STUDY PROTOCOL

Prenatal maternal stress and risk of neurodevelopmental disorders in the offspring: A systematic review and meta-analysis protocol [version 1; referees: 1 approved, 1 approved with reservations]

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Abstract

Background: Prenatal maternal stress (PNMS) is defined as the experience of significant levels of prenatal stress, depression or anxiety during pregnancy. PNMS has been associated with increased risk of autism spectrum disorder (ASD) and attention-deficit hyperactivity disorder (ADHD) in exposed offspring. However, these findings are inconsistent and other studies found no association, meaning a clear consensus on the impact of PNMS on ASD and ADHD risk is required. The purpose of this systematic review and meta-analysis is to summarize and critically review the existing literature on the effects of PNMS on ASD and ADHD risk.

Methods: Electronic databases (PubMed, PsycINFO, Web of Science, Scopus and EMBASE) will be searched for articles following a detailed search strategy. We will include cohort, case-control and cross-sectional studies that assessed maternal exposure to psychological and/or environmental stress and had ASD or ADHD as an outcome. Two reviewers will independently screen the titles, abstracts and full articles to identify eligible studies. We will use a standardised data extraction form for extracting data and a bias classification tool for assessing study quality. This systematic review will be reported according to the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA). The generic inverse variance method will be used if possible to perform meta-analyses.

Ethics and dissemination: Ethical approval is not required for this study because it will not involve the conduct or inclusion of any experimental or personal data that would require informed consent. The systematic review will be disseminated in peer-reviewed journals.

PROSPERO registration number: CRD42018084222.
Keywords
Prenatal maternal stress, Autism spectrum disorder; attention-deficit hyperactivity disorder; Systematic review; Protocol

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Competing interests: No competing interests were disclosed.

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Introduction

Description of the exposure

A growing body of evidence increasingly supports the hypothesis that indicates that early adverse experiences, including maternal stress during pregnancy, may have a significant effect on the fetal and postnatal brain development and subsequent organization of behaviour. Prenatal maternal stress (PNMS) refers to a status of low or negative well-being experienced during pregnancy. The term “stress” in this context often covers negative life events, anxiety and depressive feelings, and the prevalence of depression, anxiety, and stress in pregnant women has been estimated at 12%, 28% and 31%, respectively. A number of studies have reported an association between PNMS and increased risk of psychopathology in children. Specifically, a number of studies have reported that maternal exposure to significant life stressors (such as bereavement, job loss, divorce, trauma) at critical periods during pregnancy may change neurobehavioral outcomes in exposed offspring.

Description of the outcomes

Attention-deficit/hyperactivity disorder (ADHD) is a neurodevelopmental disorder that affects up to 1 in 20 children in the USA. It is a developmental condition characterized by inattention, hyperactivity and impulsivity behaviour. These symptoms often continue into adulthood and can significantly impact quality of life. Autism spectrum disorder (ASD) is another lifelong neurodevelopmental disorder characterized by three behaviourally altered domains including social deficits, impaired communication, and stereotyped and repetitive behaviours, and these symptoms begin in early childhood. The aetiological basis of these disorders is complex and multifactorial, and while twin studies show that genetic factors play a key role, environmental factors, such as PNMS, have also been proposed to play a role in their aetiology.

In support of this, there is a growing literature reporting an association between PNMS and increased risk of these neurodevelopmental disorders in exposed offspring. For example, Class et al. found an increased risk of ADHD and autism spectrum disorder (ASD) in children whose mothers were exposed to bereavement during the third trimester of pregnancy.

However, there are inconsistencies in the literature regarding the association between PNMS and neurobehavioral outcomes in affected offspring, with some studies finding no association between PNMS and the risk of neurodevelopmental disorders in the offspring. Moreover, for those studies that have reported an association between PNMS and adverse neurodevelopmental outcomes in the offspring, there is no clear consensus on the period of gestation that is most critical.

