Identifying the contribution of prenatal risk factors to offspring development and psychopathology: what designs to use and a critique of literature on maternal smoking and stress in pregnancy

Abstract

Identifying prenatal environmental factors that have genuinely causal effects on psychopathology is an important research priority but it is crucial to select an appropriate research design. In this review we explain why and what sorts of designs are preferable and focus on genetically informed/sensitive designs. In the field of developmental psychopathology, causal inferences about prenatal risks have not always been based on evidence generated from appropriate designs. We focus on reported links between maternal smoking during pregnancy and offspring ADHD or conduct problems. Undertaking a systematic review of findings from genetically informed designs and 'triangulating' evidence from studies with different patterns of bias, we conclude that at present findings suggest it is unlikely that there is a substantial causal effect of maternal smoking in pregnancy on either ADHD or conduct problems. In contrast, for offspring birth weight (which serves as a positive control) findings strongly support a negative causal effect of maternal smoking in pregnancy. For maternal pregnancy stress, too few studies use genetically sensitive designs to draw firm conclusions but continuity with postnatal stress seems important. We highlight the importance of moving beyond observational designs, for systematic evaluation of the breadth of available evidence and choosing innovative designs. We conclude that a broader set of prenatal risk factors should be examined including those relevant in low and middle income contexts. Future directions include a greater use of molecular genetically informed designs such as Mendelian Randomization to test causal hypotheses about prenatal exposure and offspring outcome.

Keywords: prenatal, stress, smoking, genetically sensitive, causal

Introduction

There is considerable interest in the possibility that exposure to events during intra-uterine life can influence subsequent development. Indeed, if early environmental exposures have causal effects on the likelihood of psychopathology later in life, this has clear implications for early intervention and prevention. The teratogenic effects of thalidomide, rubella, high levels of alcohol and most recently Zika virus infection on the fetus are well known (Rasmussen, Jamieson, Honein, & Petersen, 2016; Thapar & Rutter, 2009). In more recent years, the effects of exposures to a broader set of prenatal risks on the development of the offspring have been examined. These risks include exposures such as maternal smoking during pregnancy, maternal depression, anxiety and stress during pregnancy, inadequate maternal nutrition, certain types of medication (e.g. antidepressants), toxins (e.g. lead) and maternal physical illness (e.g. autoimmune diseases) (Instanes et al., 2017). The hypothesized causal mechanisms include direct toxic effects on the fetal brain, hypoxia, disrupted placental function, immune and inflammatory processes and "developmental programming" that leads to later adult disease. The developmental origins of adult disease (Barker, 2007) is a hypothesis that was first considered in relation to ischemic heart disease and type 2 diabetes and subsequently has received considerable attention. It suggests that intrauterine exposure to adversity (e.g. under-nutrition) during a sensitive period of development (fetal life) leads to potentially permanent alterations in the structure, physiology and metabolism of the organism and this in turn increases susceptibility to later disease (e.g. ischemic heart disease). Nonetheless, as documented in detail elsewhere, there are numerous challenges in establishing whether environmental exposures exert true causal risk effects on developmental outcomes (D'Onofrio, Class, Lahey, & Larsson, 2014; Gage, Munafo, & Davey-Smith, 2016; Rutter, Pickles, Murray, & Eaves, 2001; Rutter & Thapar, 2016; Thapar & Rutter, 2015). These include reverse causation, continuing adversity following the initial exposure and measured and unmeasured confounding. Reverse causation highlights the possibility that the outcome might cause the exposure rather than the other way round. The classic example of this relates to the re-investigation of socialization effects as child effects on parents (Bell, 1968). There are now many examples of instances where children's behavior and psychopathology has effects on parents (Anderson, Hytton, & Romney, 1986; Sellers et al., 2016). Often exposures of interest are associated

with continuity over time, for instance it may be difficult to disentangle the risk effects of exposure to stress in utero from stress exposure later in development (D. Lawlor et al., 2017; Thapar & Rutter, 2009). In the case of confounding, seemingly causal links can be explained by confounding variables that are associated with both exposure and outcome and it is not necessarily possible to measure or test for all possible confounders meaning that residual confounding is a serious problem for observational studies. Residual confounding therefore refers to confounding that remains even when the effect of measured confounders is included in statistical analyses and arises because of measurement error in confounders and unmeasured confounding (Fewell, Davey Smith, & Sterne, 2007). This means that erroneous conclusions about causality can be and are drawn from such designs.

One key challenge to rule out is the possibility that an observed association is due to person-environment correlation as this is potentially an important source of confounding in relation to psychopathology; for example where maternal characteristics influence the exposure (e.g. diet during pregnancy) and outcome variables (e.g. her offspring's behavior). Passive gene-environment correlation (rGE) is a special instance of a person-environment correlation, where the prenatal environment is indexed in part by maternal characteristics including genetic factors that are transmitted to the offspring (mothers and offspring share 50% of their genome; Figure 1). Thus, observational studies that find association between a prenatal exposure and offspring psychopathology are liable to identifying associations that are not necessarily causal. However there are designs that enable more robust assessments of causal inference (Davey-Smith, 2008; Gage et al., 2016; Rutter & Thapar, 2016; Thapar & Rutter, 2015). Genetically informed designs are especially attractive because they separate the genetic and environmental contributions to the association between intrauterine exposure and offspring outcome. The relevance of genetic designs for assessing environmental risk is now widely appreciated in the field of developmental psychopathology. However it is not always recognized that the designs that distinguish relevant genetic and environmental contributions differ for prenatal and postnatal exposures (see Figure 1 and Table 1); we describe these in detail in this review. The genetic and environmental contributions that need to be

separated when investigating prenatal risks are those shared between parents and offspring. For prenatal/intrauterine exposures the contribution of maternal behaviors and genes is especially important. In this review we focus on the genetically informed family-based comparison designs where either the degree of genetic relatedness differs between types of mother-offspring pair or the genetic relationship is held constant and the intrauterine environment varies (Figure 1; Table 1). These sorts of designs have been used widely to examine questions about the causal relationship between specific prenatal exposures and offspring outcomes and they allow inferences to be made about separating the contribution of the maternal genome from the intrauterine environment. We note however that there are other types of genetically informed designs (e.g. Mendelian Randomization, the polygenic transmission disequilibrium test) (Davey Smith & Hemani, 2014; Weiner et al., 2017) that use information on the specific genetic variants involved in a trait (as opposed to inferring the effects of the entire maternal genome). These sorts of designs have not yet been widely used for prenatal exposures and offspring outcomes and currently capture a small proportion of the genetic variation involved. They are however, likely to become more important in the future as genome wide association studies identify increasing numbers of genetic variants that are robustly associated with psychopathology and health-related behaviors. These sorts of designs are also useful for triangulation of evidence (see below for definition).

There is good evidence from observational studies, including meta-analyses, that a number of different exposures during prenatal development show association with psychopathology in offspring (Abraham et al., 2017; Rice, Jones, & Thapar, 2007; Ruisch, Dietrich, Glennon, Buitelaar, & Hoekstra, 2017; Talge et al., 2007). One of the most widely examined exposures is maternal smoking during pregnancy which has been observed to be associated with increased symptoms of ADHD and conduct problems in offspring (Huizink & Mulder, 2006; Langley, Rice, van den Bree, & Thapar, 2005; Linnet et al., 2003). Other studies have focused on severely restricted maternal nutrition which is associated with an increased risk of psychosis and depression in offspring when they reach adult life (Brown et al., 2000; St Clair et al., 2005) and maternal stress which is associated with a wide range of symptoms of psychopathology in offspring (Rice et al., 2008; Talge et al., 2007). Much recent interest has focused on maternal use of medications during pregnancy including

antidepressants and acetaminophen (paracetamol) as well as maternal chronic illnesses (Avella-Garcia et al., 2016; A. S. Brown et al., 2016; H. K. Brown et al., 2017; Grzeskowiak et al., 2016; Instanes et al., 2017; Man et al., 2017; Rai et al., 2017; Stergiakouli, Thapar, & Davey Smith, 2016). However, it is unclear to what extent these observed associations are due to prenatal causal risk effects or other factors including familial and genetic confounding. Some investigators explicitly acknowledge this (e.g. Instantes et al., 2017), others do not. Fortunately, there is growing interest in alternative methods for assessing causality. The importance of considering and testing for the possibility that observed associations between prenatal exposures and offspring outcomes may not be causal has been highlighted for scientific reasons. It is also important for practical and policy reasons including ensuring that pregnant women receive clear and appropriate as well as accurate advice and guidance, providing antenatal care that is consistent with current scientific evidence and avoiding the possibility of wasting resources on ineffective intervention (Gage et al., 2016; Thapar & Rutter, 2009; Rutter et al., 2007).

