CHILDHOOD PSYCHIATRIC SYMPTOMS: GENETIC ARCHITECTURE AND INTERGENERATIONAL CONTRIBUTIONS

Eshim S. Jami

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Jami bibi and *Jami sahab*,
this is for you

CONTENTS

Chapter 1

Introduction

Context

Symptoms of many psychiatric disorders emerge in childhood or adolescence and can show a chronic course across the lifespan[1,2]. Understanding the aetiology of early symptoms, before they develop into disorders, is important for leveraging prevention and early intervention strategies. This is of high societal importance, as psychiatric disorders are highly prevalent worldwide and a leading contributor to the global burden of disease[3,4]. Traditional quantitative genetics methods have highlighted the contribution of genetic factors in explaining individual differences in psychiatric behaviours, and the current era of molecular genetic research has made tremendous progress in identifying the genetic basis of adult psychiatric disorders in recent years. Less is known about the genetic architecture of childhood symptoms and the role of genetic variants over their developmental course. Knowledge is also limited about the extent to which intergenerational contributions to childhood psychiatric behaviours are explained by genetic relatedness or environmental effects. Various parental characteristics are predictive of offspring psychiatric risk, but genetic and environmental mechanisms that explain associations between parents and children are intertwined and need to be disentangled. Recent advances (including the availability of genetic data within longitudinal birth cohorts and the development of novel family-based genetic designs) provide new opportunities for addressing these gaps in our knowledge. This thesis uses novel molecular genetic statistical designs to investigate the genetic architecture of common childhood psychiatric symptoms, and examine the mechanisms and impact of intergenerational contributions.

Genetic architecture of childhood psychiatric symptoms

Psychiatric behaviours are complex traits that are influenced by a multitude of genetic and environmental factors, as well as their interplay. The relative importance of genetic and environmental influences in explaining individual differences in psychiatric behaviours can be estimated in twin and family-based designs, without knowledge or measurement of specific genes or environments affecting the trait. The classical twin method compares resemblance between monozygotic (identical; share 100% of their genes) and dizygotic twins (non-identical; share 50% of their genes on average) to estimate the extent to which variance in a target trait within a studied population is due to genetic factors, the shared environment (common influences that increase resemblance between twins, e.g. the home environment, school, neighbourhood), and the non-shared environment (unique influences that make twins differ from one another)[5].

Findings from twin literature show that commonly occurring childhood psychiatric symptoms are substantially heritable; i.e. differences in these traits amongst the population are explained by differences in genetic factors[6] (Table 1). Shared environmental influences seem important for some traits (internalising and externalising symptoms), but not others (attention-deficit hyperactivity disorder symptoms)[6]. Beyond the estimation of variance components, twin studies provide important clues about the contribution of genetic and environmental influences across development. For internalising and externalising symptoms, the period from early childhood to late adolescence sees an overall rise in heritability and a decline in the influence of the shared environment[7–9], indicating an increasing importance of genetic influences over development. New genetic influences (genetic innovation) emerge over development, particularly during the transition from childhood to adolescence for internalising and externalising symptoms, while earlier genetic influences seem to become less important (genetic attenuation)[10,11]. Stability in both internalising and externalising symptoms over age is largely explained by stable genetic effects, while changes seem to be partly driven by genetic innovation, and more so by non-shared environmental influences[10]. These findings highlight the importance of considering a developmental perspective when exploring the effects of specific genetic (or environmental) risk factors on psychiatric behaviours.

Table 1 Common psychiatric traits in childhood, and the underlying variance components explaining individual differences in studied populations. (Sources: Polderman et al.[6], Nikolas et al.[12])

Childhood psychiatric trait	Description	Variance explained by:		
		Genetic factors (A)	Shared environment (C)	Non-shared environment (E)
Internalising symptoms	Internally-focused behaviours, such as anxiety and depression	40-50%	20%	30-40%
Externalising symptoms	Externally-focused behaviours with poor impulse control, such as aggression, conduct problems and hyperactivity	47-49%	16-19%	32-37%
Attention-deficit hyperactivity disorder (ADHD) symptoms	Externalising behaviours, characterised by hyperactivity and an inability to sustain attention	71-73%	~0	27-29%

Molecular genetic designs

While twin studies reveal an important role of genetic factors in explaining individual differences in psychiatric traits, identifying specific genetic variants that influence their development and progression requires the use of molecular genetic methods. Due to major advances in genomic methodologies and the increased availability and affordability of genotyping arrays, it is now possible to systematically study the effects of measured genome-wide DNA variation on complex traits. The most common type of genetic variation (and the exclusive focus of this thesis) is a single nucleotide polymorphism (SNP), which is a single base pair change at a specific location in the DNA sequence. A genome-wide association study (GWAS) can identify the involvement of specific genetic variants in a hypothesis-free way by estimating associations between millions of SNPs and a target phenotype (trait) in a large sample of unrelated individuals[13]. This represents a significant shift from the study of related individuals in twin and family-based designs. As very large sample sizes are required to detect the small effects of individual genetic variants, consortiums such as the Psychiatric Genomics Consortium (PGC) were formed to pool data and facilitate large-scale global collaboration for genetic discovery. Subsequently, GWASs of psychiatric disorders in adult populations have seen tremendous success in recent years, leading to the identification of many genetic loci with robust associations[14]. The results of GWASs are being followed up with functional analyses to understand the biological mechanisms through which genetic variants ultimately act on psychiatric traits[15]. It is hoped that uncovering pathways from SNPs to biological processes, will help to optimise drug treatments and identify new targets for drug development.

The availability of large samples of genotyped individuals has led to the development of other genomic methods which provide further insight into the genetic architecture of psychiatric traits. This includes the estimation of the proportion of variance in a trait that is attributable to genome-wide SNPs (SNP-based heritability), and the extent to which phenotypic similarity in two traits is due to overlapping genetic effects (genetic correlation). Both of these can be estimated by using either individual-level data within a sample or summary statistics from GWASs. Molecular genomic research over the past decade shows that the genetic architecture of psychiatric disorders is highly polygenic, in that they are under the influence of many variants of small effects[15]. The cumulative effects of common genetic variants included in GWASs explain considerable proportions of variance in major psychiatric disorders, currently accounting for 28% of the twin-heritability estimate of Major Depressive Disorder and 32% for ADHD[16]. These figures are likely to rise with the inclusion of more samples, but

will have a ceiling limit as variance explained by rare and unmeasured variants will not be captured. Genetic studies have also revealed substantial genetic correlations amongst psychiatric disorders, which can help to explain their frequent comorbidity in clinical populations[17]. These genetic correlations point to pleiotropic genetic effects (the same genes contributing to multiple traits) and could imply common biological processes underlying distinct disorders.

Application to childhood psychiatric symptoms

While the overall field of psychiatric genetics is making rapid progress in understanding the genetic basis of psychiatric disorders, a developmental perspective is necessary for understanding the involvement of common genetic variants in the emergence, maintenance and co-occurrence of symptoms from childhood to adulthood. Age-specific estimates of SNP-based heritability for common childhood psychiatric symptoms are generally low and non-significant, likely due to lack of power in most studies[18]. Even so, studies have indicated genetic commonalities both within childhood psychiatric symptoms, and between childhood symptoms and adult psychiatric disorders[18]. This indicates that common genetic variants are important for explaining comorbidities amongst childhood psychiatric traits, and stability of psychiatric symptoms over the lifespan. GWAS discovery for common childhood psychiatric traits has been largely unsuccessful so far due to limited sample sizes, although genetic variants associated with ADHD were identified by combining childhood and adult samples[18]. As twin literature points to sensitive periods of development for genetic influences on internalising and externalising symptoms, age-stratified GWASs in childhood and adolescent samples are needed to understand when in development specific genetic variants exert an effect, which genetic variants have a stable effect over time, and which genetic variants show a limited effect at a specific developmental period. Other important questions that need addressing are understanding the extent to which genome-wide SNPs explain individual differences in common childhood psychiatric behaviours, and how much genetic overlap is present amongst childhood psychiatric symptoms and between childhood symptoms and adulthood disorders.

Intergenerational contributions to childhood psychiatric symptoms

Classical twin studies demonstrate the involvement of both genetic and environmental influences in explaining individual differences in common childhood psychiatric symptoms. The role of parents is ubiquitous in these influences, as parents transmit their genes to their offspring, and provide a rearing environment. Intergenerational associations (between parent and offspring traits) can therefore arise from genetic transmission, when genes present in both parent and child account for the association, as well as environmental transmission, when the environment provided by the parent affects the child's outcome. Genetic and environmental effects can also be correlated; when a child's inherited genes are associated with the parentally-provided rearing environment they experience. This gene-environment correlation can be passive - when parents pass on both trait-associated genes and environment to the child – and/or active and/or evocative - when a child seeks or elicits a parental behaviour due to their own genetically influenced traits[19].

The involvement of genes in parent-offspring associations poses a challenge for conducting and interpreting research investigating phenotypic associations between parental traits and offspring psychiatric outcomes. For example, various parental risk factors (including parental psychiatric history and parenting behaviours) have been linked to the psychiatric traits of their offspring in broader psychiatric literature[20,21]. These associations may reflect genuine environmental effects of parental traits on the child's psychiatric outcome through environmental mechanisms. Alternatively, such associations may be driven by common genetic factors that simultaneously influence both the parental exposure and the child's outcome. As many complex traits show genetic overlap[22], associations between both similar and dissimilar parent and offspring phenotypes could be explained by genetic variance shared by parents and children. When the involvement of genes is not accounted for, studies may come to inflated or inaccurate conclusions about the effect of the environment. Disentangling genetic and environmental mechanisms in intergenerational associations is not straightforward and can only be done with the use of appropriate study designs that account for genetic effects, hence described as genetically informative designs.

Many genetically informative designs (e.g. adoption, children-of-twins, sibling comparison) are available that leverage the approximate degree of relatedness between family members to model or account for genetic effects when

estimating associations between specific parental traits and offspring outcomes (see Chapter 3 for detailed descriptions of these designs)[23–30]. As well as identifying whether parent-offspring associations are accounted for by shared genetic effects, studies using these designs provide valuable knowledge about the types of parental traits that could be associated with offspring psychiatric outcomes through environmental mechanisms. This information may be relevant for identifying modifiable targets for intervention in parents that could potentially improve psychiatric outcomes in children. However, knowledge from most genetically informative literature is restricted in two ways. First, as studies focus on specific parental factors, it is hard to gauge the overall importance of parents in explaining individual differences in childhood psychiatric symptoms. Second, it is difficult to discern a parent-driven direction of effect for most findings. For example, a correlation between parental hostility and offspring aggression could be observed, but the parent's hostility could be both a cause or a reaction to the aggression of the child. Opportunities to disentangle child-to-parent effects from parent-to-child effects are afforded by some designs (e.g., extended children-of-twins method and cross-lagged or stratified adoption studies)[31,32], but this requires additional data that is often not available. A new way of estimating parental effects and disentangling genetic and environmental effects, whilst resolving the direction of effect in parent-offspring associations is through the use of family-based genetic designs.

Family-based molecular genetic designs

Novel family-based genetic designs have leveraged family data to show that the genotype of parents can influence offspring traits both *directly,* through genetic transmission, and *indirectly,* through heritable parental traits that shape the child's environment[33–36]. For instance, children inherit depression associated genes from their parents, and these genes have a direct effect on their own depression sensitivity. Meanwhile, parental genes may affect the child's depression sensitivity indirectly, through the environment created by the parent that is under the influence of the parental genome. An influential study from 2018 used the term *genetic nurture* to demonstrate and describe indirect genetic effects that operate through the environment[33]. These effects can be studied by examining the influence of parental genotypes on an offspring phenotype over and above that which results from the transmission of genes from parent to child. As the parental effect is indexed by their genotype, on which offspring phenotype cannot exert an effect, the direction of effect is evident.

Family-based genetic designs can offer unique insight into the extent to which overall parental factors contribute to individual differences in children's psychiatric outcomes. Shared environment estimates from twin studies provide an overall estimate of the influence of the common environment, but this is not limited to the effect of parents. Additionally, parental factors can also influence offspring through the non-shared environment, if siblings are treated differently by parents[37]. Family-based genetic designs can estimate the variance in an offspring trait that is explained by the overall contribution of parental genetic nurture captured by their genome-wide SNPs[34,35,38]. This is done by incorporating parent and offspring genotypes in the same model to estimate multiple variance components. The direct genetic effect estimates the variance in a behaviour explained by offspring genetic effects, after accounting for the indirect parental genetic effect. In the presence of genetic nurture, this estimate would differ from the SNP-based heritability calculated using only population-based data from unrelated individuals. The contribution of genetic nurture is indexed by the variance in an offspring trait that is explained by the additional effect of parental genotype, after accounting for the direct effect of genes that are also present in the offspring. Additionally, the covariance between direct and indirect genetic effects is used to index a passive gene-environment correlation effect. This captures the effect of genetic variants present in both the offspring and parent, that exert a direct effect through the offspring's genotype, and a genetic nurturing effect through the parent.

Family-based genetic designs can also be used to assess mechanisms of transmission between parent and offspring traits by utilising polygenic scores, which aggregate GWAS effect sizes for alleles to provide an overall index of genetic liability towards a trait for a given individual[39]. Note that polygenic scores have broader applications outside of family-based designs and are commonly used to address many research questions about genetic influences on traits[40]. In family-based designs, parental polygenic scores are used to index their phenotype. This reduces the burden of phenotyping, as the parental trait under study does not need to be measured directly within the study population. Genetic transmission is studied by estimating the effect of genetic variants transmitted from parents to children, which would act on the offspring's phenotype through their genes[33]. Genetic nurture is studied by estimating the effect of parental alleles not passed on to the child[33,36]. The effect of these non-transmitted alleles on an offspring trait can only take place through the environment, via genetically-influenced parental traits.

Application to childhood psychiatric symptoms

Decades of psychiatric research has shown associations between various parental risk factors and childhood psychiatric symptoms. This includes parental psychiatric traits[20], parenting behaviours directed towards the child (e.g. authoritarian parenting, hostility, over-monitoring)[21], broader parental factors (e.g. marital conflict)[41], and family-level characteristics (e.g. divorce, socio-economic status)[42,43]. Without the use of genetically informative designs, it is impossible to determine whether these parental risk factors represent genuine environmental effects which could be targeted for intervention. Novel family-based genetic designs offer new ways of studying mechanisms of transmission within families, with the use of genotypic data. This does not require the measurement of any specific parental variables, as environmental effects are indexed by the parental genome. The application of these designs has identified a robust effect of parental genetic nurture on their offspring's educational attainment, that is partly explained by the family's socio-economic status[44]. Due to their novelty, family-based genetic designs have scarcely been applied to investigate intergenerational contributions to childhood psychiatric outcomes, but could support and expand knowledge from other studies. The foremost question that warrants investigation is whether genetic nurture effects can help to explain phenotypic variance in childhood psychiatric symptoms. Another important research avenue is the application of family-based genetic designs to investigate whether reported associations between parental traits and offspring mental health outcomes in psychiatric literature reflect genuine environmental effects, or arise from shared genes.

Outline of this thesis

This thesis aims to investigate the genetic architecture of childhood psychiatric symptoms and disentangle the mechanisms and impact of intergenerational contributions. Chapter 2 focuses on the genetic architecture of childhood and adolescent internalising symptoms. Chapter 3 is a literature review of genetically informative studies investigating associations between parental characteristics and offspring mental health and related outcomes. Chapters 4 to 6 are family-based genetic studies that primarily focus on understanding intergenerational contributions to common childhood psychiatric symptoms, but also provide insight into their genetic architecture. Chapter 7 is a general discussion that brings together the findings of this thesis. A brief description of each chapter is outlined below.

Chapter 2 is a GWAS aiming to identify genetic loci associated with the development and course of internalising symptoms in childhood and adolescence. Data from 22 cohorts and ~65,000 individuals (with ~250,000 observations), aged between 3 and 18 were combined. All results were meta-analysed to produce a GWAS of overall internalising symptoms, while stratified analyses were used to investigate age effects, as well as rater, and instrument-specific genetic effects. The GWAS results were followed up with preliminary functional analyses and genetic correlations with external traits, with a focus on psychiatric phenotypes.

Chapter 3 is a systematic literature review of genetically informative studies investigating associations between parental characteristics and offspring mental health and related outcomes, published over a 6-year period from 2014 to June 2020. For each parent-offspring association, we reported whether there was evidence of genetic overlap, environmental transmission, and gene-environment interplay. The paper also provides a broad overview of genetically informative designs that can be applied to investigate mechanisms of transmission within families.

Chapter 4 estimates offspring genetic effects and parental genetic nurture effects on childhood internalising symptoms using an approach called M-GCTA (maternal-effects genome-wide complex trait analysis). The effects of both maternal and paternal genetic nurture on anxiety and depressive symptoms in 8-year-olds from the Norwegian Mother, Father and Child study (MoBa) were investigated. M-GCTA results were compared to a non-genomic pedigree-based model.

Chapter 5 is a replication and extension of the earlier M-GCTA study presented in Chapter 4, in a larger sample of genotyped trios from MoBa. The study investigated offspring genetic effects and maternal or paternal genetic nurture on childhood externalising symptoms and ADHD symptoms, in addition to depressive symptoms.

Chapter 6 is a within-family polygenic score study examining the association between parental wellbeing and offspring psychiatric symptoms. Results from two cohorts (ALSPAC; Avon Longitudinal Study of Parents and Children, and NTR; Netherlands Twin Register) were meta-analysed to investigate whether associations between parental wellbeing and childhood internalising,

externalising, and ADHD-related symptoms were explained by shared genetic effects and/or genetic nurture. The results are also informative about pleiotropic genetic effects between wellbeing and childhood psychiatric symptoms.

Chapter 7 concludes this thesis with a general discussion of the overall findings, their implications, and directions for future research.

Chapter 2

Genome-wide association meta-analysis of childhood and adolescent internalising symptoms

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Supplementary Note and Tables:

ABSTRACT

Objective: To investigate the genetic architecture of internalising symptoms in childhood and adolescence.

Method: In 22 cohorts, multiple univariate genome-wide association studies (GWASs) were performed using repeated assessments of internalising symptoms, in a total of 64,561 children aged between 3 and 18. Results were aggregated in meta-analyses that accounted for sample overlap, first using all available data, and then using subsets of measurements grouped by rater, age and instrument.

Results: The meta-analysis of overall internalising symptoms ($INT_{overall}$) detected no genome-wide significant hits and showed low SNP heritability (1.66%, 95% confidence intervals 0.84-2.48%, $N_{effective}$=132,260). Stratified analyses indicated rater-based heterogeneity in genetic effects, with self-reported internalising symptoms showing the highest heritability (5.63%, 95% confidence intervals 3.08-8.18%). The contribution of additive genetic effects on internalising symptoms appeared stable over age, with overlapping estimates of SNP heritability from early-childhood to adolescence. Genetic correlations were observed with adult anxiety, depression, and the wellbeing spectrum ($|r_g|$> 0.70), as well as with insomnia, loneliness, attention-deficit hyperactivity disorder, autism, and childhood aggression (range $|r_g|$=0.42-0.60), whereas there were no robust associations with schizophrenia, bipolar disorder, obsessive-compulsive disorder, or anorexia nervosa.

Conclusions: Genetic correlations indicate that childhood and adolescent internalising symptoms share substantial genetic vulnerabilities with adult internalising disorders and other childhood psychiatric traits, which could partially explain both the persistence of internalising symptoms over time and the high comorbidity amongst childhood psychiatric traits. Reducing phenotypic heterogeneity in childhood samples will be key in paving the way to future GWAS success.

INTRODUCTION

Internalising disorders, including anxiety and depression, are substantial contributors to the global burden of disease[45,46]. Whilst the estimated 12-month prevalence of depression and anxiety disorders in adults is 15%[47], internalising disorders are also present in early life, with an estimated prevalence of 2-3% of depression and 6-7% of anxiety in childhood and adolescence[48]. Prior to the diagnosis of internalising disorders, as many as one in five children self-report internalising symptoms[49]. These early symptoms of anxiety and depression appear to pose a long-term risk, as longitudinal studies show that internalising symptoms in childhood are associated with mood disorders, anxiety, and suicidality in adulthood[50–52]. Findings from twin research show that internalising symptoms have a moderately strong genetic component. 40-50% of individual differences in internalising symptoms are explained by genetic factors[6,53,54]. Moreover, research suggests that both stability and change in anxious and depressive symptoms from early childhood to adulthood are genetically influenced[10,54–56]. However, unlike adult anxiety and depression, investigation of the molecular genetic architecture of internalising symptoms in early life has received little attention thus far and to date, only two studies have applied a genome-wide approach[57,58].

Published in 2013 and 2014, the first genome-wide association studies (GWASs) on childhood internalising symptoms did not identify any genome-wide significant hits for maternal-reported anxiety-related behaviours in children aged seven (N=2,810)[57], or internalising problems in children aged three (N=4,596)[58]. Estimates of SNP-based heritability (the proportion of phenotypic variance captured by single nucleotide polymorphisms (SNPs) included in the GWAS), using genome-wide complex trait analysis (GCTA), were not robust in both studies[57,58]. Other GCTA studies similarly show mostly inconsistent and broad estimates of SNP heritability, mainly due to small sample sizes[59–64]. Large-scale GWASs have led to significant discoveries in adult samples, with now 102 variants identified for depression[65] and 5 variants for anxiety[66]. Given the comparable heritability estimates of adult and childhood internalising phenotypes, the next step in this line of research is to increase childhood sample sizes in order to generate sufficient power to capture the small effects of common variants that have been observed in adult studies.

Here, we present a genome-wide association meta-analysis which aims to identify common genetic variants associated with the development and course of internalising symptoms. The study combines repeated measurements of

dimensional symptom scores from 22 independent cohorts of European ancestry, resulting in an overall sample of 64,641 individuals and 251,152 observations in children and adolescents aged between 3 and 18. All datasets were combined to produce a GWAS of overall internalising symptoms ($INT_{overall}$), with an effective sample size of 132,260. Stratified analyses were used to investigate age, rater, and instrument-specific genetic effects. The overall GWAS of $INT_{overall}$ was followed up with gene-based analyses. Genetic overlap with external traits was examined by computing genetic correlations, with a focus on psychiatric phenotypes. Non-psychiatric traits were also investigated if they were previously found to be genetically correlated with adult anxiety and depression[65–67]. Finally, polygenic scores were computed to test prediction of internalising symptoms in independent samples. With this study, we aim to gain insight into the genetic underpinnings of internalising symptoms throughout childhood and adolescence in order to improve our understanding of the development and progression of internalising disorders.

METHODS

This project was pre-registered at the Open Science Framework (https://osf.io/edas6). Minor deviations from the pre-registration are explained in the Supplementary Note.

Sample and univariate analyses

The sample includes cohorts that are part of the EArly Genetics and Lifecourse Epidemiology (EAGLE) consortium behaviour and cognition working group (https://www.eagle-consortium.org/)[68] and additional cohorts with appropriate data. In total, 22 cohorts of European ancestry participated in the study. Ethical approval was provided by local committees at cohort level. Many cohorts were longitudinal birth or childhood cohort studies with long-term follow-up and multiple raters, e.g., mother, father, self and teacher. Repeated assessments of internalising symptoms within childhood and adolescence, from age 3 to age 18, were included. All cohorts performed univariate GWASs stratified by (i) age, (ii) rater, and (iii) instrument, with a minimum of 450 observations in each analysis. In the absence of diagnostic data, Internalising symptoms were dimensionally measured and positively scored on continuous scales, with higher scores indicating more internalising symptoms. Data was not dichotomized into a case-control design as this would have resulted in a reduction of statistical power[69].

Detailed descriptions of the cohorts, phenotypic measures, and genotyping and imputation procedures can be found in Supplementary Tables 1-6 and the Supplementary Note.

Cohorts that included only unrelated subjects applied a linear regression model. Cohorts with a sample of related individuals corrected for non-independence of observations by either applying a mixed linear model or a sandwich correction of the standard errors. Sex (ascertained through genotype) was included as a covariate in all univariate analyses. Further details about the univariate GWASs are provided in the Supplementary Note and cohort-specific covariates are listed in Supplementary Table 6.

In total, 125 univariate GWASs were collated, with 251,152 observations based on 64,641 unique participants. The observations included ratings by mothers (40.7%), fathers (6.8%), teachers (18.3%), self (19.7%) and siblings (0.7%). An additional 13.8% of ratings were parental reports, where the informant was either the mother or the father. 15.1% of observations were in early-childhood (3 to 6 years), 36.0% in mid-childhood (7 to 10 years), 18.4% in late-childhood (11 to 12 years), and 30.0% in adolescence (13 to 18 years). Twelve instruments were used to measure internalising symptoms, of which the most commonly used were the Strengths and Difficulties Questionnaire (SDQ; 38.2%)[70], Achenbach System of Empirically Based Assessment (ASEBA; 36.7%)[71] and Rutter Children's Questionnaires (8.2%)[72,73].

Meta-analyses and the calculation of SNP heritabilities stratified by age, rater, and instrument

Quality control for each univariate GWAS was performed using EasyQC (Supplementary Text)[74]. After QC, most cohorts retained between 3.4 and 7.1 million autosomal SNPs per GWAS (Supplementary Table 7). An exception was the Philadelphia Neurodevelopmental Cohort which retained fewer SNPs after merging data from different genotyping platforms. To account for dependency of repeated measurements of internalising symptoms within cohorts, the N-weighed meta-analysis approach was applied[75,76]. In short, two N × N matrices, representing sample overlap and phenotypic covariance within cohorts, were created, where N was the total number of univariate GWASs. As there was no overlap across cohorts, sample overlap and phenotypic covariance between cohorts were set to zero. Using the observed sample overlap within cohorts and their phenotypic covariance matrices, expected pairwise cross-trait intercept (CTI) values between GWASs were calculated. The pairwise CTI is approximately equal to the covariance between the test statistics from univariate

GWASs. N-weighted meta-analyses were performed to obtain a multivariate test statistic per SNP, which represents a weighted sum of test statistics, adjusted by the CTI in order to account for sample overlap between GWASs. Formulas for the calculation of the multivariate test statistic for each SNP in the meta-analyses, the CTI between GWASs and estimation of effective sample size to account for repeated measurements (N_{eff}) are provided in Ip et al. supplementary text[76].

A meta-analysis was performed based on the results of all available GWASs on internalising symptoms: $INT_{overall}$. SNP-based heritabilities (h^2) were estimated using linkage disequilibrium score regression (LDSC)[77], first for $INT_{overall}$, and next based on results of meta-analyses stratified according to rater, age, rater-by-age, and instrument (Supplementary Table 8). To ensure that the stratified analyses had sufficient power, a sample size threshold was set so that the total number of observations (N_{obs}) for each meta-analysis was at least 15,000. Rater-specific SNP heritabilities were estimated using assessments from parents (mother and/or father), mothers, fathers, teachers, and self, respectively. Age-specific SNP heritabilities focused on internalising symptoms during early childhood (3 to 6 years), mid-childhood (7 to 10 years), late-childhood (11 to 12 years), and adolescence (13 to 18 years). Rater-by-age SNP heritabilities assessed age effects within and between raters, provided that the univariate N_{obs} exceeded 15,000. Lastly, instrument-specific SNP heritabilities were calculated for SDQ, ASEBA, and Rutter for which the N_{obs} exceeded 15,000.

Genetic correlations across stratified GWAMAs were calculated using LDSC, but only if the z-score of the heritability estimate was ≥ 4, given that the heritability z-score is a good indicator of power and a score less than 4 is considered too noisy for meaningful estimates[78].

SNPs with minor allele frequency < 5% or N_{eff} < 15,000 were removed from further analyses.

Gene-based analysis

Using summary statistics for $INT_{overall}$, a MAGMA[79] gene-based test (v1.8, implemented in FUMA[80]) was performed to identify genes with a significant effect on internalising symptoms. The gene-based test applies a multiple regression model in which p-values from individual SNPs in a gene are combined into a test-statistic for each gene, while accounting for linkage disequilibrium between SNPs. European populations from the 1000 Genomes Phase III reference panel were used to estimate linkage disequilibrium. A total of 18,592

protein-coding genes were assessed for an association with internalising symptoms. A Bonferroni correction was applied to correct for multiple testing ($\alpha = 0.05 / 18{,}592$; $p < 2.69\times10^{-06}$).

Tissue expression and gene-set analyses

Tissue enrichment and gene-set analyses were conducted in FUMA. The tissue enrichment analyses used two types of tissues from GTEx version 8: 30 general tissue types from multiple organs and 53 specific tissue types within these organs. A MAGMA gene-property test was performed to test one-sided relationships between cell type-specific gene expression and disease–gene associations. Bonferroni corrections were applied to correct for multiple testing for the general ($\alpha = 0.05 / 30$; $p < 1.7\times10^{-04}$) and specific ($\alpha = 0.05 / 53$; $p < 9.4\times10^{-04}$) tissue types.

The gene-set analysis was performed with default parameters in MAGMA v1.8. Gene-based P-values were converted to Z values and a between-gene correlation matrix was used as input to perform gene-set enrichment tests. Predefined gene sets from the molecular signature database MsigDB v7.0 were used. In total, 15,484 gene sets were tested. A Bonferroni correction was applied to correct for multiple testing ($\alpha = 0.05 / 15{,}484$; $p < 3.2\times10^{-06}$).

Genetic correlations with external traits

Genetic correlations between internalising symptoms and other phenotypes were investigated using publicly available summary statistics for a curated set of traits (N=27). These primarily included adult psychiatric traits, in addition to other phenotypes selected based on previously identified correlations with adult anxiety and depression[65–67]. Additionally, we obtained summary statistics from the GWA meta-analyses of overall and rater-specific childhood and adolescent aggression[76], that were based on overlapping cohorts and similar statistical methods, and calculated genetic correlations with these traits. The external traits and source studies are summarised in Supplementary Table 9. Summary statistics from $INT_{overall}$ and INT_{self} (for which the z-score of the h2 was ≥ 4[78]) were used. Genetic correlations were calculated using LDSC[77], which calculates genetic covariance between two traits based on all polygenic effects captured by included SNPs. Overlapping samples or population differences in GWAS summary statistics do not bias the computation of genetic correlations in LDSC. LDSC corrects for sample overlap by including a covariance matrix of the cross-trait LD score intercept, which is an estimate of sample overlap and phenotypic correlation. The genetic correlation estimate was based on the estimated slope from regressing the product of z-scores from two GWASs on the LD score. The

LD scores used were computed using 1000 Genomes Phase III European data[78]. Genetic correlations were considered significant at $p < 9.26\times10^{-04}$, after applying a Bonferroni correction for 54 independent tests.

Sensitivity analysis: polygenic score prediction

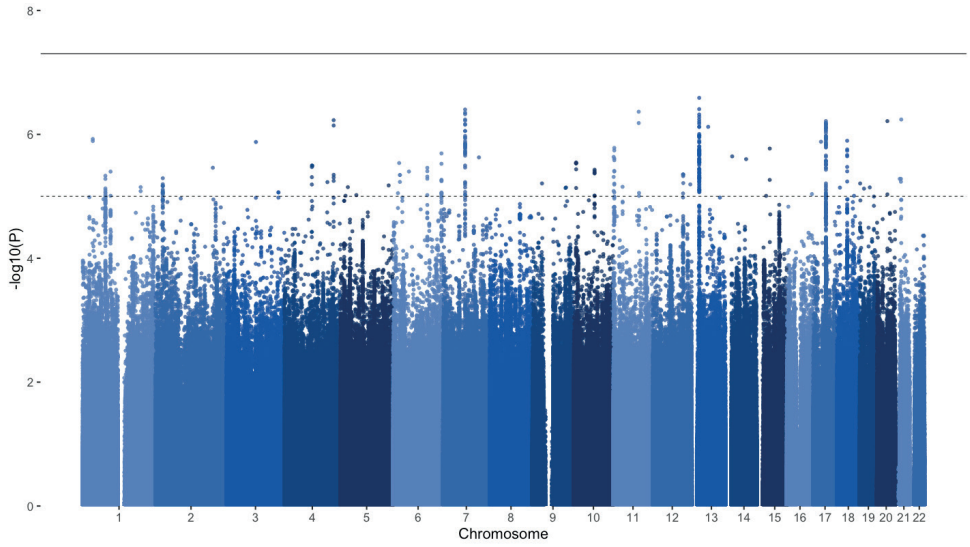
Polygenic score prediction of $INT_{overall}$ was tested as a sensitivity analysis. The Netherlands Twin Register (NTR) was used as the target sample to examine prediction of internalising symptoms in childhood and adolescence. We considered maternal-reported internalising symptoms at age 7 (N=3,845), and self-reported internalising symptoms during adolescence (age 13 to 18, N=2,679), using the ASEBA Child Behaviour Checklist and the Youth Self Report scales[71], respectively. A leave-one-cohort-out meta-analysis omitting data from NTR was performed for $INT_{overall}$. The NTR target dataset was restricted to SNPs with minor allele frequency > 5% and imputation quality of R^2 > 90%. Polygenic scores were constructed using LDpred[81], using a prior value of 0.5 to account for high polygenicity. Associations between polygenic scores of internalising symptoms and internalising problems were examined using Generalized Estimating Equations as implemented in the "gee" package in R (version 3.5.2). To account for relatedness in the target sample, the exchangeable working correlation matrix in gee was used, which applies a sandwich correction over the standard errors to account for clustering in the data. Age, sex, genotyping array, and the first 10 genetic principal components were included as covariates. Polygenic prediction was considered significant at $p < 0.025$, after applying a Bonferroni correction for 2 independent tests.

RESULTS

Overall meta-analysis of childhood and adolescent internalising symptoms

The genome-wide association meta-analysis of $INT_{overall}$ found no genome-wide significant hits (Figure 1). Assuming a N_{eff} of 132,260, SNP-based heritability of $INT_{overall}$ was estimated at 1.66% (95% confidence interval (CI) 0.84-2.48%). The mean chi-squared statistic was 1.086, with an LDSC-intercept of 1.043 (standard error (SE)= 0.0075), indicating that a small part of the inflation in test statistics might have been due to confounding biases, such as population stratification.

Figure 1 Manhattan plot of overall meta-analysis for childhood and adolescent internalising symptoms ($INT_{overall}$).



The solid line represents the significance threshold ($p < 5\times10^{-08}$) and the dotted line represents the suggestive threshold ($p < 1\times10^{-05}$).

Stratified SNP heritabilities and within-trait genetic correlations

Estimates of SNP heritability from stratified meta-analyses are shown in Figure 2 and Supplementary Table 8. In rater-specific meta-analyses, self-reported internalising symptoms showed the highest heritability (5.63%; 95% CI 3.08–8.18%), followed by teacher, maternal, and parental report, which were all significant. Although father-reported internalising symptoms had the highest SNP heritability in rater-specific analyses (8.98%), the wide confidence intervals overlapped zero (-0.06–18.02%). In age-specific meta-analyses, SNP h^2 for internalising symptoms in adolescence was highest (1.97%, 95% CI 0.30–3.64%), whereas estimates for early childhood, mid-childhood, and late childhood were similar, but not robust to significance testing. In rater-by-age meta-analyses, self-reported internalising symptoms during adolescence showed the highest SNP h^2 (3.20%, 95% CI 0.34–6.06%). Instrument-specific meta-analyses showed that variance in internalising symptoms explained by ASEBA and SDQ scales were comparable, ~3%. The estimate for Rutter was smaller (.3%), but the difference was not substantial, based on the overlapping confidence intervals.

$INT_{overall}$ and self-reported internalising symptoms were highly genetically correlated (r_g= 0.84, SE= 0.12, p=2.08×10^{-12}). The other stratified meta-analyses were insufficiently powered to estimate genetic correlations (heritability z-score < 4).

Gene-based analysis, tissue expression and gene-set analyses

The genome-wide gene-based analysis did not reveal any genes significantly associated with internalising symptoms, but the top 10 genes are reported in Supplementary Table 10. MAGMA tissue expression analyses of 30 general and 53 specific tissue types did not show any statistically significant associations with internalising symptoms (Supplementary Table 11). The gene-set analysis did not show any significant associations (Supplementary Table 12).

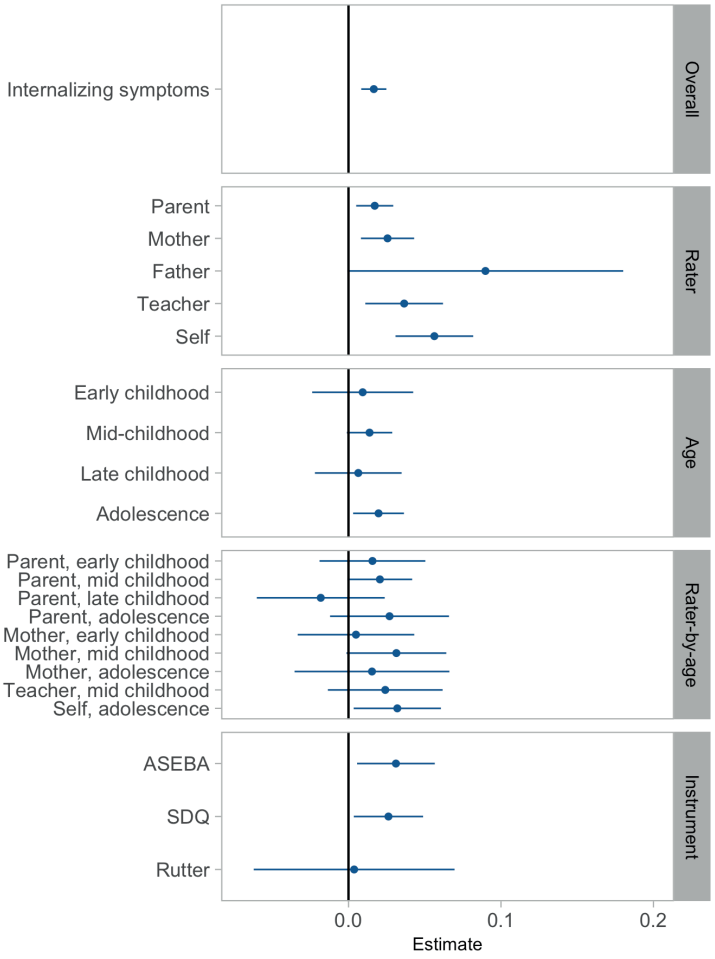
Genetic correlations with external traits

Genetic correlations between $INT_{overall}$ and INT_{self} (for which the z-score of the h2 was ≥ 4[78]), and a set of preselected external traits are shown in Figure 3 and Supplementary Table 13. $INT_{overall}$ held strong positive genetic correlations (r_g > 0.7) with Major Depressive Disorder, anxiety, and neuroticism, and a strong negative correlation (r_g < -0.7) with the wellbeing spectrum. High correlations ($|r_g|$ > 0.5) with other adult and childhood psychiatric and psychological traits, including attention-deficit hyperactivity disorder (ADHD), autism spectrum disorder (ASD), depressive symptoms, loneliness, and overall and maternal-reported aggression were found. Moderate genetic correlations ($|r_g|$ > 0.3) with insomnia, age at first birth, cigarettes per day, educational attainment, and intelligence were also observed. INT_{self} showed a similar pattern, but generally weaker genetic associations with external traits, with some exceptions. ASD, overall and maternal-reported aggression, age at first birth, and intelligence were correlated with $INT_{overall}$, but showed weaker correlations with INT_{self}, whereas self-reported aggression, smoking initiation and body-mass index (BMI) were correlated with INT_{self}, but showed weaker or no correlation with $INT_{overall}$.

Polygenic score prediction

Prediction of internalising symptoms in childhood and adolescence by polygenic scores based on $INT_{overall}$ are shown in Supplementary Table 14. After correction for multiple testing, polygenic scores for $INT_{overall}$ (N_{eff}=132,260) were significantly associated with maternal-reported internalising in 7-year-olds, and explained up to 0.38% of the phenotypic variance. Polygenic scores for $INT_{overall}$ were not associated with self-reported internalising problems in adolescence.

Figure 2 SNP heritabilities based on N-weighted meta-analyses of internalising symptoms.

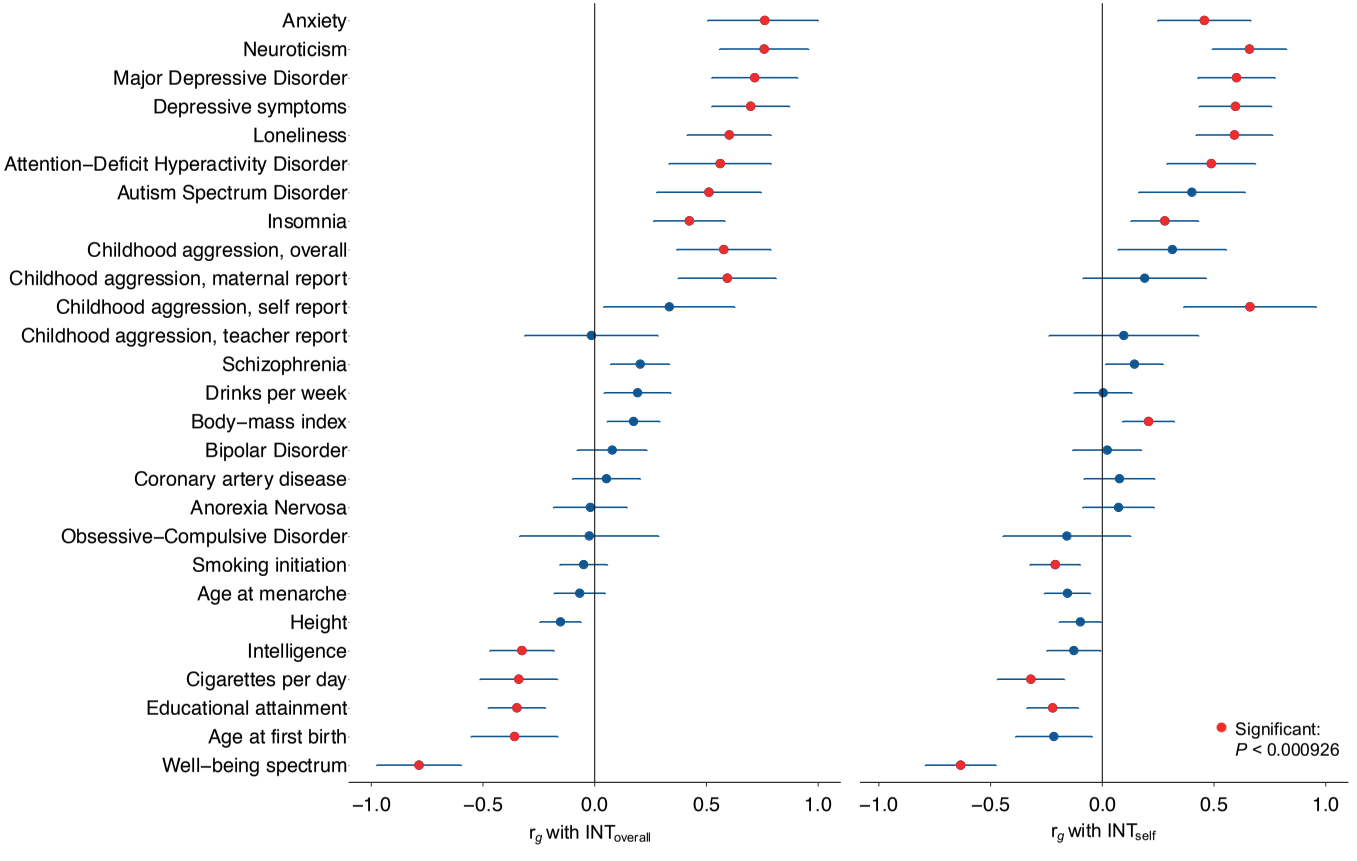


Error bars represent 95% confidence intervals.

DISCUSSION

This genome-wide association meta-analysis of childhood and adolescent internalising symptoms included data from 64,641 individuals aged between 3 and 18. The overall meta-analysis showed low SNP heritability (1.66%) and did not identify genome-wide significant loci or biological pathways for early-life internalising symptoms. Still, strong genetic correlations with external traits were observed, suggesting that childhood and adolescent internalising symptoms share substantial genetic vulnerabilities with adult internalising

Figure 3 Genetic correlations with external phenotypes.



The left panel shows genetic correlations with the meta-analysis for overall internalising symptoms in childhood and adolescence ($INT_{overall}$) and the right panel shows genetic correlations with self-reported internalising symptoms (INT_{self}). Error bars represent 95% confidence intervals. Correlation points in red are statistically significant after correction for multiple testing.

disorders and other childhood psychiatric traits, which could partially explain both the persistence of internalising symptoms over time and the high comorbidity amongst childhood psychiatric traits. A more detailed look into the results of stratified analyses pointed to rater-based heterogeneous effects, indicating that in addition to further increases of sample size, approaches that reduce heterogeneity will be essential in future GWAS investigations.

The most striking findings of this study are the direction and strength of genetic correlations with external traits, which point to an overlapping genetic architecture between internalising symptoms and other traits. This may initially be surprising given the low SNP-based heritability observed here, but while SNP heritability estimates the overall variance in a trait explained by genome-wide SNPs, a genetic correlation reflects the extent to which the same set of genetic factors are involved in two traits. As such, even traits with low SNP heritability can have high genetic correlations if the underlying set of genetic factors influencing the traits are overlapping. Strong genetic correlations ($|r_g| > 0.7$) with adult depression, anxiety, neuroticism, and the wellbeing spectrum were of note, and suggest a substantial shared genetic etiology between childhood internalising symptoms and adult internalising disorders and related traits, that has also been observed in previous studies[82–84]. Viewed in combination with the overlapping estimates of SNP heritability from early-childhood to adolescence in this study, these findings point to a stable set of genetic factors that partially explain the persistence of symptoms over time.

