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Roles of an HDL-Associated Anti-inflammatory Protein, Progranulin, in Atherosclerosis and Acute Coronary Syndrome

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COI Disclosure
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① ***Consultation fees:*** **Kowa, Skylight Biotec, Astellas-Amgen Japan, Sanofi**

② ***Stock ownership/profit:*** **none**

③ ***Patent fees:*** **none**

④ ***Remuneration for lecture:*** **MSD, Bayer, Kowa**

⑤ ***Manuscript fees:*** **none**

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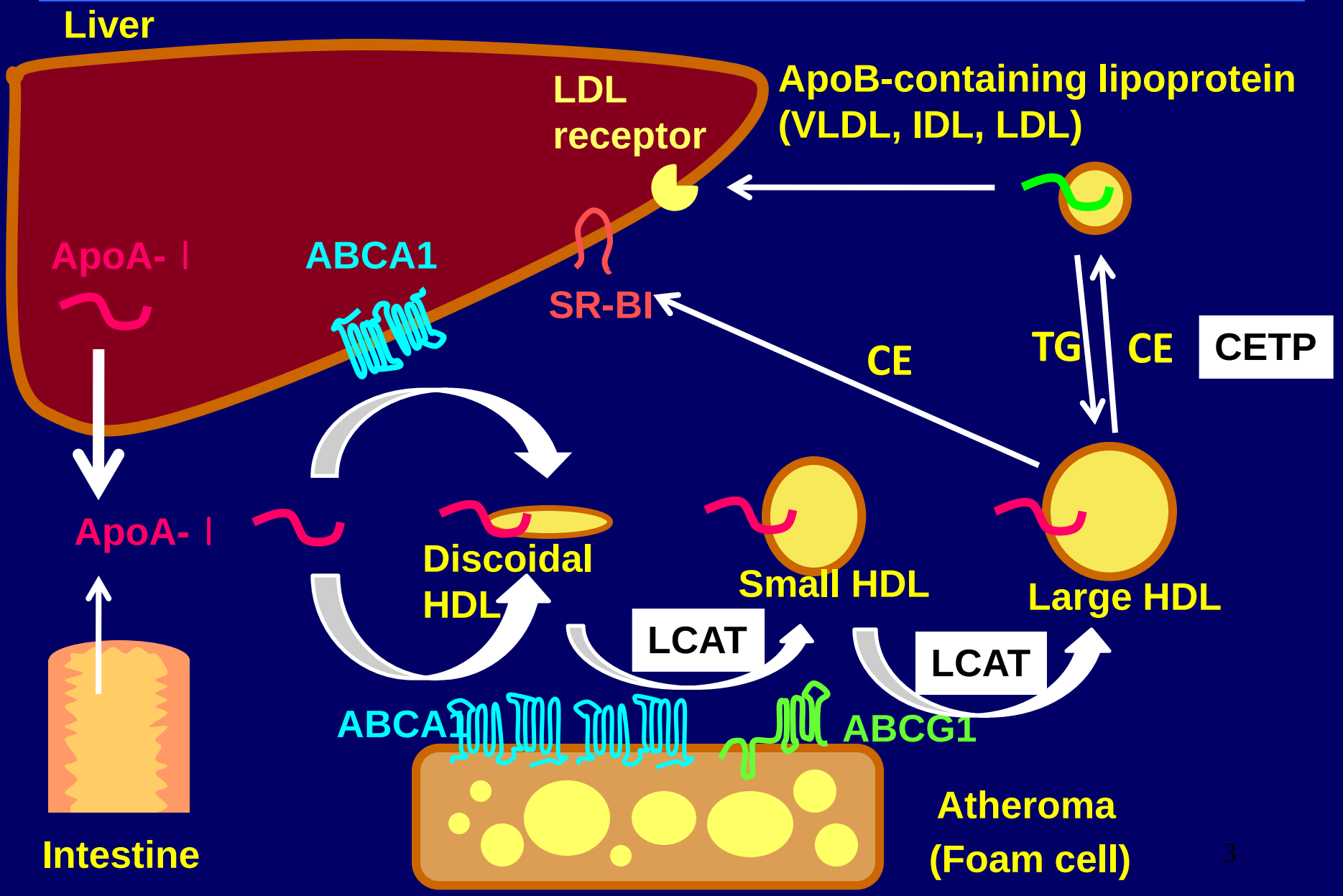
⑦ ***Scholarship fund:*** **none**

⑧ ***Affiliation with endowed department:*** **Izumisano City, Kaizuka City**

⑨ ***Other remuneration such as gifts:*** **none**

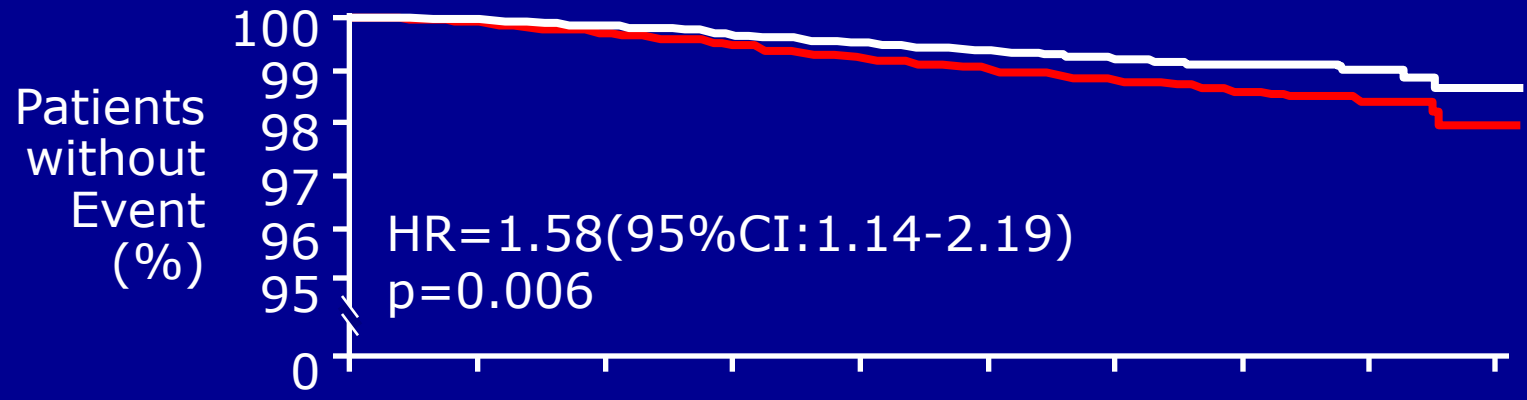
REVERSE CHOLESTEROL TRANSPORT



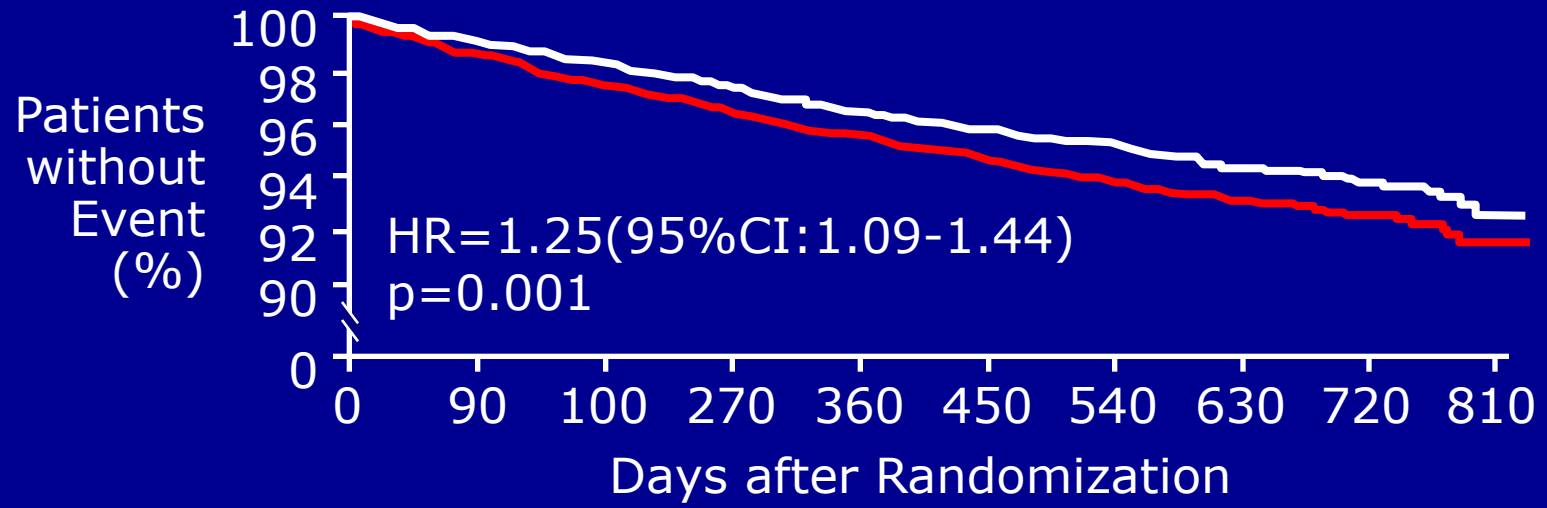
Failure of CETP Inhibitor (ILLUMINATE Study)

Effects of Torcetrapib in Patients at High Risk for Coronary Events

- Death from Any Cause



- Primary composite outcome(Major Cardiovascular Events)



: Atorvastatin(n=7,534) : Atorvastatin+Torcetrapib(n=7,533)

Barter PJ et al for the ILLUMINATE Investigators: N Engl J Med 2007; 357 :2109-2122

