psychiatric genetics,* 29**,** 63-78.

Parade, s. H., ridout, k. K., seifer, r., armstrong, d. A., marsit, c. J., mcwilliams, m. A. & tyrka, a. R. 2016. Methylation of the glucocorticoid receptor gene promoter in preschoolers: links with internalizing behavior problems. *Child dev,* 87**,** 86-97.

Peterson, r. E., kuchenbaecker, k., walters, r. K., chen, c.-y., popejoy, a. B., periyasamy, s., lam, m., iyegbe, c., strawbridge, r. J., brick, l., carey, c. E., martin, a. R., meyers, j. L., su, j., chen, j., edwards, a. C., kalungi, a., koen, n., majara, l., schwarz, e., smoller, j. W., stahl, e. A., sullivan, p. F., vassos, e., mowry, b., prieto, m. L., cuellar-barboza, a., bigdeli, t. B., edenberg, h. J., huang, h. & duncan, l. E. 2019a. Genome-wide association studies in ancestrally diverse populations: opportunities, methods, pitfalls, and recommendations. *Cell,* 179**,** 589-603.

Pidsley, r., cc, y. W., volta, m., lunnon, k., mill, j. & schalkwyk, l. C. 2013. A data-driven approach to preprocessing illumina 450k methylation array data. *Bmc genomics,* 14**,** 293.

Pidsley, r., zotenko, e., peters, t. J., lawrence, m. G., risbridger, g. P., molloy, p., van djik, s., muhlhausler, b., stirzaker, c. & clark, s. J. 2016. Critical evaluation of the illumina methylationepic beadchip microarray for whole-genome dna methylation profiling. *Genome biol,* 17**,** 208.

Pingault, j.-b., viding, e., galera, c., greven, c. U., zheng, y., plomin, r. & rijsdijk, f. 2015. Genetic and environmental influences on the developmental course of attention-deficit/hyperactivity disorder symptoms from childhood to adolescence. *Jama psychiatry,* 72**,** 651-658.

Popejoy, a. B. & fullerton, s. M. 2016. Genomics is failing on diversity. *Nature,* 538**,** 161-164.

Posserud, m. B., lundervold, a. J. & gillberg, c. 2006. Autistic features in a total population of 7-9-year-old children assessed by the assq (autism spectrum screening questionnaire). *J child psychol psychiatry,* 47**,** 167-75.

Power, c. & elliott, j. 2006. Cohort profiles. *International epidemiological association international journal of epidemiology,* 35**,** 34-41.

Price, m. E., cotton, a. M., lam, l. L., farré, p., emberly, e., brown, c. J., robinson, w. P. & kobor, m. S. 2013. Additional annotation enhances potential for biologically-relevant analysis of the illumina infinium humanmethylation450 beadchip array. *Epigenetics chromatin,* 6**,** 4.

Purcell, s. M., wray, n. R., stone, j. L., visscher, p. M., o'donovan, m. C., sullivan, p. F. & sklar, p. 2009. Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. *Nature,* 460**,** 748-52.

Quach, a., levine, m. E., tanaka, t., lu, a. T., chen, b. H., ferrucci, l., ritz, b., bandinelli, s., neuhouser, m. L., beasley, j. M., snetselaar, l., wallace, r. B., tsao, p. S., absher, d., assimes, t. L., stewart, j. D., li, y.,

hou, l., baccarelli, a. A., whitsel, e. A. & horvath, s. 2017. Epigenetic clock analysis of diet, exercise, education, and lifestyle factors. *Aging (albany ny),* 9**,** 419-446.

Raffington, l., mallard, t. & harden, k. P. 2020. Polygenic scores in developmental psychology: invite genetics in, leave biodeterminism behind. *Https://doi.org/10.1146/annurev-devpsych-051820-123945,* 2**,** 389-411.

Rakyan, v. K., down, t. A., balding, d. J. & beck, s. 2011. Epigenome-wide association studies for common human diseases. *Nat rev genet,* 12**,** 529-41.

Rice, f., riglin, l., thapar, a. K., heron, j., anney, r., o'donovan, m. C. & thapar, a. 2019. Characterizing developmental trajectories and the role of neuropsychiatric genetic risk variants in early-onset depression. *Jama psychiatry,* 76**,** 306-313.

Riglin, l., collishaw, s., richards, a., thapar, a. K., maughan, b., o'donovan, m. C. & thapar, a. 2017. Schizophrenia risk alleles and neurodevelopmental outcomes in childhood: a population-based cohort study. *The lancet psychiatry,* 4**,** 57-62.

Rijlaarsdam, j., cecil, c. A. M., relton, c. L. & barker, e. D. 2022. Epigenetic profiling of social communication trajectories and co-occurring mental health problems: a prospective, methylome-wide association study. *Dev psychopathol,* 34**,** 854-863.

Ripke, s., neale, b. M., corvin, a., walters, j. T. R., farh, k. H., holmans, p. A., lee, p., bulik-sullivan, b., collier, d. A., huang, h., pers, t. H., agartz, i., agerbo, e., albus, m., alexander, m., amin, f., bacanu, s. A., begemann, m., belliveau, r. A., bene, j., bergen, s. E., bevilacqua, e., bigdeli, t. B., black, d. W., bruggeman, r., buccola, n. G., buckner, r. L., byerley, w., cahn, w., cai, g., campion, d., cantor, r. M., carr, v. J., carrera, n., catts, s. V., chambert, k. D., chan, r. C. K., chen, r. Y. L., chen, e. Y. H., cheng, w., cheung, e. F. C., chong, s. A., cloninger, c. R., cohen, d., cohen, n., cormican, p., craddock, n., crowley, j. J., curtis, d., davidson, m., davis, k. L., degenhardt, f., del favero, j., demontis, d., dikeos, d., dinan, t., djurovic, s., donohoe, g., drapeau, e., duan, j., dudbridge, f., durmishi, n., eichhammer, p., eriksson, j., escott-price, v., essioux, l., fanous, a. H., farrell, m. S., frank, j., franke, l., freedman, r., freimer, n. B., friedl, m., friedman, j. I., fromer, m., genovese, g., georgieva, l., giegling, i., giusti-rodríguez, p., godard, s., goldstein, j. I., golimbet, v., gopal, s., gratten, j., de haan, l., hammer, c., hamshere, m. L., hansen, m., hansen, t., haroutunian, v., hartmann, a. M., henskens, f. A., herms, s., hirschhorn, j. N., hoffmann, p., hofman, a., hollegaard, m. V., hougaard, d. M., ikeda, m., joa, i., et al. 2014. Biological insights from 108 schizophrenia-associated genetic loci. *Nature,* 511**,** 421-427.

Rojas, d., rager, j. E., smeester, l., bailey, k. A., drobná, z., rubio-andrade, m., stýblo, m., garcía-vargas, g. & fry, r. C. 2015. Prenatal arsenic exposure and the epigenome: identifying sites of 5-methylcytosine alterations that predict functional changes in gene expression in newborn cord blood and subsequent birth outcomes. *Toxicol sci,* 143**,** 97-106.

Ronkainen, j., heiskala, a., vehmeijer, f. O. L., lowry, e., caramaschi, d., estrada gutierrez, g., heiss, j. A., hummel, n., keikkala, e., kvist, t., kupsco, a., melton, p. E., pesce, g., soomro, m. H., vives-usano, m., baiz, n., binder, e., czamara, d., guxens, m., mustaniemi, s., london, s. J., rauschert, s., vääräsmäki, m., vrijheid, m., ziegler, a.-g., annesi-maesano, i., bustamante, m., huang, r.-c., hummel, s., just, a. C., kajantie, e., lahti, j., lawlor, d., räikkönen, k., järvelin, m.-r., felix, j. F. & sebert, s. 2022. Maternal haemoglobin levels in pregnancy and child dna methylation: a study in the pregnancy and childhood epigenetics consortium. *Epigenetics,* 17**,** 19-31.

Sallis, h., szekely, e., neumann, a., jolicoeur-martineau, a., van, i. M., hillegers, m., greenwood, c. M. T., meaney, m. J., steiner, m., tiemeier, h., wazana, a., pearson, r. M. & evans, j. 2019. General psychopathology, internalising and externalising in children and functional outcomes in late adolescence. *J child psychol psychiatry,* 60**,** 1183-1190.

Salum, g. A., isolan, l. R., bosa, v. L., tocchetto, a. G., teche, s. P., schuch, i., costa, j. R., costa mde, a., jarros, r. B., mansur, m. A., knijnik, d., silva, e. A., kieling, c., oliveira, m. H., medeiros, e., bortoluzzi, a., toazza, r., blaya, c., leistner-segal, s., salles, j. F., silveira, p. P., goldani, m. Z., heldt, e. & manfro, g.

G. 2011. The multidimensional evaluation and treatment of anxiety in children and adolescents: rationale, design, methods and preliminary findings. *Braz j psychiatry,* 33**,** 181-95.

