Using genetic designs to identify likely causal environmental contributions to psychopathology

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Abstract

The multifactorial nature of psychopathology, whereby both genetic and environmental factors contribute risk, has long been established. In this paper, we provide an update on genetically informative designs that are utilized to disentangle genetic and environmental contributions to psychopathology. We provide a brief reminder of quantitative behavioral genetic research designs that have been used to identify potentially causal environmental processes, accounting for genetic contributions. We also provide an overview of recent molecular genetic approaches that utilize genome-wide association study data which are increasingly being applied to questions relevant to psychopathology research. While genetically informative designs typically have been applied to investigate the origins of psychopathology, we highlight how these approaches can also be used to elucidate potential causal environmental processes that contribute to developmental course and outcomes. We highlight the need to use genetically sensitive designs that align with intervention and prevention science efforts, by considering strengths-based environments to investigate how positive environments can mitigate risk and promote children's strengths.

Using genetic designs to identify likely causal environmental contributions to psychopathology

Over the last 40 years, there have been immense gains in our knowledge of how genes and environment contribute to psychopathology (Plomin, 1990; Rutter, 2004; Smoller, 2019). We have evolved from a time when genetic risk factors were considered to be of minimal or no importance for child development and psychopathology (e.g., Thapar & Rutter, 2021) to an era where it is well-recognized not only that both genes and environment contribute in complex ways, but that their contributions are closely inter-related (Rutter, 2015; State & Thapar, 2015). We also have learnt that no single genetic (or environmental) risk factor on its own explains the development of psychopathology, and that there are many different biological and developmental routes that lead to the same outcome; equifinality is a concept that has long been familiar to those in developmental psychopathology (Cicchetti & Rogosch, 1996). We also know that the same genes or set of genetic risks, like environmental risks, can lead to very different characteristics, behaviors and outcomes. For example, the same genetic variants contribute risks for Attention Deficit Hyperactivity Disorder (ADHD), Autism Spectrum Disorder (ASD), Depression, Bipolar Disorder, and Schizophrenia (Lee et al., 2019). Pleiotropy refers to the multiple different effects of the same gene/genetic risks, and has been found to be extensive for human traits (Visscher & Yang, 2016). Whilst the discovery of extensive pleiotropy for psychopathology is relatively recent, the concept of multifinality again is well established in the field of developmental psychopathology (Cicchetti & Rogosch, 1996). In short, genetic discoveries have progressed, and genetic susceptibility does not seem to operate in ways that are fundamentally different from other types of risk and protective factors.

In this paper, we first outline how quantitative behavioral genetic research designs can be utilized to disentangle genetic and environmental contributions to psychopathology. Although most research in developmental psychopathology has used traditional quantitative behavioral genetic approaches, research designs also are now starting to incorporate molecular genetic approaches. We therefore briefly discuss newer molecular genetic approaches and their relevance to research in

developmental psychology. As our focus is on conceptual issues, we refer readers elsewhere for detailed descriptions of the strengths and limitations of different genetic designs used to identify causal environmental factors (Davey Smith, Richmond & Pingault, 2021; Knopik et al., 2017; Smith et al., 2021). Most of these designs have been used to identify likely causal environmental risk factors (Pingault et al., 2018) but they can also be utilized in strengths-based research that seeks to identify protective factors and moderators that attenuate risk. Research on these topics is highly relevant for clinical practice and policymakers.

1. Research designs based on relatives: quantitative behavioral genetic research designs

Genetic studies of child psychopathology began with research designs that included relatives of varying degrees of familial and genetic relatedness (State & Thapar, 2015). These comprised family-, twin-, twin-extensions (e.g. children of twins), adoption and IVF-based designs among others (Davey Smith et al., n.d.; Liu & Neiderhiser, 2017). Such studies highlighted that between-person variation in psychopathology is explained by both genetic and non-genetic contributions and include environmental, measurement error and stochastic effects.

Twin studies were used to generate heritability estimates for different forms of psychopathology. Heritability refers to the proportion of observed variation in a specific phenotype that is attributed to genetic variation. Misconceptions of heritability remain. For example, high heritability estimates do not mean an attribute is predetermined: high heritability is not equivalent to immutability. Moreover, heritability is population specific because environmental contexts can alter genetic expression, even for phenotypes that are typically viewed as highly heritable such as height and IQ (Sellers et al., 2019); Shanahan et al., 2005; Turkheimer et al., 2003). Last, it is also important to emphasize that heritability estimates are population based statistics, and refer to genetic contributions to variance within a *population,* rather than estimates of genetic influences for specific individuals. Whilst heritability estimates have their limitations (see Tenesa & Haley, 2013 for a full explanation; see also table 1), early studies using heritability estimates were nevertheless important for highlighting that genetic factors contribute to variation across different types of

psychopathology. In general, twin studies suggested that genetic influences accounted for around 70 to 90% of total variance for neurodevelopmental disorders such as Autism Spectrum Disorder (ASD) and Attention Deficit Hyperactivity Disorder (ADHD) (Rutter, 2000; Thapar, 2018; Thapar & Rutter, 2021) and for major mental illnesses such as schizophrenia and bipolar disorder (Sullivan et al., 2018; Sullivan & Geschwind, 2019). Lower heritability estimates and larger environmental contributions were found for depression, anxiety, and antisocial behavior/conduct disorder (Burt, 2009; Polderman et al., 2015).

Although the initial relative-based research designs were used to examine the familial and genetic contributions to psychopathology, later these designs were employed to elucidate environmental processes. One consistent observation to emerge from these traditional relative-based designs was that many environmental factors (e.g. bullying, parenting, maltreatment) also appeared to be influenced by genetic factors, a phenomenon called gene-environment correlation (*r*GE: Knafo & Jaffee, 2013; Rutter, 2015; Scarr & McCartney, 1983; see Table 2). Findings emphasized that genes and environment not only worked together but were interdependent (Broderick & Neiderhiser, 2019; Rutter, 2007a). The observation of *r*GE also highlighted the possibility that some of the associations between environmental factors and psychopathology could be accounted for by shared genes between the parent and child. These associations could have arisen because of so-called “genetic confounding” (Rutter, 2007b). Genetically informed designs thus became important for differentiating likely causal environmental processes involved in the development of psychopathology from genetic processes (Arseneault et al., 2008; Caspi et al., 2004; Thapar & Rutter, 2019).

