

HDL, signalisation cellulaire et pathologie cardio-vasculaire

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Auriac-sur-Vendinelle



janvier 2007

et maintenant que vais-je faire . . . ?



Le Bikini

mars 2009



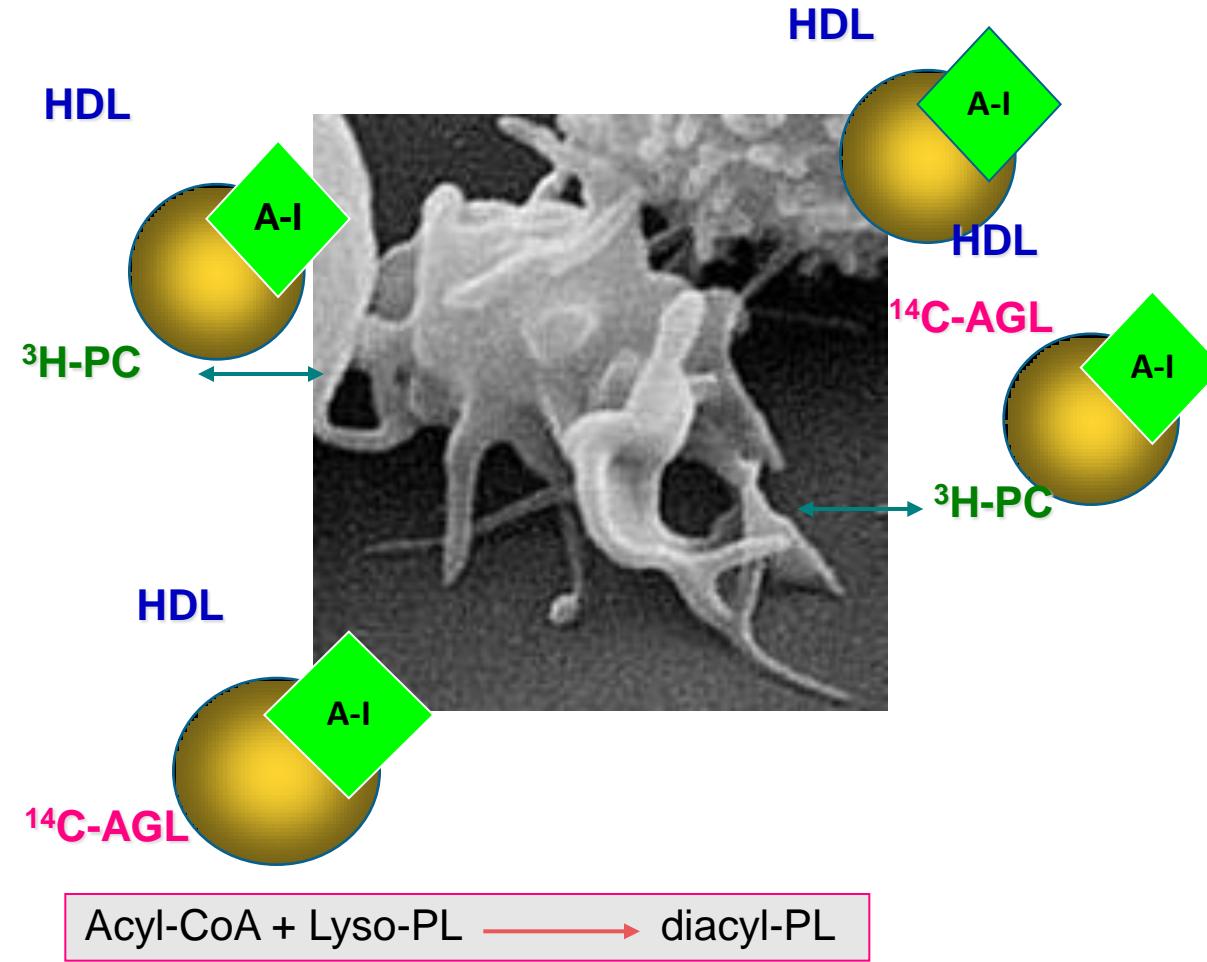
Le Bikini
IN BIKINI DURA ROCK

*Hugues Chap,
A great group leader*





Echanges de lipides entre lipoprotéines et plaquettes



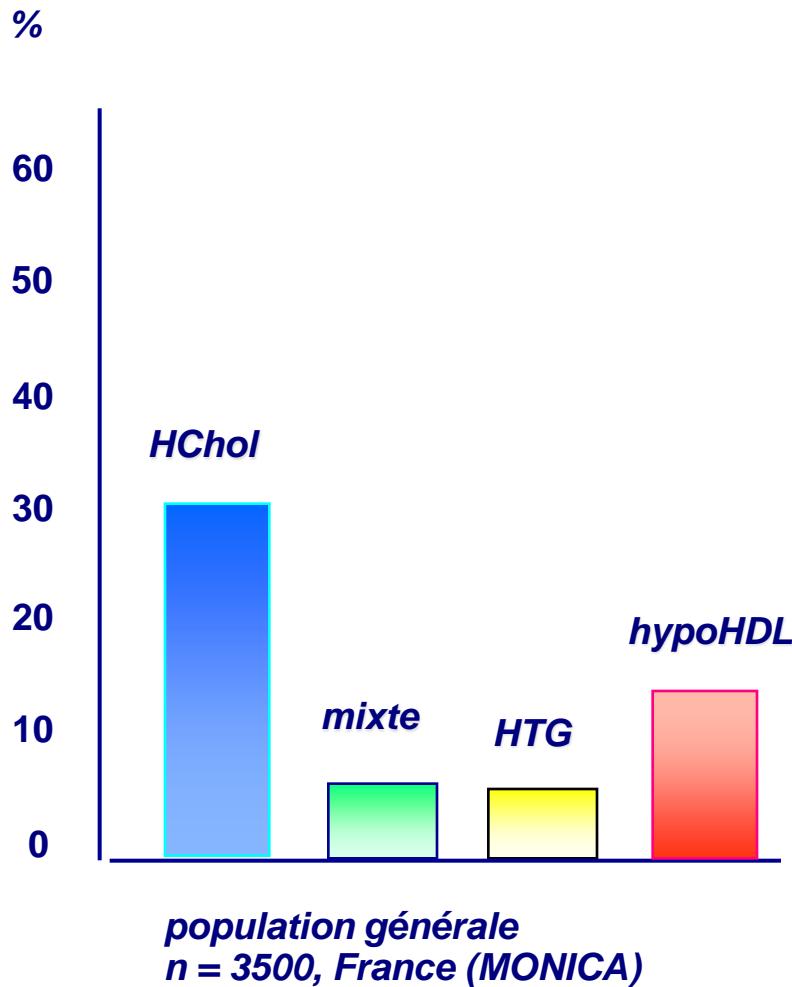
Inverse relation of HDL-C and incidence of coronary heart disease

| | Incidence of coronary events | | Mortality | |
|--------------|------------------------------|------------------|-----------|------------------|
| Men | % | p | % | p |
| FHS | - 1.90 | 0.025 | - 1.47 | 0.19 |
| CPPT | - 2.33 | 0.005 | - 0.87 | 0.59 |
| MRFIT | - 2.05 | <0.001 | - 1.22 | 0.15 |
| LRCF | | | - 3.60 | <0.001 |
| Women | | | | |
| FHS | - 3.24 | 0.011 | - 4.44 | 0.03 |
| LRCF | | | - 4.72 | 0.002 |

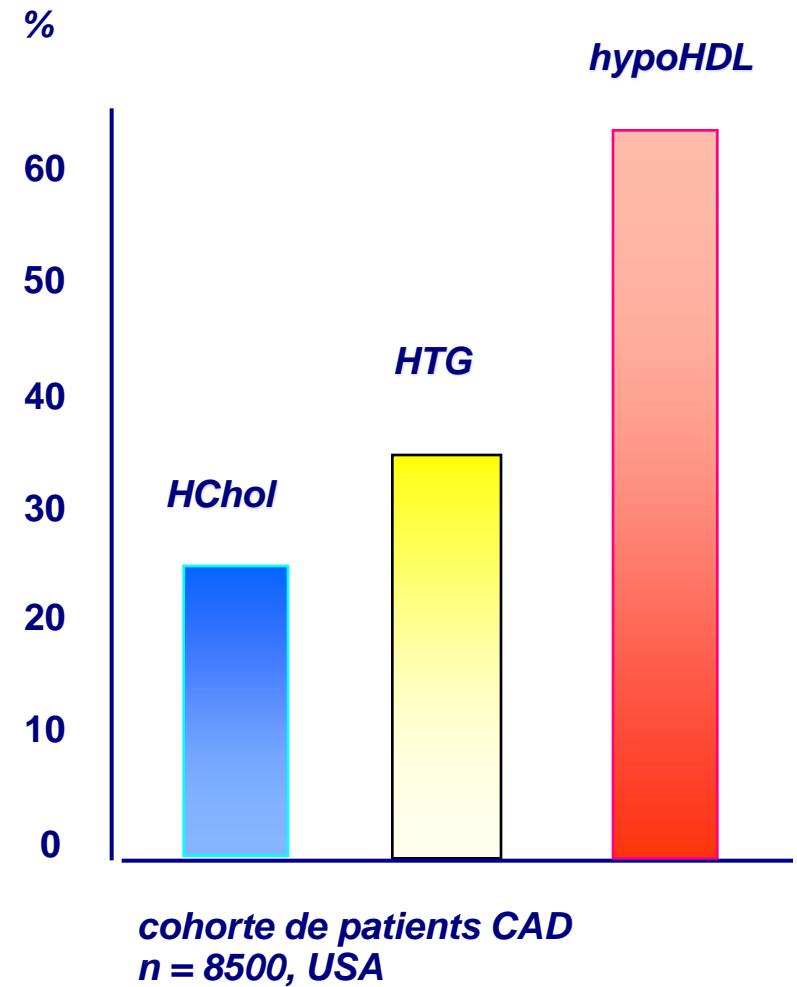
From Gordon, D.J. et al. 1989 Circulation 79: 8-15

Prospective studies: 0.01 g/l increment in HDL-C is associated with significant ~2% (men) and ~3% (women) decrements in coronary heart disease risk

Prévalence des dyslipidémies

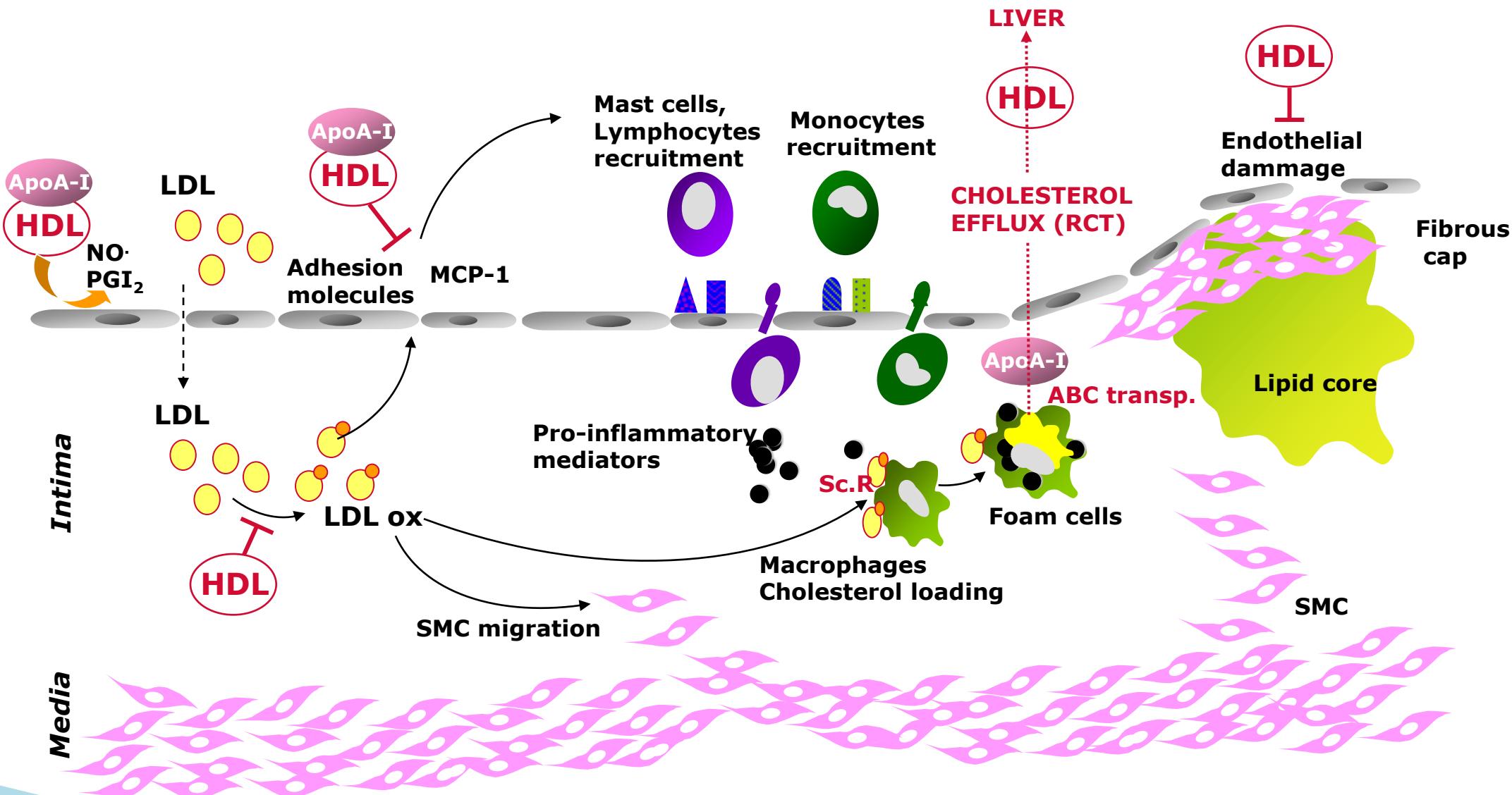


Ferrières J, 2005, Arch Mal Cœur 98, 127



Rubins HB, 1995, Am J Cardiol 75, 1196

Effets protecteurs des HDL sur la paroi vasculaire



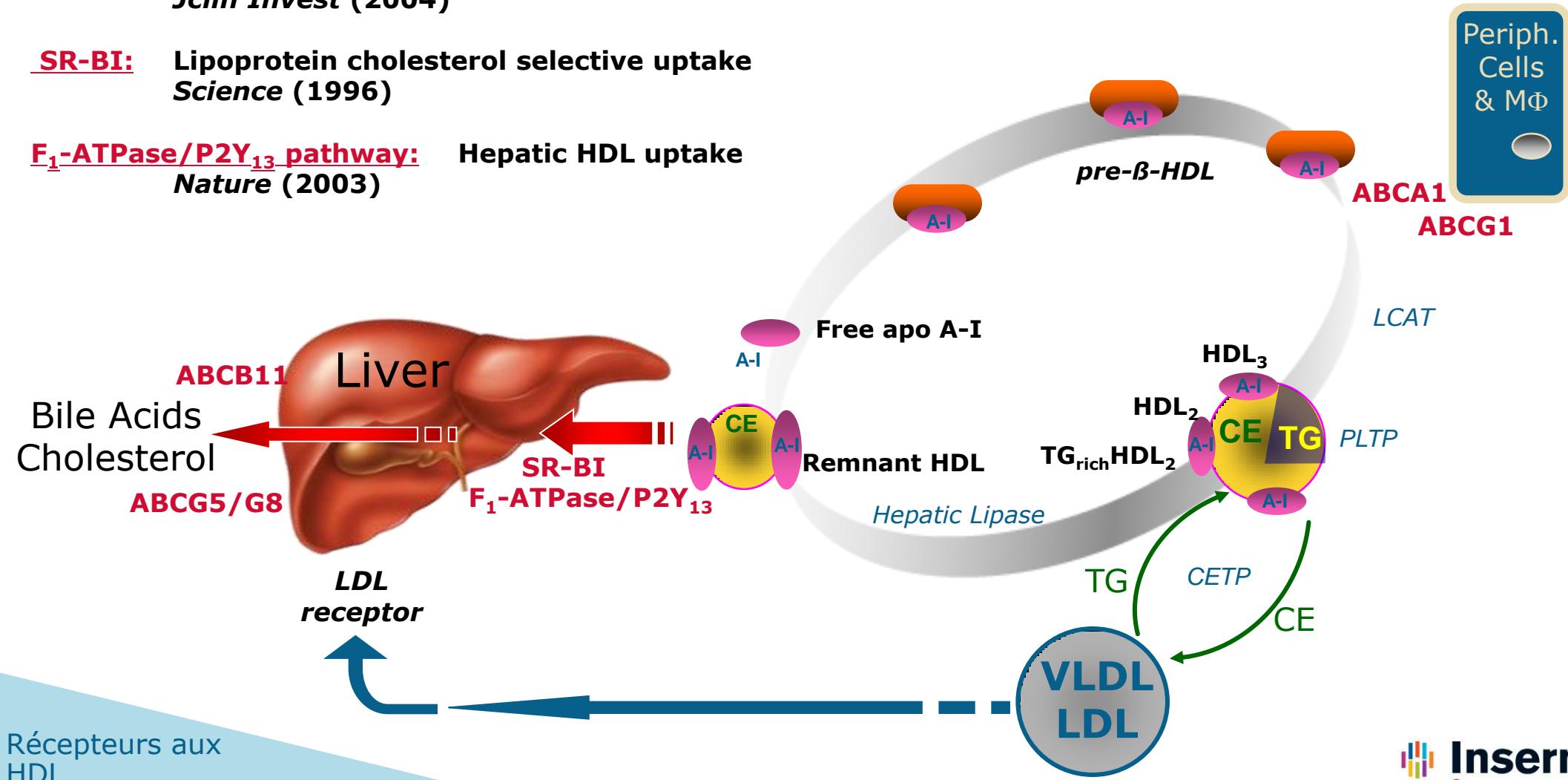
Reverse cholesterol transport and HDL/Cell interactions