Schoeman, r. & liebenberg, r. 2017. The south african society of psychiatrists/psychiatry management group management guidelines for adult attention-deficit/hyperactivity disorder. *South african journal of psychiatry,* 23**,** 14-14.

Scott, j. G., mihalopoulos, c., erskine, h. E., roberts, j. & rahman, a. 2016. Childhood mental and developmental disorders. *Disease control priorities, third edition (volume 4): mental, neurological, and substance use disorders***,** 145-161.

Shaw, m., hodgkins, p., caci, h., young, s., kahle, j., woods, a. G. & arnold, l. E. 2012. A systematic review and analysis of long-term outcomes in attention deficit hyperactivity disorder: effects of treatment and non-treatment. *Bmc med,* 10**,** 99.

Smith, g. 2018. Step away from stepwise. *Journal of big data,* 5**,** 32.

Sohani, z. N., sarma, s., alyass, a., de souza, r. J., robiou-du-pont, s., li, a., mayhew, a., yazdi, f., reddon, h., lamri, a., stryjecki, c., ishola, a., lee, y. K., vashi, n., anand, s. S. & meyre, d. 2016. Empirical evaluation of the q-genie tool: a protocol for assessment of effectiveness. *Bmj open,* 6.

Solmi, m., radua, j., olivola, m., croce, e., soardo, l., salazar de pablo, g., il shin, j., kirkbride, j. B., jones, p., kim, j. H., kim, j. Y., carvalho, a. F., seeman, m. V., correll, c. U. & fusar-poli, p. 2022. Age at onset of mental disorders worldwide: large-scale meta-analysis of 192 epidemiological studies. *Molecular psychiatry,* 27**,** 281-295.

Solovieff, n., cotsapas, c., lee, p. H., purcell, s. M. & smoller, j. W. 2013. Pleiotropy in complex traits: challenges and strategies. *Nat rev genet,* 14**,** 483-95.

Stein, d. J., koen, n., donald, k. A., adnams, c. M., koopowitz, s., lund, c., marais, a., myers, b., roos, a., sorsdahl, k., stern, m., tomlinson, m., van der westhuizen, c., vythilingum, b., myer, l., barnett, w., brittain, k. & zar, h. J. 2015. Investigating the psychosocial determinants of child health in africa: the drakenstein child health study. *J neurosci methods,* 252**,** 27-35.

Stergiakouli, e., davey smith, g., martin, j., skuse, d. H., viechtbauer, w., ring, s. M., ronald, a., evans, d. E., fisher, s. E., thapar, a. & st pourcain, b. 2017. Shared genetic influences between dimensional asd and adhd symptoms during child and adolescent development. *Molecular autism,* 8**,** 1-13.

Strandberg, t. E., andersson, s., järvenpää, a. L. & mckeigue, p. M. 2002. Preterm birth and licorice consumption during pregnancy. *Am j epidemiol,* 156**,** 803-5.

Suarez, a., lahti, j., czamara, d., lahti-pulkkinen, m., girchenko, p., andersson, s., strandberg, t. E., reynolds, r. M., kajantie, e., binder, e. B. & raikkonen, k. 2018a. The epigenetic clock and pubertal, neuroendocrine, psychiatric, and cognitive outcomes in adolescents. *Clin epigenetics,* 10**,** 96.

Suarez, a., lahti, j., czamara, d., lahti-pulkkinen, m., knight, a. K., girchenko, p., hämäläinen, e., kajantie, e., lipsanen, j., laivuori, h., villa, p. M., reynolds, r. M., smith, a. K., binder, e. B. & räikkönen, k. 2018b. The epigenetic clock at birth: associations with maternal antenatal depression and child psychiatric problems. *J am acad child adolesc psychiatry,* 57**,** 321-328.e2.

Sudre, g., sharp, w., kundzicz, p., bouyssi-kobar, m., norman, l., choudhury, s. & shaw, p. 2020. Predicting the course of adhd symptoms through the integration of childhood genomic, neural, and cognitive features. *Molecular psychiatry*.

Sullivan, p. F. & geschwind, d. H. 2019. Defining the genetic, genomic, cellular, and diagnostic architectures of psychiatric disorders. *Cell,* 177**,** 162-183.

Sumner, j. A., colich, n. L., uddin, m., armstrong, d. & mclaughlin, k. A. 2019. Early experiences of threat, but not deprivation, are associated with accelerated biological aging in children and adolescents. *Biol psychiatry,* 85**,** 268-278.

Szyf, m. & bick, j. 2013. Dna methylation: a mechanism for embedding early life experiences in the genome. *Child dev,* 84**,** 49-57.

Tekola-ayele, f. & rotimi, c. N. 2015. Translational genomics in low- and middle-income countries: opportunities and challenges. *Public health genomics,* 18**,** 242-7.

Thapar, a., martin, j., mick, e., vasquez, a. A., langley, k., scherer, s. W., schachar, r., crosbie, j., williams, n., franke, b., elia, j., glessner, j., hakonarson, h., owen, m. J., faraone, s. V., o'donovan, m. C., holmans, p. & consortium, i. 2016. Psychiatric gene discoveries shape evidence on adhd's biology. *Molecular psychiatry,* 21**,** 1202-1207.

Tishkoff, s. A., reed, f. A., friedlaender, f. R., ehret, c., ranciaro, a., froment, a., hirbo, j. B., awomoyi, a. A., bodo, j. M., doumbo, o., ibrahim, m., juma, a. T., kotze, m. J., lema, g., moore, j. H., mortensen, h., nyambo, t. B., omar, s. A., powell, k., pretorius, g. S., smith, m. W., thera, m. A., wambebe, c., weber, j. L. & williams, s. M. 2009. The genetic structure and history of africans and african americans. *Science,* 324**,** 1035-44.

Tollenaar, m. S., beijers, r., garg, e., nguyen, t. T. T., lin, d. T. S., macisaac, j. L., shalev, i., kobor, m. S., meaney, m. J., o'donnell, k. J. & de weerth, c. 2021. Internalizing symptoms associate with the pace of epigenetic aging in childhood. *Biological psychology,* 159**,** 108021.

Van dongen, j., nivard, m. G., willemsen, g., hottenga, j. J., helmer, q., dolan, c. V., ehli, e. A., davies, g. E., van iterson, m., breeze, c. E., beck, s., suchiman, h. E., jansen, r., van meurs, j. B., heijmans, b. T., slagboom, p. E. & boomsma, d. I. 2016. Genetic and environmental influences interact with age and sex in shaping the human methylome. *Nat commun,* 7**,** 11115.

Viechtbauer, w. 2010. Conducting meta-analyses in r with the metafor. *Journal of statistical software,* 36**,** 1-48.

Walton, e., pingault, j. B., cecil, c. A., gaunt, t. R., relton, c. L., mill, j. & barker, e. D. 2017. Epigenetic profiling of adhd symptoms trajectories: a prospective, methylome-wide study. *Mol psychiatry,* 22**,** 250-256.

Whiteford, h. A., degenhardt, l., rehm, j., baxter, a. J., ferrari, a. J., erskine, h. E., charlson, f. J., norman, r. E., flaxman, a. D., johns, n., burstein, r., murray, c. J. & vos, t. 2013. Global burden of disease attributable to mental and substance use disorders: findings from the global burden of disease study 2010. *Lancet,* 382**,** 1575-86.

Wichstrøm, l., berg-nielsen, t. S., angold, a., egger, h. L., solheim, e. & sveen, t. H. 2012. Prevalence of psychiatric disorders in preschoolers. *Journal of child psychology and psychiatry,* 53**,** 695-705.

Wolf, e. J., logue, m. W., morrison, f. G., wilcox, e. S., stone, a., schichman, s. A., mcglinchey, r. E., milberg, w. P. & miller, m. W. 2019. Posttraumatic psychopathology and the pace of the epigenetic clock: a longitudinal investigation. *Psychol med,* 49**,** 791-800.

Wray, n. R., lee, s. H., mehta, d., vinkhuyzen, a. A., dudbridge, f. & middeldorp, c. M. 2014. Research review: polygenic methods and their application to psychiatric traits. *J child psychol psychiatry,* 55**,** 1068-87.

Wray, n. R., lin, t., austin, j., mcgrath, j. J., hickie, i. B., murray, g. K. & visscher, p. M. 2021. From basic science to clinical application of polygenic risk scores: a primer. *Jama psychiatry,* 78**,** 101-109.

