ENVIRONMENTAL-RISK (E-RISK) LONGITUDINAL TWIN STUDY
CONCEPT PAPER FORM

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Provisional Paper Title: Associations between childhood victimisation, inflammatory biomarkers and psychotic phenomena in childhood and young adulthood: a longitudinal twin cohort study.

Date: 11th February 2019

Objective of the study and its significance:

The main objective of this study is to investigate whether higher levels of inflammatory biomarkers (CRP, IL-6 and suPAR) are detectable in children and young adults experiencing psychotic phenomena who have been victimised during childhood compared to those without such experiences.

Background

Childhood adversity is the exposure to single or multiple events during childhood (including victimisation) that exceed the child's coping resources and lead to prolonged challenges in emotional and physical well-being [1]. Childhood adverse experiences predict a third of psychiatric disorders, and an even higher percentage (44%) of disorders with childhood onset [2]. General population studies have suggested that childhood adversity may also be associated with the presence of psychotic symptoms in childhood and young adulthood [3-5]. [Such early psychotic symptoms represent a developmental risk for adult schizophrenia [6] and thus provide a framework for investigating etiological factors for later psychosis [7] as well as being of particular interest for its prevention.] However, very little is known about how exposure to childhood adversity is translated into biological risk for psychosis. Therefore, research is needed to identify mechanisms that could explain *why psychotic symptoms are more prevalent among children and young adults who have experienced adversity early in life.*

One potential biological mechanism is inflammation. The immune system is the body's first response against pathogenic organisms [9]. Both human and animal studies suggest that childhood exposure to stress can trigger a persistent inflammatory response, similar to the response the body has in the case of physical injury, which is linked to the onset of cardiovascular and immune diseases [10]. A common way to assess inflammatory processes is to measure concentrations of circulating pro-inflammatory cytokines, such as interleukin-6 (IL-6) and systemic markers of inflammation, such as C-reactive protein (CRP), in blood samples. A specific association between early life trauma and increased levels of C-reactive protein (CRP) has been demonstrated in adulthood [11]. Furthermore, results from the Dunedin Multidisciplinary Health and Development Study showed that cumulative exposure to childhood maltreatment was associated with significant elevation in high-sensitivity CRP (hsCRP) and soluble urokinase plasminogen activator receptor (suPAR) at the age-32 follow-up [12-13]. While CRP is a marker of acute inflammation and infections [14], suPAR reflects a person's overall level of immune activity and seems predictive of chronic inflammation and organ damage [15-16], development and progression of disease, adverse clinical outcomes, and mortality [17-18]. High suPAR is also positively correlated with CRP and IL-6, in both general and patient populations [13, 19]. Therefore, suPAR seems to be a useful addition to studies connecting childhood risk to later inflammatory burden.

Inflammation has also been linked to psychotic phenomena. For instance, the C4 gene of the major histocompatibility complex (MHC), which is involved in the immune response, has emerged as a robust signal in genome-wide association studies as a genetic risk locus for schizophrenia [8]. Previous research has also shown an abnormal biological response to stress at the onset of psychosis, and that increased IL-6 and TNF-α at illness onset also predicted a poor treatment response [20]. Therefore, it is plausible that exposure to childhood adversity increases levels of circulating inflammatory biomarkers which in turn trigger the development of psychotic phenomena. Indeed, childhood-life stress is considered to lead to a pro-inflammatory state in early adulthood, which in turn can trigger an increase in microglia activity [21]. Microglial overactivation may result in damage to stress-sensitive regions such as the prefrontal cortex and hippocampus, which have been found to be associated with symptoms of psychosis [21].

What is not clear, however, is when the impact of childhood adversity on inflammation in individuals with psychotic symptoms emerges. It is therefore important to investigate whether higher levels of inflammatory biomarkers are present among children and young adults experiencing pre-clinical psychotic phenomena who have been victimised during childhood. The significance of this question lies in its potential to uncover the origins of enduring disease vulnerability in children and adults exposed to adverse psychosocial experiences, and to suggest the best timing for effective interventions to prevent the development of psychotic disorders.

Pilot work which has led to the proposed project

Previous research conducted in the Environmental-Risk (E-Risk) Longitudinal Twin Study, has demonstrated that CRP levels were elevated in maltreated children who developed depression by age 12 compared to controls [22] and that the association persisted till young adulthood, with a dose-response effect [23].