Comparisons with other psychiatric disorders showed high genetic correlations ($|r_g| > 0.5$) with childhood-onset disorders ADHD and ASD, but no robust associations with bipolar disorder, obsessive-compulsive disorder, or anorexia nervosa. A small genetic correlation with schizophrenia was observed (r_g=0.2, p=.0025), which, albeit not significant due to the strict correction for multiple testing applied here, is in line with previous studies showing successful prediction of internalising symptoms in childhood using polygenic scores for schizophrenia[11,84–86]. The overall pattern of genetic correlations with other psychiatric traits is comparable to adult cross-disorder genetic correlations, where depression shows stronger associations with ADHD and ASD than with schizophrenia or bipolar disorder[87]. It appears that like adult depression, the broader (and perhaps also milder) symptomatology captured by dimensional measures of childhood internalising symptoms shares fewer genetic similarities with severe and less common disorders such as schizophrenia, bipolar disorder, OCD, and anorexia, but is more closely tied to childhood-onset disorders ADHD and ASD. This also resembles findings from the recent GWAS of total child

psychiatric problems, which similarly found no robust genetic correlations with less common disorders[88]. Correlations with other traits, including insomnia, loneliness, intelligence, educational attainment, cigarettes per day, and age at first birth were observed, as also seen in GWASs of adult depression and anxiety[65,66], but unlike adult depression, no robust associations with coronary artery disease, BMI, smoking initiation, or age at menarche were found. On the other hand, both BMI and smoking initiation held robust associations with INT_{self}, for which ratings were only available during adolescence. This could indicate that genetic factors during adolescence are particularly important in these associations. Age-specific genetic effects may also explain why coronary artery disease was not associated with $INT_{overall}$, in contrast to the small but robust genetic correlation that coronary artery disease shares with both adult depression and anxiety[65,66]. This may point to genetic innovation (the involvement of novel genetic variants) in adulthood which could explain the genetic commonalities between adult internalising disorders and coronary artery disease. Alternatively, the lack of genetic correlation between $INT_{overall}$ and coronary artery disease, as well as age of menarche (which also genetically correlates with adult depression), could be due to a lack of power. This is also shown by the wide confidence intervals for some genetic correlations (Figure 3), which can be a consequence of low SNP heritability.

Focusing on childhood traits, as well as sharing high genetic correlations with childhood-onset disorders ADHD and ASD, internalising symptoms were also highly correlated with childhood aggression. The high correlations observed across childhood traits indicate the presence of specific genetic effects that are common between childhood disorders within the neurodevelopmental spectrum. These shared genetic effects could partially explain the high comorbidity between psychiatric traits in childhood[89–91]. In further examining the association between childhood internalising symptoms and aggression, $INT_{overall}$ shared high genetic correlations with overall and maternal-reported aggression, but not with teacher or self-report. On the other hand, self-reported aggression and self-reported internalising symptoms were highly correlated, whereas INT_{self} did not share robust associations with overall, teacher, or maternal reported aggression. These patterns of rater-stratified genetic correlations suggest that observed genetic effects on childhood phenotypes can vary substantially due to differences in the phenotype captured by different raters, with the same set of raters showing the highest correlation between traits.

The difficulty in identifying causal loci for early-life internalising symptoms is not novel and resembles the trajectory of GWAS investigations of adult internalising disorders. GWAS studies of adult depression also made slow progress due to limited sample sizes and heterogeneity[40,92,93]. As depression has several potential sources of heterogeneity, including a diverse presentation of symptoms, large case-control sample sizes were required to achieve success in identifying specific genomic loci[65,67]. GWAS studies of anxiety similarly saw increased success as sample sizes grew[66,94]. Although the current study represents a substantial increase in sample size in comparison to previous GWASs of childhood internalising phenotypes[57,58], the availability of childhood samples is still insufficient to lead to successful 'brute force' GWASs such as those that are now available for adult internalising disorders. Furthermore, in addition to heterogeneity due to a broad symptomatology, our findings indicate that GWAS investigations of childhood internalising symptoms are further disadvantaged by rater-based heterogeneous effects. Unlike adult studies where measurements are typically self or clinician reports, childhood studies, particularly those focusing on early childhood, rely heavily on parent and teacher report, which act as an additional source of heterogeneity. Rater-based differences in genetic correlations with external traits have been discussed above. The current study also observed varying estimates of SNP-heritability in rater-stratified analyses (Figure 2). Although these estimates did not appear significantly different (likely due to sample size limitations), the partial genetic correlation between $INT_{overall}$ and INT_{self} points to incomplete overlap in relevant SNPs, indicating the presence of rater-specific genetic effects. Additionally, polygenic scores based on $INT_{overall}$ did not predict self-reported internalising symptoms in the NTR cohort, which could also indicate heterogeneity between the target and discovery traits[95]. Rater-specific genetic effects and rater disagreement on internalising symptoms are noted in previous research[96-99] and rater-based heterogeneity is also reported in the GWAS of childhood aggression[76].

Heterogeneous effects underlying childhood internalising symptoms can be accounted for in multivariate GWAS approaches, but our study shows that current childhood samples seem unable to meet the power requirements of these types of analyses. Another way of reducing heterogeneity and helping signal detection is to focus on diagnoses. The case-control approach has proven to be more successful than dimensional measures in adult studies of depression and anxiety[65,66] and overcomes the limitations of treating symptom scales as continuous traits. However, diagnostic data are currently not available for childhood phenotypes in large enough samples. Instead, we expect that reducing heterogeneity at phenotypic level will be key in paving the way to

success in future GWAS investigations in childhood samples. This could be tackled by examining symptom level phenotypes or separating childhood anxiety and depression into two distinct phenotypes. However, given the high genetic correlation between internalising symptoms and both adult depression and anxiety, a more promising approach would be to jointly study childhood anxiety and depression, whilst eliminating heterogenous effects through factor analysis. Factor analysis can be used to derive a stable phenotype which captures the core behaviour that multiple measurements (e.g., from different informants or at different time points) have in common. This eliminates variability from rater, age, or situational effects. Evidence from both twin and molecular research shows that focusing on the common part of multiple assessments results in a more reliable phenotype which shows higher heritability than that captured by individual measurements separately[82,98,100,101]. This way of managing rater bias has broader applicability in genetic studies within child psychiatry, but is dependent on the availability of multiple informants on behaviour at one time point.

The findings of this study should be interpreted in light of several limitations. First, our multivariate GWAS approach relied on the assumption that meta-analysing repeated measures of internalising symptoms would increase power to detect genome-wide significant loci. This expectation was based on the reasonably strong correlations between measurements from different raters or at different ages. Instead, the burden of heterogeneity within childhood measurements amplified noise in the dataset. Combined with sample size limitations, this resulted in reduced statistical power which is reflected in the low SNP heritability and lack of genome-wide significant findings. Second, the low estimate of SNP heritability in this study can also partly be explained by the methods - estimates of SNP heritability from summary statistics are typically lower than estimates from individual-level data and potential over-correction of biases in LDSC may have led to more conservative estimates. Third, the analyses in this study corrected for sex differences, rather than investigating them through sex-differentiated analysis. We chose this approach as current evidence suggests that sex differences in genetic effects for psychiatric traits are either absent or small[102]. However, sex-differentiated analyses in future work could provide insight into whether the influence of genetic factors on downstream biological processes or interplay between genetic risk and social environments can explain the different prevalence of internalising behaviours in males and females. Fourth, due to the limited availability of diverse samples, the current findings are restricted to individuals of white European ancestry. An important

goal for future GWASs is the funding and inclusion of multi-ancestry cohorts to allow better representation of diverse populations and ensure broader applicability of findings.

To conclude, in this large GWAS of childhood and adolescent internalising symptoms in population-based cohorts of European ancestry, no individual loci with strong associations with the outcome were detected. However, strong genetic correlations with adult internalising traits and childhood psychiatric traits indicate that there is signal buried in the noise. Future GWAS success is likely to lie in reducing heterogeneity in childhood samples by focusing on a more stable phenotype of internalising symptoms.

Chapter 3

Parental characteristics and offspring mental health and related outcomes: a systematic review of genetically informative literature

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ABSTRACT

Various parental characteristics, including psychiatric disorders and parenting behaviours, are associated with offspring mental health and related outcomes in observational studies. The application of genetically informative designs is crucial to disentangle the role of genetic and environmental factors, as well as gene-environment correlation, underlying these observations, as parents provide not only the rearing environment, but also transmit 50% of their genes to their offspring. This article first provides an overview of behavioural genetics, matched-pair, and molecular genetics designs that can be applied to investigate parent-offspring associations, whilst modelling or accounting for genetic effects. We then present a systematic literature review of genetically informative studies investigating associations between parental characteristics and offspring mental health and related outcomes, published since 2014. The reviewed studies provide reliable evidence of genetic transmission of depression, criminal behaviour, educational attainment, and substance use. These results highlight that studies that do not use genetically informative designs are likely to misinterpret the mechanisms underlying these parent-offspring associations. After accounting for genetic effects, several parental characteristics, including parental psychiatric traits and parenting behaviours, were associated with offspring internalising problems, externalising problems, educational attainment, substance use, and personality through environmental pathways. Overall, genetically informative designs to study intergenerational transmission prove valuable for the understanding of individual differences in offspring mental health and related outcomes and mechanisms of transmission within families.

INTRODUCTION

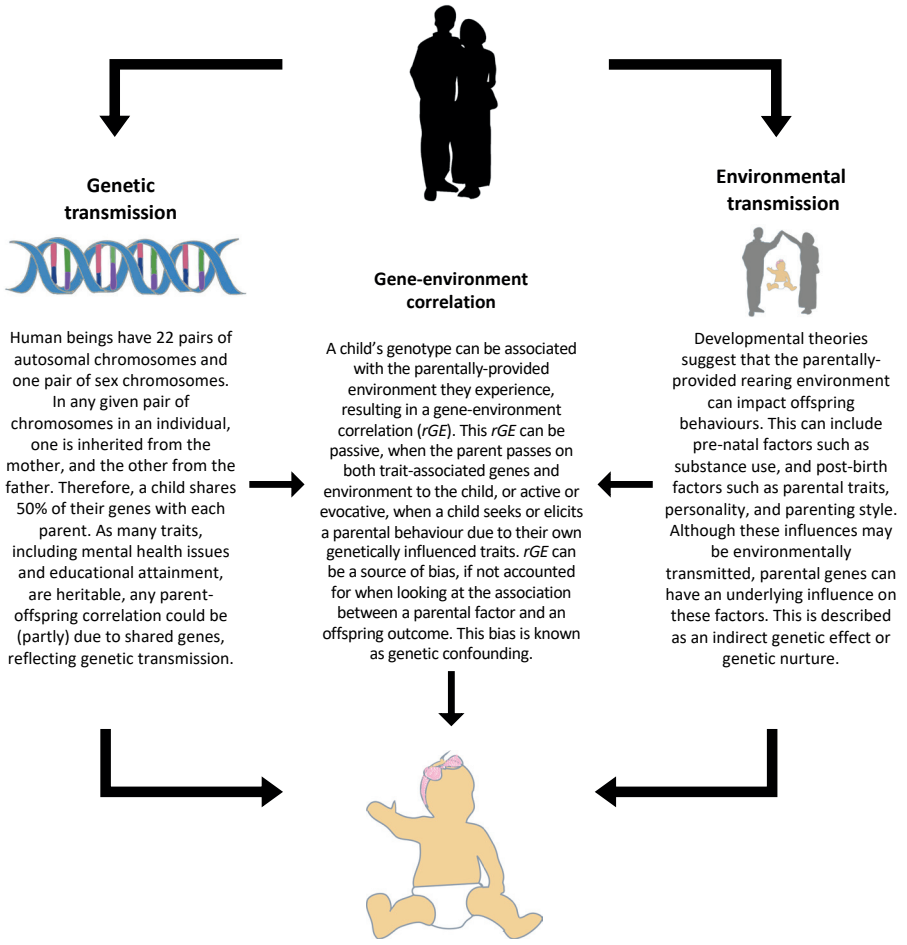
Parents are considered a driving force in the development of their children and parental factors are associated with various mental health outcomes in offspring, including emotional and behavioural problems[20]. However, although observed associations between parental factors and offspring outcomes are often interpreted as direct environmental influences, in truth parents provide both the rearing environment and genes to their children. Thus, observed parent–offspring associations may be wholly or partially explained by genetic factors shared between the parent and child; i.e., in a gene–environment correlation (*rGE*), when exposure to specific environments depends on an individual's genotype. The potential mechanisms (genetic transmission, environmental transmission and gene–environment correlation) underlying associations between parental characteristics and offspring outcomes are described in detail in Figure 1. Designs that do not account for the role of genetic factors in parent–offspring correlations can lead to biased estimates and erroneous conclusions about the extent to which these associations are causal. Genetically informative designs that explicitly model or control for potential genetic effects are essential for improving our understanding of the true effect of the parentally provided environment on offspring mental health.

In genetic epidemiology, the classical twin design is generally used to decompose the contribution of genetic and environmental effects underlying human traits[103]. Twin-based research shows that most mental health and related traits are moderately heritable (under the influence of additive genetic effects), with additional variance explained by the unique environment (which is specific to each individual), and for some traits also the shared environment (environments that the twins have in common)[6]. However, classical twin studies say little about mechanisms of transmission within families where, in addition to genetic transmission, parental effects may be transmitted through both the shared environment via parentally provided rearing factors, and to a lesser extent, the unshared environment through specific parent–child interactions. Consequently, genetically informative designs that include both the parent and offspring generations are required to disentangle genetic and environmental effects underlying parent–offspring associations.

The present review aims to synthesise literature investigating the association between parental characteristics and offspring mental health and related outcomes in genetically informative designs. An earlier systematic review published in 2014 focused on the children- of-twins method[104]. However, several

novel methodologies that investigate within-family transmission using innovative techniques have emerged in the past few years. Consequently, there is a gap in the literature for a broad systematic overview that incorporates all genetically informative designs that can be applied

Figure 1. Mechanisms underlying parent-offspring associations



A figure describing potential mechanisms (genetic transmission, environmental transmission, and gene-environment correlation) underlying associations between parental characteristics and offspring outcomes.

to study parent–offspring associations. Here, we focus on studies published from 2014 onwards, as these have not been covered by previous reviews. We first provide a brief overview of the types of genetically informative designs that can be employed to investigate parent–child associations. This is followed

by a systematic review of studies investigating associations between parental characteristics and offspring mental health and related outcomes, including internalising behaviours (such as anxiety and depression), externalising behaviours (such as attention-deficit/hyperactivity disorder), educational attainment, substance use and personality.

GENETICALLY INFORMATIVE DESIGNS

Designs that can be used to separate genetic and environmental mechanisms of transmission from parents to offspring broadly fall into the following three categories: behavioural genetics designs, matched-pair designs, and molecular genetics designs. In this section, we summarise the principles underlying these approaches (Figure 2), describe specific methods in detail and discuss their application as well as advantages and disadvantages (Table 1).

Behavioural genetics designs

Behavioural genetics designs leverage knowledge of relatedness among individuals within a family to make inferences about the contribution of genetic and environmental factors underlying parent–offspring associations. The adoption[24] and children-of-twins[23,104] designs (Figure 2) are key tools used to distinguish the effects of genetic and environmental transmission. Associations between biological parents and their adopted-away offspring suggest genetic transmission as although these parents and offspring are genetically related, the parents do not raise the child and hence have no environmental influence. On the other hand, associations between adoptive parents and offspring suggest environmental transmission as these parents and offspring are genetically unrelated, and are only connected through the environment. In children-of-twins studies, children of monozygotic twins are as genetically similar to their twin aunt/uncle as they are to their twin parent, whereas children of dizygotic twins share less genetic similarity with their aunt/uncle. Higher monozygotic than dizygotic avuncular correlations (between uncle/aunt and niece/nephew) are likely due to the higher proportion of shared genes, suggesting genetic transmission, whereas higher parent–offspring than monozygotic or dizygotic avuncular correlation indicates environmental transmission through the shared parent–child environment. Another key characteristic of adoption and children-of-twins studies is that they can be used to investigate *rGE* (Table 1). This is particularly important as even within genetically informative designs, unmeasured *rGE* can inflate estimates of genetic or environmental effects. For instance, if an observed parent–offspring association is present in both

biological and adoptive duos, but the correlation is higher in biological (shared genes plus rearing) than adoptive (rearing only) families, this indicates the contribution of both genetic and environmental effects; i.e. passive *rGE*. If unaccounted for, this *rGE*, reflected in increased similarity between biological parents and lived-with offspring, could potentially lead to an inflated estimation of genetic transmission in adoption studies.

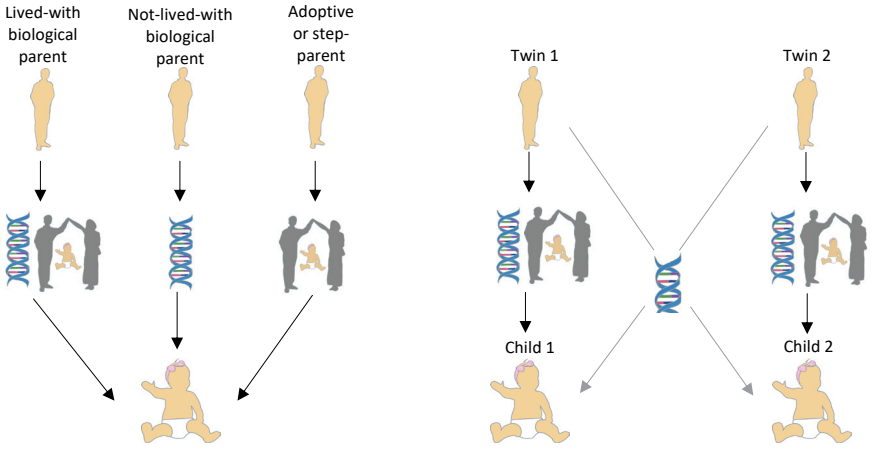
Due to modern developments in assisted reproductive technology and the availability of large-scale population-based registers, novel pseudo-adoption designs have emerged that apply the same principles (see adoption and related designs in Figure 1) to investigate genetic and environmental effects in non-adoption families. Within assisted conception[26] studies, genetically related or genetically unrelated parents are analogous to the biological and adoptive parents in an adoption design, whereas in triparental family[27] and multiple parenting relationships[25] designs, the rearing effect of step-parents and genetic effect of not-lived-with biological parents are examined (Table 1).

Matched-pair designs

Matched-pair designs strengthen the causal inference of an observed parent–offspring association by adjusting for all unmeasured genetic and environmental familial effects. In sibling comparison[28] studies (Figure 2), a sibling with no exposure to the parental candidate environment is included in the analysis as a control, as siblings are naturally matched for shared genes and the family environment. Environmental transmission is indicated if the parent–offspring association is observed only in the exposed offspring. Similarly, the case–control[30] design includes matched parent–child control pairs who share the same proportion of genetic and environmental factors as the case parent–child pairs, but do not share the candidate exposure. As the matching is done by the researchers here, it is crucial that the process is thorough so that it can be reasonably argued that unmeasured confounders are unlikely to bias the results. Matched-pairs designs cannot be used to investigate *rGE*, as they do not directly measure genetic effects. However, sibling comparison studies generally rule out passive *rGE*, as the random distribution of parental alleles across offspring ensures that siblings are equally likely to receive genes associated with the exposure in the parent, and the outcome in the offspring. Evocative *rGE* can also be ruled out if exposure to the parental characteristic definitively precedes the offspring outcome.

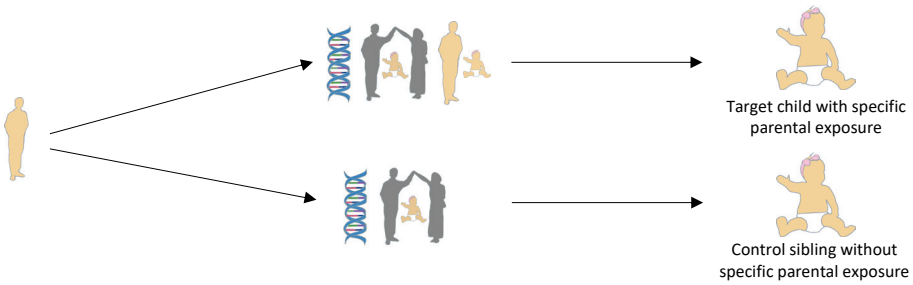
Figure 2. Schematic diagrams demonstrating the principles underlying commonly used genetically informative designs which separate genetic and environmental mechanisms of transmission in parent–offspring association

A. Behavioural genetics designs



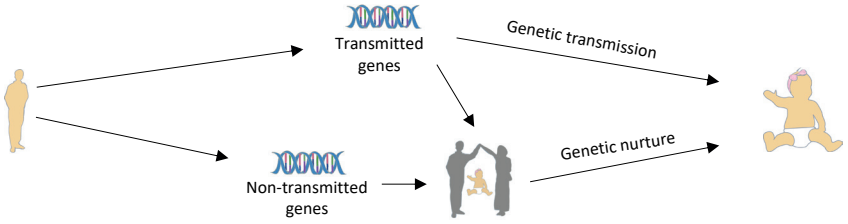
Adoption and related studies *Children-of-twin studies*

B. Matched-pair designs



Sibling comparison studies

C. Molecular genetics designs



A In adoption and related designs, knowledge of the type of relationship shared between parent and offspring is leveraged to gain insight into genetic and environmental factors underlying parent-to-offspring associations. Lived-with biological parents can influence offspring through both genetic and environmental transmission, as they provide both genes and the rearing environment. Not-lived-with biological parents who have no contact with the offspring provide only genes, indicating genetic transmission, whereas adoptive or step-parents provide only the rearing environment, indicating environmental transmission. In children-of-twins studies, children of identical (monozygotic) twins are as genetically similar to their aunt/uncle as they are to their parents (50% shared genes), whereas children of fraternal (dizygotic) twins share 25% of genes with their aunt/uncle. Higher monozygotic than dizygotic avuncular correlations (between uncle/aunt and niece/nephew, i.e., between Twin 1 and Child 2 or Twin 2 and Child 1) are likely due to a higher proportion of shared genes, suggesting genetic transmission, whereas higher parent–offspring than avuncular correlation suggests environmental transmission of a parental factor, above and beyond the effect of shared genetic or environmental effects. **B** In sibling comparison studies, the association between a specific parental factor and offspring outcome is studied in exposed versus unexposed offspring, as siblings are naturally matched for parentally provided genes and a rearing environment. Environmental transmission is indicated if the parent–offspring association is observed only in the exposed offspring. **C** In molecular genetics studies, the effect of shared parent–offspring (i.e., transmitted) genes on offspring outcome indicates the presence of genetic transmission. However, both transmitted genes and non-transmitted parental genes can also have an indirect (i.e., environmentally mediated) effect on offspring through parental traits that are genetically influenced; this is otherwise known as genetic nurture.

Molecular genomic designs

Recent advances in molecular genetics provide novel ways of investigating genetic and environment effects underlying parent–offspring associations by using genomic data. In molecular genetics studies, the effect of genetic variants transmitted from parent-to-offspring on offspring behaviour indicates the presence of genetic transmission. As described in Figures 1 and 2, parental genes can also have an indirect effect on offspring, through parental traits that are environmentally mediated but genetically influenced; a process otherwise known as genetic nurture. One way to separate genetic transmission and genetic nurture effects underlying specific parent–offspring associations is the use of polygenic scores. Polygenic scores (PGS) represent an aggregate genetic liability for a trait, determined by the presence and effect sizes of alleles associated with the trait[40]. In within-family PGS genetic sensitivity analysis, offspring PGS for exposure and outcome traits are included as covariates in the regression analyses to explore whether the association between a parental exposure variable and offspring outcome is attenuated by the offspring's PGS. If that is the case, genetic transmission explains part of the parent–offspring association[105]. Although adjusting for PGS does not entirely eliminate genetic transmission as current PGS capture only a small proportion of trait heritability, such sensitivity analyses can show whether shared genes partially account for an observed parent–offspring association. In within-family PGS genetic

nurture analyses, PGS can additionally be used to estimate the environmental influence of parental alleles not passed on to the offspring[33,36,]. If PGS based on non-transmitted parental alleles are associated with an offspring trait (transmitted/non-transmitted method in Table 1), the effect of these parental genes on offspring behaviour likely occurs via an environment pathway, i.e. genetic nurture. Similarly, if parental PGS are associated with an offspring trait after adjusting for the child's own PGS (statistical control method in Table 1), this also suggests a nurturing effect of parental genes beyond that which is due to transmitted genes. The overall contribution of genetic nurture to offspring traits can be estimated using maternal-effects genome complex trait analysis (M-GCTA)[35], relatedness disequilibrium regression (RDR)[106] or trio-GCTA[38] (Table 1). Each of these methods uses genotyped data from unrelated parent–offspring pairs to estimate the variance in offspring behaviour that is explained by their own genotype (SNP-based heritability; heritability accounted for by differences in measured genetic variants known as single-nucleotide polymorphisms) and genetic nurture (parental additive genetic effects acting via genetically influenced parental behaviours).

It is important to note that as current genetic nurture designs only index parental effects that are captured by their common genetic variation, these designs capture only a part of the overall parent-to-child environmental transmission. Parental traits that are not under the influence of common genetic variation may also influence offspring outcomes. To test whether specific parental behaviours are responsible for observed genetic nurturing effects, the parental phenotype can be included as a covariate in within-family genetic nurture analyses, M-GCTA, RDR or trio-GCTA. If a genetic nurturing effect on offspring behaviour is attenuated with the inclusion of the parental phenotype to the model, the parental phenotype is shown to be involved in the manifestation of the genetic nurturing effect. As with behavioural genetics designs, molecular genetics designs can be used to investigate rGE, by estimating covariance between additive genetic effects and indirect genetic nurturing effects (Table 1).

METHODS

We searched for articles investigating associations between parental characteristics and offspring mental health and related outcomes. We defined related traits as those that have an established link to mental health in the literature. The Web of Science database was used to conduct a systematic search of studies published from 2014 to June 2020. The search terms consisted of

Table 1. Overview of current designs that can be used to study mechanisms of transmission underlying associations between parental characteristics and offspring outcomes

	Design, reference	Genetic transmission	Environmental transmission
Behavioural genetics designs	**Adoption** [24]	Association between a biological parent and their adopted-away offspring (shared genes only) indicates genetic transmission	Adoptee's relative method: association between a parent and their adoptive offspring (rearing only) indicates environmental transmission Adoptee study method/siblings-reared-apart: higher correlation between biological parent and their lived-with offspring (genes plus rearing) than their adopted-away offspring (genes only) indicates environmental transmission
	Assisted conception [26]	Higher correlation between a genetically-related birth mother (e.g. homologous in vitro fertilisation or sperm donation) and her offspring (genes plus prenatal environment) than agenetically-unrelated birth mother and her offspring (prenatal environment only) indicates genetic transmission	Association between a genetically-unrelated birth mother (e.g. egg, oocyte or embryo donation, surrogacy) and her offspring (prenatal environment only) indicates environmental transmission
	Triparental family (offspring-focused: multiple parental relationships of one offspring) [27]	Association between an offspring and their not-lived-with biological parent (genes only) indicates genetic transmission	Association between an offspring and their step-parent (rearing only) indicates environmental transmission
	Multiple parenting relationships (parent-focused; multiple offspring relationships of one parent) [25]	Association between a parent and their not-lived-with biological offspring (genes only) indicates genetic transmission	Association between a parent and their step-child (rearing only) indicates environmental transmission
	Children-of-twins [23]	Higher monozygotic-avuncular correlation (between MZ twin uncle/aunt and niece/nephew; 50% shared genes) than dizygotic-avuncular correlation (25% shared genes) indicates genetic transmission	Higher parent-child correlation (genes plus rearing) than monozygotic avuncular correlations (genes only) indicates environmental transmission
	Extended twin (twins and their parents) [29]	Not studied, as genetic transmission is not estimated but fixed to 0.5 (50% of genes are passed on from parent to child) in the model *	The correlation between parental and offspring phenotype indicates cultural (i.e. environmental) transmission - this captures part of the shared environment effect that is explained by parent-to-child transmission

Gene-environment correlation	Advantages	Disadvantages
Higher correlation between biological and living-together parents and offspring (genes plus rearing) than adoptive parents and offspring (rearing only) suggests passive rGE Trait correlation between biological parents and their adopted away offspring (shared genes only) indicates genetic liability, and subsequent adoptee correlation with the environment provided by their adoptive parent suggests evocative rGE	- If adoption occurs at birth, passive rGE influences (on factors outside of the intrauterine environment) can be excluded as biological parents would have no rearing effect on offspring	- Generalisability to general population could be limited, as adoptees may have a higher risk of experiencing a suboptimal prenatal environment - Samples can be difficult to obtain and are usually small - Non-random process of adoption may introduce selection bias - Increase in open adoption (contact between biological and adoptive families) confounds the design
Not studied	- Effective for testing short and long-term effects of the prenatal environment	- Samples can be difficult to obtain and are usually small - Generalisability to general population could be limited - Prenatal behaviours of mothers who use assisted conception may introduce selection bias - Samples are generally very heterogeneous - Inclusion of families with within-family donation would bias the design - Design is not optimal for investigating gene-environment correlations
Higher offspring correlation with lived-with biological parent (genes plus rearing) than with their step-parent (rearing only) suggests passive rGE Offspring correlation with their not-lived-with biological parent (shared genes only) indicates genetic liability, and subsequent offspring correlation with the environment provided by their step-parent would suggest evocative rGE	- Representative of the general population as all types of parent-offspring relationships are included - Large sample sizes can be attained	- Contact with not-lived-with parents can upwardly bias estimate of genetic influences due to passive rGE - Databases with details of family structure are rare
Higher parental correlation with their lived-with biological children (genes plus rearing) than with their step-children (rearing only) suggests passive rGE	- Representative of the general population as all types of parent-offspring relationships are included - Large sample sizes can be attained	- Contact with not-lived-with parents can upwardly bias estimate of genetic influences due to passive rGE - Databases with details of family structure are rare - Cannot investigate evocative rGE as for each child in this design, information from only one parent is known
If a parental characteristic is largely estimated as heritable (under genetic influence) in a parent-based twin sample but is under the influence of the shared environment in a child-based twin sample, this suggests passive rGE Estimation of a parental characteristic as heritable (under the influence of genes) in a child-based twin sample suggests evocative rGE	- Can determine if familial correlation is due to genetic or environmental factors - Extended children-of-twins studies can incorporate siblings and other members of the pedigree and estimate additional parameters	- Samples can be difficult to obtain - Assumes that the size of the genetic contribution to variation in parent and offspring phenotype is the same - Assumes that the same genes influence the phenotype in both the parent and offspring generation
Covariance between the additive genetic effect and parental transmission suggests passive rGE	- Powerful for estimating shared environmental effects of a specific parental trait that arise due to cultural transmission or social homogamy - Design can be used to study impact of other family relationships, including siblings - Design can be used to estimate twin-based heritability	- Cultural transmission can be easily underestimated if assumptions of the design are violated or the study is under-powered

	Design, reference	Genetic transmission	Environmental transmission
Matched-pairs designs	**Sibling comparison** [28]	Not studied, as familial resemblance between full siblings could be due to genetic or environmental factors	Comparison of outcomes in children with a specific parental exposure and their unexposed full sibling who is otherwise naturally matched for familial (genetic and environmental) risk; higher outcome levels in exposed than unexposed siblings indicates environmental transmission
	Case-control [30]	Not studied, as cases and control parent-offspring pairs are matched on genetic risk	Parent-offspring pairs are manually matched on genetic risk. Outcomes are compared between children with a specific parental exposure (cases) versus unexposed children (controls); higher outcome levels in cases than controls indicate environmental transmission
Molecular genetics designs	**Within-family PGS: adjustment analyses** [105]	The disappearance of an observed parent-offspring correlation after adjusting for offspring PGS for the predictor and outcome traits indicates genetic transmission	The remaining parent-offspring correlation, after adjusting for offspring PGS for the predictor and outcome traits, estimates environmental transmission
	Within-family PGS: genetic nurture [33,36]	Association between PGS based on transmitted parental genes and offspring outcome indicates genetic transmission	Transmitted/non-transmitted method: association between PGS based on non-transmitted parental genes and offspring outcome indicates genetic nurture Statistical control method: association between parental PGS and offspring outcome, after adjusting for offspring PGS to account for shared parent-child genetic effects indicates genetic nurture
	Maternal-effects genome-wide complex trait analysis (M-GCTA) [35]	Not studied □	The overall estimated effect of maternal or paternal genetic nurture: variance in offspring outcome that is explained by the effect of maternal or paternal genotype (after accounting for transmitted genetic effects)
	Relatedness disequilibrium regression [106]	Not studied □	The overall estimated effect of parental genetic nurture: variance in offspring outcome that is explained by the effect of mid-parent genotype (after accounting for transmitted genetic effects)
	Trio-GCTA [38]	Not studied □	The estimated effect of maternal and paternal genetic nurture: variance in offspring outcome that is separately explained by the indirect effect of maternal *and* paternal genotype (after accounting for transmitted genetic effects)

rGE: gene-environment correlation; *PGS*: polygenic scores; *SNP-based heritability*: variance in a target trait that is explained by the additive genetic effect of common genetic variants known as Single Nucleotide Polymorphisms
* These designs can be used to estimate twin or SNP-based heritability for offspring outcomes, i.e., the proportion of variance in a phenotype that can be explained by genetic variation in the population under study. This does not directly index genetic transmission, although it is implicitly known that children receive their genes from their parents.

Gene-environment correlation	Advantages	Disadvantages
Not studied	- Generally excludes passive rGE as siblings typically share the same parentally provided environment - Can exclude evocative rGE within design if certain that the parental exposure precedes offspring outcome	- Requires differential exposure between siblings, which can elicit selection bias - Cannot distinguish if familial resemblance between siblings is due to genetic or environmental factors - Design is not optimal for investigating gene-environment correlations
Not studied	- Representative of general population - If matched well, ensures no effect of confounding factors	- Matching is done by the researcher and is susceptible to errors - Resources required to find matched parent-offspring pairs - Cannot investigate genetic transmission or gene-environment correlation
Reduction of parent-offspring correlation after adjusting for offspring PGS suggests passive rGE	- Can be used as sensitivity analysis to test whether parent-offspring associations are partly due to shared genes	- PGS capture only a small proportion of heritability and cannot index the effect of all shared genes - Requires well-powered GWAS summary statistics
Association between offspring PGS and parenting suggests passive rGE Association of offspring PGS with parenting, after adjusting for parental PGS suggests evocative rGE	- Can examine environmental transmission without parental phenotypic information	- Requires well-powered GWAS summary statistics - Datasets with parent-offspring genotyped duos or trios are rare
Covariance between direct genetic effect and genetic nurturing effect suggests passive rGE	- Can estimate overall impact of genetic nurture from mother or father - Representative of general population - Design can be used to estimate SNP-based heritability	- Cannot model both maternal and paternal genetic nurture effects at the same time - Large sample sizes are required to estimate multiple variance components based on genetic data - Datasets with parent-offspring genotyped duos or trios are rare
Covariance between direct genetic effect and genetic nurturing effect suggests passive rGE	- Can estimate overall impact of genetic nurture from both parents combined - Representative of general population - Design can be used to estimate SNP-based heritability	- Assumes that maternal and paternal genetic effects are the same and of equal magnitude - Large sample sizes are required to estimate multiple variance components based on genetic data - Datasets with parent-offspring genotyped trios are rare
Covariance between direct genetic effect and genetic nurturing effect suggests passive rGE	- Can estimate individual impact of genetic nurture from both parents in the same model - Representative of general population - Design can be used to estimate SNP-based heritability	- Large sample sizes are required to estimate multiple variance components based on genetic data - Datasets with parent-offspring genotyped duos or trios are rare

study design variables ("children-of-twins" or "offspring of twins" or "adoption" or "assisted conception" or "sibling comparison" or "genetic nurture" or "non-transmitted" or "polygenic score"), parent variables ("parent" or "mother" or "father" or "maternal*" or "paternal*"), offspring variables ("offspring" or "child*") and topic variables ("gene*" or "environment"). The search did not include predictor or outcome-specific search terms, so as not to limit the review to a particular set of traits. We restricted the search to scientific articles published in English. Through the results of the initial search, we identified additional designs that were relevant (Table 1), and ran separate follow-up searches for these study design variables ("extended twin" or "triparental" or "multiple parenting relationships design" or "matched pair" or "genome-wide complex trait analyses" or "relatedness disequilibrium regression"). Aside from the database searches, we scanned the references of papers for relevant studies and checked bioRxiv and medRxiv for relevant preprints.

After removing duplicates, the overall search yielded 2097 hits. Studies were included in the systematic review when the following criteria were met: the association between a parental characteristic and offspring behaviour was examined, a genetically informative design was used, and the phenotype in the offspring was a mental health or related trait. As current literature shows that most complex traits have a polygenic architecture, candidate gene studies were excluded from this review.

RESULTS

After screening and assessment of search results (Figure 3), we identified 89 articles for inclusion in this review. We present our synthesis of the literature by grouping the studies according to the offspring outcome in the following sections: internalising behaviours, externalising behaviours, educational attainment, substance use and personality. The number of studies and key findings for each outcome are summarised in Table 2. Details of all studies and their results are reported in Tables 3–7. Effect sizes showing the relative contribution of genetic and environmental factors in parent–offspring associations are included in the tables when studies provided standardised, well-interpretable statistics, i.e., odds ratios, percentage of variance explained or standardised betas.

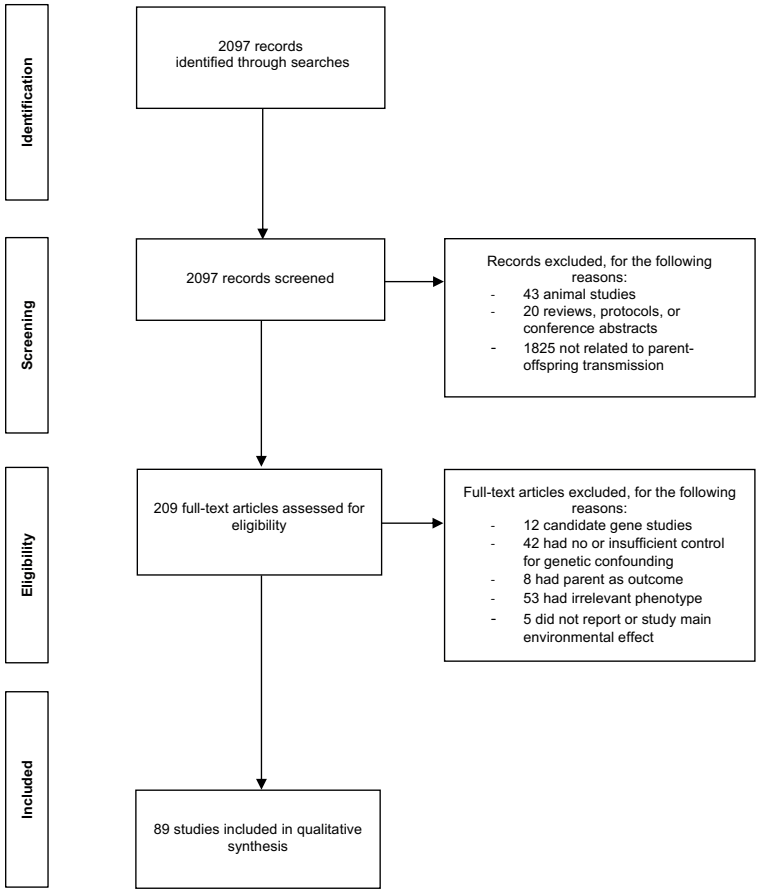
Offspring internalising behaviours

Intergenerational transmission of internalising behaviours

Studies investigating the association between parent and offspring internalising behaviours (Table 3), including depression and anxiety, showed substantial evidence of genetic transmission of depressive symptoms[107–110], and major depressive disorder (MDD) diagnosis[111]. This is in line with twin literature which shows that depression is a heritable phenotype[6]. After accounting for genetic effects, parental depression was associated with offspring internalising behaviours through environmental pathways, and these associations were observed throughout childhood[108,109,112,113], adolescence[107,114], and adulthood[111]. Similarly, associations between parental anxiety and offspring internalising behaviours also showed evidence of environmental transmission across development, from toddlerhood to early adulthood[115–120]. However, unlike depression, this association was not partly explained by shared genes, as there was no evidence of genetic overlap between parental anxiety and offspring internalising behaviours[115,117,118,120]. The lack of evidence for genetic transmission of anxiety is at odds with findings from twin literature which estimate that 40% of individual differences in anxiety are explained by genetic factors[6]. However, there are some possible explanations of why genetic transmission is not evident within the adoption and children-of-twins studies reviewed here. Measures of inherited risk in the adoption studies could lack validity, and may not adequately capture the genetic risk of anxiety from birth parents. Alternatively, as longitudinal studies show that genetic factors involved in anxiety change across the lifespan[56], different genes could be relevant for the occurrence of anxiety in early life and adulthood. Therefore, parental anxiety and offspring internalising symptoms may share fewer common genetic factors that are not easily captured using adoption or children-of-twins designs. Even if different genes are involved in childhood internalising symptoms and adult anxiety, the observed environmental association indicates that exposure to an anxious parent is a risk factor for offspring internalising symptoms.

Overall, environmental associations between parental factors and offspring internalising behaviours were generally driven by exposure to concurrent parental anxiety or depression, whereas prenatal and post-natal symptoms did not have a long-lasting effect[109,112,119,121]. This finding stands in contrast to the substantial body of literature that interprets associations between perinatal maternal distress and offspring mental health outcomes in causal terms[122]. Based on the current findings, such parent–offspring associations detected in previous observational studies are likely to be attributable to

Figure 3. Flow chart of study selection



A description of the screening and assessment procedure, reporting the number of records excluded and reasons for exclusion at each stage.

unmeasured *rGE*, or concurrent parental depression. In investigating the presence of gene–environment correlation, several adoption studies found no evidence of evocative *rGE*, although some child-to-parent effects were identified[107,116,118,120,123]. These studies highlight the dynamic nature of parent and offspring relationships, where associations can be bidirectional, with both parent and offspring behaviour influencing the other.

Parenting behaviours

Children-of-twin studies examining genetic overlap between parenting and offspring mental health found involved in parenting behaviours (such as parental criticism, parental affection and parent–child relationship quality) did not overlap

with genes involved in offspring internalising behaviours[124–127] (Table 3). After accounting for genetic relatedness, several parenting behaviours were associated with offspring internalising behaviours. Negative parenting behaviours, including over-reactive parenting[128], harsh parenting[123] and parental criticism[124,127] were associated with more offspring internalising behaviours, whereas parental expressed affection and a good parent–child relationship quality were associated with positive offspring self-worth[125], and fewer internalising problems[126], respectively. Of note, an innovative sibling comparison based on Swedish registry data identified a protective effect of adoptive parenting in children of high-risk biological parents with MDD diagnosis[129]. In interpreting associations between parenting behaviours and offspring outcomes, it is important to again note that these parent–offspring associations can be bidirectional, with each affecting the other over time. Furthermore, parenting behaviours can be evoked by the offspring's genetically influenced internalising behaviours. However, three adoption studies found no evocative *rGE* effects of offspring internalising symptoms[120,123,124], although one study reported child-to-parent effects wherein child anger predicted prospective harsh negative parenting[123].

Genetic nurture

Genetic nurture is a relatively new topic within psychiatric genetics, and as such, we identified only two studies that investigated environmentally mediated effects of parental genes on offspring internalising behaviours (Table 3). Both studies were based on the Norwegian Mother, Father and Child (MoBa) sample and estimated variance in offspring depression and anxiety symptoms that was explained by indirect parental genetic effects, over and above the transmission of genes from parent to child. The earlier study, with a smaller sample size, found no evidence of genetic nurture[63], whereas the subsequent study with three times the sample size identified a genetic nurturing effect on offspring depressive symptoms that was mediated by maternal emotional symptoms[64]. This finding is in line with the studies reviewed above which showed environmental associations between maternal depression and offspring internalising behaviours[108,109,112] and shows that seemingly environmental associations between parental factors and offspring outcomes may nonetheless be driven by genetically influenced parental traits.

Parental educational attainment

A large children-of-twins and siblings study investigating associations between parental educational attainment and offspring depressive symptoms found evidence of genetic, but not environmental transmission[130] (Table 3). Genetic overlap between education attainment and depression has been reported

previously[86], and this study highlights that without the use of genetically informative designs to account for genetic transmission, phenotypic associations between parental educational attainment and offspring internalising symptoms could be misinterpreted as causal.

Parental substance use

A large sibling comparison study investigated associations between maternal alcohol use during pregnancy and offspring emotional problems[131] (Table 3). Although exposed children were more emotionally reactive and had more somatic complaints than their unexposed siblings, associations between maternal drinking and offspring anxiety and depressive symptoms seemed to be explained by factors shared by siblings born of the same mother. Previous literature investigating the impact of drinking during pregnancy on offspring internalising behaviours shows mixed findings[131], making it difficult to make firm conclusions on whether there is an environmental association.