Wray, n. R., ripke, s., mattheisen, m., trzaskowski, m., byrne, e. M., abdellaoui, a., adams, m. J., agerbo, e., air, t. M., andlauer, t. M. F., bacanu, s. A., bækvad-hansen, m., beekman, a. F. T., bigdeli, t. B., binder, e. B., blackwood, d. R. H., bryois, j., buttenschøn, h. N., bybjerg-grauholm, j., cai, n., castelao, e., christensen, j. H., clarke, t. K., coleman, j. I. R., colodro-conde, l., couvy-duchesne, b., craddock, n., crawford, g. E., crowley, c. A., dashti, h. S., davies, g., deary, i. J., degenhardt, f., derks, e. M., direk, n., dolan, c. V., dunn, e. C., eley, t. C., eriksson, n., escott-price, v., kiadeh, f. H. F., finucane, h. K., forstner, a. J., frank, j., gaspar, h. A., gill, m., giusti-rodríguez, p., goes, f. S., gordon, s. D., grove, j., hall, l. S., hannon, e., hansen, c. S., hansen, t. F., herms, s., hickie, i. B., hoffmann, p., homuth, g., horn, c., hottenga, j. J., hougaard, d. M., hu, m., hyde, c. L., ising, m., jansen, r., jin, f., jorgenson, e., knowles, j. A., kohane, i. S., kraft, j., kretzschmar, w. W., krogh, j., kutalik, z., lane, j. M., li, y., li, y., lind, p. A., liu, x., lu, l., macintyre, d. J., mackinnon, d. F., maier, r. M., maier, w., marchini, j., mbarek, h., mcgrath, p., mcguffin, p., medland, s. E., mehta, d., middeldorp, c. M., mihailov, e., milaneschi, y., milani, l., mill, j., mondimore, f. M., montgomery, g. W., mostafavi, s., mullins, n., nauck, m., ng, b., et al. 2018. Genome-wide association analyses identify 44 risk variants and refine the genetic architecture of major depression. *Nature genetics 2018 50:5,* 50**,** 668-681.

Wray, n. R., yang, j., hayes, b. J., price, a. L., goddard, m. E. & visscher, p. M. 2013. Pitfalls of predicting complex traits from snps. *Nat rev genet,* 14**,** 507-15.

Zar, h. J., barnett, w., myer, l., stein, d. J. & nicol, m. P. 2015. Investigating the early-life determinants of illness in africa: the drakenstein child health study. *Thorax,* 70**,** 592-594.

Zhang, b., zhou, y., lin, n., lowdon, r. F., hong, c., nagarajan, r. P., cheng, j. B., li, d., stevens, m., lee, h. J., xing, x., zhou, j., sundaram, v., elliott, g., gu, j., shi, t., gascard, p., sigaroudinia, m., tlsty, t. D., kadlecek, t., weiss, a., o'geen, h., farnham, p. J., maire, c. L., ligon, k. L., madden, p. A., tam, a., moore, r., hirst, m., marra, m. A., zhang, b., costello, j. F. & wang, t. 2013. Functional dna methylation differences between tissues, cell types, and across individuals discovered using the m&m algorithm. *Genome res,* 23**,** 1522-40.

Zhang, f., chen, w., zhu, z., zhang, q., nabais, m. F., qi, t., deary, i. J., wray, n. R., visscher, p. M., mcrae, a. F. & yang, j. 2019a. Osca: a tool for omic-data-based complex trait analysis. *Genome biol,* 20**,** 107.

Zhang, q., vallerga, c. L., walker, r. M., lin, t., henders, a. K., montgomery, g. W., he, j., fan, d., fowdar, j., kennedy, m., pitcher, t., pearson, j., halliday, g., kwok, j. B., hickie, i., lewis, s., anderson, t., silburn, p. A., mellick, g. D., harris, s. E., redmond, p., murray, a. D., porteous, d. J., haley, c. S., evans, k. L., mcintosh, a. M., yang, j., gratten, j., marioni, r. E., wray, n. R., deary, i. J., mcrae, a. F. & visscher, p. M. 2019b. Improved precision of epigenetic clock estimates across tissues and its implication for biological ageing. *Genome med,* 11**,** 54.

Zhou, k., dempfle, a., arcos-burgos, m., bakker, s. C., banaschewski, t., biederman, j., buitelaar, j., castellanos, f. X., doyle, a., ebstein, r. P., ekholm, j., forabosco, p., franke, b., freitag, c., friedel, s., gill, m., hebebrand, j., hinney, a., jacob, c., lesch, k. P., loo, s. K., lopera, f., mccracken, j. T., mcgough, j. J., meyer, j., mick, e., miranda, a., muenke, m., mulas, f., nelson, s. F., nguyen, t. T., oades, r. D., ogdie, m. N., palacio, j. D., pineda, d., reif, a., renner, t. J., roeyers, h., romanos, m., rothenberger, a., schäfer, h., sergeant, j., sinke, r. J., smalley, s. L., sonuga-barke, e., steinhausen, h. C., van der meulen, e., walitza, s., warnke, a., lewis, c. M., faraone, s. V. & asherson, p. 2008. Meta-analysis of genome-wide linkage scans of attention deficit hyperactivity disorder. *Am j med genet b neuropsychiatr genet,* 147b**,** 1392-8.

Zieff, m. R., fourie, c., hoogenhout, m. & donald, k. A. 2022. Psychometric properties of the aseba child behaviour checklist and youth self-report in sub-saharan africa - a systematic review. *Acta neuropsychiatr***,** 1-24.

WHO (2023). Our work: communicable and noncommunicable diseases, and mental health. [online] www.who.int. Available at: https://www.who.int/our-work/communicable-and-noncommunicable-diseases-and-mental-health.

Hasin, Y., Seldin, M. and Lusis, A. (2017). Multi-omics approaches to disease. Genome Biology, 18(1). doi:https://doi.org/10.1186/s13059-017-1215-1.

Sumner, J.A., Colich, N.L., Uddin, M., Armstrong, D. and McLaughlin, K.A. (2019). Early Experiences of Threat, but Not Deprivation, Are Associated With Accelerated Biological Aging in Children and Adolescents. Biological Psychiatry, 85(3), pp.268–278. doi:https://doi.org/10.1016/j.biopsych.2018.09.008.

Appendices

Appendix 1: Chapter 2 PRS systematic review supplementary material

Table 1: Inclusion criteria (PICo) outline

Population	Studies involving human subjects, aged 2-18 years, will be eligible for inclusion
Areas of Interest	Areas of interest pertain to development and mental health in childhood and adolescence, i.e. neurodevelopmental outcomes/disorders; and internalising and/or externalising behaviours/disorders
Context	Polygenic risk scores (PRS, aka genetic risk scores) in the context of the areas of interest described above will be investigated. Including Attention deficit hyperactivity disorder (ADHD), Autism spectrum disorder (ASD), neurodevelopment and internalising/externalising behaviours/disorders etc.
Types of studies	All studies evaluating polygenic risk scores within the scope of child and adolescent development and mental health will be considered

Inclusion criteria
Full-text published studies evaluating polygenic risk scores associated with development (neurodevelopmental outcomes/disorders) and mental health (internalising and/or externalising behaviours/disorders) in childhood and adolescence will be eligible for inclusion. Neurological disorders and symptoms will be excluded.

Exclusion criteria
- Studies focusing on adult populations (aged ≥19 years old)
- Studies on animal models
- Genome-wide association studies (GWAS) without a PRS component
- Studies not published in English

Search terms for PRS systematic review

*Note: The search terms were broad in order to maintain consistency throughout all databases (i.e. highly specific search terms would likely have resulted in fewer hits and the exclusion of relevant papers). These broad search terms were also intended to capture papers on internalising and externalising behaviours. This approach was also utilised for EA systematic review.

Concepts
keywords / Mesh terms
Combine with AND or OR

Search terms to be used:
- Developmental psychopathology
- Neurodevelopment disorders (MESH)
- Polygenic risk
- Polygenic score
- Genetic risk
- Child
- Adolescent
- Young people
- Youth

Databases and sources:

- PubMed (including MEDLINE)
- Cumulative Index of Nursing and Allied Health (CINAHL) via Ebscohost
- Psych Info via Ebscohost
- Scopus
- Grey literature (Web of Science, all databases)

Table 2: Full Search Strategy for PRS systematic review

Database name	**MESH**	**Keywords**	**Final search**
PubMed	Neurodevelopment disorders	Developmental psychopathology OR Neurodevelopmental Disorders	**((Neurodevelopmental Disorders[Text Word] OR Developmental psychopathology[Text Word]) AND (Polygenic risk[Text Word] OR Polygenic risk score[Text Word] OR Genetic risk score[Text Word])) AND (Child[Text Word] OR Adolescent[Text Word] OR Youth[Text Word] OR Young people[Text Word])**
		Polygenic risk OR Polygenic risk score OR Genetic risk score	
		Child OR Adolescent OR Youth OR Young people	

Database name	MESH	Keywords	Final search
SCOPUS		"Development*" "psychopatholog*" OR "Neurodevelopment* Disorder*"	**"Development* psychopatholog*" OR "Neurodevelopment* Disorder*"AND "Polygenic risk*" OR "Polygenic risk score*" OR "Genetic risk score*" AND Child* OR Adolescen* OR Youth OR "Young people"**
		"Polygenic risk*" OR "Polygenic risk score*" OR "Genetic risk score*"	
		Child* OR Adolescen* OR Youth OR "Young people"	

Database name	**MESH**	**Keywords**	**Final search**
Psych Info (via Ebscohost)	MM (exploded) (S1) "Neurodevelopmental Disorders"	(S2) “Development* psychopatholog*” OR “Neurodevelopment* Disorder*”	**S1 AND S2 AND S3 AND S4**
		(S3) “Polygenic risk*” OR “Polygenic risk score*” OR “Genetic risk score*”	
		(S4) Child* OR Adolescen* OR Youth OR “Young people”	

