Genome editing: from modeling disease to novel therapeutics

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Disclosure:
CRISPR Therapeutics and Sana Biotechnology
How do we bridge the gap to understanding and treating metabolic disease?
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1p13 minor allele is associated with ↓ LDL-C, ↓ MI risk

<table>
<thead>
<tr>
<th>Chr</th>
<th>SNP (genes)</th>
<th>Combined P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1p13</td>
<td>rs599839 (??????)</td>
<td>8 x 10⁻¹⁶⁰</td>
</tr>
<tr>
<td>19p13</td>
<td>rs4420638 (APOE)</td>
<td>3 x 10⁻¹⁴⁰</td>
</tr>
<tr>
<td>19p13</td>
<td>rs6511720 (LDLR)</td>
<td>2 x 10⁻¹¹⁰</td>
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<tr>
<td>2p24</td>
<td>rs1367117 (APOB)</td>
<td>6 x 10⁻¹⁰⁹</td>
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<tr>
<td>2p21</td>
<td>rs6544713 (ABCG5/ABCG8)</td>
<td>4 x 10⁻⁴⁷</td>
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<tr>
<td>5q13</td>
<td>rs12916 (HMGCR)</td>
<td>1 x 10⁻⁴⁵</td>
</tr>
</tbody>
</table>

**rs599839**

mm: 16 mg/dL lower LDL-C

\[ P = 1 \times 10^{-14} \]

mm: 40% lower risk of coronary disease

![LDL-C levels graph]
1p13 Minor Allele is Associated with ↑ Gene Expression, Specific to Liver

Proposed model

SNP discovered to be associated with myocardial infarction in 2007 and LDL cholesterol levels in 2008

rs599839

SNP discovered by Musunuru et al. that may be causative variant

rs12740374

Risk G allele
G allele disrupts C/EBP binding site

CCTGAGCTGCT

Reduced transcription of SORT1 mRNA

De creased SORT1 mRNA

Increased hepatic VLDL secretion

More coronary artery disease

Protective T allele
T allele promotes binding of C/EBP

CCTGAGCTGCTCAGCA

Transcription of SORT1

Increased SORT1 mRNA

Decreased hepatic VLDL secretion

Less coronary artery disease

Linset-Nitschke et al., NEJM 2010
Contradictory conclusions from mouse studies of Sort1

Sort1 knockdown in mouse hepatocytes

- Control siRNA
- Sort1 siRNA

Histogram showing normalized level of apoB-100/total secreted protein with statistical significance $P=1 \times 10^{-4}$.

Sort1−/− mouse hepatocytes

- Sort1+/+
- Sort1−/

Bar graph showing relative intensity of apoB100 at different time points (1, 4, 12, 20 hr) with significance levels $P<0.01$.


Disease modeling: human induced pluripotent stem cells

- Patient
- Biopsy
- Reprogramming: hiPSCs
- Differentiation: disease-affected cell type
- Phenotyping

6 months $15,000
cell line?
Variability among different human pluripotent stem cell (hPSC) lines

- Differences in genetic background
- Differences in epigenetic state
- Differences in pluripotency/differentiation capacity
Disease modeling: genome editing

• no patient recruitment
• no need for quality control of pluripotency of iPSC clones
• allows for studies of multiple gene variants side-by-side
• more efficient (and cheaper)

hPSCs → genome editing → wild-type → mutant → differentiation → phenotypic comparisons
How do engineered nucleases create or repair mutations?
Genetic studies of \textit{SORT1}

- hPSCs
- hepatocytes
- adipocytes
- motor neurons
SORT1 has functions in multiple cell types

- Discovered by GWAS to be involved in hepatic lipoprotein processing
- Insulin-responsive glucose transport in adipocytes
- Mediates growth factor-induced neuronal apoptosis
SORT1−/− hepatocytes have increased secreted apoB

![Diagram showing wild-type HLCs vs. SORT1−/− HLCs with a bar graph comparing albumin levels in media for wild-type clones vs. SORT1−/− clones.](image)
SORT1−/− hepatocytes have increased secreted apoB
Reconstitution of \textit{SORT1} rescues phenotype

wild-type \hspace{0.5cm} \textit{SORT1}--/-

\begin{itemize}
  \item wild-type + GFP
  \item \textit{SORT1}--/- + GFP
  \item \textit{SORT1}--/- + \textit{SORT1}
\end{itemize}

\begin{itemize}
  \item \textalpha-\text{sortilin}
  \item \textalpha-\text{albumin}
  \item \textalpha-\text{\beta-actin}
\end{itemize}

