PCSK9 :
Un exemple de validation génétique d'une nouvelle cible thérapeutique

Pr. Catherine Boileau

LVTS Laboratory for Vascular Translational Science — Inserm UMR 1148 — Université Paris Diderot — Assistance Publique Hôpitaux de Paris — Hôpitaux Universitaires Paris Nord Val de Seine Bichat - Claude Bernard

Pr. Catherine Boileau

Disclosures

Consulting and speaking honoraria:

Sanofi/Regeneron and Amgen

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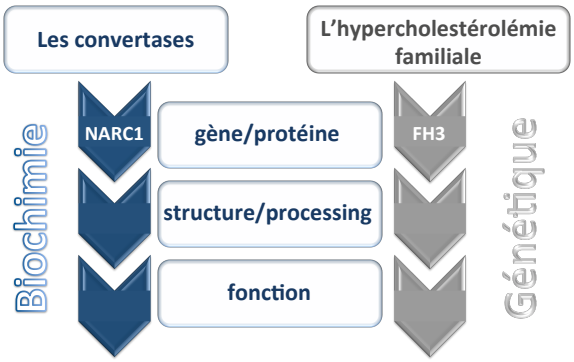
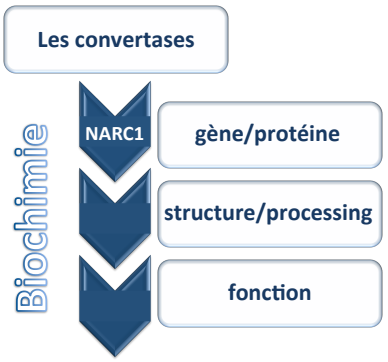


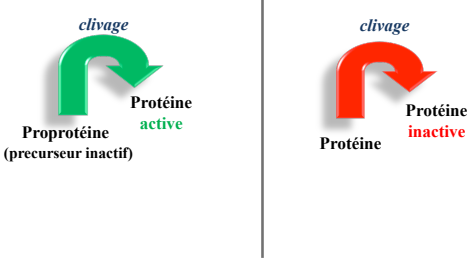
Seidah et al., 2003 *Abifadel et al., 2003*



Seidah et al., 2003

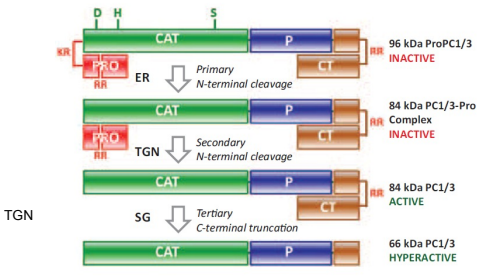
- La famille des convertases

Fonctions biologiques



- La famille des convertases

Processing et sécrétion



TGN: trans-Golgi network
SG: secretory granules

Chrétien et al., 2016

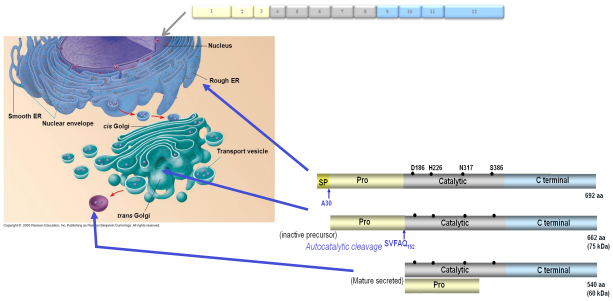
La famille des proprotéines convertases

Proprotein convertase	Typical substrates	Comments
*(R/K)X$_n$(R/K)↓**		
PC1	• PC1-specific: GHRH, ACTH, GLP1, GLP2	• Often collaborates with PC2 (for example, for insulin, TRH and MCH production)
PC2	• PC2-specific: β-endorphin, glucagon and α-MSH	• Often collaborates with PC1
Furin	• Growth factors (TGFβ) • Receptors (insulin receptor) • Adhesion molecules (α5 integrin and RGMA) • Metalloproteinases (MMP14) • Proton pump V-ATPase accessory protein Ac45 subunit • Viral glycoproteins (HIV gp160) • Bacterial toxins (anthrax pa83)	• Strong *in vitro* and *ex vivo* redundancy with PC5 and PACE4
PC4	• IGF2 and PACAP	• Cleaves substrates in gonads and placenta
PC5	• Growth factors (GDF11) • Receptors (PTPRM) • Adhesion molecules (neuronal L1CAM, α4 integrin)	• Strong *in vitro* and *ex vivo* redundancy with PC5 and PACE4
PACE4	• Growth factors (Nodal and Lefty) • Metalloproteinases (ADAM-TS4) • Viral glycoproteins (HIV protein Vpr)	• Strong *in vitro* and *ex vivo* redundancy with furin and PCSK5
PC7	• Receptors (transferrin receptor 1)	• Partial redundancy with furin, PC5 and PACE4
RX(L/I/V)X↓		
SKI-1 (also known as S1P)	• Transcription factors (SREBPs, ATF6 and CREBs) • GlcNAc-1-phosphotransferase, RGMA • Viral glycoproteins (Lassa virus glycoprotein and CCHF Gn)	• Cleaves substrates in *cis*- and *medial*-Golgi

