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HUMAN DEVELOPMENT, BIRTH TO DEATH

Moffitt & Caspi
Genes Environment Health Behavior

Concept Paper Form

Provisional Paper Title: suPAR and mental health
Proposing Author: Line Jee Hartmann Rasmussen
Author's Email: line.jee.hartmann.rasmussen@duke.edu
P.I. Sponsor: Terrie Moffitt & Avshalom Caspi (if the proposing author is a student or colleague of an original PI)
Today's Date: 2/14/2021

Please describe your proposal in 2-3 pages with sufficient detail for helpful review.

Objective of the study:

Inflammation is associated with multiple mental disorders: A growing body of evidence indicates that inflammation is non-specifically related to different mental disorders and not one diagnosis alone. The link between depression and inflammation is well-documented,[1,2] but elevated inflammation has also been observed in patients with bipolar disorder, anxiety disorders, post-traumatic stress disorder (PTSD), autism spectrum disorder, suicide, and schizophrenia.[3–5]

Pharmacological intervention studies indicate different mechanisms of causation that are suggestive of an association between inflammation and mental disorder; on the one hand, anti-inflammatory treatments (e.g., anti-cytokine treatments) could have anti-depressant effects,[6] while on the other hand some psychotropic drugs could have anti-inflammatory effects.[4,7] Similarly, prolonged systemic chronic inflammation can lead to exacerbation of sickness, sickness behaviors, and the development of symptoms of depression in vulnerable individuals.[8] For example, pro-inflammatory cytokines may induce major depressive disorder (MDD) in *physically* ill patients with no previous history of mental disorders.[8]

Despite the generally accepted link between psychopathology and inflammation, findings have been inconsistent and many studies underpowered.[4] Moreover, relevant confounders such as smoking are not consistently controlled for.[9]

Why we need an inflammatory biomarker that is better than what we currently have: In psychiatric research, inflammation has been assessed in numerous ways by the measurement of a variety of circulating inflammatory markers, including acute-phase proteins, pro- and anti-inflammatory cytokines, chemokines, white blood cells, growth factors, etc.[9] Most studies use biomarkers of acute inflammation, often leading to exclusion of participants based on acute physical illness which could actually be masking the presence of psychopathology-related chronic inflammation. While the acute-phase reactant

C-reactive protein (CRP) is commonly used as the gold standard inflammation marker both in clinical settings and in life-course research,[10] *soluble urokinase plasminogen activator receptor* (suPAR) is a newer biomarker of inflammation[11] that is thought to reflect chronic inflammation. If associated with mental disorders, suPAR could be a better measure of psychopathology-related systemic chronic inflammation than CRP and other acute inflammatory proteins that are typically used.

Why suPAR could be a more appropriate biomarker of chronic inflammation: suPAR appears to be correlated with chronic rather than acute inflammation. Although CRP and suPAR are positively correlated, they appear to capture different aspects of inflammation.[12] The blood concentration of suPAR is elevated upon activation of the immune system, and suPAR is associated with development, presence, and progression of disease[11,13,14] as well as physical and cognitive decline and accelerated biological aging.[15] Thus, suPAR levels are elevated across a wide range of diseases,[16] including cardiovascular disease,[17] type 2 diabetes,[18] cancer,[19,20] renal disease,[21,22] and infections.[23,24] In addition, suPAR is a strong predictor of mortality, both in the general population and in patient populations.[13,16]

We have recently shown that chronic inflammation in adulthood, as measured by elevated suPAR, has its origins already in early childhood. In the Dunedin Study, we showed how children who experienced more adverse childhood experiences, had lower IQ, or had poorer self-control showed elevated adult suPAR at age 38.[25] This was supported by findings in the E-Risk Study showing that children exposed to adversities and multiple forms of stress and violence had elevated suPAR levels, but not CRP or IL-6, at age 18 after adjustment for sex, BMI, and smoking.[26] Moreover, participants exposed to cumulative adverse experiences across childhood and adolescence (domestic violence or multiple types of violence in childhood and adolescence) had elevated suPAR, but not CRP or IL-6. This underlines how suPAR adds information about the health implications of stressful experiences in childhood beyond the established biomarkers CRP and IL-6. These childhood risk factors are also important predictors of mental disorder.[27]

Of note, we found that adverse experiences were prominent in the group of participants with low CRP and low IL-6 but high suPAR—a group of individuals who would have inadvertently been assigned to the low inflammation group if suPAR had not been assayed.[26]