In this review we begin by describing the phenomenon of person-environment correlation and passive rGE in detail and present new data on maternal smoking during pregnancy from the Cardiff IVF study (Thapar et al., 2007) to illustrate key points. Next we explain the genetically informative research designs that can address familial confounding and passive rGE for prenatal exposures and consider their strengths and limitations. We then systematically assess studies for two prenatal exposures where the plausible hypothesized processes underlying any potential causal association differ. The first is maternal smoking in pregnancy where any possible causal effect on offspring development and psychopathology seems likely to come about via effects of toxin exposure and/or effects secondary to this such as effects on blood flow or placental functioning that directly affect the developing brain (Ruisch et al., 2017; Slotkin, 2013). The second is maternal stress during pregnancy where developmental programming of the HPA (hypothalamic pituitary adrenal) axis is hypothesized to underlie any potential causal effect on offspring psychopathology (Talge et al., 2007). For maternal smoking during pregnancy, a large number of studies have been carried out and therefore we selected studies to review that have reported links between maternal smoking in pregnancy and offspring conduct problems and ADHD. The reason for selecting those outcomes is because reported

results have been somewhat inconsistent and misinterpreted meaning a systematic review of the findings from informative study designs would be useful and is important in the context of triangulation of evidence. Triangulation has been described as 'the practice of obtaining more reliable answers to research questions through integrating results from several different approaches where each approach has different key sources of potential bias that are unrelated to each other' (D. A. Lawlor, Tilling, & Davey Smith, 2016). Thus, it involves evaluating evidence from different studies that employ different research designs that have differing patterns of strength and weakness – where results converge that strengthens the evidence for the reasons for an observed association (causal or not), where they do not it requires careful consideration of the evidence, the likely biases involved and identification of what further research is needed (D. A. Lawlor et al., 2016). This process has some similarities with the concept of 'constructive replication' whereby replication of findings is seen to strengthen evidence only if it removes some weakness in previous studies (The Academy of Medical Sciences., 2007). Finally we highlight areas for future work.

What is person-environment correlation and why is it important for prenatal risk exposures?

Developmental science shows that people behave in ways that shape their environments and these environments have important implications for developmental psychopathology. For instance, children with antisocial behavior evoke hostile reactions from others which serve to further exacerbate that behavior in the child (Anderson et al., 1986; Ge et al., 1996; Rutter, Moffitt & Caspi, 2006). Individual differences in personality can also affect a persons' environment – for example a child concentrating and focusing on an academic task may elicit responses from a teacher that sustains that behavior (Shiner & Caspi, 2003). Person-environment correlation also applies to maternal behaviors during pregnancy in that there are measurable differences between mothers that engage in risk behaviors during pregnancy or experience stress and antenatal complications compared to those who do not. For example, mothers who smoke during pregnancy are younger, are more likely to be raising their children in a deprived socioeconomic background, have higher rates of psychopathology (depression and antisocial behavior) and substance use, report greater stress during pregnancy and are more likely to be nicotine dependent (D'Onofrio et al., 2013; Gilman et al., 2008; Gustavason et al.,

2017; Maughan et al., 2004; Rice et al., 2009). Data from the Cardiff IVF sample illustrated in Table 2 also illustrate this point in that mothers who do not smoke, abstain from smoking to prepare for pregnancy or continue smoking during pregnancy differ on socio-economic factors, psychopathology and amount smoked prior to pregnancy. Data on medical complications during pregnancy are also consistent with a different form of person effects on the prenatal environment, in terms of maternal disease liability that could be transmitted to offspring, rather than maternal behavior. For example, women who develop pre-eclampsia, gestational hypertension or abruption or infarction of the placenta are at heighted risk for later developing cardiovascular disease and diabetes after pregnancy (Ray et al., 2005; McDonald et al., 2008; Kaaja & Greer, 2005). This implies therefore that pregnancy may reveal biological vulnerabilities for chronic physical disease which lie dormant before pregnancy. Thus, if women's offspring develop similar illnesses, this could be due to inherited liability not necessarily because of prenatal exposure to the disease. These observations and data then serve to illustrate the point that maternal characteristics influence both the prenatal and the postnatal rearing environment. What implications does this have for research examining the influence of prenatal exposures on offspring development and psychopathology? One major issue is that the factors associated with these differences in the prenatal environment (e.g. for maternal smoking in pregnancy - socio-economic factors, psychopathology) are in themselves associated with developmental differences and psychopathology in offspring (D'Onofrio et al., 2013; Reppetti et al., 2001). This then raises the issue that confounding may account for associations between prenatal smoking and offspring psychopathology. For instance, it is possible that the association between prenatal smoking and offspring outcome could be due to common confounding causes including genetic ones, as highlighted earlier (see Figure 1). We will illustrate later that including measured confounders (e.g. parent psychopathology) into statistical tests of association does not remove the problem (e.g. D'Onofrio et al., 2012; Gustavson et al., 2017; Rice et al., 2009; Thapar et al., 2009).

What is passive rGE?

Gene-environment correlation (rGE) occurs when the genetic and environmental contributors to a trait, behavior or exposure are correlated. Three key types have been distinguished – passive,

evocative and active (Plomin, DeFries, & Loehlin, 1977). Here, we focus on "passive rGE" that refers to the special instance in which the child's genotype is correlated with the environment provided by his/her parents. This occurs because parents typically provide both genes and environment to their children. This means that the prenatal and the postnatal rearing environment are correlated with genetic characteristics in the parental generation, and because parents pass genes on to their offspring, also in the child generation (Figure 1). Many postnatal environmental factors that have important risk effects on psychopathology in children such as parenting style and stressful life events are influenced by parent's heritable characteristics (Jaffee & Price, 2008; Kendler & Baker, 2007; Reiss, Neiderhiser, Hetherington & Plomin, 2000). This is also true for the prenatal environment and we use the example of maternal smoking during pregnancy to illustrate the point. As described above, there are systematic differences between women who smoke and do not smoke during pregnancy (see also Table 2). Indeed, smoking behavior is a heritable trait, with twin studies showing heritability estimates of between 50-70% for smoking persistence and nicotine dependence (Kendler, Neale & Sullivan, 1999; Lessov et al., 2004; Li, Cheng et al., 2003; Maes, Sullivan et al., 2004) and genome wide association studies have identified a number of genetic loci that increase susceptibility for smoking-related behaviors (number of cigarettes smoked per day, smoking initiation and smoking cessation) (Furberg et al., 2010). The fact that smoking behavior is heritable then raises the possibility that prenatal exposure to smoking - an apparently "environmental" risk factor - is in fact a marker of maternal genetic predisposition and these same risk genotypes are then transmitted to the next generation and influence risk for psychopathology in the offspring (Figure 1). This supposition is supported by the observation that mothers who smoke and those who do not systematically differ on factors important for children's development (e.g. maternal psychopathology, substance use, maternal education) and that are heritable. More recent molecular genetic studies also find that genetic risks that contribute to smoking behavior are correlated with those that contribute to psychopathology including ADHD (Derogatis et al., 2017).

Genetically informed designs are valuable for assessing the role of unmeasured or imperfectly measured confounding including familial and genetic confounding. Confounding – where the

exposure and outcome examined have common causes – is a major threat to the validity of

observational studies. Where randomization of exposures is not possible or ethical (e.g. randomly

exposing offspring to cigarette smoke in utero) then genetically sensitive designs (and other types of

natural experiment and quasi-experimental designs) are extremely useful. Next, we provide a

description of the sorts of designs that are required to tease apart environmental and genetic factors

contributing to the association between prenatal exposures and offspring outcome because they are

different from the typical designs used to tease apart genetic and environmental influences relevant to

postnatal exposures (Figure 2; Table 1).

Which genetically informative designs are helpful for detecting familial confounding and passive rGE

for prenatal exposures?

While traditional observational studies cannot distinguish between causal intrauterine effects

and rGE, a number of designs are able to separate the prenatal environment from genetic factors

shared between parent/mother and offspring (Figure 2; Table 1). 1) The comparison of maternal vs.

paternal prenatal exposure associations with offspring outcomes. Only in the mother-child association

is there a possibility of a direct intrauterine effect but mothers and fathers both share 50% of their

genes with their offspring meaning that the extent to which association between the prenatal exposure

and offspring outcome indexes genetic effects shared between parent and child can be assessed. In

effect, the inclusion of data on paternal exposure serves as a negative control (Gage et al., 2016).

Taking the example of smoking, in the case of a causal intrauterine effect, no independent association

should be observed between paternal smoking and offspring outcome. But, if the association is due to

either unmeasured genetic factors or other confounders, the risk to offspring of an adverse outcome

should be of similar magnitudes regardless of which parent smokes (Langley et al., 2012). 2) Sibling

comparison designs where differentially exposed sibling pairs are compared e.g. where a mother

smoked for one pregnancy and not another. In effect, siblings are matched 'by nature' on many

confounders including those that are unmeasured or unknown making this a convenient method for

dealing with confounding (Sjolander & Zetterqvist, 2017). The use of the unexposed sibling group as

a control comparison allows the effect of familial confounding for all factors shared within the family

to be assessed. A comparison of differentially exposed cousins allows for the control of some shared familial cofounding but less so than for siblings. 3) The Children of Twins Design. For prenatal exposures, the comparison of the offspring of identical mother twins is most informative – where the offspring of identical twin mothers are equally related to their mother (50%) and their aunt (50%) but the cousins experience a different prenatal environment. This design has not yet been widely employed for investigating prenatal exposures on child developmental outcome (see Knopik et al (2006) and D'Onofrio et al (2003) as exceptions). 4) The IVF design where related and un-related mother-offspring pairs are compared – this is a prenatal cross-fostering design meaning some mothers experience a pregnancy for a child to whom they are not genetically related (by either egg/embryo donation or gestational surrogacy). In unrelated mother-child pairs (where an unrelated mother/surrogate experiences the pregnancy) then association between a prenatal exposure and a child outcome must come about through intrauterine effects because while the mother/surrogate experiences the pregnancy, she shares no genes with the baby meaning prenatal passive rGE is removed.