Database name	MESH	Keywords	Final search
Web of Science (all databases)	“Polygenic risk*” “Genetic risk*” “Epigenetic association*” Child* Adolescen* Young people Youth		Set 1 "polygenic risk *" OR "genetic risk *" OR “Epigenetic association*”
			Set 2 Child* OR adolescen*OR youth OR "young people"
			Set 3 "Development* psychopathology "
			#1 AND #2 AND #3

Database name	MESH	Keywords	Final search
CINAHL complete (via ebscohost)			**# 1 AND #2 AND #3**
		(#1) "Development* psychopatholog*" OR "Neurodevelopment* Disorder*"	
		(#2) "Polygenic risk*" OR "Polygenic risk score*" OR "Genetic risk score*"	
		(#3) Child* OR Adolescen* OR Youth OR "Young people"	

Table 3: Methodological quality assessment using the Q-Genie tool for PRS systematic review

Author (year)	Study rationale	Selection & definition of outcome of interest	Selection & comparability of comparison groups (if applicable)	Technical classification of the exposure	Non-technical classification of the exposure	Other sources of bias	Sample size and power	A priori planning of analyses	Statistical method & control for confounding	Testing of assumptions and inference for genetic analyses	Appropriateness of inferences drawn from results	Q score
Kwong et al 2021	4	4	4	7	7	7	3	6	5	6	6	59
Sudre et al 2020	6	5	5	5	5	3	1	5	4	5	4	48
Hannigan et al 2021	5	4	5	5	5	5	3	5	3	3	5	48
Aguilar-Lacasana et al 2020	5	5	5	3	5	3	1	5	4	3	5	44
Akingbuwa 2020	5	3	5	5	5	5	6	5	5	5	5	54
Jansen 2020	5	5	3	5	4	5	2	5	5	5	5	49
Martin et al 2015	5	4	5	5	5	5	2	3	3	4	4	45
Mistry et al (a)	3	4	5	5	5	5	2	3	3	2	3	40
Mistry (b)	5	3	5	3	5	3	2	3	2	2	3	36

Nivard et al 2017	5	3	5	5	5	5	2	5	5	2	5	47
Nudel et al 2020	5	5	5	5	5	5	5	2	5	3	7	52
Rice et al 2019	5	3	5	5	5	5	3	5	5	1	5	47
Riglin et al 2017	5	3	5	3	5	5	3	5	5	1	5	43
Riglin 2018	5	2	5	3	1	5	2	5	4	1	5	38
Riglin 2016	5	3	5	5	3	3	3	3	3	1	3	40
Salvatore2015	3	3	5	5	1	4	1	3	3	1	3	33

Poor quality: 1–2; **Good quality:** 3–4; **Very good quality:** 5–6; **Excellent quality:** 7.
For studies with control groups: scores ≤ 35 indicate poor-quality studies, > 35 and ≤ 45 indicate studies of moderate quality, and > 45 indicate good-quality studies.
For studies without control groups: scores ≤ 32 indicate poor-quality studies, > 32 and ≤ 40 indicate studies of moderate quality, and > 40 indicate good-quality studies.
NA: not applicable

Table 4. Data extraction table for PRS systematic review

Author	PMID/ DOI	Outcome	Outcome measure	Predictor	Statistical measure	Statistical test	Age	Population/cohort	Ancestry	Sample size
Kwong et al 2021	PMID: 33778956 doi:10.1111/jcpp.13422	Self-reported depressive symptoms, VS PRS for depression	Short mood & feelings questionnaire (SMFQ)	PRS for psychiatric disorders: DEP (depression) MDD (Major depressive disorder) ANX (anxiety) NEU (neuroticism) SCZ (schizophrenia)	SE; Upper and Lower limits, p-value effect size and variance % , SD p-values corrected using: Benjamini-Yekutileli method	Regression	10 to 24	(ALSPAC)	European	6302
Martin et al 2015	https://doi.org/10.1111/jcpp.12336	Parent-reported ADHD and Autistic like traits used to calculate "Neurodevelopment trait" variable Neurodevelopment i.e: hyperactivity Inattention Pragmmatic language social cognition Development and Well-Being Assessment (DAWBA): ADHD inattentive and hyperactive-impulsive traits	WISC-III : (IQ) WISC-III digit span task : verbal working memory Test of everyday attention for children (opposite worlds task) : for cognitive inhibitory control Diagnostic Analysis of the Faces subtest Counting span task Diagnostic Analysis of Nonverbal Accuracy: Facial emotional recognition Development &Well-being Assessment (DAWBA); ADHD inattentive & impulsive traits Social & communication Disorders Checklist (SCDC): social communication Children's communication Checklist (CCC): pragmatic language scales	ADHD PRS (children) derived from clinical cases	beta, P value, Variance z-score transformations	Student's t-test multivariate linear regression	7 to 10	ALSPAC	European	6832
Mistry et al 2019b	https://doi.org/10.1016/j.jad.2019.08.040	BP and cognitive function	WISC-III (processing speed, problem solving) DANVA : emotion recognition	BD-PRS	variance; CI; P value; beta	Linear regression	8	AVON longitudinal study of women and children	European	8230

		cognitive function : Verbal inteliigence Problem solving Emotion recognition Executive functioning Attention Working memory processing speed performance Intelligence quotient secondary analysis domains : SCZ,	Freedom from Distractibility index score (working memory) Test of Everyday Attention for Children (TEACh) : exective function & attention Children's test of Nonword Repetition (CTNWR): verbal learning					PRS: Psychiatrics genomics consortium (PGC-BD)		5613 to 5936
Mistry et al 2019a	https://doi.org/10.1016/j.jad.2018.12.091	BD vs child psychopathology emotional /behavioural difficulties ADHD Borderline personality disorders (BPD) traits (childhood)	Strengths and Difficulties questionnaire (SDQ): hyperactivity problems prosocial behaviour emotional difficulties conduct problems peer relationship diffictulties Development and Wellbeing Assessment (DAWBA) DSM-IV	BD-PRS	OR; CI; p value	Linear regression Logistic regression	7 to 11	Avon longitudinal study of women and children PRS: PGC	Europ ean	8230 2654 to 6111
Riglin et al 2016	doi:10.1001/jamapsychiatry.2016.2817.	ADHD symptom trajectories (childhood & adolescence) ADHD vs Neurodevelopmental disorders (multimobidity) : SCZ, BD, DEP	SDQ	ADHD PRS	CI; Mean , SE, P value, OR	Latent class growth analysis Wald test equality of means	4 to 17	Avon Longitudinal Study of Parents and Children PRS : PGC	Europ ean	9757

						(using posterior probability)				
Riglin et al 2016	doi:10.1001/jamapsychiatry.2016.2817.	Multimorbidity domains common with ADHD: ADHD symptom trajectories (childhood & adolescence)	SDQ	ADHD PRS	CI; Mean , SE, P value, OR	Latent class growth analysis	4 to 17	ALSPAC	European	9757
		IQADHD vs Neurodevelopmental disorders (multimobidity) :				Wald test equality of means		PRS PGC		
		Social communication SCZ, BD, DEP				(using posterior probability)				
		pragmatic language								
		conductMultimorbidity domains common with ADHD:								
		IQ								
		Social communication								
		pragmatic language								
		conduct								
Salvatore et al 2015	doi:10.1177/2167702614534211.	Subclinical externalizing symptoms	DSM-IV:	PRS-externalizing disorders	beta, SE , variance	Regression	12 to 17	(COGA)	European-American	n=248 adolescents
		Impulsivity-related traits	alcohol dependence & abuse criteria							
			antisocial behaviour/conduct disorder							
Salvatore et al 2015	doi:10.1177/2167702614534211.	Subclinical externalizing symptoms	Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA) adolescent versionDSM-IV:	PRS-externalizing disorders	beta, SE , variance	Regression	12 to 17 (adolescents)	(COGA)	European-American	n=248 adolescents
		Impulsivity-related traits	Achenbach Externalizing Youth self-reportalcohol dependence & abuse criteria							
			NEO conscieontiousness antisocial behaviour/conduct disorder							
			Barrat impulsiveness scale							
			Zuckerman's Sensation seeking scale (SSS-V) Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA) adolescent version							