NARC1 – la neuvième convertase

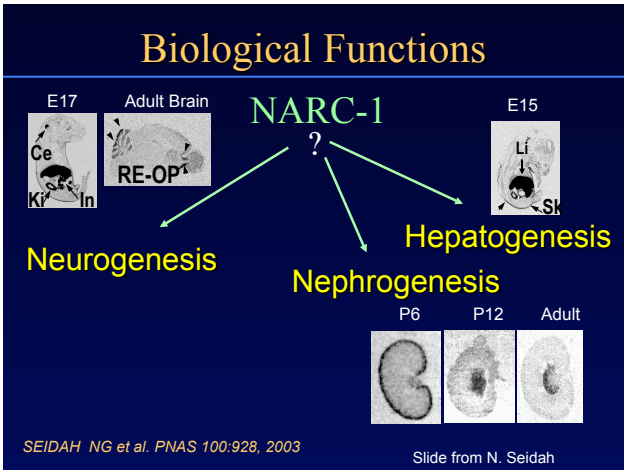
Neural Apoptosis Regulated Convertase 1

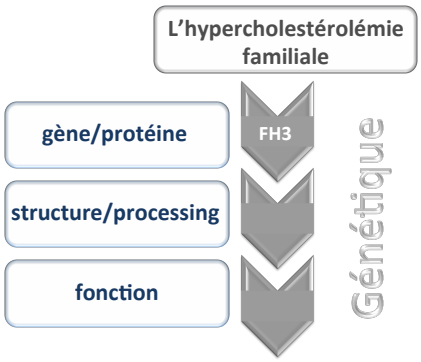
25 kb sur le chromosome 1p32



Seidah et al., 2003

Biological Functions

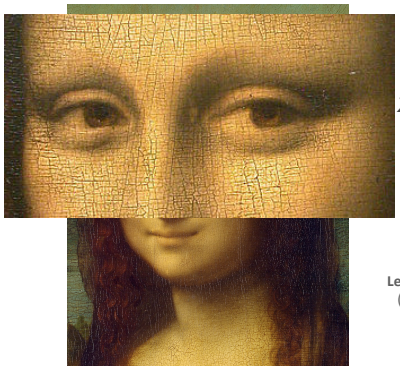




Abifadel et al., 2003

HF (Hypercholestérolemie Familiale)

.... une vieille maladie



Xanthelasmas

Leonard de Vinci
(1452 – 1519)

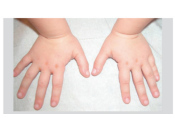
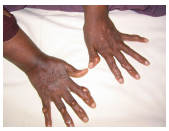
HF (Hypercholestérolemie Familiale)

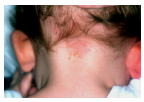
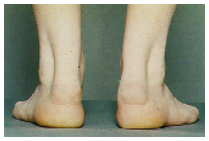
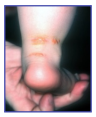
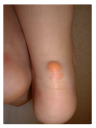
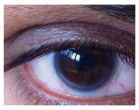
.... une vieille maladie

Frans Hals
(1582 – 1666)

Xanthomes tendineux

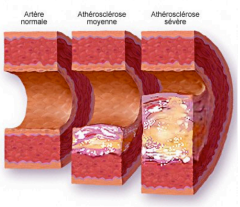
HF (Hypercholestérolemie Familiale)