ABCA1: Cholesterol efflux to apoA-1
Nature Medecine (1999)

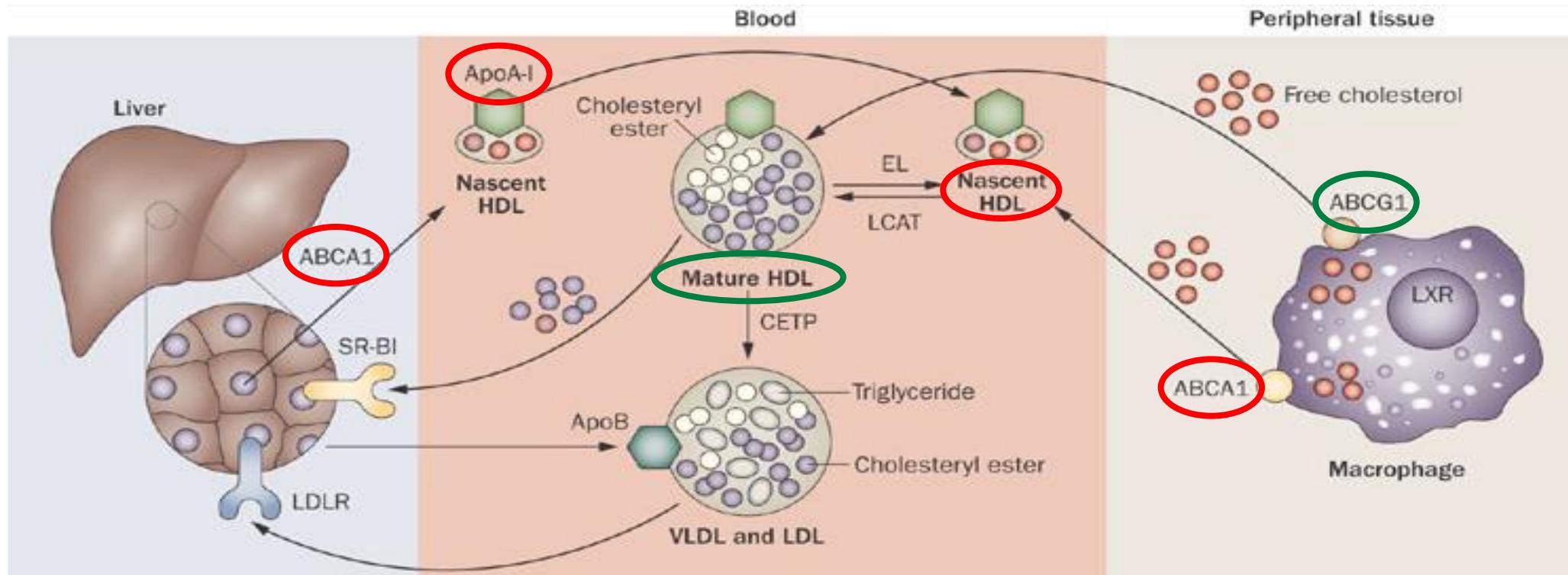
ABCG1: Cholesterol efflux to HDL
Jclin Invest (2004)

SR-BI: Lipoprotein cholesterol selective uptake
Science (1996)

F₁-ATPase/P2Y₁₃ pathway: Hepatic HDL uptake
Nature (2003)

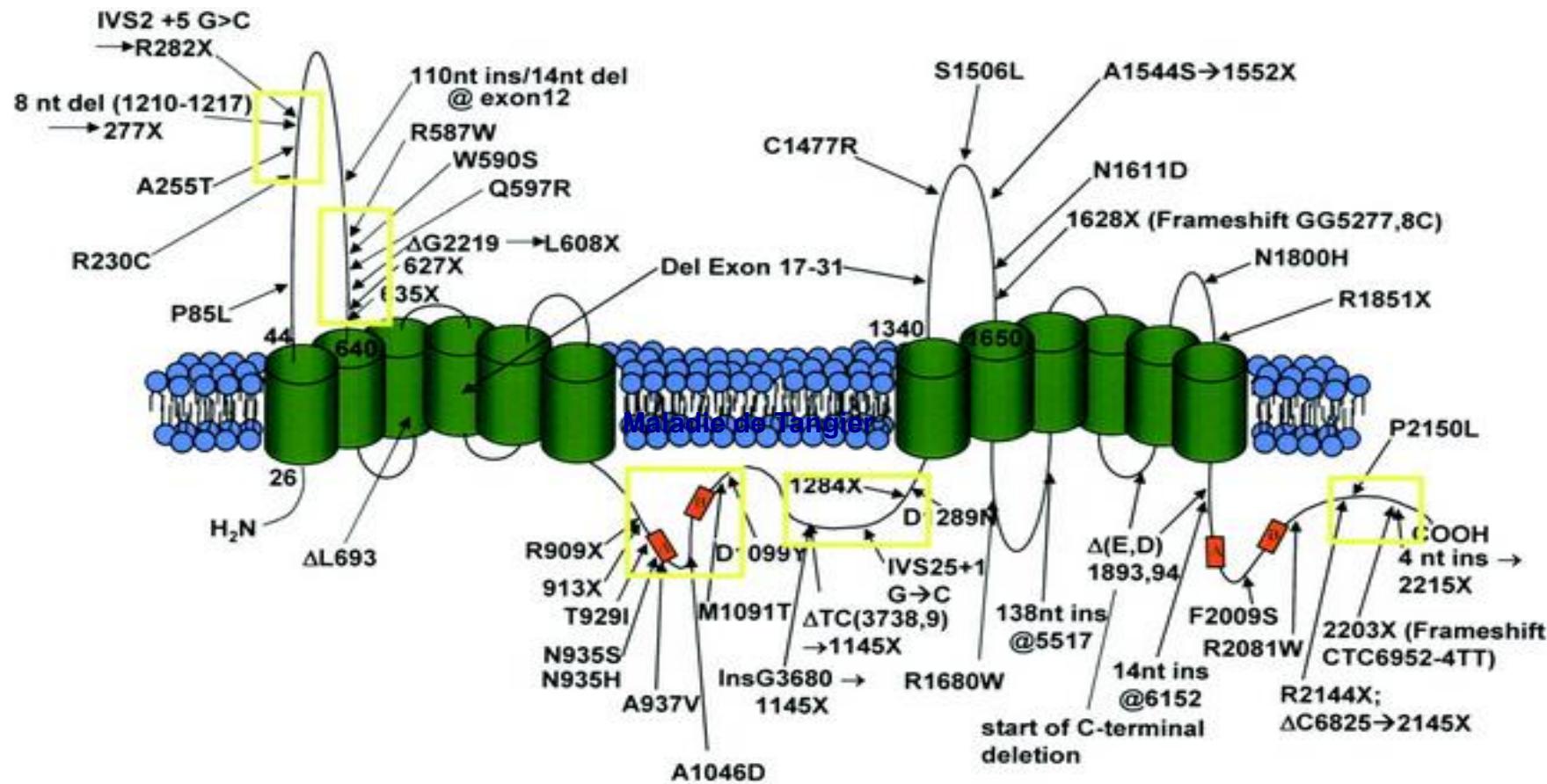


Membrane transporters in cholesterol efflux to HDL



Mutations dans ABCA1 responsables de la maladie de Tangier et hypo-HDL

ABCA1 – ATP binding cassette, sous-famille A, membre 1



★ déficience familiale en HDL (hypo-HDL^{émie})

★ maladie de Tangier

Rôle d'ABCA1 dans l'efflux de cholestérol

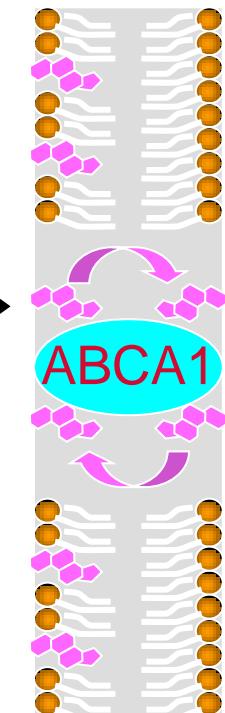
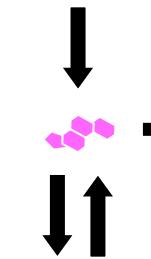
SUJET NORMAL

Intracellulaire

Extracellulaire

Apo A-I,
pauvre en lipides

Cholésterol Libre



HDL naissantes

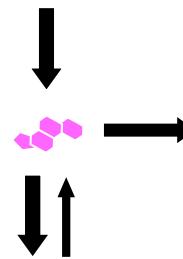
Cholestérol Esters

Patient Maladie de TANGIER

Intracellulaire

Extracellulaire

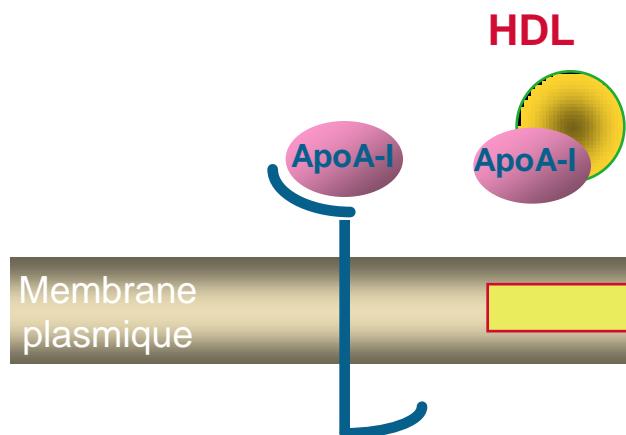
Cholésterol Libre



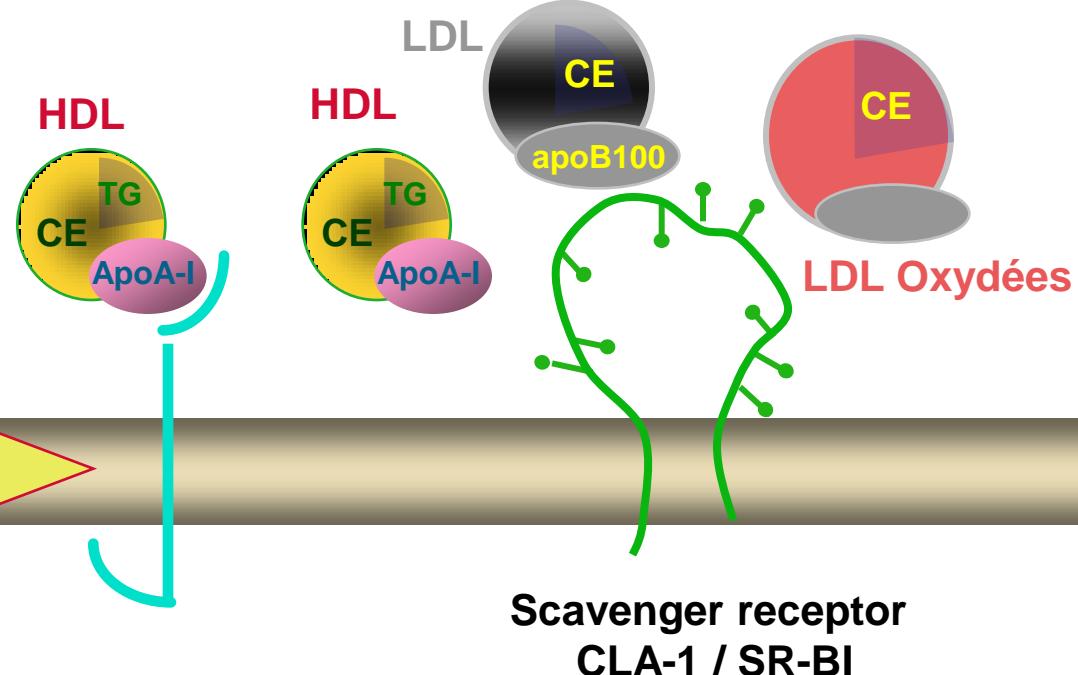
Apo A-I
catabolisée

Sites de liaison des HDL sur les hépatocytes

Haute affinité ($K_d \sim 10^{-9}$)



Basse affinité ($K_d \sim 10^{-6} - 10^{-7} M$)



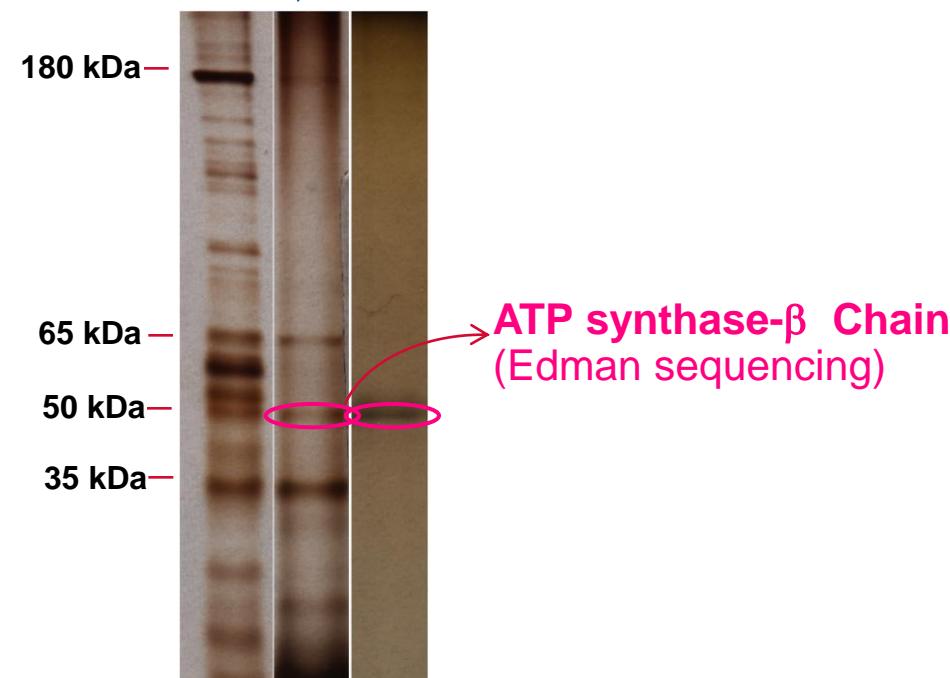
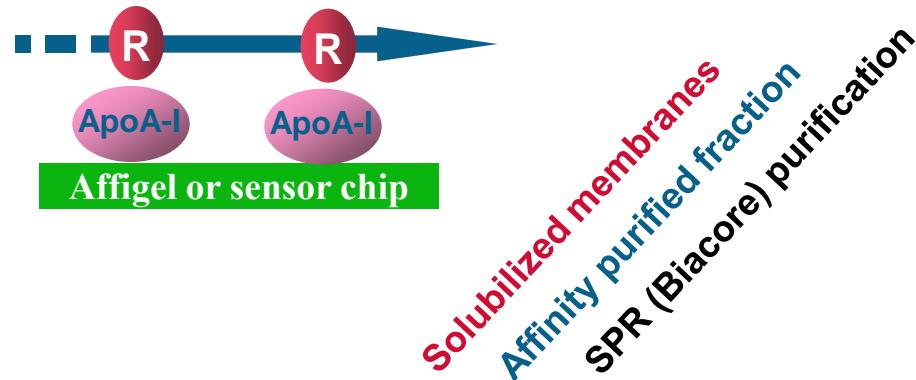
Membrane plasmique

Endocytose totale
(protéine & cholestérol)