			Parental monitoring & percieved peer substance use (adolescents)Achenbach Externalizing Youth self-report							
			NEO conscieontiousness							
			Barrat impulsiveness scale							
			Zuckerman's Sensation seeking scale (SSS-V)							
			Parental monitoring & percieved peer substance use (adolescents)							
Nivard et al 2017	doi:10.1093/schbul/sbx031	Anxiety	NTR:	Schizophrenia PRS (PGC-SCZ2)	effect sizes	Regression analyses	7 to 15	Netherlands Twin Register (NTR)		N=2588
	doi:10.1093/schbul/sbx031	Depression	Achenbach System of Empirically Based Assessment (ASEBA)		regression coefficient	multivariate meta-analytic model				
		ADHD	Development and wellbeing assessement (DAWBA)		variance	Regression analyses				
		ODD/CD			p-value			ALSPAC		N=6127
Nivard et al 2017		Anxiety	NTR:	Schizophrenia PRS (PGC-SCZ2)	beta-co-efficientseffect sizes					N=2588
		Depression	Achenbach System of Empirically Based Assessment (ASEBA)		SEregression coefficient	multivariate meta-analytic model				N=6127
		ADHD	Development and wellbeing assessement (DAWBA)		variance			Netherlands Twin Register (NTR) ALSPAC		
		ODD/CD			p-value					
					beta-co-efficients					
					SE					
Nudel et al 2020	DOI: 10.1002/aur.2211	Broad Langauge phenotypes	Danish version of the Test for Reception of Grammar, TROG-2	PRS: summary stats from :	p-value		7	Danish High risk and resilience study-VIA7		
		Narrow Language phenotypes (SLI)	Schedule for Affective Disorders and Schizophrenia of School-Age Children (KSADS) (danish V)	Genetic study of SLI in a british cohort of SLI Families	regression co-efficients	goodnes of fit model logistic regression coefficients				
		ASD (based on K-SADS lifetime criteria)	Reynolds Intellectual Screening Test (RIST) (for non-verbal intelligence)		Nagelkerke's pseudo R squared					
		ADHD (based on K-SADS lifetime criteria)			odd-ratio					18 cases GWAS N:

Nudel et al 2020	DOI: 10.1002/aur.2211	Height (negative control) Broad Langauge phenotypes	Danish version of the Test for Reception of Grammar, TROG-2	PRS: summary stats from :	p-value		7	Danish High risk and resilience study-VIA7		
		Narrow Language phenotypes (SLI)	Schedule for Affective Disorders and Schizophrenia of School-Age Children (KSADS) (danish V)	Genetic study of SLI in a british cohort of SLI Families	regression co-efficients	goodnes of fit model				
		ASD (based on K-SADS lifetime criteria)	Reynolds Intellectual Screening Test (RIST) (for non-verbal intelligence)		Nagelkerke's pseudo R squared	logistic regression coefficients				
sudre et al 2020	PMID:33173195	ADHD symptoms ADHD (based on K-SADS lifetime criteria)	Diagnostic Interview for Children and Adolescents for parents	ADHD PRS (PGC-ADHD)	t & p-values odd-ratio	mixed effect logistic regression	~8 years	Communities surrounding study site		36218 cases
		General Intelligence Height (negative control)	Wechsler scales		effect sizes	condition al random forests				
		Working memory	Beery-Butenika Developmental Test							
		visual and motor skills								
sudre et al 2020	PMID:33173195	Processing speedADHD symptoms	Woodcock Johnson III Test of Cognitive AbilitiesDiagnostic Interview for Children and Adolescents for parents	ADHD PRS (PGC-ADHD)	t & p-values	mixed effect logistic regression	~8 years	Communities surrounding study site		362
		General Intelligence	Wechsler scales		effect sizes	condition al random forests				
		Working memory	Beery-Butenika Developmental Test							
		visual and motor skills								
		Processing speed	Woodcock Johnson III Test of Cognitive Abilities							
Hanniga n et al 2021	DOI: 10.1093/schbul/sbaa193	Emotional and behavioural Psychopathology	CBCL	SCZ PRS	R-squared	latent growth models	18 months to 8 years	Norwegian Mother and child birth cohort	europ ean	15 105
		Anxiety	SCARED							
		Depressive symptoms				entropy and fit inidces				
		Disruptive behaviour disorders	RS-DBD			rubin likelihood ratio test				
Hanniga n et al 2021	DOI: 10.1093/schbul/sbaa193	Conduct problems Emotional and behavioural Psychopathology	RS-DBDCBCL	SCZ PRS	R-squared	latent growth models	18 months to 8 years	Norwegian Mother and child birth cohort	europ ean	15 105
		Hyperactivity and inattention Anxiety	RS-DBDSCARED							
		Depressive symptoms								

		Disruptive behaviour disorders	RS-DBD			entropy and fit inidces rubin likelihood ratio test				
		Conduct problems	RS-DBD							
		Hyperactivity and inattention	RS-DBD							
Aguilar-Lacasaña et al., 2020	https://doi.org/10.1017/S0033291720003189	Working memory	n-back task	ADHD PRS	beta-coeeficients	linear mixed-effects models	7 to 11			
		Attention perfromance	ANT (computer version)	ASD PRS		random effects models		Population based cohort (BREATHE project)		1667
		ADHD symptoms	DSM-IV							
Aguilar-Lacasaña et al., 2020	https://doi.org/10.1017/S0033291720003189	Working memory	n-back task	ADHD PRS	beta-coeeficients	linear mixed-effects models	7 to 11			
		Attention perfromance	ANT (computer version)	ASD PRS		random effects models		Population based cohort (BREATHE project)		1667
		ADHD symptoms	DSM-IV							
Jansen et al 2020	doi:10.1111/jcpp.12759PFI_12mmX178mm.pdf + eps format	ADHD symptoms	CBCL 1.5-5	ADHD PRS	OR	logistic regression	6 to 18	"Inside out" outpatient sample		ADHD/ASD n= 688
		ASD symptoms		ASD PRS						ADHD n= 280
				SCZ PRS						ASD n=295
Jansen et al 2020	doi:10.1111/jcpp.12759PFI_12mmX178mm.pdf + eps format	ADHD symptoms	CBCL 1.5-5	ADHD PRS	OR	logistic regression	6 to 18	"Inside out" outpatient sample		ADHD/ASD n= 688
		ASD symptoms		ASD PRS						ADHD n= 280
				SCZ PRS						ASD n=295
Akingbuwa	doi:10.1001/jamapsychiatry.2020.0527	ADHD symptoms	SDQ	MDD PRS	beta-coefficients	Multivariate meta-analysis	6 to 17	GWAS from 7 cohorts :	europ ean	42 998
		Internalising symptoms	CBCL	NEU PRS	CI			ALSPAC		

		Social problems	CBCL		SE			Child and adolescent twin study (Sweden)		
			CBCL(Youth Self Report)					Generation R		
Akingbuwa	doi:10.1001/jamapsychiatry.2020.0527	ADHD symptoms	SDQ (Conners' Parent Rating Scale)SDQ	MDD PRS	beta-coefficients	Multivariate meta-analysis	6 to 17	MoBa GWAS	european	42 998
		Internalising symptoms	Autism-Tics, ADHD and Other Comorbidities InventoryCBCL	NEU PRS	CI			Northern Finland Birth Cohort of 1986· ALSPAC		
		Social problems	Screen for Child Anxiety Repated Emotional Disorders CBCL		SE			Twins Early Development Study Child and adolescent twin study (Sweden)		
			Short Mood and Feelings Questionnaire CBCL(Youth Self Report)					Generation R		
			Screen for Child Anxiety Related Emotional DisordersSDQ (Conners' Parent Rating Scale)					MoBa		
			Short Mood and Feelings QuestionnaireAutism-Tics, ADHD and Other Comorbidities Inventory					· Northern Finland Birth Cohort of 1986		
			Rating Scale for Disruptive Behavior Disorders Screen for Child Anxiety Repated Emotional Disorders					Twins Early Development Study		
			Short Mood and Feelings Questionnaire							
			Screen for Child Anxiety Related Emotional Disorders							
			Short Mood and Feelings Questionnaire							
			Rating Scale for Disruptive Behavior Disorders							

Appendix 2: Chapter 3 EA systematic review supplementary material

Inclusion Criteria

- Full-text published studies evaluating associations between EA deviation and developmental and mental health outcomes in childhood and adolescence will be eligible for inclusion.

Exclusion Criteria

- Studies with a primary focus on adult populations (aged > 18 years old)
- Studies on animal models
- Epigenome-wide association studies (EWAS) without an EA deviation and developmental/mental health component
- Studies not published in English

Search Strategy:

Databases and sources

- PubMed (including MEDLINE)
- Cumulative Index of Nursing and Allied Health (CINAHL) via Ebscohost
- Psych Info via Ebscohost
- Scopus
- Grey literature (Web of Science, all databases)

Table 1. EA systematic review search strategy

Spider tool		Key words
Sample	Children and adolescents	"young" OR"," teen" OR "child*", "youth", "adolescent", "young people"
Phenomenon of Interest	Epigenetic age (EA) deviation i.e., EA acceleration, EA deceleration,	"Epigenetic ageing", "biological aging", Epigenetic age acceleration",
Design	All studies i.e., full-text articles	
Evaluation	Development and mental health	Internalizing disorder, externalizing disorder, neurodevelopment disorder, developmental psychopathology Cognitive development, behavioural disorders
Research type (qualitative, quantitative, mixed methods)	Mixed methods, quantitative research	

Table 2: Data extraction and appraisal for the EA deviation systematic review

Study ID	Title	Reviewer	Title of stu	Study ID (I	Aim of stu	Main findi
Suarez 2018	The epigeneti	Consensu	The epige	PMID: 300	We testec	Epigeneti
Tollenaar 2021	Internalizing s	Consensu	Internalizi	33460784	examined	Internalisi
CerveiradeBaumont 2	Telomere leng	Consensu	Telomere	33833304	We aimed	No differe
Dammering 2021	The pediatric l	Consensu	The pedia	PMID: 346	Using a pe	Children v

*This table has been embedded from an Microsoft Excel workbook.
Please click on the below text for the full document:
link to full excel spreadsheet

Appendix 3: Chapter 4 supplementary material

Correlation plots highlight non-linearity in relationship between the predictor (ADHD PRS) and the outcomes of interest (i.e. cognitive, Language and motor developmental outcomes; as well as internalising and externalising behaviour). Non-linearity in data violates the basis of linear regression models. Thus, only logistic regression models were utilised for these analyses.