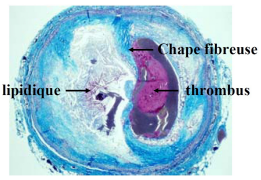



xanthelasma





arcus corneae

Cutaneous and tendineous xanthomas

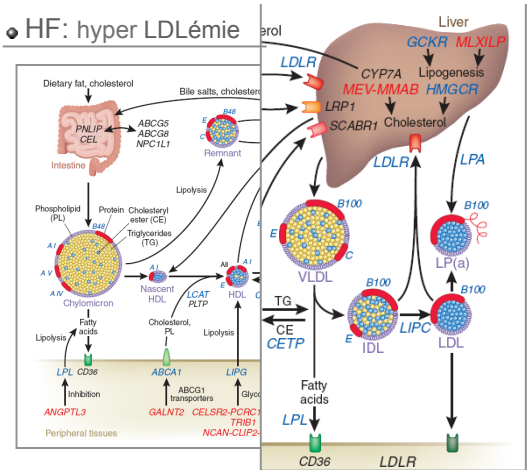
HF (Hypercholestérolemie Familiale)



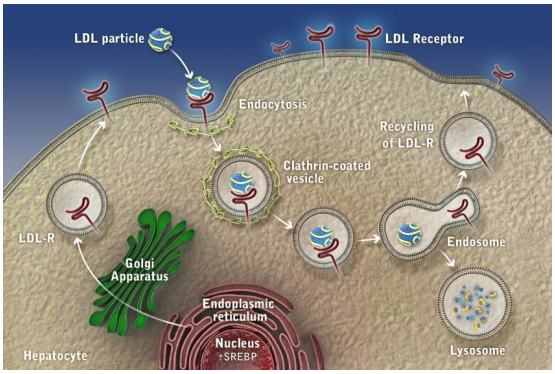


Mean onset of cardiovascular disease
40-45 yo in men
50-55 yo in women

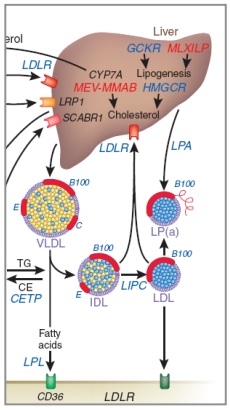
HF: hyper LDLémie

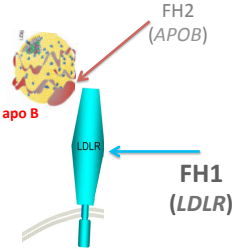


HF (Hypercholestérolemie Familiale) ….hyper LDLémie



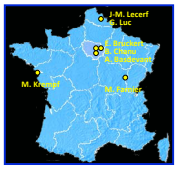
HF : un paradigme moléculaire



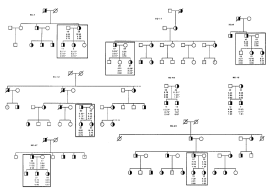


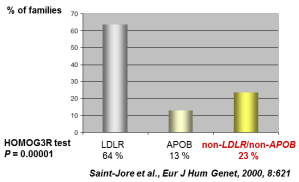
Adapted from *Seidah et al. Circulation Research, 2014*

FH: The paradigm rupture



- A nationwide research network
- Recruitment of multiplex FH families
- Molecular testing
- Maximum likelihood analyses

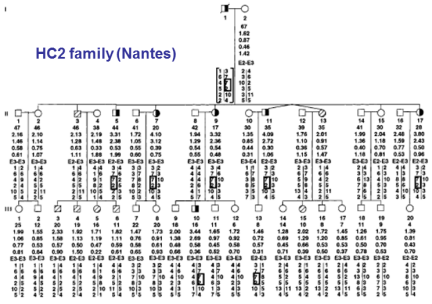




Saint-Jore et al., Eur J Hum Genet, 2000, 8:621

HF: localisation d'un nouveau gène

« Le gène FH3 est localisé en 1p34.1-p32 »



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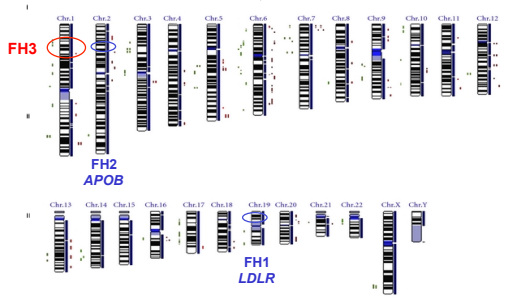
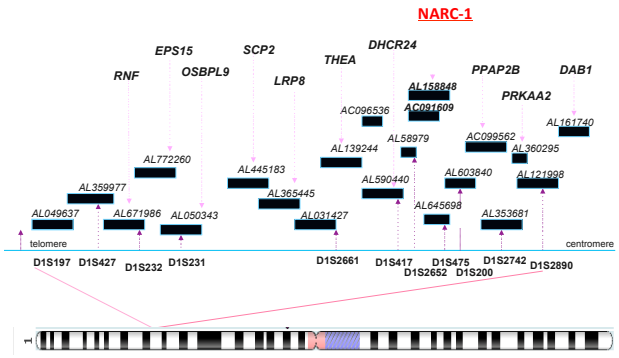


