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Maternal mental health and infant emotional reactivity: a 20-year two-cohort study of preconception and perinatal exposures

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Abstract

Background: Maternal mental health during pregnancy and postpartum predicts later emotional and behavioural problems in children. Even though most perinatal mental health problems begin before pregnancy, the consequences of preconception maternal mental health for children's early emotional development have not been prospectively studied.

Methods: We used data from two prospective Australian intergenerational cohorts, with 756 women assessed repeatedly for mental health problems before pregnancy between age 13 and 29 years, and during pregnancy and at one year postpartum for 1231 subsequent pregnancies. Offspring infant emotional reactivity, an early indicator of differential sensitivity denoting increased risk of emotional problems under adversity, was assessed at one year postpartum.

Results: Thirty-seven percent of infants born to mothers with persistent preconception mental health problems were categorised as high in emotional reactivity, compared to 23% born to mothers without preconception history (adjusted OR 2.1, 95% CI 1.4-3.1). Ante- and postnatal maternal depressive symptoms were similarly associated with infant emotional reactivity, but these perinatal associations reduced somewhat after adjustment for prior exposure. Causal mediation analysis further showed that 88% of the preconception risk was a direct effect, not mediated by perinatal exposure.

Conclusions: Maternal preconception mental health problems predict infant emotional reactivity, independently of maternal perinatal mental health; while associations between perinatal depressive symptoms and infant reactivity are partially explained by prior exposure. Findings suggest that processes shaping early vulnerability for later mental disorders arise well before conception. There is an emerging case for expanding developmental theories and trialling preventive interventions in the years before pregnancy.

Introduction

Early life environments shape patterns of childhood growth with long-lasting effects on health and human potential (Barker, 1990, Gluckman *et al.*, 2009). Effects extend to later life mental health, with early exposure to maternal mental health problems predicting later childhood emotional and behavioural problems, many of which persist into adulthood (Pearson *et al.*, 2013, Stein *et al.*, 2014, Swanson and Wadhwa, 2008). According to theories of the developmental origins of health and disease (DoHAD), in utero and postpartum development are characterised by heightened adaptive plasticity, allowing maternal transmission of environmental information to offspring to confer later developmental advantage (Gluckman and Hanson, 2004). Heightened antenatal exposure to maternal stress-related hormones and inflammatory processes (Chan *et al.*, 2017, Oberlander *et al.*, 2008), and altered caregiving postnatally (Meaney and Szyf, 2005, Newland *et al.*, 2016), have both been implicated as risk processes.

However, links between maternal mental health and offspring development may have their origins in the years before pregnancy (Keenan *et al.*, 2018). According to evolutionary developmental and life course models, maternal biology and behaviour during pregnancy and postpartum reflect experience accumulated during the preconception years (Kuzawa and Quinn, 2009). For most women, perinatal mental health problems are preceded by similar problems before pregnancy, many beginning in adolescence (Patton *et al.*, 2015). The persistence of preconception mental health problems into pregnancy may therefore affect offspring through increased exposure to antenatal and postnatal risks. Alternatively, animal studies have raised a possibility of preconception maternal mental health affecting the periconceptional environment or gamete directly, with independent effects on offspring stress responses (Zaidan *et al.*, 2013). In this latter case, it is further possible that effects previously attributed to perinatal exposures are in fact confounded by exposures occurring before pregnancy (Keenan *et al.*, 2018).

One early phenotypic indicator of infant vulnerability to later mental disorder is heightened emotional reactivity, characterised by irritability, negative mood, and intensity of reactions (Rothbart and Bates, 2006). It has been seen as an indicator of differential susceptibility to context, reflecting a greater capacity to benefit from enriched environments and interventions but also a heightened vulnerability to stress (Belsky, 2005, Boyce and Ellis, 2005, Hartman and Belsky, 2018, Slagt *et al.*, 2016). Emotional reactivity predicts mental health problems in childhood with effects varying across contexts. Four-month old infants classified by observers as highly reactive to stimuli were, for example, twice as likely to have anxious symptoms at age seven years (Kagan *et al.*, 1999). Similarly, parent-reported intensity of infant emotional reaction predicted a 1.5-fold increase in the odds of interviewer-assessed child psychiatric disorder at age seven years (Sayal *et al.*, 2014). Maternal mental health problems also predict infant emotional reactivity, leading to a suggestion that this heightened early sensitivity to environmental context may be one step in the intergenerational transmission of mental health risks (Bruder-Costello *et al.*, 2007, Davis *et al.*, 2007, Davis *et al.*, 2004, Huot *et al.*, 2004, Rouse and Goodman, 2014).

Questions remain as to the timing of these maternal effects, with implications for our understanding of the mechanisms involved and the optimal timing of interventions. In this study, using data from two longstanding Australian prospective datasets we consider the relative contributions of preconception, antenatal, and postnatal maternal mental health problems to the development of heightened emotional reactivity in infants. We further examine the extent to which any preconception associations are mediated by maternal mental health during pregnancy and in offspring infancy, as well as the extent to which any associations between perinatal mental health and offspring infant emotional reactivity are explained by a history of prior problems.

Ethical standards

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

Methods

Sample

We used data from two prospective preconception cohorts located in Australia: The Victorian Intergenerational Health Cohort Study (VIHCS) and the Australian Temperament Project, Generation 3 (ATPG3). These cohorts both assessed women's mental health before, during and after pregnancy, and offspring infant emotional reactivity at one year postpartum (appendix p 1).

VIHCS sample

The Victorian Intergenerational Health Cohort Study (VIHCS) is an ongoing prospective intergenerational study of preconception predictors of infant and child health, described elsewhere (Patton *et al.*, 2015). It arose from a cohort study commencing in 1992 in the state of Victoria, Australia (The Victorian Adolescent Health Cohort Study; VAHCS) (Patton *et al.*, 2014). Briefly, a close-to-representative sample of 1943 Victorian mid-secondary school students (1000 female) were selected via a two-stage cluster sampling design and assessed six-monthly during adolescence (VAHCS Waves 1-6: mean age 14•9-17•4 years), and three times in young adulthood (VAHCS Waves 7-9: 20•7, 24•1 and 29•1 years). VIHCS began in 2006 during the ninth wave of VAHCS. Between 2006 and 2013 (participant age 29-35 years, encompassing median maternal and paternal age for Australian births (Australian Bureau of Statistics, 2013), VAHCS participants were screened six-monthly for pregnancies via SMS, email, and phone calls. Participants reporting a pregnancy or recently born infant were invited to participate in VIHCS, and asked to complete telephone interviews in trimester three, two months' postpartum and one year postpartum for each infant born during VIHCS screening. Participants' parents or guardians provided informed written consent at recruitment into VAHCS, and participants provided informed verbal consent at every subsequent wave. Protocols were approved by the human research ethics committee at the Royal Children's Hospital, Melbourne.