Offspring externalising behaviours

Intergenerational transmission of externalising behaviours

Several adoption studies investigating the intergenerational transmission of externalising behaviours (Table 4) were based on the Early Growth and Development Study (EGDS) sample. Detection of effects in these studies seemed to correlate with sample size, indicating that power considerations are important in interpreting these results. In studies with fewer participants (up to 361 families), birth parent externalising behaviour, antisocial behaviour and self-regulation were uncorrelated with offspring externalising behaviours[132–134], suggesting no shared genetic effects. However, studies with more participants (561 families) showed correlations between birth parent and offspring externalising behaviours[135], and between birth parent antisocial behaviour and offspring callous–unemotional behaviours[136], although oppositional and attentional-deficit behaviours were uncorrelated with birth parent antisociality[136]. Findings from previous literature show substantial heritability of externalising behaviours[6] and highlight the important role of genetic transmission in explaining parent–offspring simarlity[104]. It is likely that the detection of genetic transmission in adoption studies requires more power, especially if the specific parent and offspring phenotypes under investigation are related, but not identical traits.

The role of environmental transmission in externalising behaviours has also been previously implicated[104]. Here, we identified one adoption study which found no robust evidence for an association between parent antisociality and offspring disruptive behaviours[137]. In addition, three large Swedish population-based studies of criminal behaviour found robust evidence of both genetic and environmental transmission of criminal behaviour[27,138,139] and showed that risk of criminal behaviour was strongest in families where the same parent provided both the genes and the rearing environment[27,139].

Parental anxiety or depression

Evidence from adoption and children-of-twins studies showed genetic overlap between parental depression and offspring externalising behaviours, including ADHD[107–110,140] (Table 4), whereas associations between overall parental internalising symptoms and offspring externalising symptoms showed mixed results in four adoption studies[113,128,135,141]. Genetic overlap between depression and externalising phenotypes has been reported previously[65], and the generalist-gene hypothesis suggests that the same genes may pose genetic vulnerabilities toward multiple distinct psychiatric disorders.

After accounting for genetic relatedness, exposure to parental depression was associated with offspring externalising problems in several studies[107–110,112,113,140], whereas parental anxiety[110] and overall internalising symptoms[141] were unrelated to offspring externalising behaviours. Combined with the findings described above, this indicates that exposure to a depressed parent is a risk factor for both internalising and externalising behaviours. As with childhood internalising problems, the association between maternal depression and childhood externalising problems was often observed only in relation to concurrent depressive symptoms[109,110,112], although one children-of-twins study reported an association between prenatal maternal depression and ADHD in 5-year-olds[140]. Current results mainly highlight that associations with prenatal depression in observational studies that do not control for genetic effects are likely partly explained by unmeasured *rGE*. One adoption study investigating *rGE* reported evocative effects; birth parent depression predicted offspring externalising problems, which in turn predicted adoptive parent depression[107]. As well as demonstrating how genes and environment work in combination, the study highlights the bidirectional relationship between parent and offspring mental health phenotypes.

Parenting behaviours

Genetic associations between parenting and offspring externalising behaviours were scarcely investigated (Table 4). A children-of-twins study reported no genetic overlap between parental monitoring and offspring externalising problems[142], whereas an adoption study reported that birth mother personality characteristics were partially associated with offspring callous–unemotional behaviours[143]. Previous children-of-twins studies show that it is plausible that parents with a predisposition for negative parenting behaviours have offspring predisposed to psychopathology, and subsequently both phenotypes may share a common aetiology[104].

Studies of environmental transmission found associations between both positive and negative parenting and offspring externalising behaviours. Negative parenting behaviours were associated with increased offspring externalising behaviours[133,137,143–145], but these effects were sometimes inconsistent across raters. For instance, over-reactive parenting was associated with parent-rated[128,132], but not teacher-rated[134] externalising problems. This could reflect differences in the child's behaviour observed at home by the parent, or at school by the teacher. Alternatively, these differences could be indicative of rater biases resulting from differences in the interpretation of scale items, a unique perception of the children's behaviour, or the rater's own mental health[146]. More research is required to clarify rater-specific findings. Focusing on positive parenting, factors such as parental knowledge of offspring whereabouts, good parent–child relationship quality, positive reinforcement, and warm parenting were associated with fewer externalising problems[134–136,142,147], whereas there were no associations between parental positive reinforcement and ADHD symptoms[136], or maternal support and offspring delinquent behaviour[148]. Investigation of possible gene-environmental correlation between parenting and offspring externalising behaviours in adoption samples found no passive or evocative *rGE* effects in the associations between parental knowledge and offspring externalising behaviours[142], whereas one study reported an evocative *rGE* showing that parental hostility was evoked by genetically influenced offspring behaviour[144], and another reported child-to-parent effects on maternal support and negativity[148]. As well as highlighting the bidirectionality of parent–offspring associations, these studies show that associations between parenting and offspring outcomes vary by phenotype and no single explanation fits all parenting–offspring associations.

Parental substance use

Two studies reported that parental drug abuse[149] and smoking[150] shared genetic overlap with offspring externalising behaviours (Table 4). These reports of genetic overlap are in line with classical twin studies which suggest that comorbidity between substance use and externalising behaviours is partly due to overlapping genetic factors68[151,152]. After accounting for genetic relatedness, mixed evidence for environmental associations between parental substance use and some offspring externalising behaviours was found. Maternal smoking during pregnancy was linked to offspring oppositional defiant disorder[153] and conduct problems[153], whereas a larger study showed no association with offspring disruptive behaviours[154]. Similarly, smoking during pregnancy was associated with parent-reported ADHD symptoms in one sibling comparison study[155], but not another[153], and was not associated with ADHD diagnosis in a large population-based sample[156]. Exposure to maternal alcohol use during pregnancy was linked to offspring aggression in one study[131], and to offspring ADHD symptoms in another[157], but the latter association was not reliably observed across measurement instruments, and moreover, maternal drinking was not associated with ADHD diagnosis[157] or attentional problems[131]. Studies of parental substance use during childhood found no environmental effect of parent alcohol and tobacco use[147] or drug abuse[150] on offspring externalising behaviours. The overall pattern of results indicates that prenatal exposure to substance use may be associated with some offspring externalising behaviours, but no firm conclusions can be drawn from current or previous work.

Parental education attainment

Three studies found evidence of genetic overlap between parental education attainment and offspring ADHD symptoms[105,130,158] (Table 4). Genetic overlap between educational attainment and ADHD is previously known[159], and is hypothesised to either suggests a common neurobiological process underlying both inattention symptoms and academic achievement, or an indirect mechanism through which genetically influenced inattention impacts academic achievement[160]. Both of these scenarios are feasible in the context of the observed parent–offspring associations.

Findings for an environmental pathway were mixed. Although a within-family PGS study estimated that the association between maternal education and offspring ADHD would be null after adjusting for PGS that captured all heritability based on twin-based estimates[105], a large children-of-twins study found that maternal education was associated with offspring ADHD symptoms even after accounting for genetic relatedness[130]. Parental educational attainment has been

associated with specific parenting styles[161], and it seems plausible that these parenting behaviours subsequently influence offspring ADHD. However, based on what we know from twin literature, where ADHD shows very high heritability, and little effects of the shared or unique environments[6], the overall impact of parenting behaviours on ADHD is likely to be small.

Genetic nurture

One within-family PGS study of ADHD found no genetic nurturing effect on offspring ADHD due to ADHD or educational attainment related to parental genes[158]. Although this finding requires replication, it is compatible with what we know from twin-based literature, discussed above.

Offspring educational attainment

Intergenerational transmission of educational attainment

Studies investigating intergenerational educational attainment showed consistent evidence of genetic overlap between parent and offspring educational attainment[105,130,162–164] (Table 5). Additional evidence of genetic transmission was provided by several within-family PGS studies showing that parental genetic liability for educational attainment predicted offspring educational attainment[33,105,158,165–167]. After accounting for genetic relatedness, evidence of environmental transmission of intergenerational educational attainment was observed in several studies[130,162–165]. Taken together, current literature indicates that as well as passing on education-associated genes, parents may shape the rearing environment in a way that influences the offspring's subsequent educational attainment. However, these environment influences may nonetheless be partly influenced by parental genes. In line with this, a within-family PGS study provided evidence of passive *rGE*, showing that individuals with higher PGS for educational attainment tended to grow up in better-educated households than those with lower PGS[168].

Genetic nurture

Research into genetic nurture has gained traction in the last two years, starting with the publication of three landmark studies with novel designs to identify genetic nurturing effects on offspring educational attainment[33,36,106] (Table 5). These studies have highlighted that parental genes can have an indirect (environmentally mediated) effect on offspring educational attainment through parental traits that are genetically influenced. The genetic nurturing effect on offspring educational attainment has been replicated in several samples[158,161,166,167,169–172], and a few studies reported that the observed effect

was partly explained by family socioeconomic status[36,169,172]. This finding is compatible with an adoption study which found that adoptive parents with higher income had offspring with increased educational attainment[173]. Other studies reported additional mediating effects of parental IQ[171], maternal health during pregnancy[172] and parenting behaviours[161]. The last study was the first to show that specific parenting behaviours are under the genetic influence of education-associated genes, and that these genetically influenced parenting behaviours are subsequently associated with offspring educational attainment. In addition, the study reported evidence of passive *rGE*, as mothers with higher PGS for education attainment provided home environments that were more conducive to higher educational attainment (greater cognitive stimulation, more warm and sensitive parenting, and less chaotic and safer, tidier homes)[161]. Evidence of passive *rGE* was also found for the overall genetic nurturing effect in a within-family PGS study of adoption samples, where parental PGS of educational attainment was more strongly associated with offspring educational attainment in biological families than adoptive families[167]. This particular passive *rGE* has also been reported outside of the reviewed work[174].

Maternal smoking during pregnancy

A large children-of-twins study reported genetic overlap between maternal smoking during pregnancy and offspring general cognitive ability[150] (Table 5). This finding is in line with the known negative genetic correlation between smoking and educational attainment[78] and highlights that in observational studies without genetically informative designs, this parent–offspring association explained by unmeasured genetic effects could lead to spurious conclusions. Investigations of environmental transmission did not reveal robust associations; maternal smoking during pregnancy was negatively associated with reading cognition[154], but associations with other measures of cognitive functioning[154], general cognitive ability[150], and academic achievement[150] did not remain after accounting for genetic relatedness. Previous literature on genetically informative designs suggests that familial factors, including genetic effects, account for the relationship between smoking during pregnancy and offspring cognition[175].

Offspring substance use

Intergenerational transmission of substance use behaviours

Studies investigating intergenerational transmission of substance use behaviours (Table 6) showed consistent evidence of genetic transmission of substance involvement[176], alcohol use[25,177–179], drug abuse[25,27,180,181] and smoking initiation[182].

There was also evidence of environmental transmission of many substance use behaviours, including drinking behaviour[183], alcohol use disorder[25,27,177,179], drug abuse[25,27,30,180,181,184], smoking behaviour[182,185] and addiction-prone personalities[186], whereas parental dependency on alcohol was not consistently associated with offspring alcohol involvement[176,178]. Two studies showed no long-term effects of maternal smoking during pregnancy on offspring substance use behaviours[150,187]. Although parental substance use behaviours were generally associated with an increased likelihood of substance use in offspring, an extended twin study observed negative environmental transmission of smoking behaviours, whereby parental smoking had an inhibiting effect on offspring smoking initiation[188]. The finding was marginally significant and requires replication. One study found evidence of passive *rGE* underlying parent–offspring similarity in drinking behaviours, with more similarities in biological parent–child relationships than in adoptive families[183].

Parenting behaviours

Studies investigating the associations between parenting behaviours and offspring substance use (Table 6) showed that adoptive parenting behaviours such as parental involvement[189], family care[186], family cohesion, parental monitoring, parental care and parental support[190] were associated with a lowered risk of offspring substance use behaviours, whereas adoptive parents' overprotectiveness or control had no effect[190]. In addition, children exposed to adoptive parenting had a lower risk of drug abuse than their unexposed sibling, indicating a protective effect of adoptive parenting on substance use behaviours, which was also reported for MDD above[191].

Offspring personality

There was evidence of genetic and environmental influences underlying associations between parental characteristics and offspring personality (Table 7). Parent sociability and offspring positive emotionality[192], and parent behavioural motivation and offspring social motivation[144] shared common genetic factors, whereas the intergenerational transmission of neuroticism seemed to be environmentally explained[117]. There was no evidence of an environmental association between parental traits, including anxiety[193], sociability[192], and smoking during pregnancy[154], and offspring personality traits such as sociability and temperament. In addition, an extended twin study found no evidence of environmental transmission or *rGE* underlying associations between parent and offspring dimensional personality traits[194]. However, two studies observed evocative effects of offspring social behaviours on parenting; adopted offspring's genetically influenced social behaviours predicted adoptive parent hostility[144] and

child-centred parenting[193]. Overall, current and previous literature indicates that relationships between parental factors and offspring personality vary substantially by phenotype, and can involve both genetic and environmental processes.

Table 2 Summary of findings from the reviewed studies

Trait(s)	Number of studies	Designs	Genetic overlap
Offspring internalising behaviours			
Parental internalising behaviours	19	11 adoption studies [107,108,113-116,118,120,123,128,141] 5 children-of-twins studies [107,109,110,117,195] 3 sibling comparison studies [112,119,121] 1 multiple parenting relationships study [111]	There was evidence of genetic overlap between parental depression and offspring internalising symptoms, but not parental anxiety
Parenting	8	4 children-of-twins studies [124-127] 2 sibling comparison studies [129,148] 2 adoption studies [123,128]	There was no evidence of genetic overlap between parenting factors and offspring internalising behaviours
Genetic nurture	2	1 M-GCTA study [63] 1 RDR study [64]	Not studied
Parental educational attainment	1	1 children-of-twins and siblings study [130]	There was evidence of genetic overlap between parental educational attainment and offspring depressive symptoms
Parental substance use	1	1 sibling comparison study [131]	Not studied
Offspring externalising symptoms			
Parental externalising behaviours	9	7 adoption studies [132-138] 1 multiple parenting relationships study [139] 1 triparental study [27]	There was evidence of genetic transmission of criminal behaviours; evidence for other externalising symptoms was ambiguous, although better-powered studies tended to find supportive evidence
Parental internalising behaviours	11	6 adoption studies [107,108,113,128,135,141] 3 children-of-twins studies [107,110,140] 3 sibling comparison studies [109,112,119]	There was evidence of genetic overlap between parental depression and offspring externalising symptoms
Parenting	14	11 adoption studies [128,132-137,141,143,144,147] 2 sibling comparison studies [145,148] 1 children-of-twins study [142]	There was some evidence of genetic overlap between parenting factors and offspring externalising behaviours
Parental substance use	10	7 sibling comparison studies [131,150,153-157] 3 adoption studies [135,147,149] 1 children-of-twins study [150]	There was evidence of genetic overlap between parental drug abuse and smoking and offspring externalising behaviours
Parental education	3	1 within-family PGS adjustment study [105] 1 within-family PGS genetic nurture study [158] 1 children-of-twins study [130]	There was evidence of genetic overlap between parental educational attainment and offspring externalising symptoms
Genetic nurture	1	1 within-family PGS study [158]	Not studied
Offspring educational attainment			
Parental educational attainment	9	4 adoption studies [163,164,173,196] 3 within-family PGS adjustment studies [105,165,166] 1 extended twin study [162] 1 children-of-twins and siblings study [130]	There was substantial evidence of genetic overlap between parental and offspring educational attainment
Genetic nurture	12	11 within-family PGS studies [33,36,158,161,165-169,171,172] 1 RDR study [34]	Not studied
Maternal smoking during pregnancy	2	2 sibling comparison studies [150,154] 1 children-of-twins study [150]	There was evidence of genetic overlap between maternal smoking during pregnancy and offspring cognition

	Environmental transmission	Gene-environment correlation
	There was evidence that parental anxiety and depression were associated with offspring internalising symptoms through environmental pathways	No evidence of evocative rGE was found, but child-to-parent effects were identified
	There was evidence that negative parenting behaviours were associated with more offspring internalising behaviours, and positive parenting was associated with fewer offspring internalising behaviours	No evidence of evocative rGE was found, but child-to-parent effects were identified
	There was evidence of a genetic nurturing effect on offspring depressive (but not anxiety) symptoms	One study reported a negative rGE between genetic nurture and offspring depressive symptoms
	Parental educational attainment was not associated with offspring depression through an environmental pathway	Not studied
	Maternal drinking during pregnancy was associated with emotional reactivity and somatic complaints, but associations with anxiety and depressive symptoms were confounded	Not studied
	There was evidence that parent and offspring criminal behaviours were associated through environmental pathways	Not studied
	There was evidence that parental depression was associated with offspring externalising symptoms through environmental pathways	One study reported evocative rGE effects on the association between parental depression and offspring externalising symptoms
	There was evidence that negative parenting behaviours were associated with more offspring externalising behaviours, whereas positive parenting was associated with fewer offspring externalising behaviours	There was some evidence of evocative rGE and other child-to-parent effects
	There was mixed evidence for an environmental association between parental substance use and offspring externalising behaviours	Not studied
	There was some evidence of environmental associations between maternal education and offspring attention-deficit hyperactivity disorder (ADHD)	Not studied
	No genetic nurturing effect on offspring ADHD was observed	Not studied
	There was evidence that parent and offspring educational attainment were associated through environmental pathways	One study reported passive rGE effects underlying the association between parent and offspring educational attainment
	There was evidence of a genetic nurturing effect on offspring educational attainment	There was evidence of passive rGE on offspring educational attainment
	There was little evidence of environmental associations between maternal smoking during pregnancy and offspring cognition	Not studied

Offspring substance use			
Parental substance use behaviours	15	4 children-of-twin studies [150,176,178,182] 2 adoption studies [177,183] 2 sibling comparison studies[150,187] 2 triparental studies [27,180] 2 multiple parenting relationships studies [25,181] 2 extended family designs [179,184] 1 within-family PGS study [182] 1 extended twin study [185] 1 matched-pair case-control study [30]	There was evidence of genetic overlap between parental and offspring substance use behaviours
Parenting	4	3 adoption studies [186,189,190] 1 sibling comparison study [191]	Not studied
Offspring personality			
Parental characteristics	6	3 adoption studies [144,192,193] 1 sibling comparison study [154] 1 children-of-twins study [117] 1 extended twin study [194]	There was some evidence of genetic overlap between parental characteristics and offspring personality

rGE: gene-environment correlation; *M-GCTA*: maternal-effects genome-wide complex traits analysis; *RDR*: relatedness disequilibrium regression; *PGS*: polygenic scores

	There was evidence of environmental associations between parental and offspring substance use behaviours	One study reported passive rGE underlying the association between parent and offspring substance use behaviours
	There was evidence of protective effects of several parental factors on offspring substance use behaviours	Not studied
	There was some evidence of environmental associations between parental and offspring personality	There was evidence of evocative rGE underlying associations between parenting behaviours and offspring social behaviours

DISCUSSION

This review provides a broad overview of genetically informative literature investigating associations between parental characteristics and offspring mental health and related outcomes. This is a topic of substantial interest, with 89 relevant articles published in the past 6 years. Overall, reviewed studies showed reliable evidence of genetic transmission of depression, criminal behaviour, educational attainment, and substance use behaviours from parent-to-child. Additionally, cross-phenotype genetic overlap was observed in several instances; for example, parental depression, substance use, and educational attainment were all associated with offspring externalising behaviours through genetic pathways (Table 2). After accounting for genetic transmission, parental depression or anxiety were associated with offspring internalising or externalising behaviours through environmental pathways. For maternal exposures, these associations were related to concurrent maternal symptoms, with no long-lasting effect of prenatal depression or anxiety on offspring mental health. Other environmental associations and *rGEs* were observed for parent–offspring similarity in criminal behaviours, substance use behaviours, and educational attainment. In addition, positive and negative parenting behaviours held associations with offspring internalising behaviours, externalising behaviours, substance use behaviours, and educational attainment, with some evidence of *rGE*. Finally, cross-lagged studies showed bidirectional associations between parenting traits and offspring behaviours, where parenting predicted offspring behaviours, and offspring behaviours predicted parenting.

The reviewed literature highlights that genetically informative designs must be implemented to model or control for genetic effects in studies investigating parental influences on offspring development. There was substantial evidence of genetic overlap between parental and offspring phenotypes for both similar traits (e.g. parental depression and offspring internalising symptoms)[107–111] and dissimilar traits (e.g. parental depression and offspring externalising problems)[107–110,140]. As well as indicating genetic transmission of similar traits, these findings indicate that the same genetic factors may be relevant for the development of several distinct mental health problems[78], and could also partly explain the comorbidity between mental health disorders that is widely observed in literature[197]. Without accounting for genetic transmission within families, observational studies run a serious risk of misinterpreting these associations as causal environmental influences. For instance, it was observed that after accounting for shared genetic effects, perinatal maternal depression did not hold any long-lasting associations with offspring internalising or externalising

behaviours in childhood[109,110,112,119,121]. This is in contrast to the substantial body of literature that interprets associations between perinatal maternal distress and offspring mental health outcomes in causal terms[122]. We urge future studies investigating parent–offspring associations to err on the side of caution in interpreting their results and consider evidence from multiple methodologies in forming their conclusions. Even genetically informative designs can be skewed towards non-genetic findings if there is insufficient power in the study. Triangulating evidence from multiple methodologies is required before a general conclusion can be reached on whether a given parent–offspring association is likely to be truly present, after accounting for shared genetic effects or *rGE*.

Even so, the reviewed studies indicate that both genetic and environmental factors are important in associations between parental factors and offspring mental health outcomes (Table 2). These overall findings raise two important questions; to what extent are parent–offspring associations due to genetic transmission, and to what extent does parenting truly matter? Findings from classical twin literature indicate that between 40 and 80% of individual differences in mental health phenotypes such as internalising and externalising problems between people are explained by additive genetic effects[6]. This suggests that the largest way through which parents influence offspring mental health outcomes is through the passing on of their genes. In addition, estimates of heritability for mental health phenotypes within classical twin literature tend to increase with age, while the influence of the shared family environment decreases[9]. From a developmental perspective, this indicates that genetic influences on offspring mental health become increasingly important as the child gets older while the overall environmental impact of parental characteristics on offspring behaviour is likely to be small. In the current review, effect sizes showing the relative contribution of genetic and environmental factors in parent–offspring associations were not consistently reported and the available statistics are hard to compare between studies. Some studies reported higher effect sizes for genetic or environmental transmission, while others reported equal effect sizes for genetic and environmental effects in parent–offspring associations (Tables 3–7). Based on prior knowledge, the overall effect of any single parental environmental exposure is likely to be far lower than the estimated heritability of offspring mental health and related traits, as is the effect of a single genetic variant. It is also worth highlighting that environmentally mediated influences can still be under the influence of parental genes. Previous twin literature shows that parenting behaviours are under genetic influence themselves and reflect heritable individual differences[198–200]. Genetic nurture is a new way to index the environmentally mediated effect of parental genes on offspring behaviour.

The reviewed studies provide evidence of genetic nurture effects on offspring internalising symptoms and educational attainment (Table 2). This is a promising area of research and we expect the development and application of genetic nurture designs to continue to expand in the coming years.

As well as demonstrating genetic overlap and environmental transmission within parent–offspring associations, the reviewed studies showed that confounding by passive *rGE* is also prevalent within genetically informative designs (Table 2). If unmodelled, these unmeasured effects may inflate the estimation of both genetic and environmental factors. Additionally, evocative *rGE* can also explain parent–offspring associations. The reviewed studies showed evidence of evocative *rGEs* underlying associations between parental characteristics and offspring internalising symptoms, externalising symptoms and personality (Table 2). These findings are compatible with previous literature which shows a moderate impact of offspring's genetically influenced behaviours on parenting factors[201,202]. In instances where evocative *rGE* effects were not observed, child-to-parent effects were sometimes still present[107,116,118,120,123,148]. These findings highlight the bidirectional and dynamic nature of parent–offspring associations, with child-to-parent effects, as well as parent-to-child effects, and also show the importance of cross-lagged models in modelling parent–offspring associations over time.

Reviewed findings with clinical implications are worth highlighting further. Parents with depression, anxiety, substance use problems, and externalising behaviours appeared to pass on these traits to the offspring through both genetic and environmental mechanisms. This information can be used to extend preventative and early intervention services to high-risk children of parents with internalising, externalising, or substance use disorders in healthcare settings. Family-based interventions, including cognitive, behavioural, and psychoeducational components, are already shown to be effective in children of parents with internalising and externalising disorders[203]. In addition, several reviewed studies showed that positive parental environments, such as parental warmth and positive reinforcement, were protective against externalising and substance use behaviours in children with high inherited risk[135,136,191]. Whilst preventative interventions for externalising problems already include a family component, current preventative strategies for substance use incorporate school-based and skills training approaches[204]. A family-based approach could be a valuable addition to preventative interventions of substance use behaviours in early life.

To conclude, parental factors are important predictors of offspring mental health and related outcomes. Both genetic and environmental processes are important in these associations. Further clarification of these processes requires more research. Exciting opportunities for parent–offspring research are increasingly present, with the availability of more datasets and ongoing advances in methodologies.

Table 3 Detailed characteristics of studies investigating offspring internalising behaviours (N=30)

Offspring internalising behaviours				
Study	**Design**	**Sample**	**Parental attribute (predictor)**	**Child attribute (outcome)**
Brooker et al., 2014 [115]	Adoption	*EGDS* 361 families Offspring age: 18-27 months	*Adoptive & birth parent anxiety:* self-report, BAI	*Internalising problems:* maternal and paternal-report, composite score, CBCL
Brooker et al., 2015 [116]	Adoption	*EGDS* 349 families Age: 9-27 months	*Adoptive parent anxiety:* self-report, BAI *Birth parent negative affect:* self-report, ATQ	*Negative affect:* observation and adoptive-parent report, composite score, ICQ and TBAQ
Marceau, Laurent, et al., 2015 [128]	Adoption	*EGDS* 361 families Age: 9 months-6 years	*Over-reactive parenting:* self-report, PS *Birth mother risk:* self-report, composite score, substance use, depression (BDI) & anxiety (BAI)	*Internalising behaviours:* parent-report, CBCL
McAdams et al., 2015 [107]	Adoption, Children of Twins	*Adoption: EGDS* 361 families Age: 4.5-7 years *CoT: TOSS* 287 monozygotic (MZ) & 489 dizygotic (DZ) twin families Age: 11-22 years	*Adoptive & parent depression:* self-report, BDI *Depressive symptoms (CoT):* self-report, CES-D	*Internalising problems (adoption sample):* parent-report, CBCL *Internalising problems (CoT sample):* mother, father and self-report, CBCL
Eley et al., 2015 [117]	Children of Twins	*TOSS* 387 MZ, 489 DZ families Age: 11-22 years	*Anxious personality:* self-report, KSP	*Anxiety:* mother, father and self-report, CBCL
Roos et al., 2016 [141]	Adoption	*EGDS* 293 families Age: 6-7 years	*Adoptive & birth mother internalising symptoms:* self-report, composite score, BAI and BDI *Adoptive mother uninvolved parenting:* self-report, APQ *Adoptive & birth mother processing speed:* Stroop color-word naming task	*Internalising-only problems:* parent-report, CBCL *Co-occurring internalising and externalising problems:* parent-report, CBCL
Grabow et al., 2017 [108]	Adoption	*EPoCH* 541 adoptive mother-child dyads, 126 biological mother-child dyads Age: 7 years	*Maternal trauma frequency:* repeated self-report, mean score, NLES *Adoptive & birth mother depressive symptoms:* self-report, BDI	*Internalising behaviours:* parent-report, CBCL

Control variables	Genetic overlap	Environmental transmission	G-E interplay
	No, birth parent anxiety did not predict offspring internalising problems	Yes, adoptive parent anxiety predicted offspring internalising problems (β = .25)	G×E: high birth parent anxiety x greater attention control x low adoptive parent anxiety: fewer internalising problems
Prenatal risk and obstetric complications, adoption openness	No, birth parent negative affect did not predict offspring negative affect (effect size not clear)	Yes, adoptive parent anxiety predicted offspring negative affect (effect size not clear)	No evidence of evocative rGE, but child-to-parent effects found
Adoption openness	No, birth mother risk did not predict offspring internalising behaviours (effect size not clear)	Yes, paternal (but not maternal) over-reactive parenting predicted offspring internalising behaviours (effect size not clear)	
Adoption sample: obstetric complications, adoption openness CoT sample: twin sex, age	Adoption: Birth mother depressive symptoms predicted internalising problems at age 7 (β = .15), but not age 4.5 or age 6 CoT: No shared genetic effects between parental depression and offspring internalising problems	Adoption: No, adoptive parent depression did not predict subsequent offspring internalising problems CoT: After accounting for genetic relatedness, parental depression was associated with offspring internalising problems (effect size not clear)	No evidence of evocative rGE, but child-to-parent effects found
Twin sex, age	No shared genetic effects between parental anxious personality and offspring anxiety	Yes, after accounting for genetic relatedness, parental anxiety was associated with offspring anxiety symptoms (effect size not clear)	
Child sex, child age, adoption openness, obstetric complications	Birth mother internalising symptoms and processing speed did not predict internalising-only symptoms, but processing speed was associated with co-occurring symptoms (OR = 1.88)	Adoptive parent internalising symptoms predicted internalising-only symptoms (OR = 1.17), but not co-occurring symptoms; uninvolved parenting predicted co-occurring symptoms (OR = 7.91), but not internalising-only symptoms and adoptive parent processing speed and offspring outcomes were unrelated	G×E: adoptive mother high internalising symptoms x inherited risk of slow processing speed: co-occurring symptoms
EPoCH: Timing of maternal trauma, socioeconomic status (SES), sex EGDS: Perinatal risk, adoption openness, SES, sex	Yes, birth mother depression predicted adopted-away offspring internalising behaviours (β = 0.16)	Adopted mother depression predicted offspring internalising behaviours (β = 0.15), and mediated the relationship between maternal trauma and offspring internalising behaviours	

Offspring internalising behaviours				
Study	**Design**	**Sample**	**Parental attribute (predictor)**	**Child attribute (outcome)**
Gjerde et al., 2017 [112]	Sibling comparison	*MoBa* 17,830 siblings, 11,599 families Age: 6 months to 5 years	*Maternal depression:* self-report, SCL	*Internalising problems:* maternal-report, CBCL
Bekkhus et al., 2018 [121]	Sibling comparison	*MoBa* 21,980 families with at least two siblings Age: 6 months to 3 years	*Maternal anxiety during pregnancy:* self-report, SCL (short version)	*Infant difficulties:* maternal-report, ICQ *Emotional difficulties:* maternal-report, CBCL
Bridgett et al., 2018 [123]	Adoption	*EGDS* *361 families* *Age: 4.5-6 years*	*Harsh negative parenting:* observation *Biological parent self-regulation:* Go/No Go task computerised task	*Self-regulation:* parent-report (Children's Behavior Questionnaire) and Go / No Go computerised task
Hannigan, Eilertsen, et al., 2018 [109]	Multiple children of twins and siblings	*MoBa* 22,195 mothers, 25,299 children Age: 18-60 months	*Maternal depressive symptoms:* self-report, SCL	*Internalising problems:* maternal-report, CBCL
Liskola et al., 2018 [114]	Adoption	*FAS* 548 international adopted children Age: 9-12 years	*Depressive symptoms:* self-report, GHQ	*Depressive symptoms:* self-report, CDI
Kendler Kendler, Ohlsson, Sundquist, & Sundquist, 2018 [111]	Multiple parenting relationships design	*Snr* 2,041,816 intact, 14,104 adoptive, 115,501 not-lived-with father, 57,826 stepfather, 29,205 triparental families Age: 26-56 years	*Major Depression:* diagnosis, hospital discharge and outpatient care registers	*Major Depression:* diagnosis, hospital discharge and outpatient care registers
Ahmadzadeh et al., 2019 [118]	Adoption	*EGDS* 305 families Age: 6-8 years	*Adoptive parent anxiety:* self-report, ST-AIA *Birth parents' internalising problems:* mother & father self-report, composite score, CIDI and FH-RDC	*Anxiety:* maternal and paternal report, CBCL
Gjerde et al., 2019 [110]	Multiple children of twins and siblings	*MoBa* 22,316 mothers and 35,589 offspring Age: 1.5 to 5 years	*Concurrent maternal depression symptoms:* self-report, SCL	*Emotional problems:* maternal-report, CBCL
Hails et al., 2019 [113]	Adoption	*EGDS* 561 families Age: 9 months to 6 years	*Adoptive parent depression:* self-report, BDI II *Birth mother internalising symptoms:* self-report, CIDI	*Internalising symptoms:* parent and teacher report, CBCL and (TRF

Control variables	Genetic overlap	Environmental transmission	G-E interplay
Maternal parity, maternal education, child age, and child sex	Not studied	Children exposed to concurrent maternal depression had more internalising symptoms than their unexposed siblings, but peri-natal maternal symptoms had no effect	
Maternal substance use during pregnancy, post- birth anxiety, partner disharmony, somatic disease, marital status, education, age, parity, child gestational age, birth complications, sex, birthweight	Not studied	No difference in infant or emotional difficulties between exposed and unexposed siblings	
Obstetric and neonatal complications, adoption openness, child anger (parent report), gender	Yes, birth mother self-regulation predicted adopted-away offspring's self-regulation (β = .23)	Yes, adoptive parent harsh parenting predicted poor offspring self-regulation (β = -.22 to -.25)	No evocative rGE, but child-to-parent effects of child anger found
Prenatal depression: adjusted for concurrent depression	Yes, there were shared genetic effects between maternal depression and offspring internalising problems effect size not clear)	Yes, after accounting for genetic relatedness and prenatal depression, concurrent maternal depression was associated with offspring internalising problems (effect size not clear)	
Child age, gender, age at adoption, type of placement before adoption, continent of birth, adoptive family SES	Not studied	Adoptive paternal (but not maternal) depressive symptoms were associated with offspring depressive symptoms	
None	Yes, MD status of not-lived-with biological parents was associated with offspring MD (r = 0.08)	Yes, MD status of adoptive or step-parents was associated with offspring MD (r = 0.08)	No G×E interaction found
Weighted risk score of obstetric complications, adoption openness, child sex	No, birth parents' internalising problems did not predict adopted-away offspring anxiety	Adoptive paternal anxiety (but not maternal) predicted offspring anxiety (β = .10)	No evocative rGE, but child-to-mother effects found
Child sex, maternal age	Yes, there were shared genetic effects between maternal depression and offspring emotional problems (R^2 = 21.1 to 28.5%)	Yes, after accounting for genetic relatedness, maternal depression was associated with offspring internalising problems (R^2 = 0.3 to 2.2%)	
Adoption openness, prenatal risk and obstetric complications, infant negative emotionality	No, birth mother internalising symptoms did not predict offspring internalising symptoms	Adoptive paternal (but not maternal) depression predicted parent-reported (but not teacher-reported) offspring internalising symptoms (β = .21)	

Offspring internalising behaviours				
Study	**Design**	**Sample**	**Parental attribute (predictor)**	**Child attribute (outcome)**
Field et al., 2020 [120]	Adoption	*EGDS* 561 families Age: 18 months to 4.5 years	*Adoptive and birth parent anxiety:* self-report, composite score of 2 measurements, BAI	*Anxiety symptoms:* parent report, average of maternal and paternal report, CBCL
Gjerde et al., 2020 [119]	Sibling comparison	*MoBa* 11,553 mothers and 17,724 children Age: 1.5-5 years	*Maternal anxiety*: self-report, SCL	*Child internalising problems:* maternal-report, CBCL
O'Reilly et al., 2020 [195]	Children of siblings	*Snr* 2,762,883 unique offspring Age: 12 and over	*Suicidal behaviour:* suicide attempt or death by suicide, National Patient Register and Cause of Death register, prior to offspring age 18	*Suicidal behaviour:* suicide attempt or death by suicide, National Patient Register and Cause of Death register
Horwitz et al., 2015 [124]	Extended Children of Twins	*TOSS, TCHAD* 858 twin families, 690 twin families Age: 11-22 years, 16-17 years	*Parental criticism:* self-report, EES	*Somatic symptoms:* parent and self-report, composite score, CBCL
Guimond et al., 2016 [148]	Sibling comparison	*QNTS* 164 twin pairs Age: 13-14 years	*Perceived maternal support and negativity:* child-report, NRI	*Depressive symptoms:* self-report, CDI
McAdams et al., 2017 [125]	Children of Twins	*TOSS* 387 MZ, 489 DZ families Age: 11-22 years	*Expressed affection and closeness with child:* self-report	*Self-worth:* self-report, HPCS
Hannigan, Rijsdijk, et al., 2018 [126]	Children of Twins	*TOSS* 909 twin pairs Age: 11-22 years	*Relationship quality with offspring:* maternal and paternal-report, P-CAS, EAS and P-CRQ	*Internalising problems:* self-report, CBCL
Ahmadzadeh et al., 2020 [127]	Extended Children of Twins	*TOSS, TCHAD* 876 twin families, 1030 twin families Age: 11 to 22 years	*Parental criticism:* self-report, EES	*Internalising symptoms:* parent and self-report, composite score, CBCL and YSR
Kendler et al., 2020 [129]	Sibling comparison	*Snr* 666 full sibships and 2,596 half sibships of high-risk (MDD diagnosis) biological parents Age: 15 and over	*Adoptive parenting:* protective effect of high-quality rearing environment	*Major Depression:* diagnosis, hospital discharge, outpatient care registers, primary care registry
Jami et al., 2020 [63]	M-GCTA, Children of twins and siblings	*MoBa* M-GCTA: 3,801 parent-offspring trios, extended CoT: 10,688 children Age: 8 years	*Genetic nurture*: M-GCTA, maternal and paternal genotypes *Shared maternal or paternal environment*: children of twins and siblings	*Anxiety symptoms:* maternal report, SCARED *Depressive symptoms:* maternal report, SMFQ

Control variables	Genetic overlap	Environmental transmission	G-E interplay
	No, birth parent anxiety did not predict offspring anxiety symptoms	Adoptive maternal and paternal anxiety equally predicted both offspring anxiety symptoms and change in anxiety symptoms (effect size not clear)	No evidence of evocative rGE found
Child age, child sex, maternal depressive symptoms, parity, education	Not studied	Children exposed to concurrent maternal anxiety had more internalising symptoms than their unexposed siblings, but peri-natal maternal symptoms had no effect	
Offspring: parity. Parental: age at birth, educational attainment, Swedish by birth, mental illness, criminal convictions	Yes, there were shared genetic effects between parental and offspring suicidal behaviour (effect size not clear)	Yes, after accounting for genetic relatedness, parental suicidal behaviour was associated with offspring suicidal behaviour effect size not clear)	
Age, sex, age difference for the cousin offspring in TOSS	No shared genetic effects between parental criticism and offspring somatic symptoms	Yes, after accounting for genetic relatedness, parental criticism was associated with offspring somatic symptoms (effect size not clear)	No evidence of passive or evocative rGE found
Genetically-controlled analyses using MZ twin-difference score	Not studied	No, perceived maternal support and negativity were not associated with offspring depressive symptoms	No evidence of evocative rGE, but child-to-parent effects found
Twin sex and age	No shared genetic effects between expressed affection or closeness with child and offspring self-worth	Yes, after accounting for genetic relatedness, expressed affection and closeness with child were associated with offspring self-worth (effect size not clear)	
	No shared genetic effects between parental relationship quality with offspring, and offspring internalising problems	Yes, after accounting for genetic relatedness, parental relationship quality with offspring was associated with offspring internalising problems (effect size not clear)	
Child age, sex	No shared genetic effects between parental criticism and offspring internalising symptoms	Yes, after accounting for genetic relatedness, parental criticism was associated with offspring internalising symptoms (effect size not clear)	
Parental age at birth, high-risk status of the other parent of half-sibling, child sex	Not studied	Children exposed to adoptive parenting had lower risk of MDD than their unexposed siblings, this protective effect disappeared when the adoptive family was disrupted or if there was a high-risk adoptive parent	
Sex, genotyping batch, first 10 principal components	Not studied	After accounting for shared genetic effects, maternal or paternal genes did not explain significant variance in offspring depression or anxiety symptoms, and there were no shared maternal or paternal environment effects	No evidence of rGE found

Offspring internalising behaviours				
Study	**Design**	**Sample**	**Parental attribute (predictor)**	**Child attribute (outcome)**
Cheesman et al., 2020 [64]	Relatedness Disequilibrium Regression (RDR), Children of twins and siblings	*MoBa* RDR: 11,598 parent-offspring trios, extended CoT: 26,086 pairs of relatives Age: 8 years	*Genetic nurture:* RDR, mid-parent genotype *Maternal emotional symptoms:* self-report, common factor score of 5 measurements, SCL-8 *Shared parental environment:* children of twins and siblings	*Anxiety symptoms:* maternal report, SCARED *Depressive symptoms:* maternal report, SMFQ
Lund et al., 2019 [131]	Sibling comparison	*MoBa* 14,639 mothers, 25,744 children Age: 1.5-5 years	*Maternal alcohol consumption during pregnancy:* self-report, AUDIT-C	*Emotional problems:* maternal-report, CBCL *Emotional reactivity* *Anxious/depressed* *Somatic complaints*
Torvik et al., 2020 [130]	Children of twins and siblings	*MoBa* 34,958 children Age: 8 years	*Educational attainment* (EA)*:* self-report, highest level completed	*Depression symptoms:* maternal report, SMFQ

G-E: gene-environment; *G×E*: gene-environment interaction, *rGE*: gene-environment correlation
Design= *M-GCTA*: maternal-effects genome-wide complex traits analysis
Samples= *EGDS*: Early Growth and Development Study; *EPoCH*: Early Parenting of Children study; *FAS*: Finnish Adoption Study; *MoBa*: Norwegian Mother Father and Child Study; *QNTC*: Quebec Newborn Twin Study; *Snr*: Swedish national registers*; TCHAD*: Twin Study of Child and Adolescent Development; *TOSS*: Twin Offspring Study of Sweden
Measures= *APQ*: Alabama Parenting Questionnaire; *ATQ*: Adult Temperament Questionnaire; *AUDIT-C*: Alcohol Use Disorder Identification Test-Consumption; *BAI*: Beck Anxiety Inventory; *BDI*: Beck Depression Inventory; *CBCL:* Child Behavior Checklist; *CDI*: Children's Depression Inventory*; CES-D*: Center for Epidemiological Studies Depression scale; *CIDI*; Composite International Diagnostic Instrument; *EAS*: Expression of Affection scale; *EES*: Expression Emotion scale; *FH-RDC*: Family History-Research Diagnostic Criteria; *GHQ*: General Health Questionnaire; *HPCS*: Harter Perceived Competence Scale; *ICQ*: Infant Characteristics Questionnaire; *KSP*: Karolinska Scales of Personality; *NLES*: Negative Life Events Scale; *NRI*: Network of Relationships Inventory; *P-CAS*: Parent-Child Agreement Scale; *P-CRQ:* Parent-Child Relationship Questionnaire; *PS*: the Parenting Scale*; SCARED*: Screen for Child Anxiety Related Disorders; *SCL*: Symptoms Checklist; *SMFQ*: Short Mood and Feelings Questionnaire; *S-TAIA*: State-Trait Anxiety Inventory for Adults; *TBAQ*: Toddler Behavior Assessment Questionnaire; *TRF*: Teacher Report Form; *YSR*: Youth Self Reports
Statistics= β: standardized parameter estimate; *OR*: odds ratio; R^2: percentage of variance explained; *r*: weighted tetrachoric correlation. Effect sizes are not reported for studies that did not investigate both genetic and environmental transmission.

