Kidney Glomerular Hypertrophy in HNF-1αG319S in Mice

Maryana Y. Kutuzova[1,4], Owen Barber-Dueck[2,4], Kristin L. Hunt[3], Christine A. Doucette[3,4]
[1]Bachelor of Health Sciences, Interdisciplinary Health Sciences, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, MB, Canada
[2]School of Medicine, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, MB, Canada
[3]Department of Physiology and Pathophysiology, University of Manitoba, Winnipeg, MB, Canada
[4]Children's Hospital Research Institute of Manitoba (CHRIM), Winnipeg, MB, Canada

Introduction

- In Manitoba, the incidence of type 2 diabetes (T2D) among children is 10-20-fold higher than in other regions of Canada, reaching more than 20 cases per 100,000 children.[1]
- The majority of cases (90%) are among self-declared First Nation population with a high prevalence rate in Anishininiiwuk (Oji-Cree) heritage.
- The rate of diabetic nephropathy is higher in children with T2D (13.7%, 27% and 31% at 10, 15 and 20 years of disease duration) than with type 1 diabetes mellitus (4.8%, 12.7% and 17.4% at 10, 15 and 20 years) and the onset of persistent albuminuria is as early as 0.76 yrs of mean duration (SD 1.36) suggesting that kidney malfunction occurs before diagnosis[2,3,4].
- A variant in the HNF-1α gene (HNF-1a G319S), where highly conserved guanine changed to alanine at base pair 955 of the gene, which translates to glycine to serine change at position 319, has been found in the Anishininiiwuk population, one with the highest rates of T2D worldwide and chronic kidney disease in Northern Manitoba[5,6,8]
- HNF-1α is a transcription factor found in the liver, intestine, pancreas, and kidney.
- Almost 60% of Anishininiiwuk youth T2D patients with persistent microalbuminuria renal biopsy demonstrated a presence of at least one copy of the G319S variant[7].
- Earlier preliminary RTqPCR data received in Dr. Doucette's lab by measuring gene expression on kidneys from six-month-old HNF1a female mice showed a direct negative correlation between the HNF1a expression and SGLT-2 and GLUT-2 genes expression.
- Studying of the G319S variant as a contributor to nephropathy was the aim of the research project.

Objectives

To identify whether there are changes in the histological structure of the kidneys in the presence of a genetic mutation G319S using homozygous, heterozygous, and wild type mice through the comparison of the following parameters:

- size of glomeruli;

- and the number of glomeruli.

Source: https://www.npr.org/2020/03/14/815624878/mouse-hunt-lab-races-to-grow-mice-for-covid-19-research

Materials and Methods

- CRISPR/Cas9 technology was used to knock-in the G>A single nucleotide substitution at position 955 in C57BL6 mice. Six-month-old females with the wild genotype (G/G), heterozygous (G/S), and homozygous (S/S) for G319S variant.
- Formalin-fixed paraffin-embedded (FFPE) right kidneys were sectioned and stained with Periodic Acid Schiff/hematoxylin counterstain.
- A total of nine samples was used for the project. Three from each group: G/G, G/S, and S/S.
- Microscopy was done using a Zeiss AxioCam 10.5 Light microscope with Zeiss software. Ten non-overlapping areas with the highest glomerular density in the field of view were captured at 10x magnification for the overview image and, then, 40x magnification for every glomeruli in the field.

Source: https://www.indiamart.com/carl-zeiss-india-bangalore/carl-zeiss-light-microscopes.html

- Image J 2 Fiji software was used to measure glomeruli size on the 40x magnification images. Glomerular area was contoured excluding all extraglomerular tissue. The number of pixels was converted into the factual tissue volume using the coefficient that had been calculated earlier in the Dr. Doucette's lab.
- To reduce bias, two people did measurements independently from each other as well as they were blinded in terms of which sample is G/G, G/S, or S/S.
- Average numbers were calculated using data from both measurements.
- Statistics calculated using one-way ANOVA with $p<0.05$.