Anti-atherogenic Actions of HDL

Cellular cholesterol efflux & reverse cholesterol transport

Anti-inflammatory activity

Anti-diabetic

Anti-oxidative activity

Anti-apoptotic activity

Vasodilatory activity

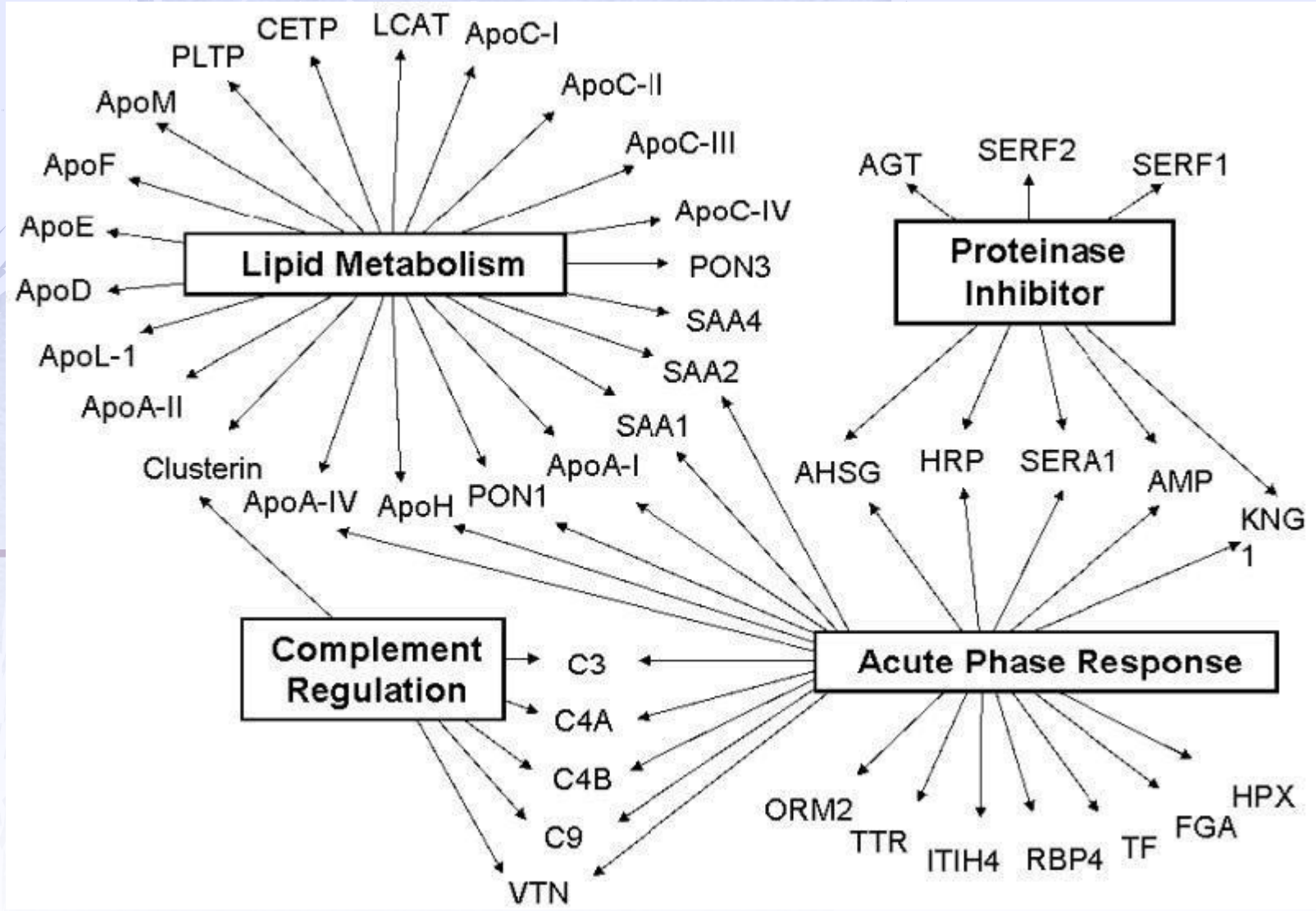
Endothelial repair

Anti-thrombotic activity

Anti-infectious activity

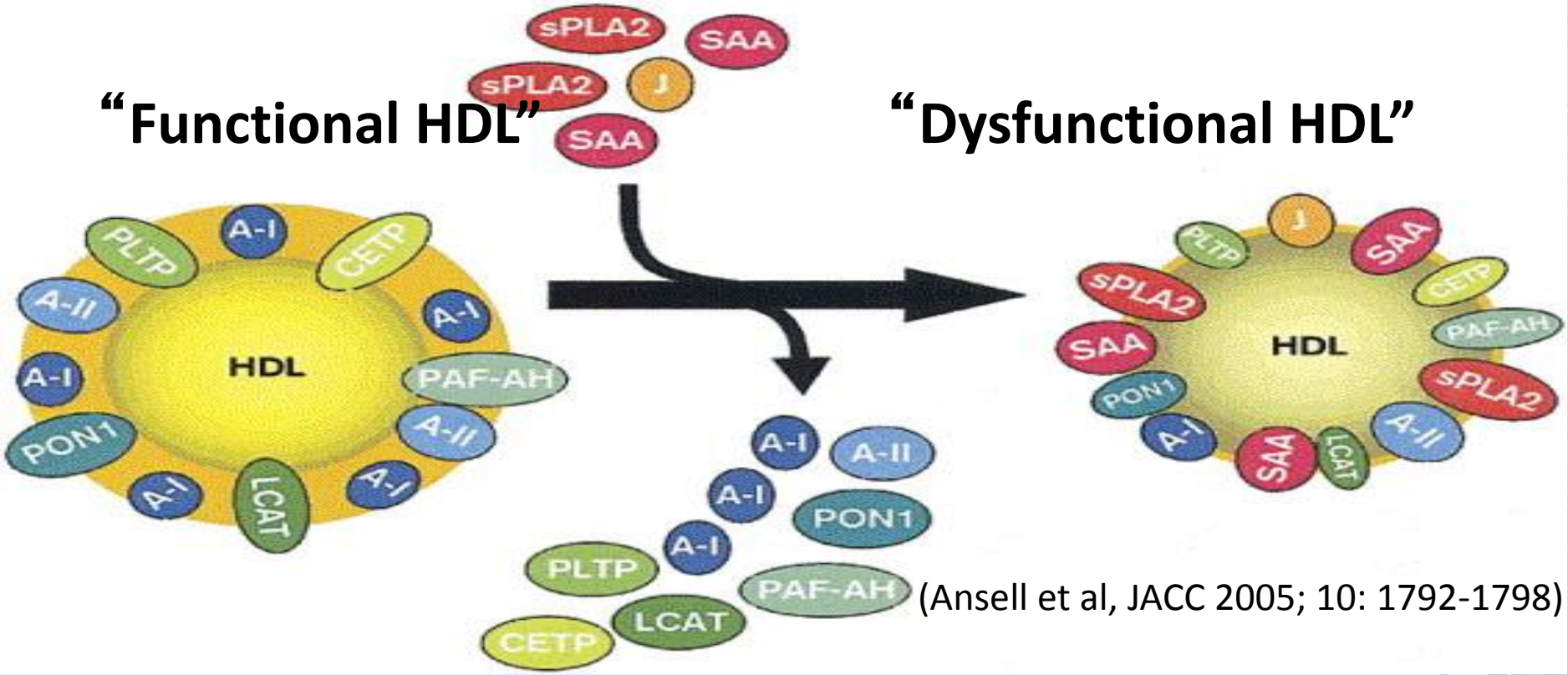
Yamashita S: J Atheroscler Thromb 17:436-451, 2010

