



STUDY PROTOCOL

**REVISED** Prenatal maternal stress and risk of neurodevelopmental disorders in the offspring: A systematic review and meta-analysis protocol [version 2; peer review: 4 approved, 1 approved with reservations]

Nicla Manzari <sup>1,2</sup>, Karen Matvienko-Sikar <sup>3</sup>, Franco Baldoni <sup>1</sup>, Gerard W. O'Keeffe <sup>4\*</sup>, Ali S. Khashan <sup>2,3\*</sup>

<sup>1</sup>Department of Psychology, University of Bologna, Bologna, Italy

<sup>2</sup>The Irish Centre for Fetal and Neonatal Translational Research (INFANT), Cork University Maternity Hospital, University College Cork, Cork, Ireland

<sup>3</sup>School of Public Health, University College Cork, Cork, Ireland

<sup>4</sup>Department of Anatomy and Neuroscience and Cork Neuroscience Centre, University College Cork, Cork, Ireland

\* Equal contributors

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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 and case-control 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.

### Open Peer Review

Reviewer Status

	Invited Reviewers				
	1	2	3	4	5
<b>version 2</b> (revision) 14 Feb 2019	 report	 report	 report	 report	 report
<b>version 1</b> 01 May 2018	 report	 report	 report	 report	 report

- Dagfinn Aune**, Imperial College London, London, UK
- Andrew P. Allen**, Maynooth University, Maynooth, Ireland
- Gabrielle Simcock**, University of the Sunshine Coast, Sippy Downs, Australia
- Mary Clarke**, Royal College of Surgeons in Ireland (RCSI), Dublin, Ireland
- Lorna M. Lopez** , Trinity College Dublin, Dublin, Ireland

**Keywords**

Prenatal maternal stress, Autism spectrum disorder; attention-deficit hyperactivity disorder; Systematic review; Protocol

Any reports and responses or comments on the article can be found at the end of the article.



This article is included in the [Maternal and Child Health](#) collection.

**Corresponding authors:** Gerard W. O'Keefe ([g.okeefe@ucc.ie](mailto:g.okeefe@ucc.ie)), Ali S. Khashan ([a.khashan@ucc.ie](mailto:a.khashan@ucc.ie))

**Author roles:** **Manzari N:** Investigation, Methodology, Project Administration, Writing – Original Draft Preparation; **Matvienko-Sikar K:** Investigation, Methodology, Writing – Review & Editing; **Baldoni F:** Investigation, Methodology, Supervision, Writing – Review & Editing; **O'Keefe GW:** Conceptualization, Investigation, Methodology, Supervision, Writing – Review & Editing; **Khashan AS:** Conceptualization, Methodology, Supervision, Writing – Review & Editing

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

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**REVISED Amendments from Version 1**

We thank the reviewers for reviewing our protocol. We addressed the comments as follows, with detailed responses to each report:

In response to Dr Lopez's and Dr Clarke's comments, we made this change in the Discussion:

*"In addition, another limitation may be the presence of confounding, such as maternal age, maternal smoking or alcohol abuse during pregnancy, paternal age, **family history of mental illness** and family's socio-economic status."*

In response to comments from Dr Simcock we made these changes in:

Introduction

*"Stressful life events may include significant daily stressors and high stress perception at individual level, but may also include massive stress events such as **natural disasters at the population level**."*

And Methods' 'Exposure' section:

*"PNMS will be defined as any psychological or environmental **type of stress** such as stressful life events, **natural disasters, significant life events**, maternal bereavement, anxiety feeling or traumatic stress measured by:"*

In response to comments from Dr Allen, we made these changes in:

Introduction:

*"This intervention has been reported to lead to long-term improvements in parenting behaviour and in child outcomes **(including but not limited to the reduction of anger and criminal behaviours)**."*

Methods:

*"Prenatal maternal depression will be defined **according to** screening scales, such as the Hamilton depression rating scale (HDRS), the Beck Depression Inventory (BDI), the Patient Health Questionnaire (PHQ), the Centre for Epidemiologic Studies Depression Scale (CES-D). **If possible, we will perform a subgroup analysis including studies on prenatal maternal depression and ASD or ADHD. This analysis will be clearly highlighted as post-hoc depending on the available data.**"*

In response to comments from Dr Aune, we made this change in the Methods:

*"Studies in other languages with an English abstract will be described and reported in the review, and **when possible, the full text of the article will be translated into English**."*

**See referee reports**

## 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<sup>1</sup>. 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<sup>2</sup>. Stressful life events may include significant daily stressors and high stress perception at individual level, but may also include massive stress events such as natural disasters at the population level. A number of studies have reported an association between PNMS

and increased risk of psychopathology in children<sup>3-6</sup>. 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<sup>7-11</sup>.