Results

HNF1a Glomerular Imaging: Six-Month-Old Females (representative images from samples with S/S and G/G genotype)

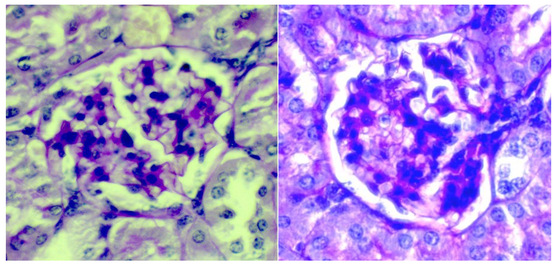


Figure 1. Six-month-old female mice S/S genotype (homozygous for G319S). Periodic Acid Schiff/hematoxylin counterstain. Zeiss AxioCam 10.5 Light microscope with Zeiss software. Representative images from two different kidney samples showing **glomerular hypertrophy**.

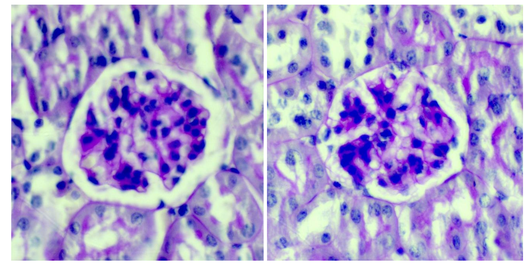


Figure 2. Six-month-old female mice G/G genotype (wild type). Periodic Acid Schiff/hematoxylin counterstain. Zeiss AxioCam 10.5 Light microscope with Zeiss software. Representative images from two different kidney samples showing **normal glomerular structure.**

HNF1a glomerular measurements: Six Month Old Females

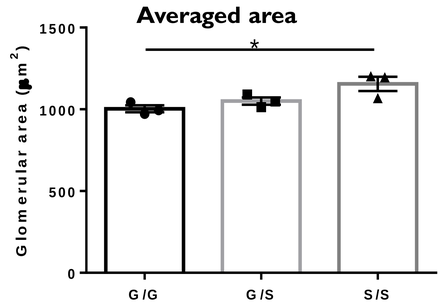
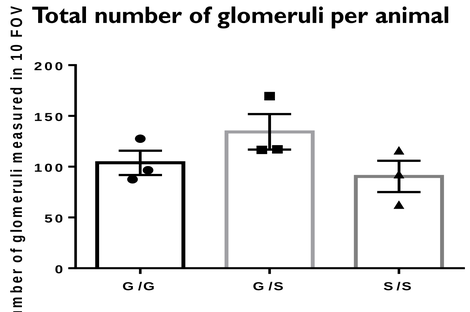


Figure 3. The graph shows the **difference in average glomerular size among G/G (wild), G/S (heterozygous), and S/S (homozygous) genotypes in the gene-dose-dependent manner.** Two sets of blinded measurement data have been used for calculations. ImageJ 2 Fiji software was used to measure glomeruli captured at 40x magnification. G/G average size is 1003 ± 20.97 (p value=0.03). S/S avarage size 1155 ± 43.57 (p value=0.03). Statistics calculated using one-way ANOVA.



Figur. The graph shoes the total number of glomeruli per animal. In the G/G genotype 112.3 glomeruli per animal, in the G/S genotype 134.7 glomeruli per animal, S/S – 90 .7 glomeruli per animal on average. Two sets of blinded measurement data have been used for calculations.

Conclusions

- Heterozygous HNF1a G319S S/S mice have a significantly larger glomerular size and the smallest number of glomeruli.
- The increased size is due to the glomerular hypertrophy.
- The glomerular hypertrophy is gene-dose-dependent.
- The number of glomeruli is not that conclusive as the highest number is noticed in heterozygous type, the smallest one in the homozygous type.

Future Directions

- Urinalysis can be done to determine if the glomerular hypertrophy is associated with the changes in kidney function,
- Assessing functions of the heparan sulphate barrier and proximal convoluted tubules reabsorption mechanism can provide an insight to the mechanism of the proteinuria.

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Contact information: – morriset@myumanitoba.ca