Apolipoprotein E isoforms in diabetic dyslipidemia and atherosclerosis

Lance Johnson
Diabetic Dyslipidemia

- Cluster of harmful changes to lipoprotein metabolism commonly noted in diabetic patients

- Characterized by high VLDL triglycerides, low HDL-cholesterol, and an increase in small, dense LDL particles

- Increases the risk of developing atherosclerosis
**Apolipoprotein E Isoforms**

<table>
<thead>
<tr>
<th></th>
<th>ApoE2</th>
<th>ApoE3</th>
<th>ApoE4</th>
</tr>
</thead>
<tbody>
<tr>
<td>position 112</td>
<td>cys</td>
<td>cys</td>
<td>arg</td>
</tr>
<tr>
<td>position 158</td>
<td>cys</td>
<td>arg</td>
<td>arg</td>
</tr>
<tr>
<td>frequency</td>
<td>7.2%</td>
<td>78.3%</td>
<td>14.3%</td>
</tr>
<tr>
<td>LDLR affinity</td>
<td>&lt;1%</td>
<td>100%</td>
<td>&gt;100%</td>
</tr>
<tr>
<td>plasma TC</td>
<td>-6%</td>
<td>ref.</td>
<td>+6%</td>
</tr>
<tr>
<td>MI risk</td>
<td>reduced (type III)</td>
<td>ref.</td>
<td>Increased</td>
</tr>
</tbody>
</table>
Plasma lipoproteins

Mice with Human apoE2 (poor ligand)

Mice with Human apoE3 (reference)

Mice with Human apoE4 (better ligand)

Normal LDLR

Increased LDLR

On a HFW diet
Figure 2 ApoE trapping. Efficient transfer of apoE4 facilitates internalization of large lipoproteins in 4m mice. In 4h mice, the efficiency of transfer is reduced because apoE4 are trapped by the increased LDLR.
Akita-diabetic mice with human apoE isoforms

Survival

With normal LDLR

With human LDLR

Percent survival

Months of age

With normal LDLR

With human LDLR

Percent survival

Months of age

Diabetic Human ApoE Mice: Survival Rate
Two of two -6mo old 4hAkita mice on a low cholesterol diet had atherosclerosis despite of low plasma cholesterol (<120 mg/dl) levels

This is the first mouse model of atherosclerosis for which diabetes is required.
Mice with Reduced Lipoic Acid Synthase

Xianwen Yi
• LA is a water- and fat-soluble antioxidant that can easily cross cell membranes.

• Removes catalysts by metal-chelating
e.g. Fenton reaction
\[ \text{H}_2\text{O}_2 + \text{Fe}^{2+} \rightarrow \text{OH}^* + \text{OH}^- + \text{Fe}^{3+} \]

• A strong reducing agent and recycles other antioxidants like reduced glutathione (GSH)
LA in diet prevents enhanced atherosclerosis induced by diabetes in apoE-/- mice

Lesion size (x 10^5 μm^2)

- LA: - - + - +
- DM: - - + + +

P < 0.001
P = 0.5
P = 0.22
P < 0.001

(5mo post STZ)
LA is an essential cofactor for several key enzyme complexes in metabolism.
Endogenous production of LA is essential

Lias-/- embryos die immediately after implantation

7.5 dpc
Lias+/-Apoe-/- mice

(129SvEvTac)
Plaque size at the aortic roots is significantly larger in *Lias*+/--*Apoe*-- males but not in females.
Increased oxidative stress in *Lias+-/Apoe-/-* mice: effects are bigger in males than in females

**Gene expression in aorta**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Male</th>
<th>Female</th>
<th><em>P</em> (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Lias+-/Apoe-/-</em> n=9</td>
<td><em>Lias+-/Apoe-/-</em> n=10</td>
<td><em>Lias+-/Apoe-/-</em> n=9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sod1</td>
<td>0.57±0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.00±0.15</td>
<td>1.01±0.12</td>
</tr>
<tr>
<td>Sod2</td>
<td>0.40±0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.00±0.16</td>
<td>0.97±0.13</td>
</tr>
<tr>
<td>Gpx1</td>
<td>0.55±0.06</td>
<td>1.00±0.26</td>
<td>0.71±0.05</td>
</tr>
<tr>
<td><em>Lias</em></td>
<td>0.40±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.00±0.13</td>
<td>0.46±0.08&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>IL-6</td>
<td>2.40±0.75</td>
<td>1.00±0.23</td>
<td>1.51±0.35</td>
</tr>
</tbody>
</table>

Blood samples are taken at the end of the study. Data shown are mean values ±SE. The number of animals in each group is in parenthesis. <sup>a</sup>: *P*<0.05 vs male *Lias+-/Apoe-/-* mice; <sup>b</sup>: *P*<0.05 vs female *Lias+-/Apoe-/-* mice.
Lias+/-Apoe-/- mice

A 50% reduction of Lias gene expression.