Functions of HDL-Associated Proteins



ATVB 2009

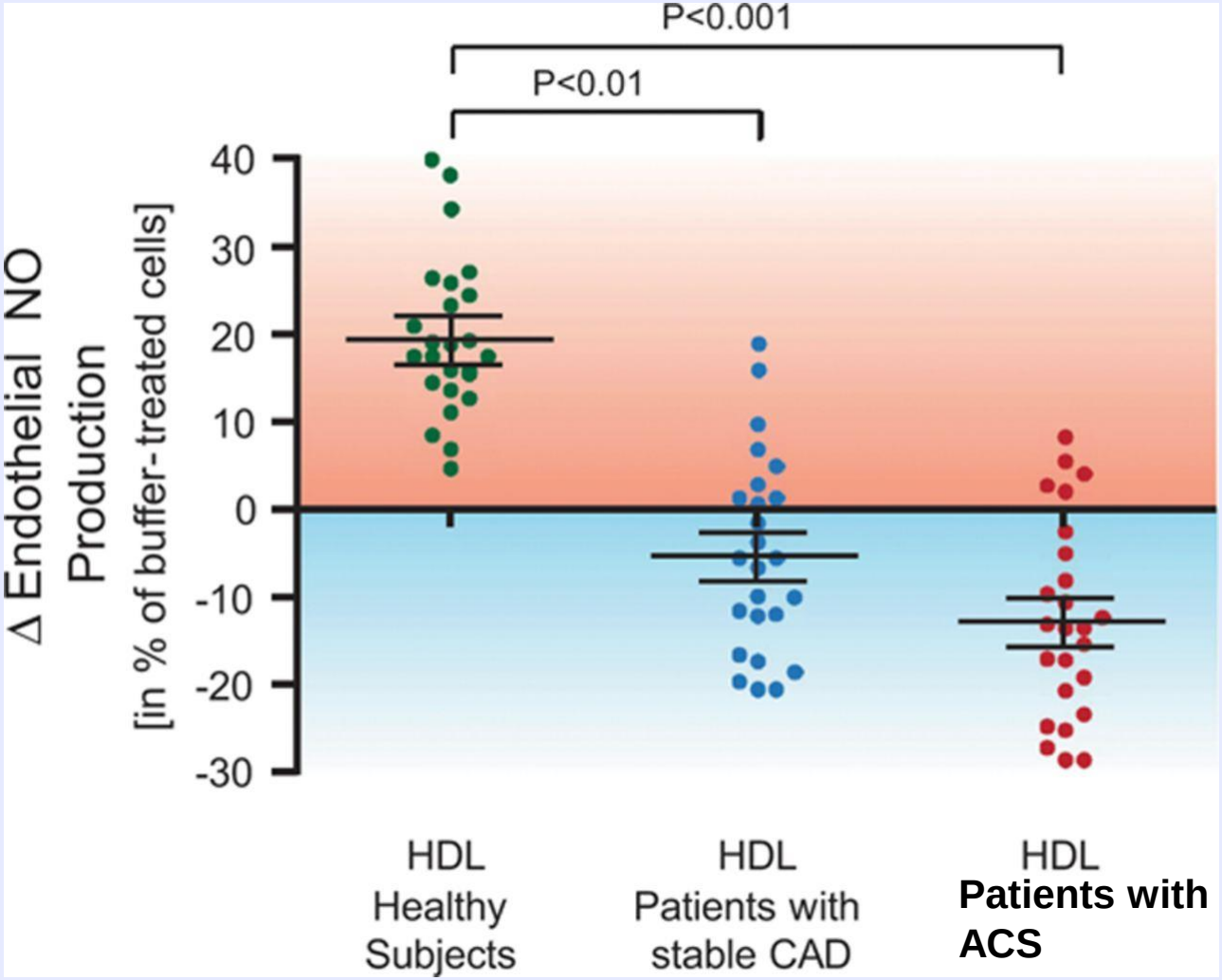
Functional HDL and Dysfunctional HDL



(Ansell et al, JACC 2005; 10: 1792-1798)

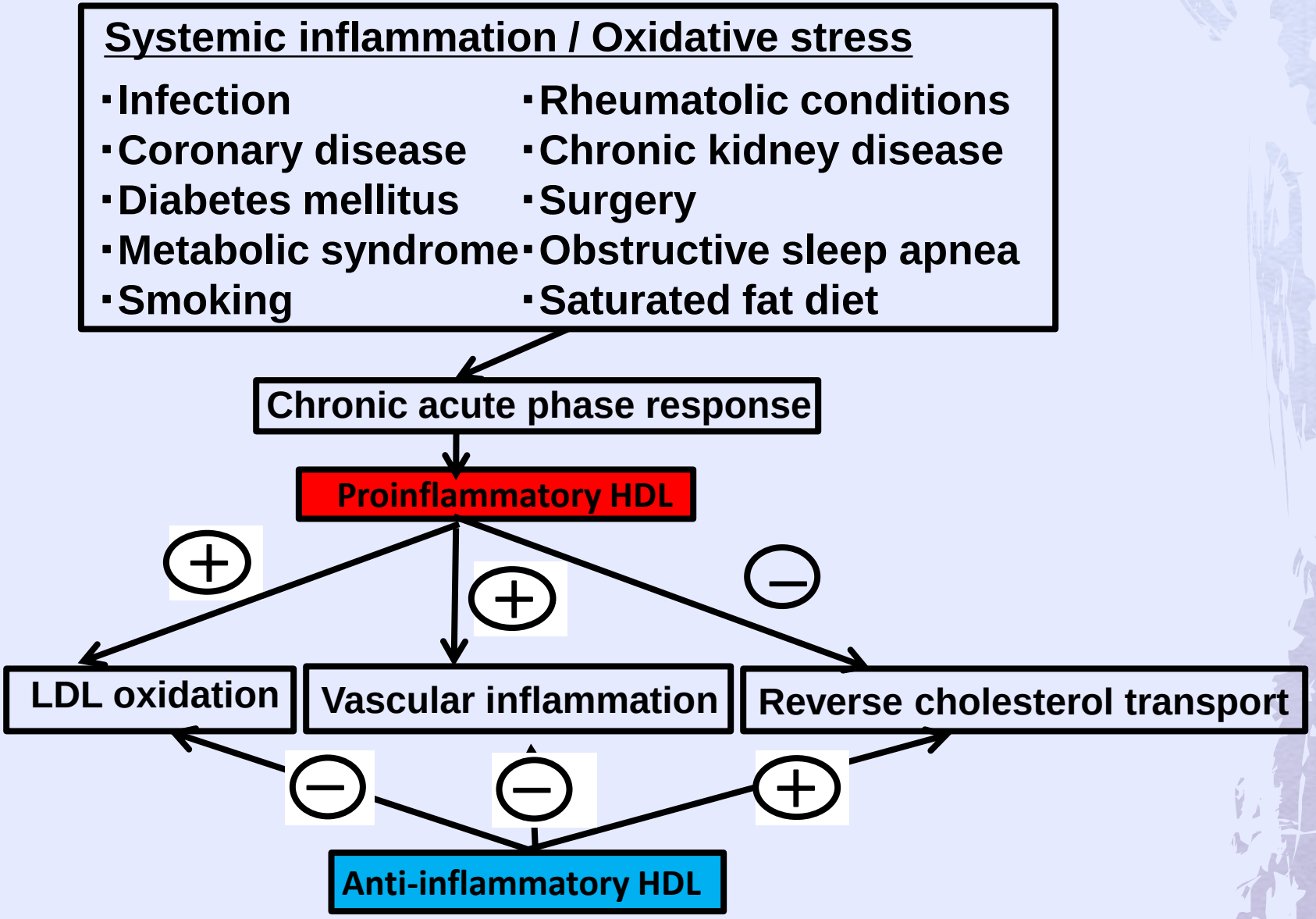
Quality is more important than Quantity?
Composition of HDL is important for playing its proper role?

Effects of HDL Obtained from Healthy Subjects, Patients with CAD or Acute Coronary Syndrome on NO Release from Human Aortic Endothelial Cells



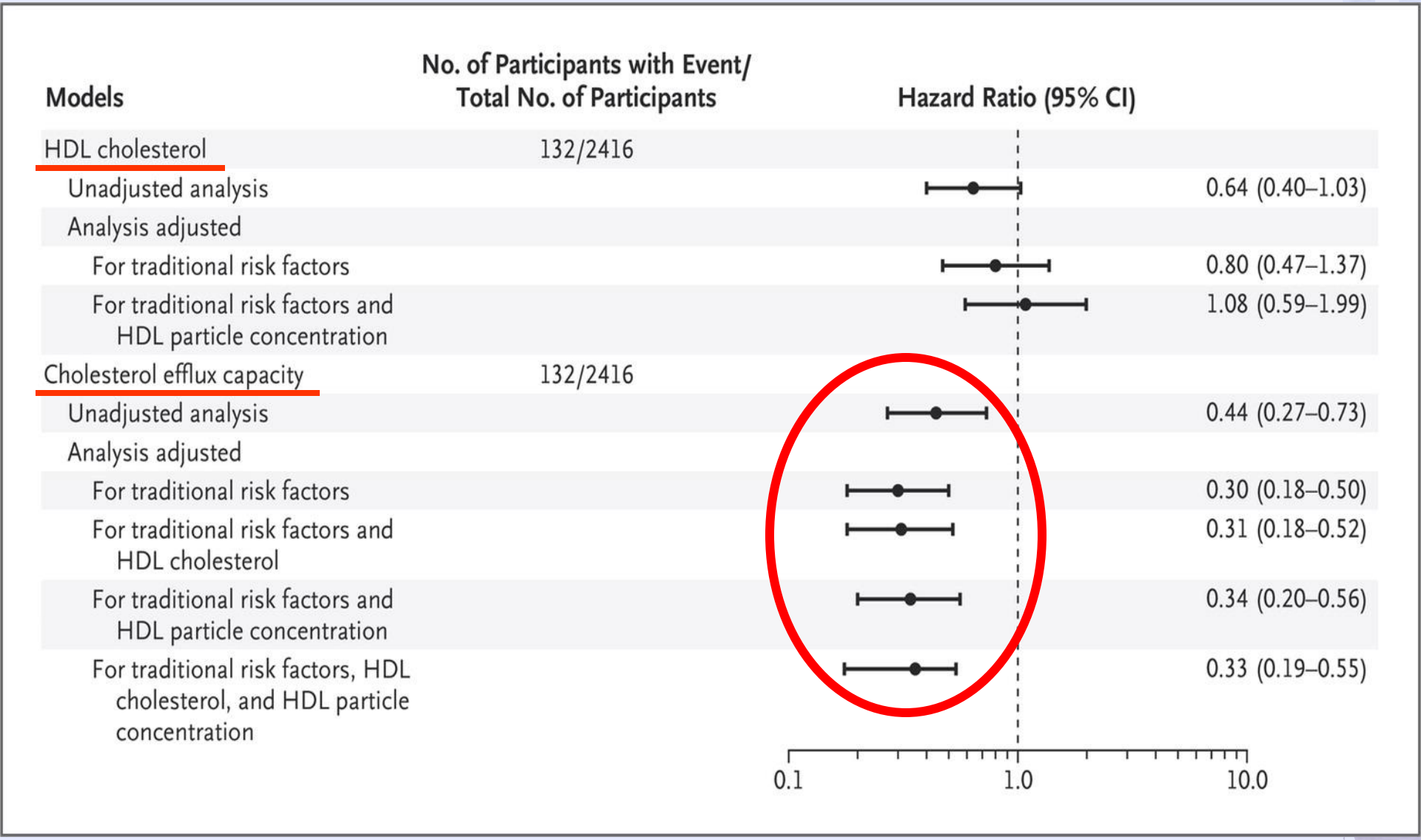
Lüscher T et al: Circulation Research 114:171-182, 2014

