Concept Paper

Provisional Paper Title: Elevated suPAR and midlife accelerated aging

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Today's Date: August 26, 2019

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

Objective of the study:

A major public health challenge is to extend healthspan in the context of an ever-expanding aging population.[1,2] Chronic inflammation is a major driver of pathogenesis and progression of common age-related, chronic diseases (e.g., cardiovascular disease, type 2 diabetes, cancer, and neurodegenerative disorders). To delay onset of common age-related diseases and to extend years lived free of disease and disability—and at the same time reduce health-care costs and disease-related deaths—interventions to slow chronic inflammation and accelerated aging must be applied *before* development of manifest disease. The identification of reliable biomarkers of chronic inflammation is therefore critical.

While the acute-phase reactant C-reactive protein (CRP) is commonly used as the gold standard inflammation marker both in the clinic and in life-course research,[3] *soluble urokinase plasminogen activator receptor* (suPAR) is a newer biomarker of inflammation[4] which 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.[5] The blood concentration of suPAR is elevated upon activation of the immune system, and suPAR is associated with development, presence, and progression of disease.[4,6,7] Thus, suPAR levels are elevated across a wide range of diseases,[8] including cardiovascular disease,[9] type 2 diabetes,[10] cancer,[11,12] renal disease,[13] and infections.[14] In addition, suPAR is a strong predictor of mortality, both in the general population and in patient populations.[6,8]

We have previously shown that exposure to childhood risk factors, including adverse childhood experiences, is associated with elevated suPAR levels later in life, even more so than were CRP and IL-6,[15,16] suggesting that exposure to stressful events might be better reflected in a person's suPAR level than in CRP or IL-6.

In addition to early-life risk factors, studies in healthy and general populations have shown that suPAR increases with age[17,18] and that major lifestyle habits (diet, smoking, alcohol, and physical activity) are reflected in suPAR, with healthy habits generally associated with lower suPAR and unhealthy habits with higher suPAR.[19]

Here, we will investigate whether the chronic inflammation marker suPAR is also associated with measures of accelerated aging. We aim to investigate the association of suPAR with measures of accelerated aging in the Dunedin study, and, further, to investigate the association between suPAR and measures of health, functional capacity, cognitive function, and lifestyle.

We hypothesize that elevated suPAR is both cross-sectionally and longitudinally associated with

adverse health outcomes, functional capacities (more physical limitations, poorer physical function), accelerated aging (accelerated Pace of Aging, Facial Aging, older Brain Age), and cognitive limitations and cognitive decline.

In addition to our primary analysis of suPAR and aging, a secondary analysis will focus on whether lifestyle changes from age 38 to age 45 are associated with parallel changes in suPAR levels.

The specific aims are to:

- Assess whether suPAR measured at age 45 is associated with health outcomes, functional capacity, accelerated aging, and cognitive status/decline.
- Assess whether suPAR measured at age 38 is associated with change from age 38 to age 45 (for those measures that have been taken on both occasions, e.g., grip strength).
- Assess whether any associations found hold over and above CRP.
- Assess whether any associations found hold over and above family history of chronic illness.
- Assess whether lifestyle changes from age 38 to age 45 are associated with parallel changes in suPAR levels.

Data analysis methods:

To address our aims, we wish to conduct the analyses described below.

1. **Is elevated suPAR at age 45 associated with concurrent poor health, poor functional status, accelerated aging, and cognitive decline?**
   We will investigate cross-sectional correlations between suPAR measured at age 45 and the following measures of health, functional capacity, aging, and cognitive status, after adjusting for sex and smoking:

   Health outcomes: (these measures will be used to describe the cohort at age 45)
   - Self-reported health
   - Blood pressure and hypertension
   - Incident chronic disease (count of illnesses)
   - Obesity

   Functional capacity:
   - Physical limitations (RAND SF-36)
   - Balance
   - Hand grip strength
   - Gait speed
   - 2-min step test
   - Chair-stand test

   Measures of aging:
   - Pace of Aging (scaled in years; longitudinal measure)
   - Facial Aging
   - Brain Age

   Cognitive status:
   - Decline in overall IQ from childhood to adulthood

- Decline in processing speed

2. **Is suPAR measured 7 years earlier (at age 38) associated with the same outcomes at age 45?**
   We will investigate whether suPAR measured at age 38 is associated with changes in these outcomes from age 38 to age 45 (depending on whether there is sufficient change to study; for example, if obesity/BMI is very stable over the 7 year period, it may not be possible, given the power that we have, to reliably estimate change). Change will be measured as a difference score or residualized change score, and associations will be investigated by multiple linear regression analyses adjusted for sex and smoking of the following outcomes (based on availability at both age 38 and 45):

   Health outcomes:
   - Self-reported health
   - Blood pressure and hypertension
   - Incident chronic disease (count of illnesses)
   - Obesity

   Functional capacity:
   - Physical limitations (RAND SF-36)
   - Balance
   - Grip strength

   Measures of aging:
   - Facial Aging

3. **Do these associations hold over and above CRP?**
   All of these analyses will be repeated controlling for hsCRP to investigate whether the potential associations between suPAR and health outcomes, functional capacity, aging, cognitive function, and lifestyle changes are independent of CRP.