Why it is important to perform this review

Developing a clear consensus on this issue is important as identifying children at increased risk of ASD and ADHD may allow for greater paediatric surveillance of those most at risk to facilitate early intensive behavioural interventions, which can improve behavioural outcomes. For example, the Early Start Denver Model is a behavioral intervention aimed at improving cognitive, social and behavioral outcomes of toddlers diagnosed with ASD. This intervention can improve cognitive and adaptive behaviour with reduction of the severity of the diagnosis. Moreover, a consensus on this issue may underscore the need for alternative interventions during pregnancy aimed at reducing stress, anxiety and depression. Currently there is only one intervention that starts during pregnancy, involving multiple home visits by qualified nurses, known as the Nurse Family Partnership. This intervention has been reported to lead to long-term improvements in parenting behaviour, as well as in child outcome, and in particular, in the reduction of criminal and antisocial behaviour, that often are consequences of ADHD and conduct disorder. Accordingly, a better knowledge of risk factors for neurodevelopmental disorders may allow increased screening and early intervention for children exposed to PNMS, with the aim of improving their long-term neurodevelopmental outcome.

Rationale for current systematic review

Evidence suggests that PNMS can have a role in increasing the risk of ASD and ADHD and that antenatal depression symptoms could be related to child behavioural problems. However, a number of studies reported no association between PNMS and adverse neurodevelopmental outcome in children. Accordingly, the aim of this systematic review and meta-analysis is to summarise the existing literature on the effects of PNMS, anxiety and depression feelings on the risk of ASD and ADHD, and if possible, to provide a quantitative estimate of this relationship.

Research questions

The primary objective of this study will be to systematically report the evidence of an increased risk of ASD and ADHD in offspring exposed to PNMS.
The specific review questions to be addressed are:

1. Is there a relationship between maternal exposure to psychological or environmental stress and the risk of ASD in the offspring?
2. Is there a relationship between maternal exposure to psychological or environmental stress and the risk of ADHD in the offspring?
3. Does the relationship between maternal exposure to stress and ASD and ADHD depend on the timing of the exposure to the stressor?
4. Does the relationship between maternal exposure to psychological or environmental stress and the risk of ASD and ADHD depend on the sex of the offspring?

Methods

Systematic review to identify eligible papers

Study registration. This study has been registered with the international Prospective Register of Systematic Reviews (PROSPERO) under the registration number CRD42018084222.

Study design. According to the Preferred Reporting Items for Systematic review and Meta-analysis protocol (PRISMA-P) guidelines, we will use a flow diagram to show the step by step study selection process and we will provide a rationale for those studies that are excluded studies. In addition we will report the descriptive characteristic of all studies included in the systematic review and we will report the meta-analysis results using a forest plot.

Ethics and dissemination. Ethical approval is not required for this systematic review and meta-analysis given that we will not include any experimental research on humans or include any identifying personal information which requires informed consent. The results of this systematic review and meta-analysis will be disseminated in peer-reviewed journals.

Types of studies. Cohort studies, cross-sectional studies and case control studies that assess maternal exposure to stressful events and have ASD and or ADHD as the outcome of interest will be included in this review. The search will not be restricted by language, although we can only include studies in English and Italian because of limited resources. Studies in other languages with an English abstract will be described and reported in the review. Only studies published in peer-reviewed journals will be considered for the systematic review. Studies published every year from database inception until March 2018 will be considered. The following inclusion criteria in accordance with the PICOS principle (population, intervention, comparison, and outcome) will be considered.

Population. Studies evaluating women who have experienced stress or anxiety or depression symptoms at any time during pregnancy or in a particular trimester will be included. The search will not be restricted to humans, although only human studies will be included in the review.

Exposure. PNMS will be defined as any psychological or environmental stress such as stressful life events, maternal bereavement, anxiety feeling or traumatic stress measured by:

- Validated self-report questionnaire
- Interview
- Physiological parameters, such as hormone levels (e.g. prolactin, corticosteroids etc.).
- Objective measures of stress and population level events

Prenatal maternal depression will be defined as a high score in screening scales, such as the Hamilton depression rating scale, the Beck Depression Inventory, the Patient Health Questionnaire, the Centre for Epidemiologic Studies Depression Scale.

