Role of Acid Sphingomyelinase Knockout Mice in Protection Against Hyperhomocysteinemia Induced Glomerular Injury

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Hyperhomocystenemia is known as a critical pathogenic factor in the progression of end stage renal disease (ESRD) and in the development of cardiovascular complications related to ESRD.

Chronic elevations of plasma Hcys levels induce proteinuria, mesangial expansion and glomerulosclerosis.

Elevated Hcys levels increase de novo ceramide synthesis in rat mesangial cells. This increased ceramide production enhances NADPH oxidase activity.

Increased NADPH oxidase activity generates superoxide production and ultimately causes glomerular injury.

However, nothing is known about hyperhomocysteinemia induced glomerular injury on mice lacking the acid sphingomyelinase gene.
Hypothesis

To explore the role of acid sphingomyelinase and NAD(P)H oxidase in the development of hHCys-induced glomerular injury in ASM mice
Biosynthesis of Ceramide

Serine + palmitoyl-CoA → 3-ketosphinganine → Sphinganine → Dihydroceramide → Ceramide → Sphingosine → Sphingosine-1-P

FF Diet → ↑hHcy → ASM/Ceramide ↑ → LR/platform → NOX/O2⁻ ↑ → Glomerular Injury
Homocysteine

\[
\text{HS} \quad \text{NH}_2 \quad \text{O} \quad \text{OH}
\]

\[
\text{S-Adenosyl-methionine} \quad \text{S-Adenosyl-homocysteine} \quad \text{Methionine} \quad \text{ATP}
\]

\[
\text{SAHH} \quad \text{CBS} \quad \text{GCS}
\]

\[
\text{B}_12 \quad \text{B}_6 \quad \text{folate}
\]

\[
\text{H}_2\text{O} \quad \text{H}_2\text{O}
\]

\[
\text{Excreted in urine} \quad \text{Cysteine} \quad \text{Excreted in urine}
\]

\[
\text{S}_4\text{O}_4^- \quad \text{Taurine}
\]
Animal Model

8-weeks old ASM mice

- **+/+** Control diet
- **+/+** Folate free diet
- **-/-** Control diet
- **-/-** Folate free diet

ASM mRNA level, Plasma Hcy, Urinary protein excretion, Superoxide production and Glomerular injury markers
Plasma Hcys concentration in mice

**Control diet**
- ASM^{+/-}
- ASM^{-/-}

**Folate free diet**
- ASM^{+/-}
- ASM^{-/-}

* vs Control diet
# vs WTFF diet
ASM mRNA expression in mice

- Control diet
- Folate free diet

ASM mRNA level

ASM\(^{+/+}\)
ASM\(^{-/-}\)
Morphological features of the glomeruli from different groups of mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Glomerular Damage Index</th>
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<tbody>
<tr>
<td>WT ND</td>
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<tr>
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<tr>
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</table>

- ASM+/+
- ASM -/-

* vs Control diet
# vs WTFF diet
Urinary total protein excretion in mice

Control diet

Folate free Diet

ASM+/+

ASM -/-
Structure and function of podocytes
Podocin expression in mice treated with control or folate free diet

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Nephrin expression in mice treated with control or folate free diet

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Desmin expression in mice treated with control or folate free diet

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**Ceramide expression in mice treated with control or folate free diet**

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ASM expression in mice treated with control or folate free diet

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Superoxide production in ASM mice

Control Diet

Folate free diet

ASM\(^{+/+}\)
ASM\(-/-\)

* vs Control diet
# vs WTFF diet
Conclusions

Mechanism:

FF Diet- ↑Hcy - ↑ ASM and Ceramide – ↑ LR platform- ↑ Nox/O₂⁻ - Glomerular injury

Conclusions:

➢ ASM gene knockout mice attenuates folate free diet induced plasma homocysteine concentration, mRNA level, urinary total protein excretion, and superoxide production compared to the wild type mice.

➢ ASM gene knockout protects against hyperhomocysteninemia induced glomerular injury.
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