Development of Human-Like genetic engineered Small Animal Models for Translational Metabolic Cardiovascular Research

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Models for Metabolic Cardiovascular Diseases: Basis of Translational Study

- Obesity
- Dyslipidemia
- Diabetes
- Hypertension

Models, models, and humanized models!

- Atherosclerosis
- Arrhythmia
- MI
- Heart Failure
- Stroke
Spontaneous Atherosclerosis in severe HTG


WT

LK+/−

LK−/−

Fraction

Triglycerides (mg/dl)

Cholesterol (mg/dl)

Lesion size (μm² × 10^3)

P < 0.001

4m 10m ≥ 15m
Weinstein MM et al: Chylocronemias elicits atherogenesis

ApoC3: inhibitor of lipoprotein lipase

GWAS study: Associated with CAD and Steatosis via TG metabolism

1. ApoC3 Tg and KO X LDLR KO mice: Atherogenesis and mechanism
2. ApoC3 Transgenic rabbits
3. ApoC3 Transgenic mini pigs

RT-PCR quantification of mRNA in the tissues

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>liver</th>
<th>intestine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-TG</td>
<td>6</td>
<td>1.5 ± 0.2</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>hApoC III-TG</td>
<td>6</td>
<td>34.0 ± 3.1**</td>
<td>13.0 ± 4.7**</td>
</tr>
</tbody>
</table>

** ± 4.7
ApoCIII transgenic rabbits: Mild HTG and impaired fat load

ApoCIII Transgenic pigs: Mild HTG and impaired fat load

Wei J et al. FEBS. J. 2012, 279(1):91-9
ApoCIII Transgenic pigs: Mild HTG and impaired fat load
Wei J et al. FEBS. J. 2012, 279(1):91-9
Fuller M et al: The Effects of Diet on Occlusive Coronary Artery Atherosclerosis and Myocardial Infarction in Scavenger Receptor Class B, Type 1/Low-Density Lipoprotein Receptor Double Knockout Mice. ATVB 2014 Nov.
Severe HTG rats

GPIHBP1 KO rats

TALEN

WT
LDL-R KO

GPIHBP1 KO rats

GPIHBP1 KO

+/-
-/-
-/-

GPIHBP1 KO rats

WT
GPIHBP1 KO

GGCCATCGCCTGCTTCCTGGGCTGCTGCTGGAG
GGCCATCGCCTGCTCCTGGGCTGCTGCTGGAG

GATTACCTGCGCTGGGTCAGACGCTTTCTGACCAGGTCCAGG
GATTACCTGCGCTTTCTGACCAGGTCCAGG
HyperChol rats

Plasma Lipids and Lps profile of ApoE and LDL-R KO rats

胆固醇

甘油三酯

小鼠

大鼠

小鼠

大鼠
Cardiomyopathy Hamsters of a Naturally occurring mutation in δ-sarcoglycan gene, the Bio 14.6 CM hamster


Type II DM Model inducible by dietary manipulations


Prion Infectable Rodents


Comparison of lipid metabolism and As among mammals

<table>
<thead>
<tr>
<th></th>
<th>Mice</th>
<th>Hamsters</th>
<th>Minipigs</th>
<th>Humans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Major Lps</td>
<td>HDL</td>
<td>LDL</td>
<td>LDL</td>
<td>LDL</td>
</tr>
<tr>
<td>CETP</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Apo B Editing</td>
<td>Intestine/Liver</td>
<td>Intestine</td>
<td>Intestine</td>
<td>Intestine</td>
</tr>
<tr>
<td>Apo B 48</td>
<td>CM/CM</td>
<td>Intestine</td>
<td>CM</td>
<td>CM</td>
</tr>
<tr>
<td>Liver LDL-R</td>
<td>High</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td>Extrahepatic Ch synthesis</td>
<td>50%</td>
<td>85%</td>
<td>?</td>
<td>90%</td>
</tr>
<tr>
<td>HFHC Diet</td>
<td>insensitive</td>
<td>sensitive</td>
<td>moderate</td>
<td>sensitive</td>
</tr>
<tr>
<td>HL</td>
<td>plasma</td>
<td>EC bound</td>
<td>EC bound</td>
<td>EC bound</td>
</tr>
<tr>
<td>As</td>
<td>resistance</td>
<td>Susceptible</td>
<td>Susceptible</td>
<td>Susceptible</td>
</tr>
<tr>
<td>CAD</td>
<td>difficult</td>
<td>?</td>
<td>?</td>
<td>major lesion</td>
</tr>
<tr>
<td>Stroke</td>
<td>difficult</td>
<td>?</td>
<td>?</td>
<td>major lesion</td>
</tr>
<tr>
<td>Genetic Bcknd</td>
<td>inbreed</td>
<td>non-inbreeder</td>
<td>non-inbreeder</td>
<td></td>
</tr>
<tr>
<td>Breeding/Husb</td>
<td>easy</td>
<td>easy</td>
<td>difficult</td>
<td>few</td>
</tr>
<tr>
<td>Genetic Models</td>
<td>numerous</td>
<td>none</td>
<td>few</td>
<td></td>
</tr>
</tbody>
</table>
Comparison of Hamsters and Mice