Unlike molecular genetic studies (see Section 2, Molecular genetic approaches) that directly measure genotype, research designs based on relatives utilize variation in genetic relatedness between family members to estimate heritable and environmental influences on phenotypes. Some designs are particularly suited to disentangling genetic and environmental contributions, such as parental genetic contributions from aspects of the rearing environment. For example adoption

studies remove the confound of passive gene environment correlation (*r*GE) from estimates of rearing environment: see section on adoption study designs, and Table 2). Other designs, such as the assisted conception design, are suited to separating parental genetic contributions from prenatal environments for child outcomes (see Rice, Langley, Woodford, Davey Smith, & Thapar, 2018). Yet other study designs allow estimation of the impact of offspring genetically influenced characteristics on their environments (evocative or active *r*GE; e.g., twin study). As the rationale of genetically informative research designs have been outlined in detail elsewhere (e.g., Harold, Leve, Sellers, 2017; Knopik et al., 2017; Liu & Neiderhiser, 2017; Thapar & Rutter, 2015, 2016, 2019), we provide only a brief overview of quantitative behavioral genetic research designs that elucidate environmental processes that may inform tractable intervention and prevention sites (see Table 1 for a summary of research designs, and their assumptions and limitations).

One of the most commonly used designs based on relatives is the twin design. Twin designs take advantage of monozygotic (MZ) twins sharing 100% of their segregating genes, and dizygotic (DZ) twins sharing on average 50%. The twin design operates under the equal environments assumption (EEA) – that environments of MZ twins are no more similar than the environments of DZ twins. The EEA would be violated, for example, if parents of MZ twins treat their children the same way because they expect the children to be identical (rather than due to the actual behavior), while parents of DZ twins treat their children differently because they expect their children to be different since they are not genetically identical. If this assumption is violated, MZ twin correlations could be inflated and increase heritability estimates.

When phenotypic similarity between twins (concordance) depends on their genetic relatedness, then genetic contributions to the phenotype are inferred. However if phenotypic similarity does not vary across MZ and DZ twin pairs, then shared environmental factors are indicated (Harold, Leve, Sellers, 2017; Knopik et al., 2017; Thapar & Rutter, 2015). Twin designs can also estimate genetic and environmental contributions between multiple constructs by comparing cross-twin cross-trait correlations for two different measures across MZ and DZ twin pairs. For

example, if the correlation between ADHD and depressive symptoms is approximately two times higher for MZ twins than DZ twins, then their covariance is due, at least in part, to shared genetic factors (Faraone & Larsson, 2019). Shared and nonshared environmental contributions to covariance between constructs can also be estimated by examining differences in MZ and DZ twin correlations. The twin design has also been employed to examine associations between environmental exposures (e.g., parenting) and outcomes (e.g., conduct problems), decomposing covariance into genetic and environmental components (Broderick & Neiderhiser, 2019; J. B. Pingault et al., 2018). For example, covariation between harsh discipline/corporal punishment and child antisocial behavior has been found to be partially accounted for by genetic factors (Jaffee et al., 2004). Conversely, associations between maltreatment and child antisocial behavior have been found to be largely explained by family-wide or shared environmental factors (Jaffee et al., 2004).

An extension of the classic twin design, Children of Twins (CoT) studies is better suited to examining cross-generational transmission. CoT studies take advantage of the fact that children of MZ and DZ twins are socially cousins, but children of MZ twins are as similar as half-siblings, sharing 25% of their segregating genes while children of DZ twins share 12.5% like any cousin pair. Children of MZ twins are therefore as genetically related to their parents as they are to their twin's sibling (i.e., their uncle/aunt; see McAdams et al., 2014, 2018; Thapar & Rutter, 2015; Sellers et al., 2019). Thus, the CoT design provides the opportunity to examine whether intergenerational transmission within families is explained by genetic factors, environment factors, or both (see D'Onofrio et al., 2007; Thapar & Rutter, 2015). A limitation of the CoT design, however, is that is does not take into account the possibility that associations between parent and child characteristics may be due to reverse causation (i.e., child effects on parents). The Extended Children of twins (ECOT) addresses this limitation (Narusyte et al., 2008). Comparing the results from child- and parent-twin samples can also be useful in identifying the relevance of passive and non-passive rGE for phenotypes. Using this approach Neiderhiser and colleagues' (2004, 2007) found evidence that different types of rGE may operate for different mothering constructs (e.g., passive rGE indicated for mother's positivity and

monitoring, and nonpassive rGE indicated for mother's negativity: Neiderhiser et al., 2004). However, this approach can only be suggestive of the types of rGE correlation that are present. Other designs are needed before drawing robust conclusions.

Other research designs have been used to examine prenatal risk factors; for example, the comparison of maternal and paternal exposure during pregnancy and associations with offspring psychopathology has been used as a method to examine intrauterine effects, separate from genetic or household-level confounders (Thapar & Rutter, 2015, 2019). Associations are examined between maternal and paternal exposures during pregnancy and offspring outcomes. If an association between exposure and child outcome is causal (via intrauterine effects), a stronger association would be found for maternal exposure relative to paternal exposure, as only the mother provides the intrauterine environment. If associations are observed between paternal exposure and child outcomes, this increased risk is assumed to be due to (genetic and/or environmental) confounding. Limitations to this design include the fact that it is confined to exposures that both parents could experience in pregnancy (see Thapar & Rutter, 2019). The discordant sibling design also is useful for disentangling genetic from prenatal environmental risks by examining the relationship between prenatal exposures and offspring outcomes where siblings have been differentially exposed (i.e., discordant for a specific exposure). Maternal genetic contribution is held constant (genetic factors are held constant at the level of mother-child genetic relationships: full siblings share 50% of their genes with their mother), but intrauterine environment can vary across pregnancy. For example, studies of siblings discordant for exposure to maternal smoking during pregnancy suggest that associations between maternal smoking during pregnancy and offspring ADHD may be due to early unmeasured confounding, rather than direct effects (Gustavson et al., 2017; Obel et al., 2011; Rice et al., 2018). Limitations of this research design include the fact that where associations are explained by confounding, it is not clear whether confounding is due to genes, shared environment, or both. There are also problems with selection bias as mothers are behaving differently in different pregnancies. For example, the samples include a group of mothers who are able to stop smoking