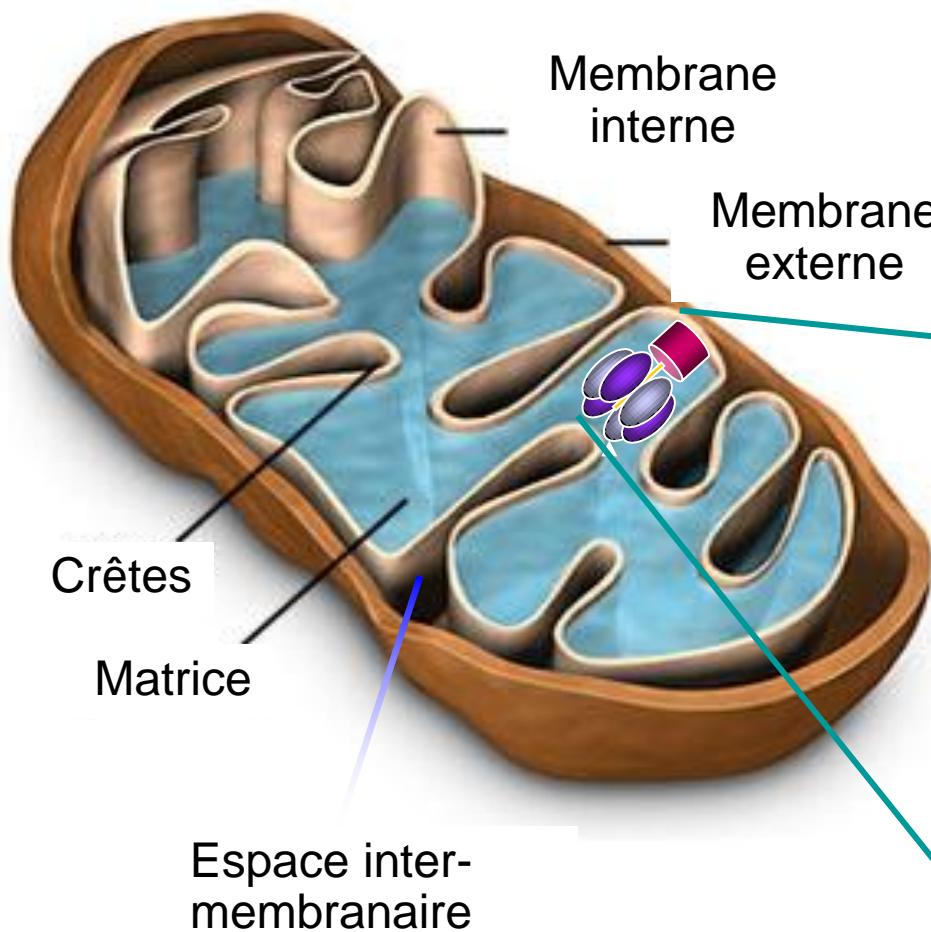
Cognition sélective du
cholestérol estérifié (CE)

Purification of high-affinity HDL receptor from pig liver plasma membranes

Solubilized liver plasma membranes



l'ATP synthase mitochondriale

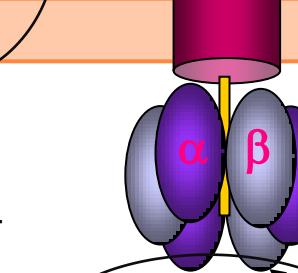


Espace
Inter-membranaire

matrice

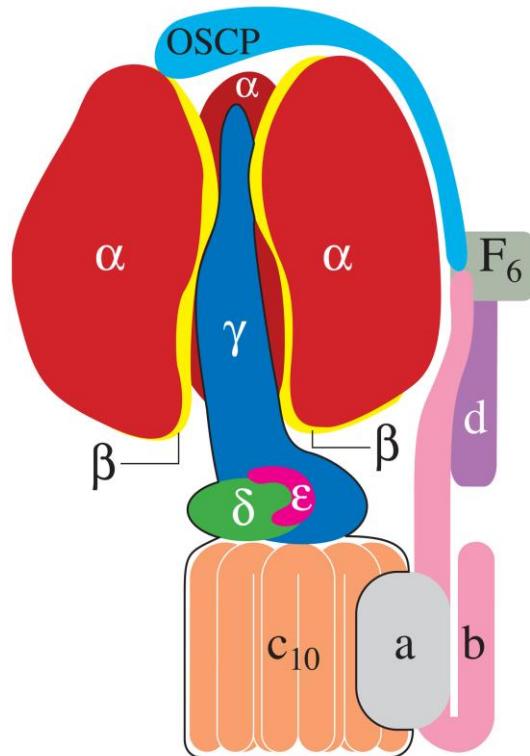
3H^+

ATP



$\text{ADP} + \text{Pi}$

Mitochondrial ATP synthase

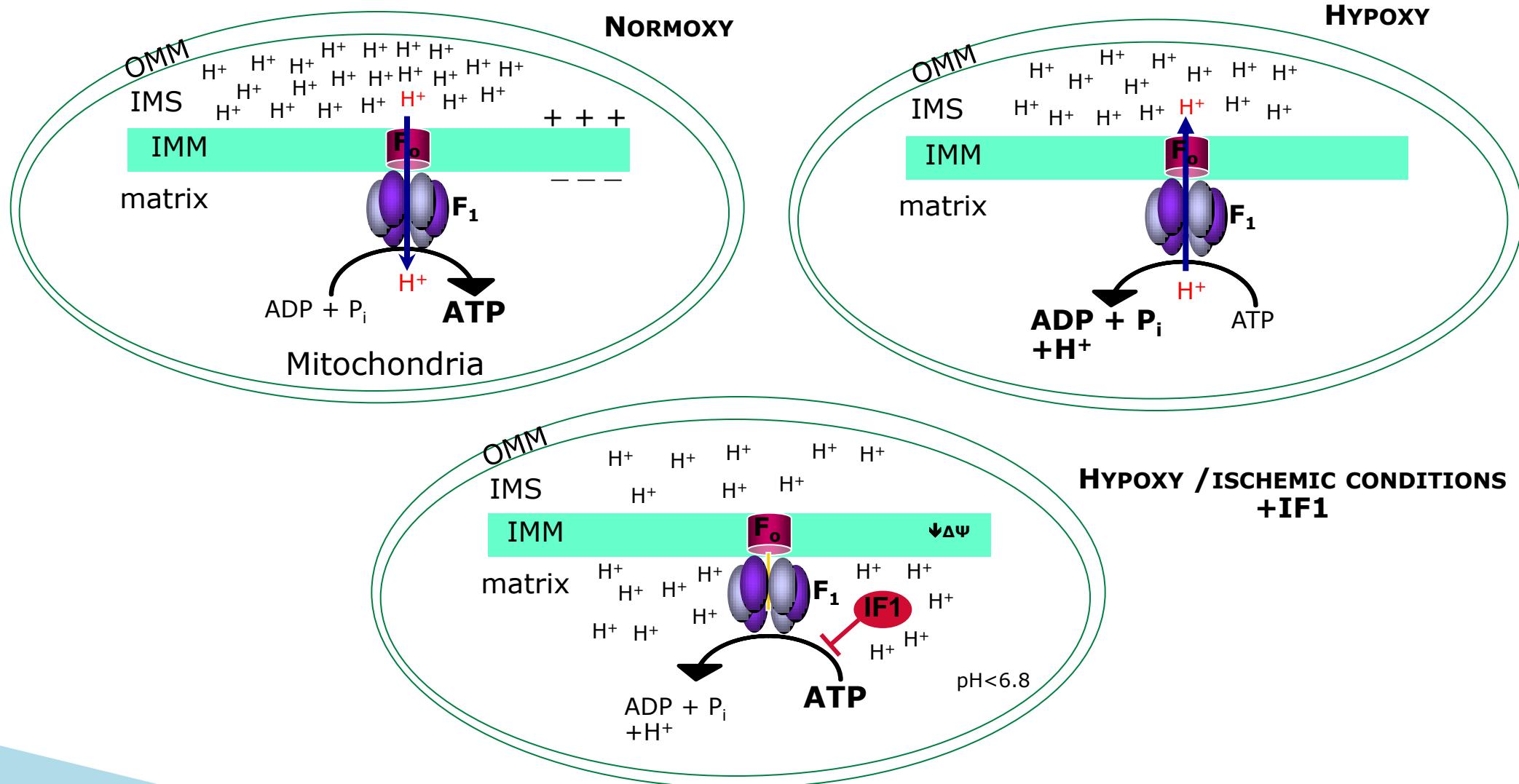


Mitochondrial ATP synthase

| | |
|---|------------------|
| Alpha (x3) ATP5A (55,264; pI=8.44; pept signal 43AA: 4559Da) | 165,792 |
| Beta (x3) ATP5B (51,705; pI=5.21; pept signal 46AA: 4596Da) | 155,115 |
| Gamma ATP5C | 30,141 |
| Delta ATP5D, Epsilon chez E.Coli | 15,065 |
| Epsilon ATP5E, absent chez E.Coli | 5,652 |
| F1 TOTAL | pI = 5.44 |
| a ATP6 (mito) | 24,788 |
| b ATP5F1 | 24,668 |
| c (x10) ATP5G1 | (7,608) |
| d ATP5H | 18,561 |
| e ATP5i | 8,189 |
| f | 10,412 |
| g ATP5L | 11,286 |
| OSCP ATP5O Delta chez E.Coli | 20,928 |
| F6 ATP5J | 8,958 |
| A6L ATP8 (mito) | 7,937 |
| Fo TOTAL | 211,807 |
| F1Fo TOTAL | pI = 6.87 |
| Inhibitor ATPIFI (pI=8.30; pept signal 25AA: 2750Da) | 9,516 |
| F1Fo + Inhibitor | 593,153 |

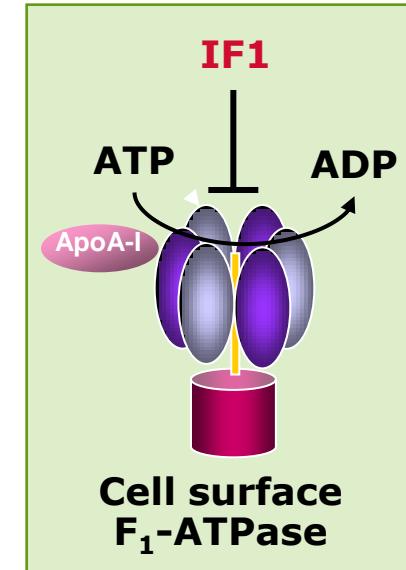
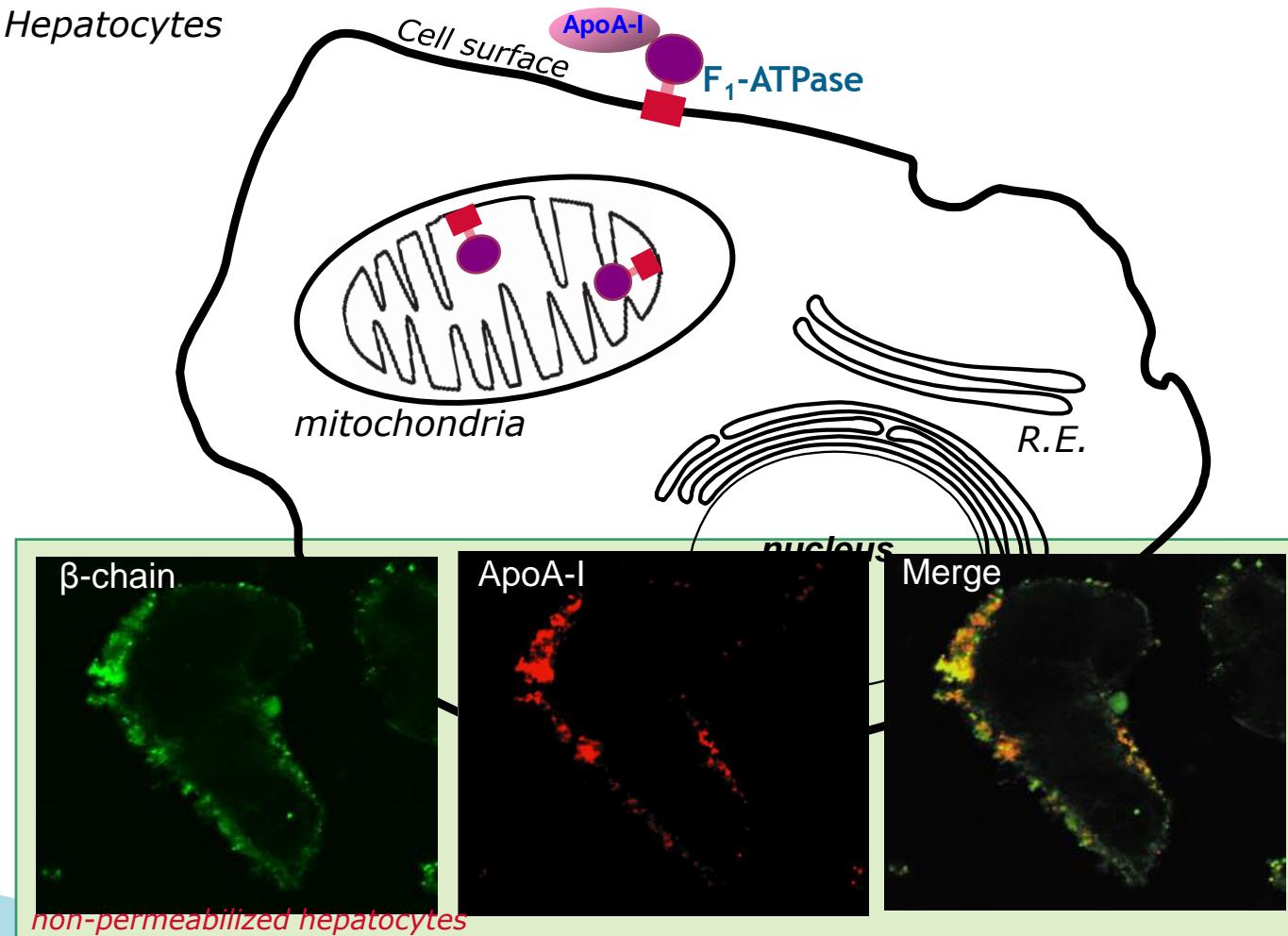
Régulation of F₁-ATPase by IF1

Function of mitochondrial IF1



F_1 -ATPase/P2Y₁₃ colocalizes with apo A-I

ATP synthase (ecto- F_1 -ATPase) =
receptor for HDL and its main apolipoprotein, apoA-I

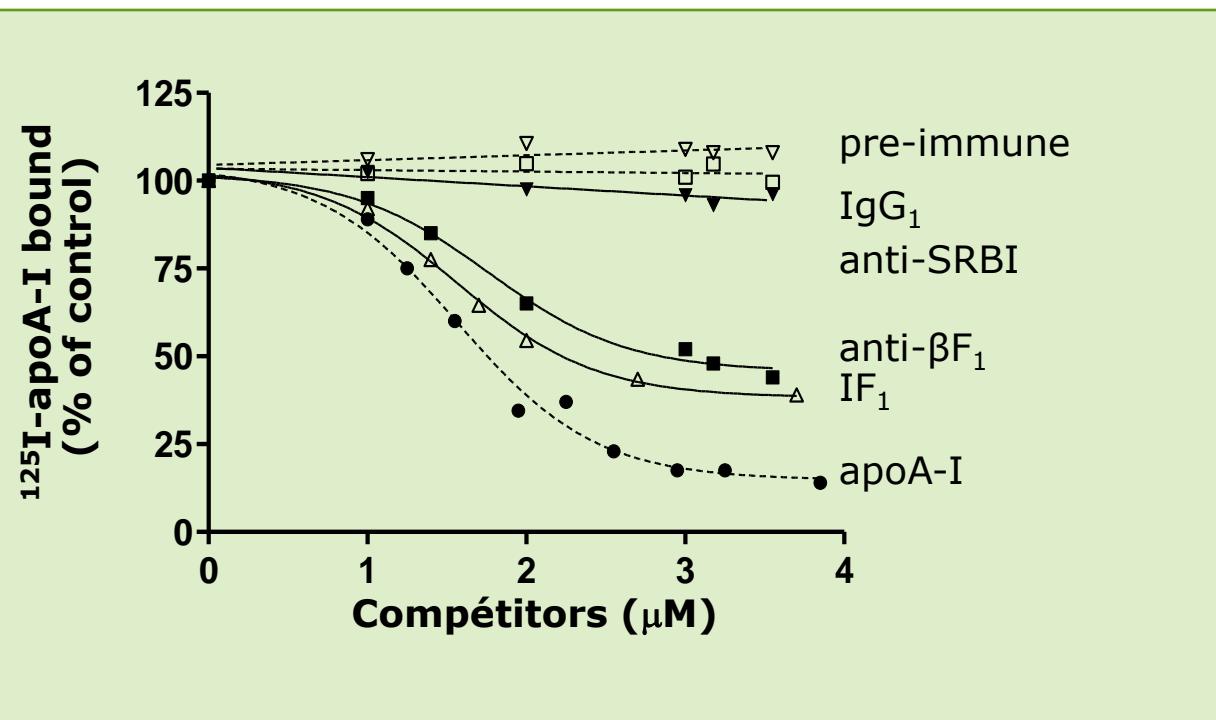
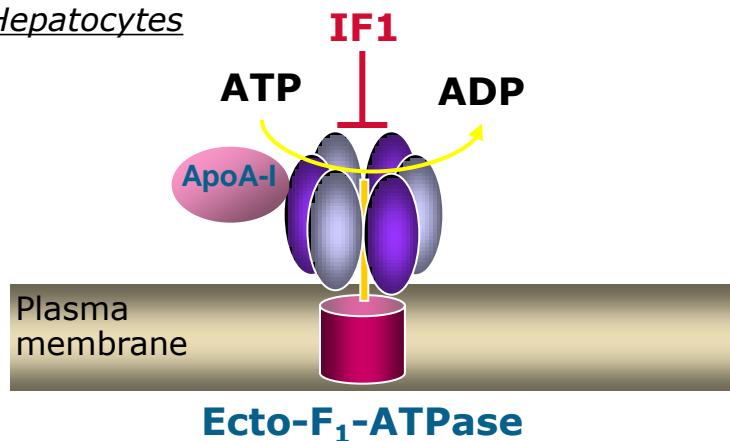


IF1 is a 10 kDa protein, classically known to inhibit mitochondrial ATP synthase, but exogenous IF1 also inhibits ecto- F_1 -ATPase.

