Genes – Association and Cause

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Disclosure / Conflict of Interest

• UBC has filed a patent application based on PCSK9 data. Keith Walley, Jim Russell, John Boyd are inventors.
• Walley, Russell, Boyd founded Cyon Therapeutics which licensed the UBC patent.
Approaches to infer causality

• Lots of gene association studies
  – Which ones count?

• Instrumental variables
  – Mendelian Randomization

• Mechanism of action
  – Inhibition, over-expression
Instrumental Variables → Cause

Unobserved alternate explanation (Confounder)

Environment Pollution

Smoking

Exposure

Cancer

Outcome
Instrumental Variables ➔ Cause

Unobserved alternate explanation (Confounder)

Tobacco tax

Instrument

Smoking

Environment Pollution

Cancer

Exposure

Outcome
Mendelian Randomization

Unobserved alternate explanation (Confounder)

Genotype → Protein

Instrument → Exposure

Environment

Protein → Phenotype

Outcome

Instrument

Environment

Protein

Phenotype

Genotype

Unobserved alternate explanation (Confounder)
Mendelian Randomization

Confounders

? 

IL-1ra 

Sepsis survival
Mendelian Randomization

IL1RN genotype ~ Sepsis survival

![Graph showing survival analysis](image)

- **CC**
- **CT**
- **TT**

*Graph legend: p = 0.028 adjusted for APACHE II score and genetic ancestry*

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Mendelian Randomization

Mechanism $\Rightarrow$ Cause
PCSK9 Loss-of-Function (LOF)

![Graph A](image1)
VASST
$p=0.0054$

![Graph B](image2)
SPH
$p=0.022$
Low LDL drives ↑PCSK9?
Elevated PCSK9 is Bad

PCSK9 Inhibitors increase lipid clearance by LDL Receptors

- Sanofi/Regeneron: alirocumab (Praluent)
- Amgen: evolocumab (Repatha)
Pathogen lipid clearance

Could PCSK9 inhibition increase pathogen lipid clearance?
**Pcsk9 knockout mice**

↑ LPS clearance, ↓ inflammation

Walley KR et al. Science Translational Medicine. 6(258):258ra143
Pcsk9 knockout mice ↓ physiologic response
PCSK9 antibody: ↑ CLP survival

p=0.034

TNFα (p=0.027), IL-6 (p=0.051), IL-10 (p=0.068), JE (p=0.0085) and MIP-2 (p=0.040)
Replication and extension

Dwivedi DJ, et al. Shock. 2016 Jul 11
LPS uptake by hepatocytes

PCSK9 inhibition increases hepatic LDL receptor numbers and may increase pathogen lipid clearance.
Approaches to infer causality

• Lots of gene association studies
  – Which ones count?

• Instrumental variables
  – Mendelian Randomization

• Mechanism of action
  – Inhibition, over-expression
<table>
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<tr>
<th>UBC co-investigators</th>
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<tbody>
<tr>
<td>John Boyd</td>
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Keith.Walley@hli.ubc.ca
Human PCSK9 genetic variants

Autocleavage site

Signal peptide
Prodomain
Subtilisin-like catalytic domain
Carboxy terminal domain

NH₂

1 31 147 425 692

R46L
A53V
V474I
G670E

1.6% 13% 18% 3.6%

MAF¹

LDL

↓20%²  ↓20%³  ↓10%³  ↑50%⁴

LOF  LOF  LOF  GOF

PCSK9 Loss-of-Function (LOF)

↑ Human septic shock survival

A

VASST

p = 0.0054

Survival

Days

Number at Risk:

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B

SPH

p = 0.022

Survival

Days

Number at Risk:

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C

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<td>Any LOF</td>
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log₂(Hazard Ratio)
“Normal subjects”, 1 ng/kg LPS i.v., IL-6 peak at 4 hours
LOF: 114 pg/mL    No LOF: 147 pg/mL    p=0.019

Walley KR et al.  Science Translational Medicine. 6(258):258ra143
Replication and extension

Dwivedi DJ, et al. Shock. 2016 Jul 11
Bacterial load

Figure 2. Influence of PCSK9 on the cellular uptake of lipoteichoic acid. HepG2 hepatocytes were pre-treated with 10 μg/mL of either recombinant wildtype or 3 different PCSK9 LOF variants, rs11583680, rs11591147 and rs562556, 4 hours before the treatment of the fluorescently labeled LTA (BODIPY-LTA, 2 μg/mL). A) Cells untreated with BODIPY-FL-LTA were imaged as a negative control. Cells treated with BODIPY-FL-LTA along B) without PCSK9 or with C) wildtype, D) rs11583680, E) rs11591147 and F) rs562556 PCSK9. Nuclei were stained with Hoescht 33342 shown in blue and BODIPY-FL-LTA fluorescence from is shown in green. Scale bars represent 10 µm.
Gram positive septic shock

Control – critically ill, no sepsis

A

B

Days

Survival

p=0.008

p=0.046

Days

PCSK9 LOF (n=79)

PCSK9 wildtype (n=51)

PCSK9 rs644000, n=225

Gram positive septic shock

PCSK9 LOF (n=75)

PCSK9 wildtype (n=95)
Was it via the LDL receptor?

Conclusions

• PCSK9 inhibition increases LDL receptor density on hepatocytes

• This increases clearance of pathogen lipids (e.g. endotoxin) from LDL cholesterol particles

• Genetic PCSK9 Loss-Of-Function suggests that PCSK9 inhibition may improve outcomes in human sepsis
Mechanism ➔ Cause

Late modulation of host response doesn’t work (e.g. rhAPC, anti-TNF)
Deans KJ et al. J Trauma, 58:867-874, 2005

Clearing the pathogen works (e.g. antibiotics)
Kumar A et al Crit Care Med, 34:1589-1596, 2006

Delayed antibiotic administration after onset of hypotension increases mortality by 6% per hour.
Are Plasma Lipids Involved In Sepsis?

Low LDL/HDL levels in sepsis

Low LDL drives ↑PCSK9