during pregnancy but another group that has not. In addition, siblings born at different times will be exposed to different family- and population-level risks (see Thapar & Rutter, 2019).

Children who have been conceived using assisted reproductive technologies (ART; Thapar, Harold et al., 2007) also provide an opportunity to examine associations between parents and children who differ in genetic relatedness to one or both of their rearing parents (”adoption at conception”; Harold et al., 2012). This allows the examination of whether associations between parents and children are primarily genetically mediated, environmentally mediated, or a combination of the two. Egg donation and gestational surrogacy allow the examination of prenatal influences separate from genetic influences (see: Thapar et al., 2009; Thapar & Rutter, 2019). This research design is particularly informative for partitioning genetic and intrauterine influences, which is not possible in twin or adoption studies. ART designs have provided further evidence that smoking during pregnancy is not causally associated with child ADHD (Rice et al., 2018; Thapar & Rice, 2021; Thapar et al., 2009).

Finally, the adoption study design provides an opportunity to disentangle heritable from postnatal effects on phenotypes, but this design cannot disentangle heritable and prenatal environmental effects on phenotypes (see Thapar et al., 2019; Thapar & Rutter, 2015). It is an especially powerful design for identifying the contributions of the rearing environment on child outcomes. Where adopted children are placed with genetically unrelated adoptive parents at birth, associations between adopted children and their adoptive/rearing parents are attributed to environmental processes (unconfounded by shared genetic factors between parent and child, i.e., passive rGE; e.g., Leve et al., 2019; Rhea et al., 2013). Conversely, similarities between adopted children and their biological parents are attributed to shared genes (and, specific to birth mother: intrauterine influences). Evocative *r*GE can also be tested by examining associations between genetically influenced child characteristics and responses from others. Thus, the adoption design provides insights into how children’s genetically influenced behaviors can evoke specific behaviors in genetically unrelated rearing (adoptive) parents. For example, work using this design suggests that

adoptive parents' hostile parenting have an environmentally mediated impact on child behavioral problems. At the same time, children's early impulsivity/activation (ADHD-like features) may elicit more hostile parenting (evocative *r*GE) (Sellers et al., 2020) that in turn contributes to later ADHD symptoms (Harold et al., 2013). The adoption design can also be employed to test for gene-environment interactions (GxE): testing whether environmental factors can modify the expression of genetically influenced risks or propensities (see Rutter, 2012). Adoption studies have shown, for example, that the effects of specific aspects of parenting on toddler behavior may vary as a function of genetic risk (as indicated by birth parent risk: Leve et al., 2009; Ganiban et al., 2021).

Overall, research designs that include relatives who differ with regard to genetic relatedness addresses several core processes that are not discernable in non-genetically informed studies: (1) associations between environmental processes and child psychopathology may be partially explained by common genetic factors shared between parents and children rather than solely through environmental effects (passive *r*GE); (2) children may evoke specific responses from those in their environment due to their own genetic propensities (evocative *r*GE); and (3) inherited aspects of the child may interact with their environment such that the effects on child outcomes are not the same for all children (gene-environment interaction, GxE: see Table 2). Genetically informative designs such as adoption studies, twin and CoT studies, can also be used to examine selection effects due to genetic propensities (active rGE, see Rutter, 2007a). For example, evidence suggests that active rGE may, at least in part, explain selection of a deviant peer group (TenEyck & Barnes, 2015; Vitaro et al., 2021), as well as prosocial leadership (see Knafo-Noam et al., 2018). In designs that remove the confound of genetic contributions, findings provide a better understanding of malleable environmental factors that could be targeted to reduce adverse outcomes for children (see Harold & Sellers, 2018).

2.Molecular genetic approaches

In the 21st Century, we have witnessed the advent of a different and direct approach to investigating genetic contributions to psychopathology: large-scale molecular genetic studies of psychopathology that have led to an increasing number of genetic discoveries at the level of DNA variation. Here, scientists have sought to identify genetic contributions directly, rather than indirectly via an average measure of genetic sharing between different relatives (State & Thapar, 2015). These genome-wide association studies (GWAS) studies test for association between multiple genetic markers-DNA variation- and psychopathology, mainly with case-control designs but also testing for association with trait measures. Tens of thousands to millions of DNA variants (Verlouw et al., 2021) across every chromosome are tested. This results in a very large multiple testing burden, which is why GWAS need to be very large and include tens to hundreds of thousands of participants to identify genomic variants that withstand appropriate correction for this testing and that are genome-wide statistically significant. There are many different types of DNA variation, although most (99.9%) of our genomes do not show variation between different individuals. Gene discovery studies have examined DNA variation that is common (>1% frequency in the population; single nucleotide polymorphisms -SNPs) and rare genetic variants (<1% frequency). Rare genetic variants include deletions and duplications of DNA stretches (copy number variants; CNVs) and variation in DNA sequence within protein-coding regions of genes (exome sequencing studies) (see State & Thapar, 2015). More recent studies are moving to sequencing variation across non-coding regions too (whole genome sequencing). These studies have shown that multiple gene variants contribute to risk of psychopathology. Thousands of common gene variants of small effect size and rare gene variants of larger effect size (e.g., odds ratios of 3-50) (Singh et al. 2022) appear to be especially important for risk of neurodevelopmental disorders [e.g., intellectual disability (Vissers, Gilissen, & Veltman, 2016); ASD (Thapar & Rutter, 2021), ADHD (Thapar, 2018), Tourette's syndrome (Huang et al., 2017) and schizophrenia (Rees, O'Donovan, & Owen, 2015; Trubetskoy et al. 2022; Singh et al. 2022)], although not exclusively to these conditions. However, these discoveries on