ATPG3 sample

The Australian Temperament Project Generation 3 (ATPG3) study is an ongoing prospective study of infants born to a 35-year, 15-wave, population-based cohort. The study has tracked the social and emotional health and development of the main cohort (Generation 2) since they were 4-8 months of age in 1983, along with their parents (Generation 1). The original sample (N=2443 G2 infants and their G1 parents) were recruited through maternal and child health centres in 20 urban and 47 rural local government areas in the state of Victoria, Australia. The sample paralleled population characteristics at the time (Prior *et al.*, 2000). Families were since invited to participate via mail survey every 1-2 years until 19-20 years and every 4 years thereafter. In 2012, the study commenced recruitment of the Generation 3 (G3) infant offspring born to G2 participants and their partners, with a similar design to VIHCS. Identification of pregnancies occurred via participant email or phone every six months between 2012 and 2018, representing the peak period of first births in Australia when participants were aged 29-36 years. Telephone or web interviews were conducted in trimester three, two months postpartum and one year postpartum. Consent was provided by Generation 1 participants from Waves 1-7, and additionally by Generation 2 participants from Waves 8-15, using consent forms approved by the relevant ethics committees. Generation 2 then provided informed written consent again on recruitment to the Generation 3 component of the study. Dependent on wave of data collection, study protocols were variously approved by human research ethics committees at the University of Melbourne, the Australian Institute of Family Studies and the Royal Children's Hospital, Melbourne.

Measures

Preconception maternal mental health problems were assessed during VAHCS Waves 2-7 (participant ages 14-21 years) using the Revised Clinical Interview Schedule (CIS-R) (Lewis *et al.*, 1992), a structured psychiatric interview designed to assess symptoms of anxiety and depression in community samples. The CIS-R has been validated for use with adolescent populations (Patton *et al.*, 1999). At each wave the total score was dichotomised at ≥12 to identify mixed depression-anxiety symptoms at a level lower than major depressive or anxiety disorder, but which a general practitioner would view as clinically significant (Lewis *et al.*, 1992). At Waves 8 and 9 (participant ages 24 and 29), symptoms of psychological distress were assessed with the 12-item General Health Questionnaire (GHQ-12), a screening measure widely used to assess psychiatric illness in the general population. Total scores were dichotomised at ≥3, a threshold that has been found to indicate psychological distress with sensitivity 76% and specificity 83% (Donath, 2001, Goldberg *et al.*, 1997), and corresponds to a CIS-R threshold of ≥12 (Lewis *et al.*, 1992).

Preconception maternal mental health problems in the ATP study were measured in adolescence and young adulthood using age-appropriate scales. Depressive symptoms were assessed in waves 10-12 (participant ages 13 to 18) using the 13-item Short Mood and Feelings Questionnaire (Turner *et al.*, 2014). At each wave the total score was dichotomised at ≥11 to identify moderate to severe depressive symptoms (Turner *et al.*, 2014). Anxiety symptoms were assessed using adapted versions of the Revised Behavior Problem Checklist Short Form in wave 10 (age 13-14) (Letcher *et* *al.*, 2012, Quay and Peterson, 1987) and the Revised Children's Manifest Anxiety Scale (Letcher *et al.*, 2012, Reynolds and Richmond, 1978) in waves 11-12 (ages 15-18). For each scale, respondents rated frequency of anxious feelings on a scale from 0 'never/rarely' to 1 'sometimes' to 2 'often/almost always', with mean scores > 'sometimes' denoting moderate to severe symptoms. At each wave, a summary variable was derived denoting presence of depressive and/or anxious symptoms. At waves 13-15 (ages 19-28), symptoms of depression and anxiety were assessed using the 21-item Depression Anxiety and Stress Scale (DASS-21; Antony *et al.*, 1998, Lovibond and Lovibond, 1995). The DASS-21 comprises three 7-item subscales measuring depression, anxiety, and stress. It has good psychometric properties and can distinguish symptoms of clinical-level severity (Antony *et al.*, 1998). Participants rated their psychological distress and physiological symptoms on a scale from 0 'did not apply to me at all' to 3 'applied to me very much or most of the time'. The depression, anxiety, and stress subscale scores were dichotomised at their respective thresholds for moderate to severe symptoms (≥7, ≥6, ≥10), and for each wave a summary variable was derived denoting presence of symptoms on one or more subscales.

For each cohort, we constructed variables denoting presence of any mental health problems at ≥1 adolescent wave (VAHCS Waves 2-6, ATP Waves 10-12), and ≥1 young adult wave (VAHCS Waves 7-9, ATP Waves 13-15). Based on these dichotomous variables, we created a four-level variable denoting continuity of mental health problems ('none', 'adolescent only', 'young adult only', and 'both adolescent and young adult').

Antenatal and postnatal maternal depressive symptoms were assessed in both VIHCS and ATPG3 in trimester three and at one year postpartum for each pregnancy, using the Edinburgh Postnatal Depression Scale (EPDS) (Cox *et al.*, 1987). The EPDS is a 10-item rating scale designed to screen for postpartum depression, which has also been validated for antenatal use (Murray and Cox, 1990). The total score (range 0-30) at each wave was dichotomised at a threshold (≥10) that is appropriate for use in community samples and when administered via telephone (de Figueiredo *et al.*, 2015, Gibson *et al.*, 2009). This cut-off has been found to indicate depressive disorder with sensitivity 76% and specificity 94% (Bergink *et al.*, 2011).

Infant offspring emotional reactivity was assessed in both VIHCS and ATPG3 via maternal report at one year postpartum using the Short Temperament Scale for Toddlers (STST), a 30-item survey designed to assess temperament in toddlers aged 1-3 years (Fullard *et al.*, 1984, Prior *et al.*, 1989). The reactivity subscale comprises eight items. High scores indicate a tendency to react negatively to unpleasant experiences (e.g. cries after a fall or bump), intensity of reaction (e.g. responds to frustration intensely (screams, yells)), and high activity levels (e.g. plays actively (bangs, throws, runs) with toys indoors). Parents rate the frequency of each item along a Likert scale, from 1 (almost never) to 6 (almost always). We calculated standardised mean scores for each individual, such that mean effects can be interpreted in units of standard deviations. In the absence of an established threshold we defined heightened emotional reactivity as an unstandardised mean score of ≥4 ("usually does").

Covariates. Our conceptual causal model included factors that were potential confounders of the associations between maternal mental health at each phase and offspring infant emotional reactivity. These were selected based on prior evidence in the literature, and included socioeconomic circumstances, maternal substance use, and offspring birth order and outcomes. Each of these potential confounding factors are associated with maternal mental health, and may affect offspring socio-emotional development through alternative pathways including effects on fetal neurodevelopment, parenting behaviour, and/or broader environmental exposures. Binary variables were constructed as follows: *Family of origin and adolescent characteristics*: mother's parents' high school completion (neither parent v. at least one parent completed) and divorce/separation before or during mother's adolescence (ever v. never divorced/separated), mother's high school completion (never v. ever completed), mother's adolescent smoking (daily smoking at one or more adolescent wave v. no daily smoking), and mother's history of divorce or separation (ever v. never divorced/separated); *pregnancy characteristics*: mother's periconceptional smoking (≥ v. < daily smoking immediately prior to pregnancy recognition), household perinatal poverty (< v. ≥ AUD $40,000/annum), and mother's primiparity (first v. subsequent liveborn infant); and *birth characteristics:* infant low birthweight (< v. ≥ 2.5kg), and premature birth (< v. ≥ 37 weeks).

Statistical analysis

Given that the cohorts were drawn from similar populations and employed similar offspring sampling and assessment procedures, the primary analyses used an integrated dataset that combined participant-level data from each cohort in order to increase sample size and statistical precision (Curran and Hussong, 2009, Hofer and Piccinin, 2009, Hutchinson *et al.*, 2015). We used linear and logistic regression to estimate the association between maternal mental health problems at each time-point (preconception, antenatal, and postnatal) and offspring infant reactivity at one year postpartum. Each model was fitted within a generalised estimating equation (GEE) framework to account for correlation between outcomes due to within-family clustering, and adjusted for cohort and background covariates occurring prior to or at the time of exposure. The antenatal and postnatal models were then progressively adjusted further for prior mental health problems. In supplementary analyses we repeated these analyses for each cohort separately.