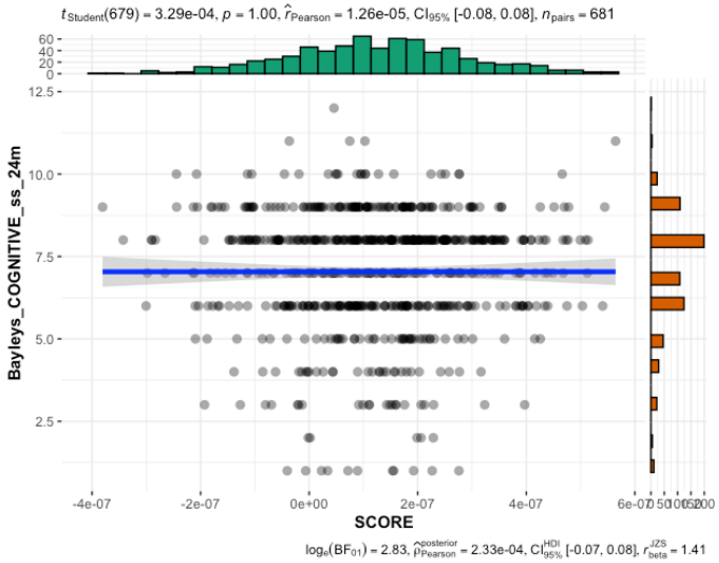

Figure 1: Correlation plot of cognitive development vs ADHD PRS. The outer axis display distribution of the data

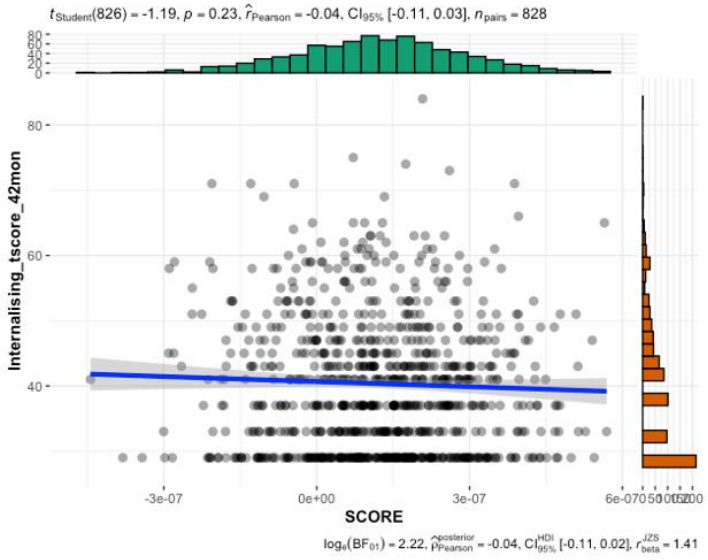

Figure 2: Correlation plot language development vs ADHD PRS. The outer axis displays distribution of the data

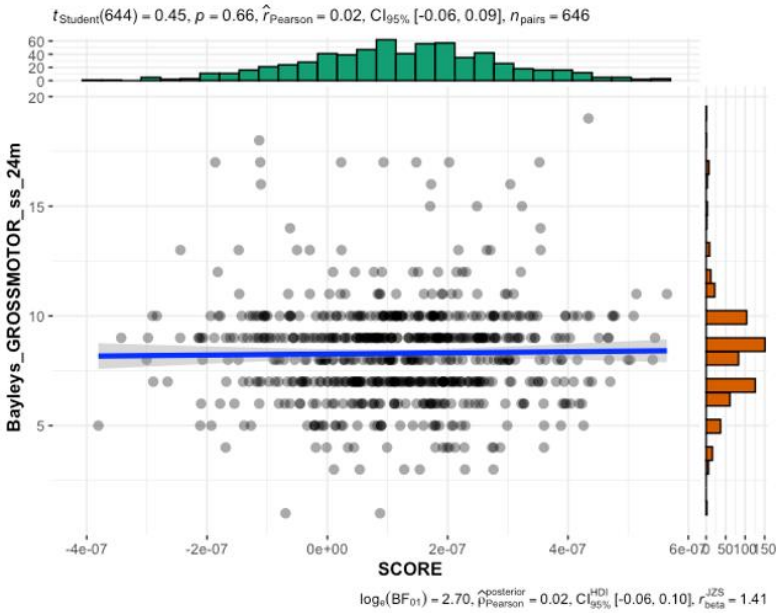

Figure 3: Correlation plots of motor development vs ADHD PRS. The outer axis displays distribution of the data

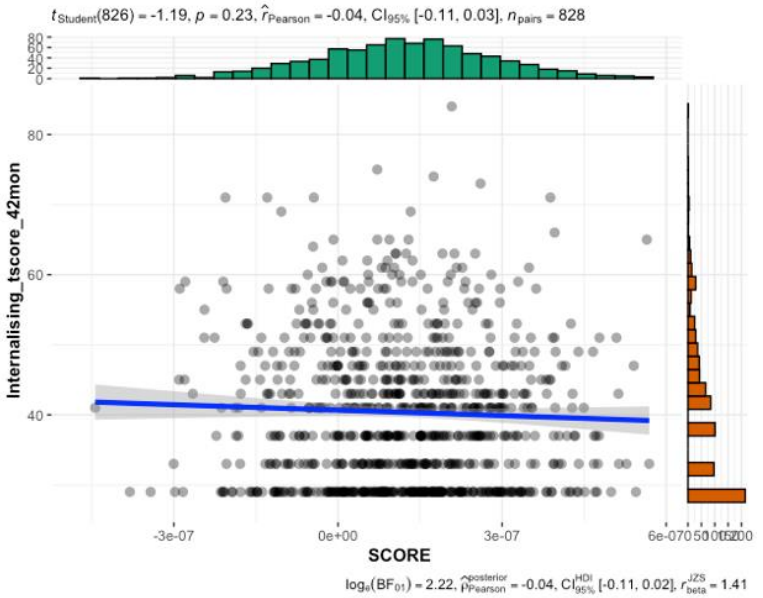

Figure 4: Correlation plots of Internalising symptoms vs ADHD PRS. The outer axis displays distribution of data

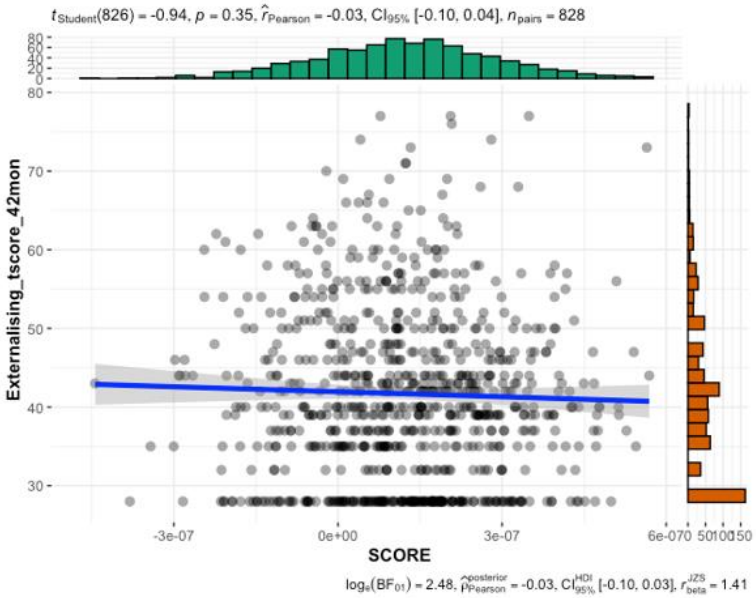

Figure 5: Correlation plots of externalising symptoms vs ADHD PRS. Outer axis displays the distribution of the data