Varret et al., Am J Hum Genet, 1999, 64:1378

L'étude génomique

Carte des gènes exprimés en 1p32 en 2003



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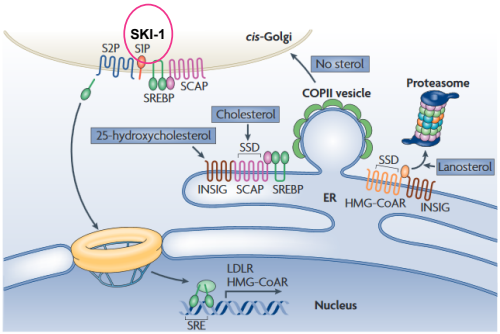
SREBP2 et régulation du métabolisme du cholestérol



SREBP
(Sterol regulatory element binding protein)

Ikonen, Nat Rev Mol Cell, 2008

L'étude génomique polymorphismes dans NARC-1

Exon	Nucleotidic Variation	Position	Amino acid variation	Number of individuals tested	Frequency
1	C → T	- 64 from ATG	5' UTR	113	0.119
	Leucine stretch 31	287_290insCTG	15_16insL (+ L)	113	0.168
	G → T	381	R46L ←	113	0.022
	C → T	385	S47S	113	0.016
	C → T 6	402	A53V	113	0.124
2	T → C	- 161	Intronic	100	0.040
3	G → A	- 201	Intronic	25	0.016
4	C → T	- 90	Intronic	100	0.548
	G → C	- 68	Intronic	100	0.547
	G → A	- 11	Intronic	100	0.063
	G → A	+ 9	Intronic	100	0.052
	C → A	+ 76	Intronic	100	0.076
	A → G 6	+ 82	Intronic	100	0.382
5	G → A	- 35	Intronic	23	0.020
	C → T	- 7	Intronic	23	0.280
	A → G	+ 3	Intronic	23	0.240
	A → C 8	+ 64	Intronic	23	0.170
8	T → C	+ 102	Intronic	20	0.175
9	T → C	- 56	Intronic	113	0.137
	G → A	1624	V460V	113	0.128
	A → G 4	1664	I474V ←	113	0.141
10	C → T	+ 63	Intronic	24	0.040
	A → G	+ 64	Intronic	24	0.146
11	A → G	+ 94	Intronic	20	0.030
12	A → G	2253	E670G	79	0.082

Association entre LDL-cholesterol

et I474V *(Shioji et al., 2004)*

et R46L *(Cohen et al., 2006)*

Abifadel et al., 2003

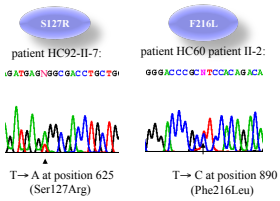
NARC-1 est le gène FH3

Les deux premières mutations GOF* dans l'HF

Mutations in *PCSK9* cause autosomal dominant hypercholesterolemia

Marianne Abifadel[1,2], Mathilde Varret[1], Jean-Pierre Rabès[1,3], Delphine Allard[1], Khadija Ouguerram[4], Martine Devillers[1], Corinne Cruaud[5], Suzanne Benjannet[6], Louise Wickham[6], Danièle Erlich[1], Aurélie Derré[1], Ludovic Villéger[1], Michel Farnier[7], Isabel Beucler[8], Eric Bruckert[9], Jean Chambaz[10], Bernard Chanu[11], Jean-Michel Lecerf[12], Gerald Luc[12], Philippe Moulin[13], Jean Weissenbach[5], Annick Prat[6], Michel Krempf[4], Claudine Junien[1,3], Nabil G Seidah[6] & Catherine Boileau[1,3]