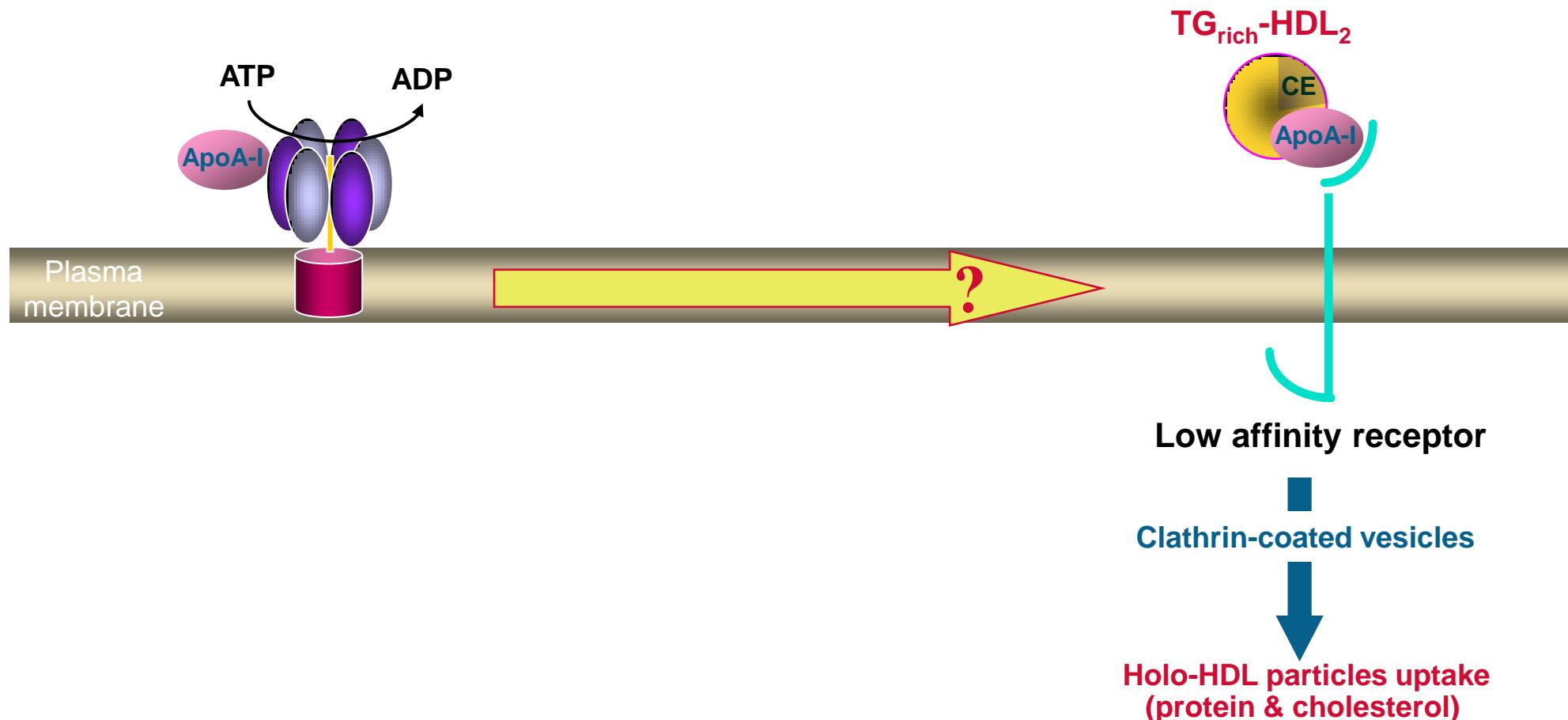
Competitors of apoA-I binding on hepatocytes

Method: ^{125}I -apoA-I binding (4° C) on cells

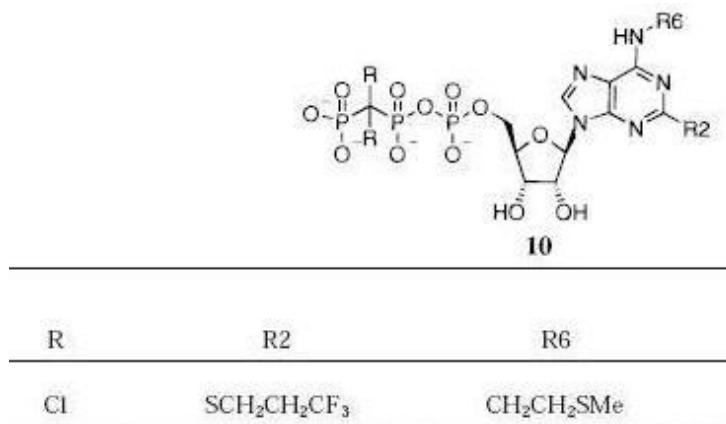
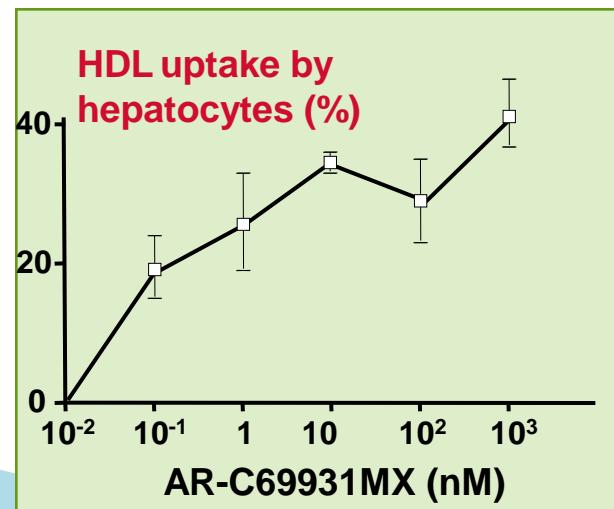
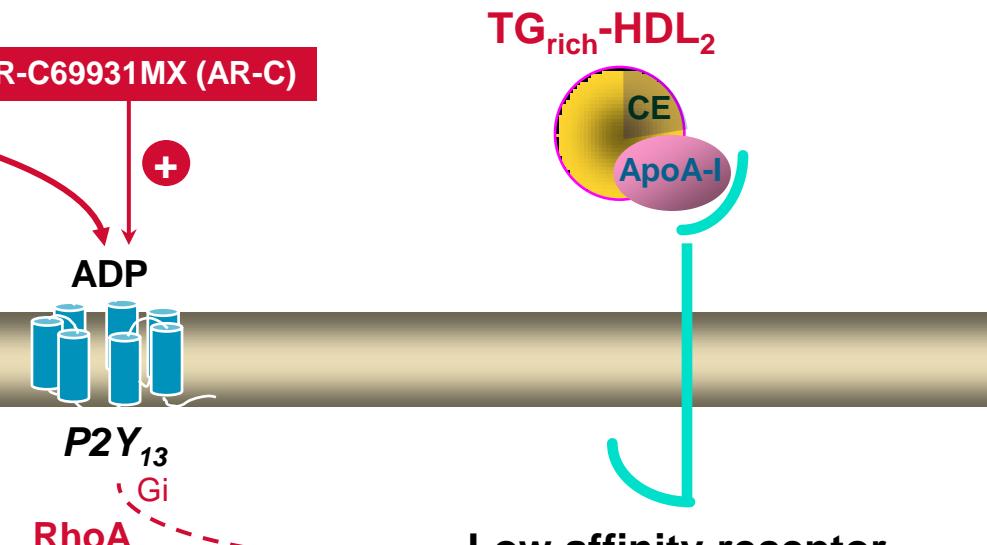
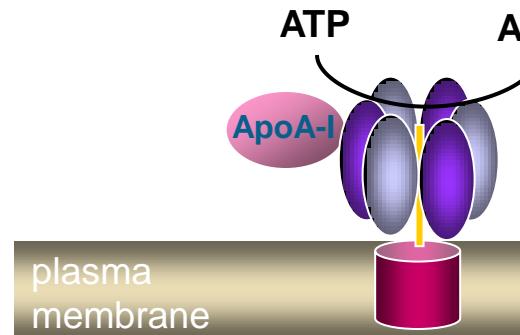
Hepatocytes



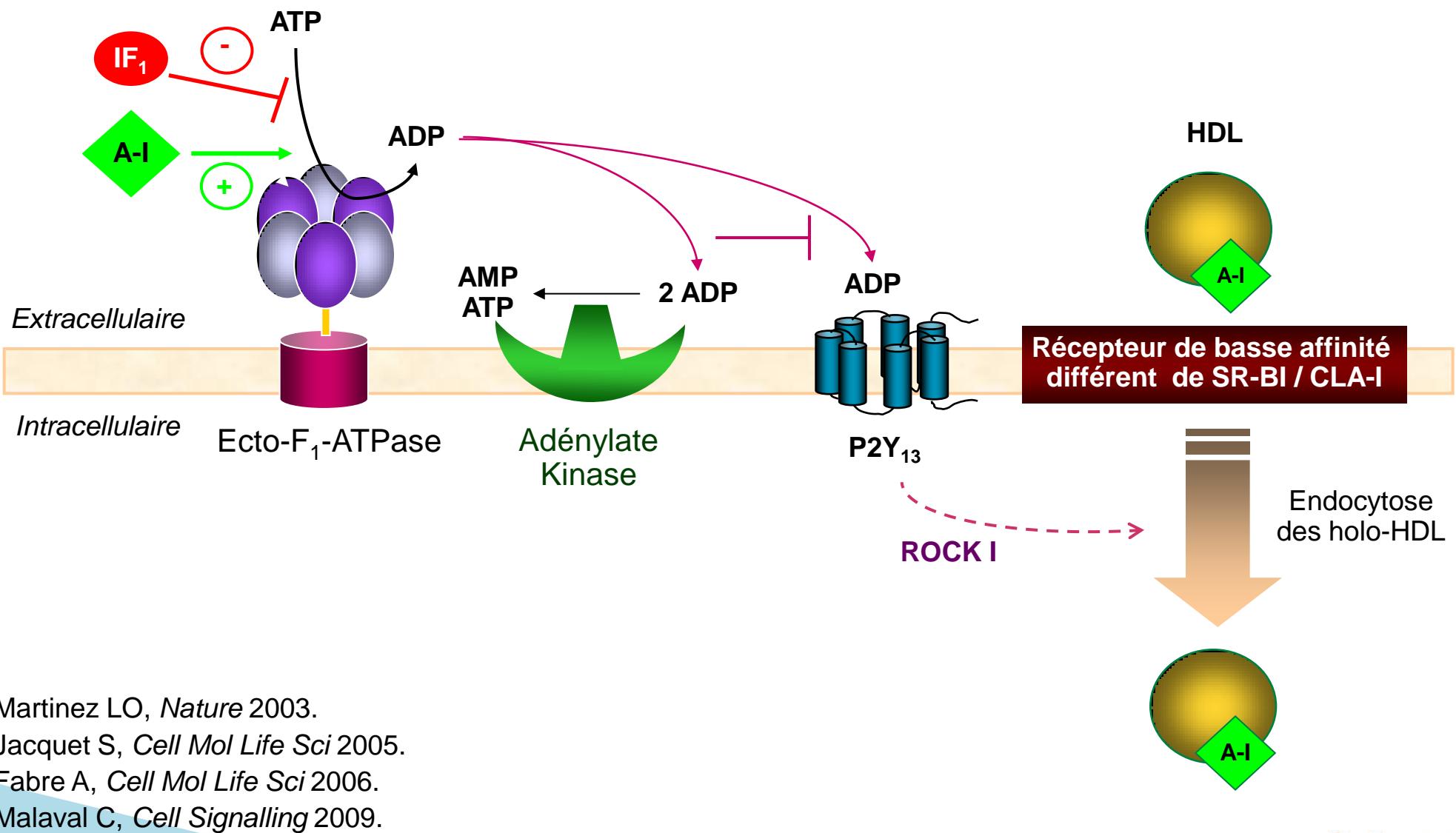
Mécanismes moléculaires de l' endocytose des HDL



Mécanismes moléculaires de l'endocytose des HDL

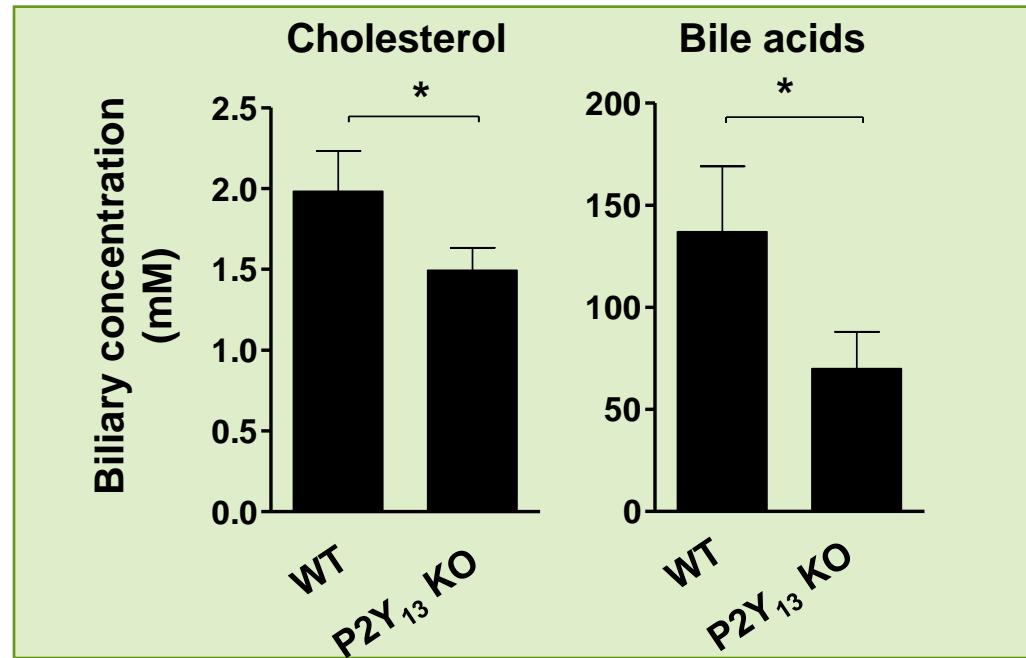


Voie de l'ecto- F_1 -ATPase / P2Y₁₃ sur le contrôle de l'endocytose des HDL



Rôles physiologiques du récepteur P2Y₁₃

Method: Gallbladder cannulation and bile collection for 1 hour



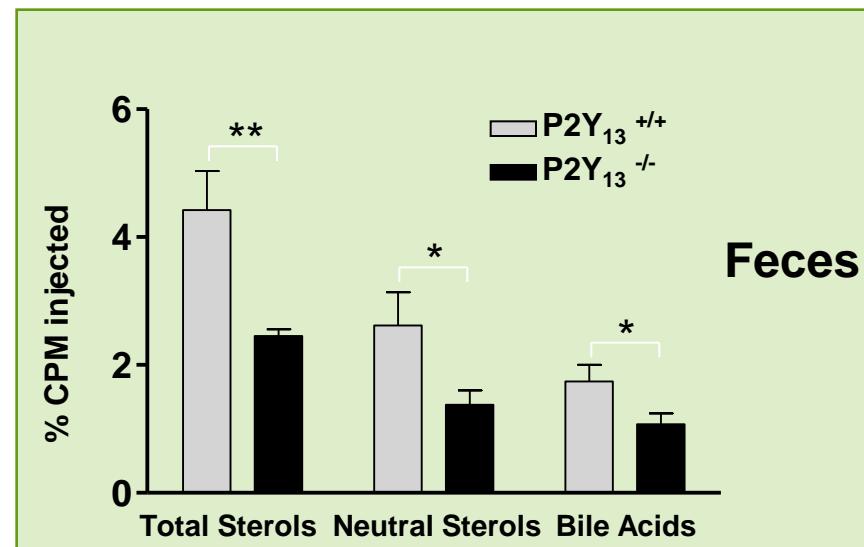
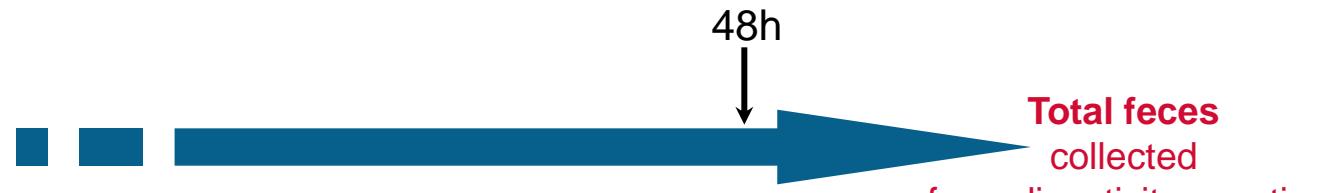
Rôle physiologique du récepteur P2Y₁₃