	Control variables	Genetic overlap	Environmental transmission	G-E interplay
	Child sex. RDR: 10 principal components and genotyping batch	Not studied	After accounting for shared genetic effects, parental genes explained significant variance in offspring depression (but not anxiety) symptoms, this effect was partly mediated by maternal emotional symptoms Shared parental environmental effect was observed for offspring depression (but not anxiety) symptoms	Negative rGE between genetic nurture and offspring depressive symptoms
	Parity, unplanned pregnancy, daily smoking, pre-pregnancy abstinence from alcohol	Not studied	Exposed children were more emotionally reactive and had more somatic complaints, but did not have more anxious depressive symptoms, than their unexposed siblings	
		Yes, there were shared genetic effects between parental EA and offspring depression symptoms effect size not clear)	No, after accounting for genetic relatedness, parental EA was not associated with offspring depression	

Table 4 Detailed characteristics of studies investigating offspring externalising behaviours (N=36)

Offspring externalising behaviours				
Study	**Design**	**Sample**	**Parental attribute (predictor)**	**Child attribute (outcome)**
Bornovalova et al., 2014 [137]	Adoption	*SIBS* 402 adoptive, 204 biological families Age: 11-21 years	*Antisociality:* interview, SCI	*Maladaptive parenting:* self-report, PEQ *Marital discord: self-report or marital status,* MRS *Antisociality:* interview, SCI
Kendler et al., 2014 [138]	Adoption	*Snr* 18,070 adoptees, and their biological (79,206) and adoptive (47,311) relatives Age: adoption until 20 years old	*Adoptive parental/sibling criminal behaviour risk:* composite score, criminal behaviour, alcohol use disorder (AUD), drug abuse, psychiatric illness, parental divorce *Biological parent/sibling criminal behaviour risk:* composite score, criminal behaviour, AUD, drug abuse, psychiatric illness, parental educational attainment (EA), maternal divorce, age at birth	*Criminal behaviour:* register-based, any conviction
Lipscomb et al., 2014 [132]	Adoption	*EGDS* 233 families Age: 9 months to 6 years	*Adoptive parent over-reactive parenting:* self-report, PS *Birth parent self-regulation:* self-report, ATQ	*Externalising behaviour:* parent-report, CBCL
Kendler, Ohlsson, Morris, Sundquist, & Sundquist, 2015 [139]	Multiple parenting relationships design	*Snr* 2,111,074 intact, 155,121 not-lived-with father, 10,194 not-lived-with mother, 107,163 stepfather, 17,637 stepmother, 10,038 adoptive families Age: 15+	*Criminal behaviour:* Swedish Crime register	*Criminal behaviour:* Swedish Crime register
Kendler, Ohlsson, Sundquit, et al., 2015 [27]	Triparental family design	*Snr* 41,360 triparental families (mother, not-lived-with biological father, stepfather) Age: 15+	*Criminal behaviour:* Swedish Crime register	*Criminal behaviour:* Swedish Crime register
Hyde et al., 2016 [136]	Adoption	*EGDS* 561 families Age: 18-27 months	*Adoptive mother positive reinforcement:* observation *Birth mother antisocial behaviour:* self-report, DIS	*Externalising behaviours:* maternal-report, CBCL *Callous-unemotional behaviours* *Oppositional behaviours* *Attention-deficit behaviours*

	Control variables	Genetic overlap	Environmental transmission	G-E interplay
	Mother and father age, parental education, child ethnicity, child adoptive status, family-based clustering correction, child sex, age	Not studied	Adoptive maladaptive parenting and marital discord (but not antisociality) were associated with offspring disruptive behaviours	Parental antisociality & child disruptive behaviour disorders were associated in biological families, but not adoptive families. The authors interpret this as passive rGE, but it may be only indicative of genetic overlap
	Sex of the adoptee, birth year, age at first cohabitation with adoptive parents	Criminal behaviour of not-lived-with biological parent and siblings was associated with offspring criminal behaviour (OR = 1.5)	Criminal behaviour of adoptive family and siblings was associated with offspring criminal behaviour (OR range = 1.3-1.4)	No evidence of G×E interaction found
	Prenatal and obstetric complications, birth mother IQ, adoptive family SES, adoption openness, child age, sex, age of entry & time spent in early care	No, birth parent self-regulation did not predict offspring externalising behaviours	Yes, over-reactive adoptive parenting was associated with externalising behaviours (β = .14)	G×E: low birth parent self-regulation & exposure to early care-centre x over-reactive parenting: more externalising problems
	Criminal behaviour status of all other relevant biological and step-parents	Yes, criminal behaviour of not-lived-with biological parents was correlated with offspring criminal behaviour (HR = 1.56)	Yes, criminal behaviour of adoptive or step-parent was correlated with offspring criminal behaviour (HR = 1.28)	
		Yes, criminal behaviour of not-lived-with biological parents was correlated with offspring criminal behaviour (HR = 1.46)	Yes, criminal behaviour of adoptive or step-parent was correlated with offspring criminal behaviour (HR = 1.30)	
	Child sex, openness/contact in the adoption, perinatal risk index	Birth mother antisocial behaviour predicted offspring callous-unemotional behaviours (β = .16), but not oppositional or attention-deficit behaviours	Adoptive mother positive reinforcement was protective against callous-unemotional (β = -.19) and oppositional (β = -.15), but not attention-deficit behaviours	G×E: high birth mother antisociality x low adoptive mother positive reinforcement: callous-unemotional behaviours

Offspring externalising behaviours				
Study	**Design**	**Sample**	**Parental attribute (predictor)**	**Child attribute (outcome)**
Stover et al., 2016 [133]	Adoption	*EGDS* 361 families Age: 9 months to 6 years	*Marital hostility:* self & spouse-report, BARS *Hostile parenting:* self-report, IFIRS *Birth mother antisociality*: self-report, composite score, delinquency (EYQ), substance use (CIDI), antisocial behaviour (CDIS)	*Aggression:* parent-report, CBCL
Reuben et al., 2016 [134]	Adoption	*EGDS* 361 families Age: 26 months to 7 years	*Warm parenting:* self-report, IFIRS *Over-reactive parenting:* self-report, PS *Birth mother externalising problems:* self-report, composite score, delinquency (ESBQ), novelty seeking (TCI), & drug dependence	*Externalising behaviour:* teacher-report, TRF *Effortful control:* shape Stroop task and gift delay task, composite score
Marceau et al., 2019 [135]	Adoption	*EGDS* 561 families Age: 4.5-8 years	*Adoptive parent warmth and hostility:* self-report, IWHS *Birth mother substance use during pregnancy:* study design cannot distinguish G and E effects *Birth mother internalising & externalising problems:* composite score, number of symptoms, diagnoses, age of onset, first degree relatives with psychopathology	*Conduct problems:* maternal-report, Preschool Age Psychiatric Assessment
Marceau, Laurent, et al., 2015 [128]	Adoption	*EGDS* 361 families Age: 9 months to 6 years	*Over-reactive parenting:* self-report, PS *Birth mother risk:* self-report, composite score, substance use, depression (BDI) and anxiety (BAI)	*Externalising behaviours:* parent-report, CBCL

	Control variables	Genetic overlap	Environmental transmission	G-E interplay
	Adoption openness	No, birth mother antisociality was not associated with offspring aggression	Adoptive parent hostile parenting and marital hostility were associated with offspring aggression (β range = -0.5 to .09)	
	Prenatal risk and obstetric complications, adoption openness, birth mother externalising problems, child sex	No, birth mother externalising problems did not predict offspring externalising behaviour or effortful control	Adoptive maternal warm parenting (but not paternal, or over-reactive parenting) was associated with offspring externalising behaviours (β = -.18), and this association was moderated by offspring effortful control	
	Adoption openness, child sex, and earlier externalising problems	Birth mother externalising and internalising problems were associated with fewer conduct problems in boys (β range = -0.09 to -0.15) but not girls	Adoptive parent warmth and hostility were not associated with offspring conduct problems after controlling for earlier externalising problems	G×E: birth mother externalising problems x adoptive parent warmth and hostility (boys only)
	Adoption openness	No, birth mother risk did not predict offspring externalising behaviours (effect size not clear)	Yes, maternal (but not paternal) over-reactive parenting predicted offspring internalising behaviours (effect size not clear)	

Offspring externalising behaviours				
Study	**Design**	**Sample**	**Parental attribute (predictor)**	**Child attribute (outcome)**
McAdams et al., 2015 [107]	Adoption, Children of Twins	*Adoption: EGDS 361 families* Age: 4.5 - 7 years *CoT: TOSS* 287 MZ and 489 DZ twin families Age: 11-22 years	*Adoptive & birth parent depression:* self-report, BDI *Depressive symptoms (CoT sample):* self-report, CES-D	*Externalising problems (adoption sample):* parent-report, CBCL *Externalising problems (CoT sample): m*other, father & self-report, CBCL
Roos et al., 2016 [141]	Adoption	*EGDS* 293 families Age: 6-7 years	*Adoptive & birth mother internalising symptoms:* self-report, composite score, BAI and BDI *Adoptive mother uninvolved parenting:* self-report, APQ *Adoptive & birth mother processing speed*: Stroop color-word naming task	*Externalising-only problems:* parent-report, CBCL *Co-occurring internalising and externalising problems:* parent-report, CBCL
Grabow et al., 2017 [108]	Adoption	*EGDS, EPoCH* 541 adoptive mother-child pairs, 126 biological mother-biological child pairs Age: 7 years	*Maternal trauma frequency:* repeated self-report, mean score, NLES *Adoptive & birth mother depressive symptoms:* self-report, BDI	*Externalising behaviours:* parent-report, CBCL, age 7
Gjerde et al., 2017 [112]	Sibling comparison	*MoBa* 11,599 families with 17,830 full siblings Age: 6 months to 5 years	*Maternal depression:* self-report, SCL	*Externalising problems:* maternal-report, CBCL
Hannigan, Eilertsen, et al., 2018 [109]	Multiple children of twins and siblings	*MoBa* 22,195 mothers and 25,299 children Age: 18-60 months	*Maternal depressive symptoms:* self-report, SCL	*Externalising problems:* maternal-report, CBCL
Gjerde et al., 2019 [110]	Multiple children of twins and siblings	*MoBa* 22,316 mothers and 35,589 offspring Age: 1.5 to 5 years	*Concurrent maternal depression symptoms:* self-report, SCL	*Behavioural problems:* maternal-report, CBCL

	Control variables	Genetic overlap	Environmental transmission	G-E interplay
	Adoption sample: Obstetric complications, adoption openness CoT sample: twin sex, age	Adoption sample: Birth mother depressive symptoms predicted externalising problems at age 4.5 and 7 (β range = .13 to .16), but not age 6 CoT sample: No shared genetic effects between parental depression and offspring externalising problems	Adoption sample: No, adoptive parent depression did not predict subsequent offspring externalising problems CoT sample: Yes, after accounting for genetic relatedness, parental depression was associated with offspring externalising problems (effect size not clear)	Evocative rGE: birth mother depression predicted child externalising problems, which predicted adoptive parent depression
	Child sex, child age, adoption openness, obstetric complications	Birth mother internalising symptoms and processing speed did not predict externalising-only symptoms, but maternal processing speed was associated with co-occurring symptoms (OR = 1.88)	Adoptive parent internalising symptoms, uninvolved parenting, and processing speed did not predict externalising-only problems, but uninvolved parenting was associated with co-occurring symptoms (OR = 7.91)	G×E: adoptive mother high internalising symptoms x inherited risk of slow processing speed: co-occurring symptoms
	EPoCH: Timing of maternal trauma, SES, child sex EGDS: Perinatal risk, adoption openness, SES, child sex	Yes, birth mother depression predicted adopted-away offspring externalising behaviours (β = .22)	Adopted mother depression predicted offspring externalising behaviours (β = .40), and mediated the relationship between maternal trauma and offspring externalising behaviours	
	Maternal parity, maternal EA, child age, child sex	Not studied	Children exposed to concurrent maternal depression had more externalising symptoms than their unexposed siblings, but peri-natal maternal symptoms had no effect	
	Prenatal analyses: adjusted for concurrent depression	Yes, shared genetic effects between maternal depression and offspring externalising problems explained 37% of variance (R^2) in offspring externalising problems	No, after accounting for genetic relatedness, maternal depression was not associated with offspring externalising problems	
	Child sex, maternal age	Yes, there were shared genetic effects between maternal depression and offspring behavioural problems (R^2 = 14.2 to 29.3%)	Yes, after accounting for genetic relatedness, maternal depression was associated with offspring behavioural problems (R^2 = 0.4 to 1.3%)	

Offspring externalising behaviours				
Study	**Design**	**Sample**	**Parental attribute (predictor)**	**Child attribute (outcome)**
Hails et al., 2019 [113]	Adoption	EGDS 561 families Age: 9 months to 6 years	*Adoptive parent depression:* self-report, BDI-II *Birth mother internalising symptoms:* self-report, CIDI	*Externalising symptoms:* parent and teacher report, CBCL and TRF
Eilertsen et al., 2020 [140]	Children of twins and siblings	*MoBa* 17,070 extended-family units Age: 5 years	*Parental prenatal depression symptoms:* self-reported at pregnancy week 30 for mothers, week 17 for fathers, Symptom Checklist	*ADHD symptoms:* maternal-report, CPRS
Gjerde et al., 2020 [119]	Sibling comparison	*MoBa* 17,724 offspring and 11,553 mothers Age: 1.5 to 5 years	*Maternal anxiety symptoms:* self-report, SCL	*Externalising problems:* maternal-report, CBCL
Samek et al., 2014 [147]	Adoption	*SIBS* 525 adopted and 323 biological offspring Age: 16.5 years and older	*Parent-child relationship quality:* offspring-report, PEQ *Alcohol and tobacco use:* mother & father report, composite score, SAM and CSUA	*Externalising behaviours:* latent factor based on antisocial behaviour (self-report, SCI), risky sexual behaviour (self-report, LEI), & alcohol and tobacco use (self-report, SAM)
Elam et al., 2014 [144]	Adoption	*EGDS* 316 families Age: 27 months to 4.5 years	*Adoptive parent hostility:* self-report, IFIRS	*Disruptive peer behaviour:* parent-report, PIPPS
Marceau, Narusyte, et all., 2015 [142]	Extended Children of Twins	*NEAD, TOSS* 408 twin/sibling pairs, 854 twin families Age: 11-22 years	*Parental knowledge:* mother, father and self-report, composite score, CMS	*Externalising problems:* mother, father, and self-report, composite score, ZBPI (NEAD sample), CBCL (TOSS sample)
Guimond et al., 2016 [148]	Sibling comparison	*QNTS* 164 twin pairs Age: 13-14 years	*Perceived maternal support and negativity:* child-report, NRI	*Delinquent behaviours:* self-report, S-RDQ
Plamondon et al., 2018 [145]	Sibling comparison	*KFP* 397 families, 920 children Age: 1.5-4 years	*Maternal negativity:* self-report, NLSCY	*Child disruptive behaviour:* mother & father report, mean score, OCHS
Trentacosta et al., 2019 [143]	Adoption	*EGDS* 561 families Age: 18 months to 4.5 years	*Adoptive parent harsh parenting:* self-report, PS *Inherited risk:* self-report, birth mother fearlessness (BISS) and interpersonal affiliation (HAS-PP)	*Callous-unemotional behaviours:* parent-report, CBCL
Ellingson et al., 2014 [154]	Sibling comparison	*CNLSY* 10,251 children of 4,827 mothers Age: 4-14 years	*Smoking during pregnancy:* self-report, mean number of packs smoked per day	*Disruptive behaviour:* maternal-report, BPI

Control variables	Genetic overlap	Environmental transmission	G-E interplay
Adoption openness, prenatal risk and obstetric complications, infant negative emotionality	Yes, birth mother's internalising symptoms predicted parent (but not teacher) rated offspring externalising symptoms (β = .11)	Adoptive maternal (but not paternal) depression predicted offspring externalising symptoms (β = .11)	
	Yes, there were shared genetic effects between parental depression and offspring ADHD symptoms (β = .42)	After accounting for genetic relatedness, maternal (but not paternal) prenatal depression was associated with offspring ADHD symptoms (β = .07)	
Child age, sex, maternal depressive symptoms, parity, and education	Not studied	No difference in externalising problems between exposed children and their unexposed siblings	
Child age, sex, ethnicity, SES	Not studied	Adoptive parent relationship quality with child (but not alcohol and tobacco use) was associated with offspring externalising behaviours	Study states that it provides evidence against passive rGE, but in fact the adoption-at-birth design excludes passive rGE
Prenatal risk and obstetric complications, adoption openness	Not studied	Adoptive mother-child and father-child hostility predicted offspring disruptive peer behaviours	Evocative rGE: birth mother low behavioural motivation predicted toddler low social motivation, which predicted adoptive parent-child hostility
Age, sex, age difference between non-twin siblings and cousins	No, there were no shared genetic effects between parental knowledge and offspring externalising problems	Yes, after accounting for genetic relatedness, parental knowledge was associated with offspring externalising problems (effect size not clear)	No passive or evocative rGE found
Genetically-controlled analyses using MZ twin-difference score	Not studied	No, perceived maternal support and negativity were not associated with offspring delinquent behaviours	No evocative rGE, but child-to-parent effects found
Maternal EA, child sex and child age	Not studied	Exposed children showed more disruptive behaviours than their unexposed offspring	
Pregnancy and obstetric complications, adoption openness, child gender, oppositional behaviour	No difference in callous-unemotional behaviours in children with high or low inherited risk	Adoptive parent harsh parenting was associated with callous-unemotional behaviours at 54, but not at 27 months (β range = .12 to .15)	G×E: high inherited risk (high birth mother fearlessness and low affiliation) x adoptive father harsh parenting: callous-unemotional behaviours
Maternal age at birth, EA, intelligence, delinquency, offspring sex, birth order, ethnicity, household income, geographic location	Not studied	No difference in disruptive behaviours between exposed children and their unexposed siblings	

Offspring externalising behaviours				
Study	**Design**	**Sample**	**Parental attribute (predictor)**	**Child attribute (outcome)**
Kuja-Halkola et al., 2014 [150]	Sibling comparison, Children of Twins	*Snr* 2,754,626 children Age: up to 20 years	*Maternal smoking during pregnancy:* self-report	*Criminality:* national crime register, any conviction
Kendler, Ohlsson, Sundquist, & Sundquist, 2016 [149]	Adoption	*Snr* 1,010 intact, 9,944 triparental, 56,906 not-lived-with father, 6,141 not-lived-with mother, 25,027 stepfather, 5049 stepmother, 837 adoptive families Age: not reported	*Drug abuse:* Swedish medical registers, the Suspicion Register, the Crime Register, drug-related driving offenses, and the Prescribed Drug Register	*ADHD:* Hospital Discharge Register, the Outpatient Care Register, and the Prescribed Drug Register
Obel et al., 2016 [156]	Sibling comparison	*DNR* Families of 17,381 children with ADHD Age: 3 years to diagnosis	*Maternal smoking during pregnancy:* self-report	*ADHD:* diagnosis of hyperkinetic disorder, or prescription of ADHD medication for at least 6 months
Knopik et al., 2016 [155]	Sibling comparison	*MO-MATCH study* 173 mothers and their offspring Age: 10-12 years	*Smoking during pregnancy:* maternal-report, MAGIC-PC	*ADHD symptoms:* parent and teacher-report, CRS
Estabrook et al., 2016 [153]	Sibling comparison	*MIDS* 299 families Age: 3-18 years	*Maternal smoking during pregnancy:* self-report	*ADHD:* SBSC *Oppositional Defiant Disorder (ODD):* SBSC *Conduct Disorder (CD):* SBSC
Eilertsen et al., 2017 [157]	Sibling comparison	*MoBa* 16,407 mothers and 34,283 children Age: 5 years	*Maternal alcohol use during pregnancy:* AUDIT-C	*ADHD symptoms:* maternal-report, revised CRS and CBCL *ADHD diagnosis:* diagnosis
Lund et al., 2019 [131]	Sibling comparison	*MoBa* 14,639 mothers, 25,744 children Age: 1.5-5 years	*Maternal alcohol consumption during pregnancy:* self-report, AUDIT-C	*Behavioural problems: maternal-report, CBCL* *Attention problems* *Aggressive behaviours*
Pingault et al., 2019 [105]	Within-family PGS: adjustment analyses	*TEDS* 3,663 to 4,693 individuals Age: 8-16 years	*Maternal EA:* self-report, 8 levels	*ADHD:* maternal, report, mean score, CRS-Revised

Control variables	Genetic overlap	Environmental transmission	G-E interplay
Maternal age at childbirth, child sex, birth year	Yes, there were shared genetic effects between maternal smoking during pregnancy and offspring criminality (effect size not clear)	No, exposed children did not differ from their unexposed siblings, and after accounting for genetic relatedness, maternal smoking was not associated with offspring criminality	
	Yes, birth parent drug abuse was associated with offspring ADHD (HR range = 2.06 to 2.48)	No, adoptive or step-parent drug abuse was not associated with offspring ADHD	
Maternal age, parity, child sex, year of birth	Not studied	No difference in ADHD diagnosis between exposed or unexposed siblings	
Maternal marital status at birth, food stamp usage at delivery, exposure to paternal smoking during pregnancy, child birth order, sex	Not studied	Exposed children had more parent-reported (but not teacher-reported) ADHD symptoms than their unexposed siblings	
Offspring age, sex, parental history of antisocial behaviour (Antisocial Behaviour Questionnaire)	Not studied	Exposed children were more likely to show oppositional defiant disorder and conduct disorder (but not ADHD) than their unexposed siblings	
Parental EA, parental income, maternal smoking during pregnancy, children's birth order, gender	Not studied	Exposed children had more ADHD symptoms (according to CPGS-R, but not CBCL) than their unexposed siblings, but did not differ in ADHD diagnosis	
Parity, unplanned pregnancy, daily smoking, pre-pregnancy abstinence from alcohol	Not studied	Exposed children were more aggressive, but did not have more attentional problems, than unexposed children	
Sex, age and 10 principal components of ancestry, PGS for EA and ADHD	Yes, association between maternal EA and offspring ADHD decreased after adjusting for EA and ADHD PGS (from β = -0.13 to β = -0.11)	Under a twin-heritability scenario, the association between maternal EA and offspring ADHD was expected to be null if EA and ADHD PGS captured all heritability	

Offspring externalising behaviours				
Study	**Design**	**Sample**	**Parental attribute (predictor)**	**Child attribute (outcome)**
Torvik et al., 2020 [130]	Children of twins and siblings	*MoBa* 34,958 children Age: 8 years	*Educational attainment:* self-report, highest level completed	*ADHD symptoms:* maternal report, RSDBDs
de Zeeuw et al., 2020 [158]	Within-family PGS	*NTR* 5,900 offspring, 2,649 families Age: 10-12, 25-64 years	*Genetic transmission:* effect of transmitted alleles PGS for EA and ADHD *Genetic nurture:* effect of non-transmitted alleles PGS for EA and ADHD	*ADHD symptoms:* parent and teacher report, at-home and at-school symptoms, CBCL and TRF

G-E: gene-environment; *G×E*: gene-environment interaction, *rGE*: gene-environment correlation
Design= CoT; Children-of-twins; *PGS:* polygenic scores
Samples= *CNLSY*: Children of the National Longitudinal Survey of Youth; *EGDS*: Early Growth and Development Study; *Dnr*: Danish national registers; *EPoCH*: Early Parenting of Children study; *MIDS:* Midwest Infant Development Study; *KFP:* Kids, Families, and Places study; *MoBa*: Norwegian Mother Father and Child Study; *MO*-MATCH: Missouri Mothers and Their Children study; NEAD*:* Nonshared Environment in Adolescent Development Study; *NTR:* Netherlands Twin Register; *QNTC*: Quebec Newborn Twin Study; *SIBS:* Sibling Interaction and Behaviour Study; *Snr*: Swedish national registers*; TEDS:* Twins Early Development Study; *TOSS*: Twin Offspring Study of Sweden
Measures= *APQ*: Alabama Parenting Questionnaire; *ATQ*: Adult Temperament Questionnaire; *AUDIT-C*: Alcohol Use Disorder Identification Test-Consumption; *BAI*: Beck Anxiety Inventory; *BARS:* Behavior Rating Scale; *BDI*: Beck Depression Inventory; *BISS*: Behavioral Inhibition System scale; *BPI*: Behaviour Problem Index; *CBCL:* Child Behavior Checklist; *CDIS:* Computerized Diagnostic Interview Schedule; *CES-D*: Center for Epidemiological Studies Depression scale; *CIDI*; Composite International Diagnostic Instrument; *CMS*: Child Monitoring Scale; *CRS*: Conner's Rating Scale; *CSUA*: Computerized Substance Use Assessment; *DIS*: Diagnostic Interview Schedule*; ESBQ:* Elliott Social Behavior Questionnaire; *EYQ:* Elliott Youth Questionnaire; *HAS-PP*: Harter Adult Self-Perception Profile scale; *IFIRS:* Iowa Family Interaction Rating Scales; *IWHS:* Iowa Warmth and Hostility Scales; *LEI*: Life Events Interview; *MAGIC-PC*: Missouri Assessment of Genetics Interview for Children – Parent on Child; *MRS:* Marital Relationship Questionnaire; *NLES*: Negative Life Events Scale; *NRI*: Network of Relationships Inventory; *NLSCY*: negativity scale from the National Longitudinal Survey of Children and Youth; *OCHS*: conduct disorder-aggression scale from the Ontario Child Health Study; *PEQ:* Parental Environment Questionnaire; *PIPPS*: Penn Interactive Peer Play Scale; *PS*: the Parenting Scale*; RSDBD*: Rating Scale for Disruptive Behavior Disorders; *SAM*: Substance Abuse Module; *SBSC*: Stony Brook Symptom Checklist; *SCI:* Structured Clinical Interview for DSM-III-R; *SCL*: Symptoms Checklist; *S-RDQ*: Self-Report Delinquency Questionnaire; *TCI:* Temperament Characteristic Inventory; *TRF*: Teacher Report Form; *ZBPI*: Zill Behavior Problems Inventory
Statistics= β: standardized parameter estimate; *OR*: odds ratio; *HR*: hazard ratio; R^2: percentage of variance explained. Effect sizes are not reported for studies that did not investigate both genetic and environmental transmission.

	Control variables	Genetic overlap	Environmental transmission	G-E interplay
		Yes, there were shared genetic effects between parental EA and offspring ADHD symptoms (effect size not clear)	Yes, after accounting for genetic relatedness, parental EA was associated with offspring ADHD (effect size not clear)	
	Sex, year of birth (for EA), interaction between sex and year of birth (for EA), 10 principal components, genotyping platform	EA and ADHD PGS based on transmitted parental alleles were associated with offspring ADHD symptoms at home and at school (R^2 = 0.8 to 2%)	EA and ADHD PGS based on non-transmitted parental alleles were not associated with offspring ADHD symptoms at home and at school	

Table 5 Detailed characteristics of studies investigating offspring educational attainment and cognition (N=21)

Offspring educational attainment and cognition				
Study	**Design**	**Sample**	**Parental attribute (predictor)**	**Child attribute (outcome)**
Kendler, Turkheimer, Ohlsson, Sundquist, & Sundquist, 2015 [196]	Adoption (siblings-reared-apart)	*Snr* 436 sibships, one member reared by biological, other by adoptive parents Age: 18-20 years	*EA:* highest education achieved by both parents, 5-point-scale	*IQ:* Military Conscription Register, standardised test
Conley et al., 2015 [165]	Within-family PGS	*FHS, HRS* 6,186 individuals from 4,867 households Mean age: 39.49 years (FHS), 68.17 years (HRS)	*Parental education* *Genetic transmission:* effect of parental EA PGS *Genetic nurture:* effect of parental EA PGS, after adjusting for child EA PGS	*EA:* self-report, highest grade completed
Ayorech et al., 2017 [162]	Extended twin, Within-family PGS	*TEDS* Twin analyses: 6,105 twin pairs PGS analyses: 5,825 individuals Age: 18 years	*EA (extended twin):* self-reported highest qualification *Genetic transmission (within-family PGS):* effect of parental EA PGS	*EA:* self or parent report, A Levels qualification *Intergenerational EA (extended twin):* similarity between parental and offspring EA, 2 levels *Intergenerational EA (within-family PGS):* similarity between parental and offspring EA, 4 levels
Scheeren et al., 2017 [173]	Adoption	*NLnr* 1,792 adopted children, 424,928 biological children Age: 15 years	*EA:* register-based, highest education level *Parental income:* yearly household income	*EA:* level of enrolment in secondary school, 4 levels
Bates et al., 2018 [36]	Within-family PGS	*BATS* 2,335 children and their genotyped parents Age: 17 years	*Genetic nurture:* effect of EA PGS based on non-transmitted alleles *SES:* ASI-2006	*EA:* Queensland Core Skills Test
Belsky et al., 2018 [170]	Within-family PGS	*E-RISK, NLAAH* 1,574 & 5,526 individuals Age: 18 years, late 20s to early 30s	*Genetic nurture:* effect of parental EA PGS, after adjusting for child EA PGS	*EA:* GCSE attainment; 4 levels
Kong et al., 2018 [33]	Within-family PGS	*deCODE* 21,637 probands with at least one genotyped parent Age: not reported	*Genetic transmission:* effect of EA alleles PGS based on transmitted alleles *Genetic nurture:* effect of EA PGS based on non-transmitted alleles	*EA*

Control variables	Genetic overlap	Environmental transmission	G-E interplay
Clustering of siblings within biological families	Not studied	Yes, adoptive parent EA predicted offspring IQ	
Child sex, age	Yes, parental EA PGS predicted offspring EA (effect size not clear)	After controlling for offspring EA PGS, parental EA was still associated with offspring EA, no evidence of genetic nurture as parental EA PGS was not associated with offspring EA after controlling for offspring EA PGS (effect size not clear)	No G×E interaction found between maternal EA and offspring PGS
PGS analyses: Previous school performance (GCSE grades)	Twin analyses: Yes, additive genetic effects underlying intergenerational EA were found (R^2 = ~50%) PGS analyses: Yes, parental EA PGS was associated with intergenerational EA	Twin analyses: Yes, shared environmental effects underlying intergenerational EA were found (R^2 = ~40%) PGS analyses: Not studied	
Father and mother year of birth, family structure, number of children in household, observation year, adoption age, country of adoption, gender	Not studied	Adoptive parents' income (but not EA) predicted offspring EA	Passive rGE: family income was more strongly associated with offspring EA in biological families than adoptive families
Sex, age at test, offspring EA PGS	Not studied	PGS for EA based on non-transmitted alleles were associated with offspring EA, but this relationship disappeared after adjusting for parental SES	No G×E interaction found between PGS and SES
Genetic principal components	Not studied	Yes, parental EA PGS was associated with offspring EA after adjusting for offspring EA PGS	Passive rGE: individuals with higher PGS grew up in better-educated households
Sex, year of birth, interaction between sex and year of birth, 100 principal components	Yes, EA PGS based on transmitted parental alleles was associated with offspring EA (direct effect explained 70% of the overall observed effect of EA PGS)	Yes, EA PGS based on non-transmitted parental alleles was associated with offspring PGS (genetic nurture explained explaining 22.4% of the overall effect of EA PGS)	

Offspring educational attainment and cognition				
Study	**Design**	**Sample**	**Parental attribute (predictor)**	**Child attribute (outcome)**
Liu et al., 2018 [166]	Within-family PGS	*FHS, HRS* 8,639 individuals from three generations & 9,342 individuals over age 50 Age: not reported	*Genetic transmission (FHS sample):* effect of parental EA PGS *Genetic nurture (FHS sample):* effect of parental EA PGS, after adjusting for child PGS *EA (HRS sample):* self-report, years of education	*EA* *FHS:* self-report, years of education completed *HRS:* parent-report
Young et al., 2018 [34]	Relatedness disequilibrium regression	*deCODE* 12,035 individuals who had parents and grandparents genotyped Age: not reported	*Genetic nurture:* estimated variance in offspring trait explained by parental genes acting indirectly via the environment	*Educational attainment:* self-report, number of years of schooling
Pingault et al., 2019 [105]	Within-family PGS	*TEDS* 3,663 to 4,693 individuals Age: 8-16 years	*Maternal EA:* self-report, 8 levels	*EA:* mean of 3 standardised tests
Bates et al., 2019 [169]	Within-family PGS	*BATS* 2,335 children and their genotyped parents Age: 17 years	*Genetic nurture:* effect of parental EA PGS based on non-transmitted alleles *SES:* ASI-2006	*EA:* Queensland Core Skills Test
Willoughby et al., 2019 [171]	Within-family PGS	*MCTFR* 1223 families, 2446 offspring Age: varied	*Genetic nurture:* effect of parental EA PGS, on top of child EA PGS *SES:* composite score, family income, parent education level, parent occupation level *Parental IQ:* WIS	*Years of education:* self-report, mean age 29 *High-school grade-point-average:* self-report, age 17 *IQ:* WIS, mean age 14.4
Armstrong-Carter et al., 2020 [172]	Within-family PGS	*BiBs* 2,077 mother-child dyads Age: 7 years	*Genetic nurture*: effect of maternal EA PGS, after adjusting for child EA PGS *Maternal health:* composite score, self-reported mental health, smoking, indirect smoke exposure, alcohol and drug use, vitamin use, sleep problems, and BMI *SES*: composite score, self-reported education, cohabitation status, employment, maternity leave, governmental benefits, perceived financial difficulty, and governmental index of neighbourhood-level deprivation	*Academic performance:* standardised national exam at age 7
Borriello et al., 2020 [163]	Adoption	*EGDS* 195 families Age: 7 years	*Mathematical achievement:* standardized scores on the mathematics fluency subtest of WJ-III	*Mathematical achievement:* standardized scores on the mathematics fluency subtest of the WJ-III

	Control variables	Genetic overlap	Environmental transmission	G-E interplay
	7 principal components HRS sample: child's EA PGS	Yes, parental EA PGS was associated with offspring EA (FHS sample; β = .345), and offspring EA PGS attenuated the association between parental and offspring EA (HRS sample; from β = .314 to β = .292)	Yes, parental EA PGS was associated with offspring EA, after adjusting for offspring EA PGS (β = .076)	
	Sex, year of birth	Not studied	Yes, after accounting for shared genetic effects, parental genes explained variance in offspring EA	
	Sex, age and 10 principal components of ancestry, PGS for EA	Yes, association between maternal EA and offspring EA decreased after adjusting for EA PGS (from β = 0.40 to β = 0.33)	Under a twin-heritability scenario, the association between maternal and offspring EA was expected to be null if EA PGS captured all heritability	
	Sex, age at test, offspring EA PGS	Not studied	PGS for EA based on non-transmitted alleles were associated with offspring EA, but this relationship disappeared after adjusting for parental SES	No G×E interaction found between PGS and SES
	Height and BMI used as negative controls	Not studied	Genetic nurture: Yes, parental EA PGS was associated with offspring EA traits after adjusting for offspring EA PGS, and this association was mediated by parental SES and IQ	
	Child EA PGS, maternal age, first 10 principal components	Not studied	Yes, maternal EA PGS was associated with offspring academic performance, after adjusting for offspring EA PGS, and this association was mediated by maternal health and SES during pregnancy	
	Obstetric complications, adoption openness, parent education level, non-mathematical cognitive skills	Yes, birth parent and offspring mathematic achievement were correlated (β = 0.17)	Yes, paternal (but not maternal) mathematic achievement was correlated with adopted-offspring mathematical achievement (β = 0.15)	No G×E interaction found

Offspring educational attainment and cognition				
Study	**Design**	**Sample**	**Parental attribute (predictor)**	**Child attribute (outcome)**
Domingue & Fletcher., 2020 [167]	Adoption	*WLS* 855 adopted and 20,939 biological offspring Age: Not reported	*Genetic transmission:* association between parental EA PGS and EA of biological offspring *Genetic nurture:* association between parental EA PGS and EA of adoptive offspring	*Educational attainment:* parent-reported, highest grade of school attended
de Zeeuw et al., 2020 [158]	Within-family PGS	*NTR* 5,900 offspring from 2,649 families Age: 10-12, 25-64 years	*Genetic transmission:* effect of EA and ADHD ADHD PGS based on transmitted alleles *Genetic nurture:* effect of EA and ADHD PGS based on non-transmitted alleles	*Childhood academic achievement:* nationwide standardised test at age 12 *Adult EA:* self-report, highest degree; 4 levels
Halpern-Manners et al., 2020 [164]	Adoption	*EGDS* 340 families Age: first-graders (6-7 years)	*Adoptive and birth parent education attainment*: self-report, highest level of education completed by adoptive or birth parents	*Early educational achievement*: WJ-III
Torvik et al., 2020 [130]	Children of twins and siblings	*MoBa* 34,958 children Age: 8 years	*Educational attainment:* self-report, highest level completed	*Academic problems:* maternal report, 3-point scale
Ellingson et al., 2014 [154]	Sibling comparison	*CNLSY* 10,251 children of 4,827 mothers Age: 4-14 years	*Smoking during pregnancy:* self-report, mean number of packs smoked per day	*Cognitive functioning:* PPVT-R (math, reading and reading Recognition subtests) and digit span test
Kuja-Halkola et al., 2014 [150]	Sibling comparison, Children of Twins	*Snr* 2,754,626 children Age: up to 20 years	*Maternal smoking during pregnancy:* self-report	*Academic achievement*: class 9 records *General cognitive ability*: Military conscription register, 9 levels
Wertz et al., 2019 [161]	Within-family PGS	*E-RISK* 860 mothers and their children Age: 18 years	*Genetic nurture:* effect of maternal EA PGS, after adjusting for child PGS *Parenting behaviour:* mother, child and interviewer report, *cognitive stimulation, warmth and sensitivity, household chaos, and safety and tidiness of the family home*	*EA:* self-report, highest educational attainment, 18 years

G-E: gene-environment; *G×E*: gene-environment interaction, *rGE*: gene-environment correlation
Design= *PGS:* polygenic scores
Samples= *BATS*: Brisbane Adolescent Twin Study; *BiBs*: Born in Bradford study; *CNLSY*: Children of the National Longitudinal Survey of Youth; *EGDS*: Early Growth and Development Study; *deCODE*: Icelandic Genealogy Database; *FHS:* Framingham Heart Study, *HRS:* Health Retirement Study; *MoBa*: Norwegian Mother Father and Child Study; *MCTFR:* Minnesota Center for Twin

	Control variables	Genetic overlap	Environmental transmission	G-E interplay
	Child sex, age, 10 principal components	Yes, parental EA PGS was associated with EA of biological offspring (effect size not clear)	Yes, parental EA PGS was associated with EA of adoptive offspring (effect size not clear)	Passive rGE implied: higher association in biological families than adoptive families
	Sex, birth year (EA), interaction between sex and birth year (EA), 10 principal components, genotyping platform	EA PGS based on transmitted parental alleles were associated with offspring academic achievement in childhood and EA in adulthood (R^2 = 5.7-7.6%) but there was no association with ADHD PGS	EA PGS based on non-transmitted parental alleles were associated with offspring EA in adulthood (R^2 = 1.7%), but not academic achievement in childhood (which was also not associated with non-transmitted PGS for ADHD)	
	Obstetric complications, adoption openness, child sex, child and adoptive parents' ethnicity, adoptive parents' age, type of adoption agency	Yes, birth parent EA was associated with offspring EA (effect size not clear)	Yes, adoptive parent EA was associated with offspring EA (effect size not clear)	No G×E interaction
		Yes, there were shared genetic effects between parental EA and offspring academic problems (effect size not clear)	Yes, after accounting for genetic relatedness, parental EA was associated with offspring academic problems (effect size not clear)	
	Maternal age at birth, EA, intelligence, delinquency, offspring sex, birth order, ethnicity, household income, geographic location	Not studied	Exposed children had poorer reading recognition than their unexposed siblings, but there were no other group differences	
	Maternal age at childbirth, child sex, birth year	Yes, there were shared genetic effects between maternal smoking during pregnancy and offspring EA traits (effect size not clear)	No, exposed children did not differ from their unexposed siblings, and after accounting for genetic relatedness, maternal smoking was not associated with offspring EA traits	
	Sex, first 10 principal components, offspring EA PGS	Yes, controlling for offspring EA PGS attenuated the association between parenting behaviours and offspring EA (from β range = .33-.52 to β range = .30-.48)	Genetic nurture: Yes, maternal EA PGS was associated with offspring EA after adjusting for offspring EA PGS (β = .11), and this effect was mediated by parenting behaviours including cognitive stimulation, household chaos and a safe, tidy home (but not parental warmth)	Evocative rGE: mother and offspring PGS for EA predicted cognitive stimulation and warm, sensitive parenting

and Family Research; *NLNR:* Dutch national registers; *NLAAH:* National Longitudinal study of Adolescent to Adult Health; *NTR:* Netherlands Twin Register; *SNR*: Swedish national registers*; TEDS:* Twins Early Development Study; *WLS*: Wisconsin Longitudinal Study
Measures= ASI: Australian Socioeconomic Index occupational status scale; *PPVT-R*: Peabody Picture Vocabulary Test-Revised; *QCST*: Queensland Core Skills Test; *WIS*: Weschler Intelligence Scale; *WJ-III*; Woodcock-Johnson Test of Achievement III
Statistics= β: standardized parameter estimate; R^2: percentage of variance explained. Effect sizes are not reported for studies that did not investigate both genetic and environmental transmission.