What these designs have in common is that they allow the effect of the intrauterine environment to be differentiated from genetic factors that mothers share with their offspring (Table 1; Figure 2). In essence, the designs do this in one of two ways: complete separation of the maternal genome shared with the offspring and the prenatal environment (IVF prenatal cross-fostering design) or by varying the prenatal environment (e.g. across different pregnancies in the same mum or in the separate pregnancies of identical twin mums) while holding the mother-child genetic relationship constant (Figure 2). These are the crucial aspects of addressing passive gene-environmental correlation for prenatal environmental exposures and as such the family-based genetically sensitive methods differ for prenatal and postnatal exposures. The comparison of maternal and paternal prenatal exposure associations also provides a useful type of negative control because only in mothers is it plausible that there is an intrauterine effect of the exposure variable (for smoking - in the absence of a substantial passive smoking effect (Gage et al., 2016; Langley, Heron, Smith, & Thapar, 2012). We next explain why the genetically sensitive designs typically used for separating genetic and environmental contributions to postnatal environments (adoption-studies-after-birth and twin studies)

are inappropriate for prenatal risks before describing the strengths and limitations of the appropriate prenatal genetically sensitive designs.

Why adoption after birth studies are not informative for identifying prenatal passive rGE

As described above, the key requirement for detecting passive rGE in the case of prenatal exposure variables is that the effect of the intrauterine environment can be isolated from genetic factors that mothers share with their offspring. This requirement means that many of the usual genetically sensitive designs such as twin studies and adoption studies where children are adopted after birth, are not useful for detecting passive gene environment correlation for prenatal environmental exposures. Adoption-after-birth studies can instead be used to examine whether the postnatal rearing environment has any moderating effect on the relationship between a prenatal exposure and an offspring outcome (Gaysina et al., 2013; Rice, Jones, et al., 2007). For standard adoption designs where the genetic mother experiences the pregnancy but the child is adopted after birth, there is no separation of the intrauterine environment from (biological) mother provided genetic effects - because biological mother provides genes and the prenatal environment to her offspring, even though she does not provide the postnatal rearing (Table 1). This means that the basic comparison between prenatal exposure and offspring outcome in the genetic mother whose child is then adopted is essentially exactly the same as it would be in a standard observational design. Unfortunately, this failure of adoption studies to address prenatal passive gene-environment correlation has not always been understood or clearly explicated meaning that erroneous conclusions may have been made (Dolan et al., 2016; Gage et al., 2016; Gaysina et al., 2013; Slotkin, 2013). Thus, although adoption studies are thought to lead to the removal of passive gene-environment correlation, this only refers to passive rGE for the *postnatal rearing environment* (Rutter et al., 2001). It is also known that mothers whose children are adopted are systematically different from mothers who do not (Rutter et al., 2001). It is likely that this creates differences in the prenatal environment of children who are adopted compared to children who continue to live with their biological parent(s). Thus, mothers whose children are adopted after birth show higher rates of smoking, alcohol use and illicit substance use during pregnancy and have higher rates of psychopathology, including ADHD and conduct problems,

than mothers whose children continue to reside with them after birth (Gaysina et al., 2103). This creates a situation where biological mothers whose offspring are adopted away are a group at high background risk for psychopathology and risky prenatal exposures and some of this risk will be due to dispositional and genetic factors which biological mothers share with their offspring. This means the degree of familial confounding is potentially higher than is typical. It then follows that if passive rGE for the prenatal environment and child outcome applies, one would expect to see stronger association in an adoption-study-after-birth (when the prenatal exposure is assessed in the biological mother) than in a standard epidemiological design. In fact, this is what has been observed for prenatal smoking and offspring conduct problems when those adopted-after-birth (b=4.27, 95% CI= -.90, 9.44 adjusted) are compared to those reared by their biological parents in the same cohort (b=.82, 95% CI= .08, 1.56 adjusted) and from a meta-analysis (b=2.17, 95% CI= .72, 3.62 adjusted reared by adoptive parents; b=1.13, 95% CI= .02, 2.24 adjusted reared by biological parents) (Gaysina et al., 2013). This observation therefore provides indirect evidence that there is passive rGE that applies to the link between maternal smoking during pregnancy and offspring conduct problems.

Why twin studies are not informative for identifying prenatal passive rGE

The standard twin design involves comparing the phenotypic similarity of identical (monozygotic; MZ) and non-identical (dizygotic; DZ) twins. MZ twins share all their genes in common and DZ twins share, on average, half their genes in common. Thus, comparing the similarity of MZ and DZ twins allows the variance of a trait to be decomposed into the proportions due to additive genetic effects, shared environmental effects (environmental influences that make members of a twin pair more similar) and unique or non-shared environmental effects (environmental effects that make members of a twin pair different). In the standard twin design and its extensions such as identical twin differences, it is not possible to identify twin pairs differentially exposed to a prenatal exposure because twins share a prenatal environment (at least as far as is typically measured) and all types of twin share exactly half their genes with their biological mother. This means that each member of a twin pair will be equivalently exposed to a prenatal exposure e.g. smoking in pregnancy and that the genetic relationship between mother and twin offspring does not differ across twin pairs.

The standard twin design is therefore uninformative for the separation of genetic and environmental contributions to a prenatal exposure and offspring outcome. However it can be used for assessing the role of perinatal risk factors on which twins may differ such as birth weight (Tully et al., 2004).

Strengths and limitations of the prenatal genetically informative designs

Four genetically sensitive designs for assessing the familial/genetic and environmental contributions to *prenatal* risk exposures and offspring outcome were described. Each has strengths and limitations which we review briefly below. 1) The comparison of prenatal exposures in mothers and fathers is a convenient approach that controls for the genetic relationship between parent and child since children share exactly half of their genes with each parent. However, it is potentially contaminated by assortative mating, shared couple behaviors, the shared postnatal family environment and its assumptions can be violated if the confounding structure of the maternal and paternal exposures differ (Keyes et al., 2014). For some exposures e.g. cigarette smoking, passive exposure to paternal risks is a potential problem (e.g. father or other household members continue to smoke and mother and baby are exposed). 2) The sibling comparison study is a convenient way of controlling for confounding factors shared by family members and the existence of large population registers in many Scandinavian countries has meant that extremely large sample sizes representative of the general population are available. This is an important strength. However, only siblings that have different prenatal risk exposures contribute to the meaningful comparison in discordant sibling comparisons and therefore such designs are susceptible to confounding by nonshared factors that might lead to such changes in the mother (Frisell et al., 2012). Also there is the problem of carry over effects where the exposure and outcome of one offspring affects the exposure and outcome of their siblings (Sjölander et al., 2017). One instance where carry-over effects might exist would be if Caesarean section was the exposure variable, where a Caesarean section in one pregnancy might well affect the likelihood of exposure in a subsequent pregnancy. Nonetheless, tests of carry over effects to date have not found this to be an issue for maternal smoking during pregnancy and offspring conduct problems or ADHD (D'Onofrio et al., 2010; D'Onofrio, Van Hulle, Goodnight, Rathouz, & Lahey, 2012; Skoglund, Chen, D'Onofrio, Lichtenstein, & Larsson, 2014). 3) The children of twin

mothers design provides an opportunity for investigating the effects of the prenatal environment controlling for shared maternal genes. Strengths include the ability to estimate genetic and environmental influences in the parent and child generation in addition to genetic and environmental transmission paths (using structural equation modelling) without the need for strong assumptions (D'Onofrio et al., 2003) and the existence of statistical models to test a variety of extensions to the design (Silberg, Maes, & Eaves, 2010). Limitations include the need to consider paternal effects, assortative mating, the need for sufficient numbers of similarly aged offspring from identical twin mothers and the need for large sample sizes. 4) The IVF design allows unambiguous separation of the prenatal environment from the maternal genome making it a powerful approach for detecting prenatal passive rGE. One main limitation is generalizability – are those that conceive via IVF similar to the population that conceives naturally? The evidence shows that for parental psychopathology, child psychopathology and the family environment the answer to this question is yes (Golombok, 2017; Golombok & MacCallum, 2003; Shelton et al., 2009). However, those conceiving via IVF are at elevated risk of perinatal complications and the rates of exposure for some prenatal risks (e.g. maternal smoking during pregnancy) are low. Another limitation is that sample sizes of the informative groups (i.e. unrelated mother child pairs) are also small given the considerable effort involved in identifying these groups. As is the case for all studies, indicators of study quality such as reliability and validity of measurement, adequate sample size and tests that the assumptions of the design are met also apply to genetically sensitive designs and consideration of these issues is informative for 'triangulation' of findings.

As described elsewhere, each of the quasi-experimental, genetically informed designs we have highlighted has a different set of strengths and weaknesses and none is without limitations (Rutter & Thapar, 2016). Nonetheless, the value of 'natural experiments' that tease apart variables that usually go together has been noted as providing important additional leverage in answering questions of environmental causation. A number of other 'natural experiment' approaches that do not directly distinguish the intrauterine environment from genetic factors shared between mother and offspring but that can be informative have been discussed in detail elsewhere (Academy of Medical Sciences, 2007; Gage et al., 2016; Rutter, 2007; Rutter & Thapar, 2016; Thapar & Rutter, 2015).