We then performed a causal mediation analysis to examine the extent to which associations between persistent preconception mental health problems and offspring infant reactivity were mediated by antenatal or postnatal maternal depressive symptoms. We used a potential outcomes framework, specifically an interventional effects approach, which is considered appropriate given correlated, sequential mediators, and exposure-induced confounding of mediator-outcome associations (Moreno-Betancur and Carlin, 2018, Vansteelandt and Daniel, 2017).
An illustrative example of the conceptual model, with two mediators and two post-exposure confounders, is shown in Figure 1. The interventional *indirect effect* via a mediator is defined as the change in the mean standardised outcome score if, hypothetically, we could change the distribution of the mediator in the exposed group to that in the unexposed group, while holding the distribution of any descendent mediator(s) to that in the unexposed group. This amounts to removing changes in mean standardised outcome score that arise via the pathways from exposure via the mediator but not via its descendants. The interventional *direct effect* is defined as the magnitude of the exposure-outcome effect that would remain if, hypothetically, we could change the joint distribution of all mediators in the exposed group to that in the unexposed group. The component effects sum to the total marginally-adjusted effect (as opposed to the conditionally-adjusted GEE effect estimate), allowing us to determine the percentage via each component.

Insert Figure 1 about here

The mediation model was adjusted for background demographic characteristics, post-exposure pregnancy and birth characteristics (perinatal poverty and preterm birth), and cohort. Because the post-exposure characteristics may be influenced by the exposure and in turn may influence the outcome, they were treated technically as mediators in the model. We estimated interventional effects as standardised mean differences using regression-standardisation methods based on Monte Carlo simulation (43, 44). Inferences were based on the non-parametric bootstrap.

All analyses included participants who responded at least once in each phase (adolescent, young adult, and perinatal). Among these, there were low levels of missing data on most variables (<10%). However, due to challenges detecting pregnancies, a greater proportion missed the antenatal interview (36%). Incomplete data were handled using multiple imputation by chained equations (White *et al.*, 2011). We imputed 35 complete datasets separately for each cohort, based on the proportion of participants with missing data (Bodner, 2008). Parameter estimates were obtained by pooling results across imputed datasets using Rubin's rules (Rubin, 1987). We performed supplementary analyses using available case data. To assess potential for participation bias, we compared characteristics of participants in each cohort with those who were either not screened for pregnancies due to prior study withdrawal, or who were screened and eligible but did not participate. We used Stata 15 (StataCorp, 2015).

Results

The flow of participants through each study is presented in appendix p 2. In total, 398 women participated in VIHCS with 609 infants and 395 in ATPG3 with 676 infants. Of these, 37 ATPG2 women did not participate in adolescence and were excluded from the analysis sample, leaving 358 ATPG2 women with 622 ATPG3 infants, and a combined analysis sample of 756 women with 1231 infants who participated at least once in each phase (adolescence, young adulthood, and perinatally). Comparisons of women screened versus not screened and participating versus eligible non-participants are presented in appendix pp 3-4. Women who participated were broadly representative of those with live births during screening on measured baseline characteristics in each study, but there were some differences between those screened and not screened due to prior loss to follow-up. The ATP women screened were less likely to have parents born outside of Australia, but remained similar to the original ATP sample on the level of parental education. The VAHCS women screened were less likely to have engaged in frequent adolescent drinking, but there were no other notable differences on measured demographic, mental health or risky behaviours in adolescence at VAHCS study entry.

Table 1 summarises infants' and their mothers' characteristics, by cohort and combined. The majority of infants (61%; [95% CI 58-64]) had mothers who reported preconception mental health problems at least once in adolescence and/or young adulthood; of these, most were adolescent-onset. Post conception, 14% of women reported antenatal depressive symptoms and 10% reported postpartum depressive symptoms. Because 4% of women reported depressive symptoms at both timepoints, the overall rate of antenatal and/or postnatal depression was 20%. There were negligible differences between cohorts on most variables, consistent with expectations given the samples were drawn from similar populations, though rates of perinatal depressive symptoms were slightly higher in the ATPG3 than in VIHCS.

Insert Table 1 about here

Table 2 shows estimated associations of preconception, antenatal and postnatal maternal mental health problems with offspring infant reactivity. The estimated proportion of infants with heightened reactivity was higher in infants of mothers with both adolescent and young adult mental health problems than in infants of those without (37% [31- 44] vs. 23% [19-27]). After adjusting for background demographic characteristics and cohort, preconception maternal mental health problems that persisted across adolescence and young adulthood predicted a twofold increase in the odds of heightened infant reactivity (adjusted OR 2.1 [1.4-3.1]), compared with those with no preconception mental health problems. Similarly, in linear regression analyses, we found a mean difference in infant reactivity scores of 0.38 standard deviations between offspring of mothers with persistent preconception mental health problems and those with no preconception mental health problems. Maternal mental health problems antenatally and at one year postpartum were similarly associated with offspring infant reactivity, but the magnitude of these perinatal associations reduced somewhat after adjustment for prior exposure. Available case analyses of the combined cohorts yielded a similar pattern of results (appendix p 5). In supplementary analyses we repeated these analyses in each cohort (appendix pp 6-7). Preconception, antenatal and postpartum effects were evident in both cohorts. Postpartum effects were somewhat weaker in VIHCS linear models than ATPG3 linear models, but consistent across cohorts in the logistic models. In fully adjusted models, cohort was not associated with infant reactivity.

Insert Table 2 about here

Table 3 shows the results of the mediation analysis as depicted in Figure 1, examining the extent to which associations between persistent preconception mental health problems and offspring infant reactivity are mediated by antenatal or postnatal exposure. The marginally-adjusted total effect of persistent maternal preconception mental disorder on offspring infant reactivity was 0.42 of a standard deviation (0.41-0.44). Of this, around 1% was mediated by poverty alone. A further 6% was mediated by antenatal depression and not depression at one year postpartum, and 7% was mediated by depression at one year postpartum. The percentage mediated by preterm birth and not postpartum depression was -2%, slightly reducing the overall mediated effect size via an opposite pathway. The remaining 88% of the total effect was a direct effect of persistent maternal preconception mental health problems on offspring infant reactivity; not mediated by perinatal poverty, preterm birth, or maternal depressive symptoms antenatally or at one year postpartum.

Insert Table 3 about here

Discussion

Mothers with persistent mental health problems before pregnancy had twice the odds of having an infant with high emotional reactivity. This effect was robust across two independent samples, and is similar in size to the effects found for antenatal and postnatal maternal depressive symptoms, in this and prior studies (Davis *et al.*, 2007, Davis *et al.*, 2004, Huot *et al.*, 2004). Despite strong continuities between maternal preconception and perinatal mental health, the effects of preconception maternal mental health problems on offspring infant reactivity were, for the most part, not mediated through greater offspring exposure to maternal depressive symptoms during pregnancy or postpartum. Furthermore, at least part of the associations between perinatal depression and infant emotional reactivity are accounted for by preconception exposure. Infants of mothers with preconception mental health problems may have greater emotional reactivity due to greater exposure during pregnancy and after birth but also through risk processes well before the recognition of the pregnancy.

