Proteoglycan 4 (PRG4) Deficiency in Mice Results in Decreased Survival Probability and Sexual Dimorphism in Blood Parameters

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INTRODUCTION: Proteoglycan 4 (PRG4, also known as lubricin) is a mucin-like glycoprotein best, and historically, known for its mechanical function as a cartilage boundary lubricant (1, 2). However, it also has anti-inflammatory (3-5), anti-fibrotic (6), and immunomodulatory properties (4, 7). Loss-of-function mutations in the PRG4 gene in humans causes Camptodactyly-Arthropathy-Coxa vara-Pericarditis (CACP), a rare disease in which patients often require joint replacements at young ages (8). There are limited studies that have explored the role of PRG4 during aging, and it is unknown both if circulating PRG4 levels can change during aging and how PRG4 deficiency impacts the aging process. As such, the objective of this study was to assess how the serum concentration of PRG4 changed during aging in mice, as well as how PRG4 deficiency impacts survival probability, blood gas analysis, and complete blood count.

METHODS: Mice. Wild type (WT) young (3-month-old) C57BL/6 male mice were purchased from Jackson Laboratories and aged (19-month-old) C57BL/6 male mice were obtained from the National Institute on Aging rodent colony. Prg4 GT mice (9) (JAX #025740) were back crossed onto WT C57BL/6J mice for 6 generations to generate Prg4 GT mice on a C57BL/6J background. All animal studies were conducted in accordance with protocols approved by the University of Connecticut Health Center IACUC. Serum analysis was performed on young (3-month-old) and aged (19-month-old) male and female Prg4 GT and WT mice, and blood analyses were performed on middle-aged (9-14 months) male and female Prg4 GT and WT mice. PRG4 serum concentration. Blood was collected via intracardiac puncture from 3-month-old (young) and 19-month-old (aged) C57BL/6J male mice immediately following euthanasia by CO2 inhalation. The concentration of PRG4 was measured using a custom AlphaLISA inhibition assay (10) (N=4, using full length recombinant human PRG4 (rhPRG4), Lubris, LLC) as a standard. Survival. Prg4 GT and WT mice were aged under normal living conditions with standard diet and water ad libitum. Kaplan-Meier survival analysis was employed to assess the probability of survival. N=8-11 male (15-44 weeks old at time of analysis), N=15-17 female (18-50 weeks old at time of analysis). Blood Analysis. Blood was obtained via submandibular bleeds on middle-aged Prg4 GT and WT mice between 40 and 62 weeks of age. Blood gas and electrolyte analysis (N=8) was done using Element POC (Heska Corporation, USA) and complete blood count (N=8) was done using the Vetscan HM5 Hematology Analyzer (Abaxis, Inc., USA). Statistics: The logrank test (Mantel-Cox method) was employed for the analysis of the Kaplan Meier survival curves. In evaluating serum PRG4 concentration, blood gas analysis, and complete blood count, Prg4 GT and WT mice were compared to each other within sex. T tests with Welch’s correction were used to determine statistical significance (p<0.05).

RESULTS: PRG4 serum concentration. Aged C57BL/6J mice (19-months-old) had a significantly lower mean serum PRG4 concentration than young mice (3-months-old) (1.178±0.09 vs. 1.43±0.04 µg/ml, respectively, p<0.05) (Fig 1). Survival. Both male and female Prg4 GT mice had significantly lower survival probability compared to WT mice (p<0.05 male, p<0.01 female; Fig 2A-B). The probability of survival for male Prg4 GT mice dropped below 50% after 40 weeks, while male WT mice remained at 100%. Similarly, the probability of survival for female Prg4 GT mice also dropped below 50% nearing 50 weeks while remaining for 100% for female WT mice. Blood Gas and Electrolytes. Only male Prg4 GT mice had significantly higher hematocrit % (HCT%) and hemoglobin (Hgb) than male WT mice. However, female Prg4 GT mice had significantly higher blood pH (7.33±0.01) than female WT mice (7.23±0.02), in addition to significantly higher total carbon dioxide (TCO2), bicarbonate (HCO3−), base excess in blood (be(b)), base excess in extracellular fluid (be(ecf)), and oxygen saturation (sO2%), as well as lower ionized calcium (Ca++, glucose (Glu), and lactate (Lac). Complete Blood Count. Middle aged male Prg4 GT mice had significantly lower mean corpuscular volume (MCV) than male WT mice, with a lower platelet number (PLT) trending toward significance (P=0.055). Female Prg4 GT mice had a lower mean significantly lower monocyte number (MON) and mean corpuscular volume (MCV) compared to female WT mice, in addition to a significantly higher red blood cell number (RBC) and red blood cell distribution width (RDW%).

DISCUSSION: This study is the first exploration into the relationship between PRG4, aging, and survivability, and its findings provide the foundation for further in-depth exploration into this area. With the development of our custom AlphaLISA PRG4 detection assay, capable of quantifying PRG4 in small (<5µl) volumes of biological fluids, we found that serum PRG4 is lower in aged mice than young mice. Kaplan-Meier survival analysis indicated a significantly reduced survival probability in the GT mice compared to WT mice, with GT survival probability dropping below 50% before 1-year-old. This is far sooner than is to be expected in WT C57BL/6J mice, which have a mean survivorship of >28 months. This data suggests PRG4 deficiency significantly impacts survivability during maturation and aging. Blood gas and electrolyte analysis and complete blood count revealed that primarily middle-aged female Prg4 GT mice were impacted, with differences including pH, glucose, lactate, and red blood cell number relative to middle aged WT mice. While the decreased survival probability findings in Prg4 GT mice are novel, and perhaps unexpected, the mechanism behind the decreased survival probability in PRG4 deficient mice is yet to be elucidated.

SIGNIFICANCE: Changes in survivability probability demonstrates PRG4 deficiency poses a health risk to the mice and supports the hypothesis that PRG4 has physiological functions well beyond that of lubrication of synovial joints. These results provide the foundation for future studies to expand on this work mechanistically, consider therapeutic potential of rPrG4, and assess clinical relevance in those affected by CACP and in aging populations.


ACKNOWLEDGEMENTS: This work was supported by Biomedical Engineering Department startup funds (TAS), a Convergence Grant from the Research Excellence Program at UConn Health (TAS, JL), MB is supported by R01AI173305 (NIH/NIAID, PI: Bartley) and the UConn Claude D. Pepper Older Americans Independence Center (NIH/NIA P30AG067988, PI: Kuchel and Fortinsky). The authors also thank Sun-Kyeeong Lee for critical insight to the project.

Fig 1. Serum PRG4 concentration in young and aged mice. Blood was obtained via cardiac puncture from young (3 month) and aged (19 month) C57BL/6 male mice and analyzed for PRG4 concentrations. N=4. Data are mean ± SEM. *P<0.05.

Fig 2. Kaplan-Meier curves of Prg4 deficient (GT) and wild type (WT) mice. A) Probability of survival of male mice. B) Probability of survival of female mice. N=8-11 male, 15-17 female.