Table 6 Detailed characteristics of studies investigating offspring substance use behaviours (N=19)

Offspring substance use behaviours				
Study	**Design**	**Sample**	**Parental attribute (predictor)**	**Child attribute (outcome)**
McGue et al., 2014 [183]	Adoption	*SIBS* 409 adoption and 208 biological families Age: 10-28 years	*Drinking behaviour:* self-report, composite score, CSUA and SAM	*Drinking behaviour:* self-report, composite score, CSUA and SAM
Waldron et al., 2014 [176]	Children of Twins	*MATCH, PACER* 1318 offspring of twin parents Age: 11-24 years	*Substance dependence:* self-report, SAGA *Parental separation:* study design cannot distinguish G and E effects	*Offspring substance involvement:* self-report, SAFA
Kuja-Halkola et al., 2014 [150]	Sibling comparison, Children of Twins	*Snr* 2,754,626 children Age: up to 20 years	*Maternal smoking during pregnancy:* self-report	*Drug/alcohol misuse:* register based, diagnosis, or drug-related conviction
Kendler, Ji, et al., 2015 [177]	Adoption	*Snr* 18,115 adoptees, 171,989 not-lived-with parent, and 107,699 stepparent families Mean age: 33.9 years	*AUD:* Swedish Hospital Discharge Register, the Swedish Prescribed Drug Register, the Outpatient Care Register, the Primary Health Care Register, and the Swedish Crime and Suspicion Register	*AUD:* Swedish Hospital Discharge Register, the Swedish Prescribed Drug Register, the Outpatient Care Register, the Primary Health Care Register, and the Swedish Crime and Suspicion Register
Grant et al., 2015 [178]	Children of Twins	*VET* 1828 offspring of male twin parents Age: Not reported	*Parental alcohol or drug dependency:* diagnosis, DIS *Parental separation:* study design cannot distinguish G and E effects	*Alcohol involvement:* self-report, SAGA
Kendler, Ohlsson, Sundquist, et al., 2015 [27]	Triparental family design	*Snr* 41,360 triparental families (mother, not-lived-with biological father, and stepfather) Age: 15+	*Drug abuse:* medical registries, the Crime Register, the Suspicion Register, drug-related driving offenses, and the Prescribed Drug Register *AUD:* medical and mortality registries, the Suspicion Register, the Crime Register, and the Prescribed Drug Register	*Drug abuse:* medical registries, the Crime Register, the Suspicion Register, drug-related driving offenses, and the Prescribed Drug Register *AUD:* medical and mortality registries, the Suspicion Register, the Crime Register, and the Prescribed Drug Register
Kendler, Ohlsson, Sundquist, & Sundquist, 2015 [180]	Triparental family design	*Snr* 2,111,074 offspring in intact families 155,121 not-lived-with father, 10,194 not-lived-with mother, 107,163 stepfather, 17,637 stepmother 10,038 adoptive families Age: 15+	*Drug abuse:* medical registers, the Crime Register, the Suspicion Register, and drug-related driving offences	*Drug abuse:* medical registers, the Crime Register, the Suspicion Register, and drug-related driving offences

	Control variables	Genetic overlap	Environmental transmission	G-E interplay
	Parent gender, and child gender	Not studied	Yes, adoptive parent drinking behaviour was associated with offspring drinking behaviour	Passive rGE implied: parent-offspring association was greater in biological pairs than adoptive pairs
	Parent or offspring comorbid psychopathology, twin sex, twin age, twin EA, child sex, age	Substance dependence: Yes, there were shared genetic effects between parental substance dependence and offspring substance involvement (effect size not clear)	Substance dependence: after accounting for genetic relatedness, parental substance dependence was not associated with offspring substance involvement with the exception of cannabis use which was associated with offspring smoking behaviour (effect size not clear)	
	Maternal age at childbirth, child sex, birth year	Yes, there were shared genetic effects between maternal smoking during pregnancy and offspring drug/alcohol misuse (effect size not clear)	No, exposed children did not differ from their unexposed siblings, and after accounting for genetic relatedness, maternal smoking was not associated with offspring drug/alcohol misuse	
		Yes, birth parent AUD predicted offspring AUD (OR = 1.46)	Yes, adoptive parent AUD predicted offspring AUD (OR = 1.40)	No G×E interaction observed
	Maternal alcohol dependency, heavy cannabis use, family income, child sex, age, history of psychiatric problems and traumatic life events, inattention, hyperactivity and oppositional defiant disorder	Substance dependency: Yes, there were shared genetic effects between parental substance dependence and offspring alcohol involvement (effect size not clear)	Substance dependency: Yes, after accounting for genetic relatedness, parental substance dependency was associated with offspring alcohol involvement (effect size not clear)	
		Yes, drug abuse and AUD registration of not-lived-with biological parents were correlated with offspring drug abuse and AUD (HR range = 1.84-2.45)	Yes, drug abuse or AUD registration of adoptive or step-parent correlated with offspring drug abuse or AUD (HR range = 1.27-1.99)	
	Drug abuse status of all other relevant biological and step-parents	Yes, drug abuse behaviour of not-lived-with biological parents were correlated with offspring drug abuse (HR = 2.73)	Yes, drug abuse behaviour of adoptive or step-parent correlated with offspring drug abuse (HR = 1.79)	

Offspring substance use behaviours				
Study	**Design**	**Sample**	**Parental attribute (predictor)**	**Child attribute (outcome)**
Bidwell et al., 2017 [187]	Sibling comparison	*MO-MATCH* 173 mothers and their offspring Age: 7-15 years	*Smoking during pregnancy:* self-report, MAGIC-PC	*Substance use:* self-report, DUSI
Kendler, Ohlsson, Edwards, et al., 2017 [179]	Extended family design	*Snr* 38,373 offspring of not-lived-with fathers and 9,711 offspring of step-fathers Age: 15 years to age of first registration or end of follow-up	*AUD:* medical registries, the Prescribed Drug Register, two or more convictions of drunk driving in the Crime register	*AUD:* medical registries, the Prescribed Drug Register, two or more convictions of drunk driving in the Crime register
Treur et al., 2018 [182]	Children of Twins, Within-family PGS	*NTR* CoT sample: 712 twins, 723 children PGS sample: 4072 individuals Age: Not reported	*Smoking initiation (CoT sample):* self-report *Exposure to smoking (PGS sample):* offspring-reported exposure as a child (up to age 18)	*CoT sample smoking initiation:* self-report *PGS sample smoking behaviour:* self-report, smoking initiation and smoking heaviness
Maes et al., 2018 [185]	Extended twin	*V-30, A-25* 22,393 twins and their families Age: 18+	*Smoking initiation:* self-report	*Smoking initiation:* self-report
Kendler, Ohlsson, Sundquist, & Sundquist, 2018 [181]	Multiple parenting relationships design	*Snr* 2,111,074 intact, 41,360 triparental, 113,762 not-lived-with father, 10,194 not-lived-with mother, 65,803 step-father, 17,637 step-mother, 10,038 adoptive families Age: Not reported	*Drug abuse:* medical and mortality registries, the Suspicion and Crime registers, drug-related driving offences, and the Prescribed Drug Register	*Drug abuse:* medical and mortality registries, the Suspicion and Crime registers, drug-related driving offences, and the Prescribed Drug Register
Kendler et al., 2019 [30]	Matched-pairs case-control	*Snr* 65,006 parent-offspring, sibling, and cousin pairs Age: 19-23 years	*Drug abuse:* medical registers, the Crime Register, the Suspicion Register, and drug-related driving offences	*Drug abuse:* medical registers, the Crime Register, the Suspicion Register, and drug-related driving offences in offspring whose parents had a drug abuse incident 1-3 years ago

	Control variables	Genetic overlap	Environmental transmission	G-E interplay
	Maternal age, marital status, EA, qualification for food stamps at time of delivery, parental substance use outside of pregnancy, child birth order, sex, exposure to paternal smoke during pregnancy	Not studied	No difference in substance use behaviours between exposed children and their unexposed siblings	
	AUD in the biological mother, and offspring sex	Yes, not-lived with father AUD (including age of registration, recurrence and number of AUD registrations) predicted offspring AUD (HR not reported)	Yes, step-father AUD (including number of registrations that occurred while co-offspring with offspring) predicted offspring AUD (HR = 1.03)	
	CoT: twin sex, twin age, child sex, age, family-based clustering correction PGS: sex, year of birth, 10 principal components, family clustering correction	CoT sample: Yes, there were shared genetic effects between parent and offspring smoking initiation (effect size not clear) PGS sample: Not studied	CoT sample: Yes, after accounting for genetic relatedness, parent smoking initiation was associated with offspring smoking initiation (effect size not clear) PGS sample: Yes, after adjusting for smoking PGS, exposure to smoking during childhood was associated with smoking initiation (OR = 1.68)	G×E: high PGS for smoking initiation & heaviness x childhood exposure to smoking: smoking heaviness (no interaction for smoking initiation)
	Age	Not studied	There were shared environmental effects underlying parent-offspring similarity in smoking initiation (negative cultural transmission)	Passive rGE: negative covariance between additive genetic effects and parental smoking
		Yes, drug abuse behaviour of not-lived-with biological parents were correlated with offspring drug abuse (*r* range = 0.13-0.19)	Yes, drug abuse behaviour of adoptive or step-parent correlated with offspring drug abuse (*r* range = 0.06-0.09)	
	Control parent-child pairs matched on sex, parent and child year of birth, country of birth, SES, number of lifetime drug abuse registrations, medical or criminal registration, parental EA	Not studied	Yes, exposed offspring were at increased risk of drug abuse than matched control offspring who were unexposed to parental drug registration	

Offspring substance use behaviours				
Study	**Design**	**Sample**	**Parental attribute (predictor)**	**Child attribute (outcome)**
Kendler et al., 2019 [25]	Multiple parenting relationships design	*Snr* 475,000 parent-offspring pairs Age: 15 and over	*Drug abuse:* medical registries, the Crime Register, the Suspicion Register, drug-related driving offenses, and the Prescribed Drug Register *AUDs:* medical and mortality registries, the Suspicion Register, the Crime Register, and the Prescribed Drug Register	*Drug abuse:* medical registries, the Crime Register, the Suspicion Register, drug-related driving offenses, and the Prescribed Drug Register *AUDs:* medical and mortality registries, the Suspicion Register, the Crime Register, and the Prescribed Drug Register
Kendler et al., 2020 [184]	Extended family design	*Snr* 44,250 children of high-risk parents (affected with drug abuse), and offspring of discordant sibling or sibling-in-law Age: 15 and over	*Drug abuse* and *alcohol use disorder:* medical registries, the Crime Register, the Suspicion Register, drug-related driving offenses, and the Prescribed Drug Register *Criminal behaviour:* Swedish Crime register *Psychiatric registration:* any mental disorder	*Drug abuse:* medical registers, the Crime Register, the Suspicion Register, drug-related driving offenses, and the Prescribed Drug Register
Cea & Barnes, 2014 [190]	Adoption	*VFS* 328 biological and 77 adoption families Age: 14-33 years	*Parenting styles:* offspring-report, *family cohesion* (FACES-II), *mother & father care, mother & father overprotectiveness* (PPBI), *parental monitoring, mother and father support, mother and father control* (GBF)	*Polysubstance use:* self-report, composite score, alcohol composition (Volume-Variability Index), smoking, and other drug usage at Time 1 (T1: 14-25 years) and T2 (21-33 years)
Cea & Barnes, 2015 [186]	Adoption	*VFS* 328 biological and 77 adoption families Age: 14-33 years	*Addiction-prone personality:* self-report, APP-21 *Familial care factor:* mother, father, & offspring-report, PPBI and FACES-II	*Addiction-prone personality:* self-report, APP-21
Samek et al., 2015 [189]	Adoption	*SIBS* 568 adopted and 412 biological offspring Age: 11-25.5 years	*Parental involvement:* offspring-report, average of maternal and paternal score, PEQ	*Substance use:* self-report, CSUA
Kendler et al., 2016 [191]	Sibling comparison	*Snr* 1161 full-sibships and 3085 half-sibships of high-risk biological parents; one sibling reared by biological, other by adoptive parents Age: 15 and over	*Adoptive parenting:* protective effect of high-quality rearing environment	*Drug abuse:* medical registers, the Suspicion Register, the Crime Register, drug-related driving offenses, and the Prescribed Drug Register

G-E: gene-environment; *G×E*: gene-environment interaction, *rGE*: gene-environment correlation
Design= *PGS:* polygenic scores
Samples= *A-25*: Australia 25,000 study; MATCH: Mothers and Their Children study; *MO-MATCH*: Missouri Mothers and Their Children study; *PACER:* Parent Alcoholism and Child Environmental Risk study; *SIBS:* Sibling Interaction and Behaviour Study; *Snr*: Swedish national registers*; VET:* Vietnam Era Twin registry; VFS: Vancouver Family Survey; *V-30*: Virginia 30,000 study

	Control variables	Genetic overlap	Environmental transmission	G-E interplay
	Drug abuse or AUD status of all other relevant biological and step-parents, offspring year of birth, and offspring sex	Yes, drug abuse and AUD registration of not-lived-with biological parents were correlated with offspring drug abuse and AUD (*r* range = 0.14-0.16)	Yes, drug abuse or AUD registration of adoptive or step-parent correlated with offspring drug abuse or AUD (*r* range = 0.04-0.10)	
	Child sex, year of birth	Not studied	Yes, after accounting for genetic relatedness, parent (and step-parent) drug abuse, AUD, criminal behaviour and psychiatric registration was associated with offspring drug abuse	
	Age, gender, and adoption status	Not studied	At T1, adoptive family cohesion, parental monitoring, maternal and paternal positive parenting, and father overprotection were associated with offspring substance use (maternal and paternal coercion, maternal overprotectiveness coercion were not). At T2, only cohesion, maternal coercion and overprotection were significant	
	Adoption status, and child gender	Not studied	Adoptive parent addiction-prone personality and familial care factor were associated with offspring addiction-prone personality	
	Earlier substance use	Not studied	Yes, adoptive parental involvement was negatively associated with offspring substance use	No evidence of passive rGE found
	Parental age at birth, high-risk status of the other parent of half-sibling, child gender	Not studied	Children exposed to adoptive parenting had lower risk of drug abuse than their unexposed siblings, this protective effect disappeared when the adoptive family was disrupted or if there was a high-risk adoptive parent	

Measures= *APP-21:* Addiction-Prone Personality-21 scale; *CSUA*: Computerized Substance Use Assessment; *DIS*: Diagnostic Interview Schedule; *DUSI*: revised Drug Use Screening Inventory; *FACES-II:* Family Adaptability and Cohesion Evaluation Scales II; *GBF*: Grace Barnes and Farrell's 1982 study*; MAGIC-PC*: Missouri Assessment of Genetics Interview for Children – Parent on Child; *PEQ*: Parental Environment Questionnaire; *PPBI*: Parker Parenting Bonding Instrument; *SAGA*: Semi-structured Assessment of the Genetics of Alcoholism
Statistics= *OR*: odds ratio; *HR*: hazard ratio; *r*: weighted tetrachoric correlation. Effect sizes are not reported for studies that did not investigate both genetic and environmental transmission.

Table 7 Detailed characteristics of studies investigating offspring personality (N=6)

Offspring personality				
Study	**Design**	**Sample**	**Parental attribute (predictor)**	**Child attribute (outcome)**
Elam et al., 2014 [144]	Adoption	*EGDS* 316 families Age: 27 months to 4.5 years	*Adoptive parent hostility:* self-report, Iowa Family Interaction Rating Scales *Birth mother low behavioural motivation:* self-report, BIBA	*Toddler low social motivation:* observation & parent-report, composite score
Ellingson et al., 2014 [154]	Sibling comparison	*CNLSY* 10,251 children of 4,827 mothers Age: 4-14 years	*Smoking during pregnancy:* self-report, mean number of packs smoked per day, reported after pregnancy	*Temperament/personality:* maternal report, CBQ
Van Ryzin et al., 2015 [192]	Adoption	*EGDS* 361 families Age: 9 months to 6 years	*Responsive parenting:* observation & self-report, composite score, Home Observation for Measurement of the Environment *Birth parent sociability:* parental self-report, composite score, ATQ	*Social competence:* parent and teacher-report, composite score, SSRS and SCSA
Eley et al., 2015 [117]	Children of Twins	*TOSS* 387 MZ, 489 DZ families Age: 11-22	*Neuroticism:* self-report, EPQ	*Neuroticism:* self-report, EPQ
Brooker et al., 2016 [193]	Adoption	*EGDS* 505 families Age: 9-18 months	*Child-centred parenting:* observation, 3 independent coders *Adoptive and birth parent anxiety symptoms:* self-report, BAI	*Social inhibition:* observation, independent coders
Kandler et al., 2019 [194]	Extended twin	*SPAD* 573 twins and their families Mean age: ~39 years	*Personality dimensions:* self-report, HEXACO, 6 dimensions: *honesty-humility, emotionality, extraversion, agreeableness, conscientiousness, opennness*	*Personality dimensions:* self-report, HEXACO, 6 dimensions: *honesty-humility, emotionality, extraversion, agreeableness, conscientiousness, opennness*

G-E: gene-environment; *G×E*: gene-environment interaction, *rGE*: gene-environment correlation
Samples= *CNLSY*: Children of the National Longitudinal Survey of Youth; *EGDS*: Earl y Growth and Development Study; *SPAD*: Study of Personality Architecture and Dynamics; *TOSS*: Twin Offspring Study of Sweden
Measures= *ATQ*: Adult Temperament Questionnaire; *BAI*: Beck Anxiety Inventory; *BIBA*: Behavioral Inhibition/Behavioral Activation scales; *CBQ*: Children's Behavior Questionnaire; *EPQ*: Eysenck Personality Questionnaire; *HEXACO*: HEXACO Personality Inventory-Revised; *IFIRS:* Iowa Family Interaction Rating Scales; *SSRS:* Social Skills Rating System; *SCSA*: Social Competence and School Adjustment
Statistics= β: standardized parameter estimate. Effect sizes are not reported for studies that did not investigate both genetic and environmental transmission.

	Control variables	Genetic overlap	Environmental transmission	G-E interplay
	Prenatal risk and obstetric complications, and adoption openness	Yes, birth mother low behavioural motivation predicted toddler low social motivation (β = .17)	Yes, adoptive parent hostility predicted offspring disruptive peer behaviour (β = .11-.28)	Evocative rGE: birth mother low behavioural motivation predicted toddler low social motivation, which predicted adoptive parent-child hostility
	Maternal age at birth, EA, intelligence, delinquency, offspring sex, birth order, ethnicity, household income, geographic location	Not studied	No difference in temperament/ personality between exposed and unexposed siblings	
	Openness/contact in the adoption, prenatal risk index, child positive emotionality at 9 months	Birth parent sociability predicted offspring social competence, (β = .17) but this association did not remain after adjusting for child positive emotionality	Adoptive responsive parenting did not predict offspring social competence	G×E: birth parent sociability x adoptive parent responsive parenting: offspring social competence
	Twin sex, and age	No shared genetic effects between parental and offspring neuroticism	Yes, after accounting for genetic relatedness, parental neuroticism was associated with offspring neuroticism (effect size not clear)	
	Prenatal risk and obstetric complications, adoption openness, adoptive parent EA, and child sex	No, birth parent anxiety did not predict offspring social inhibition	No, adoptive parent child-centred parenting or anxiety did not predict offspring social inhibition	Evocative rGE: birth parent anxiety and child social inhibition predicted adoptive mother child-centred parenting G×E: birth parent anxiety x adoptive father child-centred parenting: social inhibition
	Age, sex	Not studied	No, maternal or paternal shared environment effects were not associated with offspring personality	No evidence of passive rGE found

Chapter 4

Maternal and paternal effects on offspring internalising problems: results from genetic and family-based analyses

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Supplementary materials:

ABSTRACT

It is unclear to what extent parental influences on the development of internalising problems in offspring are explained by indirect genetic effects, reflected in the environment provided by the parent, in addition to the genes transmitted from parent to child. In this study, these effects were investigated using two innovative methods in a large birth cohort. Using maternal-effects genome complex trait analysis (M-GCTA), the effects of offspring genotype, maternal or paternal genotypes, and their covariance on offspring internalising problems were estimated in 3,801 mother–father–child genotyped trios. Next, estimated genetic correlations within pedigree data, including 10,688 children, were used to estimate additive genetic effects, maternal and paternal genetic effects, and a shared family effect using linear mixed effects modelling. There were no significant maternal or paternal genetic effects on offspring anxiety or depressive symptoms at age 8, beyond the effects transmitted via the genetic pathway between parents and children. However, indirect maternal genetic effects explained a small, but nonsignificant, proportion of variance in childhood depressive symptoms in both the M-GCTA (~4%) and pedigree (~8%) analyses. Our results suggest that parental effects on offspring internalising problems are predominantly due to transmitted genetic variants, rather than the indirect effect of parental genes via the environment.

INTRODUCTION

A key issue yet to be resolved in child psychiatry is to what extent associations between parental factors and offspring internalising problems, such as anxiety and depression, are due to genetic effects, direct environmental effects, or both. Well established risk factors for childhood internalising problems include exposure to maternal or paternal psychiatric disorders[205–207], the parentally provided rearing environment that the child experiences (e.g., parenting style or harsh punishment)[208] and the broader family environment (e.g., marital instability or financial hardship)[209,210]. While these associations may be explained by direct environmental effects from parent to child, the relationship is likely to be confounded by shared genetics as each parent passes on 50% of their DNA to their offspring. Moreover, parental environmental effects may still be mediated by the parental genome, acting over and above the transmission of genes from parent to child[211]. These non-transmitted parental genetic effects may act via the intrauterine environment or the rearing environment that the parent provides for the child. Insight into mechanisms underlying parental influences on offspring internalising problems is of importance as it could inform both prevention and treatment strategies. Disentangling the effect of transmitted and non-transmitted genetic components, and other environmental sources of variation can only be resolved by genetically informative designs. This study incorporates two novel methodologies to investigate maternal and paternal genetic effects on offspring internalising problems.

So far, knowledge on genetic and environmental parental influences on offspring internalising symptoms has largely relied on twin and family-based designs rooted in quantitative genetics. Findings from 50 years of twin research estimate that ~40% of the variance within individual differences in childhood internalising problems is due to genetic factors and up to ~36% is due to the common family environment, which encompasses parental factors that account for similarities within the offspring[6,98,101]. The remaining variance is explained by unique environment effects (unshared between twins and siblings), which can also include parental factors. Studies using family-based designs show evidence of environmental transmission of depressive and anxious symptoms from parent to child, over and above the influence of shared genes[107,109,112,119,212,213]. In terms of specific parenting behaviours, genetically sensitive designs indicate that over-reactive parenting[128], harsh parenting[123], and parental criticism[214] are associated with more internalising problems in the offspring, whereas parental expressed affection[125] and a good parent–child relationship quality[126] are associated with positive offspring self-worth and fewer internalising problems respectively.

This body of literature highlights that the parentally provided environment is an important contributor to the development of offspring internalising problems. However, such environmental effects on offspring behaviour may have an underlying genetic contribution in the parents[215], which can be investigated by incorporating information from the parental genome in a parent–offspring design.

In the current genomics era of research, the latest developments in methods of polygenic analyses provide new ways to improve our understanding of the mechanisms underlying parental influence on offspring internalising problems. Genome-wide complex trait analysis (GCTA) is used to investigate the impact that variation in measured genetic factors has on behaviour[216,217]. Using genome-based restricted maximum likelihood (GREML) analyses, common genetic variants are studied to examine the extent to which genetic similarity between unrelated individuals is associated with phenotypic similarity. The additive genetic effect of measured single nucleotide polymorphisms (SNPs) currently explains up to 14% of variance in stable emotional problems during childhood[82]. In samples that, along with data on offspring genotypes and phenotypes, have data available on parental genotypes, a novel extension of the approach used in GCTA can be applied to additionally estimate the contribution of parental genotype to offspring behaviour.

Maternal-effects GCTA (M-GCTA)[35] uses SNP data to investigate whether variance in an offspring trait can be explained by the effect of the maternal genotype, over and above the transmission of genes from mother to child. In other words, this maternal effect captures the environmental influence of the mother on offspring behaviour through genetically influenced maternal traits, for example, through the intrauterine environment or postnatal care. Additionally, M-GCTA uses the covariance between the direct effect of the offspring genotype and the indirect effect of the maternal genotype to estimate whether genes that contribute to the maternal effect when present in the mother also contribute to the additive genetic effect when present in the offspring. It therefore tests for a passive gene–environment correlation wherein the maternal environment a child is exposed to is correlated with the child's genotype. The M-GCTA method has not been applied to investigate parental influences on behavioural traits in offspring thus far, but could be a useful technique to capture the impact of parental genetic effects on offspring internalising behaviours.

Indirect parental genetic effects can also be investigated by a quantitative genetics approach making use of large-scale family data and extended pedigree information[218]. Using estimated genetic correlations between known relatives, we examine parental genetic effects on internalising problems in children and test whether M-GCTA results replicate. In previous studies, maternal genetic effects on offspring phenotypes were examined by using an extended children-of-twins design to estimate the covariance between pairs of individuals with different degrees of relatedness[219,220]. For instance, it is known that children of monozygotic twins are as genetically similar to their aunt or uncle as they are to their mother or father[104]. By comparing the phenotypic covariance between full siblings or children of monozygotic twins (who have 100% of maternal or paternal genetic factors in common) to those whose mothers or fathers are full siblings (share 50% of maternal or paternal genetic factors) or half-siblings (share 25% of maternal or paternal genetic factors), while taking into account the covariance explained by the other parent and the shared environment for children living in the same family, family data can be used to investigate maternal or paternal genetic effects on offspring behaviour.

The aim of this study is to investigate the environmental effect of non-transmitted maternal and paternal genetic factors on offspring internalising problems. We use data from the Norwegian Mother, Father and Child study (MoBa), a distinctive cohort with extensive data available on over 75,000 complete family trios (mothers, fathers and offspring), including 11,000 genotyped trios. The MoBa dataset provides the unique opportunity to simultaneously study both maternal and paternal influences on offspring behaviour. We first use the M-GCTA method to decompose genetic effects by estimating how variance in offspring internalising problems is explained by offspring genetic effects, non-transmitted maternal or paternal genetic effects, and a gene–environment correlation between the two. Next, we construct familial genetic correlations using large-scale pedigree data to clarify the effects of offspring genes, maternal or paternal genetic effects, and shared family effects.

METHODS

Sample

The Norwegian Mother and Child Cohort Study (MoBa) is a population-based pregnancy cohort study conducted by the Norwegian Institute of Public Health. Participants were recruited from all over Norway from 1999 to 2008[221]. The women consented to participation in 41% of the pregnancies. The cohort now

includes 114,500 children, 95,200 mothers, and 75,200 fathers[222]. The current study is based on version 10 of the quality-assured data files released for research in 2018. After birth, information on offspring and maternal outcomes was gathered through maternal-rated questionnaires at regular follow-up intervals, currently up to age eight. Parent and infant DNA samples were collected at birth and stored in a biobank[223]. Of these, 11,000 randomly selected trios (mother, father, offspring) were genotyped as part of the HARVEST project[222]. We identified 4,645 families with data on internalising problems available at age 8 and restricted the M-GCTA analyses to these individuals.

We linked the MoBa dataset to the Medical Birth Registry of Norway (MBRN) to identify siblings among the parents participating in the MoBa study. The MBRN contains a record of all births in Norway from 1967 onward. For same-sex twin pairs in the parents and offspring generations, zygosity was determined via either genotyping or a twin questionnaire. After exclusion of individuals without any relatives or with missing phenotype data at age eight, the final sample for the pedigree analyses included 10,688 children from 1,552 independent pedigrees (no shared grandparents).

The establishment and data collection in MoBa are based on regulations related to the Norwegian Health Registry Act. The current study was approved by The Regional Committee for Medical Research Ethics (REK 2013/863). Details of all available data are available on the Norwegian Institute of Public Health's website (https://www.fhi.no/en/studies/moba/for-forskere-artikler/questionnaires-from-moba/).

Measures

We investigated two maternally rated internalising phenotypes at age 8: childhood depression and anxiety symptoms. Childhood depressive symptoms were measured using the parent version of the Short Mood and Feelings Questionnaire (SMFQ)[224]. The 13-item scale is based on DSM-III-R criteria for depression and consists of descriptive phases regarding how the child had felt or behaved recently. Childhood anxiety symptoms were measured using Birmaher's shortened version of the Screen for Child Anxiety-Related Disorders (SCARED) consisting of five items[225]. SCARED is a multidimensional questionnaire designed to measure DSM-defined anxiety symptoms. For both scales, mothers rated how true statements describing their child's recent behaviours were using a 3-point scale (1 = Not true, 2 = Sometimes true, 3 = True). Based on these measures, childhood depression and anxiety scores were calculated with maximum

allowed missingness of two items from the SMFQ and one item from the SCARED questionnaire, per individual. Missing items were imputed with the mean of the non-missing responses.

Genotyping

MoBa parents and offspring were genotyped using Illumina Human Core Exome Bead chips 12 version 1.1 and 24 version 1.0 and imputed based on the Haplotype Reference Consortium[226] reference set. Pre-imputation quality control procedures and imputation processes are described in detail elsewhere[227]. Post-imputation, genetic data from the two chips was merged based on overlapping SNPs, according to the procedure used by Fedko et al.[97]. Four and a half million high quality SNPs (imputation info score > 0.9, minor allele frequency > 0.05) were used in downstream analyses.

Statistical analyses

GCTA and extended GCTA analyses

GCTA[217] was used to estimate the proportions of variance in depressive and anxiety symptoms that were explained by genome-wide SNPs in the offspring. First, a genetic relationship matrix (GRM) was calculated to estimate the genetic relationships between pairs of unrelated children based on all autosomal SNPs in the imputed genotype dataset. Cryptic relatedness in the sample was removed using a genetic correlation cut-off threshold of 0.025. GREML analyses, performed in GCTA, were used to estimate the variance in childhood depression and anxiety symptoms that was explained by the genotyped SNPs[216]. The analysis adjusted for gender, genotyping batch effects, and the first 10 principal components to account for population stratification.

To resolve non-transmitted maternal and paternal genetic effects, the imputed genotype dataset was split into mother–offspring and father–offspring datasets using Plink 1.96[228]. The M-GCTA tool[229] was used to construct GRMs indicating genetic similarity between unrelated offspring, unrelated mothers or unrelated fathers, and unrelated mother–offspring or father–offspring pairs. A correlation cut-off threshold of 0.025 was applied to exclude cryptic relatedness within the groups of mothers, fathers, and offspring. GREML analyses were carried out to examine the extent to which genetic similarity between unrelated parents, as well as unrelated parent–offspring pairs, was associated with similarity in offspring internalising behaviours. If unrelated parents that were more similar genetically had offspring that were more similar than expected based on the offspring genetic similarity, this would indicate an effect of the non-transmitted

parental genotype on offspring internalising problems. We estimated the proportion of variance in childhood depression and anxiety symptoms that was explained by the offspring's genotype (A), maternal or paternal genotype (M/F), the covariance between the offspring and maternal or paternal genotypes (Q), and the residual environmental component (E). To test for significance, this full model was tested against the classical GCTA AE model. The analysis was performed separately for mother–offspring and father–offspring pairs for childhood depression and anxiety. Gender, genotyping batch, and the first 10 principal components based on the offspring GRM were included as covariates in the analyses.

Pedigree analyses

Using linkage between MoBa and MBRN, we derived expected genetic correlations among known relations of children in the sample (e.g., Figure 1). To capture offspring additive genetic effects, we made use of monozygotic and dizygotic twin correlations, as well as correlations between siblings, half-siblings, cousins, and half-cousins (children of half-siblings). Maternal effects were examined by comparing correlations between children of the same mother and children whose mothers were monozygotic twins (these children share 100% of maternal genetic effects) to children whose mothers were full siblings (share 50% of maternal genetic effect) and children whose mothers were half-siblings (share 25% of maternal genetic effect). If children who shared the same mother, or whose mothers were monozygotic twins, were more alike than children whose mothers were full siblings or half-siblings, this would indicate a maternal genetic effect on offspring internalising problems. To account for influences due to the other parent and the shared family environment, we further tested for a shared family effect, which was shared among children of the same mother and father. In a separate model, paternal effects were examined using the same structure, but focusing on fathers of children instead of mothers. The number of different correlations within each type of effect are tabulated in Table 1.

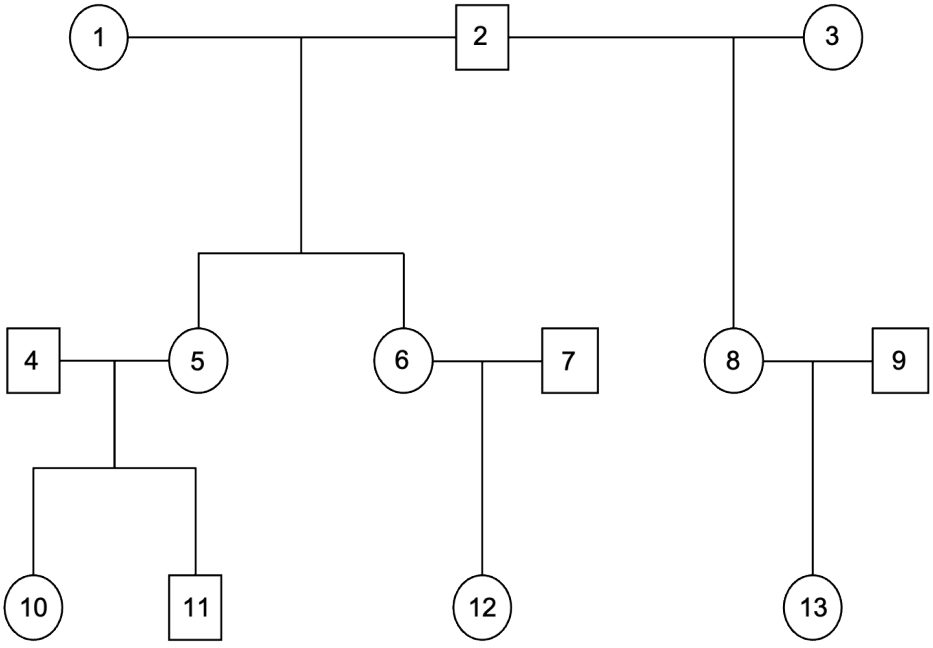
We modelled the covariance structure among the childhood phenotypes, depression and anxiety symptoms, as arising from offspring additive genetic effects (A), indirect maternal and paternal genetic effects (M/F), shared family effects (C), and environmental effects unique to the individual (E). While individuals could be correlated with each other within each type of effect, the different types of effects were assumed to be independent of each other, that is, no gene–environment correlation. Variance components associated with the different types of random effects were estimated using a linear mixed effects model [230] in software package R, version 3.4.4. In all analyses, gender of offspring was included as a covariate.

Table 1 Number of distinct correlations between pairs of children for each of the included random effects

Type of effect	1/16	1/8	1/4	1/2	1
Additive genetic	95	2339	101	4235	116
Maternal genetic	0	0	57	1154	4411
Paternal genetic	0	0	30	857	4382
Shared environment	-	-	-	-	4351

Additive genetic effect: '1' = monozygotic twins, '2' = dizygotic twins or full siblings, '1/4' = half-siblings, '1/8' = cousins, '1/16' = half-cousins. Maternal or paternal genetic effect: '1' = full siblings or children of monozygotic twins, '1/2' = children of full siblings, '1/4' = children of half-siblings. Shared family effect: '1' = children with the same mother and father (full siblings)

Figure 1 Pedigree figure showing an example of relations between children of siblings included in the pedigree analyses.



Individuals 10–13 represent the offspring generation, 4–9 represent their parents, and 1–3 represent their grandparents. Offspring 10 and 11 are full siblings. As Mothers 5 and 6 are full siblings, Offspring 12 is the cousin of Offspring 10 and 11. As Mother 8 is the half-sibling of Mothers 5 and 6, Offspring 13 is the half-cousin of Offspring 10, 11, and 12. Offspring 10 and 11 share 50% of additive genetic effects, 100% of maternal effects, 100% of paternal effects, and 100% shared family effects. With Offspring 12, they share 25% of additive genetic effects, 50% of maternal genetic effects, no paternal effects, and no shared family effects. With Offspring 13, Offspring 10, 11, and 12 share 12.5% of additive genetic effects, 25% of maternal genetic effects, no paternal effects, and no shared family effects

RESULTS

After quality control procedures, the extended GCTA analyses included data on up to 3,801 trios, while data on 10,688 children were included in the pedigree analyses. Sample descriptive statistics are shown in the Supplementary Information.

GCTA and extended GCTA analyses

We present the results of the GCTA analyses in Table 2. In the standard GCTA models, offspring additive genetic effects from measured SNPs explained close-to-significant variance in childhood depressive symptoms (0.10, 95% confidence intervals [CI]: −0.3 to 0.23) and significant variance in childhood anxiety symptoms (0.17, 95% CI: 0.03–0.31). The extended GCTA models including the parental effects did not show a better fit than the standard AE model. The CI showed that none of the variance components were significant, although maternal and paternal genotypes explained small proportions of variance in childhood depressive symptoms (0.04, 95% CI: −0.17 to 0.26 and 0.06, 95% CI: −0.16 to 0.28, respectively).

Pedigree analyses

Table 3 shows correlations in anxiety and depressive scores between related individuals. There were no shared family effects on offspring depression or anxiety symptoms; therefore, the shared family effect was omitted from both models (Table 4). Offspring additive genetic effects were present for both depression and anxiety symptoms, as model fitting showed that omitting the offspring genetic effect significantly worsened model fits (depressive symptoms: $\chi^2 = 338.38$, $p < 2e\text{-}16$, anxiety symptoms: $\chi^2 = 166$, $p < 2e\text{-}16$). The maternal effect explained a small percentage of variance in offspring depressive symptoms (7.6%), but this was not significant as the model including the maternal effect was no different to the model which only included offspring genetic effects ($\chi^2 = 1.71$, $p = .19$). There was no paternal effect on offspring depressive symptoms, and no maternal or paternal effects on offspring anxiety symptoms.

Table 2 Results from GCTA and extended GCTA analyses

	A (SE)	M/F (SE)	Q (SE)	G (SE)	E	df	p	N
Depressive symptoms (SMFQ)								
Standard GCTA	0.10 (0.07)	-	-	0.10 (0.07)	0.90	1	0.053	3794
Maternal effects GCTA	0.14 (0.11)	0.04 (0.11)	0.00 (0.09)	0.18 (0.12)	0.82	2	0.4	3030
Paternal effects GCTA	0.11 (0.11)	0.06 (0.11)	0.00 (0.08)	0.17 (0.12)	0.83	2	0.4	3059
Anxiety symptoms (SCARED)								
GCTA	0.17 (0.07)	-	-	0.17 (0.07)	0.83	1	0.007	3801
Maternal effects GCTA	0.16 (0.11)	0.00 (0.10)	0.00 (0.08)	0.16 (0.12)	0.84	2	0.5	3038
Paternal effects GCTA	0.03 (0.11)	0.00 (0.11)	0.06 (0.09)	0.09 (0.12)	0.91	2	0.3	3067

Model parameters are: *A* variance due to direct additive genetic ("offspring" effects), *M* variance due to indirect maternal genetic effects on offspring phenotype ("maternal effects"), *F* variance due to indirect paternal genetic effects on offspring phenotype ("paternal effects"), *Q* phenotypic variance due to covariance of direct and indirect genetic effects, *G* variance due to combined direct and indirect genetic effects and the residual *E* ("unique environmental effects"). SE: standard error, p = p value, N = sample size. The p-value is calculated by comparing the full model to the model with the offspring component only.

Table 3 Phenotypic correlations between children that were present in the pedigree analyses

	Depression symptoms (95% CI)	Anxiety symptoms (95% CI)	N
Monozygotic twins	0.553 (0.412 - 0.668)	0.674 (0.560 - 0.763)	116
Dizygotic twins	0.162 (0.046 - 0.273)	0.211 (0.097 - 0.320)	282
Full siblings	0.272 (0.242 - 0.302)	0.152 (0.120 - 0.183)	3702
Half siblings	-0.029 (-0.333 - 0.281)	0.345 (0.041 - 0.590)	45
Cousins	0.053 (-0.012 - 0.117)	0.018 (-0.047 - 0.082)	917
Half-cousins	0.283 (0.016 - 0.512)	-0.005 (-0.272 - 0.263)	54

N = number of pairs used to calculate each correlation. *95% CI* = 95% confidence intervals. Pairwise correlations presented are indicative, but not representative of all data within the analyses. Correlations were calculated by using at most one pair from a nuclear family and with each individual only able to partake in one pairing per correlation. Thus, children with more than one sibling, half-sibling, cousin or half-cousin are under-represented in this table but are included in the linear mixed effects model.

Table 4 Results from the pedigree analyses

Phenotype	Model	A (SE)	M/F (SE)	C (SE)	E (SE)
Depression symptoms	Maternal effects	0.419 (0.12)	0.076 (0.06)	0.000 (0)	0.505 (0.06)
	Paternal effects	0.554 (0.11)	0.000 (0)	0.006 (.06)	0.440 (0.05)
Anxiety symptoms	Maternal effects	0.377 (0.03)	0.000 (0)	0.000 (0)	0.623 (0.03)
	Paternal effects	0.377 (0.03)	0.000 (0)	0.000 (0)	0.623 (0.03)

Model parameters are: *A* variance due to direct additive genetic ("offspring" effects), *M* variance due to maternal environmental effect ("maternal effects"), *F* variance due to paternal environmental effect ("paternal effects"), *C* variance due to the shared family effect and the residual *E* ("unique environmental effects"). *SE*: Standard error.

DISCUSSION

We set out to resolve the impact of non-transmitted parental genetic factors on offspring internalising problems during childhood using two complementary approaches: M-GCTA analyses and pedigree analyses. The extended GCTA analyses used molecular data from genotyped trios to estimate the contribution of maternal and paternal genetic effects on offspring internalising problems, beyond the effects of transmitted genes from parents to offspring, and further investigated whether there was evidence of a passive gene–environment correlation. The pedigree analyses investigated maternal and paternal genetic effects using estimated genetic correlations from rich family data, and additionally examined whether there was a shared family effect in full siblings. In both the M-GCTA and pedigree analyses, there were no significant non-transmitted maternal or paternal genetic effects on childhood depression or anxiety symptoms. The M-GCTA analyses showed no evidence of a passive gene–environment correlation for childhood depression or anxiety symptoms, and the pedigree analyses found no shared family effect.

Focusing on the results for offspring depressive symptoms, findings from the M-GCTA and pedigree analyses converged to show that a small proportion of variance (between 4 and 8%) was explained by non-transmitted maternal genetic effects, although the estimate was not significant in either of the analyses. The contribution of these maternal genetic effects led to an increased proportion of variance explained in the extended GCTA (18%), compared to when maternal genetic effects were not included in the analyses (10%). While the large confidence intervals signify insufficient power, the consistency of the estimate using two independent methodologies suggests that the true contribution of

maternal genetic effects on offspring depressive symptoms is likely not far from this estimate. Therefore, we predict that although a larger sample size would be required to find a significant maternal genetic effect on symptoms of depression, the size of this effect is likely to remain relatively small. Previous family based studies have found small (0.05)[212] to moderate (0.28)[107] direct environmental effects of concurrent maternal depression, but no effect of prenatal depressive symptoms[109], on offspring internalising problems after taking into account confounding due to shared mother–offspring genes. Bearing these results in mind, the findings of the current study suggest that maternal genetic factors may account for a small proportion of the overall environmental effects on offspring behaviour that arise due to the mother. With regard to paternal genetic effects on offspring depressive symptoms, results from the two methodologies were discrepant. A small effect was observed in the M-GCTA analyses (explaining 6% of the variance), but was not replicated in the pedigree analyses. As paternal effects are rarely studied, in part due to limited availability of paternal data, more research is required to interpret this inconsistent finding and elucidate the impact of paternal genome on offspring depression symptoms.

Results from the M-GCTA and pedigree analyses converged again when looking at non-transmitted parental genetic effects on offspring anxiety symptoms. There were no effects of maternal or paternal genotype on anxiety symptoms, using either of the methodologies. There are two possible explanations for this; there may have been insufficient power to detect indirect parental genetic effects on anxiety symptoms, or childhood symptoms of anxiety may be unaffected by indirect parental genetic effects. Further research is required to clarify which of these is the case. However, if the latter is true, it may hold implications for research on parental influences on internalising problems that group anxious and depressive symptoms together, as there may be different effects underlying the parent–offspring associations. Indeed, it has previously been suggested that while genetic influences underlying anxiety and depression are not disorder-specific, environmental effects could be specific and unshared across the two disorders[231]. Therefore, it may be that genetically influenced parental characteristics have some influence on offspring depressive symptoms, but not anxiety symptoms. This requires further investigation, as although the current findings suggest a small indirect maternal genetic effect on offspring depressive symptoms, the results were not statistically significant.

Previous research found gene–environment correlation effects on internalising problems in childhood[32,126]. However, as the parental effects were nonsignificant in the current study, it was impossible to detect such an effect, even if it were

present. More power in the M-GCTA analyses would be needed to detect whether gene–environment interplay underlying offspring internalising problems arises due to the indirect effect of the parental genome. Alternatively, it is also possible that the gene–environment correlations observed in offspring internalising problems within existing research do not act via parental factors that are genetically influenced. The current study also did not find a shared family effect (reflecting the influence of the other parent and the shared family environment) on depression or anxiety symptoms, within the pedigree-based analyses. Within previous research, estimates of variance explained by the common family environment are broad and range from 0 to 0.32[6,98,101]. The ability to detect the effect varies, depending on the population and sample size. Finally, the pedigree analyses in the current study showed that large amounts of variance in depressive and anxious symptoms were explained by unique environmental effects. It is important to note that these may include the effects of parental behaviours toward the child that are not genetically influenced, and are child specific.

In the context of broader literature, estimates of the contribution of additive genetic effects to variance in depression (45%) and anxiety (38%) from the pedigree analyses were in line with existing findings which estimate that ~40% of the variance in internalising problems in childhood is due to genetic factors[6]. Our results confirm that individual differences in childhood anxiety and depression in childhood have a substantial underlying genetic component. In molecular research, the maximum estimate of SNP heritability of internalising problems from previous research is 14%[82]. The estimates from the current study are close to this, with measured genetic variants explaining 10% of the variance in depressive symptoms (not significant) and 17% of the variance in anxiety symptoms (significant). The gap in heritability estimates based on the pedigree analyses versus GCTA analyses is not unexpected, and is widely recognized in existing literature[62,232,233].

The current study has a number of strengths. We used methodological triangulation in investigating our research question to determine whether results from quantitative and molecular genetics approaches converged. To our knowledge, this is the first application of the M-GCTA technique to examine parental genetic effects on mental health outcomes, as the method has previously only been applied to study physical characteristics such as birth length and weight[35,229,234]. Furthermore, much of the research investigating parental contribution to offspring internalising problems in childhood has primarily focused on mothers[235], even though paternal factors also exert

an influence on offspring behaviour. This study pays equal attention to the contribution of maternal and paternal influences. The study design is resourceful as it does not require direct measurement of parental phenotypes in order to study parental influences on offspring internalising problems. This is an advantageous approach for cohorts that do not have measurements of parental behaviours, to still answer research questions investigating parental effects on offspring behaviour. The approach is also useful when the mechanisms through which parents have an effect are unclear and the relevant variables cannot be easily identified.

The results of this study should be considered in the context of certain limitations. First, the M-GCTA analyses were underpowered to detect maternal or paternal genetic effects on offspring internalising problems. Despite a large sample of genotyped trios available (11,000), after quality control procedures and exclusion of missing data, the sample was limited to between 3,000 and 3,800 pairs per analysis. This yielded limited power (0.57) to detect a maternal or paternal genetic effect of 0.05, in proportion of variance explained. It is now estimated that at least 10,000 pairs are required to detect maternal or paternal genetic effects[236]. Second, in cohort studies with long-term follow-up such as MoBa, biases in study participation can impact the results. It has already been shown that participation at baseline was related to maternal education[237]. Furthermore, there was substantial study dropout as only 47% of the original sample had data available at age 8[221]. If families of children with internalising problems withdrew from the study or were less likely to participate, this would reduce coverage of the higher end of the distribution within the sample. This could be important if severe cases have different underlying mechanisms. In investigating this, we found that children whose mothers answered questions on internalising behaviours at two measurement points (age 3 and 8) showed fewer internalising symptoms on average, than those who responded at one time point, either age 3 or age 8 (Supplementary Information). Based on this selective nonresponse bias, the current findings may not extend to individuals with more severe internalising problems, if they are differentially impacted by indirect parental genetic effects. Finally, although the use of maternal ratings to define offspring internalising behaviours is beneficial as mothers are considered good informants on early life behaviours among children[238], it could also be a potential limitation. In using maternal ratings of offspring behaviour to identify maternal effects, we are restricted in our ability to distinguish real environmental effects from rater bias effects. Sources of rater bias are stereotyping, employing different normative standards, or having certain response styles (e.g., judging problem behaviours more or less severely). Previous twin research shows that

10–20% of the variance in internalising behaviours is accounted for by rater bias[98,99,101]. A large study with behavioural observations would be an opportunity to overcome these effects of rater bias, although these observations might also be biased and are not feasible in large population-based cohorts.

There are several additional avenues for future investigations in light of the current findings. We first note that larger sample sizes are needed to generate enough power to adequately estimate internalising problems variance components based on SNP effects. To achieve this, it would be beneficial to combine data from multiple cohorts in order to maximize the number of genotyped individuals available. In cohorts with large amounts of family data available, the influence of other family members, such as siblings or adoptive parents, could additionally be studied using the M-GCTA technique. The method would also very well compliment other recently developed genetic nurture methodologies, such as exploring the effect on non-transmitted parental alleles on offspring behaviour[33]. Finally, the current study specifically focuses on non-transmitted maternal and paternal genetic effects on offspring internalising problems. Future research may wish to focus on other mechanisms that account for the influence of parental factors on offspring internalising problems. For instance, in animal models mother–offspring interactions have been shown to influence DNA methylation in the offspring, leading to changes in gene expression, that may be related to offspring behaviour[239,240].

In summary, we applied two distinct methodologies to investigate maternal and paternal genetic effects on offspring internalising problems during childhood. Genetic variation in offspring internalising problems was predominantly due to offspring additive genetic effects rather than indirect maternal or paternal genetic sources of variation. However, the pattern of results suggests that indirect maternal genetic effects may account for a small proportion of variation in offspring depressive symptoms in childhood.

Chapter 5

Do environmental effects indexed by parental genetic variation influence common psychiatric symptoms in childhood?

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ABSTRACT

Background: Parental genes can indirectly influence the offspring through the environment that parents create for their children. Novel genomic methods can uncover the effect of indirect parental genetic effects (known as genetic nurture) on offspring behaviour, in addition to the effect of offspring's own genotype. This study estimates the overall contribution of offspring genetic effects and genetic nurture on common childhood psychiatric symptoms using measured genotypes from mothers, fathers, and offspring.

Methods: Genome-based restricted maximum likelihood (GREML) models estimate the overall variance in a given trait that is explained by common genetic variation. This study analysed data from up to 10,499 children, 5,990 mother-child pairs, and 6,222 father-child pairs from the Norwegian Mother Father and Child Study. GREML models were applied using software packages GCTA and M-GCTA to estimate variance in depressive, externalising, and ADHD symptoms at age 8 that was explained by offspring genetic effects and maternal or paternal genetic nurture.

Results: There was no strong evidence of genetic nurture in this study, although a suggestive paternal genetic nurture effect on offspring depressive symptoms and a suggestive maternal genetic nurture effect on ADHD symptoms were observed.

Conclusion: Parental genetic nurture effects could be of importance in explaining individual differences in some childhood psychiatric symptoms, but application of GREML-based models in better-powered samples is required to robustly estimate their contribution.