What is known about suPAR and mental disorders: A few studies have investigated suPAR in the context of mental disorders. For example, patients with MDD,[28,29] schizophrenia,[30,31] or attempted suicide[28] have been found to have elevated suPAR levels compared to healthy controls. Similarly, incident use of anti-depressants or incident hospital diagnosis of depression were associated with elevated suPAR among blood donors.[32] Whereas suPAR remained significantly associated with schizophrenia in adjusted analyses, CRP was no longer significantly associated when controlling for BMI.[31] Moreover, suPAR (area under the curve [AUC] 0.92) was better at discriminating between controls and patients who had attempted suicide than CRP (AUC 0.72, p .0053).[28] However, findings have been inconsistent with some studies not finding any associations between suPAR and schizophrenia[33] or reporting lower suPAR in patients with bipolar disorders,[34] and some studies lack appropriate controls for covariates, most importantly smoking.[28,32]

What this study will add: Here, we will test the hypothesis that elevated levels of the chronic inflammation marker suPAR are broadly associated with mental disorders in three

independent cohorts: (i) the 29K suPAR cohort (Denmark) of unselected acute medical patients with mental health data and suPAR registered at time of admission to the Emergency Department; (ii) the longitudinal population-representative Dunedin birth cohort (New Zealand), which offers life-course mental health data and suPAR measured at two time-points; and (iii) the population-representative E-Risk cohort (UK), which has suPAR and mental health data measured in young adults at age 18. In addition to our primary analysis of suPAR and mental disorders, a secondary analysis will focus on testing the same associations between CRP and mental disorders. Thus, this study gives us the opportunity to test broad associations of suPAR with a variety of mental disorders in patients and community samples, to see if this could be a potential new marker for psychopathology-related inflammation.

Hypotheses:

- Main hypothesis: Elevated suPAR is associated with mental disorders in acute medical patients and general populations.
- Patients with F-diagnoses (International Classification of Diseases (ICD-10) diagnoses from Chapter V: Mental and behavioral disorders, F00-F99) have higher suPAR levels than patients without F-diagnoses (both compared to all patients without F-diagnoses and to patients with a Charlson score of 0, i.e., no chronic illnesses).
- Elevated suPAR is associated with higher *p* factor (a general psychopathology factor, independent of covariates.
- Elevated suPAR is non-specifically associated with mental disorders, grouped by externalizing, internalizing, and thought disorders, as well as individual disorders.

Data analysis methods:

Cohorts:

- The 29K suPAR cohort (n~29,000 patients with suPAR measured at admission to the Emergency Department, Hvidovre Hospital, and with data on F-diagnoses from the Danish National Patient Registry)
    - Cases: Patients with any F-diagnoses
    - Controls:
        - Patients without any F-diagnoses *or*
        - Patients without any F-diagnoses and without any chronic illness
- The Dunedin Multidisciplinary Health and Development Study (n=875 with suPAR measured at age 45, n=895 with suPAR measured at age 38)
- The Environmental Risk (E-Risk) Longitudinal Twin Study (n=1447 with suPAR measured at age 18)

A P value < 0.05 is a priori designated as statistically significant. Bonferonni-corrected P levels will be reported.

Primary analyses:

Aim 1a: Do acute medical patients with mental disorders have higher suPAR levels than patients without mental disorders?

We will test whether patients with mental disorders (defined as any ICD-10 F-diagnoses recorded in the Danish National Patient Registry in the past 10 years) have higher suPAR levels than patients without mental disorders, defined as i) all patients without F-diagnoses,

or ii) patients with a Charlson score of 0 (i.e., no chronic illnesses), with controls for sex, age, and Charlson score.

Aim 1b: Is elevated suPAR associated with higher *p* factor?
We will investigate cross-sectional associations between *p* factor and suPAR at age 45 in Dunedin and age 18 in E-Risk with controls for sex, BMI, and smoking.

Secondary analyses: (depending on sufficient power for each analysis)
Aim 2: Is elevated suPAR non-specifically associated with mental disorders?
We will investigate cross-sectional associations between individual blocks of ICD-10 F-diagnoses or disease groups (e.g., mood disorders, substance use disorder, schizophrenia) and suPAR in the patients from the 29K suPAR cohort with controls for sex, age, and Charlson score.

We will investigate cross-sectional associations between higher order factors (internalizing, externalizing, thought disorders) and suPAR at age 45 in Dunedin and age 18 in E-Risk with controls for sex, BMI, and smoking.

Aim 3: Is elevated suPAR associated with age of onset, persistence/recurrence, and comorbidities?
If elevated suPAR is found to be associated with the *p* factor in the Dunedin Study, we will further test associations between suPAR at age 45 with age of onset, recurrence of mental disorders, and number of comorbidities, hypothesizing that elevated suPAR would be associated with earlier age of onset (longer duration of illness), more waves with presence of mental disorders, and with more comorbidities.

Aim 4: Is elevated CRP associated with mental disorders in acute medical patients and general populations?
In parallel analyses, we will investigate associations of CRP with F-diagnoses in the 29K suPAR patient cohort and with *p* factor, internalizing, externalizing, and thought disorders in Dunedin and E-Risk

Sensitivity analyses: (can be published in supplement)
Aim 5. Do acute medical patients with mental disorders within the past year have higher suPAR levels than patients without mental disorders during the past year?
As a sensitivity analysis, we will test associations between suPAR at admissions and F-diagnoses within the past year.