Associations between both antenatal and postnatal maternal depressive symptoms and heightened infant reactivity are consistent with prior work. However, a finding of a similar-sized and largely direct effect of exposure to persisting maternal mental health problems prior to pregnancy is new. We cannot exclude confounding by genetic susceptibility (Luciano *et al.*, 2018), though 'children of twin' studies indicate that independent links between parent depressive symptoms and offspring internalising or externalising problems persist after accounting for genetic transmission (McAdams *et al.*, 2015). We have considered a range of baseline confounders related to family background, as well as those that might confound the relationship with mediators including perinatal household poverty and infant prematurity. It nevertheless remains possible that other unmeasured contextual factors have confounded the observed associations. These may include stressful life events, family violence or other childhood trauma, caregiver and peer relationship quality, or perceived social support (Stein et al., 2014, Yehuda and Meaney, 2018).

Potential mechanisms

We considered the possibility that preconception mental health problems might affect offspring infant reactivity through persistence of maternal symptoms into the antenatal and postnatal periods (Meaney and Szyf, 2005). However, preconception exposure effects on infant reactivity were largely direct, with mediation through antenatal and postnatal processes relatively small.

Although it is possible that a failure to fully identify maternal antenatal and postnatal mental health problems has led to an underestimation of mediation effects, depressive symptoms are the commonest perinatal mental health problem and prevalence at each timepoint in our study was consistent with previous meta-analyses in high-income countries (Woody *et al.*, 2017).

It is also possible that chronic preconception mental health problems might have an enduring effect on maternal endocrine and immune-inflammatory physiology, affecting the fetal environment even when mothers report few perinatal depressive symptoms (Moog *et al.*, 2018). One recent study linked maternal abuse in childhood to increased placental hormone production during later pregnancies, providing preliminary evidence that maternal stress before conception may influence offspring neurodevelopment through changes to the *in utero* environment (Moog *et al.*, 2016). We assessed antenatal maternal depressive symptoms in the third trimester and may not have captured periconceptional exposure including during embryogenesis and implantation, both sensitive to environmental influence including maternal stress (Ord *et al.*, 2017). Brain regions integral to stress response regulation and susceptible to excess exposure to maternal hormones are identifiable by eight weeks gestation (Gunnar and Davis, 2013). Similarly, preconception mental health problems may also be linked to infant emotional reactivity through increased risk of other exposures during pregnancy and postpartum, including health-related behaviours such as maternal substance use or diet, or social factors such as perceived social support, maternal attachment style, partner relationship quality and conflict, or family violence (Howard *et al.*, 2014).

A final possibility is that persistent maternal mental health problems prior to pregnancy might directly affect the maternal germline with persisting effects on offspring stress response and reactivity (Chan *et al.*, 2017). The epigenetic profile of gamete DNA can be altered by parental exposure to stress (Klengel *et al.*, 2015) but until recently these alterations were thought to be completely erased during embryonic development. There is now evidence that some epigenetic marks persist after fertilisation (Klengel *et al.*, 2015). Animal data support the intergenerational transfer of stress-related behaviours through epigenetic modifications to the paternal germline (Klengel *et al.*, 2015). Though studies of maternal germline transmission are limited, evidence is emerging that stress reactivity traits may also be maternally transmitted by epigenetic modifications to methylation of gamete genes associated with altered stress response (Mitchell *et al.*, 2016). Non-epigenetic gametic alterations, such as the accumulation of metabolites and proteins in oocyte cytoplasm, may also influence fetal development and offspring phenotype (Kovalchuk, 2012).

Developmental origins of mental health and disease: a role for preconception influences

Heightened reactivity in response to ante- and postnatal stress may have predictive adaptive utility, altering stress physiology and brain structure to confer survival advantage in environments characterised by scarcity or threat (Gluckman *et al.*, 2009, Sheriff *et al.*, 2017). For example, evidence suggests that infants exposed to maternal depressive symptoms during only one perinatal timepoint (either pregnancy or postpartum) demonstrate lower mental development at one year postpartum compared to infants not exposed at either timepoint or exposed at both timepoints (Sandman *et al.*, 2011). The current study raises the question about whether predictive adaptive responses might arise prior to pregnancy, with longer-term maternal stress prior to conception providing a more stable source of environmental information (Kuzawa and Quinn, 2009). Yet such adaptations might come at a cost with reactive infants having greater susceptibility to childhood emotional and behavioural problems (Belsky, 2005, Boyce and Ellis, 2005, Bylsma *et al.*, 2008, Hartman and Belsky, 2018, Slagt *et al.*, 2016).

Strengths and limitations

This study drew together data from two rare prospective intergenerational studies, with repeated assessment across adolescence and young adulthood, and during pregnancy and postpartum of the next generation, allowing us to examine the relative contribution of mental health problems at each phase. Combining data allowed us to achieve greater precision estimates via pooled analyses, and to examine the consistency of findings across intergenerational samples. The two studies maintained high retention rates, and 85% and 88% of women with live births during the VIHCS and ATPG3 recruitment phases respectively participated in the intergenerational studies. However, a number of limitations should be noted. First, despite consistency in most measures in VIHCS and ATPG3 (i.e., mediators, outcomes and most covariates), measurement of preconception mental health varied between studies. Nonetheless, the prevalence of preconception mental health problems and demographic characteristics were similar across cohorts; the overall pattern of results was similar in the pooled and within cohort analyses; and adjustment for cohort in the models did not alter effect estimates. Sample loss and related bias are further potential limitations. Aside from loss of a small number of women with frequent adolescent drinking (VIHCS) or parents born outside Australia (ATPG3), those screened for and participating in each study remained broadly similar to the original and eligible study samples on measured characteristics at baseline. Even so, it is possible that the achieved sample differed on unmeasured confounders with some effect on associations found. There were low levels of missing data at most waves, in both cohorts;

however, around one third of antenatal interviews were missed due to difficulties in detecting eligible pregnancies. We addressed potential biases due to missing data using multiple imputation. We also only included infants born to women aged 29-36 years. This included the median maternal age at birth in Australia and maximised the number of included births, but it remains possible that the risk profiles of older and younger mothers may differ from those in focus in this study.

Finally, infant emotional reactivity was assessed by maternal report and usefully draws on a mother's knowledge of her baby's usual behaviour across contexts, particularly relevant for the study of phenotypic traits such as emotional reactivity (Bates et al., 2014, Shiner and Caspi, 2003). Maternally reported infant reactivity predicts later child social and emotional problems, with effect sizes similar to studies of independently assessed infant reactivity (Kagan *et al.*, 1999, Sayal *et al.*, 2013). However, maternal report of infant outcomes may be affected by a mother's mental state such that depressed mothers perceive their infant as more reactive (Luoma *et al.*, 2004, Najman *et al.*, 2001). We investigated this possibility by including maternal depressive symptoms at the time of the outcome in our mediation model. The association between preconception maternal mental health and offspring infant emotional reactivity was overwhelmingly independent of maternal depressive symptoms at the time of the outcome, suggesting minimal role of maternal reporting bias due to concurrent depression. These findings align with previous research indicating that depression-related biases explain only a small proportion of variance in maternally reported child behavioural traits (Bagner et al., 2013, Goodman et al., 2011, Rothbart and Bates, 2006).