which variants are associated with psychopathology do not in themselves tell us which genes and proteins are involved or explain the underlying biology or the mechanisms that lead to psychopathology. They represent only the first and distal step towards many more investigations. While the specifics of gene discovery and subsequent biological investigations may not interest most in the field of developmental psychopathology, some of these discoveries are currently being utilized to examine practice-relevant questions and processes relevant to psychopathology. We will discuss these newer molecular genetic approaches in brief and how they are relevant to research in developmental psychopathology.

Polygenic risk scores

Although the main objective of GWAS is to discover genetic variants for specific characteristics or traits including psychopathology, as with studies based on relatives, GWAS findings have also been used to test genetic as well as environmental contributions psychopathology. One approach has involved generating a composite measure of common gene variants known as polygenic scores (PGS). A "discovery" GWAS, which must be large, is used to identify nominally associated common gene variants (thousands of single nucleotide polymorphism: SNPs). The PGS are then calculated in an independent "target" sample by summing these "risk" or "protective" alleles and their effect sizes obtained from the discovery data set. These scores can be calculated for every individual in the independent genotyped "target" sample and their summed effects (PGS) provide a direct indicator of individual genetic propensity for the trait or disorder in question (Bogdan et al., 2018; Murray et al., 2021). PGS can be generated from a "discovery" GWAS that can include measures of any trait or categorically defined characteristic (e.g. height, blood pressure, neuroticism, diabetes, depression, reported maltreatment). PGS have been generated for multiple physical health conditions, different types of psychopathology, traits such as height, and environmental measures among many other measured characteristics. As PGS are a sum of common variants (alleles; single nucleotide polymorphisms-SNPs) that are nominally associated with the characteristic in question, they include alleles that are not genome-wide significant or causal. PGS

are being used increasingly in the field of developmental psychopathology because they provide a useful indicator of genetic propensity/liability in populations and samples that are not otherwise genetically informative (i.e., they do not contain related individuals).

As is true for other research designs (see Table 1), the use of PGS does have limitations. First, they provide a weak indicator of genetic predisposition/liability and explain only a small proportion of variance in psychopathology (e.g. 4% variance of ADHD, 11% in schizophrenia) and only a fraction of twin heritability. Although they become more powerful when GWAS are larger, they are still weak predictors on their own currently and do not capture all relevant genetic variation for any given phenotype. Second, as we might expect from relative-based study findings, PGS do not show specificity because of extensive pleiotropy for different mental health conditions. For example, schizophrenia PGS not only predict schizophrenia but also are associated with depression, anxiety and bipolar disorder. This pattern of findings likely reflects the well-reported genetic overlap between different psychopathologies. New methods are being developed to differentiate shared and specific genetic variance across multiple psychopathologies (e.g., genomic structural equation modeling: Grotzinger et al., 2019; Peyre et al., 2021). To some extent, this pattern may also reflect symptom overlap between current diagnostic categories. Third, PGS do not replicate well in samples that differ from the original discovery sample. The biggest source of difference here is ancestry. It is a serious concern in the field of genetics that nearly all the largest GWAS have been generated using people of European ancestry. PGS derived from these GWAS do not consistently generalize to people of other ancestries. This has led to calls for many more genetic studies of ethnically diverse populations. Without use of more diverse samples, the likely future beneficial impacts of genetic discoveries on healthcare, will lead to further social and healthcare inequities. Nevertheless, provided these limitations are understood, PGS can provide a useful indicator of genetic susceptibility. In addition, there is growing interest as to whether and when to combine PGS with family history and social/environmental measures to inform practice. For example, by combining these sources of data, practitioners could be helped in selecting the most appropriate intervention

for individual children and families (Murray et al., 2021). Multiple recent research studies also have shown that many social environmental measures are associated with PGS for psychopathology, in keeping with findings of gene-environment correlation from previous relative-based studies. For example, maltreatment and bullying victimization have been found to be associated with ADHD and depression PGS (Schoeler et al., 2019; Warrier et al., 2021). If parents have also been genotyped it is possible to test associations with parent PGS, allowing for the fact that parent-child PGS are correlated (i.e., controlling for passive *r*GE). Relatedly, one study observed that a number of prenatal environmental exposures (e.g., maternal smoking in pregnancy) were associated with maternal ADHD PGS (Leppert et al. 2019).

A separate question is whether PGS be used to test gene-environment interaction. The previous approach commonly used for identifying candidate genes (i.e., picking DNA variants in genes thought to be involved) has been shown to be flawed. False positives are easily generated. As already mentioned, with millions of DNA variants, sample sizes need to be enormous to identify genome-wide significant variants - the chances of a false positive are too high otherwise (Zammit, Owen & Lewis, 2010; Thompson, 1991). While testing candidate gene variant x environment was popular, because of non-replications such findings are now regarded with suspicion. A more recent approach is using PGS to test G x E. Whilst PGS are more robust than candidate gene variants, challenges remain. First, we have to take account of *r*GE before testing interactions as G x E effects can be observed in error if *r*GE is present but not taken into account (Rutter, Moffit, & Caspi, 2006). Second, there are no biologically plausible reasons for testing PGS x environment interactions because they are a sum of different genetic variants for multifactorial, complex phenotypes (Murray et al., 2021; Zammit et al., 2010): PGS are derived from genome wide inquiry, taking a composite score of genes based on the extent to which genes are correlated with a specific phenotype (Zhang & Belsky, 2022). Third, multiple testing increases the potential for false positives when investigating a large number of environmental factors. Also, environmental exposures need to be assessed using high-quality measures and at developmentally appropriate times. Whilst G x E is intuitively attractive