Outcomes. A diagnosis of ASD or ADHD.

Method for identifying studies for inclusion

Electronic searches. Two authors (NM and GWOK) will independently perform the search for relevant articles using PUBMED, PsycINFO, Web of Science, EMBASE and SCOPUS databases. In addition we will hand-check the bibliographies of studies selected for inclusion to identify if there are any other potentially eligible studies for inclusion.

Search strategy. We will use the following searching strategy:

(Prenatal OR Antenatal OR Pregnant) AND (Stress) OR (Distress) OR (Anxiety) OR (Bereavement) AND (Offspring OR Child) AND (Autism spectrum disorder) OR (Attention deficit/hyperactivity disorder).

The detailed search strategy is presented in Supplementary File 1.

Data collection, extraction and assessment

Study selection. Two authors (NM and GWOK) will independently screen the titles and the abstracts for all articles, in order to identify studies that potentially meet the inclusion criteria outlined above. The titles and abstracts obtained from each database will be collected and processed in Endnote X8®. Duplicates will be excluded using the Endnote function “remove duplicates”. The full texts of potentially relevant articles will be examined independently by both authors and it will be a requirement that all eligibility criteria be met in order to select each study for review.

Data extraction. Two authors (NM and FB) will independently extract data using a standardised data extraction form (see Supplementary File 2). Data extraction will include author and year of publication, study outcome, exposure (type, timing and duration), study design, offspring gender, data sources, cohort size, definition of the outcome used, exclusion criteria, crude and adjusted estimates [if reported, including the relative risk (RR), odds ratio (OR), hazard ratio (HR) and 95% confidence interval (CI)]. If disagreements occurs a third reviewer (ASK) will be included to resolve them where necessary. If the situation arises where data reported in the manuscripts selected for
inclusion are insufficient or unclear for the purposes of analysis, NM will contact the authors of these studies by e-mail to request clarification or the missing data.

**Assessment of risk of bias.** A quality assessment tool will be used to critically appraise all included studies by two independent review authors (NM and KMS) (see Supplementary File 3). Risk of bias will be assessed with the Cochrane collaboration’s tool for observational studies\(^{40}\), considering six domains: selection bias, exposure, outcome measurement, statistical analysis, study attrition and confounding. Each domain will be assessed and study bias will be classified as having a high, moderate, low, minimal or unreported risk of bias for each domain. Each study will then be rated as having high, moderate or low risk of bias. For example, those with all six domains rated as minimal or low will be classified studies having a low-risk of bias.

**Quantitative data synthesis.** If possible, a meta-analysis will be performed in order to calculate a quantitative estimate of the relationship between PNMS and the risk of ASD and ADHD. The meta-analysis will be performed on all studies that report crude and/or adjusted estimates in the form of an OR, IR or RR, with 95% CIs. The generic inverse variance method will be used for crude and adjusted results. In particular, studies reporting adjusted estimates will undergo a separate in unadjusted and adjusted models in order to evaluate potential study confounders. The analysis will be performed for ASD and ADHD separately.

The Review Manager (Cochrane Collaboration Software, RevMan) 5.3 software will be used to perform statistical analysis and to combine results in a forest plot, using a fixed-effect model if heterogeneity is low. If heterogeneity is high, we will use a random-effect model. The pooled OR and standard error (SE) will be used to generate a funnel plot in order to estimate the likelihood of publication bias.

**Statistical methods**

**Assessment of heterogeneity.** Statistical heterogeneity will be assessed using the I\(^2\) statistic with an alpha of 0.05 for statistical significance, as per the Cochrane Handbook for Systematic Reviews threshold recommendations\(^{45}\). I\(^2\) reflects the percentage of total variation across studies that is attributable to heterogeneity rather than chance. An I\(^2\) negative or up to 0% suggests no observed heterogeneity. I\(^2\) between 0% and 25% represents low heterogeneity; I\(^2\) from 25% to 50% represents moderate heterogeneity; and 50% to 75% represents substantially high heterogeneity\(^{46}\).