INTRODUCTION

Offspring psychiatric symptoms are often linked to parental characteristics. These intergenerational associations are not necessarily causal with the parental characteristics having a direct effect on the offspring symptoms. Instead, parent-offspring associations can be explained by genetic as well as environmental factors[241]. Insight into these mechanisms is important to provide families and children with adequate information about the etiology of children's symptoms and for the development of interventions targeting modifiable factors.

That both genetic and environmental factors play a role in childhood psychiatric symptoms is confirmed by classical twin studies, which compare similarities between identical versus non-identical twins. They show that large proportions of variance (40-80%) in childhood psychiatric traits such as depressive, externalising and attention-deficit hyperactivity disorder (ADHD) symptoms are explained by genetic factors[6]. As genes are inherited from parents, this suggests that genetic transmission is a key factor in explaining associations between parental characteristics and offspring psychiatric symptoms. Twin studies also show a role of the shared environment (the environment shared between children in the same family) in explaining childhood psychiatric symptoms. This effect explains individual differences in depressive and externalising symptoms up to age 12[98,101], after which the influence of the shared environment decreases[7,8]. An important aspect of the shared environment is the environment created by the parents, which includes the effect of parental characteristics. Studies show that several parental characteristics, such as parental psychiatric traits[109,181] and parenting behaviours[128,145] are associated with offspring psychiatric symptoms after accounting for the role of genetic transmission[241]. However, even though they exert their influence through the environment, these parental characteristics may still have an underlying genetic component. A meta-analysis of twin and adoption studies in parent-based samples showed that parenting behaviours are themselves heritable and under the influence of parental genes[199]. Recent genetic studies use the terms *genetic nurture* or *indirect genetic effect* to describe such a phenomenon, in which parental genes indirectly influence the offspring by acting on the environment that parents create for their children[33].

Family-based molecular genetics designs use the most common type of DNA sequence variation, known as single nucleotide polymorphisms (SNPs) to study the overall impact of genetic nurture on an offspring trait by modelling the cumulative effect of millions of parental SNPs on offspring behaviour[35,38,106].

This technique does not involve measured parental behaviours, but examines the effect of parental genetic variants as a proxy for the parentally-provided environment. Designs that estimate the overall effect of genetic nurture in this way are an extension of an established molecular genetics technique known as genome-based restricted maximum likelihood (GREML) estimation, which estimates the overall variance in a given trait that is explained by common genetic variation (i.e., SNP-based heritability). This is done by exploring whether unrelated individuals that are more similar genetically also show more similarity in the trait of interest[217]. However, family-based genetic studies indicate that SNP-based heritability estimates from the standard GREML approach may index the indirect genetic effect of relatives, as well as the direct genetic effect of the individual's own genetic variation[106]. Extended GREML designs, which include both offspring and parental genotype in the same model, can be used to estimate both direct and indirect genetic effects on a phenotype[34,35,38]. In other words, this analysis allows the partition of variance into the effect of the offspring's genotype (direct genetic effect) the effect of the parent's genotype (indirect genetic effect, i.e., genetic nurture), and the effect of the covariance between the two. The covariance term reflects genes present in both the parent and offspring, which exert both a direct genetic effect through the offspring and a genetic nurture effect through the parent. In quantitative genetics, this type of an effect is described as a passive gene-environment correlation, when parents pass on both trait-associated genes and environment to the child[19]. Hence, extended GREML models also offer a novel way of estimating the contribution of at least a part of the passive gene-environment correlation that explains individual differences in offspring behaviour.

Given their recent development, the application of these models to study genetic nurture effects in childhood psychiatry is rare. Our previous work used an extended GREML method called M-GCTA[35] (maternal-effects genome-wide complex trait analysis) to separately estimate maternal and paternal genetic nurture effects on depressive and anxiety symptoms in 8-year-olds[63]. We found non-significant but suggestive maternal and paternal genetic nurturing effects on offspring depressive symptoms[63]. A subsequent study in the same sample used a similar method to estimate an overall parental effect, and found that parental genetic nurture explained 14% of the variance in offspring depressive symptoms[64]. In contrast, no genetic nurturing effects on childhood anxiety symptoms were observed in either study. GREML-based results in both studies were supported by the pattern of results in pedigree-based models, which reinforced the findings from these designs[63,64]. Beyond the described studies, GREML-based methods have not been applied to examine childhood psychiatric

outcomes to our knowledge. The genetic nurture findings on childhood depression require replication, and moreover, broader application of extended GREML methods is required to estimate the overall effect of genetic nurture on other common childhood psychiatric problems, such as externalising and ADHD symptoms.

The investigation of parental genetic nurture in extended GREML models requires large samples with genotypic information on both parents and offspring and phenotypic information on offspring. The Norwegian Mother Father and Child cohort study (MoBa) is a population-based sample with an extensive, and growing, number of genotyped families. With its unique combination of large-scale genetic and behavioural data, the dataset offers the ideal opportunity to study genetic nurture effects on childhood psychiatric symptoms. This study estimates offspring genetic effects, parental genetic nurture effects, and passive gene-environment correlation effects on offspring depressive, externalising, and ADHD symptoms that are captured by common genetic variance in a large sample of Norwegian families.

METHODS

Sample

The Norwegian Mother, Father and Child Cohort Study (MoBa) is a population-based pregnancy cohort study conducted by the Norwegian Institute of Public Health. Pregnant women were recruited from all over Norway from 1999-2008[221]. The women consented to participation in 41% of the pregnancies. The cohort now includes 114,500 children, 95,200 mothers and 75,200 fathers[222]. After birth, information on offspring outcomes was gathered through parental questionnaires at regular follow-up intervals. Parent and infant DNA samples were collected at birth and stored in a biobank[223]. Of these, genotyped data from approximately 33,000 trios (including mothers, fathers and offspring) is currently available in MoBa Genetics. The current study is restricted to a subset of this sample consisting of 14,064 unique individuals in the offspring generation, for whom data on psychiatric symptoms was also available. This was linked to parental genotype data from 13,690 mothers and 13,299 fathers.

The establishment and data collection in MoBa is based on regulations related to the Norwegian Health Registry Act. The current study was approved by The Regional Committee for Medical Research Ethics (REK 2013/863) and is based on version 11 of the quality-assured data files released for research in 2018.

Measures

The outcome measures were maternally-rated depressive, externalising, and ADHD symptoms in 8-year-olds. Depressive symptoms were measured using the parent version of the Short Mood and Feelings Questionnaire (SMFQ)[224]. The 13-item scale is based on DSM-III-R criteria for depression. Externalising symptoms were measured using the Parent/Teacher Rating Scale for Disruptive Behaviour Disorder (RS-DBD)[242]. The oppositional defiant and conduct disorder subscales, consisting of 8 items each, were combined to measure externalising symptoms. The ADHD subscale of the RS-DBD, consisting of 18 items, was used to measure ADHD symptoms. For all measures, mothers rated how true statements describing their child's recent behaviours were using 3- or 4-point Likert scales. Childhood depressive, externalising, and ADHD symptom scores were calculated with maximum allowed missingness of two items for the SMFQ, three items for the RD-DBD externalising scale and four items for the RS-DBD ADHD subscale. Missing items were imputed with the mean of the non-missing responses.

Genotyping

The current release of the MoBa Genetics dataset consists of approximately 32,000 trios who were genotyped as part of a collaborative research effort, consisting of four major research projects. Genotyping, quality control and imputation procedures were performed separately for each subproject according to standard practices and are described in detail elsewhere (https://github.com/folkehelseinstituttet/mobagen). After imputation of missing genotypes, all datasets were merged to create the MoBa Genetics dataset. Using this dataset as the starting point, we conducted post-imputation quality control to select high quality SNPs for analysis. SNPs were selected if they met the following standard criteria: Hardy-Weinberg equilibrium $p < 1x10^{-6}$, 90% genotyping rate, minor allele frequency > 0.05, high imputation quality (INFO score > 0.9 on average across batches), non-multiallelic, and non-duplicated. 5.1 million SNPs were retained for subsequent analysis.

Statistical analyses

To first obtain estimates of the variance in childhood psychiatric symptoms explained by offspring genotype without correcting for parental genotypes (offspring model), the GCTA software package was used[217]. A genomic relatedness matrix (GRM) was constructed to index genetic similarity between the 14,064 genotyped offspring in the dataset. Based on this GRM, a correlation cut-off threshold of 0.025 was applied to exclude excessive relatedness, as the presence of closely related individuals can bias variance estimates. This resulted in a reduced sample size of up to 10,499 individuals. A GREML model was run

in GCTA to estimate variance in offspring depressive, externalising and ADHD symptoms explained by offspring genetic variants (V_o). This variance component in the offspring model represents the standard SNP-based heritability based on a sample of unrelated individuals.

The M-GCTA software package[229] implements extended GREML models to estimate variance in offspring phenotype that is explained by direct offspring genetic effects, genetic nurture and gene-environment correlation. As M-GCTA estimates maternal and paternal effects in separate models, the overall genotyped dataset was first split into separate mother-child and father-child datasets using Plink 1.96[228]. Using the mother-child dataset, M-GCTA was then used to construct multiple GRMs indexing genetic similarity between: 1) individuals within the offspring generation, 2) individuals within the maternal generation, and 3) unrelated mother-child pairs (i.e., offspring from X family and mother from Y family). The same, but for fathers, was repeated for individuals in the father-child dataset. A correlation cut-off threshold of 0.025 was applied using each of the constructed GRMs to exclude excessive relatedness within the offspring generation, the parental generation, and between unrelated pairs across the generations in the mother-child and father-child datasets. After this step, 5,990 pairs in the mother-child and 6,222 pairs in the father-child dataset were retained.

Using the mother-child dataset (maternal model), extended GREML analyses were carried out in M-GCTA to estimate the proportion of variance in childhood depressive, externalising and ADHD symptoms that was explained by offspring genotype (V_o; corrected for maternal genetic nurture effect), maternal genotype (V_m i.e., maternal genetic nurture) and the covariance between offspring and maternal genotypes (V_{om} i.e., passive gene-environment correlation between offspring genetic effects and maternal genetic nurture). To test for significance, the full model was compared to a model that only estimated the effect of offspring genotype, after correcting for maternal genetic nurture. The analyses were repeated using the father-child dataset (paternal model) to estimate the variance explained by offspring genotype (V_o; corrected for paternal genetic nurture effect), paternal genotype (V_f i.e., paternal genetic nurture) and the covariance between offspring and paternal genotypes (V_{of} i.e., passive gene-environment correlation between offspring genetic effects and paternal genetic nurture).

The following covariates were regressed out of the outcomes in all analyses: sex, genotyping batch and ten genetic principal components based on offspring genotype (to correct for population structure).

RESULTS

The overall sample of children had a mean age of 8.08 with a standard deviation (SD) of 0.67. There were slightly more boys (52%) than girls in this study. The mean SMFQ score for depressive symptoms was 14.85 (SD = 2.45). The mean RS-DBD score for externalising symptoms was 20.28 (SD = 4.28). The mean score RS-DBD score for ADHD symptoms was 26.72 (SD = 7.61). Sex differences in all scales were observed, with boys scoring slightly higher than girls ($p = 0.004$ for depressive symptoms, $p < 0.001$ for externalising and ADHD symptoms). Moderate correlations between symptom scores were observed; depressive symptoms were correlated with externalising symptoms ($r = 0.52$, $p < .001$) and ADHD symptoms ($r = 0.53$, $p < .001$), which in turn were also correlated with each other ($r = 0.59$, $p < .001$).

GREML models

Full results for the offspring, maternal and paternal GREML models are presented in Table 1.

The offspring models estimated variance in childhood psychiatric symptoms explained by child genotype, without correcting for parental genotype. In these models, offspring genotype explained 5% of variance in their depressive symptoms (95% confidence interval (CI) = 0 – 11%), 3% of variance in their externalising symptoms (95% CI = -2 – 8%), and 10% of variance in ADHD symptoms (95% CI = 4 – 16%).

After correcting for maternal or paternal genetic nurture effects, variance explained by direct effects of offspring's own genetic effects varied, with inconsistent estimates and wider confidence intervals (Table 1; maternal and paternal models). There was no strong evidence of maternal or paternal genetic nurture effects on childhood psychiatric symptoms. However, estimates for paternal genetic nurture effects on offspring depressive symptoms (10%, 95% CI = -1 - 21%) and maternal genetic nurture effects on offspring ADHD symptoms (8%, 95% % CI = -3 – 20%) were higher than others, pointing to suggestive genetic nurture effects that could be different from zero with more power.

Table 1 Estimates of variance explained, standard errors, and sample sizes of the fitted models

	V_o (SE)	$V_{m/f}$ (SE)	$V_{om/pf}$ (SE)	G (SE)	logL	*p*	N
Depressive symptoms							
Offspring model	**0.053** (0.027)	-	-	0.053 (0.027)	-5250.08	0.021	10475
Maternal model	0.002 (0.057)	0.029 (0.060)	0.076 (0.045)	0.107 (0.064)	-2828.09	0.312	5964
Paternal model	0.074 (0.059)	0.098 (0.057)	0.000 (0.044)	0.172 (0.062)	-2932.84	0.181	6184
Externalising symptoms							
Offspring model	0.029 (0.026)	-	-	0.030 (0.027)	-5111.40	0.128	10493
Maternal model	0.029 (0.060)	0.041 (0.060)	0.000 (0.047)	0.070 (0.064)	-2750.72	0.263	5966
Paternal model	0.087 (0.061)	0.019 (0.058)	0.001 (0.047)	0.108 (0.063)	-2718.52	0.369	6196
ADHD symptoms							
Offspring model	**0.101** (0.029)	-	-	0.102 (0.029)	-5215.83	< 0.001	10499
Maternal model	0.063 (0.060)	0.084 (0.058)	0.000 (0.048)	0.155 (0.065)	-2737.43	0.069	5972
Paternal model	0.000 (0.056)	0.000 (0.058)	0.038 (0.045)	0.038 (0.060)	-2833.18	0.500	6204

V_o = variance explained by offspring genetic effects, $V_{m/f}$ = variance explained by maternal or paternal genetic nurture, $V_{om/of}$ = variance explained by a passive gene-environment correlation between offspring genetic effects and maternal or paternal genetic nurture, **G** = variance explained by combined direct and indirect genetic effects, **SE** = standard error, **logL** = log-likelihood value, ***p*** = p-value, **N** = sample size. Results in bold show models that were significant at $p < 0.05$.
Note: the offspring model estimating standard SNP-based heritability was implemented in GCTA, and maternal and paternal models were implemented in M-GCTA.

As genetic nurture effects were not robust, we cannot meaningfully interpret the observed covariances between offspring genetic effects and parental genetic nurture estimates.

DISCUSSION

This study used parent and offspring genotypic data to estimate maternal and paternal genetic nurture effects on childhood depressive, externalising, and ADHD symptoms in a Norwegian population-based sample. There was no strong evidence of genetic nurture in this study, although a suggestive paternal genetic nurture effect on offspring depressive symptoms and a suggestive maternal genetic nurture effect on ADHD symptoms were observed. This indicates the need to investigate these effects in well-powered samples.

To our knowledge, this is the first study to estimate the overall variance in childhood externalising and ADHD symptoms that was explained by parental genetic nurture effects. Although we did not observe clear statistical evidence of an effect, there was some indication of maternal genetic nurture effects on offspring ADHD symptoms. This observation initially stands in contrast to findings from classical twin studies, which indicate little to no effects of the shared environment on ADHD[6]. However, environmentally-driven effects on ADHD symptoms have been observed in previous studies. For instance, a large genetically informative study in MoBa showed that after accounting for shared parent-offspring genetic effects, maternal educational attainment was associated with offspring ADHD symptoms through an environmental pathway[130]. It has been suggested that methodological issues (such as lack of power) could account for the lack of shared environment effect on ADHD behaviours in many twin studies[243]. It is, therefore, possible that parental genetic nurture effects on ADHD symptoms are present, and are mediated by parental educational attainment. This hypothesis requires formal investigation and the suggestive genetic nurture effect requires replication in a larger sample.

Some evidence of genetic nurture effects on offspring depressive symptoms was found in this study, which is in line with our previous work[63] and corroborates evidence of genetic nurture found in a larger study[64]. As current evidence points towards genetic nurturing effects on depressive symptoms being present, the next step in this line of research is to identify mediating factors that may account for these effects. A mediating role of maternal anxiety and depression was already identified, but did not account for the entire genetic nurture effect[64]. Associations between parental characteristics and offspring depressive symptoms in previous literature could guide research on other mediating factors. For instance, genetically informative studies have shown associations between positive and negative parenting behaviours and offspring internalising behaviours after accounting for shared genetic effects[123,124,126–128]. Identifying whether these parenting behaviours partly account for the genetic nurturing effect on depressive symptoms would help to clarify the direction of effect for these observed associations. Additionally, identifying the contribution of broader environments provided by parents (e.g., socioeconomic status) is also warranted as genetic nurture may also encapsulate such effects[44].

The estimates of offspring genetic effects on depressive and ADHD symptoms in the offspring models are in line with previous literature, showing SNP heritability estimates of between 0-17% for depressive and 0-34% for ADHD symptoms[18]. Similarly, the low estimate of SNP heritability for externalizing symptoms (3%;

non-significant) matches the estimate from the recent GWAS of childhood aggression (3%; significant)[76]. The considerable range within estimates of SNP heritability can be explained by factors including the method, sample size, selection of SNPs, and genomic relatedness threshold, all of which can have an impact on the estimation of variance components using genetic data. It should be noted that these estimates of SNP heritability in the offspring models could include potential indirect genetic effects from relatives[106]. Even though clear evidence of parental genetic nurture was not identified in the current study, there are hints towards its involvement, especially based on previous work on childhood depressive symptoms[64]. Larger family-based genetic studies are needed to accurately assess the contribution of both direct and indirect genetic effects.

This study and design have certain limitations that should be acknowledged. First, power is a considerable concern for extended GREML analyses. The GWAS era of research has highlighted that the polygenic nature of complex traits makes it important to accumulate large samples in order to study the small effects of individual SNPs. This becomes of even more importance when estimating multiple variance parameters from genetic data in the same model. Unfortunately, the availability of large-scale datasets with genotypic information on both parents and children is currently limited, especially for datasets that also have data on childhood psychiatric phenotypes. Second, SNP effects can be diluted by biases related to the measurement of psychiatric symptoms[244]. Whilst parental characteristics were indexed by their genome, measurement of offspring psychiatric symptoms was through maternally-reported questionnaires, and could be susceptible to rater bias[146]. Third, while the M-GCTA method estimates maternal and paternal effects in separate models, recent work indicates that modelling maternal and paternal effects together will provide more accurate estimates of offspring and parental genetic effects[245]. A new method called trio-GCTA provides a framework for the joint estimation of maternal and maternal effects in the same model[38].

This study used GREML models to estimate variance in childhood depressive, externalising and ADHD symptoms that could be explained by common genetic variance in children and their parents. While there was some indication of parental genetic nurture effects, follow-up analyses in better-powered samples are required to obtain more reliable estimates. If robust genetic nurture effects on childhood psychiatric symptoms are identified, the subsequent step in this line of research would be to identify mediating factors that account for these effects and represent modifiable targets for intervention.

Chapter 6

Using parent and offspring genotypes to study the relationship between parental wellbeing and childhood psychiatric symptoms

Submitted as: **Jami, E. S.**, Sallis, H. M., Hammerschlag, A. R., Pearson, R. M., Havdahl, A., Pingault, J. B., Ystrøm, E., Munafò, M. R., Middeldorp, C. M., & Bartels, M. Using parent and offspring genotypes to study the relationship between parental wellbeing and childhood psychiatric symptoms.

ABSTRACT

Background: Parental wellbeing is associated with the mental health of their offspring, but it is unclear whether this is due to the environment created by the parent, or a result of genetic variance shared between parents and children. This study used genotyped data from families to examine whether associations between parental wellbeing and common childhood psychiatric symptoms were explained by shared genetic effects and/or genetic nurture - an environmentally-mediated effect of parental genotype on offspring behaviour.

Methods: The study sample consisted of two European cohorts: the Netherlands Twin Register and the Avon Longitudinal Study of Parents And Children. Internalising, externalising, and ADHD-related symptoms scores in 7 to 8-year-olds were regressed on wellbeing spectrum polygenic scores, calculated using genetic data from parents and offspring. Two methods were used to estimate genetic nurture effects and results across the two cohorts were meta-analysed.

Results: Offspring and parental wellbeing polygenic scores were negatively associated with childhood internalising, externalising, and ADHD-related symptoms, indicating shared genetic effects between wellbeing and childhood psychiatric symptoms that partly explain these parent-offspring correlations. After accounting for genetic effects, parental polygenic scores for wellbeing did not strongly predict childhood psychiatric symptoms, providing no clear evidence of genetic nurture.

Conclusions**:** Associations between parental wellbeing and childhood psychiatric symptoms are at least partly due to overlapping genetic factors, indicating that intergenerational studies must account for genetic effects when studying the effect of parental wellbeing on offspring mental health.

INTRODUCTION

Wellbeing refers to the perception of one's own happiness and life satisfaction, and is conceptually and empirically tied to psychiatric behaviours. For instance, greater wellbeing and life satisfaction are correlated with reduced likelihood of experiencing psychiatric symptoms such as depression[246,247]. Intergenerational research links the wellbeing of parents with the mental health of their offspring[248,249]. As wellbeing and psychiatric traits have overlapping genetic factors[246,250,251] and children inherit their genes from their parents, it follows that associations between parental wellbeing and offspring psychopathology may be partially explained by genetic variance shared between parents and children. Designs that can disentangle genetic and environmental mechanisms are required to uncover whether parental wellbeing and offspring psychiatric symptoms are correlated due to shared genes between parents and children, or are causally related through the environment created by the parent. If the latter is true, then strategies to improve wellbeing in parents could lead to improved psychiatric outcomes in children.

Novel family-based genetic designs can assess mechanisms of transmission between parent and offspring traits by leveraging genotypic data from parents and children[33,36]. Each parent passes on half of their genes to their offspring and the influence of these transmitted genes on offspring behaviour shows a genetic effect. However, parental genotypes that are not passed on to the child can also influence offspring traits via the environment, through characteristics that are under the influence of parental genes. This environmentally-mediated effect of parental genes on offspring behaviour is referred to as *genetic nurture*[33]. Genetic nurture effects on offspring behaviour can be formally investigated in within-family polygenic score studies which include polygenic scores based on parental and on offspring genotypes. Polygenic scores aggregate the small effects of genetic variants from genome-wide association studies (GWASs) to quantify an individual's genetic susceptibility towards a given trait[252]. Genetic nurture is modelled by estimating whether polygenic scores reflecting non-transmitted parental alleles are associated with the offspring phenotype[33,36]. As these alleles are not transmitted to the child, their effect on offspring phenotype can only occur through the environment. Given their recent development, no studies thus far have applied within-family polygenic score methods to study whether associations between parental wellbeing and childhood psychiatric symptoms are explained by shared genetic effects, genetic nurture, or both. Using this approach will allow us to study the impact of parental wellbeing, without specifically measuring it within this study.

A consideration for using a within-family polygenic score design is the low predictive power of polygenic scores. A powerful GWAS is required to provide enough signal to detect the small effects of common genetic variants known as single nucleotide polymorphisms (SNPs). The wellbeing spectrum is a data-driven approach that indexes the full range of positive and negative wellbeing by capturing the phenotypic and genetic overlap between subjective wellbeing, neuroticism, and depressive symptoms[251]. The most recent large-scale GWAS of the wellbeing spectrum combined four closely related traits: life satisfaction, positive affect, neuroticism, and depressive symptoms. With over 2 million observations, the study identified 304 SNPs significantly associated with the wellbeing spectrum[75]. The success of this GWAS is on par with the GWAS of educational attainment[253], which has been extensively used to uncover mechanisms underlying the intergenerational transmission of educational attainment. We therefore expect this GWAS to provide a powerful genetic index for wellbeing.

This study utilises within-family polygenic score methods to investigate whether associations between parental wellbeing and offspring psychiatric symptoms are explained by shared genetic effects or genetic nurture. We focus on three commonly occurring psychiatric behaviours in childhood: internalising, externalising, and Attention Deficit Hyperactivity Disorder (ADHD) related symptoms. Findings from two multi-generational cohorts - the Netherlands Twin Register (NTR) and the Avon Longitudinal Study of Parents and Children (ALSPAC) - with trio (mothers, fathers, and offspring) data are presented. To test the consistency of genetic nurture findings, results from two polygenic score approaches are compared. The non-transmitted genotype method uses trio data to extract parental alleles which are not present in the offspring. The effect of these non-transmitted parental genotypes on offspring behaviour indexes genetic nurture[33,36]. The statistical control method investigates genetic nurture by estimating the residual effect of parental genotype on offspring behaviour, after statistically controlling for the effect of child genotype to account for genetic transmission[254,255].

METHODS

Sample

This study includes data from two multi-generational European cohorts. NTR is a longitudinal Dutch cohort study that follows twins from birth onwards[256]. Surveys were completed by parents up until the child was aged 12. Genotyped

data of approximately 21,000 individuals is currently available, including twin pairs, non-twin siblings, and parents. This study included 4,035 children for whom both genotypic and phenotypic data at age 8 were available. Maternal and paternal genotypes were available for a subset of these (2,466 mothers and 2,125 fathers). The NTR study was approved by the Central Ethics Committee on Research Involving Human Subjects of the Vrije Universiteit Medical Centre, Amsterdam.

ALSPAC is a population-based birth cohort study that follows children born between 1990 and 1991 in the Avon county region of the United Kingdom, and their mothers[257,258]. Genotype data of 8,237 children, 8,196 mothers and 1,722 fathers are currently available for analysis. This study included 6,578 children for whom both genotypic and phenotypic data were available. Maternal and paternal genotypes were available for a subset of these (4,905 mothers and 1,544 fathers). Ethical approval for the study was provided by the ALSPAC Ethics and Law Committee and the Local Research Ethics Committee.

Measures

Childhood psychiatric symptoms

Maternally-rated measures of offspring internalising, externalising, and ADHD-related symptoms at ages 7 to 8 were included. Internalising symptoms are internally-focused behaviours, such as anxiety and depression, while externalising symptoms reflect disinhibited and disruptive behaviours such as aggression and conduct problems. We characterised ADHD-related symptoms as symptoms of inattention and/or hyperactivity.

In NTR, ratings from the Child Behavior Checklist (CBCL/6-18) were used[71]. Sumscores for the internalising, externalising, and attention problems syndrome scales at age 7 were utilised. In ALSPAC, ratings were based on the Strengths and Difficulties Questionnaire (SDQ)[70], measured at 97 months (approximately 8 years). The emotional symptoms, conduct problems, and hyperactivity/inattention subscales were used. All scales were positively coded so that higher scores indicated more childhood psychiatric symptoms.

Genotyping

Genotyping, quality control, and imputation procedures within both cohorts were performed according to standard practices and are described in detail elsewhere[158,259]. The current analyses were restricted to SNPs which passed post-imputation quality control if they met the following criteria: genotype

call rate > 0.99, Hardy-Weinberg equilibrium $p < 1x10^{-6}$, minor allele frequency > 0.05, high imputation quality (INFO score > 0.9), non-multiallelic, and non-duplicated. Mendelian errors were set to missing. After post-imputation quality control procedures, between 4.5-5.7 million SNPs in ALSPAC and 4.9-5.3 million SNPs in NTR were available for analysis.

Non-transmitted genotypes and polygenic scores

Non-transmitted parental genotypes were calculated in plink 1.07[228] using the tucc flag, as described in de Zeeuw et al., 2020[158]. This method uses the genotypes of complete trios (mother, father, child) to generate a new dataset with a pseudo-control sample containing all of the non-transmitted alleles.

Wellbeing spectrum polygenic scores were calculated for offspring, mothers, and fathers, as well as for the non-transmitted parental genotype. Summary statistics from the latest multivariate GWAS of the wellbeing spectrum were used[75]. To avoid potential overlap between discovery (GWAS) and target (childhood outcomes) samples, polygenic scores within each cohort were constructed using summary statistics which excluded that cohort's data from the GWAS. Polygenic scores were calculated using LDpred[81] by computing the sum of alleles that an individual had, weighted by the effect sizes from the wellbeing spectrum GWAS**.** LDpred accounts for the linkage disequilibrium between SNPs to avoid inflation of effect sizes. Polygenic scores were calculated at the following priors: 0.01, 0.1, 0.3, 0.5 and 0.75. Priors represent the fraction of SNPs in the GWAS that are thought to be causal, and testing a range of priors allows the downstream selection of the prior with the most optimal prediction.

Statistical analyses

Within each cohort, regressions were performed to test associations of polygenic scores based on offspring, maternal, paternal, and non-transmitted genotypes at each prior, with each childhood psychiatric outcome (internalising, externalising and ADHD-related symptoms). As the NTR included data on related individuals, family relatedness was accounted for by using the exchangeable model within R package gee (http://cran.r-project.org/web/packages/gee/). Wellbeing spectrum polygenic scores and childhood psychiatric symptoms were standardised to a mean of 0 and standard deviation of 1, allowing for comparison of betas between the two cohorts. Sex, 10 principal components based on offspring and parental genotypes (to correct for population stratification), and genotyping batch and/or chip were included as covariates in the regression analyses.

Genetic effects were assessed by regressing the psychiatric outcome scores on the wellbeing polygenic scores based on maternal, paternal, and offspring genotypes. Associations between parent and offspring wellbeing polygenic scores and childhood psychiatric symptoms would indicate shared genetic effects between wellbeing and childhood psychiatric symptoms.

Two within-family polygenic score methods were used to assess whether associations between parental wellbeing and childhood psychiatric symptoms were explained by genetic nurture. In the non-transmitted genotypes method, non-transmitted wellbeing polygenic scores were used to predict childhood psychiatric symptoms. As these parental alleles are not present in the offspring, association between non-transmitted wellbeing polygenic score and childhood psychiatric symptoms would indicate genetic nurture. In the statistical control method, wellbeing polygenic scores of each parent were used to predict childhood psychiatric symptoms, after controlling for the polygenic scores of the offspring and the other parent. Genetic nurture was indicated if maternal or maternal polygenic scores were still associated with offspring psychiatric symptoms, after adjusting for polygenic scores of the offspring and the other parent.

Meta-analyses

Results across the two cohorts were meta-analysed to obtain overall estimates. For univariate associations within both cohorts, the most predictive prior (explaining the highest percentage of variance) was selected for meta-analysis. The rma.uni() command in R package metafor[260] was used to conduct 18 meta-analyses (6 predictors x 3 outcomes). A Bonferroni correction was applied to account for multiple testing (0.05/18; α= 2.78E-03).

RESULTS

This study had an overall sample size of 10,613 children from two European cohorts; NTR and ALSPAC. Cohort-specific descriptive statistics are provided in Table 1. Meta-analytic results reporting associations between wellbeing spectrum polygenic scores and childhood psychiatric symptoms are reported in Table 2.

Table 1 Descriptive statistics

	NTR	ALSPAC
N	4035	6578
Age mean (SD)	7.48 (0.40)	8.20 (0.25)
N males (%)	1933 (47.91%)	3348 (50.90%)
Childhood psychiatric symptoms		
	Mean CBCL score (SD)	*Mean SDQ score (SD)*
Internalising symptoms	4.56 (4.78)	1.67 (1.82)
Externalising symptoms	6.37 (6.41)	1.48 (1.46)
ADHD-related symptoms	3.01 (3.13)	3.31 (2.45)

NTR = Netherlands Twin Register, *ALSPAC* = Avon Longitudinal Study of Parents And Children, *N* = sample size, *SD* = standard deviation, *CBCL* = Child Behavior Checklist, *SDQ* = Strengths and Difficulties Questionnaire

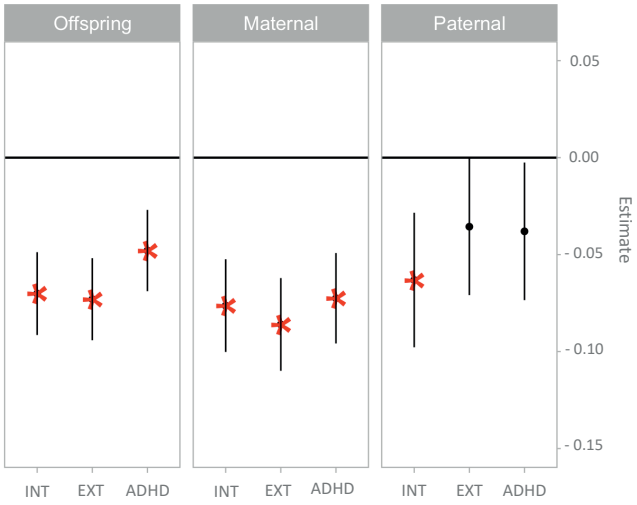
Shared genetic effects

Higher offspring and maternal wellbeing spectrum polygenic scores were strongly associated with fewer internalising, externalising, and ADHD-related symptoms. These associations between polygenic scores and outcomes show evidence of shared genetic effects between the wellbeing spectrum and these childhood psychiatric traits. Based on lower numbers of genotyped fathers, paternal polygenic scores for wellbeing were also associated with fewer childhood psychiatric symptoms, but associations with externalising and ADHD-related symptoms were not robust to significance testing (Figure 1, Table 2).

Genetic nurture

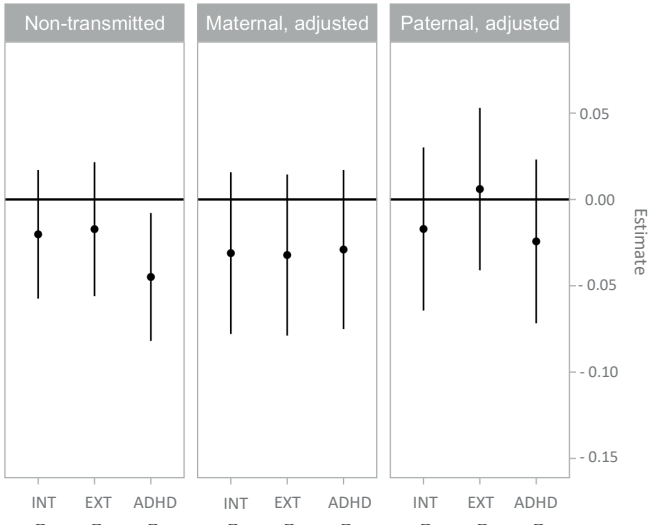
In the non-transmitted genotype method, polygenic scores based on non-transmitted genotype were not strongly associated with any of the childhood psychiatric traits, showing no clear evidence of genetic nurture of parental wellbeing on childhood internalising, externalising, and ADHD-related symptoms In the statistical control method, we observed a similar pattern of results when using paternal and paternal polygenic scores, after controlling for the polygenic scores of the offspring and other parent (Figure 2, Table 2).

Figure 1 Shared genetic effects between wellbeing and childhood psychiatric symptoms



INT: internalising/emotional symptoms, **EXT**: externalising/conduct problems, **ADHD**: ADHD-related symptoms
Red stars show estimates that were statistically significant after correction for multiple testing. Error bars represent 95% confidence intervals.
Associations based on offspring and parental polygenic scores denote shared genetic effects between wellbeing and childhood psychiatric symptoms.

Figure 2 Genetic nurture effects on childhood psychiatric symptoms



INT: internalising/emotional symptoms, **EXT**: externalising/conduct problems, **ADHD**: ADHD-related symptoms
Error bars represent 95% confidence intervals.
Associations based on non-transmitted genotype polygenic scores denote genetic nurture effects of parental wellbeing and childhood psychiatric symptoms (non-transmitted method). The adjusted maternal and paternal polygenic scores (statistical control method) capture genetic nurture effects arising from mothers or fathers.

Table 2 Meta-analytic results; associations between wellbeing spectrum polygenic scores and childhood psychiatric symptoms

Predictor (polygenic score)	Childhood psychiatric outcome	β	SE	p	R^2	N
Offspring	Internalising symptoms	-0.0701	0.0109	**1.22E-10**	0.4914	9227
	Externalising symptoms	-0.0730	0.0108	**1.62E-11**	0.5329	9293
	ADHD-related symptoms	-0.0479	0.0107	**8.26E-06**	0.2294	9308
Maternal	Internalising symptoms	-0.0763	0.0122	**3.50E-10**	0.5822	7334
	Externalising symptoms	-0.0860	0.0122	**1.59E-12**	0.7396	7362
	ADHD-related symptoms	-0.0725	0.0119	**1.04E-09**	0.5256	7370
Paternal	Internalising symptoms	-0.0631	0.0177	**4.00E-04**	0.3982	3639
	Externalising symptoms	-0.0356	0.0180	4.82E-02	0.1267	3664
	ADHD-related symptoms	-0.0380	0.0181	3.61E-02	0.1444	3668
Non-transmitted genotype	Internalising symptoms	-0.0202	0.0190	2.87E-01	0.0408	3020
	Externalising symptoms	-0.0172	0.0198	3.84E-01	0.0296	3038
	ADHD-related symptoms	-0.0449	0.0189	1.77E-02	0.2016	3043
Maternal: adjusted*	Internalising symptoms	-0.0311	0.0239	1.93E-01	0.0967	2988
	Externalising symptoms	-0.0322	0.0238	1.77E-01	0.1037	3007
	ADHD-related symptoms	-0.0290	0.0235	2.17E-01	0.0841	3012
Paternal: adjusted*	Internalising symptoms	-0.0171	0.0241	4.77E-01	0.0292	2988
	Externalising symptoms	0.0060	0.0240	8.03E-01	0.0036	3007
	ADHD-related symptoms	-0.0243	0.0242	3.15E-01	0.0590	3012

β = beta, SE = standard error, *p* = p-value, R^2 = percentage of variance explained, N = cumulative sample size. * = adjusted for the wellbeing spectrum polygenic score of the offspring and the other parent. Results in bold are statistically significant after correction for multiple testing.

DISCUSSION

This study used novel family-based genetic designs to study the relationship between parental wellbeing and offspring psychiatric symptoms. In two multi-generational European cohorts, results showed evidence of shared genetic effects between wellbeing and childhood psychiatric symptoms. In both cohorts and using two complementary methods, no strong evidence of genetic nurturing effects of wellbeing spectrum-associated genes on childhood psychiatric symptoms was found, although we noted lower power for these analyses based on the number of genotyped trios available. Our results indicate that associations between parental wellbeing and offspring psychiatric symptoms in childhood are at least partly explained by shared genetic effects.

Genetic overlap between wellbeing and adult psychiatric disorders has been reported before[250,251]. This study extends previous work by showing that wellbeing also shares genetic commonalities with childhood psychiatric symptoms, including internalising, externalising and ADHD-related symptoms. Most notably, our results show that these shared genetic effects partly account for associations between parental wellbeing and offspring psychiatric symptoms. This finding highlights an important confounder in existing intergenerational studies that do not account for the role of genes when investigating associations between parental wellbeing and offspring mental health[248,249]. When unaccounted for, genetic effects may lead to inflated or inaccurate conclusions about the direct environmental impact of parental characteristics on offspring behaviour. While the current study focused on wellbeing, various parental and parenting factors have been linked to offspring psychiatric outcomes in previous studies[41,261]. Our findings highlight that genetically informative methods are required to uncover whether associations between parental and offspring variables reflect genuine environmental effects or are confounded by genetics[241].

Although we showed that associations between parental wellbeing and childhood psychiatric symptoms can be partly explained by genetic relatedness, the findings of this study cannot be used to dismiss the possibility of causal relationships between parental wellbeing and childhood psychiatric symptoms. While we did not find robust evidence of genetic nurture effects, there was some indication that these estimates could be significantly different from zero in better-powered samples. For example, some estimates of parental genetic nurture were similar to estimates of genetic associations, but had distinctly wider confidence intervals that overlapped zero. This could be explained by sample size differences; due to fewer numbers of genotyped parents, sample sizes were lowest for analyses that required trio data and highest when only offspring genotypes were required. Recent simulations indicate that trio data from 10,000 families would provide greater than 70% power to detect paternal genetic nurture effects that explain 0.1% of variance in the child's phenotype[262]. Given these large sample size requirements, the limited availability of trio data is a substantial barrier to family-based genetic studies that seek to uncover genetic nurture effects on offspring phenotypes. Pooling trio data from multiple datasets will be of importance for investigating the presence of parental genetic nurture effects in future family-based genetic studies.

A genetic index for wellbeing was used to study environmentally-mediated effects in this study. This can be seen as a limitation, as parental wellbeing is not directly measured in this approach and is only indexed by common

genetic variants included in the GWAS of the wellbeing spectrum[75]. The validity of our findings therefore relies on the power of the GWAS, and how well it captures the trait under study. The four traits captured in the wellbeing spectrum GWAS showed a high mean genetic correlation (r_g = 0.7), and were measured using well-established scales[75]. With a large sample size and a high number of genome-wide significant SNPs, the GWAS was sufficiently powered for the current analyses. Still, more refined polygenic scores will become available with the inclusion of more samples and rare genetic variants, in which case these analyses can be repeated for increased accuracy. Even so, using a genetic index for wellbeing restricts our findings to genetically-influenced wellbeing effects and excludes the non-genetic component of parental wellbeing. To consider the overall effect of parental wellbeing, other genetically informative designs (such as adoption or the children-of-twins method)[24,104] can be employed that make use of measured parental traits. As these designs also have their own limitations, triangulating findings from multiple designs will be necessary for gaining confidence in findings[263].

Future studies can extend the work presented here by examining whether shared genetic effects also partly or wholly account for when the parent-offspring association is observed in the opposite direction, i.e., when offspring psychiatric symptoms are associated with lower parental wellbeing. Bi-directional associations between parents and offspring traits have been noted with regards to wellbeing[264]. Family-based genetic designs could be applied to interrogate child-to-parent associations, for instance by investigating whether polygenic scores for offspring traits predict parental wellbeing, after controlling for the polygenic scores of the parents.

This study shows that the relationship between parental wellbeing and offspring psychiatric symptoms is at least partly explained by shared genetic effects. No genetic nurturing effects of wellbeing associated genes on offspring psychiatric symptoms were detected using the sample sizes available, calling for more follow-up studies to investigate whether parental wellbeing and childhood psychiatric symptoms are related through the environment.

Chapter 7

Discussion

This thesis is formed of a collection of studies that applied molecular genetic statistical designs with the aim of understanding the genetic architecture of childhood psychiatric symptoms, and disentangling the mechanisms and impact of intergenerational contributions. This chapter summarises the findings of this thesis, discusses their clinical and research implications, and offers insight into possible directions for future research.

SUMMARY

The introduction to this thesis (**Chapter 1**) described how a key gap within psychiatric genetics is the absence of well-powered GWAS investigations of most childhood psychiatric symptoms. As twin literature points to sensitive periods of development for genetic influences on psychiatric symptoms, genetic studies in childhood samples are necessary for uncovering the involvement of genetic variants over the developmental course of psychiatric symptoms. In **Chapter 2**, we sought to address this gap in the literature by carrying out a GWAS of internalising symptoms across childhood and adolescence, designed to study both changes and stability in genetic effects over time. This large-scale collaborative study, with ~250,000 observations from 64,641 individuals in 22 European cohorts, represents a substantial sample size increase compared to previous GWASs of internalising symptoms in infanthood (N = 4,596)[58], and anxiety symptoms in childhood (N = 2,810)[57]. The meta-analysis of overall internalising symptoms detected no genome-wide significant hits and showed low overall SNP-based heritability. We inferred that the power in this study was diluted by substantial phenotypic heterogeneity, and heterogeneity was inflated by combining measurements from multiple raters. Age-stratified analyses showed that the contribution of additive genetic effects on internalising symptoms appeared stable over age, with overlapping estimates of SNP heritability from early-childhood to adolescence. Substantial genetic correlations with adult internalising disorders and other childhood-onset psychiatric traits were observed, suggesting that genetic effects could partially explain the persistence of internalising symptoms over time and the high comorbidity amongst childhood psychiatric traits.

The next four chapters of this thesis focused on intergenerational contributions to childhood psychiatric symptoms. Broader psychiatric research shows that various parental risk factors are associated with childhood psychiatric symptoms[20,21,41,42]. However, as parents typically provide offspring with both their genes and a rearing environment, associations between parent and offspring

traits could be due to both genetic overlap or environmental transmission. Disentangling the mechanisms underlying parent-offspring associations requires the use of genetically informative designs. In **Chapter 3**, we presented an overview of three types of designs (behavioural genetics, matched-pair and molecular genetics designs) that can be used to study environmental associations between parents and offspring, whilst modelling or accounting for genetic effects. We then systematically aggregated and summarised findings from genetically informative literature that investigated associations between parental characteristics and offspring mental health and related outcomes, published between 2014 and June 2020. Eighty-nine relevant studies were identified. Our synthesis of the results showed evidence of genetic transmission from parents to offspring for both similar and dissimilar psychiatric traits. There was also evidence of shared genetic effects between offspring psychiatric traits and parental traits outside the psychiatric domain, such as educational attainment and parenting. After accounting for genetic effects, parental traits (including psychiatric traits, parenting behaviours, and educational attainment) were reported to be associated with offspring psychiatric traits through environmental pathways. However, the size of these effects were mostly unclear, and the direction of effect (from parent-to-child or vice versa) was often not established.