These include utilizing naturally occurring situations that have involved universally introduction or removal of prenatal risk. The best example here being the Dutch Hunger Winter and Chinese famine studies which suggest that extreme prenatal nutritional adversity has likely causal risk effects on later schizophrenia (Lumey, Stein, & Susser, 2011; St Clair et al., 2005; Susser et al., 1996). Other methods not yet mentioned include using changes in policy as natural experiments, instrumental variable approaches other than Mendelian Randomization, and cross-cultural comparisons where the confounding structure of exposure variables differs (Davey-Smith & Hemani, 2014; Gage et al., 2016; Thapar & Rutter, 2015). Animal studies that enable experimental design can also be helpful but here there is the difficulty in assuming that offspring behavior in other species can be equated to child psychopathology (Thapar & Rutter, 2015). Comparing prenatal factors in siblings with and without a psychiatric diagnosis can be informative (Grizenko et al., 2012; Oerlemans et al., 2015) but population-based registers are needed to overcome issues of ascertainment and retrospective recall bias. We do not directly include studies using these methods in our review of prenatal smoking and gestational stress and offspring psychopathology.

Method

The effects of maternal smoking during pregnancy have been examined for a wide range of developmental outcomes including child psychopathology. For some outcomes the findings from such studies appear inconsistent. For the outcome of offspring birth weight, findings from a range of genetically informative designs including multiple maternal vs. paternal comparisons, discordant sibling studies, a children of twins study and an IVF study are remarkably consistent and consistent with a causal interpretation in that regardless of familial confounding or genes shared between mother and child, birth weight is reduced in the infants of mothers that smoked during pregnancy (D'Onofrio et al., 2003; Ellingson, Goodnight, Van Hulle, Waldman, & D'Onofrio, 2014; Gilman, Gardener, & Buka, 2008; Kuja-Halkola, D'Onofrio, Larsson, & Lichtenstein, 2014; Langley et al., 2012; Obel et al., 2016; Rice et al., 2009). We carried out a systematic search for studies of maternal smoking during pregnancy and offspring conduct problems and ADHD where results from genetically informative studies appear to be less consistent. We sought to identify studies using informative

research designs (i.e. paternal vs maternal smoking during pregnancy comparisons; discordant sibling and/or cousin comparisons; IVF design which includes unrelated 'prenatal' mother-child pairs; children of twin studies). Figure 3 illustrates a flow chart of the search process and full details can be found in Appendix 1. The results of the identified genetically informed family-based studies are summarized in Table 3. In our interpretation of results we consider the following: magnitude of effect sizes, precision of effect sizes (i.e. the width of confidence intervals), the extent to which results are consistent across indicators of the same construct and analytical options (also referred to as vibration of effects) (Button et al., 2013), and consistency in the pattern of results when negative controls are used.

Results

Maternal smoking during pregnancy and offspring conduct problems: findings from genetically informed family-based designs

Nine studies utilized an approach that should be robust to genetic and some other sources of confounding and examined offspring conduct problems or antisocial behavior in childhood, adolescence and adult life. Two publications included the same IVF data set and assessment outcome (Gaysina et al., 2013; Rice et al., 2009) meaning that eight independent studies remained although some studies used the same sample but assessed conduct problems at a later time point e.g. (Gilman, Gardener, et al., 2008) – childhood; (Paradis, Shenassa, Papandonatos, Rogers, & Buka, 2017) – adolescence/adulthood. Differences in how the dependent and independent variables were assessed and in the analytical procedures employed complicate direct comparisons of the effect sizes observed in different studies. In analyses without controls for familial/genetic factors, the studies included in this systematic review report correlation coefficients (or b or β coefficients) between .1 and .3 (D'Onofrio et al., 2008; Ellingson et al., 2014; Gaysina et al., 2013) and odds ratios or hazard ratios of between 1.01 and 3.43 depending on the outcome and scaling of the exposure variable (D'Onofrio et al., 2010; D'Onofrio et al., 2012; D'Onofrio et al., 2008; Kuja-Halkola et al., 2014; Paradis et al., 2017). These effect sizes are similar to that reported in a meta-analysis of observational studies (odds ratio = 2.06, 95% CI= 1.67, 2.54) (Ruisch et al., 2017). Seven studies reported that the association

between maternal smoking and offspring conduct problems was mainly attributable to familial or genetic confounding. For instance, Rice and colleagues (2009) using an IVF design (n=779) observed an association between maternal smoking during pregnancy (defined by an amalgamation of data from self-report and antenatal records) in genetically related mother-child pairs (Cohen's d = .527). However there was no association between maternal smoking during pregnancy and offspring conduct problems in the group of mothers who experienced the pregnancy but were genetically unrelated to their child (Cohen's d = -.210). The magnitude of association was greater in the related mother-child pairs than in the un-related mother-child pairs (test for difference in strength of association F=4.106, p=.04). These results are therefore consistent with the association between maternal smoking during pregnancy and offspring conduct problems being due to passive gene-environment correlation although the sample size, particularly the unrelated pregnancies exposed to maternal smoking during pregnancy, was unsurprisingly small. Importantly, in this study as in others (D'Onofrio et al., 2010; D'Onofrio et al., 2012; D'Onofrio et al., 2008), including measured confounders, such as maternal antisocial behavior, did not alter association findings, highlighting the need for genetically-informative designs because including measured confounders in analyses of observational data does not circumvent the problem of passive gene-environment correlation. In a different analysis of the same IVF data set, Gaysina et al (2013) examined the relationship between maternal reported number of cigarettes smoked and offspring conduct problems. These authors also included an adoption-at-birth sample and observational cohort data. Consistent with what had been published previously (Rice et al., 2009), in the analysis of the IVF unrelated mother-child pairs no association between maternal smoking and offspring conduct problems was found and in fact the correlation coefficient was zero for this group (r=.00, p=.98). These observations fail to support the hypothesis that there is a causal effect of maternal smoking during pregnancy on offspring conduct problems and suggest that genes shared between mother and child are important in explaining associations reported in observational studies. However, it is worth noting that the findings reported in one study (Gaysina et al., 2013) have been interpreted by others as being consistent with a causal effect (Dolan et al., 2016; Slotkin, 2013) despite not reporting data supporting such an interpretation as highlighted by Thapar & Rutter (2015). This is likely due to confusion in assumptions that data from adoption- after- birth studies

enable causal inferences for prenatal exposures - they do not. This misinterpretation highlights the need for systematic review and clear reporting of findings (Academy of Medical Sciences, 2007). As described earlier, the association between prenatal smoking and offspring outcome in an adopted-after-birth study is uninformative regarding differentiating the influences of intrauterine and maternal genetic effects (because the biological mother who shares genes with the adopted away offspring experiences the pregnancy).

Results from six discordant sibling studies (D'Onofrio et al., 2010; D'Onofrio et al., 2012; D'Onofrio et al., 2008; Ellingson et al., 2014; Gilman, Gardener, et al., 2008; Kuja-Halkola et al., 2014) report findings that are inconsistent with a causal effect of maternal smoking during pregnancy on offspring conduct problems. Some studies use partly overlapping samples but assess different offspring outcomes. These studies have tended to be based on large samples including two studies of Swedish population-wide registries (sample sizes of 609,372 and 2,754,626) which are representative of the population as a whole, three of a representative population US sample (sample sizes of 6,066, 10,251 and 11,192) and one of a large US volunteer sample (sample size 52,919). Each of these six studies observe an association between maternal smoking during pregnancy and offspring conduct problems in the full population sample but for the sibling comparisons that control for shared familial confounding the association is substantially attenuated. For instance, D'Onofrio and colleagues (2010; 2012) reported results consistent with familial confounding for adult criminal behavior and adolescent antisocial behavior. For adult violent criminal convictions the hazard ratio for association with high levels of maternal smoking during pregnancy was 3.43. In sibling comparison models, the hazard ratio was 1.03. For high adolescent antisocial behavior, the hazard ratio was 1.34 in the full sample and 0.67 in the sibling comparison. Similarly, Gilman and colleagues (2008) reported a dose response relationship for amount mothers smoked during pregnancy and number of offspring conduct problems in the full sample ($F=20.4$, $p<.001$) but no dose-response relationship in the sibling analysis ($F=.5$, $p=.665$). Those authors concluded that the results observed suggested that such effects were either: 'not present, not readily distinguishable from a broader range of familial factors associated with maternal smoking or were not detectable using the assessment methods available at the time of the study'. The findings from these discordant sibling studies are therefore also inconsistent with

inferring a causal effect of maternal smoking during pregnancy on offspring conduct problems. Only one study in Table 3 reported evidence partially consistent with a causal effect of prenatal smoking on offspring antisocial behavior in a genetically informed design (Paradis et al., 2017) – which was a discordant sibling study of a US cohort. That study was a sub-sample (sample size ranged from 1883 to 3447 depending on the outcome) of a much larger study (n=52,919) which reported results inconsistent with a causal effect of maternal smoking during pregnancy on childhood conduct problems measured at age 7 (Gilman, Gardener, et al., 2008). In the study by Paradis et al., (2017) the within family effect tended to be larger than the between family effect for the six offspring antisocial behavior outcomes examined. However, the confidence intervals for the within family effects were very wide and results fluctuated depending on the outcome variable and how it was defined. Of note, the total effect tended to be fairly low and to some extent, this is what would be expected when the exposure variable is common in the general population as was the case in this sample where the prevalence of maternal smoking during pregnancy (in women pregnant between 1959 and 1966) was 59%. Indeed, attitudes to smoking have become less permissive over time which has had the effect that, in more recent cohorts, smoking behavior has become increasingly associated with psychiatric vulnerability and lower socio-economic status (Gilman, Breslau, Subramanian, Hitsman, & Koenen, 2008; Talati, Keyes, & Hasin, 2016; Talati et al., 2013). At time periods when maternal smoking during pregnancy was more normative, attenuated associations with offspring antisocial behavior may therefore be expected in the full population. In summary, all but one of the reports based on appropriate genetically informative designs reported no association between maternal smoking during pregnancy and offspring antisocial behavior during childhood, adolescence and adulthood once familial/genetic confounding had been controlled. These results are therefore inconsistent with a causal effect on prenatal smoking on offspring conduct problems.