**Analysis of subgroups or subsets.** If there are sufficient numbers of studies and data available, we will perform the following subgroup analysis:

1. For timing of stress exposure (e.g. First trimester vs Second trimester vs. Third trimester);
2. According to gender (male vs female);
3. According to different type of stress (e.g. objective vs subjective stress);
4. According to the study design setup (e.g. cohort studies vs case-control studies);
5. According to study quality (e.g. those with minimal or low quality vs moderate to high quality).

Any additional analyses will be described as post-hoc. Additional subgroup and sensitivity analyses will be performed as appropriate to assess the causes of heterogeneity as appropriate.

**Assessment of reporting biases.** If a sufficient number of studies is available (at least 10 studies), we will assess publication bias using a funnel plot and Egger test. A funnel plot will be produced to estimate the likelihood of publication bias using the pooled OR and SE.

**Current study status**

The protocol was finalised in March 2018, the literature search was completed in April, data extraction and risk of bias assessment will be completed in May, the statistical analysis will be completed in June and we expect to finish the study in July 2018.

Any protocol amendments will be stated in the review article. Any additional analyses will be reported in the review article and stated post-hoc.

**Discussion**

The goal of this systematic review and meta-analysis will review and systematically analyse the scientific literature examining the association between PNMS and risk of ASD and ADHD in exposed offspring. The underlying aim of this study is to identify potential aetiologies and possible contributors to ASD and ADHD outcomes in childhood. This may highlight the need for further work defining the impact of stress-reducing interventions in pregnancy on these adverse neurodevelopmental outcomes\(^{36-42}\). These findings may also highlight the importance of routine screening of PNMS in pregnancy to allow for greater paediatric surveillance of offspring exposed to high levels of PNMS. The identification of high-risk infants may allow earlier behavioural intervention, which can improve neurodevelopmental outcome\(^{36-34}\).

**Potential limitations**

This study may have several limitations. First, the search will be restricted to English and Italian language literature only. Second, we will not include unpublished literature, possibly resulting in an increased risk of publication bias for the included studies, given that reports that describe a significant finding or a positive outcome have a greater probability of being published when compared to those that do not. However, it should be considered that unpublished studies (and studies written in languages other than English) are more likely to report non-significant or small effect sizes, thereby leading to a weakening of the overall estimate. Where possible, we will make a funnel plot for assessing the presence of publication bias. In addition, another limitation may be the presence of confounding, such as maternal age, maternal smoking or alcohol abuse during pregnancy and family’s socio-economic status. We will make separate
analyses for adjusted and unadjusted results and subgroup analyses in order to evaluate the potential role of these confounders.

**Data availability**
No data are associated with this article.

**Competing interests**
No competing interests were disclosed.

**Author information**
ASK and GWOK conceived the idea of the study. All authors contributed to the study protocol design and NM drafted the protocol manuscript. KMS, FB, GWOK and ASK revised the manuscript critically. NM, KMS, GOK and ASK developed of the search strategy. Selection of the studies to include will be performed by NM and GWOK. Extraction of data from studies will be conducted by NM and FB. Statistical analyses will be performed by NM and ASK. All authors will be involved in the interpretation and analysis of the results, while an appraisal of study quality will be performed by NM and KMS. The results and subsequent manuscript will be written and edited by all authors. All authors approved this submission. NM and ASK are the guarantors of the review.

**Supplementary material**

Supplementary File 1: Strategies to search PUBMED, PsycINFO, Web of Science, EMBASE to identify cohort, cross-sectional and case–control studies to determine the association prenatal maternal stress and offspring neurodevelopmental disorders.
Click here to access the data.