Etiology of Dysfunctional HDL



Ansell et al, Curr Opin Lipidol 18: 157-163, 2007

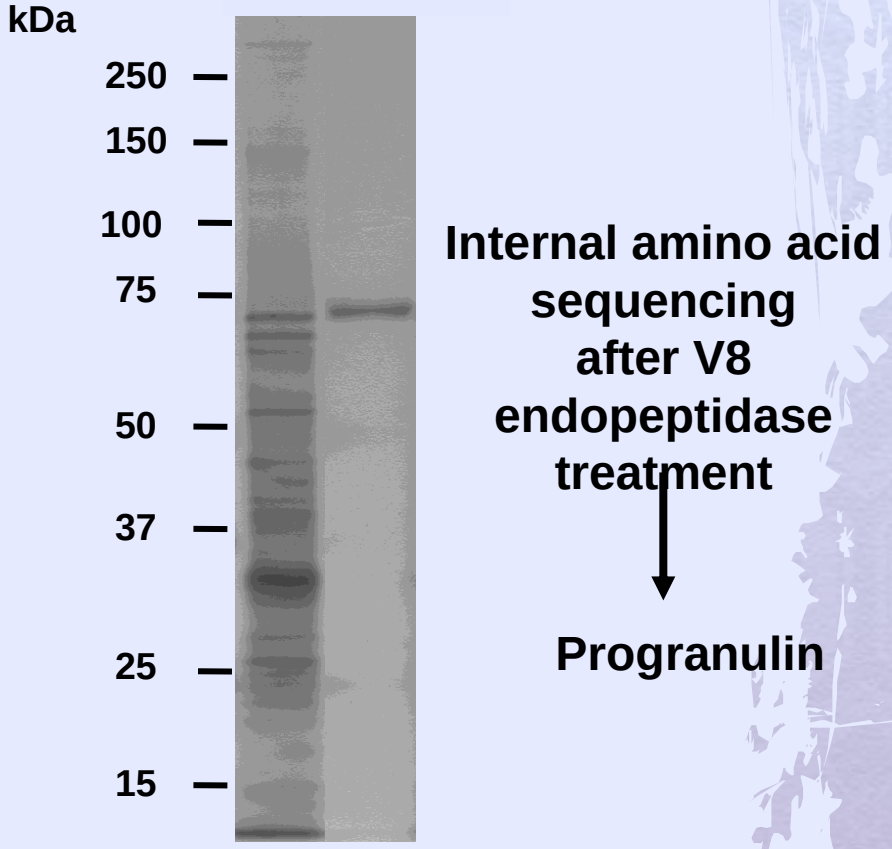
HDL Cholesterol Efflux Capacity and Incident Cardiovascular Events



Rohatgi A, et al: NEJM 371:2383-2393, 2014

HDL/Apolipoprotein A-I Binds to Macrophage-Derived Progranulin and Suppresses its Conversion into Proinflammatory Granulins

Okura H, Matsuyama A, Yamashita S, et al: Journal of Atherosclerosis and Thrombosis 17: 2010



HDL/Apolipoprotein A-I Binds to Macrophage-Derived Progranulin and Suppresses Its Conversion into Proinflammatory Granulins

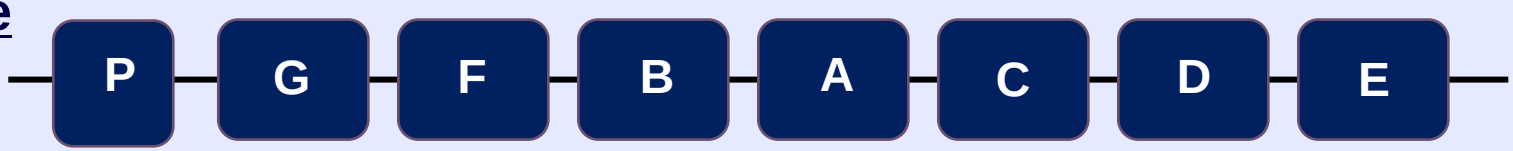
- Progranulin derived from macrophages is bound to Apolipoprotein A1
- Granulin, which is the cleaved product of progranulin, released proinflammatory cytokines from macrophages
- Progranulin associated with apolipoprotein A1 might suppress the conversion into proinflammatory granulin, leading to inhibition of inflammation

Okura H et al: Journal of Atherosclerosis and Thrombosis 2010

Progranulin Is a Multifunctional Protein

< Progranulin >

Structure



Tissue Distribution

Progranulin ubiquitously expresses in almost all tissues

Expression levels of PGRN are increased in some stress conditions such as hypoxia, acidosis and age

Functions

Growth factor (neuronal growth factor)	BBRC 1990
Cancer progression	J Mol Med 2003
Wound healing	Nat Med 2003
Systemic inflammation	Cell 2002

Phenotypes of Reduced Progranulin Levels in Humans and Mice

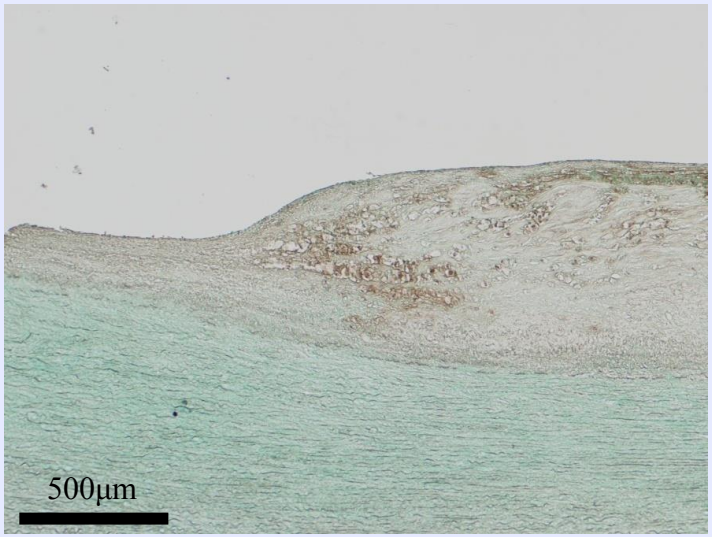
Consequenses of Reduced Progranulin Levels

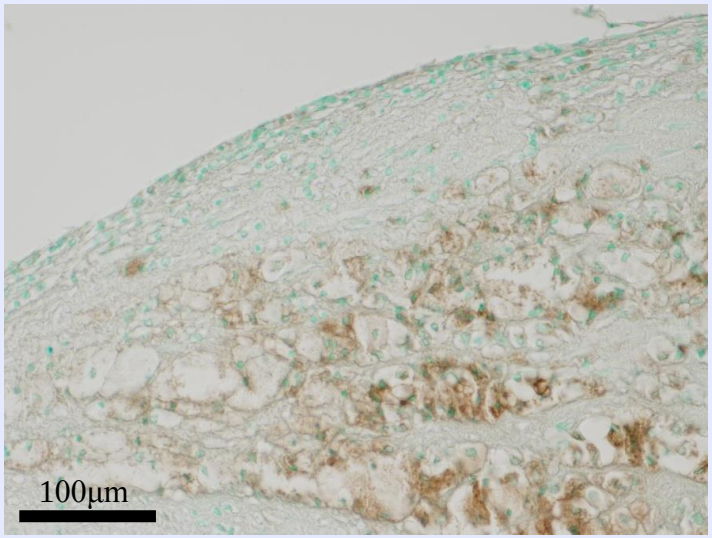
- **Cause Neurological Diseases (Affected Neurons and Microglia)**
  - Heterozygous deficiency causes frontotemporal dementia in humans (Nature 2006)
  - Homozygous deficiency causes neuronal ceroid lipofuscinosis in mice (Am J Hum Genet 2012)
  - Reduced progranulin levels might be a risk factor for Alzheimer disease (JAMA Neurol 2013)
- **Modulate Metabolic Diseases (Affected Adipocytes and Macrophages)**
  - Homozygous deficiency protects against diet-induced obesity and insulin resistance in mice (Cell Metab 2012)