Maternal smoking during pregnancy and offspring ADHD problems: findings from genetically informed family-based research designs

Twelve informative studies examined offspring ADHD as an outcome (Table 3). In analyses without controls for familial/genetic factors, the studies included in this systematic review report

correlation coefficients (or β or b coefficients) between .10 and .32 and odds ratios or hazard ratios of between 1.48 and 2.86 depending on the outcome and scaling of the exposure variable. These effect sizes are similar to that reported in a pooled analysis of observational studies (odds ratio = 2.39, 95% CI= 1.61, 3.52) (Langley et al., 2005). Eleven studies reported no evidence of a causal association between maternal smoking during pregnancy and offspring ADHD diagnosis or symptoms. These include an IVF study, 8 discordant sibling studies and 2 maternal vs paternal comparisons. A study using the IVF design reported results inconsistent with a causal effect of maternal smoking during pregnancy on offspring ADHD (Thapar et al., 2009). These authors observed association between maternal smoking during pregnancy and offspring ADHD in the genetically related mother-child pairs only. The magnitude of association was greater in the related compared to unrelated mother-child pairs (test for difference in strength of association β= -.10, p <.05). In addition, the study by Thapar and colleagues (2009) also carried out sensitivity analyses of paternal smoking during pregnancy for related and unrelated fathers (a different set of parent couples to the previous analysis) and reported findings consistent with a shared genetic influence on paternal smoking and offspring ADHD (similar to that for maternal smoking during pregnancy) such that there was only an association between paternal smoking during pregnancy and offspring ADHD when the father was genetically related to the child. These results are therefore consistent with passive rGE.

Eight discordant sibling studies report results showing that the association between maternal smoking during pregnancy and offspring ADHD was largely due to familial or genetic confounding (D'Onofrio et al., 2008; Ellingson et al., 2014; Gustavson et al., 2017; Knopik et al., 2016; Lindblad & Hjern, 2010; Obel et al., 2011; Obel et al., 2016; Skoglund et al., 2014). These include discordant sibling studies of whole population registries and extremely large samples that are representative of the local population as a whole (sample sizes between 100,000 and 1,000,000) (Gustavson et al., 2017; Obel et al., 2011; Obel et al., 2016; Skoglund et al., 2014). For instance, in the study by Obel and colleagues (2016) of a Danish national register-based cohort, in the full sample, the adjusted hazards ratio of ADHD contingent on exposure to maternal smoking during pregnancy was 2.01, 95% CI= 1.94, 2.07. In contrast, in the discordant sibling comparison (where the rate of ADHD in the

exposed and unexposed siblings are compared), the hazards ratio was substantially attenuated 1.07, 95% CI= 0.94, 1.22. This suggests that most of the observed association between maternal smoking during pregnancy and offspring ADHD is due to familial confounding. Similar results were reported in a Swedish national register-based cohort (Skoglund et al., 2014) such that the level of maternal smoking during pregnancy substantially increased risk of offspring ADHD in conventional observational tests (hazard ratios = 1.89 moderate smoking 2.50 high smoking). This association was reduced somewhat when controlling statistically for measured confounds but was substantially attenuated for cousin (hazard ratios 1.45 moderate smoking and 1.69 high smoking) and sibling comparisons (hazard ratios 0.88 moderate smoking and 0.84 high smoking). Gustavson et al (2017) found similar results in a using a discordant sibling design. There are four published studies that have used the comparison of maternal and paternal smoking during pregnancy (Gustavson et al., 2017; Keyes, Davey Smith, & Susser, 2014; Kovess et al., 2015; Langley et al., 2012). Two studies report findings inconsistent with a causal effect of maternal smoking during pregnancy on offspring ADHD (Gustavson et al., 2017; Langley et al., 2012) and two studies report findings that are at least partially consistent with a causal effect (Keyes et al., 2014; Kovess et al., 2015). In the large study by Gustavson and colleagues, three negative control variables were included (paternal smoking during pregnancy, maternal grandmother smoking during pregnancy and maternal smoking during previous pregnancies). Results showed that associations between maternal smoking during pregnancy (where a intrauterine effect is plausible) on offspring ADHD diagnosis were of a similar magnitude when compared to each of the three negative control variables (HR maternal smoking =1.48, 95% CI= 1.30, 1.68; HR paternal smoking 1.28, 95% CI= 1.16, 1.42; HR maternal grandmother smoking = 1.28 95% CI= 1.15, 1.42; HR maternal previous smoking = 1.53 95% CI= 1.33, 1.75). These results are therefore inconsistent with a causal intrauterine effect of maternal smoking during pregnancy on offspring ADHD because a similar effect size is seen regardless of which parent smoked and the timing of maternal smoking (during the index pregnancy or a different pregnancy). Similarly, the UK study by Langley and colleagues (2012) showed no difference in the magnitude of association of maternal (β = .25, 95% CI= .18, .32) and paternal smoking (β = .21, 95% CI= .15, .27; test for difference in strength of association F=.21, p=.65) during pregnancy and offspring ADHD and results were therefore inconsistent with a true intrauterine effect. These two studies included data on

mothers' and fathers' own reports (where available) of their smoking behavior assessed contemporaneously during pregnancy (Gustavson et al., 2017; Langley et al., 2012). While the results of Keyes et al (2014) were inconsistent with a potentially causal effect when no statistical adjustments for measured confounders (the magnitude of association for maternal (β =.22, 95% CI=.11, .33) and paternal smoking (β = .18, 95% CI=.07, .30) was very similar), when statistical adjustments for measured confounders were made there was an attenuation of the association between paternal smoking during pregnancy (β =.25, 95% CI= .09, .40 maternal; (β = .02, 95% CI= -.20, .24 paternal). That finding therefore suggests that maternal smoking during pregnancy may be more important than paternal smoking during pregnancy – consistent with a causal hypothesis. Nevertheless, in the same study, maternal quitting smoking <u>prior</u> to pregnancy was associated with offspring ADHD to the same extent as maternal smoking <u>during</u> pregnancy. That finding is therefore consistent with dispositional factors that affect the likelihood of women smoking being important in the association with offspring ADHD as opposed to a true intrauterine risk effect. In conclusion, the findings from that study are ambiguous. Results from the study by Kovess et al (2015) are similarly difficult to interpret – in that the authors observed an association for both maternal and paternal smoking during pregnancy and offspring ADHD in unadjusted associations (OR= 1.82, 95% CI= 1.45, 2.29 maternal; OR= 1.53, 95% CI= 1.25, 1.86 paternal) which were attenuated in both groups (OR= 1.44, 95% CI= 1.06, 1.96 maternal; OR= 1.17, 95% CI= 0.92, 1.49 paternal) (slightly more so in the fathers) when statistical adjustment for potential confounders was made. The adjusted association between maternal (and paternal) smoking during pregnancy were attenuated further when teacher reports of ADHD problems were used (OR= 1.33, 95% CI= 0.96, 1.84 maternal; OR= 1.10, 95% CI= 0.86, 1.40 paternal). One methodological issue to note is that these two studies relied on maternal retrospective reports of paternal smoking during pregnancy at child age 10 (Keyes et al., 2014) and in a sample of children aged 6-11 years (Kovess et al., 2015). The reliability of a mother retrospectively reporting on their partner's smoking behavior during pregnancy once a relatively long period has elapsed is not known.

In summary, a body of evidence from a series of studies using innovative research designs suggests that it is unlikely that there is a substantial environmental causal effect of maternal smoking

during pregnancy on offspring ADHD or conduct problems. Nevertheless, it is important to note that

the results of maternal and paternal comparison studies are inconsistent and methodological tests of

the reliability and validity of maternal retrospective reports of paternal smoking during pregnancy are

required. There is a need for further studies that include reports on smoking behavior from mothers

and fathers assessed during pregnancy rather than after the child is born. The vast majority of

genetically informative studies use maternal reports of smoking behavior. There is good evidence that

these are reliable and valid: maternal retrospective reports of smoking status correlate highly with

contemporaneous reports during pregnancy (Rice, Lewis, et al., 2007) and with plasma cotinine levels

which index recent exposure to nicotine in tobacco smoke (George, Granath, Johansson, &

Cnattingius, 2006). The best evidence for the validity of maternal reported smoking is the consistent

evidence for correlations with objective measures of infant birth weight. Indeed, there is strikingly

consistent evidence from genetically sensitive study designs that maternal smoking during pregnancy

reduces offspring birth-weight in a way that is consistent with a causal effect (Gustavson et al., 2017;

Langley et al., 2012; Obel et al., 2016; Rice et al., 2009; Thapar et al., 2009) illustrating that studies

using these methods are able to detect potentially causal intrauterine effects when they are present.

Importantly, this same pattern of findings for maternal smoking during pregnancy and infant birth

weight emerges from studies using alternative methods (with differing patterns of bias) including

randomized controlled trials of smoking cessation and Mendelian randomization (Tyrrell et al., 2012;

Veisani, Jenabi, Delpisheh, & Khazaei, 2017).