VOLUME 34 | NUMBER 2 | JUNE 2003 NATURE GENETICS



GOF = Gain Of Function*

NARC-1 devient PCSK9

Human Genome Organisation

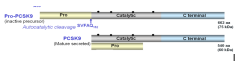
PCSK9

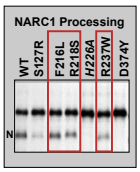
Approved Symbol	PCSK9
Approved Name	proprotein convertase subtilisin/kexin type 9
HGNC ID	HGNC:20001
Previous Symbols & Names	HCHOLA3, "hypercholesterolemia, autosomal dominant 3"
Synonyms	FH3, NARC-1
Locus Type	gene with protein product
Chromosomal Location	1p34.1-p32

Abifadel et al., 2003

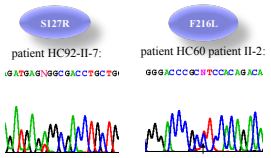
PCSK9 est le produit du FH3

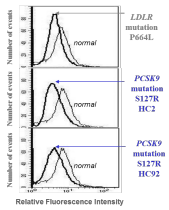
Les deux premières mutations GOF* dans l'HF





Effets paradoxaux sur le clivage autocatalytique

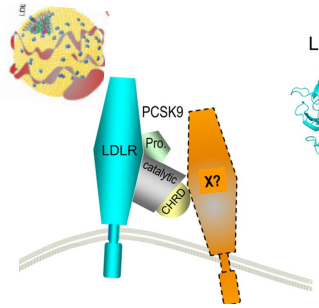


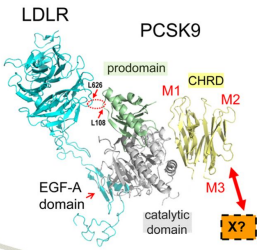


Diminution du nombre de récepteurs LDL chez les sujets FH3

Benjannet et al., 2004

Interaction LDL récepteur et apo B et PCSK9

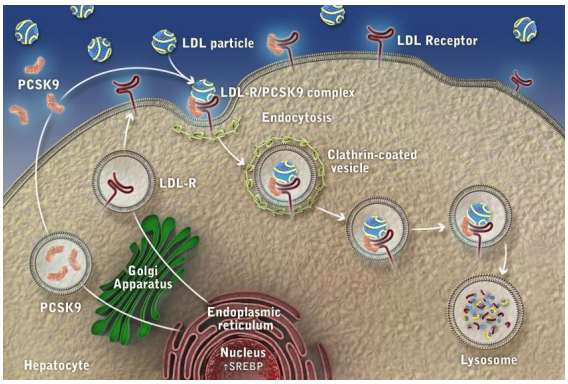




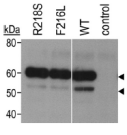
From *Seidah et al. Circulation Research, 2014*

Crystal structure of *PCSK9* determined by:
Piper et al. *Structure 2007*
Hampton et al. *PNAS 2007*
Kwon et al., *PNAS, 2008*

PCSK9 et régulation des taux de LDLR

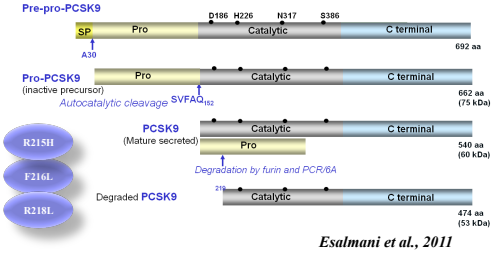


Mutations GOF dans in PCSK9 : résistance à la furine

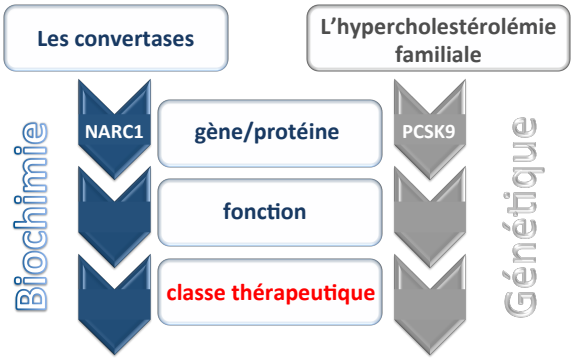


In Vivo Evidence That Furin from Hepatocytes Inactivates PCSK9

Rachid Essalmani[1], Delia Susan-Resiga[1], Ann Chamberland[1], Marianne Abifadel[2], John W. Creemers[3], Catherine Boileau[2], Nabil G. Seidah[1], and Annik Prat[1]