This raises the question of how much parental factors contribute to individual differences in children's psychiatric outcomes. This was the focus of Chapters 4 and 5. These studies used a novel family-based genetic design to estimate the overall contribution of parental genetic nurture (the effect of parental phenotypes indexed by their genome) on common childhood psychiatric symptoms. **Chapter 4** focused on anxiety and depressive symptoms in 8-year-olds within the Norwegian Mother Father and Child Study (MoBa). Maternal-effects genome-wide complex trait analysis (M-GCTA) was used to estimate variance in childhood symptoms that was due to maternal or paternal genetic nurture. No strong evidence of genetic nurture was found, although suggestive maternal and paternal genetic nurture effects explained 4-6% of variance in childhood depressive symptoms. A similar maternal effect was also observed in a pedigree-based model. We concluded that while there was some indication of genetic nurture effects on childhood depressive symptoms, more power would be needed to obtain accurate estimates.

In **Chapter 5**, we utilised a larger sample of genotyped individuals in MoBa to estimate the contribution of parental genetic nurture on childhood depressive, externalising, and ADHD symptoms using M-GCTA. We did not include anxiety

symptoms, as no parental genetic nurture effects were indicated in Chapter 4. The results showed no strong evidence of genetic nurture, but a suggestive paternal genetic nurture effect on childhood depressive symptoms and a suggestive maternal genetic nurture effect on ADHD symptoms were observed, explaining 10% and 8% of variance respectively. The study reiterated the need for larger samples to be able to accurately estimate the contribution of genetic nurture to individual differences in childhood psychiatric symptoms. In the discussion, we speculated about possible mediating factors that may explain potential genetic nurture effects (if present) on childhood ADHD and depressive symptoms.

Chapter 6 focused on the association between parental wellbeing and offspring internalising, externalising and ADHD-related symptoms. We used genotype data from parents and children within two population-based European cohorts (ALSPAC; Avon Longitudinal Study of Parents and Children, and NTR; Netherlands Twin Register) to examine whether associations between parental wellbeing and offspring psychiatric symptoms were explained by shared genetic effects and/or genetic nurture. We observed evidence of shared genetic effects between parental wellbeing and childhood internalising, externalising and ADHD symptoms. No strong evidence of genetic nurture effects was found, although we observed lower power for these analyses based on the number of genotyped parent-offspring trios that were available. Our results show that findings from studies that do not account for the role of genes when investigating associations between parental wellbeing and offspring mental health could be confounded.

GENERAL DISCUSSION

This section discusses the findings of this thesis in the context of broader literature, describes clinical and research implications, and provides directions for future research. It is divided into two subsections, with the first focusing on the genetic architecture of childhood psychiatric symptoms (with a focus on internalising symptoms), and the latter on intergenerational contributions to childhood psychiatric symptoms.

Genetic architecture of childhood psychiatric symptoms

A developmental view

The influence of genetic factors can vary over the developmental course of psychiatric problems,[10,54] but little is known about the involvement of specific genetic variants at different developmental stages. To this end, we carried out age-stratified GWASs of childhood and adolescent internalising symptoms to understand when in development specific genetic variants exert an effect, which genetic variants have a stable effect over time, and which genetic variants show a limited effect at a specific developmental period (**Chapter 2**). While we did not identify the involvement of specific genetic variants at any developmental stage, we observed high genetic correlations between childhood and adolescent internalising symptoms and adult anxiety (r_g = 0.76) and depression (r_g = 0.70). This points to the presence of a set of genetic variants that exert stable effects on internalising behaviours across the lifespan. Our findings are supported by polygenic score studies showing genetic overlap between childhood internalising symptoms and adult depression,[18,83] and twin literature findings showing that stability in internalising symptoms over time is largely explained by stability in genetic factors[10,54]. Discovering which specific genetic variants (and how many) exert a stable effect on internalising symptoms over the life-course is an important next step for future work and calls for longitudinal GWASs that include measurements from all life stages. Following up on genetic variants with stable effects in subsequent functional analyses and drug target research could potentially lead to therapeutic discoveries that are relevant for both children and adults. Meanwhile, knowledge that internalising symptoms in childhood reflect substantial genetic risk for adult internalising disorders reiterates the importance of identifying at-risk individuals early in life, as early detection and treatment could break the chronicity of symptoms and prevent the developmental progression of symptoms to disorders.

An improved understanding of how the involvement of genetic variants is linked to the developmental trajectory of psychiatric symptoms over the life-course could help to pinpoint processes and mechanisms that contribute to stability, severity and changes in symptom profiles. For instance, one study looking at trajectories of depressive symptoms from childhood to young adulthood found that polygenic scores for adult depression were associated with both persistent and adult-onset internalising symptoms, but not with symptoms that were limited to either childhood or adolescence[265]. This could indicate that childhood- or adolescence-limited symptoms have a partly different genetic aetiology to stable or late-onset depressive symptoms. Such trajectories, capturing phenotypic stability and changes in internalising symptoms, could be used as target phenotypes in future developmentally oriented GWASs to identify the involvement of genetic variants for stable versus childhood- or adolescent-limited symptoms[266]. In the meantime, polygenic scores based on both adult and childhood GWASs could be leveraged to examine genetic contributions to developmental stability and changes in childhood psychiatric symptoms[267,268].

Uncovering the genetic (and environmental) aetiology of internalising behaviours

Many psychiatric symptoms share phenotypic and genetic overlap, but a special relationship amongst psychiatric traits is the close link between anxiety and depression, which are highly comorbid and have a partly overlapping symptomology that is especially characterised by negative affect[269,270]. Symptoms of anxiety and depression in very early life are difficult to distinguish from one another and are often grouped under the internalising domain. In older children and adults, it is possible to assess anxiety and depression separately, and as a result, research is often conducted within symptom or diagnostic boundaries. It is useful to consider how the grouping or separation of anxiety and depression phenotypes could impact knowledge on genetic and environmental influences on these internalising behaviours. A notable twin study found that while genetic influences on anxiety and depressive symptoms were largely overlapping, environmental influences were trait-specific and unshared[231]. Subsequent research and the results of this thesis seem to corroborate this finding. **Chapter 2** showed that childhood and adolescent internalising symptoms share substantial genetic correlations with both adult anxiety and depression, which themselves are highly genetically correlated[65,66,271] and have large overlap in the specific genetic variants and genomic regions that are implicated[272]. In **Chapter 4**, we observed that parental genetic nurture effects explained some variance in individual differences in depressive, but not anxiety symptoms, pointing to differences in the influence of environmental factors

for these two traits, which was replicated in another study[64]. The substantial genetic overlap between anxiety and depression can be leveraged to accelerate knowledge of the common genetic aetiology of internalising symptoms/disorders by combining measurements of anxiety and depression within GWASs through factor analysis[272,273]. Meanwhile, research on environmental influences *should* distinguish between anxiety and depression, as potentially important information about distinct environmental influences would be lost by using a higher-order phenotype, i.e., internalising symptoms. An obstacle to this approach is that commonly used measurement instruments in childhood cohorts do not necessarily have separate subscales for anxiety and depression[71,274]. This could be tackled by using item-level data to extract latent variables representing the distinct symptomologies of anxiety and depression from a broader scale[53].

Missing heritability of childhood psychiatric traits

While our overall understanding of the genetic architecture of childhood psychiatric traits is increasing, the variance in these behaviours that is explained by genome-wide SNPs is still small. Most estimates of SNP-based heritability of childhood psychiatric symptoms based on individual-level data are low and non-significant[18]. This includes our estimates of the heritability of depressive symptoms (**Chapter 4**) and externalising symptoms (**Chapter 5**) in 8-year-olds. Low and non-significant estimates reflect lack of power in individual studies, especially when analyses are focused on a single age[18]. One expectation of genetic research is that large samples lead to more power for the estimation of heritability and the detection of genome-wide significant loci. However, efforts to accumulate large sample sizes by pooling data from repeated assessments (at different ages and by different raters) did not lead to success in GWASs of childhood internalising symptoms (**Chapter 2**, SNP h^2 = 1.7%) and aggression (SNP h^2 = 3.3%)[76]. Instead, power in these studies was diluted by heterogeneity from multiple measurements, in which variability due to rater-based differences was particularly noticeable. This problem is specific to the investigation of childhood symptoms, which relies on information from informants in the absence of diagnostic data.

Creating more homogenous phenotypes for childhood psychiatric phenotypes, whilst continuing to accumulate sample sizes, will be important for increasing power in future GWAS studies. One way of doing this, that can utilise existing measurements, is to use factor analysis to extract a stable phenotype that captures the behaviour that multiple assessments have in common[82]. This approach was shown to eliminate variability due to age and rater differences, leading to increased estimates of both twin[98,101] and SNP-based[82] heritability of

childhood internalising symptoms. A potential downside of this approach is that a stable phenotype cannot be used to examine the influence of genetic variants on variable developmental trajectories of symptoms. For this, stratified analyses may still be necessary, but efforts can be made to address noise from rater-based variance.

The broader issue of missing heritability is a prevailing issue in psychiatric genetics research[233], which so far has focused largely on the effects of common genetic variation. This places a ceiling limit on SNP-based heritability estimates, as the effects of rare genetic variants (and untagged common SNPs) are not accounted for. This is likely to change in the future, with the increased availability and affordability of deep genotyping technologies. A recent study using whole genome sequence data recovered most of the heritability of height and BMI[275]. Future availability of whole-genome sequence data in datasets who also have information on childhood traits will help to bridge the twin/SNP heritability gap for childhood psychiatric symptoms and provide a more holistic view of their genetic architecture[275].

Addressing environmental sources of bias

A recently identified challenge for uncovering the genetic aetiology of psychiatric traits is the confounding effect of environmental factors within GWASs of complex traits. Innovative family-based genetic designs that have revealed the occurrence of genetic nurture also show that SNP estimates from population-based GWASs of unrelated individuals may also capture indirect genetic effects of close relatives,[33,106] in addition to potential population effects from assortative (non-random) mating and population stratification[276]. All factors other than the direct genetic effect can bias SNP estimates in GWASs, as well as calculations of heritability, genetic correlations, polygenic scores, and other downstream analyses[276]. A clever solution for accounting for non-genetic effects is the inclusion of sibling genotypes in GWASs[106,277]. Siblings are naturally matched on shared environmental influences, and potential bias due to assortative mating and population stratification is eliminated in within-family analysis[106,277]. To facilitate large-scale investigation of direct genetic effects for complex traits that could be sensitive to environmental sources of bias, a new collaborative initiative called the Within Family Consortium is focusing on collecting sibling genotypes. A preprint of their first study is now online, which includes within-sibship GWASs of 25 phenotypes, including depressives symptoms[276]. The authors report that within-sibship GWAS estimates differ from usual GWAS estimates, which seem to overestimate direct genetic effects on depressive symptoms[276]. It is useful to consider the relevance of these findings

for the genetic investigation of childhood internalising symptoms. While there is little evidence of assortative mating for anxiety and depression[278], there was some indication of residual population stratification in our GWAS (inflated LDSC intercept, **Chapter 2**). Additionally, family-based genetic studies (e.g. **Chapter 4 and 5**) point to indirect genetic effects on childhood depressive symptoms[63,64]. On balance, it is plausible that SNP effects in population-based genetic studies of internalising symptoms in childhood could be inflated. This extends to other psychiatric traits in childhood, where indirect genetic effects are, or could be, implicated. The work in this thesis is placed at the cusp of a paradigm shift, where for some psychiatric traits, the inclusion of family data in GWAS studies will become necessary for uncovering genetic architecture without bias from non-genetic sources. These non-genetic effects are of interest in their own right, and offer novel insight into the entangled relationship between genes and environment.

Intergenerational contributions to childhood psychiatric symptoms

Shared genetic effects between parent and offspring phenotypes

Just as genetics research needs to take environmental sources of bias into account, studies on intergenerational risk factors need to account for genetic relatedness. Our review of genetically informative literature provided evidence that many associations between various parental traits (including their own psychiatric behaviours, educational attainment, and positive and negating parenting behaviours) and offspring psychiatric traits are partly explained by shared genetic effects between parents and offspring (**Chapter 3**). This was also observed in our own research, where we found evidence of shared genetic effects between parental wellbeing and childhood internalising, externalising and ADHD-related symptoms in childhood (**Chapter 6**). That genes underlie associations between both similar and dissimilar parent-offspring psychiatric traits, and traits within and outside of the psychiatric domain shows the pervasiveness of genetic pleiotropy (as also indicated by the genetic correlations in **Chapter 2)**. This has serious implications for the validity of results from observational studies reporting associations between parental risk factors and childhood psychiatric symptoms[21,41–43]. Findings from such studies should be followed up using genetically informative designs to identify whether they represent a genuine environmental association or are confounded by genetic relatedness.

A challenge in this is that shared genetic effects between parent and offspring traits can be hard to detect, even when prior knowledge indicates that they are relevant. For instance, no evidence of genetic overlap between parental anxiety and childhood internalising symptoms was found within the adoption and children-of-twins studies reviewed in **Chapter 3**, even though internalising symptoms are partly characterised by anxiety and both are known to be under the influence of genes[6,279]. Factors that influence the ability to detect shared parent-offspring genetic effects include the overall power of the study, heritability of the traits, the validity of the measurements and how well they capture inherited risk, and the extent to which genetic overlap is an important factor in explaining the particular parent-offspring association under investigation. For genetic studies, such as the within-family polygenic score study in **Chapter 6**, this also includes the power of the GWAS indexing the parental trait, and the size and population of the target sample in which the effect is being studied. Consideration of these factors is important when designing and interpreting findings of genetic contributions to an intergenerational effect. Power analyses can help to gauge the validity of a null result, and comparing findings from multiple designs (i.e. triangulation) will be necessary for drawing conclusions[263].

Environmental effects

Triangulation is also necessary for assessing whether an environmental parent-offspring association is present and if it is parentally-driven, in which case it may be a relevant target for intervention to improve psychiatric outcomes in children. The convergence of findings across multiple genetically informative designs, which otherwise each have their own set of assumptions and limitations, can strengthen the evidence base for an observed finding. An example of this is the association between maternal depression and childhood internalising symptoms, which has been widely reported in observational research[20,280]. Non-genetic genetically informative studies reviewed in **Chapter 3** showed that after accounting for genetic relatedness, prenatal maternal depression had no lasting influence on childhood internalising symptoms, whereas concurrent associations in early childhood were partly attributed to the environment[109,110,112]. However, it was unclear whether these concurrent associations reflected the influence of maternal depression on offspring internalising symptoms, or vice versa, based on the study designs. The most recent evidence from a family-based genetic design identified a parent-driven effect, whereby genetic nurture effects on childhood depressive symptoms during mid-childhood were partly mediated by maternal anxiety and depression[64].

While it is not possible to establish causality due to the nature of these study designs, such findings raise interesting lines of enquiry for both future research and the hypothetical application of parent-based clinical interventions. For instance, could treatment of maternal depression prevent or improve children's internalising symptoms and would this have a lasting effect? More longitudinal genetically informative research is first needed to assess the effect size and long-term impact of specific parental exposures. If a parental influence is only relevant for concurrent scenarios, then perhaps intervention strategies for children should focus on other factors. Additionally, to what extent is parent-offspring similarity in internalising behaviours due to a learning mechanism, or does maternal depression influence offspring internalising symptoms through mediating factors, such as caregiving behaviours[281]? Investigation of intermediate pathways in genetically informative studies could help to refine targets of intervention, for instance by informing whether implementation of parenting interventions in mothers with depression would be useful. Finally, and most importantly, to what extent could the effect of maternal depression aggregate or interact with other maternal or familial risk factors (such as parenting, lack of social support, poverty)[206] in influencing offspring internalising symptoms? It is likely that individual risk factors have a small effect, but understanding the cumulative effects of familial factors, through genetically informative designs, could lead to the development of intervention strategies and healthcare policies that have the most impact.

The example of depression/internalising symptoms is illustrative, and these are relevant questions for assessing the impact of intergenerational contributions to any childhood psychiatric trait.

Application of novel family-based genetic designs

It is useful to consider how family-based genetic designs can strengthen and expand knowledge from other genetically informative designs. Key advantages of family-based genetic designs are that child and parent-driven effects can be distinguished from one other, there is no need of measuring specific parental phenotypes to estimate genetic nurture, and it is possible to estimate the overall contribution of parental factors to variance in children's psychiatric symptoms. Notably, variance explained by genetic nurture may represent a lower bound estimate of the overall contribution of parental factors, as the effects of parental traits that are not under genetic influence (and not tagged by genotyped SNPs) are not captured in this approach. In the same way that SNP-based heritability captures a proportion of twin heritability, genetic nurture estimates will likely capture a proportion of overall parental effects.

Chapters 4 and 5 are the first studies to apply M-GCTA models to psychiatric phenotypes, as the method has previously only been used to study perinatal childhood outcomes such as birth length and weight[35,234]. Our early attempts to uncover genetic nurture effects for childhood psychiatric symptoms provide lessons that could guide future family-based genetic studies of psychiatric traits. The suggestive estimates of genetic nurture give some indication of effect sizes and can be utilised in power calculations to assess the sample sizes that will be needed to obtain robust estimates[236]. Additionally, future studies should account for the genotypes of both parents in the same model to lower the risk of biased estimates. A recent paper highlighted the possibility of loss of power and potential bias from unmodelled effects in within-family polygenic score studies that only include the genotype of one parent[262]. In principle, the same limitation applies to M-GCTA, and the exclusion of one parent's genotype from the model could affect the estimates of direct offspring effects and indirect genetic effects of the other parent. Trio-GCTA and RDR (relatedness disequilibrium regression) are alternative extended GREML approaches that include both parents in the same model[34,38].

Family-based genetic designs hold great potential for answering many questions regarding intergenerational transmission. However, power is a substantial barrier to the wide-spread implementation of family-based genetic designs. The estimation of multiple parameters in extended GREML designs (**Chapters 4 and 5**) and the application of within-family polygenic score designs (**Chapter 6**) require huge numbers of genotyped families with data on both parents and offspring[236,262]. As large-scale genotyping efforts thus far have prioritised the collection of unrelated samples, the availability of big datasets with genotyped families is limited. New genotyping of parents and children on a large scale is an important goal, but it may take many years before such datasets become available. In the meantime, novel methods can help to maximise the potential of existing data. Two new software packages called IMPISH[245] and SNIPar[282] impute virtual parental genotypes based on the genotypes of offspring pairs (i.e. siblings). This allows the estimation of indirect parental genetic effects without the requirement of physically genotyped parents. Other promising methods focus on taking advantage of pre-existing summary-level data (i.e. GWAS summary statistics) to disentangle direct and indirect genetic effects[283,284]. Finally, as genetic nurture estimates can potentially include population effects or the effect of assortative mating, two recent papers outline a structural equation modelling framework for how these factors can be dealt with to obtain unbiased estimates[285,286].

Family-based approach to prevention and early intervention

Strong evidence of intergenerational transmission of psychiatric traits[287–291], be it due to genetic or environmental mechanisms, places emphasis on utilising the family setting to reduce the potential burden of childhood and long-term psychiatric symptoms. There are several ways in which families could be a strategic target for prevention, early detection, and treatment approaches. First, parents, and other primary caregivers, are in the position to assess offspring traits throughout development, but one of the barriers to children's access to treatment is poor parental knowledge and understanding of mental health problems[292]. An increased awareness of common childhood psychiatric symptoms in caregivers (including knowledge of the presentation of early symptoms, their persistence over time, and their aggregation in families) could help families to recognise early symptoms and seek early diagnosis and treatment. Second, children of parents with psychiatric disorders represent a vulnerable and at-risk population that can be specifically targeted for early screening and prevention efforts. In recognition of this, some countries (Finland, Sweden, and Norway) have made changes to legislation so that healthcare providers are obligated to report whether their adult patients have children, and assess whether the child needs support[293,294]. Third, if offspring psychiatric symptoms are explained by exposure to specific parental factors, these parental exposures could be targeted for intervention. Current parent-based prevention and treatment approaches show variable efficacy[295–299], and could be improved by incorporating knowledge from genetically-informative research (see section on environmental effects). Finally, if intergenerational associations are explained mostly by genetic transmission, family-based interventions could potentially be applied to address the psychiatric needs of both parents and children.

Conclusion

Progress and innovations in the rapidly-evolving field of psychiatric genetics bring the potential to enrich our understanding of not only the genetic architecture of childhood psychiatric symptoms, but also the mechanisms and impact of intergenerational contributions. Continued investigation of the genetic aetiology of childhood psychiatric symptoms is of importance, and could lead to novel therapeutic discoveries or clinical implications that reduce the burden of psychiatric disorders. New research should strive to include participants of non-European ancestries so that findings can benefit wider populations. The occurrence of genetic nurture means that efforts to uncover the genetic etiology of childhood psychiatric traits need to consider and account for bias from non-genetic sources. The use of family-based methods such as within-sibship GWAS are relevant for this, and could be combined with deep genotyping

technologies, such as whole genome sequencing, to accelerate genetic discoveries in the future. It will be important that improvements in genetic technologies and methodologies are matched with improvements in our use of phenotypic measurements. Careful ways of modelling phenotypic data will enable researchers to maximise genetic signal, gain specificity in phenotypes, and reduce noise from external sources, such as rater variance. Beyond the role of genes, more genetically informative research is needed to understand the contribution of non-genetic factors to the aggregation of psychiatric traits in families. The moderate to high twin heritability estimates of childhood psychiatric symptoms signal that the primary way in which parents may contribute to children's psychiatric symptoms is through the passing on of their genes. Still, it is feasible that individual parental risk factors have small effects on children's psychiatric outcomes. More research into cumulative effects will improve our understanding of how intergenerational genetic and environmental factors accumulate, correlate, and interact with each other to influence children's psychiatric symptoms. A developmental view is of importance here, as the contribution of both genetic and environmental influences may vary over time.

REFERENCES

1. Costello, E. J., Mustillo, S., Erkanli, A., Keeler, G. & Angold, A. Prevalence and development of psychiatric disorders in childhood and adolescence. *Arch. Gen. Psychiatry* 60, 837–844 (2003).
2. Copeland, W. E., Shanahan, L., Costello, E. J. & Angold, A. Childhood and adolescent psychiatric disorders as predictors of young adult disorders. Arch. Gen. Psychiatry 66, 764–772 (2009).
3. Whiteford, H. A. et al. Global burden of disease attributable to mental and substance use disorders: findings from the Global Burden of Disease Study 2010. Lancet 382, 1575–1586 (2013).
4. Sansone, R. A. & Sansone, L. A. Psychiatric disorders: a global look at facts and figures. Psychiatry (Edgmont) 7, 16–19 (2010).
5. Neale, M. C. & Cardon, L. R. Methodology for genetic studies of twins and families. (1992).
6. Polderman, T. J. C. et al. Meta-analysis of the heritability of human traits based on fifty years of twin studies. Nat. Genet. 47, 702–709 (2015).
7. Lamb, D. J. et al. Heritability of anxious-depressive and withdrawn behavior: age-related changes during adolescence. J. Am. Acad. Child Adolesc. Psychiatry 49, 248–255 (2010).
8. Wesseldijk, L. W. et al. Genetic and environmental influences on conduct and antisocial personality problems in childhood, adolescence, and adulthood. Eur. Child Adolesc. Psychiatry 27, 1123–1132 (2018).
9. Bergen, S. E., Gardner, C. O. & Kendler, K. S. Age-related changes in heritability of behavioral phenotypes over adolescence and young adulthood: a meta-analysis. Twin Research & Human Genetics: the Official Journal of the International Society for Twin Studies 10, 423–33 (2007).
10. Hannigan, L. J., Walaker, N., Waszczuk, M. A., McAdams, T. A. & Eley, T. C. Aetiological influences on stability and change in emotional and behavioural problems across development: a systematic review. Psychopathol. Rev. 4, 52–108 (2017).
11. Nivard, M. G. et al. Genetic overlap between schizophrenia and developmental psychopathology: longitudinal and multivariate polygenic risk prediction of common psychiatric traits during development. Schizophr. Bull. 43, 1197–1207 (2017).
12. Nikolas, M. A. & Burt, S. A. Genetic and environmental influences on ADHD symptom dimensions of inattention and hyperactivity: a meta-analysis. J. Abnorm. Psychol. 119, 1–17 (2010).
13. Uffelmann, E. et al. Genome-wide association studies. Nat. Rev. Methods Primers 1, 59 (2021).
14. Horwitz, T., Lam, K., Chen, Y., Xia, Y. & Liu, C. A decade in psychiatric GWAS research. Mol. Psychiatry 24, 378–389 (2019).
15. Visscher, P. M. et al. 10 years of GWAS discovery: biology, function, and translation. Am. J. Hum. Genet. 101, 5–22 (2017).
16. Baselmans, B. M. L., Yengo, L., van Rheenen, W. & Wray, N. R. Risk in Relatives, Heritability, SNP-Based Heritability, and Genetic Correlations in Psychiatric Disorders: A Review. Biol. Psychiatry 89, 11–19 (2021).
17. Smoller, J. W. et al. Psychiatric genetics and the structure of psychopathology. Mol. Psychiatry 24, 409–420 (2019).
18. Akingbuwa, W. A., Hammerschlag, A. R., Bartels, M. & Middeldorp, C. M. Systematic review: molecular studies of common genetic variation in child and adolescent psychiatric disorders. *J. Am. Acad. Child Adolesc. Psychiatry* (2021). doi:10.1016/j.jaac.2021.03.020
19. Plomin, R., DeFries, J. C. & Loehlin, J. C. Genotype-environment interaction and correlation in the analysis of human behavior. Psychol. Bull. 84, 309–322 (1977).
20. McLaughlin, K. A. et al. Parent psychopathology and offspring mental disorders: results from the WHO World Mental Health Surveys. Br. J. Psychiatry 200, 290–299 (2012).
21. Yap, M. B. H. & Jorm, A. F. Parental factors associated with childhood anxiety, depression, and internalizing problems: a systematic review and meta-analysis. J. Affect. Disord. 175, 424–440 (2015).

22. Plomin, R., DeFries, J. C., Knopik, V. S. & Neiderhiser, J. M. Top 10 replicated findings from behavioral genetics. Perspect. Psychol. Sci. 11, 3–23 (2016).
23. McAdams, T. A. et al. Revisiting the Children-of-Twins Design: Improving Existing Models for the Exploration of Intergenerational Associations. Behav Genet 48, 397–412 (2018).
24. Cadoret, R. J. Adoption studies. Alcohol Health and Research World 19, 195 (1995).
25. Kendler, K. S., Ohlsson, H., Sundquist, J. & Sundquist, K. Parent-offspring transmission of drug abuse and alcohol use disorder: Application of the multiple parenting relationships design. Am. J. Med. Genet. B, Neuropsychiatr. Genet. 180, 249–257 (2019).
26. Thapar, A. et al. Prenatal smoking might not cause attention-deficit/hyperactivity disorder: evidence from a novel design. Biol. Psychiatry 66, 722–727 (2009).
27. Kendler, K. S., Ohlsson, H., Sundquist, J. & Sundquist, K. Triparental families: a new genetic-epidemiological design applied to drug abuse, alcohol use disorders, and criminal behavior in a Swedish national sample. Am. J. Psychiatry 172, 553–560 (2015).
28. Lahey, B. B. & D'Onofrio, B. M. All in the Family: Comparing Siblings to Test Causal Hypotheses Regarding Environmental Influences on Behavior. Curr. Dir. Psychol. Sci. 19, 319–323 (2010).
29. Keller, M. C. et al. Modeling extended twin family data I: description of the Cascade model. Twin Res Hum Genet 12, 8–18 (2009).
30. Kendler, K. S., Ohlsson, H., Sundquist, J. & Sundquist, K. A Contagion Model for Within-Family Transmission of Drug Abuse. Am. J. Psychiatry 176, 239–248 (2019).
31. O'Connor, T. G., Deater-Deckard, K., Fulker, D., Rutter, M. & Plomin, R. Genotype-environment correlations in late childhood and early adolescence: antisocial behavioral problems and coercive parenting. Dev. Psychol. 34, 970–981 (1998).
32. Narusyte, J. et al. Testing different types of genotype-environment correlation: an extended children-of-twins model. Dev. Psychol. 44, 1591–1603 (2008).
33. Kong, A. et al. The nature of nurture: Effects of parental genotypes. Science 359, 424–428 (2018).
34. Young, A. I. et al. Relatedness disequilibrium regression estimates heritability without environmental bias. Nat. Genet. 50, 1304–1310 (2018).
35. Eaves, L. J., Pourcain, B. S., Smith, G. D., York, T. P. & Evans, D. M. Resolving the effects of maternal and offspring genotype on dyadic outcomes in genome wide complex trait analysis ("M-GCTA"). Behav Genet 44, 445–455 (2014).
36. Bates, T. C. et al. The Nature of Nurture: Using a Virtual-Parent Design to Test Parenting Effects on Children's Educational Attainment in Genotyped Families. Twin Res Hum Genet 21, 73–83 (2018).
37. Plomin, R. & Daniels, D. Why are children in the same family so different from one another? Int. J. Epidemiol. 40, 563–582 (2011).
38. Eilertsen, E. M. et al. Direct and Indirect Effects of Maternal, Paternal, and Offspring Genotypes: Trio-GCTA. Behav Genet 51, 154–161 (2021).
39. Wray, N. R., Goddard, M. E. & Visscher, P. M. Prediction of individual genetic risk to disease from genome-wide association studies. Genome Res. 17, 1520–1528 (2007).
40. Wray, N. R. et al. Research review: Polygenic methods and their application to psychiatric traits. J. Child Psychol. Psychiatry 55, 1068–1087 (2014).
41. Yap, M. B. H., Pilkington, P. D., Ryan, S. M. & Jorm, A. F. Parental factors associated with depression and anxiety in young people: a systematic review and meta-analysis. J. Affect. Disord. 156, 8–23 (2014).
42. Fendrich, M., Warner, V. & Weissman, M. M. Family risk factors, parental depression, and psychopathology in offspring. Dev. Psychol. 26, 40–50 (1990).
43. Peverill, M. et al. Socioeconomic status and child psychopathology in the United States: A meta-analysis of population-based studies. Clin. Psychol. Rev. 83, 101933 (2021).
44. Wang, B. et al. Robust genetic nurture effects on education: A systematic review and meta-analysis based on 38,654 families across 8 cohorts. Am. J. Hum. Genet. 108, 1780–1791 (2021).
45. Erskine, H. E. et al. A heavy burden on young minds: the global burden of mental and substance use disorders in children and youth. Psychol. Med. 45, 1551–1563 (2015).
46. World Health Organization. *Depression and other common mental disorders: global health estimates*. (World Health Organization, 2017).

47. Steel, Z. et al. The global prevalence of common mental disorders: a systematic review and meta-analysis 1980-2013. Int. J. Epidemiol. 43, 476–493 (2014).
48. Polanczyk, G. V., Salum, G. A., Sugaya, L. S., Caye, A. & Rohde, L. A. Annual research review: A meta-analysis of the worldwide prevalence of mental disorders in children and adolescents. J. Child Psychol. Psychiatry 56, 345–365 (2015).
49. Husky, M. M. *et al.* Self-reported mental health in children ages 6–12 years across eight European countries. *European child & adolescent psychiatry* 1–11 (2017).
50. Weissman, M. M. et al. Children with prepubertal-onset major depressive disorder and anxiety grown up. Arch. Gen. Psychiatry 56, 794–801 (1999).
51. Roza, S. J., Hofstra, M. B., van der Ende, J. & Verhulst, F. C. Stable prediction of mood and anxiety disorders based on behavioral and emotional problems in childhood: a 14-year follow-up during childhood, adolescence, and young adulthood. Am. J. Psychiatry 160, 2116–2121 (2003).
52. Gregory, A. M. & Eley, T. C. Genetic influences on anxiety in children: what we've learned and where we're heading. Clin. Child Fam. Psychol. Rev. 10, 199–212 (2007).
53. Franić, S., Dolan, C. V., Borsboom, D., van Beijsterveldt, C. E. M. & Boomsma, D. I. Three-and-a-half-factor model? The genetic and environmental structure of the CBCL/6-18 internalizing grouping. Behav Genet 44, 254–268 (2014).
54. Nivard, M. G. et al. Stability in symptoms of anxiety and depression as a function of genotype and environment: a longitudinal twin study from ages 3 to 63 years. Psychol. Med. 45, 1039–1049 (2015).
55. Franić, S., Middeldorp, C. M., Dolan, C. V., Ligthart, L. & Boomsma, D. I. Childhood and adolescent anxiety and depression: beyond heritability. J. Am. Acad. Child Adolesc. Psychiatry 49, 820–829 (2010).
56. Kendler, K. S., Gardner, C. O. & Lichtenstein, P. A developmental twin study of symptoms of anxiety and depression: evidence for genetic innovation and attenuation. Psychol. Med. 38, 1567–1575 (2008).
57. Trzaskowski, M. et al. First genome-wide association study on anxiety-related behaviours in childhood. PLoS One 8, e58676 (2013).
58. Benke, K. S. et al. A genome-wide association meta-analysis of preschool internalizing problems. J. Am. Acad. Child Adolesc. Psychiatry 53, 667–676.e7 (2014).
59. Trzaskowski, M., Dale, P. S. & Plomin, R. No genetic influence for childhood behavior problems from DNA analysis. J. Am. Acad. Child Adolesc. Psychiatry 52, 1048–1056.e3 (2013).
60. Pappa, I. et al. Single Nucleotide Polymorphism Heritability of Behavior Problems in Childhood: Genome-Wide Complex Trait Analysis. J. Am. Acad. Child Adolesc. Psychiatry 54, 737–744 (2015).
61. Sallis, H. et al. Genetics of depressive symptoms in adolescence. BMC Psychiatry 17, 321 (2017).
62. Cheesman, R. et al. Childhood behaviour problems show the greatest gap between DNA-based and twin heritability. Transl. Psychiatry 7, 1284 (2017).
63. Jami, E. S. et al. Maternal and paternal effects on offspring internalizing problems: Results from genetic and family-based analyses. Am. J. Med. Genet. B, Neuropsychiatr. Genet. 183, 258–267 (2020).
64. Cheesman, R. et al. How important are parents in the development of child anxiety and depression? A genomic analysis of parent-offspring trios in the Norwegian Mother Father and Child Cohort Study (MoBa). BMC Med. 18, 284 (2020).
65. Howard, D. M. et al. Genome-wide meta-analysis of depression identifies 102 independent variants and highlights the importance of the prefrontal brain regions. Nat. Neurosci. 22, 343–352 (2019).
66. Purves, K. L. et al. A major role for common genetic variation in anxiety disorders. Mol. Psychiatry 25, 3292–3303 (2020).
67. Wray, N. R. et al. Genome-wide association analyses identify 44 risk variants and refine the genetic architecture of major depression. Nat. Genet. 50, 668–681 (2018).
68. Middeldorp, C. M. et al. The Early Growth Genetics (EGG) and EArly Genetics and Lifecourse Epidemiology (EAGLE) consortia: design, results and future prospects. Eur J Epidemiol 34, 279–300 (2019).

69. van der Sluis, S., Posthuma, D., Nivard, M. G., Verhage, M. & Dolan, C. V. Power in GWAS: lifting the curse of the clinical cut-off. Mol. Psychiatry 18, 2–3 (2013).
70. Goodman, R. The strengths and difficulties questionnaire: A research note. J Child Psychol & Psychiat 38, 581–586 (1997).
71. Achenbach, T. M. *Achenbach system of empirically based assessment (ASEBA) Development, findings, theory, and applications*. (University of Vermont, Research Center of Children, Youth & Families, 2009).
72. Rutter, M. A children's behaviour questionnaire for completion by teachers: preliminary findings. Journal of child Psychology and Psychiatry 8, 1–11 (1967).
73. Rutter, M., Tizard, J. & Whitmore, K. Education, Health and Behaviour. (1970).
74. Winkler, T. W. et al. Quality control and conduct of genome-wide association meta-analyses. Nat. Protoc. 9, 1192–1212 (2014).
75. Baselmans, B. M. L. et al. Multivariate genome-wide analyses of the well-being spectrum. Nat. Genet. 51, 445–451 (2019).
76. Ip, H. F. et al. Genetic association study of childhood aggression across raters, instruments, and age. Transl. Psychiatry 11, 413 (2021).
77. Bulik-Sullivan, B. et al. LD Score regression distinguishes confounding from polygenicity in genome-wide association studies. Nat. Genet. 47, 291–295 (2015).
78. Bulik-Sullivan, B. et al. An atlas of genetic correlations across human diseases and traits. Nat. Genet. 47, 1236–1241 (2015).
79. de Leeuw, C. A., Mooij, J. M., Heskes, T. & Posthuma, D. MAGMA: generalized gene-set analysis of GWAS data. PLoS Comput. Biol. 11, e1004219 (2015).
80. Watanabe, K., Taskesen, E., van Bochoven, A. & Posthuma, D. Functional mapping and annotation of genetic associations with FUMA. Nat. Commun. 8, 1826 (2017).
81. Vilhjálmsson, B. J. et al. Modeling linkage disequilibrium increases accuracy of polygenic risk scores. Am. J. Hum. Genet. 97, 576–592 (2015).
82. Cheesman, R. et al. Extracting stability increases the SNP heritability of emotional problems in young people. Transl. Psychiatry 8, 223 (2018).
83. Akingbuwa, W. A. et al. Genetic Associations Between Childhood Psychopathology and Adult Depression and Associated Traits in 42 998 Individuals: A Meta-analysis. JAMA Psychiatry 77, 715–728 (2020).
84. Musci, R. J. et al. The effects of the interplay of genetics and early environmental risk on the course of internalizing symptoms from late childhood through adolescence. Dev. Psychopathol. 28, 225–237 (2016).
85. Riglin, L. et al. Investigating the genetic underpinnings of early-life irritability. Transl. Psychiatry 7, e1241 (2017).
86. Jansen, P. R. et al. Polygenic scores for schizophrenia and educational attainment are associated with behavioural problems in early childhood in the general population. J. Child Psychol. Psychiatry 59, 39–47 (2018).
87. Cross-Disorder Group of the Psychiatric Genomics Consortium. Genomic Relationships, Novel Loci, and Pleiotropic Mechanisms across Eight Psychiatric Disorders. Cell 179, 1469–1482. e11 (2019).
88. Neumann, A. *et al.* A genome-wide association study of total child psychiatric problems scores. *medRxiv* (2020). doi:10.1101/2020.06.04.20121061
89. Rhee, S. H., Lahey, B. B. & Waldman, I. D. Comorbidity Among Dimensions of Childhood Psychopathology: Converging Evidence from Behavior Genetics. Child Dev Perspect 9, 26–31 (2015).
90. Allegrini, A. G. et al. The p factor: genetic analyses support a general dimension of psychopathology in childhood and adolescence. J. Child Psychol. Psychiatry 61, 30–39 (2020).
91. Bartels, M. et al. Childhood aggression and the co-occurrence of behavioural and emotional problems: results across ages 3-16 years from multiple raters in six cohorts in the EU-ACTION project. Eur. Child Adolesc. Psychiatry 27, 1105–1121 (2018).
92. Levinson, D. F. et al. Genetic studies of major depressive disorder: why are there no genome-wide association study findings and what can we do about it? Biol. Psychiatry 76, 510–512 (2014).

93. Cai, N., Choi, K. W. & Fried, E. I. Reviewing the genetics of heterogeneity in depression: operationalizations, manifestations and etiologies. Hum. Mol. Genet. 29, R10–R18 (2020).
94. Otowa, T. et al. Meta-analysis of genome-wide association studies of anxiety disorders. Mol. Psychiatry 21, 1485 (2016).
95. de Vlaming, R. et al. Meta-GWAS Accuracy and Power (MetaGAP) Calculator Shows that Hiding Heritability Is Partially Due to Imperfect Genetic Correlations across Studies. PLoS Genet. 13, e1006495 (2017).
96. Ask, H., Torgersen, S., Seglem, K. B. & Waaktaar, T. Genetic and environmental causes of variation in adolescent anxiety symptoms: a multiple-rater twin study. J. Anxiety Disord. 28, 363–371 (2014).
97. Fedko, I. O. et al. Estimation of genetic relationships between individuals across cohorts and platforms: application to childhood height. Behav Genet 45, 514–528 (2015).
98. Wesseldijk, L. W. et al. Psychopathology in 7-year-old children: Differences in maternal and paternal ratings and the genetic epidemiology. Am. J. Med. Genet. B, Neuropsychiatr. Genet. 174, 251–260 (2017).
99. Bartels, M., Boomsma, D. I., Hudziak, J. J., van Beijsterveldt, T. C. E. M. & van den Oord, E. J. C. G. Twins and the study of rater (dis)agreement. Psychol. Methods 12, 451–466 (2007).
100. Lubke, G. H. et al. A powerful phenotype for gene-finding studies derived from trajectory analyses of symptoms of anxiety and depression between age seven and 18. Am. J. Med. Genet. B, Neuropsychiatr. Genet. 171, 948–957 (2016).
101. Fedko, I. O. et al. Heritability of Behavioral Problems in 7-Year Olds Based on Shared and Unique Aspects of Parental Views. Behav Genet 47, 152–163 (2017).
102. Martin, J. et al. Examining Sex-Differentiated Genetic Effects Across Neuropsychiatric and Behavioral Traits. Biol. Psychiatry 89, 1127–1137 (2021).
103. Martin, N. G. & Eaves, L. J. The genetical analysis of covariance structure. Heredity 38, 79–95 (1977).
104. McAdams, T. A. et al. Accounting for genetic and environmental confounds in associations between parent and child characteristics: a systematic review of children-of-twins studies. Psychol. Bull. 140, 1138–1173 (2014).
105. Pingault, J.-B. *et al.* Estimating the sensitivity of associations between risk factors and outcomes to shared genetic effects. *BioRxiv* (2019). doi:10.1101/592352
106. Young, A. I., Benonisdottir, S., Przeworski, M. & Kong, A. Deconstructing the sources of genotype-phenotype associations in humans. Science 365, 1396–1400 (2019).
107. McAdams, T. A. et al. The relationship between parental depressive symptoms and offspring psychopathology: evidence from a children-of-twins study and an adoption study. Psychol. Med. 45, 2583–2594 (2015).
108. Grabow, A. P. et al. Using an adoption-biological family design to examine associations between maternal trauma, maternal depressive symptoms, and child internalizing and externalizing behaviors. Dev. Psychopathol. 29, 1707–1720 (2017).
109. Hannigan, L. J. et al. Maternal prenatal depressive symptoms and risk for early-life psychopathology in offspring: genetic analyses in the Norwegian Mother and Child Birth Cohort Study. Lancet Psychiatry 5, 808–815 (2018).
110. Gjerde, L. C. *et al.* Associations between maternal depressive symptoms and risk for offspring early-life psychopathology: the role of genetic and non-genetic mechanisms. *Psychol. Med.* 1–9 (2019). doi:10.1017/S0033291719003301
111. Kendler, K. S., Ohlsson, H., Sundquist, K. & Sundquist, J. Sources of Parent-Offspring Resemblance for Major Depression in a National Swedish Extended Adoption Study. JAMA Psychiatry 75, 194–200 (2018).
112. Gjerde, L. C. et al. Maternal perinatal and concurrent depressive symptoms and child behavior problems: a sibling comparison study. J. Child Psychol. Psychiatry 58, 779–786 (2017).
113. Hails, K. A. et al. Interaction between adoptive mothers' and fathers' depressive symptoms in risk for children's emerging problem behavior. Social Development 28, 725–742 (2019).
114. Liskola, K., Raaska, H., Lapinleimu, H. & Elovainio, M. Parental depressive symptoms as a risk factor for child depressive symptoms; testing the social mediators in internationally adopted children. Eur. Child Adolesc. Psychiatry 27, 1585–1593 (2018).