Trends in Endocrinology & Metabolism 2013

1. Roles of an HDL-associated Anti-inflammatory Protein, Progranulin, in Atherosclerosis

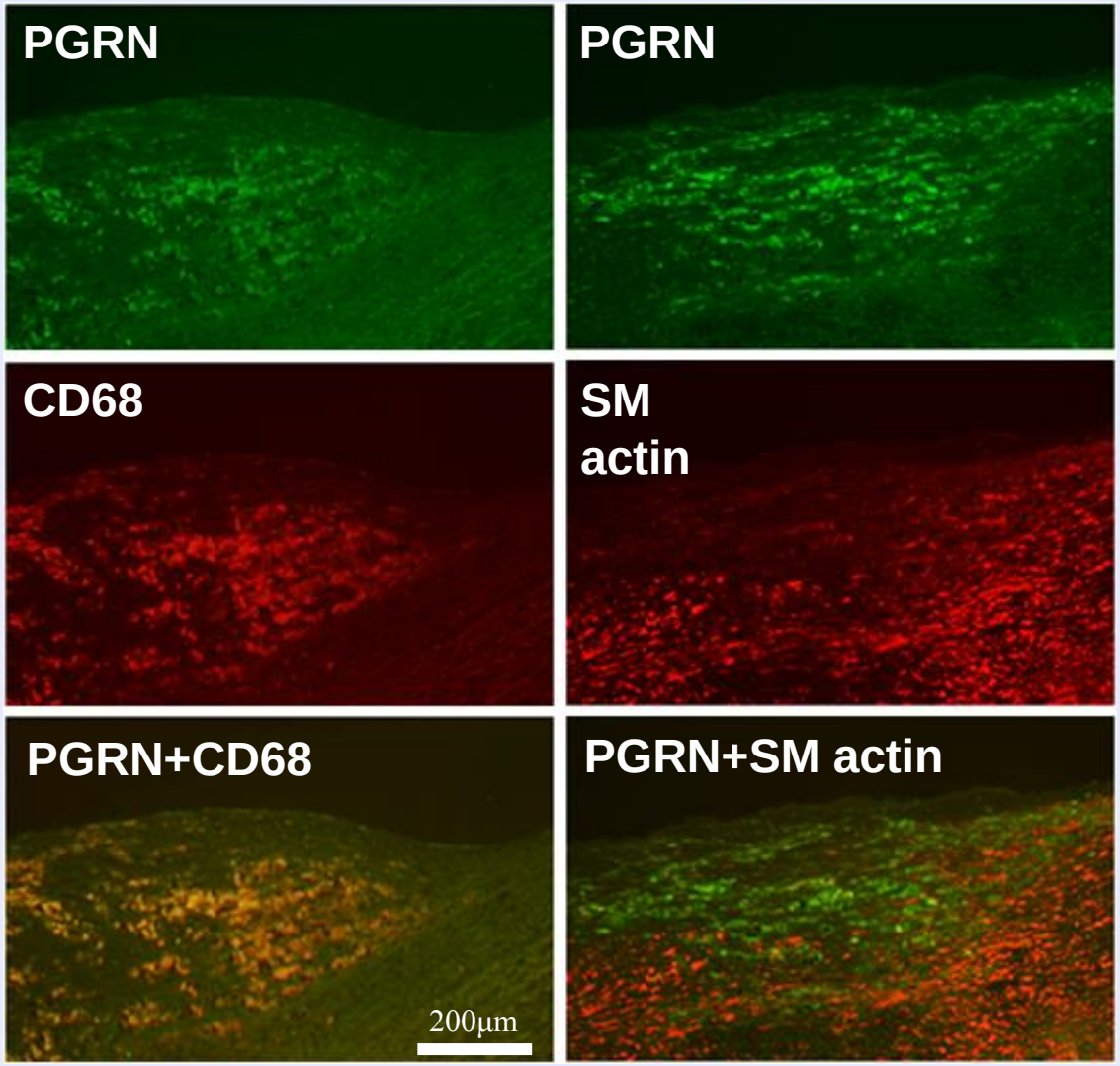
PGRN Was Expressed in Foam Cells of Human Aortic Atherosclerotic Plaques





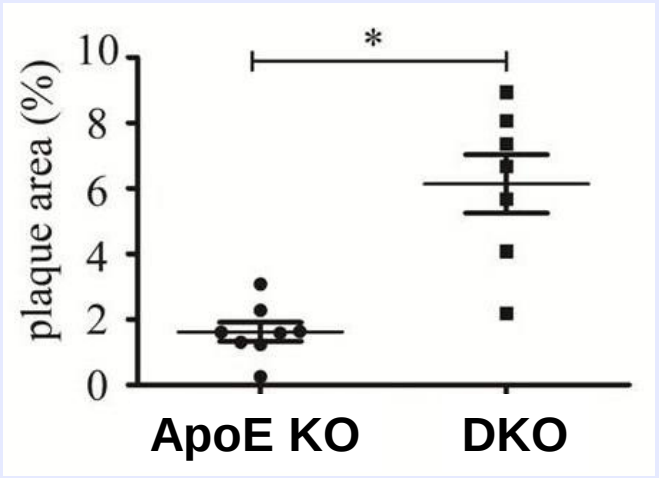
Kawase R, et al: Cardiovasc Res 2013

PGRN Is Co-localized with CD68 to a Greater Extent Than with Smooth Muscle Actin



Kawase R, et al: Cardiovasc Res 2013

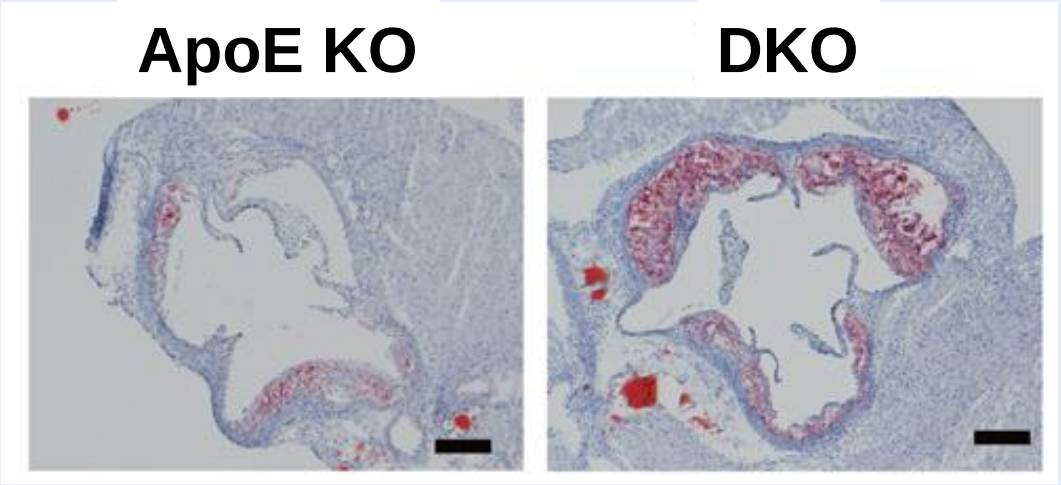
Deletion of Progranulin Exacerbates Atherosclerosis (1)



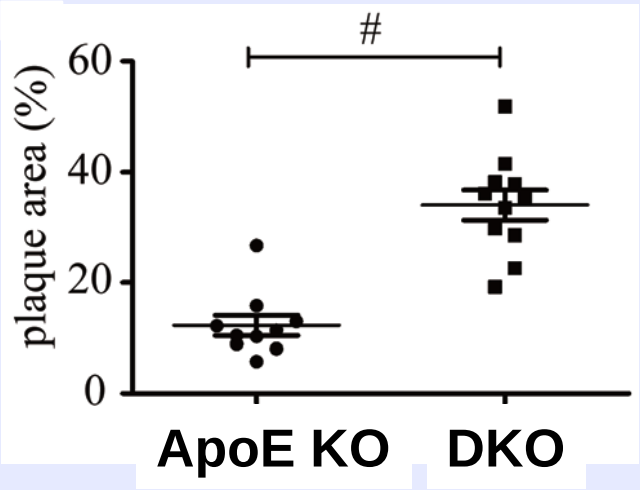
* p<0.0005

Kawase R, et al: Cardiovasc Res 2013

Deletion of Progranulin Exacerbates Atherosclerosis (2)



Bar: 500μm

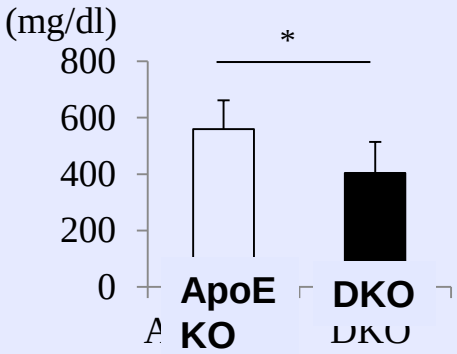


# p<0.0001

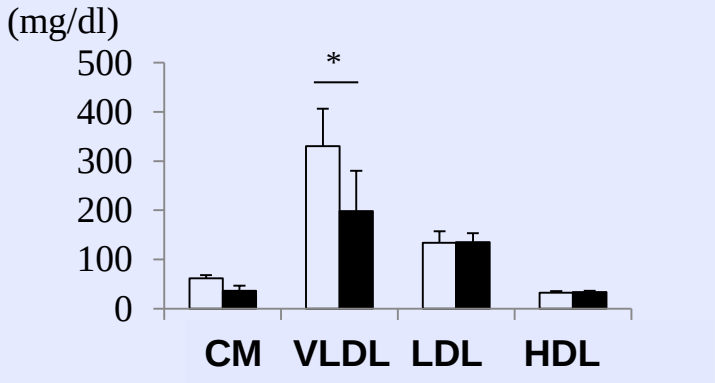
Kawase R, et al: Cardiovasc Res 2013

Deletion of PGRN Leads To Severe Atherosclerosis Despite Lower Plasma Cholesterol Level