Significantly increased atherosclerosis in males but not in females.

Higher plasma cholesterol, TG and glucose, and increased body weight --- increased food intake?
LDLR gene expression in the liver is reduced.

Increased oxidative stress markers.

These effects are present in both in males and females, but more pronounced in males.
Lias+/--Akita mice

$Lias^{+/-}$

(129)

$\times$

$Ins^{2Akita/+}$

(C57BL/6J)
Increased Kidney Damage

A. $\text{Lias}^{+/+} \text{Ins2}^{\text{Akita}/+}$ mice

B. $\text{Lias}^{+/+} \text{Ins2}^{\text{Akita}/+}$ mice

Plasma glucose (mg/dl)

<table>
<thead>
<tr>
<th></th>
<th>+/-</th>
<th>+/+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Value</td>
<td>19</td>
<td>22</td>
</tr>
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</table>

$P < 0.01$

Urine albumin ($\mu$g/day)

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<td>Value</td>
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<td>21</td>
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</table>

$P < 0.01$

MME score

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<tbody>
<tr>
<td>Value</td>
<td>19</td>
<td>22</td>
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</table>

$P < 0.05$
Increased oxidative stress markers in the kidneys
Podocyte Effacement

Lias+/+Akita

Lias+-Akita
Mitochondrial damage in the proximal tubular cells

$Lias^+/+Akita$  $Lias^+/-Akita$
Table 2 Gene expression in kidney

<table>
<thead>
<tr>
<th>Gene</th>
<th>$\text{Lias}^{+/+}\text{Ins2}^{\text{Akita}+/+}$ n=15</th>
<th>$\text{Lias}^{+/+}\text{Ins2}^{\text{Akita}+/+}$ n=14</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neprhin</td>
<td>0.48±0.07</td>
<td>1.00±0.14</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Sod1</td>
<td>0.66±0.11</td>
<td>1.00±0.12</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Sod2</td>
<td>0.65±0.10</td>
<td>1.00±0.11</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Sod3</td>
<td>0.80±0.10</td>
<td>1.00±0.12</td>
<td>0.09</td>
</tr>
<tr>
<td>Col I</td>
<td>1.27±0.18</td>
<td>1.00±0.23</td>
<td>NS</td>
</tr>
<tr>
<td>Col IV</td>
<td>1.44±0.19</td>
<td>1.00±0.10</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Tgfβ1</td>
<td>1.56±0.16</td>
<td>1.00±0.12</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Lias</td>
<td>0.35±0.03</td>
<td>1.00±0.10</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

mRNA levels in kidney cortex are expressed as mean ± SE relative to the mean levels of the control without LA as 1.00.
Conditional Reduction of Lipoic Acid Synthase

Xianwen Yi, Nobuyo Maeda
University of North Carolina

Racheal Wallace
Jackson Lab
Figure 8. Conditional reduction of the Lias gene expression in mice. A, Genomic structure of the 3' end of the mouse Lias gene. B, Targeting construct (see text). Mice carrying the modified Lias gene (Lias-Mod) will produce Lias transcript containing the natural 3'UTR sequence (C). Breeding Lias-Mod mice with Cre-mice will generate Hypo-Lias mice in which the Lias 3'UTR-Neo cassette excised. Hypo-Lias mice will produce Lias transcripts with cFOS 3'UTR sequence.
Graded changes of the *Lias* expression in the liver

\[ Lias^{+/} \times Lias^{low/+} \]

**129** X **B6**

![Graph showing graded changes of *Lias* expression](image)
Conclusions and next directions

Mice expressing human apoE4 and human LDLR develop atherosclerosis when they are diabetic even on a low cholesterol diet.
--- postprandial lipid clearance ?
--- vascular cells ?

A reduced *Lias* gene expression increases diabetic kidney complications.
--- mitochondrial function in proximal tubules?
--- altered metabolism?
--- relationships with atherosclerosis?