Conclusion

Maternal mental health problems remain one of the most significant early life risk factors for childhood emotional and behavioural problems. The current findings do not detract from the importance of antenatal and infancy phases as intervention points for both mothers and offspring, to improve mental health outcomes for infants higher in emotional reactivity (Belsky, 2005, Boyce and Ellis, 2005, Slagt *et al.*, 2016). Indeed, highly reactive children encountering few challenges may have a lower likelihood of externalising problems, and greater prosocial behaviours, school engagement and cognitive competence than low-reactive children (Obradović *et al.*, 2010, Slagt *et al.*, 2016). Yet the current study suggests that intervention in the perinatal period alone is unlikely to be sufficient to eliminate risks for the offspring of women with persistent mental health problems prior to pregnancy. It is perhaps one reason why the effects of existing postnatal interventions on maternal depression have been mixed (Poobalan *et al.*, 2007, Stein *et al.*, 2018). There is now a need to further explore whether the effects of maternal preconception mental health problems extend to higher rates of emotional and behavioural problems in later childhood, as well as understand the processes whereby preconception exposure leads to heightened infant reactivity. Even so, the current findings suggest that a reorientation of clinical services and public health responses to the years prior to pregnancy is warranted. Current approaches to preconception care, for example, have largely focused on contraception (Patton *et al.*, 2018) with little attention to maternal mental health. The growing calls for preconception health care around other aspects of health and health risk (Barker *et al.*, 2018) should also extend to mental health (Wilson *et al.*, 2018). It is likely that the benefits will extend beyond women themselves to their children's emotional development.

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Conflict of interest

None.

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Figure 1. Directed acyclic graph illustrating the causal pathways estimated in the two-mediator model

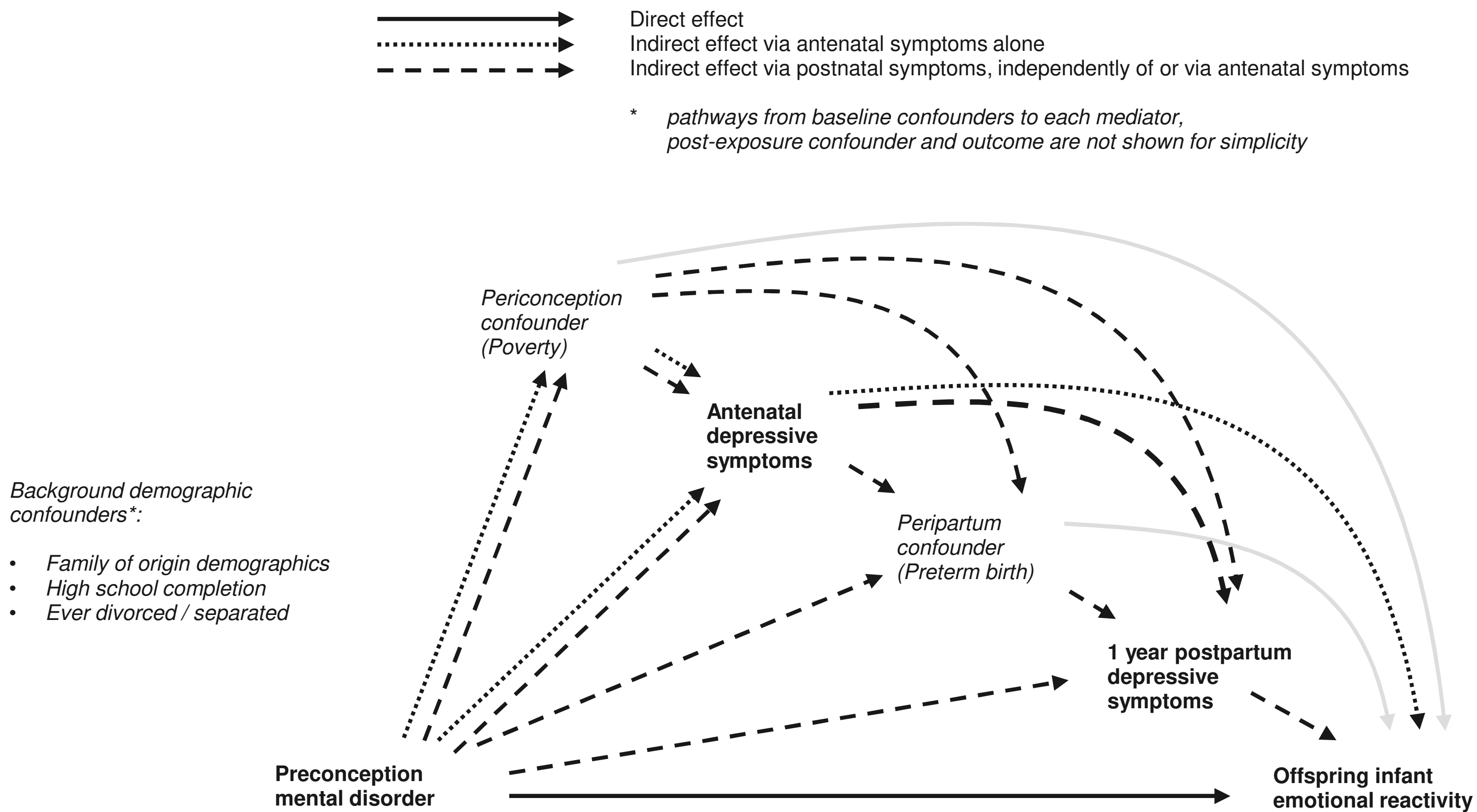


Table 1. Estimated preconception and perinatal sample characteristics of infants and mothers, in each cohort and combined.

	VIHCS N=609		ATPG3 N=622		Combined N=1231	
Preconception	n	(%)	n	(%)	n	(%)
Family background						
Mother's parents divorced / separated	110	(18)	100	(16)	210	(17)
Mother's parents didn't complete high school	225	(37)	150	(24)	375	(30)
Mother's preconception characteristics						
Ever separated or divorced	90	(15)	82	(13)	172	(14)
Never completed high school	43	(7)	29	(5)	73	(6)
Any daily cigarette smoking in adolescence	122	(20)	99	(16)	221	(18)
Mother's mental health problems						
Any adolescent mental health problems	301	(49)	306	(49)	606	(49)
Any young adult mental health problems	224	(37)	228	(37)	452	(37)
Continuity of mental health problems						
None	242	(40)	243	(39)	485	(39)
Adolescent only	143	(23)	150	(24)	294	(24)
Young adult only	67	(11)	73	(12)	139	(11)
Both adolescent and young adult	157	(26)	156	(25)	313	(26)
Perinatal						
Mother's periconceptional characteristics						
Primiparous	282	(46)	281	(45)	563	(46)
Household perinatal poverty	41	(7)	33	(5)	74	(6)
Daily cigarette smoking	76	(12)	60	(10)	135	(11)
Mother's mental health problems						
Antenatal depressive symptoms (third trimester)	76	(12)	100	(16)	175	(14)
Postnatal depressive symptoms (1 year)	49	(8)	70	(11)	119	(10)
Infant characteristics						
Female sex	307	(50)	316	(52)	623	(51)
Pre-term birth (< 37 weeks)	37	(6)	48	(8)	85	(7)
Low birthweight (< 2.5 kg)	29	(5)	43	(7)	72	(6)
Infant emotional reactivity (mean, sd)	2.46	(0.65)	2.64	(0.57)	2.54	(0.62)

Frequency estimates were calculated from imputed percentage estimates and total number of infants. VIHCS=The Victorian Intergenerational Health Cohort Study. ATPG3=The Australian Temperament Project, Generation 3. The difference in mother's parents' secondary completion reflects between-cohort differences in the original study data capture, with VIHCS capturing non-completion of secondary school and ATPG3 capturing non-completion of post-secondary school qualifications. Other covariates were assessed consistently across the two cohorts.