Based on these findings, I hypothesized that victimised children who also experienced psychotic symptoms at the time of inflammation assessment would show elevated hsCRP levels. In preparation for fellowship applications, I undertook pilot work to explore this question using a sample of 172 children from the E-Risk Longitudinal Twin Study on whom data was available on hsCRP levels, victimisation, and psychotic symptoms at age 12. The preliminary results (see Figure 1) showed that severely victimised children with psychotic symptoms at age 12 had a significant mean elevation in hsCRP levels compared with victimised children without psychotic symptoms [t(98)=3.04, p=0.003]. In contrast, non-victimised children with psychotic symptoms showed mean hsCRP levels similar to their peers without psychotic symptoms [t(70)=0.40, p=0.687]. These results provide the first evidence that the origins of abnormalities in stress-sensitive systems seen in individuals experiencing psychotic phenomena who have been exposed to early adversity could be traced back to the childhood years.

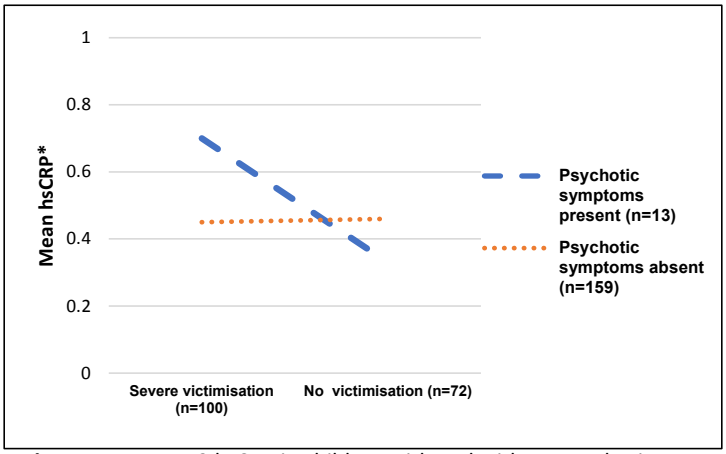

Fig 1. Mean age-12 hsCRP in children with and without psychotic symptoms exposed to any severe victimisation compared to those not exposed. *log-transformed.

The proposed study will extend the previous pilot work to explore the associations between childhood victimisation (severe physical abuse, severe sexual abuse, severe emotional abuse or neglect, severe physical neglect, severe bullying, or severe exposure to domestic violence by age 12) and a broader range of inflammatory biomarkers, including CRP, IL-6, and SuPAR collected at age 12 (only CRP) and 18 in the E-Risk sample and utilise the assessments of psychotic phenomena obtained at these ages.

First, I will test whether victimised children with psychotic symptoms at age 12 have higher age-12 CRP levels than those victimised but without psychotic symptoms, as well as non-victimised children with psychotic symptoms, and those not exposed to victimisation and without psychotic symptoms.
Second, I will investigate childhood victimisation and psychotic symptoms at age 12 predicts higher CRP, IL-6, and SuPAR levels at age 18.

Finally, I will also test whether the association between childhood victimisation and age-18 CRP, IL-6, and SuPAR levels at age 18 is stronger in those with psychotic phenomena at age 18 than in those without age-18 psychotic phenomena, even after controlling for individual and family characteristics, age-12 psychopathology, and proxy genetic risk factors.

This study could provide further evidence of how early traumatic events might impact on the stress response system and subsequently lead to the emergence of psychotic phenomena.

Statistical analyses:

In line with previous analyses conducted on the E-Risk sample, CRP, IL-6, and SuPAR will be log-transformed to improve the normality of their distribution [16]. All analyses (aside from the discordant twin analysis) will be corrected for the non-independence of twin observations using the Huber-White variance estimator.

First, for the cross-sectional analyses at age 12, I will calculate mean differences in age-12 CRP across the following four groups and compare then using t-tests:

1) children with no severe victimisation and without psychotic symptoms (controls)
2) children with no severe victimisation and with psychotic symptoms (psychotic symptoms-only),
3) children with any severe victimisation and without psychotic symptoms (childhood adversity-only),
4) children with any severe victimisation and with psychotic symptoms (childhood adversity and psychotic symptoms).

