“PCSK9: from Discovery to Therapeutic Applications”
I have no disclosures or conflicts of interest to report.
Learning Objectives:

At the end of this presentation, participants will be able to:

• understand if the major function of PCSK9 requires protease activity
• understand how PCSK9 lead to LDLR degradation
• understand the current strategy for PCSK9 inhibition and what the CVD outcomes are
100 g cholesterol / 70 kg body weight

Cellular membranes:
control substances that enter or leave cells

Hormones:
testosterone & estradiol

Vitamines:
vitamin D

Biliary acids:
cholic acid

Cholesterol: 75% synthesized by the human body
25% obtained from ingested food
Xanthoma/Xanthelasma are associated with hyperlipidemia

Franz Hals (1583-1666)
Noble Haarlem woman

Leonardo da Vinci (1452-1519)
Mona Lisa (died at 37 years)
Familial Hypercholesterolemia

FH is essentially due to mutations in the LDLR gene

C. MULLER 1939
POMERANTZ et al. 1951
BROWN & GOLDSTEIN 1974...
EPSTEIN et al. 1959
FREDERICKSON et al. 1967
K. B. TUTTLE 1950
PIPER & ORRILD 1956
P. ADH 1964
EPSTEIN et al. 1959

AKIRA ENDO
Compactin, 1982
INNERARITY 1987

LDLR > 97%
APOB < 3%

ABIFADEL et al. 2003

Lovostatin
(Commercialization Merck) 1986

Statins save lives 1992

BROWN & GOLDSTEIN 1974...

LDLR
APOB
PCSK9
LDL-cholesterol clearance

LDLs accumulate in blood!

Seidah NG et al. PNAS (2003) 100, 928

PCSK9 (NARC-1) is the 9th and last member of the PC family


PCSK9 is the third locus linked to ADH
The LDL-cholesterol regulating PC: PCSK9
Proprotein Convertases of the Subtilisin/Kexin type (PCSK)

Phylogenetic analysis of the nine PCs including PCSK9

Seidah NG. et al. Pharmacological Reviews (2017) 69, 33
Autocatalytic processing of proPCSK9 and section of PCSK9-prosegment complex

HEK293 cells
4h pulse
^{35}S-Met/Cys
C-terminal V5 tag

Autocatalytic cleavage
Inactivating furin cleavage

Seidah NG et al. PNAS (2003) 100, 928
The prosegment is
– essential (cannot be deleted)
– an intramolecular chaperone
– an inhibitor

Prosegment neutralization requires
– a second cleavage (except for PC4; PC7)
– acidic compartments
– partners, e.g. 7B2 for PC2

PCSK9 is a class of its own

ER

Golgi, cell surface, SG

Final destination

inactive

PC4 & PC7

active

pro

catalytic

PCSK9

inactive

active

inhibited

Furin

inactive PCSK9
Cellular and tissue expression of PCSK9

Seidah NG et al. PNAS (2003) 100, 928
**PCSK9 biological functions?**

**Clues**
1. Rich in liver & intestine
2. Chrom. 1p32

“The best way to have a really good idea is to have lots of ideas”

Linus Pauling
Familial Hypercholesterolemia

Physical map at 1p32 in 2002

≈ 3 Mb

M. Varret

≈ 3 Mb

M. Abi Fadel

HCHOLA3 – 1.6 cM
In families HC92 and HC2, PCSK9 exhibits a S127R mutation in exon 2.
In family HC60, PCSK9 exhibits a F216L mutation in exon 4

Heterozygote (C/T)

Homozygote (T/T)

T→C at position 890: Phe216Leu

Human wt sequence
ccc gag gag gac ggg acc cgct tc cac aga cag gcc agc aag tgt

Mus musculus
PEEEDGTRFHQRASKC

Rattus norvegicus
PEEEDGTRFHQRASKC

Patient T890C-F216L
ccc gag gag gac ggg acc cgct tc cac aga cag gcc agc aag tgt
**Human PCSK9 mutations**

**Cause Autosomal Dominant Hypercholesterolemia (ADH)**
or **Hypocholesterolemia**

**M. Abifadel**

**gain of function**

hypercholesterolemia

**C. Boileau**


**E32K**

**D35Y**

**L108R**

**R104C/V114A**

(16 mg/dL; 0.4 mM)

**S127R**

**R104C/V114A**

**N157K**

**Q152H**

**D199**

**H239**

**R215H**

**F216L**

**R218S**

**G236S**

**R237W**

**D374Y**

**R237W**

**R237W**

**C679X**

**R469W; R496W**

**R46L**

**G236S**

**R434W**

**C679X**

(XX Hom: 0.4 mM)