and found to be important for plants and animals raised in experimental conditions, even for physical health conditions where E and G are much better documented than for psychopathology, identifying G x E remains fraught with challenges. Whilst new methods are being developed to explore G x E using genomic data (e.g., Genome-wide by environment interaction studies, GWEIS; see Aschard et al., 2012), such an approach has a number of limitations: existing GWEIS may have reduced power to detect such effects as most large genotyped samples have limited environmental measures (Uher & Zwicker, 2017). Furthermore, they take a SNP-by-SNP approach to GxE (Assary et al., 2018; Uher & Zwicker, 2017). Finally, psychopathology is influenced by multiple risk (and protective) factors each of which has probabilistic effects where genetic variants have distal influences on outcomes. Thus, caution is warranted. Nevertheless, it will be interesting to see if it becomes possible to test biologically plausible interactions as the field moves forward.

"Genetic nurture" and Mendelian randomization

To identify environmental contributions using genomic data, two designs have emerged as potentially relevant to the field of developmental psychopathology: a mother-father-child trio design to examine nurture using genomic data ("genetic nurture") and Mendelian Randomization. The parent-child trio design assesses the effects of parents' non-transmitted (and as transmitted) alleles on their offspring to differentiate direct (inherited) and indirect (phenotypically mediated) parental impacts. As non-transmitted genetic variants are free from genetic confounding that arises from genetic variants shared between parents and offspring (akin to removing confound of passive *r*GE: Wang et al., 2021), non-transmitted alleles are assumed to be mediated by the parent's phenotype ("genetic nurture") and thus index environmental contributions (Kong et al., 2018). Using PGS, this approach has provided evidence that the intergenerational transmission of educational attainment includes both inherited and environmental components (Kong et al., 2018; Wang et al., 2021). Such designs have yet to be widely utilized in psychopathology research, although recent work has

provided evidence that ADHD cross-generational transmission is mainly attributable to inherited alleles rather than genetic nurture (de Zeeuw et al., 2020; Pingault et al., 2021; Martin et al. 2022).

Mendelian randomization (MR) is a different method that utilizes genetic variants as instrumental variables - proxies for an exposure (e.g., measured trait or environmental exposure). MR tests whether an exposure causes an outcome (vertical pleiotropy), accounting for pleiotropic effects (e.g., the same genetic factors influencing both the exposure and the outcome; horizontal pleiotropy) (Hemani, Bowden, & Davey Smith, 2018). Based on certain assumptions, MR is analogous to a randomized controlled trial (RCT) in that genetic variants (SNPs, single nucleotide variants) are randomly assigned at conception and thus differ regarding the exposure they are selected as being associated with, but not with confounders, and are therefore comparable to groups within an RCT. One common type of MR method is a two-sample MR that utilizes summary SNP-exposure and SNP-outcome data from different GWAS (Hemani et al., 2018). MR has been invaluable in some areas of medicine but is challenging to apply to psychopathology because high polygenicity, and overlapping biology place limitations on identifying strong instruments (Martin, Daly, Robinson, Hyman, & Neale, 2019). Also, many of the key assumptions are easily violated (e.g., due to *r*GE). Thus, findings using MR methods should be interpreted with caution unless they converge across many different study designs.

Several studies have now used MR to investigate potentially causal effects of environmental factors on mental health (Pingault, Cecil, Murray, Munafò, & Viding, 2017). For example, one MR study found genetic liability to years of education and body mass index to be associated with a decreased and increased likelihood of depression respectively and did not find strong evidence of a causal association for coronary artery disease (Wray et al., 2018). MR studies have also added to evidence supporting (active) *r*GE, such as work suggesting genetic liability to schizophrenia may have a causal effect on living in more densely populated areas (Colodro-Conde et al., 2018). Finally, MR has been utilized to examine causal relationships between different psychopathologies, for example, suggesting that ADHD may have a causal impact on depression (Riglin et al., 2020). Limitations of MR

include that the use of samples of unrelated individuals can result in biased results because of uncontrolled confounding from familial effects. Samples of related individuals such as siblings or parent-offspring trios can be used to control for such effects (Brumpton et al., 2020; Smith & Hemani, 2014).

3. Applying Strength-based approaches to genetically informative designs

Both relative-based and molecular genetic research designs have highlighted the complex interplay between genetics and environmental exposure and the challenges of disentangling these, especially using traditional observational data. Genetic designs were originally used to examine the contribution of genetic and environmental influences to the origins of psychopathology. However, different social and genetic factors may contribute to the developmental course, accompanying comorbidities and outcomes of psychopathology compared to those that contribute to its origins (e.g., Pingault et al., 2015) (Figure 1). While genetic designs have traditionally been used to focus on risk factors that contribute to the *origins* of psychopathology, for those seeing children and young people with psychopathology, the key question is: can we help optimize outcomes by modifying family and social contexts? If so, what aspects should we focus on?

Considering neurodevelopmental difficulties as an example, whilst psychopathologies such as ADHD, ASD and schizophrenia appear to be highly heritable, their developmental course and outcomes (e.g. mental wellbeing, physical health, anxiety and depression, gainful employment) may be influenced by different genes and environmental factors as well as moderated or shaped by social and family environments (Figure 1). Indeed, those with neurodevelopmental disorders such as ADHD are at heightened risk of later mental health problems including depression (Jaffee et al., 2002, Rice et al., 2019). Such comorbid mental health problems further impair functioning in those with a neurodevelopmental condition, yet currently there is very little evidence to guide families, practitioners and educators as to whether modifying family, educational and social environments

could help protect against the development of common mental health problems (e.g., depression, anxiety) in this high-risk group.