A **Total Cholesterol**

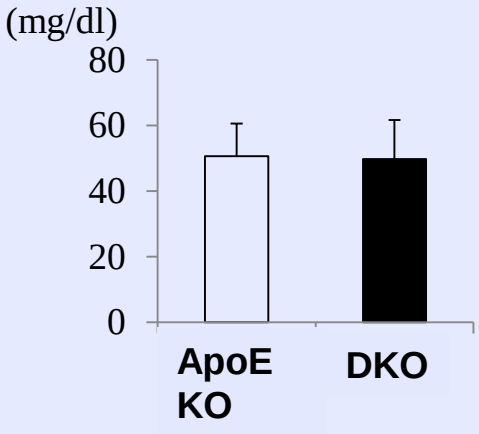


B **Cholesterol**

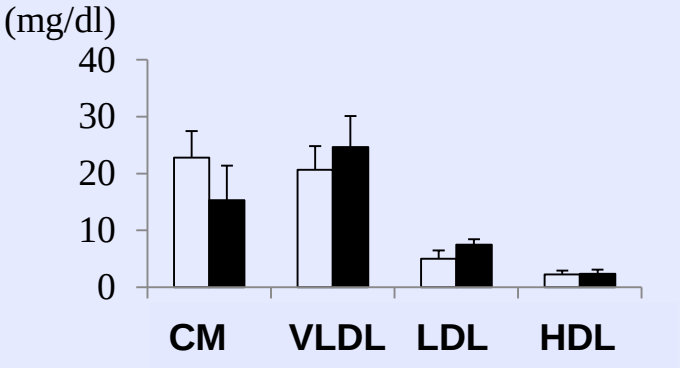


*P <0.005

C **Triglyceride**



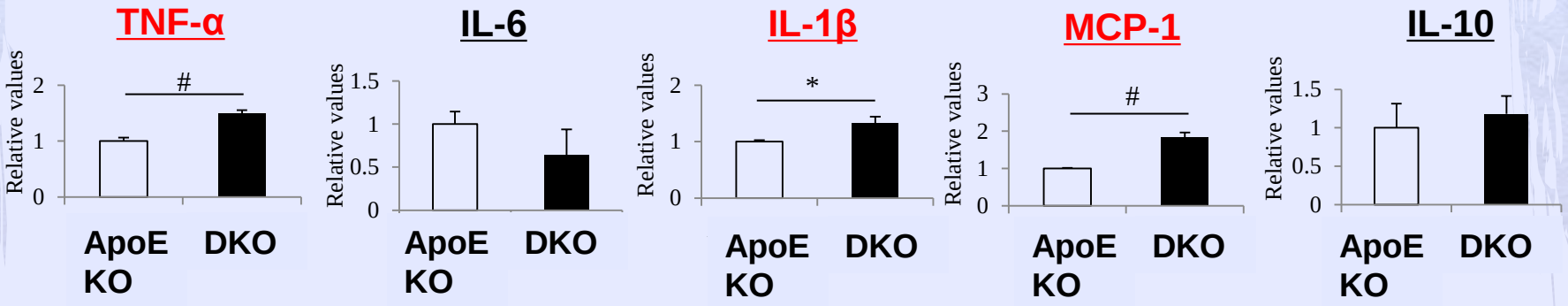
D **Triglyceride**



Kawase R, et al: Cardiovasc Res 2013

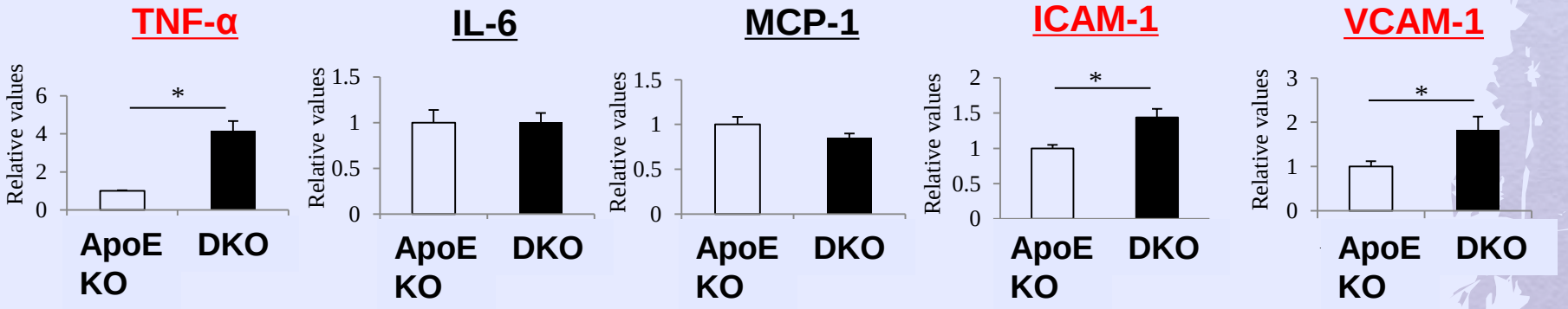
DKO Mice Exhibit Increased Expression of Proinflammatory Cytokines

A mRNA Expression Levels in the Liver



*P < 0.05 and #P < 0.01 compared with ApoE KO mice

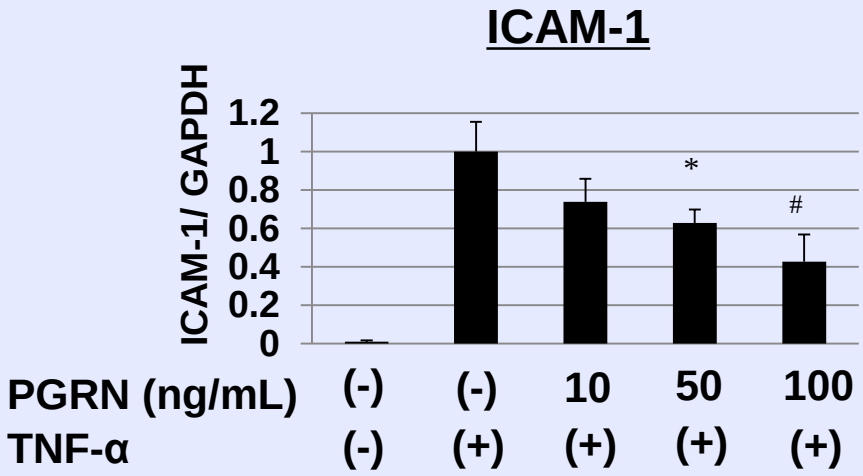
B mRNA Expression Levels in the Aorta

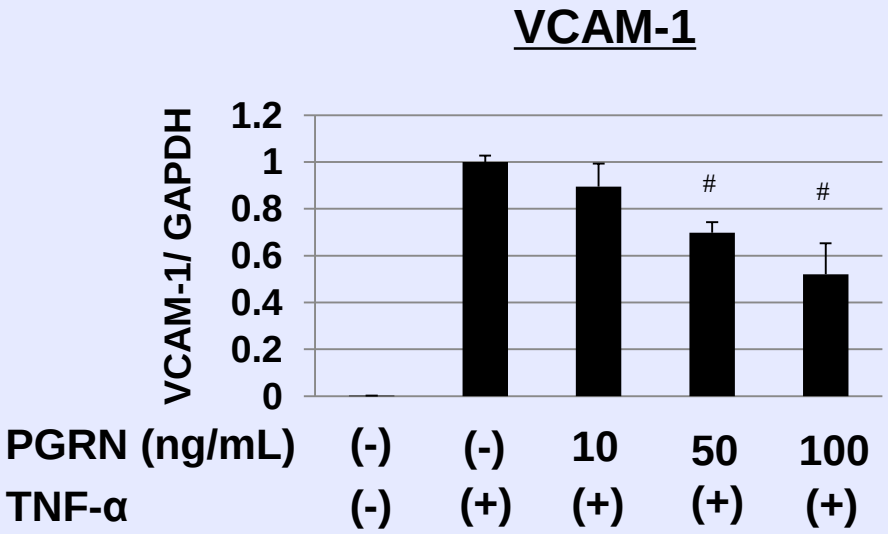


*P < 0.005, compared with ApoE KO mice

Kawase R, et al: Cardiovasc Res 2013

Progranulin Suppresses TNF-α-induced Expression of ICAM-1 and VCAM-1 in HUVEC

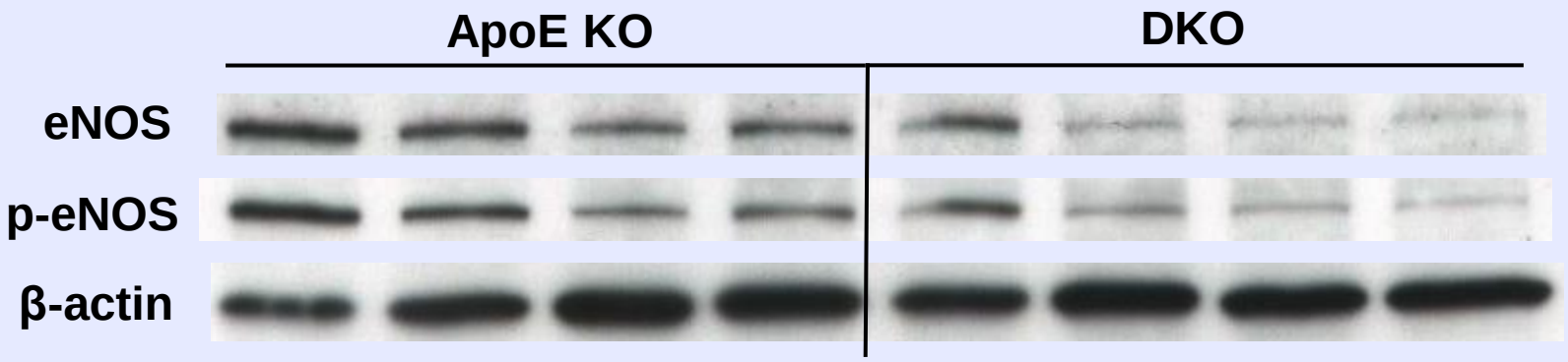




*P <0.05 and #P <0.01, compared with TNF-α treatment alone

Kawase R, et al: Cardiovasc Res 2013

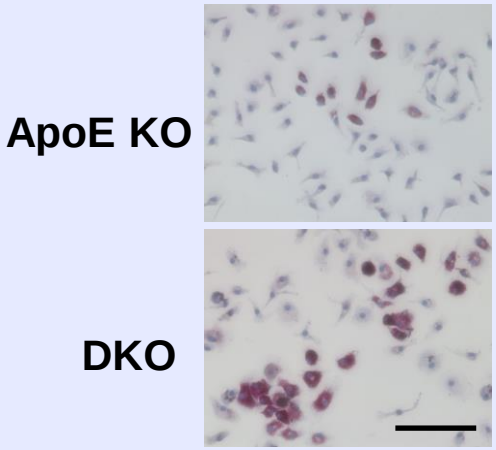
DKO Mice Exhibit Decreased Expression of Endothelial NOS (eNOS) in the Aorta

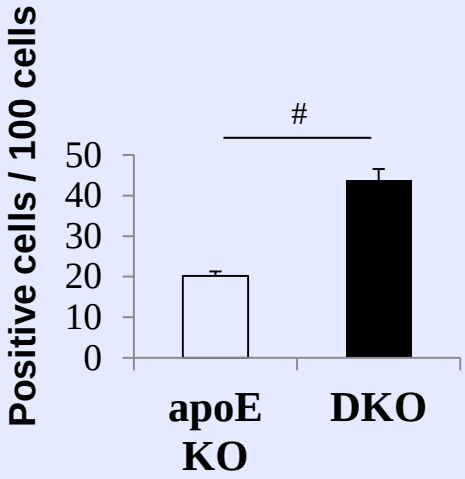


Kawase R, et al: Cardiovasc Res 2013

Lack of Progranulin Leads to Accumulation of Excessive Cholesterol in Macrophages