Supplementary File 2: Data extraction form.
Click here to access the data.

Supplementary File 3: Bias classification tool.
Click here to access the data.

**References**

Open Peer Review

Current Referee Status:  

Andrew P. Allen  
Maynooth University, Maynooth, Ireland

This protocol describes a systematic review and meta-analysis on an interesting public health question relating to prenatal maternal stress and mental health in offspring. I have a few minor comments.

Introduction: “Stress” could perhaps be a bit more precisely described in the introduction, (e.g. referring to high demands being placed on a person, not just related to negative life events but daily stressors as well), rather than overlapping with anxiety and depression (notwithstanding these are all correlated).

I would be inclined to re-think the following: “the reduction of criminal and antisocial behaviour, that often are consequences of ADHD and conduct disorder”. This statement runs the risk of conflating the association between antisocial behaviour and two distinct disorders, one of which has antisocial behaviour as a key characteristic and one (ADHD, which more relevant to this article) which doesn’t.

Method: The systematic review method is very strong in my opinion. I am not authoritative on meta-analysis, but this also looks thorough.

Can you be a bit more specific about “high score” on screening scales for depression? I believe some have ranges that would be consider “mild/moderate depression” - would this count as a “high score”?

Is the “current study status” up-to-date?

Is the rationale for, and objectives of, the study clearly described?  
Yes

Is the study design appropriate for the research question?  
Yes

Are sufficient details of the methods provided to allow replication by others?  
Yes

Are the datasets clearly presented in a useable and accessible format?  
Not applicable

Competing Interests: No competing interests were disclosed.
**Referee Expertise**: Psychology

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

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Dagfinn Aune  
Department of Epidemiology and Biostatistics, School of Public Health, Imperial College London, London, UK

This is a protocol for a review on prenatal maternal stress and neurodevelopmental disorders in the offspring. Because I'm not an expert on the specific topic assessed in this protocol I have only assessed the methodological issues in the manuscript.

Why is the inverse variance method (fixed-effects models) used? It is more appropriate to use random effects models because there is likely to be heterogeneity between studies and heterogeneity is in general expected in meta-analyses of observational studies.

Why include cross-sectional studies? It's such a weak study design that cannot say anything about causality so I would exclude such studies. If there are enough cohort studies I would also exclude case-control studies from the review.

Page 5: limitations - why not just limit the study to English literature? If you wanted to include other non-English languages it would be more appropriate to include studies in any language.

**Is the rationale for, and objectives of, the study clearly described?**  
Yes

**Is the study design appropriate for the research question?**  
Yes

**Are sufficient details of the methods provided to allow replication by others?**  
Yes

**Are the datasets clearly presented in a useable and accessible format?**  
Not applicable

**Competing Interests**: No competing interests were disclosed.

**Referee Expertise**: I have carried out a lot of reviews/meta-analyses. I've worked on nutrition, adiposity and physical activity and chronic disease risk.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.
We would like to Dr Aune for reviewing our protocol and for the very helpful feedback. Below is our response to the reviewer’s comments:

In the section "Quantitative data synthesis" we specified that we would have used a fixed effects model in case of low heterogeneity ($I^2 < 50\%$) or a random effect model in case of high heterogeneity, in accordance with the criteria proposed by Higgins (Higgins JP et al., 2011). We then actually used a random effect model because the heterogeneity was very high. Moreover, we decided to include cross-sectional studies only in the search strategy, in order to include in the review those studies that were erroneously defined as "cross-sectional", but which were actually cohort studies. As a consequence, in our review we only had cohort and case-control studies. As we planned to perform a comprehensive review we planned to include all cohort and case-control studies, however, we also planned to perform the meta-analysis for cohort and case-control studies separately.

Finally, the limitation paragraph is not correct. Initially we thought to include only studies in English and Italian (which are the languages best known by our research group), but later we decided to search for articles without language limiters, in order to obtain a more complete list of potentially eligible studies.

**Competing Interests:** No competing interests were disclosed.