4. **Do these associations hold over and above family history of chronic illness?**
   Furthermore, we will repeat the analyses with controls for family history of chronic illness, used as a proxy for the family medical record.

5. **Are lifestyle changes from age 38 to age 45 associated with parallel changes in suPAR levels?**
   We will investigate whether participants who have made healthy lifestyle changes from age 38 to age 45 have parallel changes in their suPAR level from age 38 to 45.
   For example, do participants who have made healthy lifestyle changes (e.g., smoking cessation, increased physical activity, decreased alcohol use) have smaller increases, or even decreases, in their suPAR levels from age 38 to age 45, compared with participants who have not changed their lifestyle and participants with a more unhealthy lifestyle at age 45 than age 38?

   In these analyses of change (difference between age 38 and 45), we will control for starting point. For example, for smoking, we will analyze the change in smoking from age 38 to 45 (cigarettes per day at age 45 minus cigarettes per day at age 38), controlling for the no. of cigarettes per day smoked at age 38. The analysis will be restricted to life-time ever-smokers.
   Similarly, we will investigate changes in physical activity (based on difference in Mets from age 38 to

age 45) and alcohol consumption (difference in units of alcohol from age 38 to age 45; excluding people who do not drink at all).

A P value < 0.05 is a priori designated as statistically significant.

Variables needed at which ages:

Family history of chronic age-related disease: [SUMMARY OF FAMILY HISTORY]

- Any heart disease
- Hypertension
- Stroke
- High cholesterol
- Diabetes

Childhood variables:

- Childhood IQ
- Childhood SES

Variables at both age 38 and age 45:

- Lifestyle:
    - Current smoking
    - Pack-years
    - Physical activity (Mets)
    - Anti-inflammatory medication
- Anthropometry:
    - BMI
    - Waist-hip ratio
    - Weight
- Functional capacity:
    - Physical limitations (RAND SF-36)
    - One-legged balance
    - Grip strength
    - Chair-stand test (for age 45)
    - 2-min step test
    - Gait speed (for age 45)
- Cognitive function:
    - Adulthood IQ (WAIS incl. individual domains)
    - Processing speed
- Measures of aging
    - Pace of Aging (scaled in years)
    - Facial Aging
    - Brain Age
- Inflammatory biomarkers:
    - CRP
    - Fibrinogen ?
    - IL-6
    - suPAR
    - White blood cells

- Health outcomes:
    - Blood pressure
    - Hypertension group
    - Self-reported health
    - Diabetes group
    - Cardiovascular disease group
    - Cancer
    - Count of diagnosed diseases
    - Incident chronic disease (a count of all illnesses)
    - Death between 38 and 45
    - Self-reported health
    - Obesity

Significance of the Study (for theory, research methods or clinical practice):

This study will extend our understanding of the biomarker suPAR by investigating its association with indicators of biological aging in healthy midlife adults. It will further improve our understanding of the impact of lifestyle changes and health behaviors on suPAR.

If the findings turn out to support suPAR as a robust correlate of accelerated aging, suPAR may be recommended as a useful adjunct to basic science studies of inflammaging and to intervention studies that seek to slow or reverse aging.

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Data Security Agreement

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Today's Date	August 26, 2019

Please keep one copy for your records and return one to the PI Sponsor

Please initial your agreement

LJHR	I am current on Human Subjects Training (CITI (www.citiprogram.org) or equivalent)
LJHR	My project is covered by Duke or Otago ethics committee OR I have /will obtain ethical approval from my home institution.
LJHR	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.
LJHR	I will not "sync" the data to a mobile device.
LJHR	In the event that my laptop with data on it is lost, stolen or hacked, I will immediately contact Professor Moffitt or Caspi. (919-684-6758, tem11@duke.edu, ac115@duke.edu)
LJHR	I will not share the data with anyone, including my students or other collaborators not specifically listed on this concept paper.
LJHR	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 Dunedin Study Members have not given informed consent for unrestricted open access, so we have a managed-access process. Speak to Terrie or Avshalom for strategies for achieving compliance with data-sharing policies of journals.*
LJHR	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. The data remains the property of the Study and cannot be used for further analyses without an approved concept paper for new analyses.

Signature: Line Jee Hartmann Rasmussen