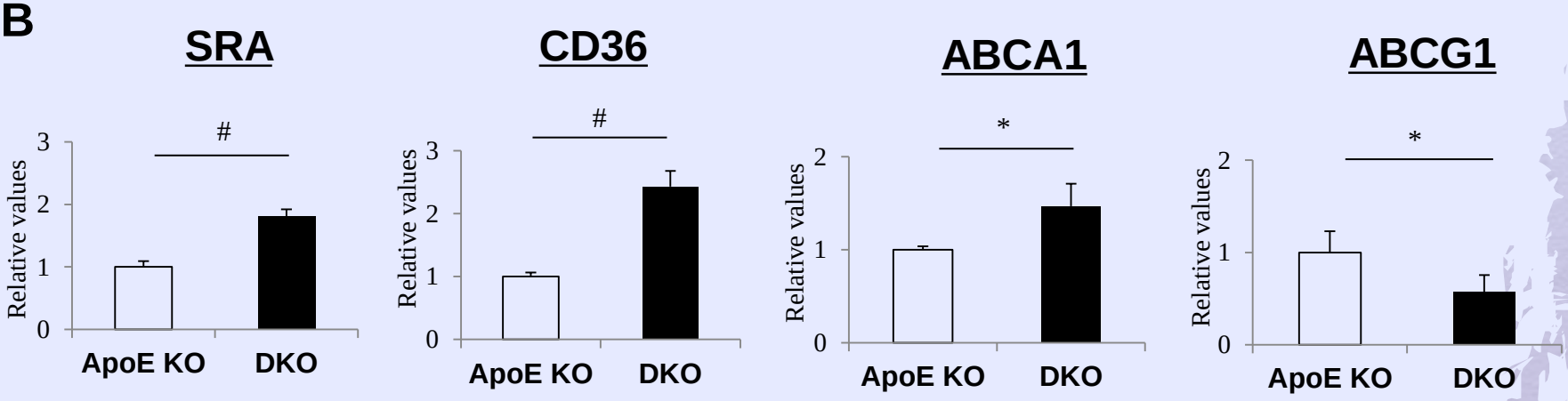
A





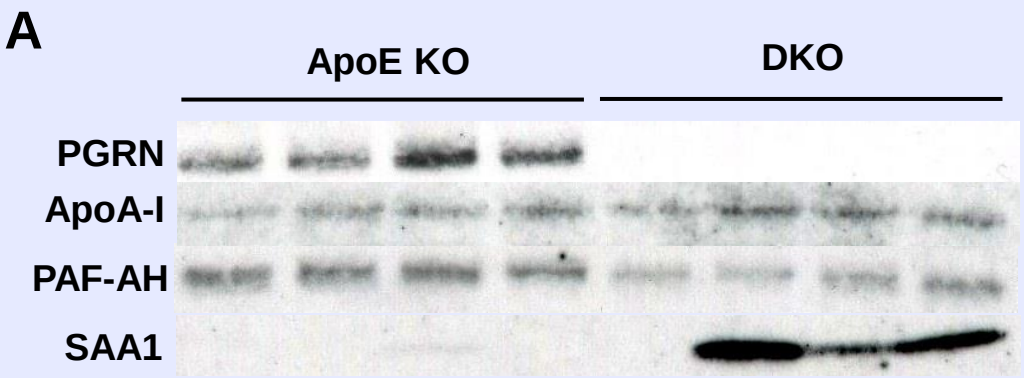
*P <0.005, #P <0.05 compared with ApoE KO mice