Most previous genetically informative research has primarily focused on a deficit-based approach, being employed to identify likely causal environmental *risks*. For example, ADHD/ ADHD genetic liability is known to elicit more hostile family relationships, and parents of children with ADHD or ASD are more likely to experience parenting stress, marital stress, and separation (Ben-Naim et al., 2019; Kousgaard et al., 2018). Previous genetically informative studies have shown evocative effects from birth parent characteristics (birth-mother ADHD) through offspring early impulsivity and activation on maternal and paternal hostile behaviors (Harold et al., 2013; Sellers et al 2020), which in turn was associated with developmental course of ADHD, as well as conduct problems (Sellers et al., 2020). Whilst a deficit model can help with addressing questions about need, deficit models do not necessarily tell us about what interventions would work (see Sellers et al., 2019), and strength-based approaches also need to be considered.

Strength-based approaches consider positive assets, behaviors, or strengths within the individual, family and/or community that may support positive outcomes, and is linked to the concept of resilience, which is a developmentally dynamic perspective whereby specific environments/characteristics can reduce background risk. A strengths-based approach aligns more closely to preventive interventions which focus on enhancing positive rearing environments to prevent or mitigate negative child outcomes. As such, applying strength-based approaches to genetically informative study designs could help provide insights into positive environments that may mitigate risk, with findings of particular importance and relevance for clinical practice and policy. Whilst it is possible to incorporate and consider processes that emphasize strengths, there is currently limited examination of the role of positive aspects of family processes (and broader environmental factors) for developmental outcomes including mental health and related aspects of functioning.

Whilst the study of protective/promotive processes for children with neurodevelopmental difficulties is in its infancy, there is some evidence for the role of specific social- and family-level systems (Dvorsky & Langberg, 2016). For example, social support and acceptance has been found to buffer against negative outcomes including poor academic attainment and co-occurring depression symptoms among children with ADHD (Dvorsky & Langberg, 2016). Positive parenting may also promote more positive outcomes (Dvorsky & Langberg, 2016). However, few studies have examined the complex interplay between biological and environmental processes when examining strengths-based processes. There is a need for future research to consider strengths-based approaches using behavioral genetic research designs, to disentangle genetic and environmental processes to support intervention and prevention science efforts.

Genetically informative designs, such as the adoption design, provide an especially powerful design for testing environmental mediators and moderators of children's early behaviors and outcomes because adoptive parents are genetically unrelated to their offspring. It therefore becomes possible to test environmental mechanisms independent of parents' genotype. A small number of studies have utilized genetically informative designs to examine the role of positive rearing environments. For example, CoT studies suggest that parent-child relationship quality is associated with positive self-worth and fewer internalising problems (see Jami et al., 2021). Using a longitudinal adoption-at-birth design, positive parenting (e.g., positive parent-child relationships, warmth parenting, and positive reinforcement) has also been associated with fewer externalising problems (see Jami et al., 2021). This suggests that positive rearing environments may provide important targets for intervention and prevention.

Genetically informative designs have also been used to examine whether positive rearing environments may modify risk. Using a home-reared and adopted away co-sibling design of individuals at high risk for major depression, a study found that those reared in adoptive homes

(selected for high-quality rearing environments) had significantly reduced risk for major depression compared to individuals raised in their home environment (Kendler et al., 2020). This protective effect was no longer evident if an adoptive parent had major depressive disorder. This suggests that positive rearing environments can mitigate risk for major depression.

Using a longitudinal adoption-at-birth design, evidence suggests that structured guidance provided a buffering effect on toddler behaviour problems in those at high genetic risk, but did not help those at low genetic risk. Conversely, positive reinforcement benefited children regardless of genetic risk (Leve et al., 2009). This specificity could help to inform interventions. Other genetically informative designs have examined the role of parenting as a moderator of genetic risk. For example, a twin study suggested that other aspects of parenting (parental warmth/rewarding parenting) may moderate the relationship between genetic risk and the developmental of callous/unemotional traits (Henry et al., 2018). This suggests that warm and rewarding parents may mitigate risk.

Parent-offspring designs (including adopted and biological children) suggested that warmth in the mother-child relationship moderated the association between harsh parenting and child externalizing problems, such that the association between harsh parenting and child externalizing problems was stronger in the context of low maternal warmth, and weaker in the context of high maternal warmth. This pattern of association was observed whether or not the mother and child were genetically related, this ruling out passive rGE. This suggests that maternal warmth may modify risk of externalizing problems in children exposed to harsh parenting (Deater-Deckard, Ivy, & Petril, 2006).

Most genetically sensitive study designs have been used to identify likely causal environmental *risk* factors, and impacts of risks on child outcomes, making it more challenging to translate such findings in prevention and intervention contexts (Sellers et al., 2019). However, genetically informative designs can be used to examine protective factors that could help improve child psychopathology outcomes, by addressing processes that are not discernable in non-

genetically informed studies: for example, considering rGE processes, and GxE. Genetically informative research designs can be utilized in strengths-based research to investigate how positive environments can mitigate risk (or promote child strengths), thus provide a better understanding of modifiable environmental factors that could inform recommendations for prevention and intervention targets, as well as address research gaps to help inform practice and policy, and ultimately reduce adverse outcomes for children. Positive measures of family life (e.g., supportive interparental and parent-child relationships) as well as across other contexts (e.g. schools) therefore need to be examined in genetically informative designs in the future to understand potentially environmental contributions to the developmental course of different mental health and functional outcomes.

Conclusion

The modern researcher is faced with a growing range of genetically informative designs available to address important questions in the field of developmental psychopathology. Many of these designs whilst first developed to identify genetic contributions, provide powerful approaches for examining environmental factors that contribute to psychopathology. There are many excellent examples of how quantitative behavioral genetics approaches have been used to test and identify prenatal, family, and social factors that contribute to the risk of psychopathology, independent of genotype. However, most of this research has focused on the origins of psychopathology, not necessarily on developmental course and outcomes. Moreover, as the predominant focus has been on a deficit approach, interventions, clinical practice, and policy that focus on supporting positive rearing environments often lack good quality evidence (Leve et al., 2010). It is crucial to align research to intervention and prevention science efforts more closely by considering strength-based environments, and how these positive environments can mitigate risk (or promote child strengths) (Sellers et al., 2019). Although there is a wide array of different genetic designs, each has different strengths and limitations (Davey Smith, Richmond & Pingault, 2021) and these are not always

appreciated. Going forward, it will be important to select the design that is most appropriate for the question and to seek replication and convergence of findings across different study designs. Robust evidence that offers complement and replication across study designs is crucial for interventions and policies to be effective.