Zimbabwe

**R104C/V114A**

(16 mg/dL; 0.4 mM)

**R46L**

**E32K**

**D35Y**

**L108R**

**R104C/V114A**

**S127R**

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**R215H**

**F216L**

**R218S**

**G236S**

**R237W**

**D374Y**

**R237W**

**C679X**

**R469W; R496W**

**R46L**

**G236S**

**R434W**

**C679X**

(XX Hom: 0.4 mM)

Zimbabwe

**loss of function**

hypocholesterolemia

Cohen et al. Nature Gen. 2005
In a non-enzymatic fashion, PCSK9 leads to LDLR degradation in lysosomes

DiI-LDL labeling of the cell surface
PCSK9 and EGFP coexpression

PCSK9 target proteins:
LDLR, VLDLR, LRP1, LRP8 (apoER2) & CD36

NH₄Cl

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LDLR


PCSK9 binds the LDLR and triggers its degradation in endosomes/lysosomes.
PCSK9 is the third gene implicated in hypercholesterolemia

- 1/500
- high LDL levels
- xanthomas
- early cardiovascular disease

FH1

FH2

FH3...

chromosome 19p13 2p23-24 1p32

gene LDLR APOB PCSK9

~67% LDLR

~14% APOB

~17% unknown genes

~2% PCSK9

(Catherine Boileau’s lab; Necker; Paris)

Seidah et al. *PNAS* 2003

Natural PCSK9 mutations

GOF
- p.E32K
- p.D35Y
- p.E54A
- p.L108R
- p.S127R
- p.D129N
- p.D129G
- p.V41
- c.61_63delCTG
- c.61_63triCTG

LOF
- p.R215H
- p.F216L
- p.R218S
- p.R237W
- p.R357H
- p.D374Y
- p.D374H
- p.H417Q

- p.N425S
- p.R469W
- p.E482G
- p.A514T
- p.F515L
- p.A522T
- p.H553R
- p.V624M

Signal peptide
- p.R46L
- p.A68fsL82X
- p.R93C
- p.R97del
- p.R104C
- p.G106R
- p.V114A
- p.Y142X
- p.Q152H
- c.61_63dupCTG

Pro-domain
- p.N157K
- p.G236S
- p.R237W
- p.L253F
- p.N354I
- p.H391N

Catalytic domain
- p.W428X
- p.R434W
- p.A443T
- p.S462P
- p.Q554E
- p.Q516L
- p.S668R
- p.C679X

Dron JS & Hegele RA. Curr Opin Lipidol (2017) 28, 161
Haplotyp e structure of PCSK9 in modern and extinct human species

Seidah NG et al. Pharmacological Reviews (2017) 69, 33
550,000 - 765,000 years ago
Neanderthal
50,000 y

380,000 - 473,000 years ago
Denisova
LOF; H449L
41,000 y
« Pinkie »
The PCSK9/LDLR paradox

Statins upregulate PCSK9 expression

HepG2 cells

PCSK9 mRNA levels

48h-atorvastatin (mM)

Cholesterol downregulates PCSK9


PCSK9 & LDLR are co-regulated by cholesterol

Attie A & Seidah NG. *Cell Metab* (2005) 1, 290

Maxwell KN et al., *JLR* (2003) 44, 2109
Rosuvastatin increased plasma concentration of PCSK9 in proportion to the magnitude of LDLc reduction.
Ser-phosphorylation of PCSK9 enhances its function on LDLR

Ben Djoudi Ouadda A. et al. ATVB (2019) (in revision)
PCSK9 animal models
**PCSK9 conditional inactivation (Cre/lox)**

Exon 1 flanked with **loxP** sites:

1. $\text{Pcsk}^\text{flox}$
2. $\text{Pcsk}^\Delta 1$

PCSK9 mRNA

Exon 1 deleted in a Cre-dependent manner → no mRNA, no protein

**PCSK9 KO mice:**
- breeding with CMV-cre mice expressing Cre ubiquitously
  - no PCSK9

**Hepatocyte-specific KO mice:**
- breeding with Tg(Albumin-cre) mice expressing Cre only in hepatocytes
  - no liver PCSK9
PCSK9 inactivation leads to severe hypocholesterolemia

- ~40% drop in TC
- ~80% drop in LDL-C

LDLR immuno
- LDLR accumulation in the liver
- LDLR accumulation at the hepatocyte cell surface