Appendix 4: Chapter 6 supplementary material

Table 1. MRS measured across different p-value thresholds, Epic array samples

P5e-06	P5e-05	P5e-04	P0.005	P0.05
0.571304815634405	4.90996228247813	30.8728391768203	131.140652454139	1144.41699524474
0.639386964096211	4.54714973650889	31.9740248099673	141.455265990179	1214.45966891798
0.441523374044248	5.32032374105467	30.5951259869944	119.732602289724	1047.21590661156
0.576170723979168	5.14585087651121	32.5350655268979	133.641630815127	1140.27800599463
0.474396161395131	4.37739155209737	30.3142190762586	127.085546624461	1136.38299383911
0.615165373627233	4.87144440442409	32.5271864254713	146.508743135752	1230.15794254915
0.495728048611788	3.92096342104163	30.4871672093683	131.347214279394	1146.82430145531
0.474839677140439	4.83288765827967	30.1471720982985	123.752083184696	1100.82342983762
0.500208965242111	4.11615651978919	29.432562089595	133.384070022268	1121.18841241671
0.598540009148466	5.01381279261309	31.201266596721	133.81025746334	1156.18900597059
0.476861103742171	4.79329743890859	31.2355789626389	129.847720581861	1080.66601003301
0.537549114219281	4.88184729527895	33.100219736383	134.367610345203	1174.68089752848
0.520879186016843	4.94251505860803	29.9596268572447	124.065614478097	1075.64353327888
0.528031594461341	4.374773212383	29.4421846893138	124.713898947321	1083.67012716547
0.489436054240095	5.06190381864663	32.9943124809231	143.864818844388	1209.29841330287
0.516078624947911	4.87118332847516	30.7224632967022	127.67189949752	1105.77978767605
0.515308280380362	3.84663479084965	28.7672407805703	121.06383313138	1034.07793353495
0.549768556277727	4.78838730682494	32.8889952793786	138.113406674884	1200.19514238782
0.557787633551554	5.17110093975454	32.1658792046066	131.169378608787	1140.11651192058
0.51828432193711	3.43520147508177	29.8178280499339	135.094387785397	1127.37243665596
0.686008245746637	4.66253726426699	29.5758600371265	124.24234550796	1126.12558525226
0.507396548544105	3.8246099330603	26.6084548350292	108.249396869887	960.201367407222
0.613367576418517	4.50568428931392	28.7393331035993	118.513128460778	1054.28790281722
0.570485254952593	4.62293341848092	29.3764885483884	122.075965519731	1091.80509873726
0.459895657980248	4.99587887072579	31.0254198313178	132.740927403377	1155.02756989577
0.433267226905301	5.13779803585357	32.1278156550805	135.357708369619	1138.94816464632
0.480301259112471	4.29860496512644	28.6742706875878	126.146605508659	1064.0909928785
0.451775040905461	4.58741415790994	29.2954679730107	113.855726259059	1040.68111753277
0.608417460827605	4.77907088246315	30.6177270807432	124.102047284387	1094.58415620636
0.574688148199544	4.80124867825569	31.0093608197414	133.563806621198	1148.75211929865
0.600463209344847	4.93292909037747	29.8079913373916	131.334228563681	1129.16625312136
0.559806385948677	4.79929860348503	31.0105879628654	135.723853685523	1169.3624595632
0.502265722782897	5.48437570526742	33.8279715275693	145.47039827919	1225.03150329849
0.478014410815048	4.49422869536935	29.7350544965245	122.378568897326	1075.05357297265
0.63492326249518	5.13469934119004	31.855762223555	136.093829975886	1148.30392791978
0.545197194696718	4.79042559777694	32.2226651539232	140.190871006542	1171.07142541113
0.580568997012316	5.05910385033566	30.6436708842041	131.384518474669	1122.648946515
0.5472068149306	4.57562823852186	29.932856019259	129.261381206955	1069.07684147249
0.560762471653617	5.40487872001575	31.432851987267	133.721750968755	1148.97147134946
0.509516675630465	5.16943464529288	31.019348700661	126.618326359293	1115.19287893158

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0.649091043904993	4.90639577880985	31.269953612814	128.832763793279	1119.25239569697
0.491189899890095	4.56843436433701	28.3575342037975	119.844061593824	1053.43072853295
0.53274948795291	5.54490244471971	33.4387600938316	139.969017071051	1208.32737472878
0.513042528504214	5.12987136836709	32.0102303009105	133.982548119071	1175.79341702649
0.533295900288315	4.68614778828033	30.9099493033406	121.582755528357	1115.68057581742
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0.484721029248685	5.11817111204609	30.1872033125973	126.299296401016	1108.24777466096
0.568283525406926	5.45365850351337	33.3041757649311	138.261468215054	1230.08102297916
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0.562515458118576	4.42470816859582	29.3334463491664	121.21553739977	1073.22830894538
0.416389376806517	4.93672447466965	29.7547690474577	122.421330186901	1086.10718404405
0.575728490871165	5.07165047290618	29.6149872740564	127.031166936532	1093.74081201191
0.667980769953425	4.95189048440574	30.3756744127763	130.564848789311	1127.70030374585
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0.491952112447406	3.7064499801907	29.1385811810063	127.767077460147	1097.9714466198
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0.633885793777805	4.60834856547139	31.657455345844	131.269349283419	1133.4032418832
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0.418223576747663	4.1451799280599	30.0955977134029	128.01503442094	1132.24940015095
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0.476045832900924	4.79861427613739	31.2713157694041	127.922544094031	1117.45478611314
0.507645087776582	5.23122649938185	31.121274870935	130.521424561253	1139.01819450637
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0.576536189217023	4.707643813027	28.18646427489	122.54974687485	1060.89769463888
0.430461386378849	4.67514171597993	31.1303909409366	126.962577149584	1131.73751269845
0.546128758483864	5.05804441671002	30.6095221916527	123.870796942063	1088.25356837462
0.533859894576686	4.59872554598666	31.3497645826621	131.657622057754	1115.37097252307
0.456408528791858	4.43628183355061	29.7018056599824	132.049034714341	1095.83252957036
0.492124777326709	5.2560652429935	31.3963305854647	130.276308446185	1123.24388990798
0.567026622599652	4.84397871170708	31.1809693582388	135.789452979422	1144.64348706148
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0.52150792962376	4.34043589061788	29.1604887526477	118.169669065912	1030.4918449947
0.534513153770571	5.56061331551119	32.1547050426648	132.639676229223	1150.96719718913
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0.453312282666418	4.25676798028359	30.2474343793259	129.352382497077	1141.43989833529
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0.493659925493592	5.03907441218711	29.2276648618575	126.336711595252	1099.63098815558
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0.457755982735566	4.75984923026592	32.4434102676632	145.263557052271	1244.24813326391
0.522081092733869	4.47768034480618	27.9560764392095	119.293866516278	1050.04224928553
0.612586293931469	4.32139595236511	30.1753869829375	124.853528480191	1082.90478629657
0.539920553698737	4.65874804967487	30.6428500443807	133.635774462593	1162.33590815173
0.515969417894419	4.84956716296119	29.8595142569102	122.002601134616	1078.05919416105
0.716006418675383	4.2070803524014	29.1440758732432	120.720731516805	1111.18021159253
0.488787465284138	4.73272074971673	30.611800332154	131.395496038091	1138.40043170427
0.520922471869047	4.59655426130942	29.5806171903781	129.782101045003	1108.31588603298
0.437929556566162	4.4068873253068	29.4796195252501	120.571648997904	1058.43514224962
0.419236485207207	5.10603821491112	30.6516725837645	124.738531695763	1103.78358891651
0.533953508035776	4.81533619994421	29.1775445870481	121.42626462122	1078.61337793461
0.513863164802069	5.29412096261348	33.1271078661313	132.431985997741	1152.31590155314