Secondly, I will examine the association between any severe childhood victimisation and increased CRP, IL-6 and SuPAR levels at age 18 in individuals presenting with and without psychotic phenomena in young adulthood. I will use linear regression to examine the association between any severe childhood victimisation and age-18 CRP, IL-6 and SuPAR levels stratifying for the presence/absence of any psychotic experiences at age 18. I will then run sensitivity analyses stratifying for the presence/absence of any clinician-verified psychotic symptoms at age 18. Additionally, I will re-run the associations substituting childhood poly-victimisation for any severe victimization and then each type of childhood victimisation separately (numbers permitting). I will also check whether adolescent victimisation modifies or confounds the association between childhood victimisation and CRP, IL-6 and SuPAR levels at age 18 in those with psychotic phenomena at age 18.

I will check whether these associations are robust after controlling for gender, age-5 IQ, family socioeconomic status, other forms of psychopathology (depression and anxiety) at age 12, and psychotic symptoms at age 12 (for the age-18 analyses). Body temperature and waist–hip ratio at age 12 (for the age-12 analyses) or 18 (for the age-18 analyses) will also be included in the adjusted model as important potential confounding variables. Additionally, we will repeat analyses adjusting for family psychiatric history and maternal psychotic symptoms to exclude their potentially confounding influence. Given previous findings in this cohort [23], I will also stratify analyses by gender to check for gender differences (numbers permitting).

Additionally, we will repeat the second part of the analyses using twins discordant for age-12 psychotic symptoms and age-18 psychotic experiences to exclude the potentially confounding influence of unmeasured family and (and at least partially) genetic factors (MZ & DZ pairs together) in the associations between childhood victimisation and age-18 CRP, IL-6 and SuPAR levels.

Lastly, all the analyses will be re-run using the retrospective self-report measure of childhood maltreatment obtained from study members at age 18 to test whether the results differ from those obtained using the prospective informant-reported victimisation measure.

Variables Needed at Which Ages (names and labels):

Study: E-Risk

Age 5

FAMILYID	Unique family identifier

ATWINID	Twin A ID (ex chkdg)
BTWINID	Twin B ID (ex chkdg)
RORDERP5	Random Twin Order
RISKS	Sample Groups
COHORT	Cohort
SAMPSEX	Sex of Twins: In sample
ZYGOSITY	Zygosity
SESWQ35	Social Class Composite
IQE5	Childhood IQ

Age 12

EX_SVE12	Exposed to severe victimization (0/1), 5-12, E-Twin
EX_SVY12	Exposed to severe victimization (0/1), 5-12, Y-Twin
POLYVE512C	Extent of Polyvictim (Truncated @3), 5-12, E-Twin
EANSEVE12	Severity of Emotional abuse/neglect of Elder twin, thru age 12, 2014
PABSEVTYE12	Physical abuse by 12, severity, Elder
PNSEVERITYE12	Physical neglect by 12, severity, Elder
SASEVTYE12	Sexual abuse by 12, severity, Elder
BULLSEVE12	Bullying victim to Age 12 - Elder
ExpV_DV510	Exposure to domestic violence, 5 to 10, 012 coding (from HonaLee)
PSYSYMP01E12	Psychosis Symptom Count-Verified Coding-Elder - 0, 1+ - Elder
PSYSYMP01Y12	Psychosis Symptom Count-Verified Coding-Elder - 0, 1+ - Younger
PSYSYM12	Mother Psychosis - Symptom Count
FHANYPM12	Proportion of family members with valid data who have any disorder
CDICATE12	Clinically significant depression (CDI >= 20) - P12 - Elder
MASCCATE12	Extreme anxiety (>= 95th percentile) - P12 - Elder
CRPEmgl	CRP mgL - Elder (previously CRPEBS)
CRPYmgl	CRP mgL - Younger (previously CRPYBS)
BM3EBS	Body temperature in celsuis – Elder
BM4EBS	Waist measurement in cms - Elder
BM5EBS	Hip measurement in cms - Elder

Age 18

Victimisation:

POLYVCTZCE18	Poly-victimisation 4 cat (0,1,2,3+) - P18 – Elder
POLYVCTZCY18	Poly-victimisation 4 cat (0,1,2,3+) - P18 –Younger
CTQCTOTE18	CTQ combined - types of abuse or neglect at mod/severe level (0-5) - P18 – Elder
CTQCTOTY18	CTQ combined - types of abuse or neglect at mod/severe level (0-5) - P18 – Younger
CTQABUCE18	CTQ abuse elder twin - P18 – Elder
CTQABUCY18	CTQ abuse - P18 – Younger

Inflammatory markers:

CRPE18	CRP concentration (mg/L) - P18 – Elder
CRPY18	CRP concentration (mg/L) - P18 – Younger
CRPmgLE18	Plasma CRP - elder twin
CRPmgLY18	Plasma CRP - younger twin
IL6pgmlE18	IL-6 - P18 – Elder
IL6pgmlY18	IL-6 - P18 – Younger
suPARngml_E	suPAR - P18 – Elder
suPARngml_Y	suPAR - P18 – Elder
PSYSYMPO1E18	Psychosis Symptom Count (0,1+) – P18 – Elder

PSYSYMPO1E18	Psychosis Symptom Count (0,1+) – P18 – Younger
PSYEXPCE18	Psychotic Experiences (cat) - P18 - Elder
PSYEXPCY18	Psychotic Experiences (cat) - P18 - Younger
Health measures:	
WAISTHIPE18	Waist Hip Ratio - P18 – Elder
WAISTHIPY18	Waist Hip Ratio - P18 – Younger
BMIE18	BMI - P18 – Elder
BMIY18	BMI - P18 – Younger
BTEMPE18	Body temperature, Celsius - P18 – Elder
BTEMPY18	Body temperature, Celsius - P18 – Younger

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Data Security Agreement

Provisional Paper Title	Associations between childhood victimisation, inflammatory biomarkers and psychotic phenomena in childhood and young adulthood: a longitudinal twin cohort study.
Proposing Author	Dr Antonella Trotta
Today's Date	07/02/2019

Please keep one copy for your records
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AT I am familiar with the King's College London research ethics guidelines (https://www.kcl.ac.uk/innovation/research/support/ethics/about/index.aspx) and the MRC good research practice guidelines (https://www.mrc.ac.uk/research/policies-and-guidance-for-researchers/good-research-practice/).

AT My project has ethical approval from my institution.

AT I am familiar with the EU General Data Protection Regulation (https://mrc.ukri.org/documents/pdf/gdpr-guidance-note-3-consent-in-research-and-confidentiality/), and will use the data in a manner compliant with its requirements.

AT My computer is (a) encrypted at the hard drive level, (b) password-protected, (c) configured to lock after 15 minutes of inactivity, AND (d) has an antivirus client which is updated regularly.

AT I will treat all data as "restricted" and store in a secure fashion.

AT I will not share the data with anyone, including students or other collaborators not specifically listed on this concept paper.

AT I will not merge data from different files or sources, except where approval has been given by the PI.

AT I will not post data online or submit the data file to a journal for them to post.
Some journals are now requesting the data file as part of the manuscript submission process. The E-Risk Study cannot be shared because the Study Members have not given informed consent for unrestricted open access. Speak to the study PI for strategies for dealing with data sharing requests from Journals.

AT Before submitting my paper to a journal, I will submit my draft manuscript and scripts for data checking, and my draft manuscript for co-author mock review, allowing three weeks.

AT I will submit analysis scripts and new variable documentation to project data manager after the manuscript gets accepted for publication.

AT I will delete the data after the project is complete.

N/A **For projects using location data:** I will ensure geographical location information, including postcodes or geographical coordinates for the E-Risk study member's homes or schools, is never combined or stored with any other E-Risk data (family or twin-level data)

N/A **For projects using genomic data:** I will only use the SNP and/or 450K data in conjunction with the phenotypes that have been approved for use in this project at the concept paper stage.

Signature: .....Antonella Trotta.....

CONCEPT PAPER RESPONSE FORM

A. To be completed by the proposing author

Proposing Author: Dr Antonella Trotta

X I have read the E-Risk data-sharing policy guidelines and agree to follow them

Provisional Paper Title: Associations between childhood victimisation, inflammatory biomarkers and psychotic phenomena in childhood and young adulthood: a longitudinal twin cohort study.

Potential co-authors: Helen Fisher, Louise Arseneault, Andrea Danese, Terrie Moffitt, Avshalom Caspi, Carmine Pariante

Potential Journals:

Intended Submission Date (month/year): June 2019

Please keep one copy for your records and return one to Louise (louise.arseneault@kcl.ac.uk)

B. To be completed by potential co-authors:

☐ Approved ☐ Not Approved ☐ Let's discuss, I have concerns

Comments:

Please check your contribution(s) for authorship:

☐ Conceptualizing and designing the longitudinal study

☐ Conceptualizing and collecting one or more variables

☐ Data collection

☐ Conceptualizing and designing this specific paper project

☐ Statistical analyses

☐ Writing

☐ Reviewing manuscript drafts

☐ Final approval before submission for publication

☐ Acknowledgment only, I will not be a co-author

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