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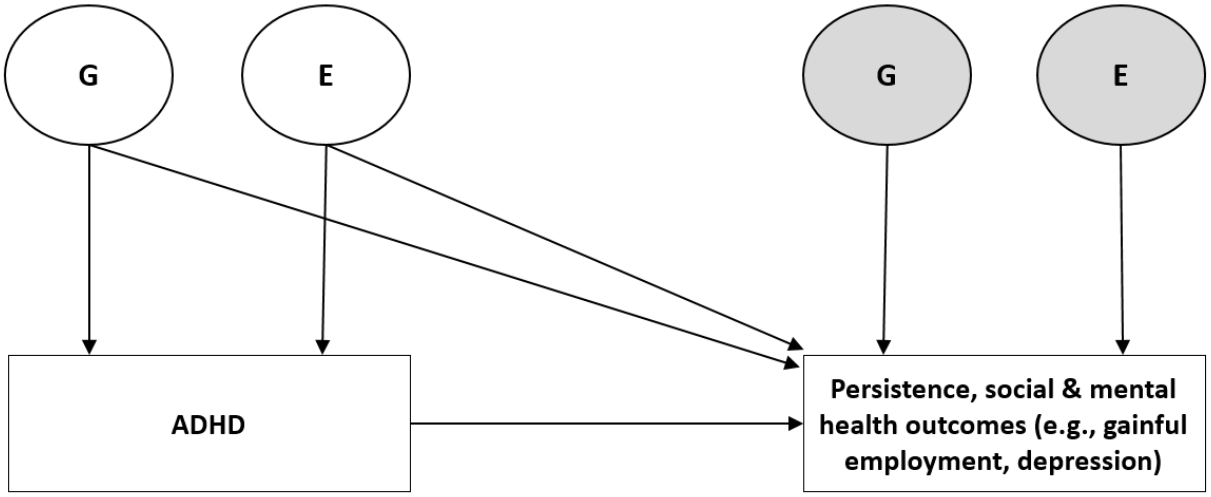

Figure 1. Genetic and environmental influences on the origins and development of later psychopathology and outcomes

Table 1. Summary of research designs

Research design	Study assumptions, strengths & limitations
QUANTITATIVE BEHAVIORAL RESEARCH DESIGNS	
Classic Twin design	Key Assumptions ● MZ twins share 100% of their segregating genes, whereas DZ twins share around 50%. ● Heritability estimates are specific to the phenotype and population from which they were derived ● Equal Environments Assumption (EEA), is the premise that MZ & DZ twin pairs share environments to the same extent. If the experiences of MZ twins are more similar, genetic influences would be overestimated ● Genetic – Environment independence (i.e., assumes no gene-environment interplay - violations may result in inaccurate estimates) Main Strengths ● Disentangle genetic and environmental factors for a trait of interest. ● Estimates proportion of variance in a trait attributable to genetic variation, shared environment or non-shared environment that includes measurement error ● Powerful tool for detecting genetic effects ● studies examining environmental exposures in child- and parent-twin samples can be used to identify the relevance of passive and non-passive (evocative or active) rGE ● Extending classic twin designs to examine longitudinal processes allows the examination of active rGE Main Limitations ● Unable to explicitly examine intergenerational effects. ● Classic twin model does not consider assortative mating (although it is possible to incorporate. See Horwitz et al., 2016) ● Possibility of passive rGE cannot be ruled out (effects of genes & environments within a related family cannot be separated). Heritability estimates capture passive rGE effects. ● Difficult to disentangle genetic from shared environmental effects when twins are reared together in the same household
Children of Twins (CoT)	Key Assumptions ● See assumptions from classic twin design ● The same genetic influences contribute across development (i.e. in different generations), and to the same extent across development i.e., no genes X age effects (Thapar & Rutter, 2019). ● Note that assumptions apply within an individual's own development, but also across a given population over time (i.e., across generations) Main Strengths ● Well-suited to examination of inter-generational transmission: opportunity to examine whether intergenerational transmission within families is explained by genes, environments, or both ● Estimates proportion of variance in a trait attributable to genetic variation, shared environment or non-shared environment for both parent twin, and offspring ● Can examine effects of passive rGE (not possible to estimate in classic twin design) ● Can examine different phenotypes in parent and child generations Main Limitations ● Difficulties accounting for spouse of twins: as spouses/partners are not included in traditional CoT Design, results may be biased due to assortative mating effects