Aim 6: Do these associations hold over and above CRP?
All of the analyses of suPAR will be repeated controlling for hsCRP to investigate whether the potential associations between suPAR and mental disorders are independent of CRP.

Variables needed at which ages:

29K suPAR	Mental health variables at admission and from the past 10 years before admission: - Any F-diagnoses - F-diagnoses grouped by ICD-10 chapters: o F00-F09 Organic, including symptomatic, mental disorders o F10-F19 Mental and behavioural disorders due to

	psychoactive substance use o F20-F29 Schizophrenia, schizotypal and delusional disorders o F30-F39 Mood [affective] disorders o F40-F48 Neurotic, stress-related and somatoform disorders o F50-F59 Behavioural syndromes associated with physiological disturbances and physical factors o F60-F69 Disorders of adult personality and behaviour o F70-F79 Mental retardation o F80-F89 Disorders of psychological development o F90-F98 Behavioural and emotional disorders with onset usually occurring in childhood and adolescence o F99-F99 Unspecified mental disorder - F-diagnoses grouped by mental disorders (list provided by Richmond-Rakerd): o Self-harm o Substance use disorders o Psychotic disorders o Mood disorders o Anxiety disorders o Physiological disturbance disorders (e.g., eating, sleep) o Personality disorders o Developmental disorders (e.g., autism) o Childhood-onset disorders (e.g., ADHD, CD) o Unspecified Systemic inflammation: - suPAR and CRP at admission Covariates: - Sex, age, Charlson score (BMI and smoking not available)
Dunedin	Mental health variables at age 38 and 45: - *p* factor - Internalizing - Externalizing - Thought disorders - Age of onset of first mental disorder - Number of waves with mental disorders - Number of comorbidities - Depression (ever) - Anxiety (ever) - Substance use disorders (ever) - ADHD (ever) - Mood disorders (ever) - Schizophrenia (ever) Systemic inflammation:

	- suPAR and CRP at 45 Covariates: - Sex, BMI, current smoking status at age 45
E-Risk	Mental health variables at age 18: - *p* factor - Internalizing - Externalizing - Thought disorders - Depression - Anxiety - Substance use disorders - ADHD - Mood disorders - Schizophrenia Systemic inflammation: - suPAR and CRP at age 18 Covariates: - Sex, BMI, current smoking status at age 18

Significance of the Study (for theory, research methods or clinical practice):
This study will extend our understanding of the biomarker suPAR by investigating its associations with psychopathology in one clinical sample of unselected acute medical patients and two community samples of population-representative young and midlife adults.

By comparing the widely used inflammation marker CRP with the novel inflammation marker suPAR in three independent population-/patient-based cohorts using both cross-sectional and longitudinal data, we hope to advance our knowledge of the usefulness of these biomarkers to assess psychopathology-related inflammation, by introducing suPAR as a more reliable biomarker of inflammation to a field that has relied on other inflammation markers like CRP.

If elevated suPAR is robustly correlated with mental disorders in these independent cohorts, there are several potential applications for suPAR in the field of psychiatry, including addition of suPAR in basic science studies of inflammation in psychopathology or in intervention studies that seek to reduce psychopathology-related inflammation, as well as clinical assessment of suPAR in patients with mental disorders to assess chronic inflammation level and the risk of comorbidity.

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Data Security Agreement

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Proposing Author: Line Jee Hartmann Rasmussen
Today's Date: 2/14/2021

☒	I am current on Human Subjects Training (CITI (www.citiprogram.org) or equivalent)
☒	My project is covered by the Duke ethics committee OR I have /will obtain ethical approval from my home institution.
☒	I will treat all data as "restricted" and store in a secure fashion. My computer or laptop is: a) encrypted (recommended programs are FileVault2 for Macs, and Bitlocker for Windows machines) b) password-protected c) configured to lock-out after 15 minutes of inactivity AND d) has an antivirus client installed as well as being patched regularly.
☒	I will not "sync" the data to a mobile device.
☒	In the event that my laptop with data on it is lost, stolen or hacked, I will immediately contact Moffitt or Caspi.
☒	I will not share the data with anyone, including my students or other collaborators not specifically listed on this concept paper.
☒	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. Study participants have not given informed consent for unrestricted open access, so we have a managed-access process. Speak to Temi or Avshalom for strategies for achieving compliance with data-sharing policies of journals.*
☒	I will delete all data files from my computer after the project is complete. Collaborators and trainees may not take a data file away from the office. This data remains the property of the Study and cannot be used for further analyses without an approved concept paper for new analyses.
☒	I have read the Data Use Guidelines and agree to follow the instructions.

Signature: February 14, 2021: *Line Jee Hartmann Rasmussen*