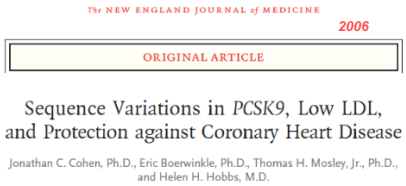


Esalmani et al., 2011

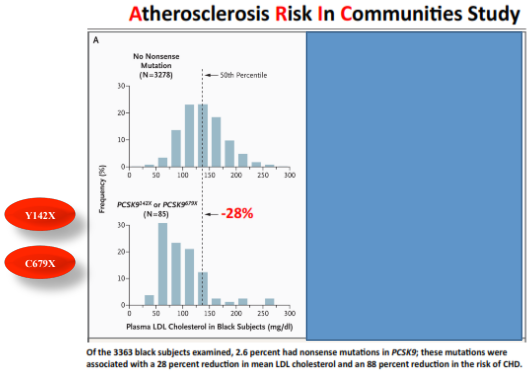


Seidah et al., 2003 *Abifadel et al., 2003*

Inhibition de PCSK9 : la preuve de concept



Mutations **perte** de fonction (LOF*) dans PCSK9



LOF = Loss Of Function*

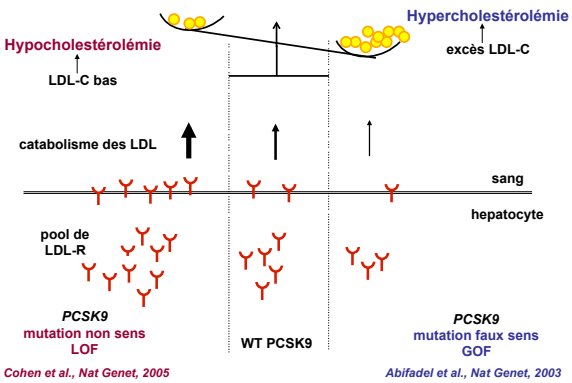
LOF* mutations in PCSK9

- Subjects with loss-of-function mutations in PCSK9 or total lack of PCSK9
  - Have naturally low levels of LDL-C and reduced Coronary Heart Disease (→ efficacy)
  - Are generally healthy with no other apparent metabolic abnormalities (→ safety)

	PCSK9 Mutation	LDL-C Reduction	CHD Reduction	Population
Benn et al. *JACC.* 2010	R46L	13%	30%	CCHS N=10,032 CGPS N=26,013 CIHDS N=9654 (Denmark)
Cohen et al. *NEJM.* 2006	R46L	15%	47%	ARIC Study (US) (Black patients N=3363; White patients N=9524)
	Y142X Or C679X	28%	88%	

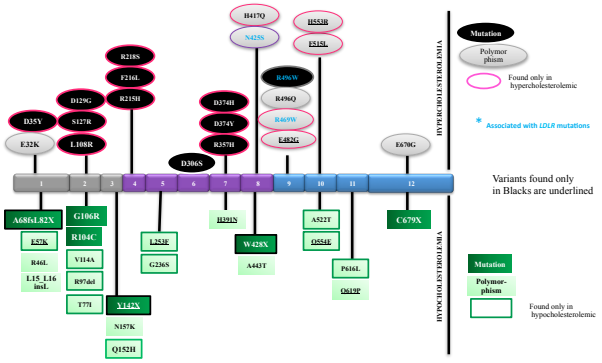
ARIC=Atherosclerosis Risk in the Community; CCHS=Copenhagen City Heart Study; CGPS=Copenhagen General Population Study; CIHDS=Copenhagen Ischemic Heart Disease Study; LDL-C=low-density lipoprotein cholesterol.
Cohen JC et al. *N Engl J Med*. 2006;354:1264-1272; Benn M et al. *J Am Coll Cardiol*. 2010; 55:2833-2842;
Catapano AL and Papadopoulos N. *Atherosclerosis*. 2013;228:18-28

Le double jeu de PCSK9



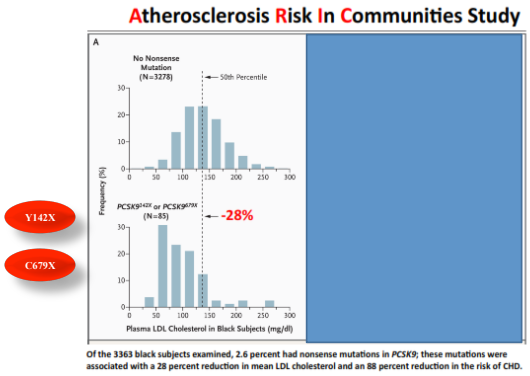
Cohen et al., Nat Genet, 2005 *Abifadel et al., Nat Genet, 2003*