Maternal prenatal stress

Our search identified only one genetically informative study that examined maternal prenatal

stress and offspring psychopathology in humans (Rice et al., 2010). That study used an IVF design, a

retrospective measure of perceived maternal stress during pregnancy which showed reliability (using

test-retest methods) and examined childhood anxiety, ADHD and conduct problems as continuous

outcomes (rated by mothers). Results differed for each childhood outcome examined. For ADHD,

results were consistent with shared genetic effects as associations were observed in related 'prenatal'

mother-child pairs only. For offspring conduct, there was some evidence consistent with a causal

intrauterine effect because similarly sized effects were observed in related and unrelated 'prenatal' mother-child pairs. For offspring anxiety, while associations with maternal prenatal stress were observed in both groups, results appeared to be primarily attributable to postnatal anxiety and postnatal anxiety mediated observed associations in both the related and unrelated groups. This study showed that results differed depending on the child outcome examined. Limitations include the retrospective assessment of perceived stress which requires validation and the possibility of shared method variance because mothers rated both the exposure and the outcome variable. There is clearly a need for further genetically informative studies focusing on maternal stress in pregnancy given the dearth of such studies. These will need to consider continuity of maternal stress to the postnatal rearing environment (O'Donnell & Meaney, 2017; Rice et al., 2010)

Discussion

After undertaking a systematic review of genetically informative studies, the findings suggest that the prenatal risk factor of maternal smoking during pregnancy has likely causal effects on infant birth weight and prematurity but that there is minimal evidence to support a causal effect on offspring ADHD or conduct problems and much evidence to suggest associations reflect familial confounding and passive gene-environment correlation. There are too few genetically informative studies of maternal stress in pregnancy to draw firm conclusions in spite of a substantial observational literature on the topic. It seems reasonable to conclude that relatively less attention has been paid to genetically informed studies that include either extremely large samples or extremely informative comparisons but report negative results compared to studies reporting apparently positive results e.g. (Obel et al., 2016; Slotkin, 2013; Thapar et al., 2009) - a problem that applies to the whole of science (Ahmed, Sutton, & Riley, 2012; Easterbrook, Berlin, Gopalan, & Matthews, 1991). Clear reporting is needed to address this (Academy of Medical Sciences, 2007). The uptake of common analytical strategies is also likely to be helpful. For prenatal risks there are examples of designs where current evidence using appropriate designs supports causal effects, such as exposure to extreme maternal under-nutrition during pregnancy and offspring psychosis risk (Mackay, Dalman, Karlsson, & Gardner, 2017). In these cases, it will be important to understand the mechanisms through which such

exposures influence offspring risk for maladaptive outcomes. In our view, for certain prenatal exposures and outcomes, especially maternal smoking in pregnancy and ADHD or conduct problems, further reports of association from observational designs will be unhelpful because of contributions of person-environment correlation, passive gene-environment correlation and the problem of residual confounding. Indeed, a key reason for identifying if early environmental exposures have causal effects on the likelihood of psychopathology later in life is to guide prevention and early intervention. Effective strategies for reducing maternal smoking remain an appropriate public health target because of the deleterious effects of smoking on fetal growth and with obstetric and perinatal complications including prematurity and miscarriage. Given that maternal smoking in pregnancy is already recognized as a health hazard, we therefore urge researchers in the field of developmental psychopathology, to investigate other environmental risk factors amenable to change. Some may be especially or exclusively relevant for mothers in low and middle income settings and studies in these contexts is a priority. Findings from genetically informative and quasi-experimental designs that are well designed will however continue to be important. Avoiding the expenditures of resources on preventive interventions that do not work becomes even more important in low resource settings. Where studies with different sets of strengths and limitations find converging evidence this adds to confidence about inferring causal effects. However what happens when findings from such studies do not 'triangulate' – as illustrated by the data presented on maternal smoking in pregnancy? Here it remains crucial that, in addition to considering the key sources of bias, the usual criteria regarding careful consideration of the scientific quality of each of the published quasi-experimental studies prevail – including appropriate design, adequate measurement of exposure and outcome, sample size and evidence that the assumptions of the method are met. Future directions in this area include a greater use of genetically informed approaches that utilize information on genetic variants associated with psychopathology to test casual inferences. As understanding of the genetic variants contributing to psychiatric disorders and health related traits increases, such approaches can potentially be applied to a large number of exposures. Indeed, genome wide association studies for psychiatric disorders have now identified many variants robustly associated with disorder. This then provides the opportunity for this information to be used to test hypotheses relevant to the causal contribution of

prenatal risk factors to offspring development and psychopathology. One important such approach is Mendelian Randomization which uses genetic variants as instrumental variables to facilitate causal inference with observational data avoiding bias due to confounding and reverse causality (Gage et al., 2015; Davey-Smith & Hemani, 2014). A number of recommendations and extensions of Mendelian Randomisation for testing the specific situation of prenatal exposures on offspring outcomes have been developed including examining data from fathers and offspring as well as the use of maternal genetic instrumental variables where the mother's allele is not transmitted to the offspring (D. Lawlor et al., 2017). However Mendelian Randomization relies on a number of assumptions (Davey-Smith & Ebrahim, 2004; Davey-Smith & Hemani, 2014) and these are not always met for psychopathology. In particular pleiotropy, where the same genetic variant has independent effects on different outcomes, may well exist for psychopathology and complicates interpretation. Care is therefore needed in conducting and interpreting Mendelian Randomization findings and triangulation of evidence is again important. Other potential future directions include application of techniques such as the polygenic transmission disequilibrium test (Weiner et al., 2017) and examination of placental functioning in the context of genetically informed designs.

Figure 1

Schematic of passive gene environment correlation for the prenatal environment
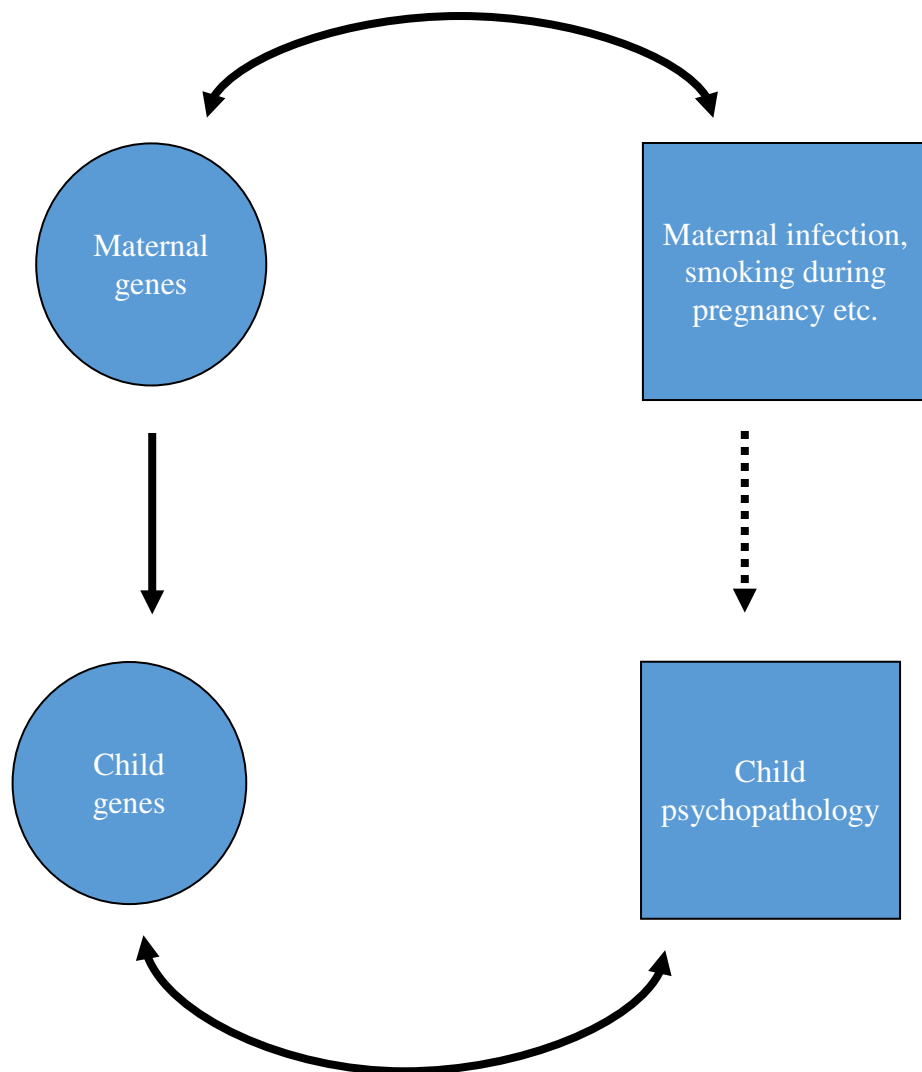
Footnote to Figure 1

A dashed arrow between prenatal exposure and child outcome and a filled arrow between maternal and child genes illustrates passive gene environment correlation (i.e. that association may arise because of genes shared between mother and child rather than a causal environmental risk effect). Double headed arrows represent correlations, directional arrows represent associations.

Figure 2

Schematic of genetically sensitive designs that separate genetic and environmental contributions to prenatal exposure and offspring outcome

1) requires the use of assisted conception designs where a mother experiences a pregnancy for a baby with whom she shares no genes.

2) Comparing maternal and paternal exposures varies intrauterine exposures (only possible for mothers) holding the parent-offspring genetic relationship constant

Intrauterine environment must either:

1) Be separated from the maternal genome or

2) Vary holding the mother-offspring genetic relationship constant *

2) In the discordant sibling design pregnancies from the same mother are compared where an intrauterine exposure (e.g. maternal infection) is different for the two pregnancies.

2) In the children of twins design the offspring outcomes are compared for twin mothers e.g. identical twin mums that are discordant for an intrauterine exposure.