Table 2. Estimated adjusted associations of preconception and perinatal maternal mental health problems with infant emotional reactivity, in combined data (N=1231 infants of 756 women).

		Offspring infant emotional reactivity							
		Logistic regression					Linear regression		
Maternal mental health problems	**n[1]**	**n[2]**	**%**	**OR**	**(95% CI)**	***p***	**β**	**(95% CI)**	***p***
Preconception[#]									
Adjusted for background characteristics									
No waves (reference)	485	109	23						
Adolescent only	294	78	27	1.3	(0.9 , 2.0)	0.226	0.11	(-0.08 , 0.30)	0.251
Young adult only	139	36	26	1.3	(0.7 , 2.1)	0.414	0.15	(-0.09 , 0.38)	0.217
Adolescent and young adult	313	115	37	2.1	(1.4 , 3.1)	<0.001	0.38	(0.20 , 0.57)	<0.001
Antenatal[†]									
Adjusted for background characteristics	175	73	42	2.2	(1.3 , 3.8)	0.003	0.37	(0.17 , 0.56)	<0.001
Further adjusted for preconception mental health	175	73	42	1.9	(1.1 , 3.3)	0.021	0.27	(0.07 , 0.48)	0.008
Postnatal*									
Adjusted for background characteristics	119	52	44	2.2	(1.4 , 3.6)	0.001	0.31	(0.10 , 0.53)	0.004
Further adjusted for preconception mental health	119	52	44	1.9	(1.2 , 3.1)	0.009	0.23	(0.01 , 0.45)	0.044
Further adjusted for antenatal mental health	119	52	44	1.7	(1.1 , 2.9)	0.030	0.18	(-0.05 , 0.41)	0.129

n[1] = number exposed; n[2] = number with exposure and outcome. Frequency estimates were calculated from imputed percentage estimates and total number of infants. Heightened infant reactivity at one year of age defined as unstandardised STST reactivity mean score ≥4. Linear regression estimates are presented as standardised mean score differences.
# Background characteristics: cohort, mother's parents high school completion and divorce, mother's high school completion, and mother's adolescent smoking.
† Background characteristics: cohort, mother's parents high school completion and divorce, mother's high school completion, mother's adolescent smoking, mother's history of separation and divorce, periconceptional smoking, perinatal poverty, and parity.
* Background characteristics: cohort, mother's parents high school completion and divorce, mother's high school completion, mother's adolescent smoking, mother's history of separation and divorce, periconceptional smoking, perinatal poverty, parity, infant preterm birth, and low birthweight.

Table 3. Estimated direct and indirect pathways from persistent preconception maternal mental health problems to offspring infant emotional reactivity at one year of age, after adjusting for baseline and intermediate confounding, in combined data (N=1231 infants of 756 women).

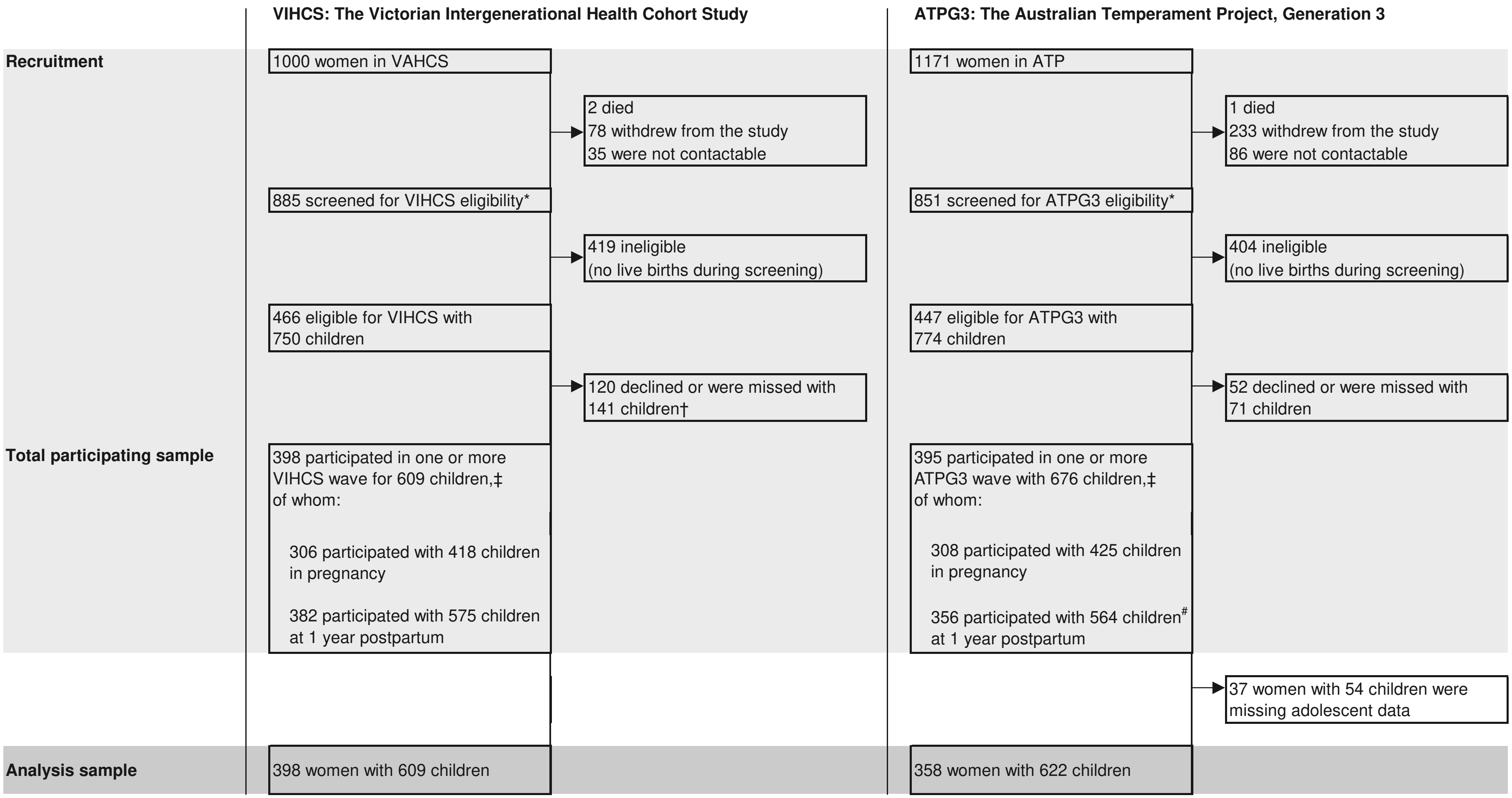
	Mean difference	(95% CI)	Proportion attributable
Direct effect $_{(interv)}$	0.37	(0.35 , 0.39)	88
Indirect effect $_{(interv)}$	0.05	(0.04 , 0.06)	12
Indirect via perinatal poverty	0.01	(0.00 , 0.01)	1
Indirect via antenatal depressive symptoms	0.02	(0.02 , 0.03)	6
Indirect via preterm birth	-0.01	-(0.01 , -0.01)	-2
Indirect via postnatal depressive symptoms	0.03	(0.03 , 0.04)	7
Total causal effect $_{(interv)}$	0.42	(0.41 , 0.44)	100

Marginally adjusted linear regression estimates are presented as standardised mean score differences. Proportion attributable was calculated as a percentage of the total effect. Persistent preconception maternal mental health problems defined as presence of mental health problems during both adolescence and young adulthood. Model adjusted for baseline confounders (cohort, mother's parents high school completion and divorce, mother's high school completion, and mother's history of separation/divorce). The postnatal estimate includes an effect via the mediator’s interdependence [see Vansteelandt, S. and R. M. Daniel (2017)] which was very small in this study.