The current molecular picture of *PCSK9*



Abifadel et al. 2015

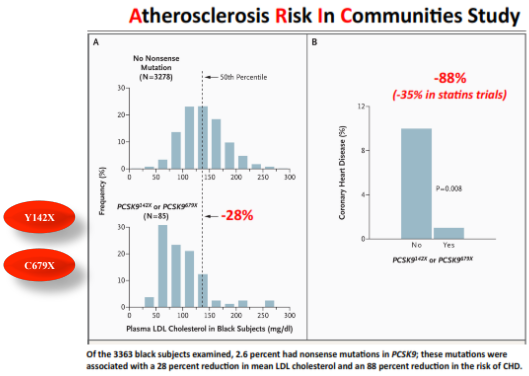
Mutations **perte** de fonction (LOF*) dans PCSK9



Of the 3363 black subjects examined, 2.6 percent had nonsense mutations in *PCSK9*; these mutations were associated with a 28 percent reduction in mean LDL cholesterol and an 88 percent reduction in the risk of CHD.

LOF = Loss Of Function*

Inhibition de PCSK9 : efficacité



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Cohen JC et al. N Engl J Med. 2006;354:1264-1272

Inhibition de PCSK9 : tolérance/sécurité

Homo et Hétérozygotes composites avec deux mutations LOF dans *PCSK9*

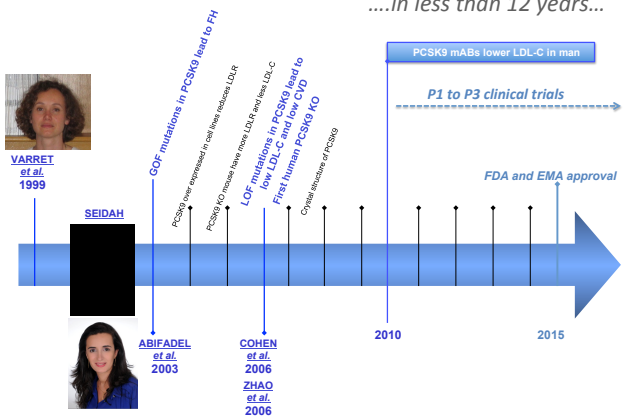
- 32 ans
- femme noire américaine
- **Pas de PCSK9 circulant,**
- **LDL-C : 0,14 g/L**
- normotensive
- fertile (2 enfants)
- fonctions hépatique et rénale normales

- femme africaine du Zimbawe
- **LDL-C : 0,15 g/L**

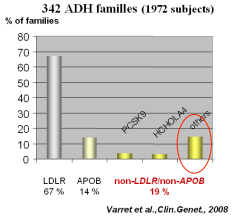
Zhao et al., 2006
Cariou et al., 2009

From genomics to bedside

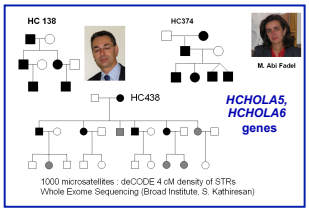
....in less than 12 years...

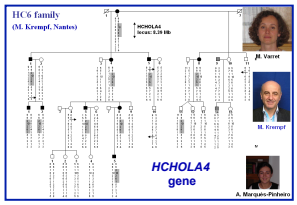


.....et la suite !