HLC lysate Western blotting

\begin{itemize}
  \item \textalpha-\text{sortilin:}
    \begin{itemize}
      \item wild-type with GFP virus
      \item \textit{SORT1}--/- with GFP virus
      \item \textit{SORT1}--/- with \textit{SORT1} virus
    \end{itemize}
\end{itemize}

ELISA of media (1 clone each)

\begin{itemize}
  \item normalized level
  \item \textapoB/albumin ratio
\end{itemize}

\begin{itemize}
  \item \textit{SORT1}--/- with \textit{SORT1} virus: P=0.02
  \item \textit{SORT1}--/- with GFP virus: P=0.05
  \item wild-type with GFP virus: P=0.02
\end{itemize}
Sort1 in mouse adipocytes

Sortilin plays a role in the formation of Glut4 storage vesicles in 3T3-L1 adipocytes

SORT1−/− adipocytes have no insulin-stimulated glucose uptake

HUES 1 adipocytes

- wild-type + control virus − insulin
- wild-type + control virus + insulin
- SORT1−/− + control virus − insulin
- SORT1−/− + control virus + insulin
- SORT1−/− + SORT1 virus − insulin
- SORT1−/− + SORT1 virus + insulin

2 clones each

P=0.05

P=0.002

normalized level

glucose uptake/protein
Sortilin is essential for proNGF or proBDNF-induced neuronal cell death
**SORT1−/− MNs are resistant to proBDNF apoptosis**

HUES 9 motor neurons

- wild-type + GDNF + BDNF
- wild-type + GDNF + proBDNF
- *SORT1−/−* + GDNF + BDNF
- *SORT1−/−* + GDNF + proBDNF

2 clones each

Motor neuron % versus normalized level

- *P* = 0.04
Sort1-/- mouse cells vs. SORT1-/- human cells

- Knock down or knock out Sort1 in mouse hepatocytes show contradictory results in terms of apoB secretion
- Knock down Sort1 in 3T3-L1 adipocytes show decreased insulin stimulated glucose uptake
- Sortilin blockade enhances mouse motor neuron survival in the presence of proBDNF
- SOORT1-/- human hepatocytes show increased apoB secretion
- SORT1-/- human adipocytes show no insulin stimulated glucose uptake
- Sort1-/- human motor neurons show enhanced survival in the presence of proBDNF

Genetic Studies of Lipids and Heart Attack

Teslovich, Musunuru, et al.
Nature 2010; 466:707-13
100s of genes and loci identified by genetics studies

<table>
<thead>
<tr>
<th>LDL-C</th>
<th>HDL-C</th>
<th>Triglycerides</th>
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<tbody>
<tr>
<td>ABCG5/8</td>
<td>ABCA1</td>
<td>ACSS2</td>
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<tr>
<td>ABO</td>
<td>ABCA8</td>
<td>AFF1</td>
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<td>SPTY2D1</td>
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High throughput phenotypic screens for understanding loci identified via genetics

RVAS for LDL/TG or MI

CRISPR/Cas

Gene Knockout

Hepatocyte-like cell (HLC)

LDL uptake
Cellular TG
Cellular Free Cholesterol
Secreted APOB
Secreted TG
High-throughput phenotypic screening

Yu et al. Nature Genetics 2017
A1CF regulates hepatic and blood cholesterol and triglyceride levels

Yu et al. *Nature Genetics* 2017

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<thead>
<tr>
<th>Gene Name</th>
<th>p-value</th>
<th>Fold Change (WT vs KO)</th>
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<tbody>
<tr>
<td>APOB100</td>
<td>0.002544</td>
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<td>SREBF1</td>
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<td>MVD</td>
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In Vivo Gene Targeting
PCSK9 inhibition lowers LDL Cholesterol
CRISPR/Cas9 ablation of PCSK9 *In Vivo* Reduces Plasma Cholesterol

Qiurong Ding et al. Circulation Research. 2014;115:488-492
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