### Description of the outcomes

Attention-deficit/hyperactivity disorder (ADHD) is a neurodevelopmental disorder that affects up to 1 in 20 children in the USA<sup>12</sup>. 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<sup>13</sup>. 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<sup>14</sup>, and these symptoms begin in early childhood<sup>15,16</sup>. The aetiological basis of these disorders is complex and multifactorial, and while twin studies show that genetic factors play a key role<sup>17,18</sup>, environmental factors, such as PNMS, have also been proposed to play a role in their aetiology<sup>19</sup>.

In support of this, there is a growing literature reporting an association between PNMS and increased risk of these neurodevelopmental disorders in exposed offspring<sup>4-6,20</sup>. For example, Class *et al.* reported an increased risk of ADHD in offspring of mothers exposed to the death of a first-degree relative during pregnancy<sup>20</sup>. Van Batenburg *et al.* found that maternal antenatal depressive symptoms were related to attention problems in affected children at 3-4 years of age<sup>21</sup>. Moreover, exposure to prenatal maternal anxiety has also been reported to be significantly associated with ADHD symptoms in early childhood<sup>22</sup>. Accumulating evidence suggests that the impact of PNMS and other environmental factors on the development of the fetal brain is dependent on the gestational stage at which the exposure occurs<sup>23-25</sup>. Accordingly, evaluating the timing of the exposure to the stressor is particularly important, because associations during a specific time increase the probability of a causal association<sup>26</sup>. For example, Li *et al.* found an association between preconception stress (experienced 0-6 months before pregnancy) and increased risk of ADHD, but only in male offspring<sup>6</sup>, while Class *et al.* found an increased risk of ASD and ADHD in children whose mothers were exposed to bereavement during the third trimester of pregnancy<sup>20</sup>.

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<sup>27-29</sup>. 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<sup>27,28,30</sup>.

### 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<sup>31-34</sup>. 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<sup>31</sup>. Moreover, a consensus on this issue may underscore the need for alternative interventions during pregnancy aimed at reducing stress, anxiety and depression<sup>35,36</sup>. Currently there is only one intervention that starts during pregnancy, involving multiple home visits by qualified nurses, known as the Nurse Family Partnership<sup>36</sup>. This intervention has been reported to lead to long-term improvements in parenting behaviour, as well as in child outcome, (including but not limited to the reduction of anger and criminal behaviours)<sup>37</sup>. 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<sup>9-11,20,38</sup> and that antenatal depression symptoms could be related to child behavioural problems<sup>10,21</sup>. However, a number of studies reported no association between PNMS and adverse neurodevelopmental outcome in children<sup>27,28,30</sup>. 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 objective or subjective stress and the risk of ASD in the offspring?
2. Is there a relationship between maternal exposure to objective or subjective 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](https://doi.org/10.1111/CRD4.2018084222).

**Study design.** According to the Preferred Reporting Items for Systematic review and Meta-analysis protocol (PRISMA-P) guidelines<sup>39</sup>, 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 and case-control studies that assess maternal exposure to stressful events and the risk of ASD and/or ADHD diagnosis in the offspring, 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, and when possible, the full text of the article will be translated into English. 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/and 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 type of stress such as stressful life events, natural disasters, significant life events, maternal bereavement, anxiety feeling or traumatic stress measured by:

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

Prenatal maternal depression will be defined according to screening scales, such as the Hamilton depression rating scale, the Beck Depression Inventory, the Patient Health Questionnaire, the Centre for Epidemiologic Studies Depression Scale. If possible, we will perform a subgroup analysis including studies on prenatal maternal depression and ASD or ADHD. This analysis will be clearly highlighted as post-hoc depending on the available data.

**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 occur 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<sup>40</sup>, 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, HR 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 random-effect models. The pooled OR and standard error (SE) will be used to generate a funnel plot in order to estimate the likelihood of publication bias if 10 or more studies are included in the meta-analysis.

### 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<sup>40</sup>.  $I^2$  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<sup>41</sup>.

**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<sup>36,42</sup>. 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<sup>31-34</sup>.

## 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 use 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, paternal age, family history of mental illness and family's socioeconomic 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.

## Author contributions

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.

## Grant information

This work was conducted with the financial support of Science Foundation Ireland in the form of a research centre grant to the Irish Centre for Fetal and Neonatal Translational Research [Grant #: INFANT-12/RC/2272].

*The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.*

## 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.](#)

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## Open Peer Review

Current Peer Review Status:



### Version 2

Reviewer Report 06 March 2019

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**Lorna M. Lopez** 

Trinity College Dublin, Dublin, Ireland

No further comments to make.