*Footnote to Figure 2: * In the discordant sibling design, the genetic relationship between biological mother and children is .5; in the children of twins design, identical twin mothers are equally related to their own child (.5) and that of their sister (.5 the avuncular relationship) because identical twin mothers share all their genes in common; in the comparison of maternal and paternal exposures, biological mother and biological father each share .5 of their genes with their child.*
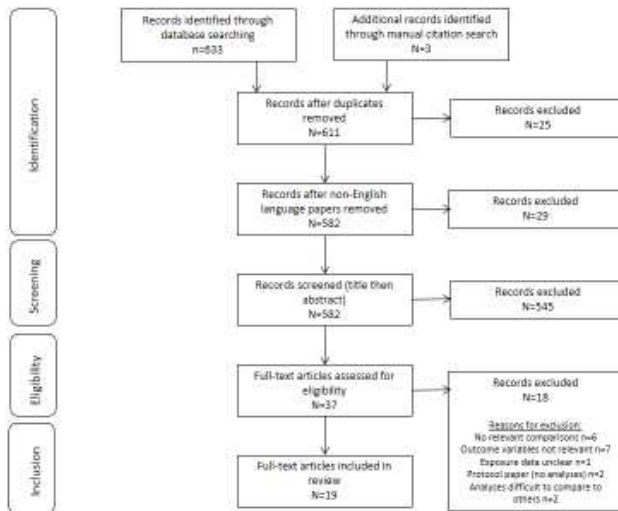
Figure 3

Figure 3a. PRISMA flow diagram detailing each stage of the review process for prenatal smoking as an exposure.
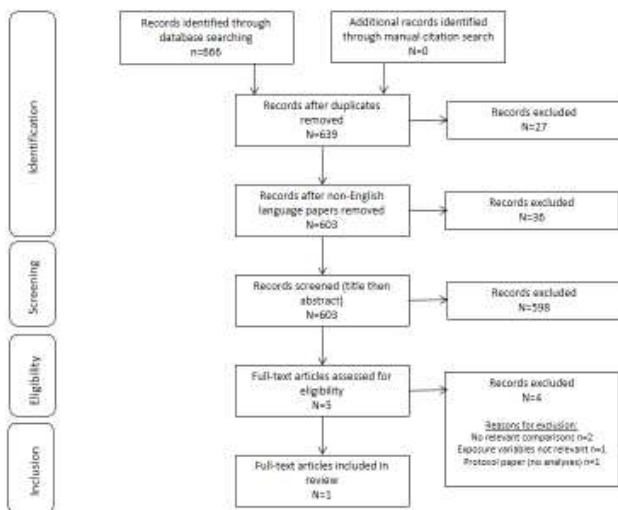

Figure 3b. PRISMA flow diagram detailing each stage of the review process with prenatal stress as an exposure.

Table 1

Summary of if and how particular research designs separate prenatal and genetic contributions to offspring outcome

	Fertilization – inheritance of genetic material from biological mother	*Gestation – provision of intrauterine environment by biological mother*	*Postnatal rearing – provided by biological mother*	*Distinguishes prenatal environment from maternal genes?*
Related mother experiences the pregnancy and rears the child (standard observational design)	√	√	√	*No*
Post-birth adoption	√	√	X	*No*
Twin study	√	√	√	*No*
Prenatal cross fostering study (IVF design – oocyte donation and embryo donation with unrelated donor)	√	X *Mother unrelated to offspring experiences the pregnancy*	X	*Yes*
Prenatal cross fostering study (IVF design – gestational surrogacy with unrelated surrogate)	√	X *Unrelated surrogate experiences the pregnancy*	√	*Yes*
Children of twins (identical twin mothers each with at least one child)	√	√ *Maternal genetic contribution held constant for own and the identical twin sister's pregnancies but intrauterine environment varies across the pregnancies*	√	*Yes, partial.*
Discordant siblings (biological mother has at least two pregnancies where a prenatal exposure (e.g. maternal smoking during pregnancy,	√	√	√	*Yes, partial.*

maternal infection) differs across the pregnancies		*Maternal genetic contribution held constant but intrauterine environment can vary across her pregnancies*		
Mother father comparison	√	√ *Parental genetic contribution held constant but effect of intrauterine environment only possible for maternal exposures (as fathers do not experience pregnancy)*	√	*Yes, partial.*

Table 2: Comparison of maternal characteristics in Cardiff IVF sample by smoking before and during pregnancy status

	1 Non-smoker (n)	2 Mother Smoked in Year Before Pregnancy Only (n)	3 Mother Smoked During Pregnancy (n)	F	*1 vs 2*	*1 vs 3*	*2 vs 3*
Maternal age at Birth (Mean ± SD)	35.52 ± 4.84 (710)	33.24 ± 3.95 (89)	33.81 ± 5.29 (48)	11.06***	.001*	.045*	.779
Highest level of maternal education (Mean ± SD)	1.90 ± 1.26 (705)	1.58 ± 1.18 (89)	1.17 ± 1.17 (48)	9.470***	.069	.001*	.148
Stress early pregnancy (Mean ± SD)	5.80 ± 2.81 (709)	5.71 ± 3.01 (89)	5.60 ± 3.32 (48)	.141	.953	.888	.978
Stress mid pregnancy (Mean ± SD)	3.96 ± 2.54 (710)	3.79 ± 2.68 (89)	4.96 ± 2.98 (48)	3.682*	.812	.027*	.031*
Stress late pregnancy (Mean ± SD)	3.63 ± 2.68 (695)	3.84 ± 3.06 (88)	4.57 ± 3.38 (47)	2.641	.779	.062	.308
Current mother depression (Mean ± SD)	4.23 ± 3.01 (705)	4.28 ± 2.82 (89)	5.92 ± 3.65 (48)	6.945**	.988	.001*	.008*
Pre-pregnancy cigarettes (Mean± SD)	0.00 ± 0.00 (710)	1.24 ± .48 (89)	1.69 ± .52 (48)	2994.03***	.001*	.001*	.001*

Footnote to Table 2: As illustrated above, mothers who smoke during pregnancy differ on socio-economic factors, psychopathology and amount smoked

prior to pregnancy from to non-smoking mothers. It can also be observed that mothers who continue to smoke during pregnancy also differ from those that

smoked only in the year before the pregnancy. These data therefore show that maternal characteristics influence both the prenatal and the postnatal rearing

environment. Maternal smoking defined using combination of antenatal records and maternal retrospective report. For further detail on the sample and the

measures included, please see Rice et al., 2009; Thapar et al., 2009; Rice et al., 2010.

Table 3: Summary of papers identified in systematic search of maternal smoking during pregnancy and offspring conduct problems or ADHD

Study	Exposure	Offspring Outcome	Design	Key comparisons	Main finding	Consistent with a causal effect?
D'Onofrio et al 2008	Maternal retrospective self-report (within 4 years after child birth)	Conduct problems Oppositional defiant problems (ODD) ADHD problems – all maternal reports on selected items from CBCL	Cohort (Offspring age 4-10 years of women in the National Longitudinal Survey of Youth 1979)	Discordant sibling design	Association in full sample (unadjusted associations: conduct problems b = .29 male, b=.18 female; ODD b=.29; ADHD b=.27) Substantially attenuated in sibling comparisons for all outcomes (unadjusted associations: conduct problems b = .06 male, b=-.01 female; ODD b=-.02; ADHD b=.07)	No
Gilman et al 2008	Maternal self-report during antenatal visits (rate of MSDP > 60%)	Conduct problems - behavioral observations by the examining psychologist at the age 7 assessment (6 items)	Cohort (the collaborative perinatal project – Boston and Providence sites) 1959-1974	Discordant sibling design	Dose response association present in the full sample (F= 20.4, p<.001). No dose response association in the discordant siblings (F= 0.5, p=.665). Effect size in discordant siblings small (maternal smoking yes/no b= .05, 95% CI= -.03, .13 maternal smoking amount b= -.00, 95% CI=-.00, .00)	No
Rice et al 2009	Maternal smoking in pregnancy (retrospective self-report plus data from antenatal records)	Conduct problems (SDQ – combined mother and father reports)	IVF Prenatal cross fostering	Association in related mother-child pairs vs association in unrelated mother-child pairs (where unrelated mother experiences pregnancy)	Association between MSDP and offspring conduct only in the related pregnancies (d=.527) and not unrelated pregnancies (d= -.210). Association in related pregnancies greater (F=4.106, p=.04).	No

Thapar et al 2009	Maternal smoking in pregnancy (retrospective self-report plus data from antenatal records) and current smoking Paternal smoking during pregnancy and current smoking - self-report	ADHD symptoms (Du Paul – mother reports)	IVF Prenatal cross fostering	Association in related mother-child pairs vs unrelated mother-child pairs Also: 1) maternal vs paternal prenatal smoking. 2) maternal current smoking. 3) related vs unrelated paternal smoking during pregnancy	Association between MSDP and offspring ADHD only in the related pregnancies (β=.10, p<.02) and not unrelated pregnancies (β=-.05 p>.1). Association in related pregnancies greater. Additional sensitivity analyses inconsistent with a causal effect: paternal SDP associated with offspring ADHD in related fathers and not unrelated fathers (β=.11, p<.05 vs β=.03, p>.1). Maternal current smoking associated with offspring ADHD in related pairs and not unrelated pairs (β=.09, p<.04 vs β= -.02, p>.1).	No
D'Onofrio et al 2010 *	Maternal self-report at first antenatal visit	Criminal convictions via the National Crime Register	Cohort – Swedish population of births 1983–1989	Discordant sibling design	Association with violent and non-violent convictions in full sample Full sample (association with violent convictions adjusted for maternal and paternal traits): moderate smoking HR = 2.47, 95% CI= 2.34, 2.60; high smoking HR = 3.43 95% CI=3.25, 3.63 Substantially attenuated in sibling comparisons: moderate HR =1.02, 95% CI=0.79, 1.30; high HR =1.03, 95% CI= 0.78, 1.37	No
Lindblad et al 2010 *	Maternal self-report at first antenatal visit	ADHD medication	Cohort–register of offspring born 1987-2000 at term and resident in Sweden in 2006 (age 6-19 years)	Discordant sibling design	Association in full sample (for >=10 cigarettes/day OR = 2.86, 95% CI = 2.66, 3.07) Substantially attenuated in sibling comparisons (for >=10 cigarettes/day OR = 1.26, 95% CI= 0.95, 1.58)	No