	● Associations between parental characteristics & child outcomes may be due to reverse causation (i.e., child effects on parents): Any child-to-parent effects will be subsumed into the parent-to-child effect estimates, and genetic confounding will appear as passive rGE. Extended CoT designs address this limitation (Narusyte et al., 2008) ● Relatively low statistical power ● Less power to detect genetic effects unique to the offspring generation than for parent generation, and less power to detect genetic effects shared between parent & child. Cannot estimate the role of environmental effects shared by siblings in the offspring generation. Recent extension of CoT – multiple children of twins (MCot) addresses some of these limitations (see McAdams et al., 2018).
Maternal vs. paternal exposure during pregnancy	Key Assumptions ● Compares maternal, paternal exposures during pregnancy & their associations with offspring outcome. Intrauterine contribution is possible for the mother-child association but not for father-child associations ● Mothers and fathers both share 50% of genetic material with their offspring (genetic contribution shared between parent & child is held constant). Main Strengths ● Can control for unknown & known confounders ● Disentangles intrauterine environment from residual confounding (biological intrauterine effects indicated by stronger maternal association, compared with the paternal association, since paternal exposures would not normally be expected to affect the intrauterine environment). ● Possible to recruit large representative samples Main Limitations ● Does not take into account assortative mating ● Limited to exposures both parents could experience in pregnancy (see Thapar & Rutter, 2019) ● Assumptions violated if confounding structure of maternal & paternal exposures differs (Stronger maternal associations may be observed if maternal confounders are more strongly related to maternal exposure than the paternal confounders are with the paternal exposure).
Discordant sibling	Key Assumptions ● Sibling comparisons assume a stable family & social context. ● Assumes that one sibling's exposure does not influence the unexposed sibling Main Strengths ● Siblings are essentially a 'matched' case-control comparison, matched for many potential confounders ● Disentangles genetic from prenatal environmental risks, and well-suited for assessing postnatal exposures ● Can employ large population-based registries Main Limitations ● Problems of selection bias (mothers are behaving differently in different pregnancies). ● Siblings born at different times will be exposed to different family- and population-level risks ● Differences between siblings may arise from another exposure. For exposures after birth, genetic differences between siblings will also contribute. ● May not be generalizable to general population ● Where results suggest findings are due to confounding, we do not know whether confounding is due to genes, environments, or both ● Can be used to examine adolescent/adult exposures and outcomes but additional limitation to interpretation includes reverse causation

Children conceived via assisted reproductive technologies (ART)	Key Assumptions ● Compares associations between pairs of mother/fathers and children who are genetically related or unrelated. This allows the examination of whether associations between parent & child are primarily genetically mediated, environmentally mediated, or a combination of the two. Egg donation and gestational surrogacy allow the examination of prenatal influences separate from genetic influences ● Considered 'adoption at conception': see adoption study design assumptions below. Main Strengths ● Unambiguously separates genetic & intrauterine influences [e.g., Gestational surrogacy (whereby children are genetically related to both parents but the prenatal environment is provided by a surrogate)] which is not possible in twin or adoption studies ● Removes confound of passive rGE Main Limitations ● Representativeness of families who have undergone IVF treatment ● Low prevalence of some type of risk factors (e.g., maternal smoking in pregnancy) ● Small sample sizes in some informative groups (e.g., unrelated mother– child pairs).
Parent-offspring adoption study design	Key Assumptions ● Compares associations between adopted children & their adoptive/rearing parents with biological parents ● Associations between biological parents and child are assumed to be due primarily to genetic influences (and prenatal influences for biological mothers) ● Associations between adoptive parents and adoptive child assumed to be due primarily to rearing environments Main Strengths ● Disentangle inherited and prenatal exposure effects from postnatal rearing environmental effects, and well-suited for assessing postnatal exposures ● Removes confound of passive rGE. ● Possible to examine influence of evocative inherited child effects on the rearing environment ● Can be used to test gene-environment interaction ● Well suited to examination of intergenerational genetic and environmental transmission Main Limitations ● Cannot disentangle prenatal from biological mother genetic effects ● Selective placements ensuring positive adoptive environments may limit ability to examine some postnatal risks
MOLECULAR GENETIC APPROACHES	
Polygenic scores (PGS)	Key Assumptions ● Risk allele effect sizes are the same in the discovery and target samples ● Risk alleles included in the polygenic scores are independent ● Samples include individuals from genetically homogenous populations Main Strengths ● Useful indicator of genetic liability in samples that are not otherwise genetically informative (i.e., they do not contain related individuals) ● Data gathered from large GWAS discovery samples can be applied to smaller target samples Main Limitations ● Requires very large discovery GWAS sample sizes

	● Requires discovery GWAS samples of similar ethnic origin ● Bias can be introduced by overlapping discovery/target samples ● Does not capture all genetic variation ● Typically small effect sizes ● PGS derived from GWAS do not reflect gene networks that code for biological functioning. Recent extensions of PGS include biologically informed PGS (e.g., Dass et al., 2019) and pathway PRS (e.g., O'Reilly et al., 2021)
Mendelian Randomization	Key Assumptions ● Genetic proxies are strongly associated with the exposure ● No unmeasured confounders of the association between genetic proxies and the outcomes ● Genetic variants are associated with the outcome only via the exposure Main Strengths ● Minimal confounding and rules out reverse causation to strengthen causal inference Main Limitations ● Dependent on large discovery GWAS sample sizes to provide strong genetic proxies ● Less powered to detect associations in the presence of horizontal pleiotropy

For further details regarding assumptions, strengths and limitations of quantitative behavioural research designs, see Thapar & Rice (2020) and Thapar & Rutter (2019), Rutter & Thapar (2016), and Knopik et al. (2017). See also Davey-Smith, Richmond and Pingault (2022) and Smith et al. (2021).

Table 2. Description of different types of gene-environment interplay

Term	Definition
Passive gene–environment correlation (*r*GE)	Where parents and children are genetically related, parents' genes (which are shared with their offspring) may be correlated with the environment they provide, confounding associations between family and child level variables. Specific environments may be markers of parental genetic risk rather than a causal environmental process.
Evocative gene-environment correlation (rGE)	Genetically influenced characteristics in a child may evoke particular responses from others. The field of intervention research, suggests specific environmental processes can be identified and made 'resilient' to child-driven effects.
Active gene-environment correlation (rGE)	A child actively selects environments that are correlated with their genetically influenced characteristics.
Gene-environment interaction (GxE)	Environmental contexts and processes may modify the manifestation of genetic liability. ● The 'diathesis-stress' model: psychopathology results from inherited risk that occurs under particular environmental risks. ● Differential susceptibility: an individual is differentially susceptible to high levels of both positive and negative environments

For more information regarding these processes see: Ge, et al., 1996; Jaffee & Price, 2008; Jaffee & Price, 2012; Knafo & Jaffee, 2013; Knopik et al., 2017; Luthar & Brown, 2007; Price & Jaffee, 2008; Reiss, Leve & Neiderhiser, 2013; Scarr & McCartney, 1983.