Method: Quantification of macrophage to feces RCT *in vivo* model

P2Y₁₃ KO or WT
male mice
8-9 weeks old
 $n \geq 6$



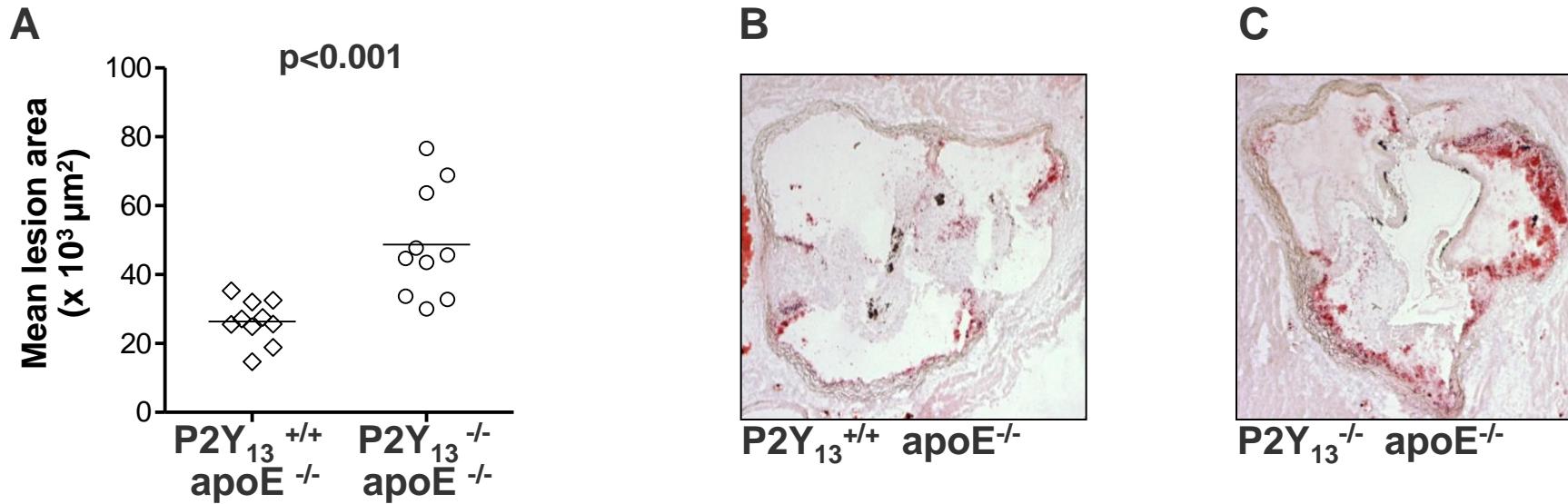
³H cholesterol-loaded
macrophages



P2Y₁₃ joue un rôle important dans le transport retour du HDL-C

Metabolic functions: role of P2Y₁₃ in RCT and atherosclerosis

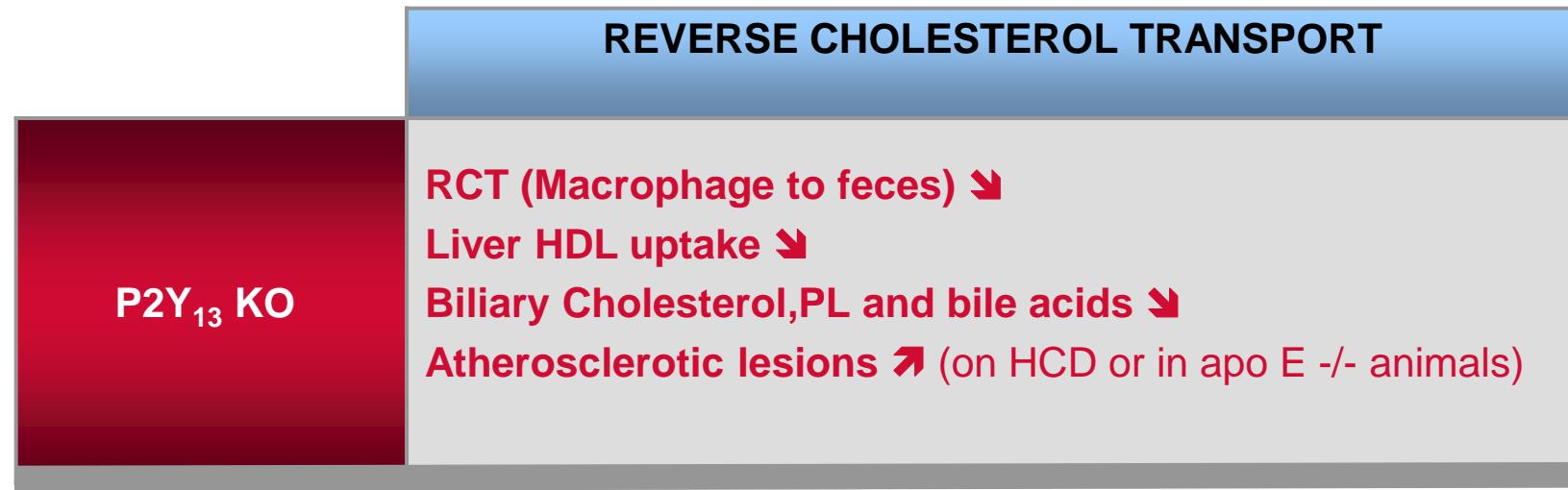
Effect of P2Y₁₃ deletion on atherosclerotic lesion in apoE^{-/-} mice



- ▶ Impaired RCT in P2Y₁₃ KO mice is associated with initiation of atherosclerosis

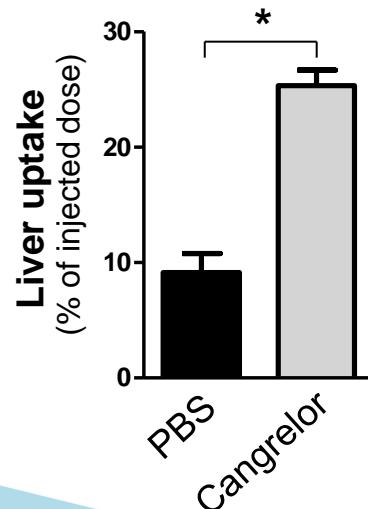
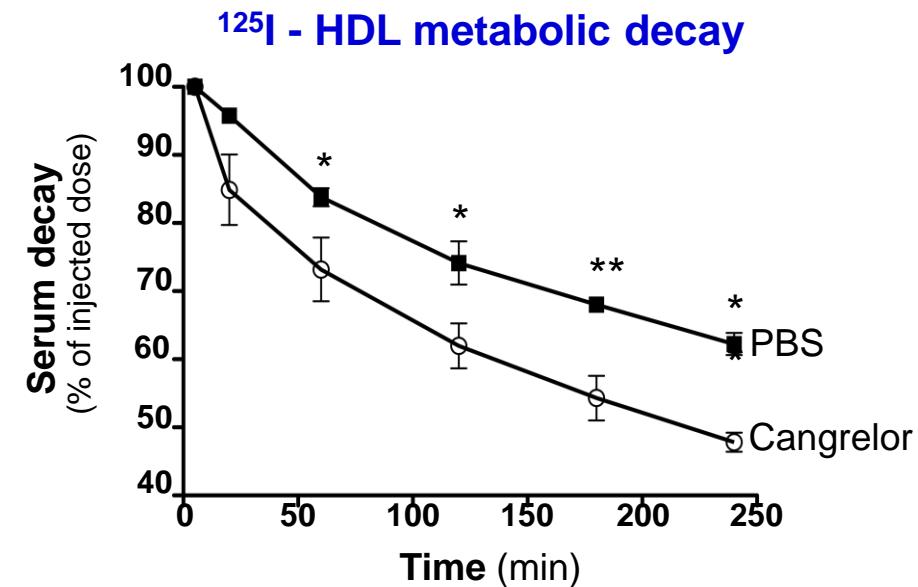
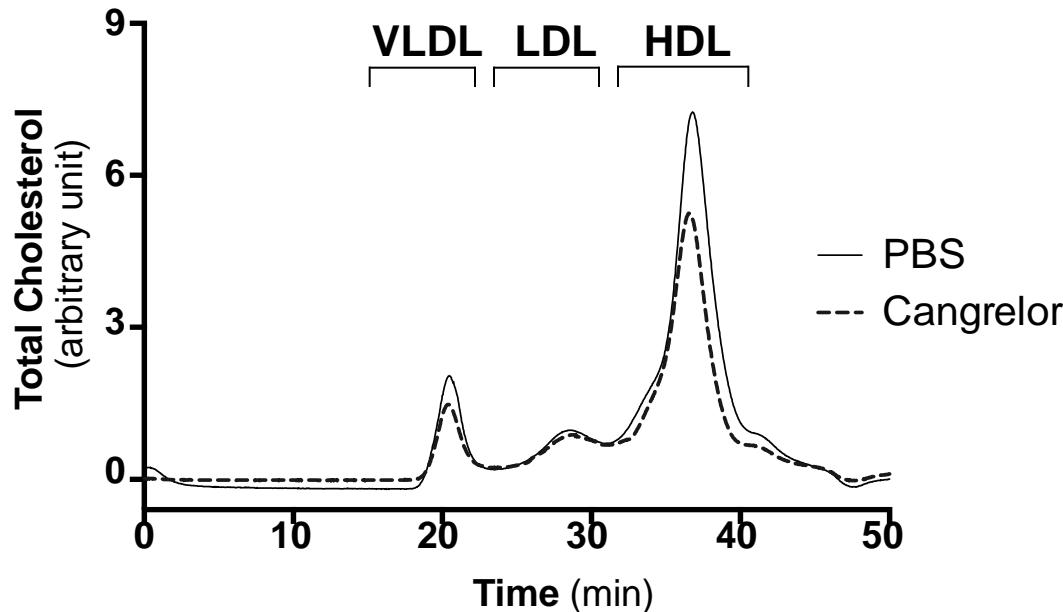
Martinez L.O et al. (2009) *ISA, Atherosclerosis suppl.*
Lichtenstein L. et al. (2015), *Cardiovasc Res.*

In vivo effects of P2Y₁₃ deficiency



→P2Y₁₃ ADP-RECEPTOR IS CRITICAL FOR REVERSE CHOLESTEROL TRANSPORT

Stratégie thérapeutique: Activation de P2Y₁₃



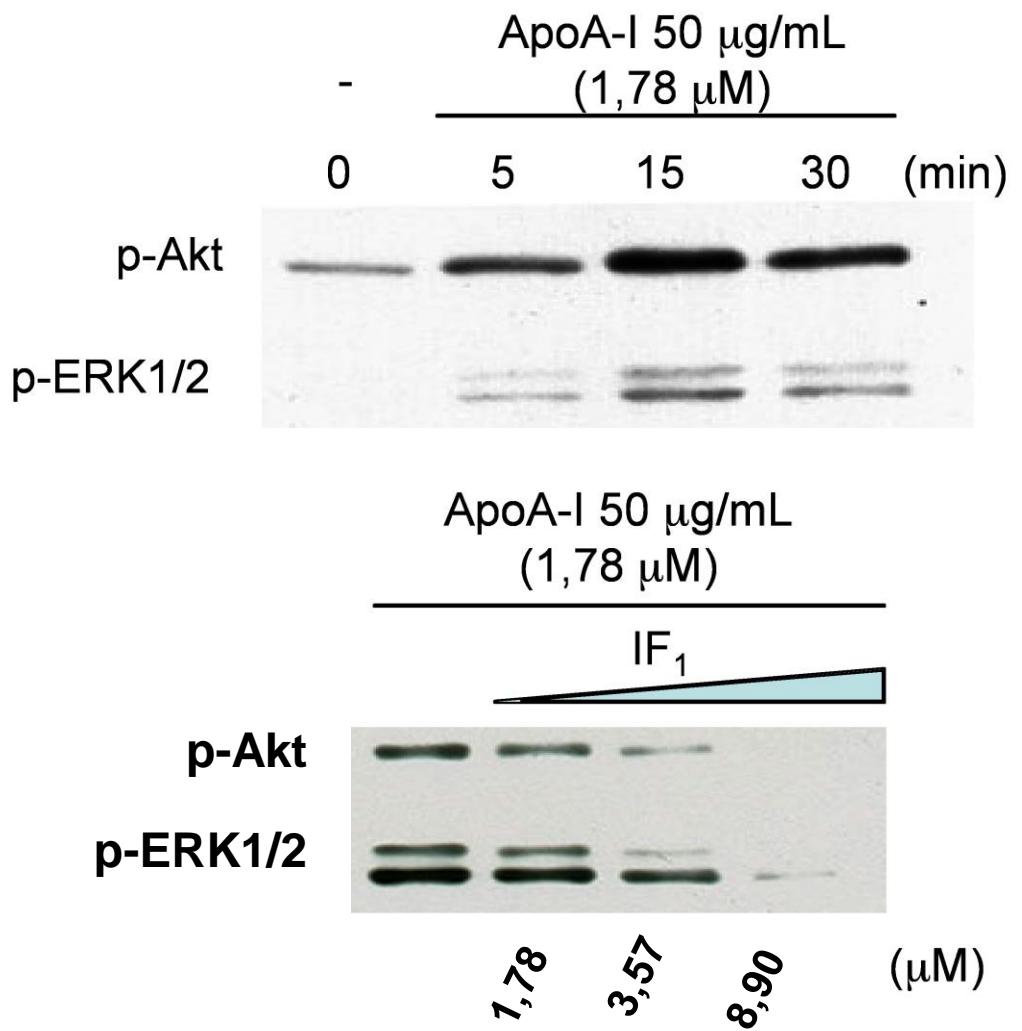
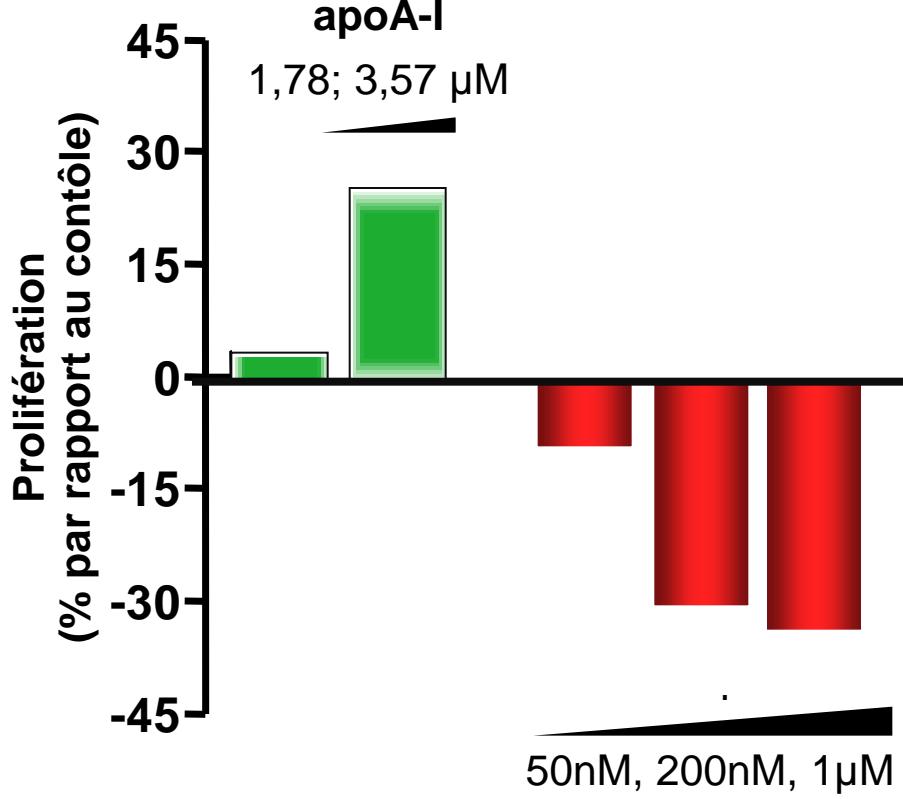
- Cangrelor increases biliary lipid secretion increases
- Cangrelor improves reverse cholesterol transport