Supplementary Table 1. Sample descriptions and included assessement waves and ages

Parent Cohorts	ATP	VAHCS
Year commenced	1983	1992
Recruited sample	A representative sample of 2443 infants (1170 female) (aged 4-8 months) in the state of Victoria, Australia	A representative sample of 1943 (1000 female) mid-secondary school students (aged 14-15) in the state of Victoria, Australia
Adolescent assessment waves	Wave 10: 13-14 years Wave 11: 15-16 years Wave 12: 17-18 years	Wave 2: 15.4 years Wave 3: 15.9 years Wave 4: 16.3 years Wave 5: 16.8 years Wave 6: 17.4 years
Young adult assessment waves	Wave 13: 19-20 years Wave 14: 23-24 years Wave 15: 27-28 years	Wave 7: 20.6 years Wave 8: 24.0 years Wave 9: 29.0 years
Offspring Cohorts	**ATPG3**	**VIHCS**
Year commenced	2011	2006
Recruited sample	395 ATP women with 676 infants	398 VAHCS women with 609 infants
Perinatal assessment waves	Wave 1: Third trimester of pregnancy Wave 3: 1 year postpartum	Wave 1: Third trimester of pregnancy Wave 3: 1 year postpartum

Supplementary Figure 1. Sampling and ascertainment of VIHCS and the ATPG3



* Eligibility defined as all live births occurring during screening (VIHCS: September 2006 - June 2013; ATPG3: December 2011 - August 2018).

† Of the 120 VAHCS women who didn't participate for 1+ eligible VIHCS children, 68 were excluded and the remaining women were recruited to participate in the study with 1+ other child.

‡ In each study many parents participated with more than one child born during the recruitment phase.

ATPG3 1 year assessments ongoing until end 2019

Supplementary Table 2a. Comparison of baseline charateristics at VAHCS study recruitment in adolescence of a) VAHCS women screened and not screened for VIHCS eligibility and b) eligible women who did and did not participate in VIHCS.

	Comparison between the VAHCS women screened and not screened for VIHCS										
	All VAHCS women[a]			Screened[b]			Not screened[c]			Screened v. not screened	Screened v. all VAHCS women
	N	*n*	(%)	*N*	*n*	(%)	*N*	*n*	(%)	χ^2 *p* -value	χ^2 *p* -value
Baseline adolescent characteristics											
Adolescent common mental disorder (CIS-R ≥ 12)	1000	342	34	885	305	34	113	37	33	0.717	0.869
Regular cigarette smoking (≥ daily)	1000	120	12	885	102	12	113	18	16	0.175	0.664
Regular cannabis use (≥ monthly)	987	69	7	876	61	7	109	8	7	0.885	0.975
Frequent drinking (> 3 times per week)	1000	27	3	885	20	2	113	7	6	0.015	0.197
Family of origin demographic factors											
Parents divorced or separated	999	221	22	885	195	22	112	26	23	0.777	0.962
Neither parent completed high school	966	364	38	870	329	38	94	35	37	0.912	0.944

	Comparison between the eligible women participating and non-participating in VIHCS										
	All eligible women[d]			Participants[e]			Eligible non-participants[f]			Participants v. eligible non-participants	Participants v. all eligible women
	N	*n*	(%)	*N*	*n*	(%)	*N*	*n*	(%)	χ^2 *p* -value	χ^2 *p* -value
Baseline adolescent characteristics											
Adolescent common mental disorder (CIS-R ≥ 12)	466	146	31	398	121	30	68	25	37	0.296	0.699
Regular cigarette smoking (≥ daily)	466	51	11	398	39	10	68	12	18	0.055	0.481
Regular cannabis use (≥ monthly)	462	30	6	394	26	7	68	4	6	0.825	0.937
Frequent drinking (> 3 times per week)	466	8	2	398	7	2	68	1	2	0.866	0.928
Family of origin demographic factors											
Parents divorced or separated	466	89	19	398	79	20	68	10	15	0.319	0.704
Neither parent completed high school	459	184	40	393	152	39	66	32	49	0.132	0.565

a. 1000 women originally recruited to VAHCS in adolescence

b. 885 women active in VAHCS at VIHCS commencement, and screened for VIHCS eligibility

c. 115 women lost to follow-up in VAHCS at VIHCS commencement, and not screened for VIHCS eligibility

d. 466 women eligible to participate in VIHCS with one or more live-born children during VIHCS screening

e. 398 women who participated in VIHCS with one or more live-born children

f. 68 women eligible to participate in VIHCS with one or more live-born children during VIHCS screening, who refused all participation or were missed.

Supplementary Table 2b. Comparison of baseline charateristics at ATP study recruitment in infancy of a) ATP women screened and not screened for ATPG3 eligibility and b) eligible women who did and did not participate in ATPG3.

	Comparison between the ATP women screened and not screened for ATPG3 eligibility							
	All ATP women[a] N=1171		Screened[b] N=851		Not screened[c] N=320		Screened v. not screened	Screened v. all ATP women
	n	(%)	*n*	(%)	*n*	(%)	χ^2 *p* -value	χ^2 *p* -value
Family of origin demographic factors								
Mother didn't complete high school	831	71	593	70	238	74	0.019	0.224
Father didn't complete high school	599	51	422	50	177	55	0.004	0.143
Mother non-Australian born	236	20	145	17	91	28	<0.001	0.023
Father non-Australian born	297	25	187	22	110	34	<0.001	0.018

	Comparison between eligible women participating and non-participating in ATPG3							
	All eligible women[d] N=447		Participants[e] N=395		Eligible non-participants[f] N=52		Participants v. eligible non-participants	Participants v. all eligible women
	n	(%)	*n*	(%)	*n*	(%)	χ^2 *p* -value	χ^2 *p* -value
Family of origin demographic factors								
Mother didn't complete high school	314	70	271	69	43	83	0.027	0.372
Father didn't complete high school	207	46	179	45	28	54	0.170	0.596
Mother non-Australian born	65	15	58	15	7	13	0.850	0.877
Father non-Australian born	92	21	81	21	11	21	0.867	0.830

a. 1171 women originally recruited to ATP in infancy

b. 851 women active in ATP at ATPG3 commencement, and screened for ATPG3 eligibility

c. 320 women lost to follow-up in ATP at ATPG3 commencement, and not screened for ATPG3 eligibility

d. 447 women eligible to participate in ATPG3 with one or more live-born children during ATPG3 screening

e. 395 women who participated in ATPG3 with one or more live-born children

f. 52 women eligible to participate in ATPG3 with one or more live-born children during ATPG3 screening, who refused all participation or were missed.

Supplementary Table 3. Estimated associations of preconception and perinatal maternal mental health problems with infant emotional reactivity in the VIHCS and ATPG3 combined, using available case data.