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

**Reviewer Expertise:** Genetics

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

Reviewer Report 28 February 2019

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**Gabrielle Simcock**

University of the Sunshine Coast, Sippy Downs, QLD, Australia

I am satisfied that the comments I made when reviewing the original article have been revised/addressed adequately in this response; and recommend indexing.

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

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



Reviewer Report 14 February 2019

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**Dagfinn Aune**

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

I only have one more comment. For consistency between the analyses it would still be better to always use a random effects model because any differences between subgroups etc. could at least partly be due to the fact that you have chosen a different model for the different analyses. When there is low heterogeneity the random effects model is in anyway showing a result more similar to a fixed effects models so you might as well use the random effects model in all analyses for consistency.

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

**I confirm that I have read this submission and 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.**

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## Version 1

Reviewer Report 29 January 2019

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**Lorna M. Lopez**

Trinity College Dublin, Dublin, Ireland

This study protocol by Manzari *et al.* is to perform a systematic review and meta analysis on the link between prenatal maternal stress and a risk of neurodevelopmental disorders in the offspring. It is certainly an important question to understand the origins of NDDs, and in particular for the existing literature to be evaluated in a systematic manner and reported in a standardised way.

The research questions are well described and the methods are thorough. One comment I have is that the authors state in the introduction "The aetiological basis of these disorders is complex and multifactorial, and while twin studies show that genetic factors play a key role<sup>17,18</sup>, environmental factors, such as PNMS, have also been proposed to play a role in their aetiology<sup>19</sup>." In order to correctly evaluate the

contribution of PNMS, as a distinct separate contributor to autism than genetics, then I think information such as family history and parental age at birth should be considered. The authors mention in the potential limitations section that "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." I think family history of ASD and ADHD, and paternal age are important considerations.

I agree with limiting the literature to English literature.

**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.

**Reviewer Expertise:** Genetics

**I confirm that I have read this submission and 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.**

Author Response 11 Feb 2019

**Ali Khashan**, University College Cork, Cork, Ireland

We would like to thank Dr Lopez for the helpful comments.

We agree that family history of ASD and ADHD, and paternal age are important considerations and may play a role in the aetiology of these disorders, but our specific focus was prenatal stress exposure.

However, we considered these types of variables to be very important and those studies that did not take these variables into account as potential confounders were considered low quality studies. We also addressed this issue in the limitations section as follows:

*"In addition, another limitation may be the presence of confounding, such as maternal age, maternal smoking or alcohol abuse during pregnancy, paternal age, **family history of mental illness** and family's socio-economic status."*

**Competing Interests:** None

Reviewer Report 28 January 2019

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### Mary Clarke

Department of Psychiatry, Royal College of Surgeons in Ireland (RCSI), Dublin, Ireland

This is a protocol for a systematic review and meta-analysis of the evidence for an association between exposure to prenatal stress and a later diagnosis of an autism spectrum disorder or a diagnosis of attention deficit disorder. It is clear and well-written and includes all the standard elements for a thorough systematic review.

I was not sure from the description of the exposure how or if exposure to maternal stress and maternal depression in-utero would be separated out. The definition of the outcome is a diagnosis of ASD or ADHD - there may very few studies in this area and allowing for assessment of outcome using standardised instruments but which are not diagnostic may give needed scope to the review.

There is a list of potential confounders mentioned at the end - an additional one is parental psychiatric history - it is very possible that antenatal stress is a proxy for psychiatric history in some cases.

I look forward to reading the results of this review.

#### **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.

**Reviewer Expertise:** Psychiatric epidemiology

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

Author Response 11 Feb 2019

**Ali Khashan**, University College Cork, Cork, Ireland

We thank Dr Clarke for the helpful comments.

We agree with that there are few studies considering the diagnosis of ASD or ADHD in relation to prenatal stress, but in our inclusion criteria we preferred to exclude studies that only considered ASD or ADHD symptoms, so we focused only on diagnoses. Given that prenatal stress as an exposure is very variable, something we mention in our review, in our view limiting variability in the outcome measure to a confirmed diagnosis, may provide new insights into how different prenatal stressors may (or may not) lead to the same outcome.

Moreover, stress and depression are distinct though highly correlated phenomena, often measured with different instruments, so we decided to evaluate them separately, when possible.

We agree with the reviewer that parental psychiatric history is a potential confounder and we took it into consideration when evaluating the quality of the studies included in the meta-analysis. We also addressed this issue in the revised protocol. Please see our response to reviewer 4 about this point.

**Competing Interests:** None

Reviewer Report 10 January 2019

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### Gabrielle Simcock

University of the Sunshine Coast, Sippy Downs, QLD, Australia

This is a protocol for a systematic literature review on the effects of prenatal maternal stress (PNMS) on childhood disorders (ADHD and ASD). This review would add to the many published PNMS reviews by focusing specifically on these developmental disorders, and hopefully clarifying disparate findings in the literature.