Serhan N. Biochim Biophys. Acta 2012

CT 1007900 reduces carotid and aorta atherosclerosis progression

Goffinet M. Plos One 2014

L'ecto-F₁-ATPase induit la prolifération des cellules endothéliales



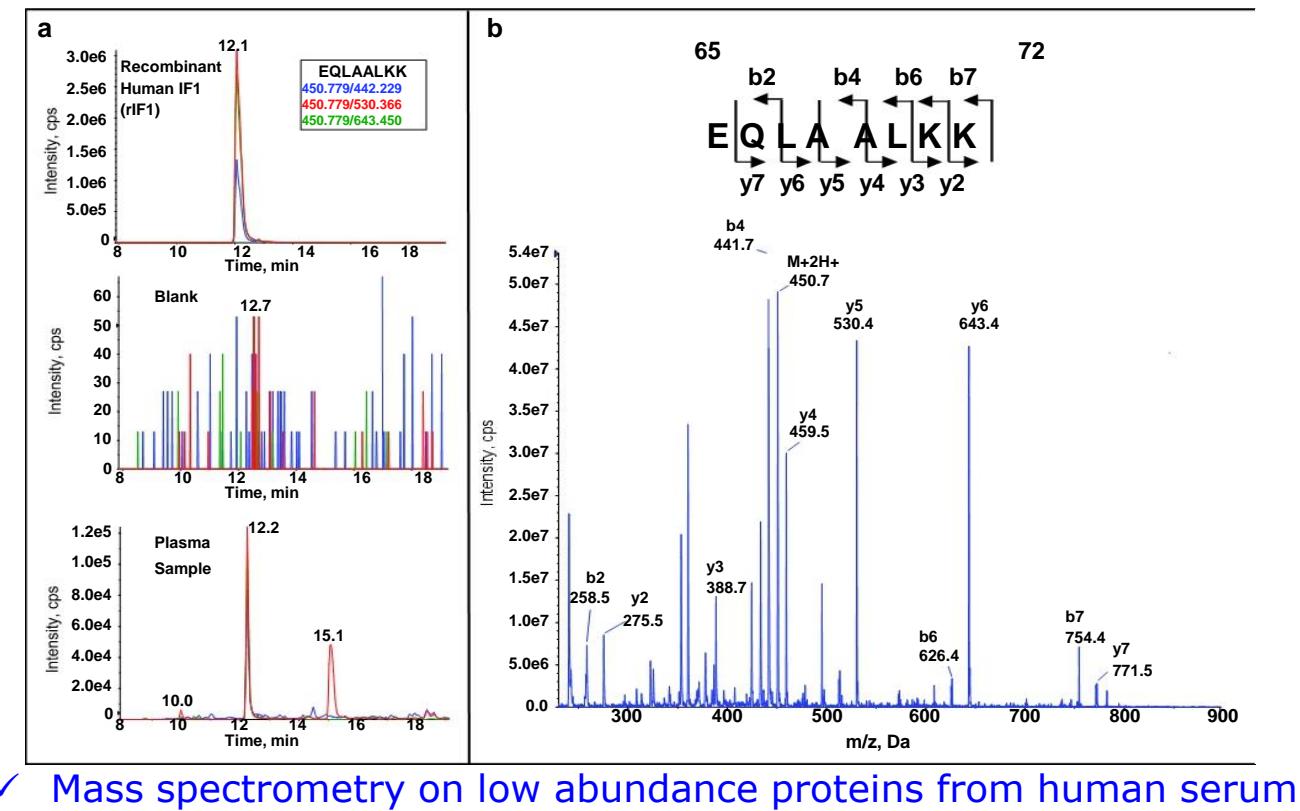
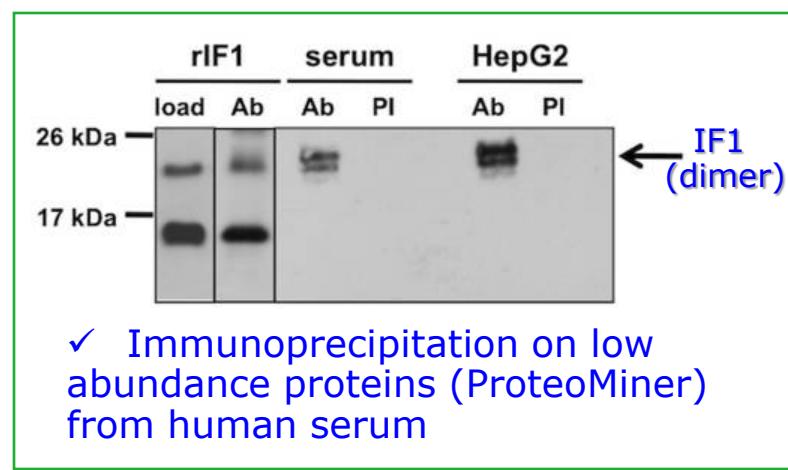
IF1, a new determinant of HDL and CHD risk

Identification of IF1 in Human serum

Identification by MS of 4 IF1 peptides in human serum:

dqsenvdrgagsireaggafgkreqaeeeeryfraqseqlaalkkhheeeivhhkkeierlqkeierhkqkikml
khdd

- Peptide 1: **EQLAALK** (38-44) 7AA
- Peptide 2: **EQLAALKK** (38-45) 8AA
- Peptide 3: **HHEEEIVHHK** (46-55) 10AA
- Peptide 4: **LQKEIER** (61-67) 7AA



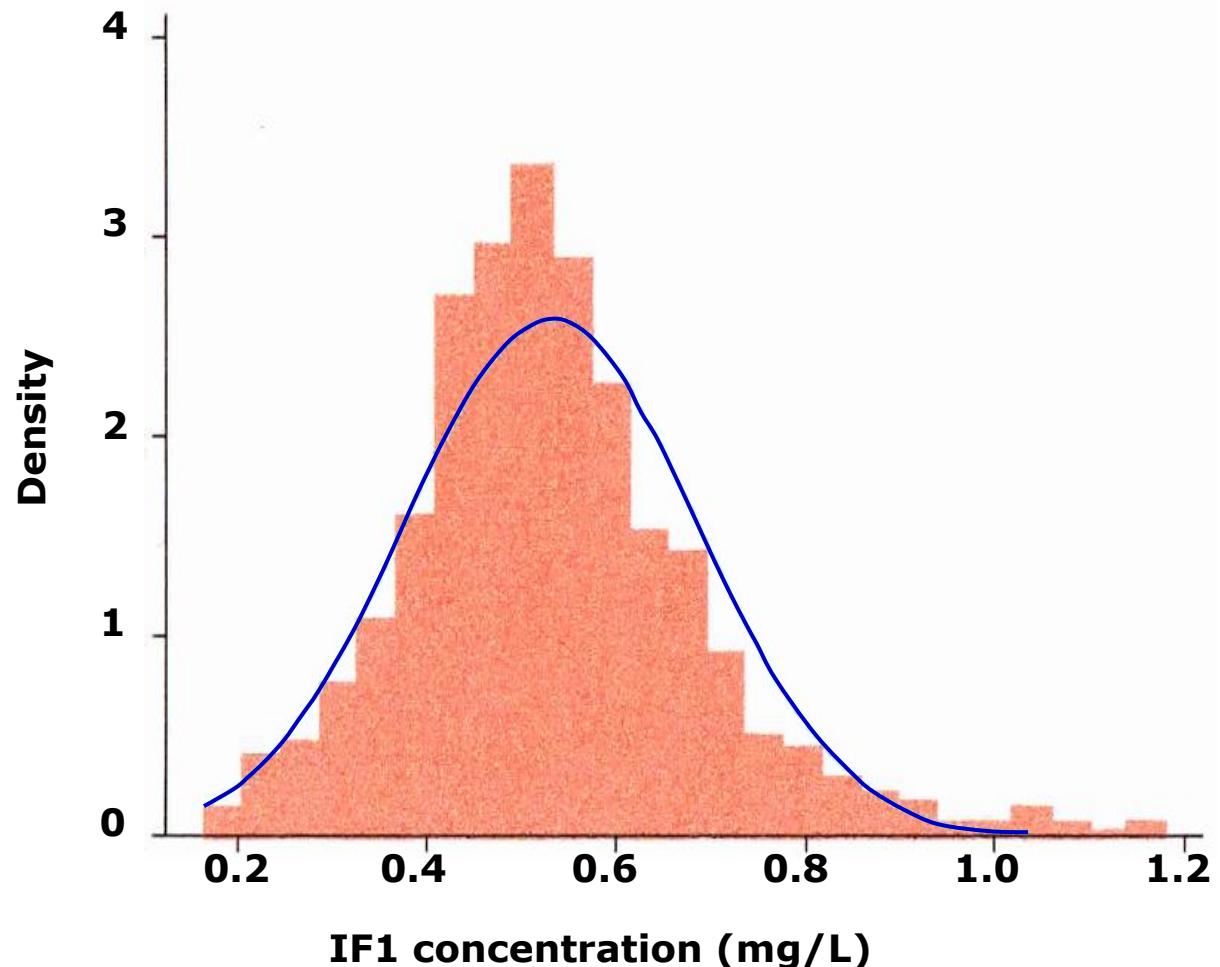
IF1 frequency distribution in the general population

n = 706 male subjects from
the general population,
selected on electoral rolls

normal distribution

median = 0.52 mg/l
(95% CI: 0.22-0.82 mg/l)

mean = 0.53 ± 0.16 mg/l



Multiple linear regression analysis of apoA-I

R² = 22.2 %

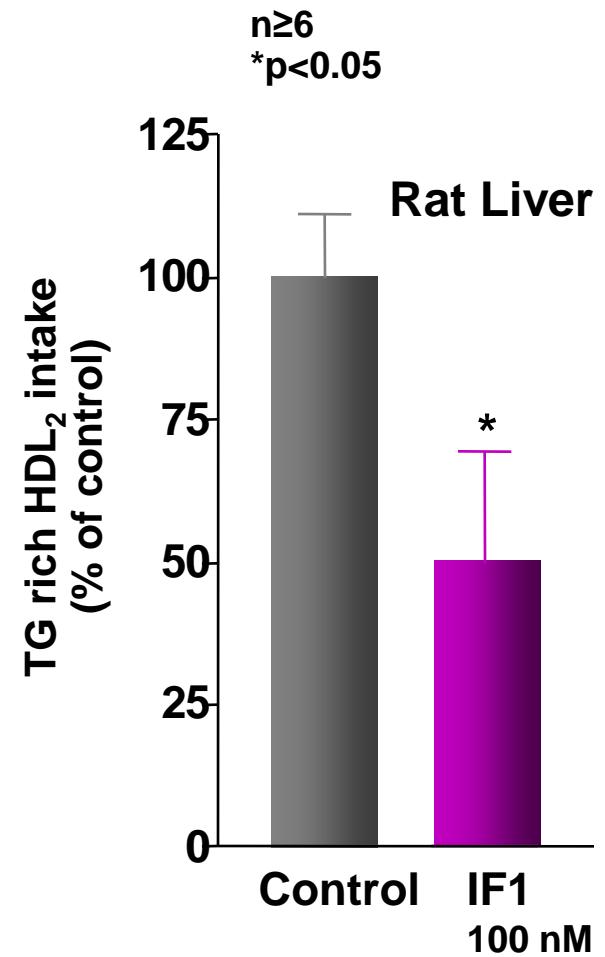
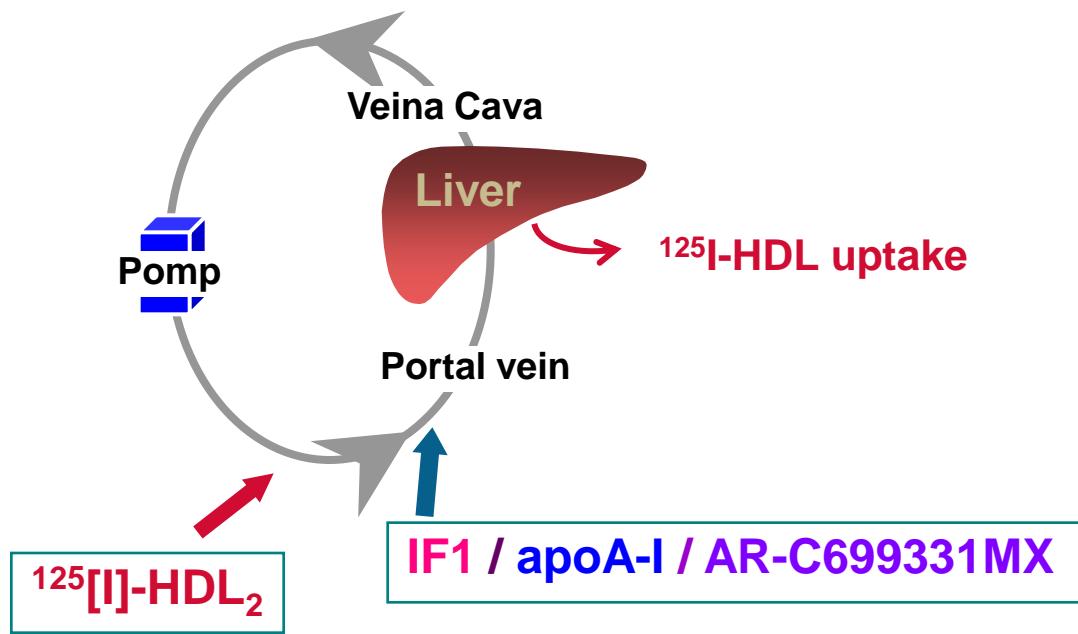
| Parameters | β | p | % of variance explained |
|--|---------------|--------------|-------------------------|
| Triglycerides (g/l) | -0.048 | 0.010 | 4.8 |
| gGT (by 10 UI/l) | 0.007 | 0.001 | 12.8 |
| CRP ≥ 5 mg/l (vs <) | -0.088 | 0.001 | 10.9 |
| BMI ≥ 30 kg/m² (vs <) | -0.069 | 0.004 | 6.3 |
| Alcohol (per 10g/day) | 0.023 | 0.001 | 26.4 |
| Physical activity (high vs low) | 0.045 | 0.010 | 4.7 |
| Current smoker (yes vs no) | -0.067 | 0.008 | 5.2 |
| IF1 (mg/l) | 0.350 | 0.001 | 28.9 |

β = linear regression coefficient

IF1 is a new independent determinant of HDL and apo A-I levels

Exogenous IF1 inhibits hepatic uptake of HDL

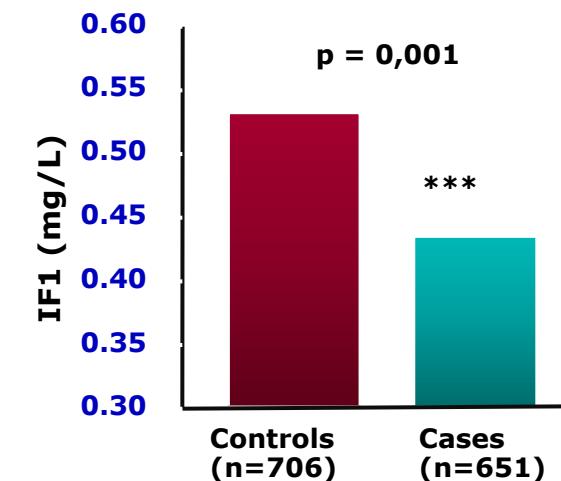
In situ rat liver perfusion with radiolabeled -HDL ± agonists/antagonists