			Offspring infant emotional reactivity							
			Logistic regression					Linear regression		
Maternal mental health problems	**N[1]**	**n[2]**	**n[3]**	**%**	**OR**	**(95% CI)**	***p***	**β**	**(95% CI)**	***p***
Preconception[#]										
Adjusted for background characteristics										
No waves (reference)										
Adolescent only	1090	266	65	24	1.1	(0.8 , 1.7)	0.508	0.05	(-0.12 , 0.22)	0.556
Young adult only	1090	123	33	27	1.4	(0.8 , 2.2)	0.232	0.18	(-0.04 , 0.40)	0.102
Adolescent and young adult	1090	251	94	37	2.1	(1.5 , 3.0)	0.000	0.34	(0.17 , 0.51)	0.000
Antenatal[†]										
Adjusted for background characteristics	680	85	36	42	2.2	(1.4 , 3.5)	0.001	0.37	(0.14 , 0.60)	0.001
Further adjusted for preconception mental health	680	85	36	42	1.8	(1.1 , 3.0)	0.017	0.27	(0.04 , 0.51)	0.023
Postnatal*										
Adjusted for background characteristics	1029	90	40	44	2.1	(1.4 , 3.3)	0.001	0.29	(0.07 , 0.50)	0.009
Further adjusted for preconception mental health	1029	90	40	44	1.8	(1.1 , 2.9)	0.013	0.21	(-0.01 , 0.42)	0.066
Further adjusted for antenatal mental health	1029	90	40	44	1.7	(1.1 , 2.7)	0.024	0.18	(-0.04 , 0.40)	0.108

N[1] = number with exposure and outcome data; n[2] = number exposed; n[3] = number with exposure and outcome. Frequency estimates were calculated from imputed percentage estimates and total number of infants. Heightened infant reactivity at one year of age defined as unstandardised STST reactivity mean score ≥4. Linear regression estimates are presented as standardised mean score differences.

Background characteristics: cohort, mother's parents high school completion and divorce, mother's high school completion, and mother's adolescent smoking.

† Background characteristics: cohort, mother's parents high school completion and divorce, mother's high school completion, mother's adolescent smoking, mother's history of separation and divorce, periconceptional smoking, perinatal poverty, and parity.

* Background characteristics: cohort, mother's parents high school completion and divorce, mother's high school completion, mother's adolescent smoking, mother's history of separation and divorce, periconceptional smoking, perinatal poverty, parity, infant preterm birth, and low birthweight.

Supplementary Table 4a. Estimated associations of preconception and perinatal maternal mental health problems with infant emotional reactivity, in 609 infants of 398 women who participated in VIHCS.

		Offspring infant emotional reactivity							
		Logistic regression					Linear regression		
Maternal mental health problems	**n[1]**	**n[2]**	**%**	**OR**	**(95% CI)**	***p***	**β**	**(95% CI)**	***p***
Preconception[#]									
Adjusted for background characteristics									
No waves (reference)									
Adolescent only	144	30	21	1.2	(0.6 , 2.3)	0.590	0.12	(-0.14 , 0.38)	0.351
Young adult only	67	19	28	1.8	(0.8 , 3.9)	0.150	0.24	(-0.09 , 0.57)	0.151
Adolescent and young adult	157	53	34	2.4	(1.3 , 4.2)	0.003	0.40	(0.15 , 0.64)	0.002
Antenatal[†]									
Adjusted for background characteristics	76	28	37	2.1	(1.1 , 4.2)	0.024	0.33	(0.04 , 0.62)	0.026
Further adjusted for preconception mental health	76	28	37	1.7	(0.9 , 3.4)	0.129	0.24	(-0.07 , 0.54)	0.126
Postnatal*									
Adjusted for background characteristics	49	21	42	2.5	(1.3 , 4.8)	0.008	0.23	(-0.09 , 0.55)	0.157
Further adjusted for preconception mental health	49	21	42	2.0	(1.0 , 3.9)	0.054	0.12	(-0.20 , 0.45)	0.464
Further adjusted for antenatal mental health	49	21	42	1.8	(0.9 , 3.6)	0.119	0.07	(-0.27 , 0.41)	0.689

n[1] = number exposed; n[2] = number with exposure and outcome. Frequency estimates were calculated from imputed percentage estimates and total number of infants. Heightened infant reactivity at one year of age defined as unstandardised STST reactivity mean score ≥4. Linear regression estimates are presented as standardised mean score differences.

Background characteristics: cohort, mother's parents high school completion and divorce, mother's high school completion, and mother's adolescent smoking.

† Background characteristics: cohort, mother's parents high school completion and divorce, mother's high school completion, mother's adolescent smoking, mother's history of separation and divorce, periconceptional smoking, perinatal poverty, and parity.

* Background characteristics: cohort, mother's parents high school completion and divorce, mother's high school completion, mother's adolescent smoking, mother's history of separation and divorce, periconceptional smoking, perinatal poverty, parity, infant preterm birth, and low birthweight.

Supplementary Table 4b. Estimated associations of preconception and perinatal maternal mental health problems with infant emotional reactivity, in 622 infants of 358 women who participated in ATPG3.

		Offspring infant emotional reactivity							
		Logistic regression					Linear regression		
Maternal mental health problems	n[1]	n[2]	%	OR	(95% CI)	*p*	β	(95% CI)	*p*
Preconception[#]									
Adjusted for background characteristics									
No waves (reference)									
Adolescent only	150	48	32	1.5	(0.8 , 2.7)	0.205	0.11	(-0.16 , 0.39)	0.417
Young adult only	73	17	23	0.9	(0.4 , 2.0)	0.825	0.08	(-0.25 , 0.41)	0.642
Adolescent and young adult	156	62	40	1.9	(1.1 , 3.4)	0.022	0.39	(0.11 , 0.66)	0.006
Antenatal[†]									
Adjusted for background characteristics	100	46	46	2.4	(1.2 , 4.9)	0.017	0.40	(0.13 , 0.68)	0.004
Further adjusted for preconception mental health	100	46	46	2.1	(1.0 , 4.5)	0.051	0.30	(0.01 , 0.60)	0.041
Postnatal*									
Adjusted for background characteristics	70	31	45	2.1	(1.1 , 4.2)	0.032	0.40	(0.09 , 0.70)	0.011
Further adjusted for preconception mental health	70	31	45	1.9	(1.0 , 3.8)	0.069	0.32	(0.01 , 0.63)	0.040
Further adjusted for antenatal mental health	70	31	45	1.7	(0.9 , 3.5)	0.132	0.27	(-0.04 , 0.59)	0.083

n[1] = number exposed; n[2] = number with exposure and outcome. Frequency estimates were calculated from imputed percentage estimates and total number of infants. Heightened infant reactivity at one year of age defined as unstandardised STST reactivity mean score ≥4. Linear regression estimates are presented as standardised mean score differences.

Background characteristics: cohort, mother's parents high school completion and divorce, mother's high school completion, and mother's adolescent smoking.

† Background characteristics: cohort, mother's parents high school completion and divorce, mother's high school completion, mother's adolescent smoking, mother's history of separation and divorce, periconceptional smoking, perinatal poverty, and parity.

* Background characteristics: cohort, mother's parents high school completion and divorce, mother's high school completion, mother's adolescent smoking, mother's history of separation and divorce, periconceptional smoking, perinatal poverty, parity, infant preterm birth, and low birthweight.