However, the authors have overlooked a body of PNMS research that fits their definition, review questions, and analysis plan (see relevant quotes below): stress in pregnancy due to natural disasters. Research show that disaster-related PNMS (e.g., experience of a severe ice storm, flood exposure, hurricanes, or an earthquake in pregnancy) is related to offspring ASD prevalence or symptoms. Importantly, the sudden onset of natural disasters in pregnancy make them ideal for studying gestational timing effects on offspring development. Additionally, stress from the disaster can be broken down into the objective events experienced (e.g., relocation to emergency shelter, death of family/friends) and the subjective emotional reactions (e.g., PTSD symptoms) to determine their specific effects. Thus, this body of research appears to be directly relevant to the purpose and scope of the proposed review.

- In the first paragraph the term PNMS is *defined* as "... 'stress' in this context often covers negative life events.... **significant life stressors**..."

- The specific *review questions* use the term “...**environmental stress**...” and propose to address the issues of timing of the stressor in pregnancy.
- The authors propose *analyses of subgroups* by “...different types of stress (e.g. **objective vs subjective stress**);”

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

Yes

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

Partly

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

Partly

**Are the datasets clearly presented in a useable and accessible format?**

Not applicable

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

**Reviewer Expertise:** Prenatal maternal stress, child development, cognition and language, developmental psychopathology

**I confirm that I have read this submission and 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.**

Author Response 11 Feb 2019

**Ali Khashan**, University College Cork, Cork, Ireland

We would like to thank Dr Simcock for reviewing our protocol and for the helpful feedback. Below is our response to the reviewer's comments:

Just to clarify, we had considered this exposure and we have now changed the text in the Introduction to reflect this and to clarify our conceptualisation of stress. The meta-analysis aims to address potential differences between types of stress, including exposure to natural disasters and objective hardship. We specifically mentioned natural disasters in the Introduction as follows:

*“Stressful life events may include significant daily stressors and high stress perception at individual level, but may also include massive stress events such as **natural disasters at the population level.**”*

And in the Exposure section as follows:

*“PNMS is defined as any type of stress such as stressful life events, **natural disasters**, significant life events, maternal bereavement, anxiety feeling or traumatic stress measured by:”*

**Competing Interests:** None

Reviewer Report 18 December 2018

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**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.

**Reviewer Expertise:** Psychology

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

Author Response 11 Feb 2019

**Ali Khashan**, University College Cork, Cork, Ireland

We would like to thank Dr Allen for reviewing our protocol and for the very helpful feedback. This is our response to the reviewer's comments:

We have modified the statement about the reduction of criminal and antisocial behaviour as suggested. The statement now reads as follows:

*"This intervention has been reported to lead to long-term improvements in parenting behaviour and in child outcomes (including but not limited to the reduction of anger and criminal behaviours)".*

Regarding depression, we aimed to use the definitions and cut-off points that were used in the papers. We modified the text now to highlight that if data allowed us, we will perform a subgroup analysis on maternal prenatal depression and that the analysis will be considered post-hoc. The text was modified as follows:

*"Prenatal maternal depression is defined according to screening scales, such as the Hamilton depression rating scale (HDRS), the Beck Depression Inventory (BDI), the Patient Health Questionnaire (PHQ), the Centre for Epidemiologic Studies Depression Scale (CES-D). If possible, we will perform a subgroup analysis including studies on prenatal maternal depression and ASD or ADHD. This analysis will be clearly highlighted as post-hoc depending on the available data."*

**Competing Interests:** None

Reviewer Report 14 August 2018

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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.

**Reviewer 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 confirm that I have read this submission and 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.**

Author Response 15 Aug 2018

**Ali Khashan**, University College Cork, Cork, Ireland

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.



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Author Response 11 Feb 2019

**Ali Khashan**, University College Cork, Cork, Ireland

We would like to thank Dr Aune for reviewing the protocol and for the very helpful feedback.

Regarding the fixed-effects models, this was changed to random-effects models and no fixed-effects models will be used. We also clarified our inclusion criteria in relation to language. The quantitative data synthesis section was modified as follows:

*“Studies in other languages with an English abstract will be described and reported in the manuscript and, when possible, the full text of the article will be translated into English”.*

We agree with the reviewer about cross-sectional studies, however, we included this study design in the search strategy in order to include in the review those studies that were possibly erroneously described as “cross-section”, but were actually cohort studies. As we planned to perform a comprehensive review, we planned to include all cohort and case-control studies. We also planned to perform the meta-analysis for cohort and case-control studies separately for the reasons highlighted by the reviewer. To avoid confusion about the study design inclusion criteria, we have now removed “cross-sectional” from the ‘Methods section’ in the Abstract and from the ‘Types of Studies section’.

**Competing Interests:** None

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