Martinez L.O. et al. (2003) *Nature*

Association between IF1 plasma level and coronaropathy risk

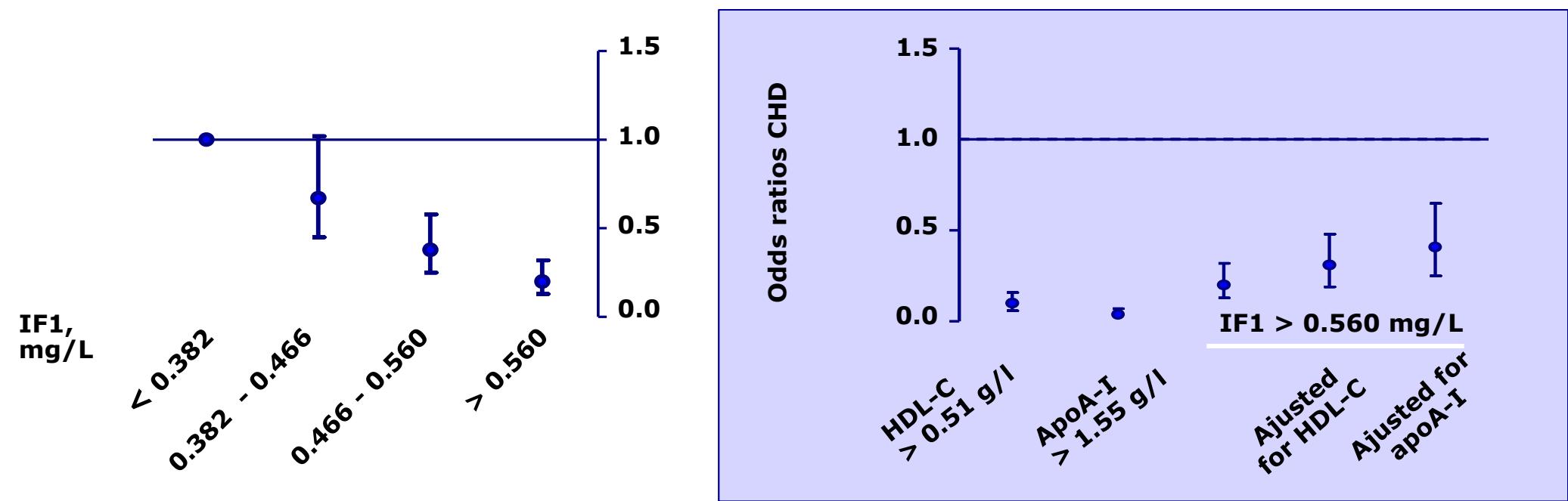
| Parameters | Cases | Controls | p |
|--------------------------------|-------------|-------------|--------------|
| Age (year) | 60.4 | 59.2 | 0.006 |
| Systolic blood pressure (mmHg) | 139.6 | 136.9 | 0.004 |
| Smoking (cig/day) | 3.2 | 1.9 | 0.001 |
| BMI (kg/m ²) | 27.4 | 26.8 | 0.001 |
| Physical activity (high level) | 1.87 | 2.22 | 0.001 |
| CRP (mg/l) | 13.1 | 3.1 | 0.001 |
| Glucose (mmol/l) | 5.94 | 5.47 | 0.001 |
| Insulin (mUI/l) | 14.7 | 10.3 | 0.001 |
| Triglycerides (g/l) | 1.70 | 1.23 | 0.001 |
| Total cholesterol (g/l) | 2.01 | 2.25 | 0.001 |
| HDL-cholesterol (g/l) | 0.39 | 0.50 | 0.001 |
| ApoA-I (g/l) | 1.24 | 1.52 | 0.001 |
| Lp AI (g/l) | 0.47 | 0.56 | 0.001 |
| IF1 (mg/l) | 0.43 | 0.53 | 0.001 |



✓ IF1 serum level is 20% lower in cases as compared to control subjects

Relative risk of coronary heart disease (CHD) as a function of IF1

Adjusted for smoking, physical activity, alcohol consumption, CRP, Lp(a), triglycerides, treatment for dyslipidemia, hypertension, diabetes.

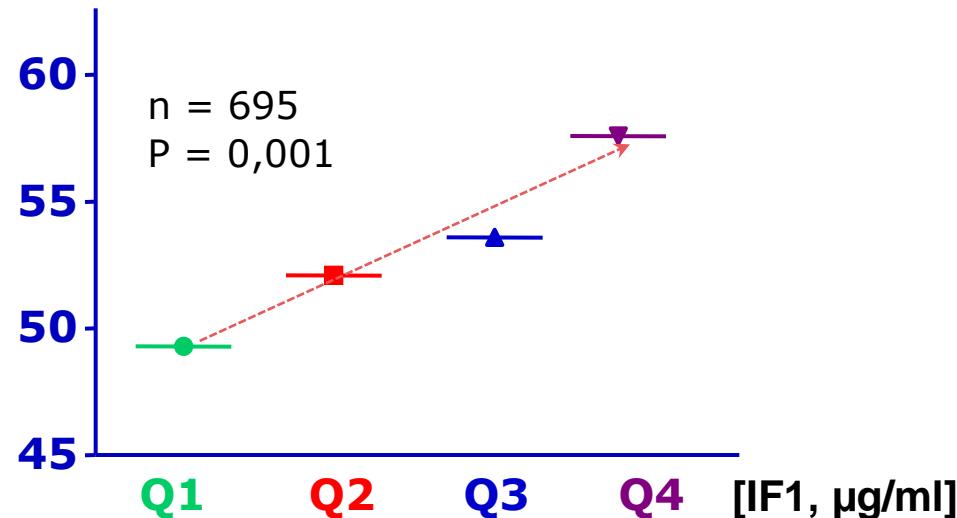


Logistic regression analyses regarding CHD status and studying IF1 as an explanatory variables:
 IF1 is a new **independent determinant of coronary heart disease risk**

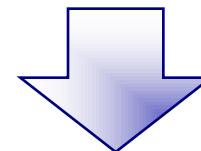
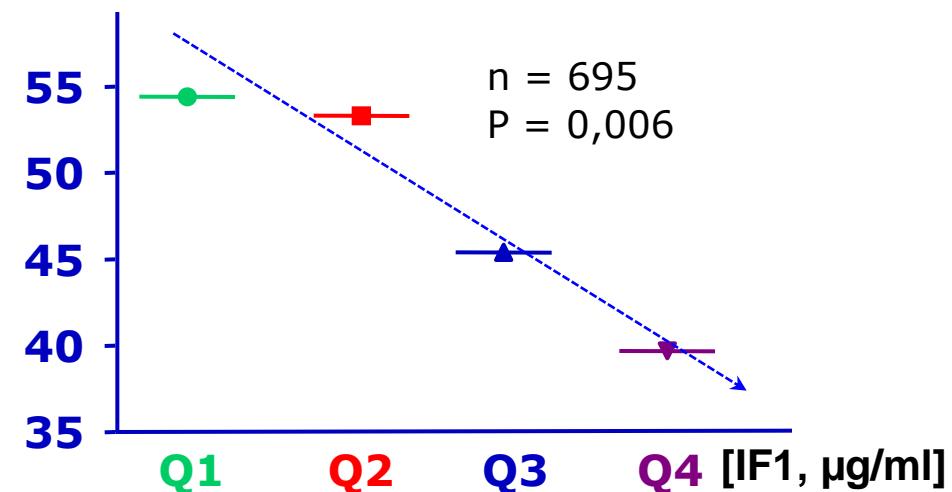
Genoux et al. (2013) J Lipid Res. 54(9):2550-8

Taux d'IF1, fonction myocardique et sévérité de la maladie coronarienne

Fraction d'éjection ventriculaire gauche (%)



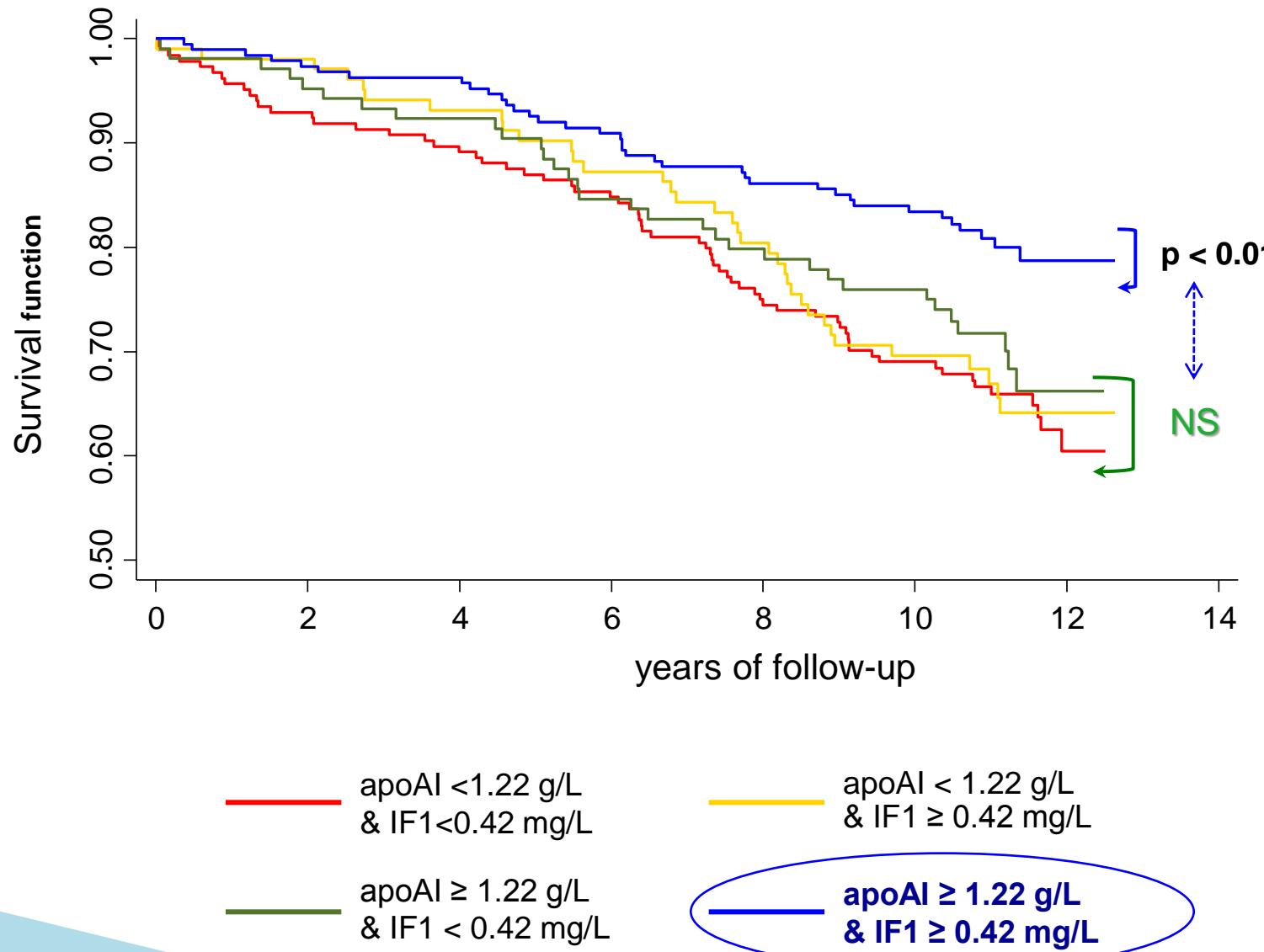
Score de Gensini



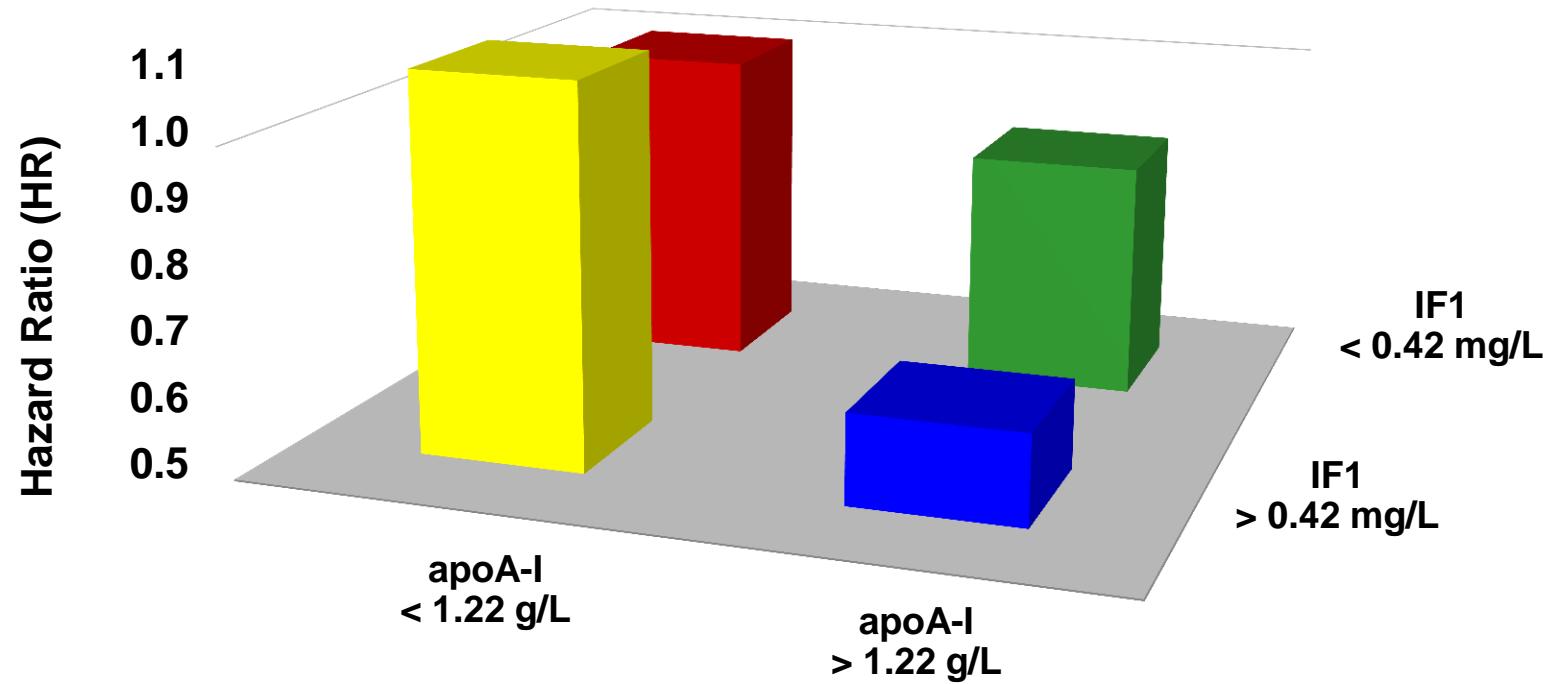
Chez les patients coronariens, le taux d'IF1 est corrélé

- positivement avec la FEVG
- négativement avec la fréquence cardiaque
- négativement avec les scores de sévérité lésionnelle