Table 2. MRS measured across different p-value thresholds, 450K array samples

P5e-06	P5e-05	P5e-04	P0.005	P0.05
0.630948644293131	-1.96578075735281	31.6878565685424	88.8126854269106	1192.53138185893
0.48285276810801	-2.28086708298588	26.3334983852869	75.8284281624256	1051.69595119809
0.513873282574568	-2.25093016154913	27.2018284021378	65.8577307695455	1051.19713862013
0.524924901278533	-2.10611305113737	30.4572957652578	84.2440798360108	1204.48202483368
0.532089587472709	-2.39152943033854	28.7294482084602	77.5291207419301	1157.8167876648
0.575012010988836	-2.36554013570775	30.0198449869014	95.0248548488385	1216.87171059518
0.549757994867172	-2.41579890435024	29.389933480562	79.0690489229929	1181.61777070778
0.527965837158117	-2.60077389032159	25.6116731444932	70.4619734103271	1016.18927037006
0.475738945918406	-2.58140685911416	29.191704062089	72.8037422296687	1145.13185833503
0.485025267726546	-2.34703496130074	29.1667695470204	84.0305137473622	1178.72595663307
0.526173037035834	-2.16076060489107	30.2735274093354	86.7002593081732	1242.69946394571
0.473891927204927	-2.32916168294994	29.3996824506175	80.4151478956677	1194.46743155075
0.593925245027116	-2.00338117729513	29.3043266511504	80.7831192986217	1156.52319365613
0.480980672559152	-2.12482632042444	28.9014841588059	75.8226934099475	1145.1021983005
0.533380447099508	-2.43878040101521	29.6928249654206	86.7606573445087	1221.9003494242
0.513794577731722	-2.24077881746907	29.0635535286614	78.7088449570793	1170.02398473013
0.495414668116453	-2.20668188035261	30.7762430862592	92.028590545838	1217.84099807263
0.536334864717957	-2.18329883806553	30.7600828114808	83.3797596944679	1179.72213765255
0.53371676635408	-2.44717287629419	29.2039205510962	75.3550045924646	1135.38656526162
0.478202120028567	-2.31839638800942	29.7788661690725	85.6812409039897	1194.8259319589
0.578822310827392	-2.16681562038086	31.7881013615708	98.0980244162092	1267.85939085066
0.54158580119532	-2.17300415867546	31.0628531187187	96.3462582253038	1280.35240357569
0.604872017631711	-2.14798716757434	30.5490043069456	88.7440409046147	1237.91522672607
0.492982206629854	-2.3927028311067	26.1140650974007	74.8017455355042	1049.73981725726
0.588217183439807	-1.53380801269288	33.1349034172432	101.987845610103	1324.9211828347
0.480628360770776	-1.6750036202698	32.3425456239551	89.1036304551755	1209.16501935229
0.468277236288356	-2.3168123337727	29.3291508534648	88.2127107199302	1181.99666307946
0.476102601415648	-2.3748031944253	28.8363964755704	84.3823679029001	1204.98952740399
0.484245819170103	-2.75221023788937	27.4095634023787	83.8609816281281	1110.36847144726
0.601662238676753	-1.88273957474423	29.696191273641	87.0207669093196	1164.02413865267
0.476413375472346	-2.41779895233175	27.6585944332568	75.3593590566469	1147.60010036999
0.447982442985635	-2.64146034230566	27.8333428777597	78.4827417810275	1092.84854879723
0.586385388826151	-2.07544472336661	30.4623291173855	84.0818565448665	1173.54477143461
0.409617542401503	-1.94679893464847	31.6695080347771	92.2651579966086	1216.11652804386
0.47582710226038	-2.34419795104934	28.4007620709932	81.5279376257304	1177.0620672869
0.517207863859029	-2.4150161666793	29.2071947256582	90.2777609001583	1245.50949485274
0.542114831961136	-1.82003952450482	30.0693128072512	85.707051890134	1191.78923819827
0.494868630189979	-2.3931870460366	28.9711566165931	82.4560378326545	1151.64742548285
0.498329402843367	-2.29298451657083	27.2191391836176	74.9435003264045	1073.26556160338
0.622366605796016	-2.12508491883512	31.3678999695609	106.160567190182	1314.34445999051

0.49119410910007	-1.84474502279803	30.0622822119273	86.576027442747	1210.91400413968
0.595985761772147	-1.81309144837929	32.6541244511939	100.560798605555	1317.00004092351
0.590445027556534	-2.01266785761266	30.5361483867289	82.5775625903383	1179.9184124551
0.569426898684602	-1.62470685334901	32.5575640411376	97.2998976041226	1304.73249783135
0.490750081432143	-1.80288742427212	32.1609455384221	89.9906442028559	1219.2159866881
0.496181641995209	-1.8044937627416	32.3984527389191	90.1185201226085	1255.77020064574
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0.497282191309378	-2.10129055261563	30.4293389262837	79.3433473898785	1170.05583656619
0.531889817612944	-2.25232154959746	29.157065407164	73.7374534333035	1130.44840352176
0.607605995685906	-1.59955968440981	29.658929112271	90.3577318701443	1211.01652415775
0.496762040715774	-2.13137112292418	31.0677626810937	91.3524890672937	1232.08571528376
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0.625070596091623	-1.87804598956275	31.1531238962548	92.8849827196986	1208.96714099281
0.486588636384743	-1.92295972109173	29.6267378746818	82.3891973945752	1192.82725111686
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0.511721149481333	-2.06119927111406	31.5516786635359	85.4576061592558	1218.5528437735
0.494108268588827	-2.72655452647318	29.0930179342805	83.5427522384739	1161.43475632603
0.463129515319372	-1.96907663762351	28.2970843667105	77.8920537998679	1154.93057071143
0.457102896769467	-2.40328710774983	27.7949926120786	65.7732431136125	1071.19972474106
0.53401888234382	-1.66218825813705	32.4063928237479	88.0837191307673	1233.56941011246
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0.47774785651345	-1.76313625646146	30.5597797744803	93.5425153370843	1228.666951464
0.547319239526708	-2.53280959492929	27.7113513957044	67.3477297841271	1078.99363089717
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0.478681188601748	-2.17562829806491	27.6724383963376	74.6100174318481	1092.86040153479
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0.571661014911642	-1.55302380576357	33.3129994892145	92.3339355649782	1266.20815054475
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0.527957494025424	-1.8188078659238	30.7421022750523	97.8251628987835	1244.12251002661

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0.4793887637625	-2.1567027282315	30.310313063639	82.7832530432639	1200.99966131122
0.456196139837035	-2.42319760698404	28.1299700245188	81.0203318439908	1157.40238342743
0.495134764337052	-2.78082707348775	29.1759099392215	86.9904143537656	1164.10276494227
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0.453674395009736	-2.36198471392604	29.4531341096409	81.8008251244854	1168.28564713415
0.539476656530717	-2.21601256572627	31.2258532639732	92.9474508789931	1220.57623442534
0.553387667500939	-2.38816393680378	29.8160650925661	82.6786786956727	1158.49813728793
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0.434077146550849	-2.28845464543562	30.1137747617274	82.186197532644	1191.6213061467
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0.468887934364463	-2.19088981441421	27.6740943766746	65.6764254371225	1071.39131993188
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0.506159501010478	-2.21182798837476	29.561140899156	75.3377568844251	1132.93037835484
0.489160656588068	-2.20001969799607	28.1694376327009	71.3374331836506	1061.2965692249
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0.568404296826359	-1.46019899919767	32.3550469251702	89.2366233258785	1226.14131116972
0.44207327576761	-2.6741109160375	29.1352229708245	78.0609935569942	1154.90044479106
0.521283037017869	-2.18119803045764	28.293350383772	61.2556379195873	1058.40820332957
0.606888594499129	-2.03564049010569	29.2537685915821	76.4342792751361	1131.19417249986
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0.46614282670405	-2.4349530470909	29.0024587571982	85.921820741938	1139.694590193

Appendix 5: Ethics paperwork

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room G50- Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6492
Email: hrec submissions@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

30 July 2021

HREC REF: 289/2021

Dr N Koen
Department of Psychiatry & Mental Health
Neuroscience Centre, GSH
Email: NasLassja.koen@uct.ac.za
Student: MYKLIH001@myuct.ac.za

Dear Dr Koen

PROJECT TITLE: GENETIC AND EPIGENETIC ASSOCIATIONS WITH DEVELOPMENTAL PSYCHOPATHOLOGY IN CHILDHOOD (LINKED TO 401/2009: DRAKENSTEIN CHILD HEALTH STUDY)-PHD CANDIDATE-MS LIHLE MOYAKHE-SUB-STUDY LINKED 401/2009

Thank you for your response letter, addressing the issues raised by the Faculty of Health Sciences Human Research Ethics Committee.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

This approval is subject to strict adherence to the HREC recommendations regarding research involving human participants during COVID -19, dated 17 March 2020, 06 July 2020 & 01 July 2021.

Approval is granted for one year until the 30 July 2022.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

The HREC acknowledge that the student: Ms Lihle Moyakhe will also be involved in this study.

Please quote the HREC REF 289/2021 in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval, where necessary, before the research may occur.

UNIVERSITY OF CAPE TOWN

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

FHS016: Annual Progress Report / Renewal

HREC office use only (FWA00001637; IRB00001938)			
This serves as notification of annual approval, including any documentation described below.			
☑ Approved	Annual progress report	Approved until/next renewal date	30.7.2024
☐ Not approved	See attached comments		
Signature Chairperson of the HREC/ Designee		Date Signed	24/7/2023

Note: Please email this form and supporting documents (if applicable) in a combined pdf-file to hrec-enquiries@uct.ac.za.
Please clarify your plan for research-related activities during COVID-19 lockdown.
Please use the latest form found on our website:
http://www.health.uct.ac.za/fhs/research/humanethics/forms

Comments to PI from the HREC

Principal Investigator to complete the following:

1. Protocol information

Date (when submitting this form)	24 July 2023		
HREC REF Number	283/2021 (linked to 401/2009	Current Ethics Approval was granted until	30 July 2023
Protocol title	Genetic and epigenetic associations with child development and mental health **(linked to HREC REF 401/2009: Drakenstein Child Health Study)**		
Protocol number (if applicable)	N/A		
Are there any sub-studies linked to this study?		☐ Yes	☑ No
If yes, could you please provide the HREC Reference number for all sub-studies? Note: A separate FHS016 must be submitted for each sub-study.	N/A		

(Note: Please complete the Closure form (FHS017) if the study is completed within the approval period)