Hepatocyte-specific \textit{Pcsk9} inactivation (hepKO)

Liver-specific PCSK9 KO:
no circulating PCSK9 (also by ELISA)
→ exclusively secreted by hepatocytes

The absence of PCSK9 unmasks an estrogen-dependent subcellular distribution of the LDLR in mouse liver.
Clinical applications of PCSK9 inhibition
Strategies to target PCSK9

1. Gene editing (CRISPR-Cas9)
2. siRNA
3. PF-06446846
4. Adnectins
5. ABD-fused Anticalin
6. mAbs against CHRD
7. mAbs
8. Vaccine
9. sdAbs

DNA → transcription → PCSK9 mRNA → mRNA degradation → translation → Endoplasmic reticulum

LDL → LDLR → LDLR recycling → Endosome → Lysosome

“Px” → LDLR degradation
Humanized PCSK9 monoclonal antibodies: Repatha (140 mg SC) versus Praluent (75 mg SC)

mAb of the human IgG2 subclass can form covalent dimers in vivo (ideal), whereas IgG1 are monomers.
Benefit of continuing aggressive lipid-lowering therapy to prevent recurrent cardiovascular events

FOURIER: Landmark analysis of fatal or nonfatal MI or stroke

Year 1: RRR 19%  >Year 1: RRR 33%

Longer duration of treatment and follow-up suggests larger risk reduction

Main Secondary Endpoints: Any Cardiovascular Event and Death, Nonfatal MI, or Nonfatal Ischemic Stroke

Composite death from:
- CAD,
- non fatal MI,
- non fatal ischemic stroke,
- or unstable angina

Alirocumab and Cardiovascular Outcomes After Acute Coronary Syndrome
After acute myocardial infarction (MI), survival is higher in PCSK9 KO mice than PCSK9 WT ones

MI is induced by ligation of the left coronary artery


Goo Taeg Oh et al. (In preparation)
Inclisiran (siRNA) efficacy (two dose-starting regimen): robust, sustained LDL-C reductions

Mean percent change (±95% CI) vs days from first injection.

- **Placebo**: No significant change.
- **100 mg**: Slight decrease over time.
- **200 mg**: Moderate decrease over time.
- **300 mg**: Significant decrease over time, especially within the first 90 days.

**Key Points**:
- **9 months time adjusted mean 50% reduction**
- **300 mg x2**: 55.5% reduction vs placebo, P-value <0.0001
- **Mean percent change**

In addition to lowering LDL-C, evolocumab reduced other atherogenic lipids and modestly increased HDL-C in patients with HeFH.

Placebo-adjusted treatment difference from baseline between weeks 10 and 12 (%)

- HDL-C*: Up to 9%
- ApoA: Up to –7%
- Triglycerides*: Up to –22%
- Lp(a)*: Up to –31%
- ApoB*: Up to –55%
- non-HDL-C*: Up to –60%
- LDL-C*: Up to –66%

*P<0.001 when compared with placebo
Under supra-physiological levels of the LDLR, as with PCSK9 mAbs, the LDLR is the receptor of Lp(a).
KO versus WT male mice (n=9) – 25 µg/mouse

- 3 to 4 months of age
- 3hrs fasting
- Injection 100 µL at 0.25 mg/mL (25 µg/mouse)
- Bleeds at t = 5, 15, 30, 120 and 360 min (re-feeding after the t = 2 h bleeding)
- Lp(a) ELISA (Mercodia)

Initial velocity of clearance is 2.5-fold faster in KO males (slope between the points 5 and 15 min: 1.56 versus 0.64 U/min)

Lp(a) half life is shorter in PCSK9 KO mice compared to WT mice

Roubstova A. et al. (in preparation)
PCSK9 binds the LDLR and triggers its degradation.


Mice solely expressing human PCSK9

Weider E. et al. JBC (2016) 291, 16659
sdAbs against human the CHRD domain of PCSK9 inhibits its activity.

Could the sdAb P1.40 compete with « protein X »?

Compact structure of the complex of CHRD & sdAb P1.40

Carole Fruchart-Gaillard & Vincent Dive (collab., France)
Autosomal dominant hypercholesterolaemia and the PCSK9 adventure
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Objective 1
• Is PCSK9 acting as a protease and why?

Objective 2
• How does PCSK9 lead to LDLR degradation?

Objective 3
• What is the current strategy for PCSK9 inhibition and what are the CVD outcomes?
merci / thank you!