Kaplan-Meier survival curves according to ApoAI and IF1 combination

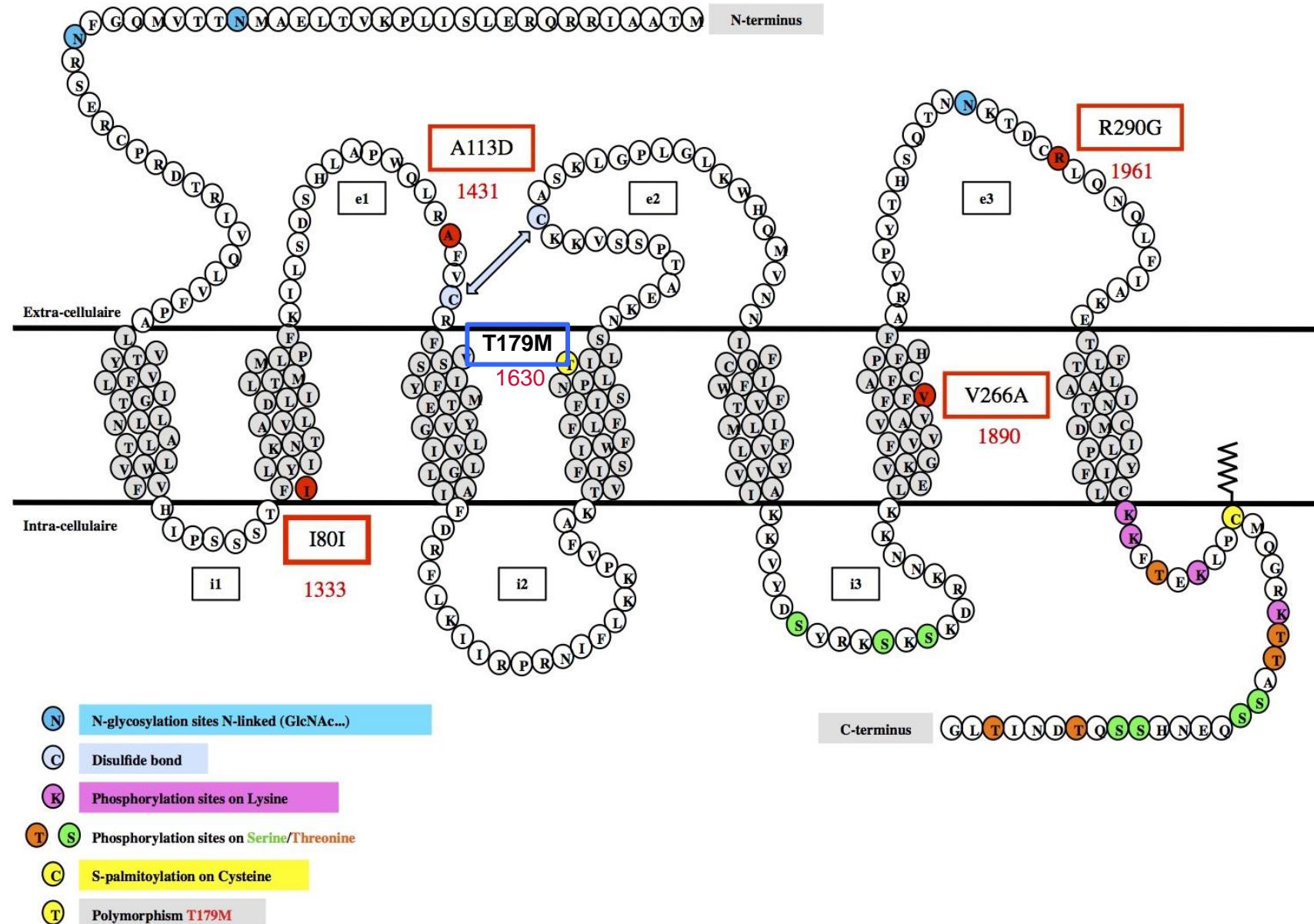


Relative risk of mortality in coronary patients as a function of apo A-I and IF1



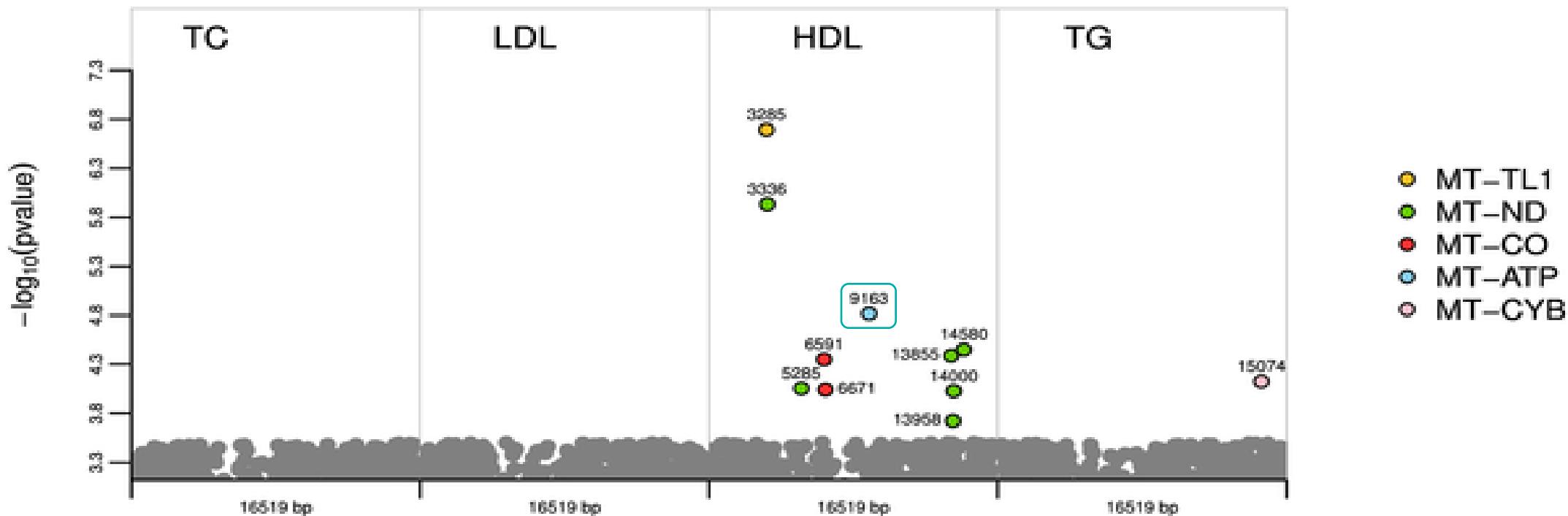
Adjusted on: treatments for dyslipidaemia, diabetes, hypertension, smoking, physical activity, age, waist, CRP, eGFR, γGT, heart rate, ankle-arm index, duration of CAD, Gensini score and LVEF

Séquence du P2Y13 et variants



- ⇒ Fréquence allélique voisine de 0.05
- ⇒ associé à
 - ⇒ augmentation de la masse grasse (**)
 - ⇒ diminution de la fréquence cardiaque (*)
 - ⇒ augmentation de la FEVG (NS)
 - ⇒ meilleur index systolique bras – cheville (*)
- ⇒ augmentation des lipoprotéines à apo AI (*)
- ⇒ augmentation des taux sériques d'IF1 (**)

Illustration by phenotype of the 11 significant mtSNPs

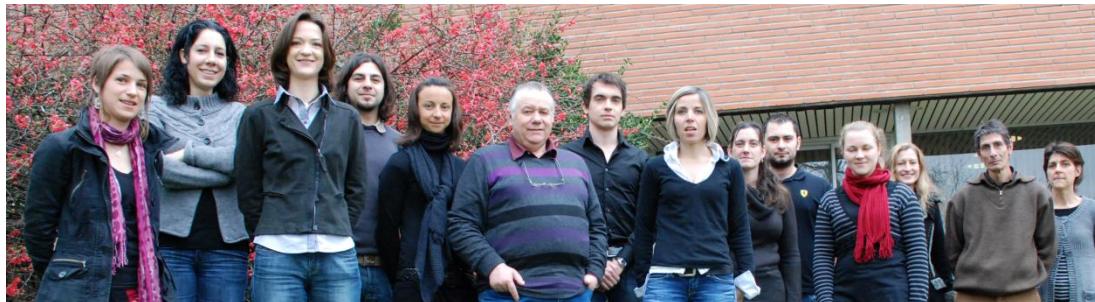


Flaquer A, Rospleszcz S, Reischl E, Zeilinger S, Prokisch H, et al. (2015) Mitochondrial GWA Analysis of Lipid Profile Identifies Genetic Variants to Be Associated with HDL Cholesterol and Triglyceride Levels. PLoS ONE 10(5): e0126294. doi:10.1371/journal.pone.0126294

- Apo A-I interagit spécifiquement avec la chaîne β de la F1-ATP_{ase}, stimulant la formation d'ADP extracellulaire
- IF1 inhibe la liaison et les effets de l'apo A-I
- Apo A-I stimule :
 - la captation hépatique des HDL via P2Y₁₃
 - la prolifération des cellules endothéliales via P2Y₁ et P2Y₁₂
- IF1 sérique est négativement associé au risque cardiovasculaire
- Apo A-I et IF1 sériques sont associés
 - positivement à la fonction myocardique
 - négativement à la sévérité de la maladie coronarienne
- Apo A-I et IF1 ont des effets additifs en prévention secondaire

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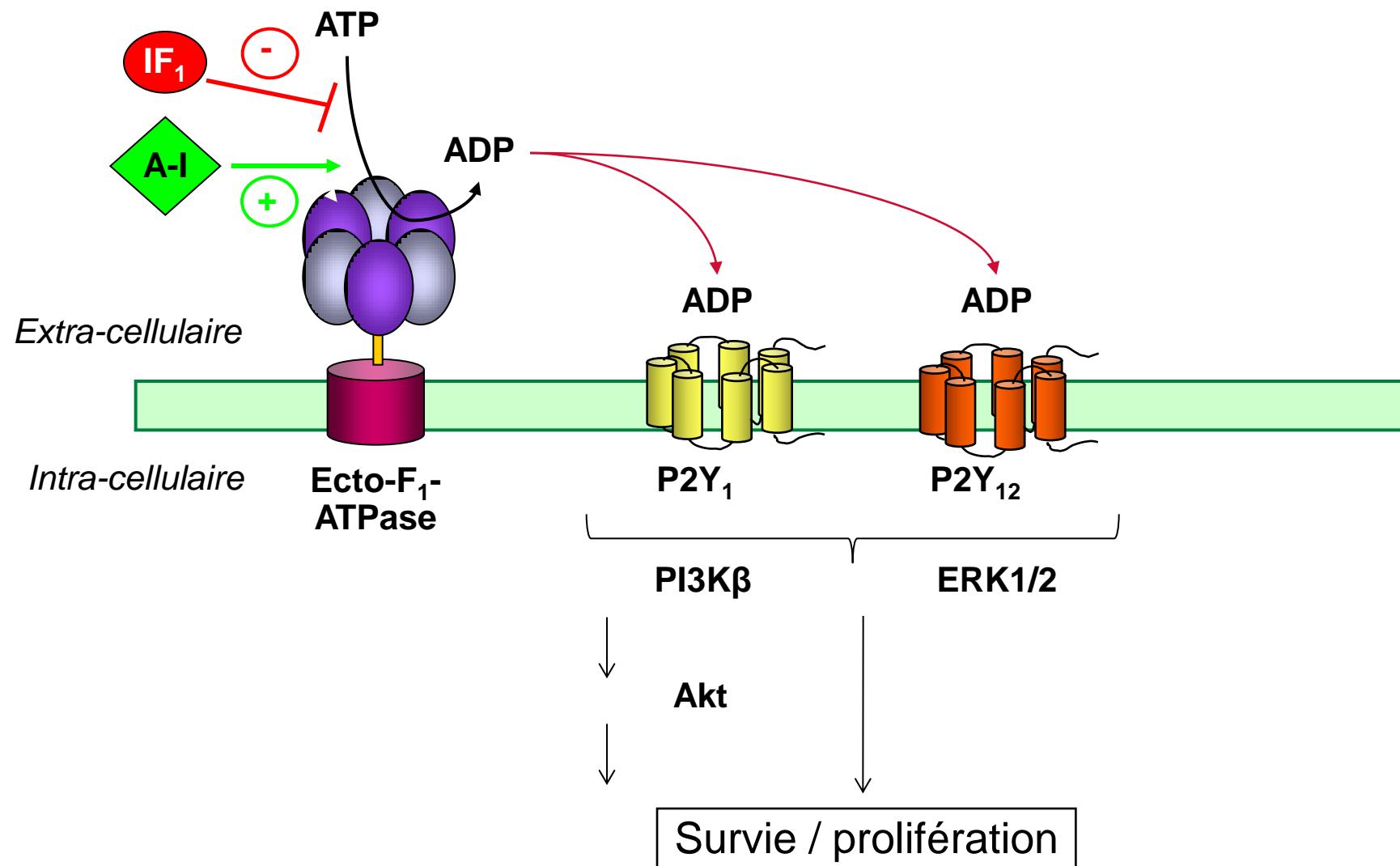
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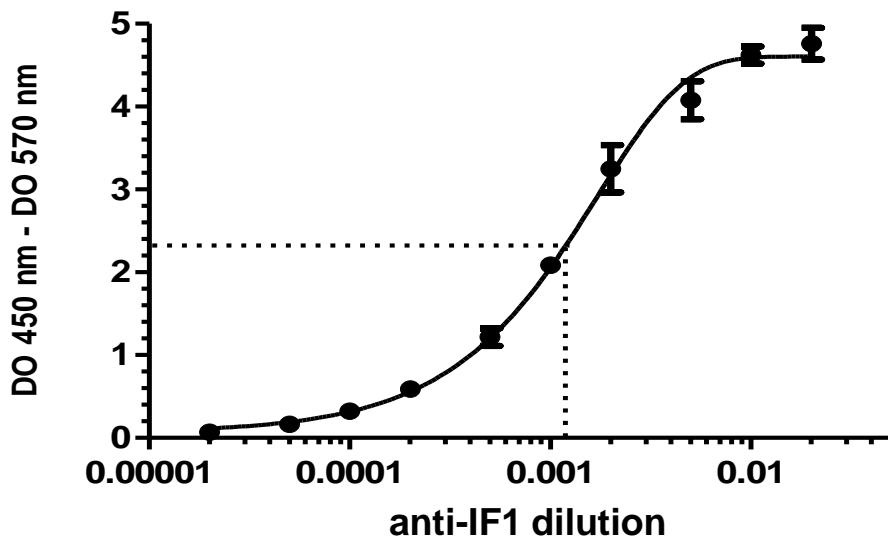
Dr. Frédéric Lopez

Mécanismes d'action de l'apo A-I sur la prolifération endothéliale

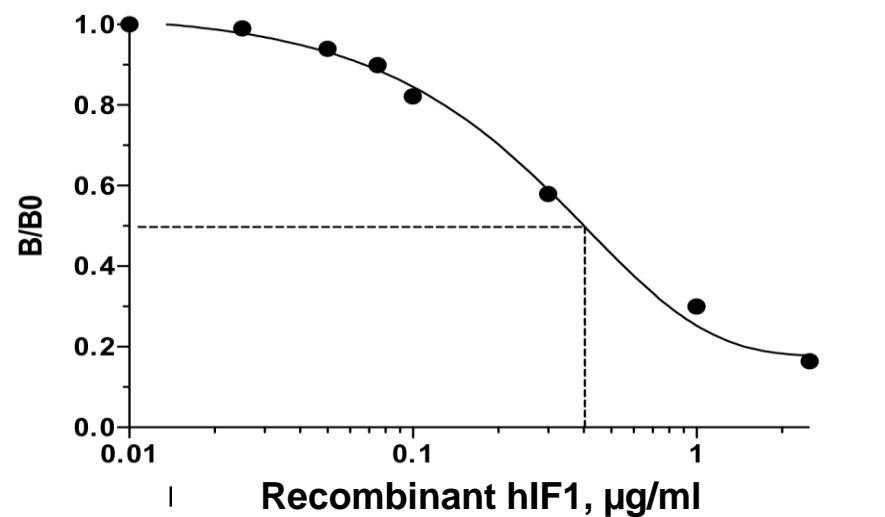


Development of a competitive ELISA immunoassay in human serum

Titration of anti-IF1 (#14J74)

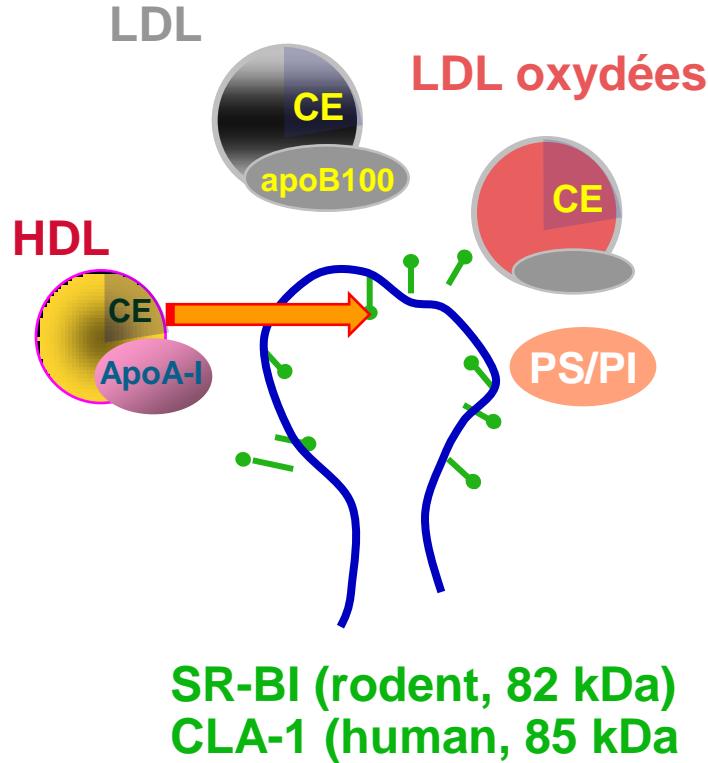


Standard curve used for IF1 quantification



- Repeatability (CV = 5.9 %), reproducibility (CV = 6.8%), competition, overloading and dilution assays, freezing and thawing

Scavenger receptor class B type 1 (SR-BI): un récepteur multi-ligand



- Glycoprotéine de la famille CD36 and LIMP II
(9 sites potentiels de N-glycosylation)
- Expression: foie et tissus stéroïdien
(ovaire, surrénales; régulée par ACTH, LH)
- Liaison des HDL, LDL, LDLac, LDLox et PL
- Impliqué dans la captation sélective du cholestérol estérifié (CE)
- Variants géniques de CLA-1 ont peu d'impact sur les taux de HDL (+ sur les LDL)