Obel et al 2011 *	Maternal self-report during 2nd trimester of routine antenatal care	Diagnosis of ADHD via psychiatric inpatient and outpatient care register (ICD-10 hyperkinetic disorder)	Cohort – Finnish population of singleton births born 1987-2001	Discordant sibling design	Association in full sample (HR = 2.01, 95% CI= 1.90, 2.12 Substantially attenuated in sibling comparison (HR = 1.20. 95% CI= 0.97, 1.49)	No
D'Onofrio et al 2012	Self-reported maternal smoking	Adolescent self-reported antisocial behavior (Self Reported Delinquency Scale) and criminal convictions	Cohort (Adolescent offspring of women in the National Longitudinal Survey of Youth 1979)	Discordant sibling design	Association with greater smoking and antisocial behavior in full sample (unadjusted ORs range from 1.15 to 1.57 depending on outcome) Association substantially weaker in sibling comparisons (unadjusted odds ratios range from 0.67 to 0.98 depending on outcome). Direction of association is in the opposite direction in discordant siblings vs the full sample.	No
Langley et al 2012	Self-reported maternal and paternal smoking assessed during pregnancy	ADHD symptoms (parent-rated symptoms from diagnostic interview DAWBA and diagnoses made on basis of teacher and parent ratings)	Cohort (ALSPAC-data from 1991-2000)	Maternal vs paternal prenatal smoking comparison	Magnitude of maternal prenatal smoking (β= .25, 95% CI= .18, .32) vs paternal prenatal smoking (β= .21, 95% CI= .15, .27) and offspring ADHD was similar. Strength of mother and father associations not substantially different (F=.21, p=.65)	No
Gaysina et al 2013	Maternal retrospective self-report of amount smoked during pregnancy	Averaged, standardized problem scores for mother /father/ teacher reports depending on sample	1) IVF Prenatal cross fostering [a] 2) Adoption after birth vs cohort comparison	Association in unrelated mother-child pairs between amount smoked in pregnancy and offspring conduct Adoption after birth vs cohort comparison to assess	No association between prenatal maternal amount smoked and offspring conduct in unrelated IVF mother-child pairs (r=.00, p=.98). No effect of the postnatal rearing environment on observed associations	No

				moderation by the *postnatal rearing environment*		
Ellingson et al 2014	Maternal retrospective self-report (within 2 years after child birth)	Maternal reports on Behavior Problem Index – biannually from child age 4 to 13. Developmental trajectory of ADHD, ODD and CD.	Cohort (Adolescent offspring of women in the National Longitudinal Survey of Youth 1979)	Discordant sibling design	In within-family analysis, little evidence of association of MSDP with the intercept (i.e. average) or slope of any externalizing outcome (ADHD, ODD, CD)	No
Keyes et al 2014	Maternal retrospective report at child age 10 years of own and father smoking during pregnancy and current smoking	Maternal report of ADHD symptoms at age 10. 8 items derived by factor analysis from a 100-item battery of child characteristics.	Cohort (CHDS – 1961-1963). Sub-sample ~10% of the original cohort who participated in two follow-up assessments.	Maternal vs paternal prenatal smoking comparison	Maternal (β=.22, 95% CI=.11, .33) and paternal (β=.18, 95% CI=.07, .30) association similar in unadjusted models but association attenuated for paternal following adjustment (β=.25, 95% CI=.09, .40 vs (β=.02, 95% CI=-.20, .24). Maternal quitting smoking prior to pregnancy also associated with offspring ADHD (β=.32, 95% CI=.01, .63). Maternal current smoking (β=.35, 95% CI=.09, .61) and lifetime smoking (β=.25, 95% CI=.03, .48) also associated.	Partially
Kuja-Halkola et al 2014 *	Maternal self-report at first antenatal visit	Criminal convictions, violent convictions and drug misuse collected via the national inpatient register and convictions of crimes in Swedish lower court	Cohort - Swedish Population born 1983-2009	Discordant sibling design (full siblings and maternal half-siblings) Also: discordant cousins and half-cousins	In all within-family analysis, little evidence that maternal smoking in pregnancy was associated with conduct problems	No

Skoglund et al 2014 *	Maternal smoking during pregnancy – antenatal record data	Diagnosis of ADHD via psychiatric inpatient and outpatient care register (ICD-10 hyperkinetic disorder, DSM-IV ADHD or medication for ADHD).	Cohort (Population born in Sweden 1992-2000)	Discordant sibling design Also: discordant cousin design	Association in full sample (HR moderate=1.89, 95% CI=1.83, 1.97) HR high 2.50, 95% CI=2.40, 2.61) Attenuated in cousin comparisons (HR moderate =1.45, 95% CI= 1.24, 1.68; HR high 1.69, 95% CI=1.40, 2.04) Completely attenuated in full-sibling comparisons (HR moderate =0.88, 95% CI=.73, 1.06); HR high 0.84, 95% CI=.65, 1.06))	No
Kovess et al 2015	Retrospective maternal report of self and father	Maternal and teacher reports of "probable ADHD" on SDQ hyperactivity scale.	Cross-sectional schools survey across six European countries	Maternal vs paternal prenatal smoking comparison	Maternal (OR=1.82, 95% CI= 1.45, 2.29) and paternal (OR=1.53, 95% CI= 1.25, 1.86) association similar in unadjusted models. Greater attenuation of association for paternal following adjustment (maternal OR = 1.44, 95% CI=1.06, 1.96 paternal OR = 1.17, 95% CI= 0.92, 1.49). CIs wide for teacher-rated ADHD.	Partially
Knopik et al 2016	Maternal report – number smoked in each trimester	ADHD symptoms – parent and teacher reports - Conners scale plus Child Behavior Checklist	Missouri Mother and their Children study (1998-2005)	Discordant sibling design – within and between family effects estimated	Within family effects very small. The one exception was for parent-rated hyperactivity/impulsivity scale but did not replicate for teacher reports or total scores.	No
Obel et al 2016 *	Maternal self-report at antenatal visit at first antenatal	Diagnosis of ADHD via psychiatric inpatient and outpatient care register (ICD-10 hyperkinetic disorder or	Cohort – Danish population of singleton births born 1991-2006	Discordant sibling design	Association in full sample (HR = 2.01, 95% CI= 1.94, 2.07) Substantially attenuated in sibling (HR = 1.07, 95% CI=0.94, 1.22) and half sibling comparisons.	No

		medication for ADHD for >6mths).				
Gustavson et al. 2017	Maternal report on own and maternal grandmother smoking during pregnancy. Maternal report on smoking during previous pregnancies. Paternal self-reported smoking.	ADHD diagnosis – Norwegian Patient Registry ADHD symptoms – maternal reports at child age 5 (6 items from Child Behavior Checklist)	Cohort (Norwegian Mother and Child Cohort Study - 1999-2008)	Discordant sibling design Also: a series of additional negative controls - maternal vs paternal vs grand-maternal vs maternal smoking in previous pregnancies comparisons.	Association between maternal smoking and offspring ADHD no stronger than paternal, grand-maternal or maternal smoking in previous pregnancies. (HR=1.48 95% CI= 1.30, 1.68 MSDP; HR=1.28 95% CI= 1.16, 1.42 FSDP; HR=1.28 95% CI= 1.15, 1.42 GSDP; HR=1.53 95% CI =1.33, 1.75 MSDPP). For discordant siblings effects inconsistent with causal interpretation (within family effect b=-.01, SE=.03, p=.58)	No
Paradis et al 2017	Maternal self-report during antenatal visits (rate of MSDP 59%)	Antisocial behavior and offences (offspring self-reported in adolescence and adulthood; official records of arrests)	Cohort (a sub-sample representing ~9% of the collaborative perinatal project) 1959-1966. Follow up in adulthood of Providence site – interview response rate ~ 40%	Discordant sibling design	Multiple outcomes examined. Within family effects tended to be larger than between family effects. Confidence intervals were wide. For the two within family effects with the strongest evidence according to p-value, the evidence for a total effect was not strong.	No

Footnote to table 3:

MSDP = mother smoking during pregnancy; FSDP = father smoking during pregnancy; MSDPP = mother smoking during previous pregnancies; GSDP = grandmother smoking during pregnancy

ODD = oppositional defiant disorder

CD = conduct disorder

ADHD = attention deficit hyperactivity disorder

HR = hazard rate

OR = odds ratio

CI = confidence interval

SDQ = Strengths and Difficulties Questionnaire

[a] Same IVF data set as Rice et al., 2009 and Thapar et al., 2009

* A full population cohort

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