115. Brooker, R. J. et al. Birth and adoptive parent anxiety symptoms moderate the link between infant attention control and internalizing problems in toddlerhood. Dev. Psychopathol. 26, 347–359 (2014).
116. Brooker, R. J. et al. Associations Between Infant Negative Affect and Parent Anxiety Symptoms are Bidirectional: Evidence from Mothers and Fathers. Front. Psychol. 6, 1875 (2015).
117. Eley, T. C. et al. The Intergenerational Transmission of Anxiety: A Children-of-Twins Study. Am. J. Psychiatry 172, 630–637 (2015).
118. Ahmadzadeh, Y. I. et al. Anxiety in the family: a genetically informed analysis of transactional associations between mother, father and child anxiety symptoms. J. Child Psychol. Psychiatry 60, 1269–1277 (2019).
119. Gjerde, L. C. et al. Maternal Perinatal and Concurrent Anxiety and Mental Health Problems in Early Childhood: A Sibling-Comparison Study. Child Dev. 91, 456–470 (2020).
120. Field, A. P. et al. Maternal and paternal influences on childhood anxiety symptoms: A genetically sensitive comparison. J Appl Dev Psychol 68, 101123 (2020).
121. Bekkhus, M. et al. Re-examining the link between prenatal maternal anxiety and child emotional difficulties, using a sibling design. Int. J. Epidemiol. 47, 156–165 (2018).
122. Glover, V. & O'Connor, T. G. Effects of antenatal stress and anxiety: Implications for development and psychiatry. Br. J. Psychiatry 180, 389–391 (2002).
123. Bridgett, D. J. et al. Contributions of mothers' and fathers' parenting to children's self-regulation: Evidence from an adoption study. Dev. Sci. 21, e12692 (2018).
124. Horwitz, B. N. et al. Parental criticism is an environmental influence on adolescent somatic symptoms. J Fam Psychol 29, 283–289 (2015).
125. McAdams, T. A. et al. Associations between the parent-child relationship and adolescent self-worth: a genetically informed study of twin parents and their adolescent children. J. Child Psychol. Psychiatry 58, 46–54 (2017).
126. Hannigan, L. J. et al. Shared genetic influences do not explain the association between parent-offspring relationship quality and offspring internalizing problems: results from a Children-of-Twins study. Psychol. Med. 48, 592–603 (2018).
127. Ahmadzadeh, Y. I. *et al.* Parental criticism and adolescent internalising symptoms: Associations remain after accounting for shared genetic effects. *medRxiv* (2020). doi:10.1101/2020.05.07.20084319
128. Marceau, K. et al. Combined influences of genes, prenatal environment, cortisol, and parenting on the development of children's internalizing versus externalizing problems. Behav Genet 45, 268–282 (2015).
129. Kendler, K. S., Ohlsson, H., Sundquist, J. & Sundquist, K. The Rearing Environment and Risk for Major Depression: A Swedish National High-Risk Home-Reared and Adopted-Away Co-Sibling Control Study. Am. J. Psychiatry 177, 447–453 (2020).
130. Torvik, F. A. et al. Mechanisms linking parental educational attainment with child ADHD, depression, and academic problems: a study of extended families in The Norwegian Mother, Father and Child Cohort Study. J. Child Psychol. Psychiatry 61, 1009–1018 (2020).
131. Lund, I. O. et al. Is the association between maternal alcohol consumption in pregnancy and pre-school child behavioural and emotional problems causal? Multiple approaches for controlling unmeasured confounding. Addiction 114, 1004–1014 (2019).
132. Lipscomb, S. T. et al. Genetic vulnerability interacts with parenting and early care education to predict increasing externalizing behavior. Int J Behav Dev 38, 70–80 (2014).
133. Stover, C. S. et al. Marital Hostility, Hostile Parenting, and Child Aggression: Associations from Toddlerhood to School Age. J. Am. Acad. Child Adolesc. Psychiatry 55, 235–242 (2016).
134. Reuben, J. D. et al. Warm Parenting and Effortful Control in Toddlerhood: Independent and Interactive Predictors of School-Age Externalizing Behavior. J. Abnorm. Child Psychol. 44, 1083–1096 (2016).
135. Marceau, K. et al. Parenting and prenatal risk as moderators of genetic influences on conduct problems during middle childhood. Dev. Psychol. 55, 1164–1181 (2019).
136. Hyde, L. W. et al. Heritable and Nonheritable Pathways to Early Callous-Unemotional Behaviors. Am. J. Psychiatry 173, 903–910 (2016).
137. Bornovalova, M. A. et al. Understanding the relative contributions of direct environmental effects and passive genotype-environment correlations in the association between familial risk factors and child disruptive behavior disorders. Psychol. Med. 44, 831–844 (2014).

138. Kendler, K. S. et al. A Swedish national adoption study of criminality. Psychol. Med. 44, 1913–1925 (2014).
139. Kendler, K. S., Ohlsson, H., Morris, N. A., Sundquist, J. & Sundquist, K. A Swedish population-based study of the mechanisms of parent-offspring transmission of criminal behavior. Psychol. Med. 45, 1093–1102 (2015).
140. Eilertsen, E. M. *et al.* Parental prenatal symptoms of depression and offspring symptoms of ADHD: A genetically informed intergenerational study. *J Atten Disord* 1087054720914386 (2020). doi:10.1177/1087054720914386
141. Roos, L. E. et al. Inherited and environmental influences on a childhood co-occurring symptom phenotype: Evidence from an adoption study. Dev. Psychopathol. 28, 111–125 (2016).
142. Marceau, K. et al. Parental knowledge is an environmental influence on adolescent externalizing. J. Child Psychol. Psychiatry 56, 130–137 (2015).
143. Trentacosta, C. J. et al. Callous-Unemotional Behaviors and Harsh Parenting: Reciprocal Associations across Early Childhood and Moderation by Inherited Risk. J. Abnorm. Child Psychol. 47, 811–823 (2019).
144. Elam, K. K. et al. Adoptive parent hostility and children's peer behavior problems: examining the role of genetically informed child attributes on adoptive parent behavior. Dev. Psychol. 50, 1543–1552 (2014).
145. Plamondon, A., Browne, D. T., Madigan, S. & Jenkins, J. M. Disentangling Child-Specific and Family-Wide Processes Underlying Negative Mother-Child Transactions. J. Abnorm. Child Psychol. 46, 437–447 (2018).
146. Hoyt, W. T. Rater bias in psychological research: When is it a problem and what can we do about it? Psychol. Methods 5, 64–86 (2000).
147. Samek, D. R. et al. General and specific predictors of nicotine and alcohol dependence in early adulthood: genetic and environmental influences. J Stud Alcohol Drugs 75, 623–634 (2014).
148. Guimond, F.-A. et al. Associations Between Mother-Child Relationship Quality and Adolescent Adjustment: Using a Genetically Controlled Design to Determine the Direction and Magnitude of Effects. Int J Behav Dev 40, 196–204 (2016).
149. Kendler, K. S., Ohlsson, H., Sundquist, K. & Sundquist, J. Cross-generational transmission from drug abuse in parents to attention-deficit/hyperactivity disorder in children. Psychol. Med. 46, 1301–1309 (2016).
150. Kuja-Halkola, R., D'Onofrio, B. M., Larsson, H. & Lichtenstein, P. Maternal smoking during pregnancy and adverse outcomes in offspring: genetic and environmental sources of covariance. Behav Genet 44, 456–467 (2014).
151. Hicks, B. M., Iacono, W. G. & McGue, M. Index of the transmissible common liability to addiction: heritability and prospective associations with substance abuse and related outcomes. Drug Alcohol Depend. 123 Suppl 1, S18-23 (2012).
152. Kendler, K. S. & Myers, J. The boundaries of the internalizing and externalizing genetic spectra in men and women. Psychol. Med. 44, 647–655 (2014).
153. Estabrook, R. et al. Separating Family-Level and Direct Exposure Effects of Smoking During Pregnancy on Offspring Externalizing Symptoms: Bridging the Behavior Genetic and Behavior Teratologic Divide. Behav Genet 46, 389–402 (2016).
154. Ellingson, J. M., Goodnight, J. A., Van Hulle, C. A., Waldman, I. D. & D'Onofrio, B. M. A sibling-comparison study of smoking during pregnancy and childhood psychological traits. Behav Genet 44, 25–35 (2014).
155. Knopik, V. S. et al. Smoking during pregnancy and ADHD risk: A genetically informed, multiple-rater approach. Am. J. Med. Genet. B, Neuropsychiatr. Genet. 171, 971–981 (2016).
156. Obel, C. et al. The risk of attention deficit hyperactivity disorder in children exposed to maternal smoking during pregnancy - a re-examination using a sibling design. J. Child Psychol. Psychiatry 57, 532–537 (2016).
157. Eilertsen, E. M. et al. Maternal alcohol use during pregnancy and offspring attention-deficit hyperactivity disorder (ADHD): a prospective sibling control study. Int. J. Epidemiol. 46, 1633–1640 (2017).
158. de Zeeuw, E. L. et al. Intergenerational transmission of education and ADHD: effects of parental genotypes. Behav Genet 50, 221–232 (2020).
159. Demontis, D. et al. Discovery of the first genome-wide significant risk loci for attention deficit/hyperactivity disorder. Nat. Genet. 51, 63–75 (2019).

160. Liu, C.-Y., Li, Y., Viding, E., Asherson, P. & Pingault, J.-B. The developmental course of inattention symptoms predicts academic achievement due to shared genetic aetiology: a longitudinal twin study. Eur. Child Adolesc. Psychiatry 28, 367–375 (2019).
161. Wertz, J. *et al.* Using DNA from mothers and children to study parental investment in children's educational attainment. *Child Dev.* (2019). doi:10.1111/cdev.13329
162. Ayorech, Z., Krapohl, E., Plomin, R. & von Stumm, S. Genetic influence on intergenerational educational attainment. Psychol. Sci. 28, 1302–1310 (2017).
163. Borriello, G. A. *et al.* The intergenerational transmission of mathematics achievement in middle childhood: A prospective adoption design. *Dev. Sci.* e12974 (2020). doi:10.1111/desc.12974
164. Halpern-Manners, A. et al. The intergenerational transmission of early educational advantages: new results based on an adoption design. Res Soc Stratif Mobil 67, (2020).
165. Conley, D. et al. Is the effect of parental education on offspring biased or moderated by genotype? J Sociological Science 2, 82 (2015).
166. Liu, H. Social and genetic pathways in multigenerational transmission of educational attainment. *Am. Sociol. Rev.* 000312241875965 (2018). doi:10.1177/0003122418759651
167. Domingue, B. W. & Fletcher, J. Separating measured genetic and environmental effects: evidence linking parental genotype and adopted child outcomes. Behav Genet 50, 301–309 (2020).
168. Belsky, J. The differential susceptibility hypothesis. JAMA Pediatr. 170, 321 (2016).
169. Bates, T. C. *et al.* Social competence in parents increases children's educational attainment: replicable genetically-mediated effects of parenting revealed by non-transmitted DNA. *Twin Res Hum Genet* 1–3 (2019).
170. Belsky, D. W. et al. Genetic analysis of social-class mobility in five longitudinal studies. Proc. Natl. Acad. Sci. USA 115, E7275–E7284 (2018).
171. Willoughby, E. A., McGue, M., Iacono, W. G., Rustichini, A. & Lee, J. J. The role of parental genotype in predicting offspring years of education: evidence for genetic nurture. *Mol. Psychiatry* (2019). doi:10.1038/s41380-019-0494-1
172. Armstrong-Carter, E. et al. The earliest origins of genetic nurture: the prenatal environment mediates the association between maternal genetics and child development. Psychol. Sci. 31, 781–791 (2020).
173. Scheeren, L., Das, M. & Liefbroer, A. C. Intergenerational transmission of educational attainment in adoptive families in the Netherlands. Res Soc Stratif Mobil 48, 10–19 (2017).
174. Cheesman, R. et al. Comparison of Adopted and Nonadopted Individuals Reveals Gene-Environment Interplay for Education in the UK Biobank. Psychol. Sci. 31, 582–591 (2020).
175. D'Onofrio, B. M., Lahey, B. B., Turkheimer, E. & Lichtenstein, P. Critical need for family-based, quasi-experimental designs in integrating genetic and social science research. Am. J. Public Health 103 Suppl 1, S46-55 (2013).
176. Waldron, M. et al. Parental separation and early substance involvement: results from children of alcoholic and cannabis dependent twins. Drug Alcohol Depend. 134, 78–84 (2014).
177. Kendler, K. S. et al. An extended Swedish national adoption study of alcohol use disorder. JAMA Psychiatry 72, 211–218 (2015).
178. Grant, J. D. et al. Parental Separation and Offspring Alcohol Involvement: Findings from Offspring of Alcoholic and Drug Dependent Twin Fathers. Alcohol. Clin. Exp. Res. 39, 1166–1173 (2015).
179. Kendler, K. S., Ohlsson, H., Edwards, A., Sundquist, J. & Sundquist, K. The clinical features of alcohol use disorders in biological and step-fathers that predict risk for alcohol use disorders in offspring. Am. J. Med. Genet. B, Neuropsychiatr. Genet. 174, 779–785 (2017).
180. Kendler, K. S., Ohlsson, H., Sundquist, K. & Sundquist, J. The causes of parent-offspring transmission of drug abuse: a Swedish population-based study. Psychol. Med. 45, 87–95 (2015).
181. Kendler, K. S., Ohlsson, H., Sundquist, K. & Sundquist, J. Sources of Parent-Child Transmission of Drug Abuse: Path Analyses of Not-Lived-With Parental, Stepparental, Triparental, and Adoptive Families. J. Nerv. Ment. Dis. 206, 239–244 (2018).
182. Treur, J. L. et al. Testing familial transmission of smoking with two different research designs. Nicotine Tob. Res. 20, 836–842 (2018).

183. McGue, M., Malone, S., Keyes, M. & Iacono, W. G. Parent-offspring similarity for drinking: a longitudinal adoption study. Behav Genet 44, 620–628 (2014).
184. Kendler, K. S., Ohlsson, H., Sundquist, J. & Sundquist, K. Facilitating Versus Inhibiting the Transmission of Drug Abuse from High-Risk Parents to Their Children: A Swedish National Study. Twin Res Hum Genet 23, 1–7 (2020).
185. Maes, H. H. et al. Cross-Cultural Comparison of Genetic and Cultural Transmission of Smoking Initiation Using an Extended Twin Kinship Model. Twin Res Hum Genet 21, 179–190 (2018).
186. Franco Cea, N. & Barnes, G. E. The development of Addiction-Prone Personality traits in biological and adoptive families. Pers. Individ. Dif. 82, 107–113 (2015).
187. Bidwell, L. C. et al. Prenatal exposure effects on early adolescent substance use: preliminary evidence from a genetically informed bayesian approach. J Stud Alcohol Drugs 78, 789–794 (2017).
188. Maes, H. H., Silberg, J. L., Neale, M. C. & Eaves, L. J. Genetic and cultural transmission of antisocial behavior: an extended twin parent model. Twin Res Hum Genet 10, 136–150 (2007).
189. Samek, D. R., Rueter, M. A., Keyes, M. A., McGue, M. & Iacono, W. G. Parent involvement, sibling companionship, and adolescent substance use: A longitudinal, genetically informed design. J Fam Psychol 29, 614–623 (2015).
190. Cea, N. F. & Barnes, G. E. Parenting styles and offspring's polysubstance use in biological and adoptive families. International Journal of Child Youth & Family Studies 5, 466-492 (2014).
191. Kendler, K. S., Ohlsson, H., Sundquist, K. & Sundquist, J. The rearing environment and risk for drug abuse: a Swedish national high-risk adopted and not adopted co-sibling control study. Psychol. Med. 46, 1359–1366 (2016).
192. Van Ryzin, M. J. et al. Genetic influences can protect against unresponsive parenting in the prediction of child social competence. Child Dev. 86, 667–680 (2015).
193. Brooker, R. J. et al. Early inherited risk for anxiety moderates the association between fathers' child-centered parenting and early social inhibition. J. Dev. Orig. Health Dis. 7, 602–615 (2016).
194. Kandler, C., Richter, J. & Zapko-Willmes, A. The nature and nurture of HEXACO personality trait differences. Z. Psychol. 227, 195–206 (2019).
195. O'Reilly, L. M. et al. The intergenerational transmission of suicidal behavior: an offspring of siblings study. Transl. Psychiatry 10, 173 (2020).
196. Kendler, K. S., Turkheimer, E., Ohlsson, H., Sundquist, J. & Sundquist, K. Family environment and the malleability of cognitive ability: a Swedish national home-reared and adopted-away cosibling control study. Proc. Natl. Acad. Sci. USA 112, 4612–4617 (2015).
197. Lai, H. M. X., Cleary, M., Sitharthan, T. & Hunt, G. E. Prevalence of comorbid substance use, anxiety and mood disorders in epidemiological surveys, 1990-2014: A systematic review and meta-analysis. Drug Alcohol Depend. 154, 1–13 (2015).
198. Plomin, R., Reiss, D., Hetherington, E. M. & Howe, G. W. Nature and nurture: genetic contributions to measures of the family environment. Dev. Psychol. 30, 32 (1994).
199. Klahr, A. M. & Burt, S. A. Elucidating the etiology of individual differences in parenting: A meta-analysis of behavioral genetic research. Psychol. Bull. 140, 544–586 (2014).
200. Vinkhuyzen, A. A. E., van der Sluis, S., de Geus, E. J. C., Boomsma, D. I. & Posthuma, D. Genetic influences on "environmental" factors. Genes Brain Behav. 9, 276–287 (2010).
201. Avinun, R. & Knafo, A. Parenting as a reaction evoked by children's genotype: a meta-analysis of children-as-twins studies. Pers. Soc. Psychol. Rev. 18, 87–102 (2014).
202. Kendler, K. S. & Baker, J. H. Genetic influences on measures of the environment: a systematic review. Psychol. Med. 37, 615–626 (2007).
203. Siegenthaler, E., Munder, T. & Egger, M. Effect of preventive interventions in mentally ill parents on the mental health of the offspring: systematic review and meta-analysis. J. Am. Acad. Child Adolesc. Psychiatry 51, 8–17.e8 (2012).
204. Stockings, E. et al. Prevention, early intervention, harm reduction, and treatment of substance use in young people. Lancet Psychiatry 3, 280–296 (2016).
205. Côté, S. M. et al. Why Is Maternal Depression Related to Adolescent Internalizing Problems? A 15-Year Population-Based Study. J. Am. Acad. Child Adolesc. Psychiatry 57, 916–924 (2018).
206. Goodman, S. H. et al. Maternal depression and child psychopathology: a meta-analytic review. Clin. Child Fam. Psychol. Rev. 14, 1–27 (2011).

207. Ramchandani, P. & Psychogiou, L. Paternal psychiatric disorders and children's psychosocial development. Lancet 374, 646–653 (2009).
208. Sangawi, H., Adams, J. & Reissland, N. The effects of parenting styles on behavioral problems in primary school children: a cross-cultural review. Asian Social Science 11, 171–186 (2015).
209. Cui, M., Donnellan, M. B. & Conger, R. D. Reciprocal influences between parents' marital problems and adolescent internalizing and externalizing behavior. Developmental psychology 43, 1544 (2007).
210. Reiss, F. Socioeconomic inequalities and mental health problems in children and adolescents: a systematic review. Soc. Sci. Med. 90, 24–31 (2013).
211. Wolf, J. B. & Wade, M. J. What are maternal effects (and what are they not)? Philos. Trans. R. Soc. Lond. B, Biol. Sci. 364, 1107–1115 (2009).
212. Rice, F., Harold, G. T. & Thapar, A. The Link between depression in mothers and offspring: an extended twin analysis. Behav Genet 35, 565–577 (2005).
213. Silberg, J. L., Maes, H. & Eaves, L. J. Genetic and environmental influences on the transmission of parental depression to children's depression and conduct disturbance: an extended Children of Twins study. Journal of Child Psychology and Psychiatry 51, 734–744 (2010).
214. Horwitz, B. N. & Neiderhiser, J. M. Gene - Environment Interplay, Family Relationships, and Child Adjustment. J. Marriage Fam. 73, 804–816 (2011).
215. McGuire, S. The heritability of parenting. Parenting: Science and Practice 3, 73–94 (2003).
216. Yang, J. et al. Common SNPs explain a large proportion of the heritability for human height. Nat. Genet. 42, 565–569 (2010).
217. Yang, J., Lee, S. H., Goddard, M. E. & Visscher, P. M. GCTA: a tool for genome-wide complex trait analysis. Am. J. Hum. Genet. 88, 76–82 (2011).
218. Merikangas, K. R. in Applied Computational Genomics (ed. Yao, Y.) 13, 17–26 (Springer Singapore, 2018).
219. Magnus, P. Further evidence for a significant effect of fetal genes on variation in birth weight. Clin. Genet. 26, 289–296 (1984).
220. Magnus, P. Causes of variation in birth weight: a study of offspring of twins. Clin. Genet. 25, 15–24 (1984).
221. Schreuder, P. & Alsaker, E. The Norwegian Mother and Child Cohort Study (MoBa) – MoBa recruitment and logistics. Nor J Epidemiol 24, (2014).
222. Magnus, P. et al. Cohort profile update: the norwegian mother and child cohort study (moba). Int. J. Epidemiol. 45, 382–388 (2016).
223. Paltiel, L. et al. The biobank of the Norwegian Mother and Child Cohort Study – present status. Nor J Epidemiol 24, (2014).
224. Angold, A., Costello, E. J., Messer, S. C. & Pickles, A. Development of a short questionnaire for use in epidemiological studies of depression in children and adolescents. *International Journal of Methods in Psychiatric Research* (1995).
225. Birmaher, B. et al. The Screen for Child Anxiety Related Emotional Disorders (SCARED): scale construction and psychometric characteristics. J. Am. Acad. Child Adolesc. Psychiatry 36, 545–553 (1997).
226. McCarthy, S. et al. A reference panel of 64,976 haplotypes for genotype imputation. Nat. Genet. 48, 1279–1283 (2016).
227. Helgeland, Ø. et al. Genome-wide association study reveals dynamic role of genetic variation in infant and early childhood growth. Nat. Commun. 10, 4448 (2019).
228. Purcell, S. et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am. J. Hum. Genet. 81, 559–575 (2007).
229. Qiao, Z. et al. Introducing M-GCTA a Software Package to Estimate Maternal (or Paternal) Genetic Effects on Offspring Phenotypes. Behav Genet 50, 51–66 (2020).
230. Pawitan, Y., Reilly, M., Nilsson, E., Cnattingius, S. & Lichtenstein, P. Estimation of genetic and environmental factors for binary traits using family data. Stat. Med. 23, 449–465 (2004).
231. Kendler, K. S., Heath, A. C., Martin, N. G. & Eaves, L. J. Symptoms of anxiety and symptoms of depression. Same genes, different environments? Arch. Gen. Psychiatry 44, 451–457 (1987).
232. Maher, B. Personal genomes: The case of the missing heritability. Nature News 456, 18–21 (2008).

233. Manolio, T. A. et al. Finding the missing heritability of complex diseases. Nature 461, 747–753 (2009).
234. Horikoshi, M. et al. Genome-wide associations for birth weight and correlations with adult disease. Nature 538, 248–252 (2016).
235. Sawyer, K. M., Zunszain, P. A., Dazzan, P. & Pariante, C. M. Intergenerational transmission of depression: clinical observations and molecular mechanisms. Mol. Psychiatry 24, 1157–1177 (2019).
236. Moen, G.-H., Hemani, G., Warrington, N. M. & Evans, D. M. Calculating power to detect maternal and offspring genetic effects in genetic association studies. Behav Genet 49, 327–339 (2019).
237. Biele, G. et al. Bias from self selection and loss to follow-up in prospective cohort studies. Eur J Epidemiol 34, 927–938 (2019).
238. Loeber, R., Green, S. M. & Lahey, B. B. Mental health professionals' perception of the utility of children, mothers, and teachers as informants on childhood psychopathology. Journal of Clinical Child Psychology 19, 136–143 (1990).
239. Jensen Peña, C. L. & Champagne, F. A. Epigenetic and neurodevelopmental perspectives on variation in parenting behavior. Parenting: Science and Practice 12, 202–211 (2012).
240. Kappeler, L. & Meaney, M. J. Epigenetics and parental effects. Bioessays 32, 818–827 (2010).
241. Jami, E. S., Hammerschlag, A. R., Bartels, M. & Middeldorp, C. M. Parental characteristics and offspring mental health and related outcomes: a systematic review of genetically informative literature. Transl. Psychiatry 11, 197 (2021).
242. Silva, R. R. et al. A rating scale for disruptive behavior disorders, based on the DSM-IV item pool. Psychiatr. Q. 76, 327–339 (2005).
243. Wood, A. C., Buitelaar, J., Rijsdijk, F., Asherson, P. & Kuntsi, J. Rethinking shared environment as a source of variance underlying attention-deficit/hyperactivity disorder symptoms: comment on Burt (2009). Psychol. Bull. 136, 331–340 (2010).
244. van der Sluis, S., Verhage, M., Posthuma, D. & Dolan, C. V. Phenotypic complexity, measurement bias, and poor phenotypic resolution contribute to the missing heritability problem in genetic association studies. PLoS One 5, e13929 (2010).
245. Hwang, L.-D. et al. Estimating indirect parental genetic effects on offspring phenotypes using virtual parental genotypes derived from sibling and half sibling pairs. PLoS Genet. 16, e1009154 (2020).
246. Bartels, M., Cacioppo, J. T., van Beijsterveldt, T. C. E. M. & Boomsma, D. I. Exploring the association between well-being and psychopathology in adolescents. Behav Genet 43, 177–190 (2013).
247. Proctor, C. L., Linley, P. A. & Maltby, J. Youth life satisfaction: A review of the literature. J. Happiness Stud. 10, 583–630 (2009).
248. Berger, Eva, Spiess & Katharina. Maternal Life Satisfaction and Child Outcomes: Are They Related? *Journal of Economic Psychology* (2011).
249. Hughes, C., Devine, R. T., Mesman, J., Blair, C. & NewFAMS Team. Parental well-being, couple relationship quality, and children's behavioral problems in the first 2 years of life. Dev. Psychopathol. 32, 935–944 (2020).
250. Okbay, A. et al. Genetic variants associated with subjective well-being, depressive symptoms, and neuroticism identified through genome-wide analyses. Nat. Genet. 48, 624–633 (2016).
251. Baselmans, B. M. L. et al. A Genetic Investigation of the Well-Being Spectrum. Behav Genet 49, 286–297 (2019).
252. Sugrue, L. P. & Desikan, R. S. What are polygenic scores and why are they important? JAMA 321, 1820–1821 (2019).
253. Lee, J. J. et al. Gene discovery and polygenic prediction from a genome-wide association study of educational attainment in 1.1 million individuals. Nat. Genet. 50, 1112–1121 (2018).
254. Tubbs, J. D., Porsch, R. M., Cherny, S. S. & Sham, P. C. The genes we inherit and those we don't: maternal genetic nurture and child BMI trajectories. Behav Genet 50, 310–319 (2020).
255. Pingault, J.-B. *et al.* Identifying intergenerational risk factors for ADHD symptoms usingpolygenic scores in the Norwegian Mother, Father and Child Cohort. *medRxiv* (2021).
256. Ligthart, L. et al. The Netherlands Twin Register: Longitudinal Research Based on Twin and Twin-Family Designs. Twin Res Hum Genet 22, 623–636 (2019).

257. Fraser, A. et al. Cohort Profile: the Avon Longitudinal Study of Parents and Children: ALSPAC mothers cohort. Int. J. Epidemiol. 42, 97–110 (2013).
258. Boyd, A. et al. Cohort Profile: the 'children of the 90s'--the index offspring of the Avon Longitudinal Study of Parents and Children. Int. J. Epidemiol. 42, 111–127 (2013).
259. Taylor, A. E. et al. Exploring the association of genetic factors with participation in the Avon Longitudinal Study of Parents and Children. Int. J. Epidemiol. 47, 1207–1216 (2018).
260. Viechtbauer, W. Conducting Meta-Analyses in R with the metafor Package. J. Stat. Softw. 36, (2010).
261. Chen, Y., Haines, J., Charlton, B. M. & VanderWeele, T. J. Positive parenting improves multiple aspects of health and well-being in young adulthood. Nat. Hum. Behav. 3, 684–691 (2019).
262. Tubbs, J. D., Zhang, Y. D. & Sham, P. C. Intermediate confounding in trio relationships: The importance of complete data in effect size estimation. Genet. Epidemiol. 44, 395–399 (2020).
263. Munafò, M. R., Higgins, J. P. T. & Smith, G. D. Triangulating Evidence through the Inclusion of Genetically Informed Designs. Cold Spring Harb. Perspect. Med. 11, (2021).
264. Knoester, C. Transitions in Young Adulthood and the Relationship between Parent and Offspring Well-Being. Social Forces 81, 1431–1458 (2003).
265. Kwong, A. S. F. et al. Genetic and environmental risk factors associated with trajectories of depression symptoms from adolescence to young adulthood. JAMA Netw. Open 2, e196587 (2019).
266. McGrath, L. M., Weill, S., Robinson, E. B., Macrae, R. & Smoller, J. W. Bringing a developmental perspective to anxiety genetics. Dev. Psychopathol. 24, 1179–1193 (2012).
267. Kwong, A. S. F. *et al.* Polygenic risk for depression, anxiety and neuroticism are associated with the severity and rate of change in depressive symptoms across adolescence. *J. Child Psychol. Psychiatry* (2021). doi:10.1111/jcpp.13422
268. van der Laan, C. M. et al. Continuity of Genetic Risk for Aggressive Behavior Across the Life-Course. Behav Genet 51, 592–606 (2021).
269. Hirschfeld, R. M. A. The comorbidity of major depression and anxiety disorders: recognition and management in primary care. Prim. Care Companion J. Clin. Psychiatry 3, 244–254 (2001).
270. Anderson, E. R. & Hope, D. A. A review of the tripartite model for understanding the link between anxiety and depression in youth. Clin. Psychol. Rev. 28, 275–287 (2008).
271. Levey, D. F. et al. Reproducible genetic risk loci for anxiety: results from ~200,000 participants in the million veteran program. Am. J. Psychiatry 177, 223–232 (2020).
272. Thorp, J. G. et al. Symptom-level modelling unravels the shared genetic architecture of anxiety and depression. Nat. Hum. Behav. 5, 1432–1442 (2021).
273. Grotzinger, A. D. et al. Genomic structural equation modelling provides insights into the multivariate genetic architecture of complex traits. Nat. Hum. Behav. 3, 513–525 (2019).
274. Rutter, M. A children's behaviour questionnaire for completion by teachers: preliminary findings. J. Child Psychol. Psychiatry 8, 1–11 (1967).
275. Wainschtein, P. *et al.* Recovery of trait heritability from whole genome sequence data. *BioRxiv* (2019). doi:10.1101/588020
276. Howe, L. J. *et al.* Within-sibship GWAS improve estimates of direct genetic effects. *BioRxiv* (2021). doi:10.1101/2021.03.05.433935
277. Selzam, S. et al. Comparing Within- and Between-Family Polygenic Score Prediction. Am. J. Hum. Genet. 105, 351–363 (2019).
278. Yengo, L. et al. Imprint of assortative mating on the human genome. Nat. Hum. Behav. 2, 948–954 (2018).
279. Ask, H. *et al.* Genetic contributions to anxiety disorders: where we are and where we are heading. *Psychol. Med.* 1–16 (2021). doi:10.1017/S0033291720005486
280. Lawrence, P. J., Murayama, K. & Creswell, C. Systematic Review and Meta-Analysis: Anxiety and Depressive Disorders in Offspring of Parents With Anxiety Disorders. J. Am. Acad. Child Adolesc. Psychiatry 58, 46–60 (2019).
281. Natsuaki, M. N. et al. Raised by depressed parents: is it an environmental risk? Clin. Child Fam. Psychol. Rev. 17, 357–367 (2014).
282. Young, A. I. *et al.* Mendelian imputation of parental genotypes for genome-wide estimation of direct and indirect genetic effects. *BioRxiv* (2020). doi:10.1101/2020.07.02.185199

283. Wu, Y. et al. Estimating genetic nurture with summary statistics of multigenerational genome-wide association studies. Proc. Natl. Acad. Sci. USA 118, (2021).
284. Warrington, N. M., Hwang, L.-D., Nivard, M. & Evans, D. M. Estimating direct and indirect genetic effects on offspring phenotypes using genome-wide summary results data: a comparison of multivariate methods. *BioRxiv* (2020). doi:10.1101/2020.11.02.365981
285. Balbona, J. V., Kim, Y. & Keller, M. C. Estimation of parental effects using polygenic scores. Behav Genet 51, 264–278 (2021).
286. Kim, Y., Balbona, J. V. & Keller, M. C. Bias and Precision of Parameter Estimates from Models Using Polygenic Scores to Estimate Environmental and Genetic Parental Influences. Behav Genet 51, 279–288 (2021).
287. Paananen, R., Tuulio-Henriksson, A., Merikukka, M. & Gissler, M. Intergenerational transmission of psychiatric disorders: the 1987 Finnish Birth Cohort study. Eur. Child Adolesc. Psychiatry 30, 381–389 (2021).
288. Johnson, S. E., Lawrence, D., Perales, F., Baxter, J. & Zubrick, S. R. Prevalence of Mental Disorders Among Children and Adolescents of Parents with Self-Reported Mental Health Problems. Community Ment. Health J. 54, 884–897 (2018).
289. Rasic, D., Hajek, T., Alda, M. & Uher, R. Risk of mental illness in offspring of parents with schizophrenia, bipolar disorder, and major depressive disorder: a meta-analysis of family high-risk studies. Schizophr. Bull. 40, 28–38 (2014).
290. Dean, K. et al. Full spectrum of psychiatric outcomes among offspring with parental history of mental disorder. Arch. Gen. Psychiatry 67, 822–829 (2010).
291. Dean, K. et al. The impact of parental mental illness across the full diagnostic spectrum on externalising and internalising vulnerabilities in young offspring. Psychol. Med. 48, 2257–2263 (2018).
292. Reardon, T. et al. What do parents perceive are the barriers and facilitators to accessing psychological treatment for mental health problems in children and adolescents? A systematic review of qualitative and quantitative studies. Eur. Child Adolesc. Psychiatry 26, 623–647 (2017).
293. Solantaus, T. & Puras, D. Caring for Children of Parents with Mental Health Problems – A Venture into Historical and Cultural Processes in Europe. International Journal of Mental Health Promotion 12, 27–36 (2010).
294. Skogøy, B. E. et al. Differences in implementation of family focused practice in hospitals: a cross-sectional study. Int. J. Ment. Health Syst. 12, 77 (2018).
295. Sandler, I. N., Schoenfelder, E. N., Wolchik, S. A. & MacKinnon, D. P. Long-term impact of prevention programs to promote effective parenting: lasting effects but uncertain processes. Annu. Rev. Psychol. 62, 299–329 (2011).
296. Barlow, J., Bergman, H., Kornør, H., Wei, Y. & Bennett, C. Group-based parent training programmes for improving emotional and behavioural adjustment in young children. *Cochrane Database Syst. Rev.* CD003680 (2016). doi:10.1002/14651858.CD003680.pub3
297. Yap, M. B. H. et al. Parents in prevention: A meta-analysis of randomized controlled trials of parenting interventions to prevent internalizing problems in children from birth to age 18. Clin. Psychol. Rev. 50, 138–158 (2016).
298. Thongseiratch, T., Leijten, P. & Melendez-Torres, G. J. Online parent programs for children's behavioral problems: a meta-analytic review. Eur. Child Adolesc. Psychiatry 29, 1555–1568 (2020).
299. Lee, P., Niew, W., Yang, H., Chen, V. C. & Lin, K. A meta-analysis of behavioral parent training for children with attention deficit hyperactivity disorder. Res. Dev. Disabil. 33, 2040–2049 (2012).

SUMMARY OF AUTHOR CONTRIBUTIONS

Chapter 2

Published as: **Jami, E. S**., Hammerschlag, A. R., Ip, H. F., Allegrini, A. G., Benyamin, B., Border, R., ... & Middeldorp, C. M. (2022). Genome-wide association meta-analysis of childhood and adolescent internalising symptoms. *Journal of the American Academy of Child and Adolescent Psychiatry.*

The study was conceptualised by Christel Middeldorp and Meike Bartels. The pre-registration document and standard operational procedure was written by me, and edited and reviewed by Christel Middeldorp, Meike Bartels and Anke Hammerschlag. Author contributions for cohort-specific analyses are listed in Table 1. I liaised with all cohorts to support their analyses, collated the results and performed quality control procedures. I performed the overall and stratified meta-analyses with the help of Hill Ip. Michel Nivard provided statistical advice. I performed the follow-up analyses, including functional analyses (implemented in FUMA), calculation of genetic correlations, and polygenic score prediction. I wrote the manuscript, with input from Christel Middeldorp, Meike Bartels and Anke Hammerschlag, and created the figures and supplementary tables. All co-authors actively participated in editing and reviewing the manuscript, and I collated and integrated their feedback before submission. Christel Middeldorp and Meike Bartels helped to review and edit the manuscript during the revisions.

Author contributions

Table 1 Author contributions, per cohort, for the univariate analyses in Chapter 2

Cohort(s)	Author	Contribution			
		Statistical analysis	Genotyping	Sample collection / organisation	Cohort design / PI / funding
1958BC	Beben Benyamin	X			
1958BC	Elina Hypponen			X	X
1958BC	Ang Zhou	X			
Add Health	Kathleen Mullan Harris		X	X	X
Add Health, PNC	K. Paige Harden	X			
Add Health, PNC	Travis T Mallard	X			
ALSPAC	Marcus R Munafò				X
ALSPAC	Hannah M Sallis	X			
BREATHE	Silvia Alemany		X	X	
BREATHE	Jordi Sunyer				X
BREATHE	Natàlia Vilor-Tejedor	X	X	X	
CATSS	Qi Chen	X			
CATSS	Ralf Kuja-Halkola		X	X	
CATSS	Henrik Larsson		X	X	X
CATSS	Yi Lu	X	X		
CATSS	Paul Lichtenstein				X
CATSS	Sebastian Lundstrom				X
CATSS	Ashley E. Tate	X			
FinnTwin12	Danielle M Dick		X	X	X
FinnTwin12	Jaakko Kaprio			X	X
FinnTwin12	Tellervo Korhonen			X	
FinnTwin12	Teemu Palviainen	X			
FinnTwin12	Richard J Rose				X
FinnTwin12	Eero Vuoksimaa			X	
FinnTwin12	Alyce M Whipp			X	
GenR	Elizabeth W Diemer	X			
GenR	Alexander Neumann	X		X	
GenR	Fernando Rivadeneira		X		X
GenR	Henning Tiemeier				X
GINIplus/LISA	Joachim Heinrich			X	X
GINIplus/LISA	Marie Standl	X		X	X
GINIplus/LISA	Elisabeth Thiering	X	X	X	

GSMS	Daniel E Adkins	X	X		
GSMS	William Copeland			X	X
GSMS	Elizabeth J Costello		X	X	X
GSMS	Andrey A Shabalin	X			
IBG	Richard Border	X			
IBG	Sandra A Brown				X
IBG	Robin P Corley			X	
IBG	Luke M Evans	X			
IBG	John K Hewitt				X
IBG	Christian Hopfer				X
IBG	Kenneth Krauter				X
IBG	Chandra Reynolds				X
IBG	Andrew Smolen		X		
IBG	Michael Stallings				X
IBG	Sally Wadsworth				X
IBG	Tamara L Wall			X	X
MoBa	Ole A Andreassen		X		X
MoBa	Helga Ask			X	
MoBa	Alexandra Havdahl				X
MoBa	Per Magnus				X
MoBa	Pål Njølstad		X		
MoBa	Ted Reichborn-Kjennerud			X	X
MoBa	Eivind Ystrom			X	X
MSUTR	S. Alexandra Burt	X	X	X	X
MSUTR	Chang Jiang	X	X		
MSUTR	Kelly L Klump				X
MSUTR	Qing Lu	X	X		
MSUTR	Xiaoran Tong	X	X		
MUSP	Anjali K Henders		X		
MUSP	Abdullah Mamun				X
MUSP	Shelby A Marrington			X	
MUSP	Jake M Najman				X
MUSP	Gail M Williams				X
NFBC	Ville Karhunen	X			
NFBC	Marjo-Riitta Jarvelin		X	X	X

Author contributions

NTR	Meike Bartels					X
NTR	Dorret I Boomsma					X
NTR	Erik A Ehli		X			
NTR	Fiona A Hagenbeek			X		
NTR	Jouke Jan Hottenga		X			
NTR	Hill F Ip	X				
NTR, ABCD, MoBa	Eshim S Jami	X				
NTR	Christel M Middeldorp					X
NTR	Michel G Nivard	X				
NTR	Catharina E van Beijsterveldt			X		
NTR, MUSP, ABCD	Anke R Hammerschlag	X				
Raine Study	Craig E Pennell		X	X		X
Raine Study	Carol Wang	X				
Raine Study	Andrew J.O. Whitehouse					X
TEDS	Andrea G Allegrini	X				
TEDS	Robert Plomin					X
TEDS	Kaili Rimfeld			X		
TRAILS	Catharina A Hartman			X		X
TRAILS	Ilja M Nolte	X				
TRAILS	Albertine J Oldehinkel			X		X
TRAILS	Harold Snieder		X			
VTSABD	Lindon J Eaves					X
VTSABD	Hermine H Maes					X
VTSABD	Judy L Silberg					X
VTSABD, GSMS	Roseann E Peterson	X	X			
YFS	Christian Hakulinen	X				
YFS	Liisa Keltikangas- Järvinen					X
YFS	Terho Lehtimäki		X	X		X
YFS	Pashupati Mishra	X				

Chapter 3

Published as: Jami, E. S., Hammerschlag, A. R., Bartels, M., & Middeldorp, C. M. (2021). Parental characteristics and offspring mental health and related outcomes: a systematic review of genetically informative literature. *Translational Psychiatry*, 11(1), 1-38.

The review was conceptualised by me and I performed the systematic search, wrote the first draft, and created the tables and figures. The illustrations in the figures were drawn by Zainab Humayun. All co-authors actively participated in editing and reviewing the first and subsequent drafts, and I collated and integrated their comments for each draft before submission and during the revision.

Chapter 4

Published as: Jami, E. S., Eilertsen, E. M., Hammerschlag, A. R., Qiao, Z., Evans, D. M., Ystrøm, E., ... & Middeldorp, C. M. (2020). Maternal and paternal effects on offspring internalising problems: Results from genetic and family-based analyses. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*, 183(5), 258-267.

The study was conceptualised by Christel Middeldorp, Meike Bartels and Eivind Ystrom. Eivind Ystrom also provided access to MoBa data, for which I conducted quality control procedures for the genetic analyses. Espen Eilertsen and I performed the statistical analyses, where I performed the GCTA and extended-GCTA analyses and Espen Eilertsen carried out the pedigree-based models. David Evans and Zhen Qiao provided statistical support for the M-GCTA analyses. I wrote the first draft of the manuscript and created the tables. Espen Eilertsen helped write the section on the pedigree analyses and created Figure 1. All co-authors actively participated in editing and reviewing the manuscript, and I collated and integrated their comments and feedback prior to submission and during the revision.

Chapter 5

Submitted as: Jami, E. S., Hammerschlag, A. R., Sallis, H. M., Qiao, Z., Andreassen, O. A., Magnus, P. M., ... & Middeldorp, C. M. Do environmental effects indexed by parental genetic variation influence common psychiatric symptoms in childhood?

The study was conceptualised by me, with input from Christel Middeldorp, Meike Bartels and Anke R Hammerschlag. Eivind Ystrom and Alexandra Havdahl provided access to MoBa data. I conducted quality control procedures for the genetic data, carried out the statistical analyses, wrote the manuscript and created the table. David Evans and Zhen Qiao provided statistical support for the M-GCTA analyses. Christel Middeldorp and Meike Bartels edited and reviewed the current version and previous drafts. All other co-authors will participate in editing and reviewing the manuscript before submission to a journal, and I will collate and integrate their comments and feedback prior to submission.

Chapter 6

Submitted as: Jami, E. S., Sallis, H. M., Hammerschlag, A. R., Pearson, R. M., Havdahl, A., Pingault, J. B., Ystrøm, E., Munafò, M. R., Middeldorp, C. M., & Bartels, M. Using parent and offspring genotypes to study the relationship between parental wellbeing and childhood psychiatric symptoms.

The study was conceptualised by me, with input from Christel Middeldorp, Meike Bartels and Anke Hammerschlag. I constructed non-transmitted genotypes, created polygenic scores and performed the statistical analyses for the NTR sample, while Hannah Sallis did the same for the ALSPAC sample with help from Rebecca Pearson. I meta-analysed the results from the NTR and ALSPAC samples, wrote the manuscript and created the figures and table. Christel Middeldorp and Meike Bartels edited and reviewed the current version and previous drafts. All other co-authors will participate in editing and reviewing the manuscript before submission to a journal, and I will collate and integrate their comments and feedback prior to submission.

LIST OF PUBLICATIONS

Jami, E. S., Hammerschlag, A. R., Ip, H. F., Allegrini, A. G., Benyamin, B., Border, R., ... & Middeldorp, C. M. (2022). Genome-wide association meta-analysis of childhood and adolescent internalising symptoms. *Journal of the American Academy of Child and Adolescent Psychiatry*.

Jami, E. S., Hammerschlag, A. R., Bartels, M., & Middeldorp, C. M. (2021). Parental characteristics and offspring mental health and related outcomes: a systematic review of genetically informative literature. *Translational Psychiatry*, *11*(1), 1-38.

Eilertsen, E. M., **Jami, E. S**., McAdams, T. A., Hannigan, L. J., Havdahl, A. S., Magnus, P., ... & Ystrom, E. (2021). Direct and indirect effects of maternal, paternal, and offspring genotypes: Trio-GCTA. *Behavior Genetics*, *51*(2), 154-161.

Ask*, H., Cheesman*, R., **Jami*, E. S**., Levey*, D. F., Purves*, K. L., & Weber*, H. (2021). Genetic contributions to anxiety disorders: where we are and where we are heading. *Psychological Medicine*, 1-16.
**All authors contributed equally to this work*

Jami, E. S., Pritchard, M., Shetty, H., Stewart, R., Young, A. H., & Heslin, M. (2021). Antidepressant and antipsychotic treatment of Psychotic Major Depression in a British mental healthcare setting. *Journal of Mental Health*, 1-7.

Rajula, H. S. R., Manchia, M., Agarwal, K., Akingbuwa, W. A., Allegrini, A. G., Diemer, E., Doering, S., Haan, E., **Jami, E. S**., ... & Middeldorp, C. M. (2021). Overview of CAPICE—Childhood and Adolescence Psychopathology: unravelling the complex etiology by a large Interdisciplinary Collaboration in Europe—an EU Marie Skłodowska-Curie International Training Network. *European Child & Adolescent Psychiatry*, 1-11.

Akingbuwa, W. A., Hammerschlag, A. R., **Jami, E. S**., Allegrini, A. G., Karhunen, V., Sallis, H., ... & Middeldorp, C. M. (2020). Genetic associations between childhood psychopathology and adult depression and associated traits in 42 998 individuals: a meta-analysis. *JAMA Psychiatry*, *77*(7), 715-728.

Jami, E. S., Eilertsen, E. M., Hammerschlag, A. R., Qiao, Z., Evans, D. M., Ystrøm, E., ... & Middeldorp, C. M. (2020). Maternal and paternal effects on offspring internalizing problems: Results from genetic and familybased analyses. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*, *183*(5), 258-267.

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PhD thesis
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