Varret et al.,Clin.Genet., 2008



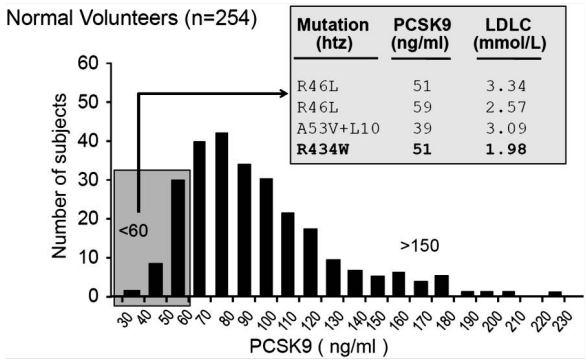


UMR 1148

FRANCE GÉNOMIQUE

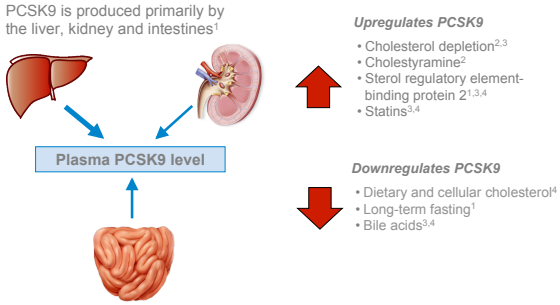
FONDATION maladies rares

Fondation Leducq



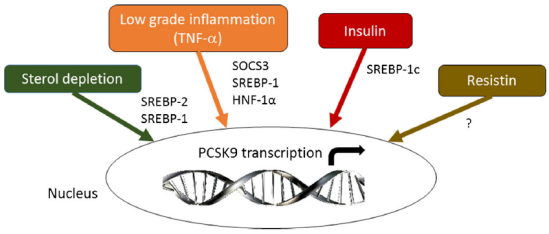
Geneviève Dubuc, Michel Tremblay, [...], and Jean Davignon, J Lipid Res

Dynamic regulation or PCSK9



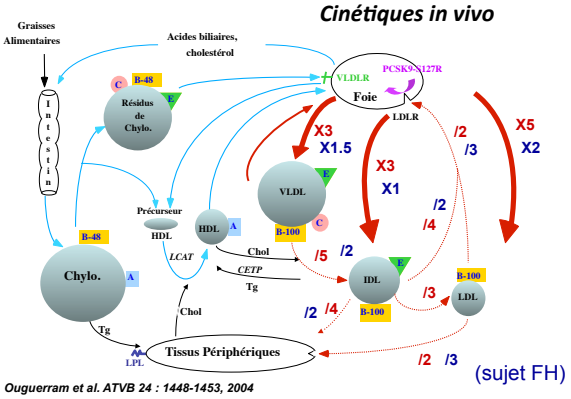
1. Horton JD, et al. *J Lipid Res.* 2009;50:S172–S177; 2. Lopez D. *Biochem Biophys Acta.* 2008;1781:184–191; 3. Abifadel M, et al. *Hum Mutat.* 2009;30: supplementary information; 4. Abifadel M, et al. In: Toth PP. *The Year in Lipid Disorders.* Vol. 2. Oxford, UK: Atlas Medical Publishing Ltd. 2010:3–23.

PCSK9 : Régulation transcriptionnelle



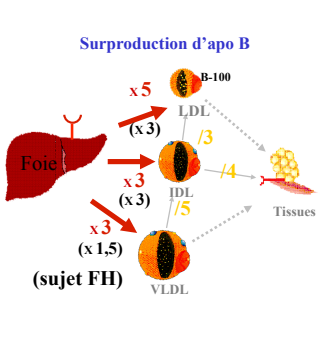
Ferri et al., 2016

Métabolisme chez les patients PCSK9-S127R

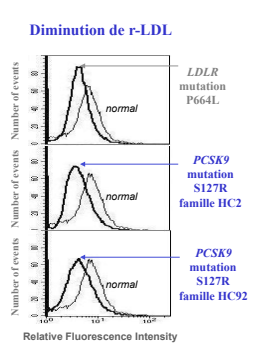


Ouguerram et al. ATVB 24 : 1448-1453, 2004

Le paradoxe des mutants HFAD naturels *PCSK9*



Ouguerram et al., 2004



Benjannet et al., 2004

Impact of PCSK9 on hepatic processing of apo B

Arteriosclerosis, Thrombosis, and Vascular Biology
Journal of the American Heart Association

American Heart Association.

S127R

Proprotein Convertase Subtilisin/Kexin Type 9 Interacts With Apolipoprotein B and Prevents Its Intracellular Degradation, Irrespective of the Low-Density Lipoprotein Receptor

Hua Sun, Amin Samarghandi, Ningyan Zhang, Zemin Yao, Monxiao Xiong and Ba-Bie Teng

Arterioscler Thromb Vasc Biol. 2012;32:1585-1595; originally published online May 10, 2012;

increases plasma cholesterol, TAG, and apoB levels. PCSK9 interacts with apoB to prevent/inhibit/decrease the mobilization of apoB toward autophagosomes for degradation via the autophagosome/lysosome pathway. This, in turn, results in increased production and secretion of apoB and apoB-containing lipoproteins. Most importantly, our study demonstrates a direct protein–protein interaction of PCSK9 and apoB in cells under physiological conditions. Taken together, these data establish that PCSK9 regulates apoB metabolism independent of the LDLR. The present study provides a new mode by which PCSK9 could regulate apoB degradation, and suggests the importance of lowering PCSK9 levels to reduce the production/secretion of apoB-containing lipoproteins.