ORO staining of Liver

Plasma Lipoprotein profiling by FPLC

Table 1
Plasma lipids (mg/dl) in hamsters and mice infected with Ad-LCAT or Ad-AP

<table>
<thead>
<tr>
<th></th>
<th>TC (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>PL (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hamster</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ad-hAP (n = 5)</td>
<td>111.6 ± 5.1</td>
<td>73.0 ± 8.8</td>
<td>199.3 ± 4.0</td>
<td>53.9 ± 3.8</td>
</tr>
<tr>
<td>Ad-hLCAT (n = 5)</td>
<td>347.2 ± 46.1</td>
<td>47.7 ± 2.2</td>
<td>289.7 ± 18.8b</td>
<td>124.0 ± 9.2b</td>
</tr>
<tr>
<td><strong>Mouse</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ad-hAP (n = 5)</td>
<td>95.0 ± 1.4</td>
<td>69.6 ± 5.9</td>
<td>275.9 ± 5.6</td>
<td>60.4 ± 1.5</td>
</tr>
<tr>
<td>Ad-hLCAT (n = 5)</td>
<td>166.2 ± 13.8b</td>
<td>51.2 ± 7.7</td>
<td>286.0 ± 11.8</td>
<td>71.3 ± 5.7</td>
</tr>
</tbody>
</table>

a $P < 0.05$, as compared with the Ad-hAP-infected control group.
b $P < 0.01$, as compared with the Ad-hAP-infected control group.


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Hepatic VLDL Secretion

A. hamsters

B. mice

Towards Generation of Transgenic Hamsters

Problems in 2 cell blockage of fertilized eggs solved

Birth of hamster pups from Surrogate Mother
EGFP vector for Lenti viral mediated Transgenesis

pGC-FU EGFP vector and division of infected fertilized eggs

<table>
<thead>
<tr>
<th>transfer/DOB</th>
<th>egg Don.</th>
<th>egg No.</th>
<th>Egg Inj.</th>
<th>Egg Tfed</th>
<th>Sorrag M</th>
<th>Dam B</th>
<th>Pups</th>
<th>PCR+</th>
<th>Southern</th>
<th>Exprs</th>
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</thead>
<tbody>
<tr>
<td>12-12-29/13-01-13</td>
<td>4</td>
<td>160</td>
<td>155</td>
<td>155</td>
<td>5</td>
<td>1</td>
<td>6</td>
<td>1</td>
<td>0</td>
<td>0</td>
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<tr>
<td>13-01-05/13-01-20</td>
<td>3</td>
<td>140</td>
<td>130</td>
<td>120</td>
<td>4</td>
<td>1</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>1</td>
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<tr>
<td>13-01-19/13-02-03</td>
<td>2</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>3</td>
<td>2</td>
<td>9</td>
<td>6</td>
<td>2</td>
<td>0</td>
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<tr>
<td>13-04-20/13-05-05</td>
<td>2</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>3</td>
<td>2</td>
<td>13</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>
PCR genotyping of new born hamster pups

Southern Blot of genomic DNA digested by EcoR1
Birth of GFP transgenic hamster:
2013-2-10 (Year of Snake) 鼠小龙诞生！

## Comparison of breeding and Husbandry expenses of common mammalian Lab animals

<table>
<thead>
<tr>
<th></th>
<th>Mice</th>
<th>rats</th>
<th>hamsters</th>
<th>rabbits</th>
<th>minipigs</th>
<th>monkeys</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breeding cycle (D)</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>n/a</td>
<td>n/a</td>
<td>28</td>
</tr>
<tr>
<td>Pups/litter</td>
<td>8</td>
<td>8</td>
<td>12</td>
<td>12</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>Pregnancy (D)</td>
<td>20</td>
<td>20</td>
<td>17</td>
<td>30</td>
<td>114</td>
<td>160</td>
</tr>
<tr>
<td>Growth of pups (D)</td>
<td>28</td>
<td>30</td>
<td>28</td>
<td>45</td>
<td>90</td>
<td>240</td>
</tr>
<tr>
<td>Sex Maturation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F (D)</td>
<td>45</td>
<td>60</td>
<td>45</td>
<td>180</td>
<td>180</td>
<td>4.0 y</td>
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<tr>
<td>M (D)</td>
<td>60</td>
<td>75</td>
<td>60</td>
<td>210</td>
<td>210</td>
<td>3.5 y</td>
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<tr>
<td>Space/10 animals (M²)</td>
<td>0.1</td>
<td>0.3</td>
<td>0.2</td>
<td>5</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Cost/animal/day (Beijing, RMB)</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>5</td>
<td>20</td>
<td>50</td>
</tr>
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</table>
Tennis for Cardiovascular Health!