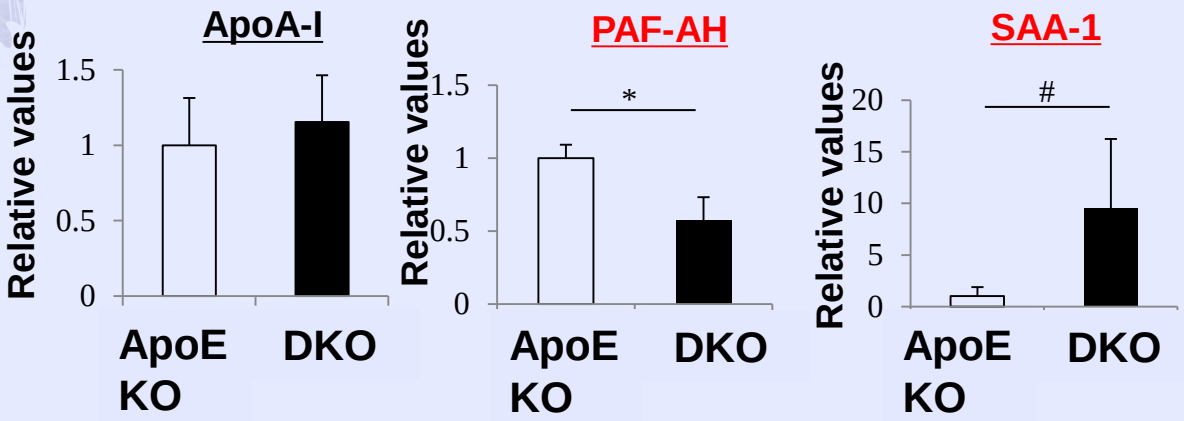
B

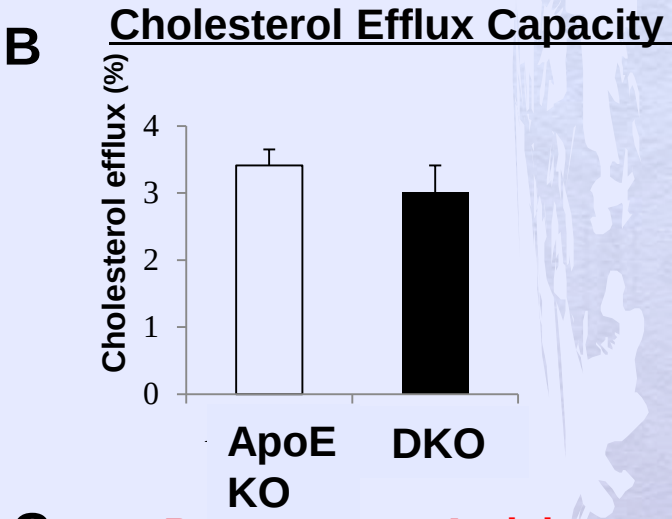


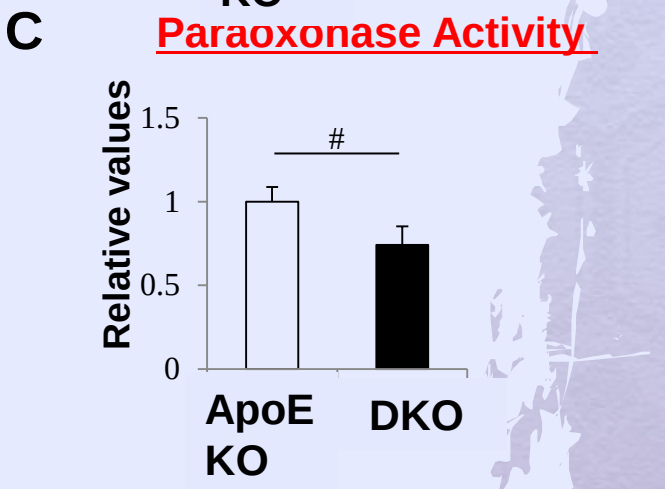
Kawase R, et al: Cardiovasc Res 2013

Lack of Progranulin Altered the Proportion of HDL-Associated Proteins





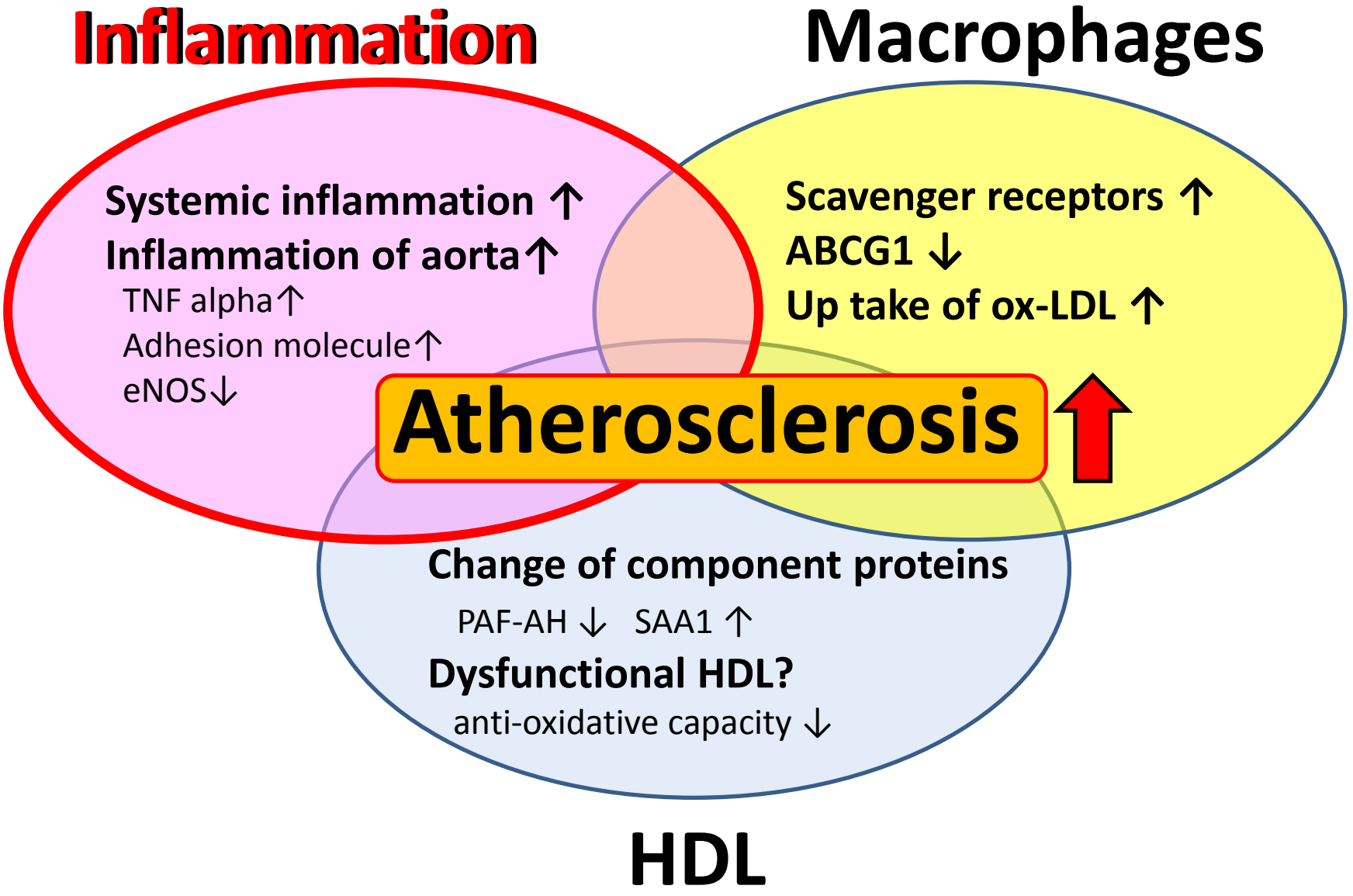




*P <0.005, #P <0.05 compared with ApoE KO mice

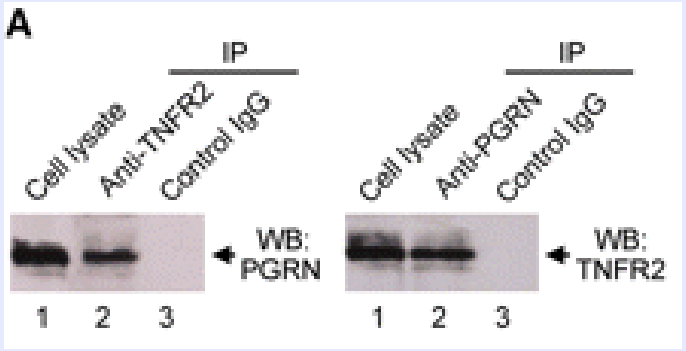
Kawase R, et al: Cardiovasc Res 2013

Suggested Mechanisms for Atherosclerosis in Progranulin Deficiency



PGRN Directly Binds to TNFR and Antagonizes TNFα Actions

A



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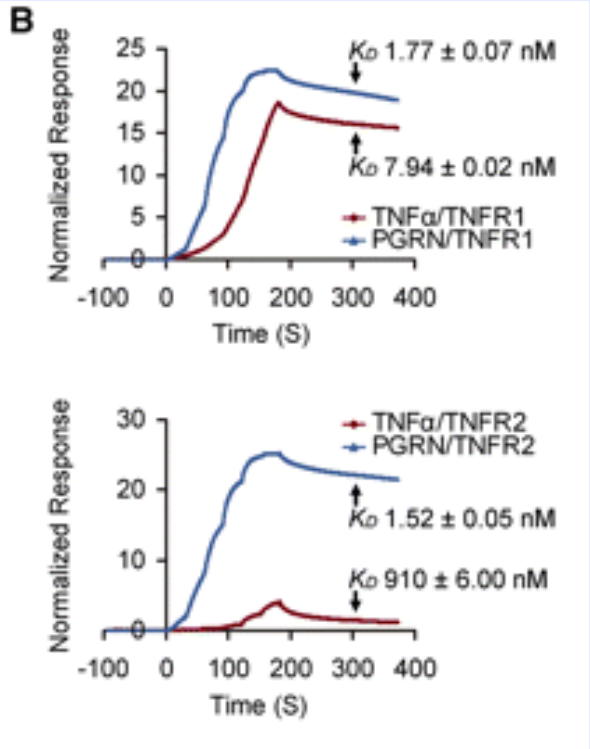
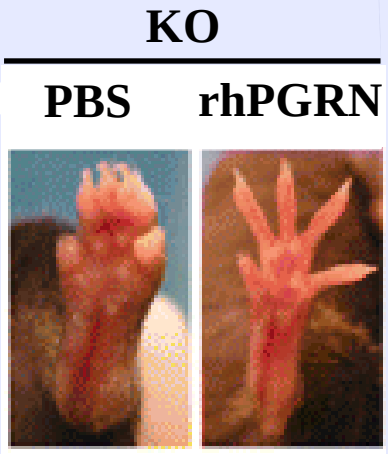


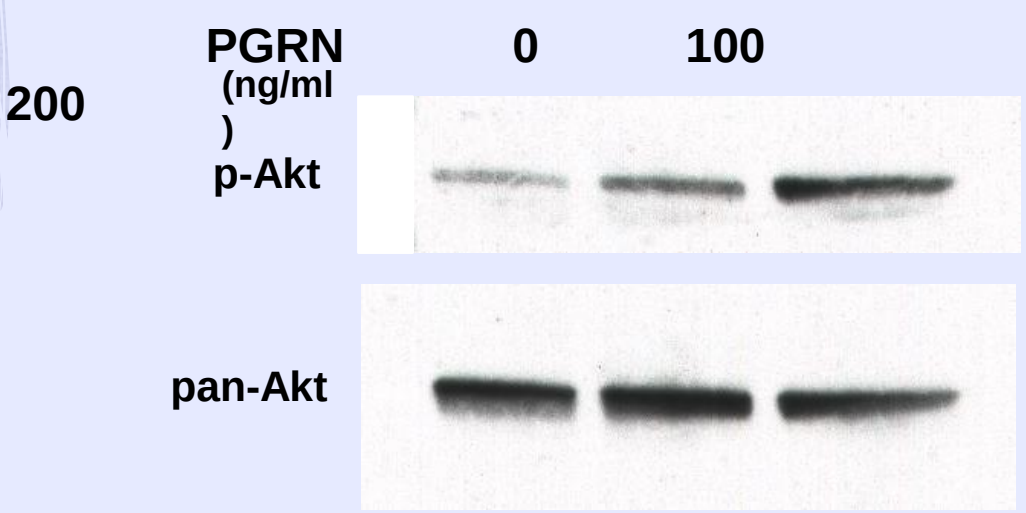
C

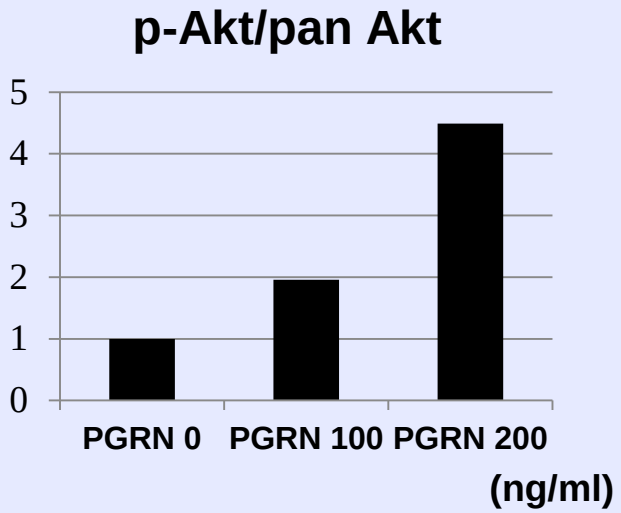


Science, 2011

Possibility of Receptors Other Than Tumor Necrosis Factor (TNF) Receptors

(Using peritoneal macrophages in TNF receptor 1/2 KO mouse)





Kawase R, Unpublished data

Summary 1

- ✓ **Deletion of progranulin exacerbates atherosclerosis partly through suppressing inflammation**
- ✓ **Progranulin might be a promising therapeutic target for atherosclerotic cardiovascular diseases**

2. Roles of an HDL-associated Anti-inflammatory Protein, Progranulin, in Acute Coronary Syndrome

Hypothesis

We hypothesized that progranulin may play some roles in the coronary plaque stability in patients with acute coronary syndrome (ACS)

Subjects & Methods (1)

We enrolled consecutive 51 patients with ACS who underwent emergent PCI at Saiseikai Senri Hospital and 158 controls without CAD

《Clinical characteristics of ACS patients》

	All	Male	Female
n	51	40	11
Age(y)	65.6±10.3	62.7±9.1	76.0±7.2
BMI(kg/m^2)	24.5±3.6	24.7±3.6	23.9±3.5
Hypertension	57%(29)	53%(21)	73%(8)
Diabetes	35%(18)	38%(15)	27%(3)
Dyslipidemia	53%(27)	53%(21)	55%(6)
Smoker	67%(34)	80%(32)	18%(2)
Previous MI	4%(2)	5%(2)	0%(0)

《PGRN concentration in peripheral vein in both groups》

	Control	ACS	
n	158	51	
male / female	79 / 79	40 / 11	
Age (y)	61.8±13.5	65.6±10.3	
PGRN (ng/ml)	2.9±0.6	3.0±0.5	n.s.

Subjects & Methods (2)

We obtained peripheral venous blood samples, arterial blood samples as well as the aspirated coronary arterial blood samples obtained from the culprit lesion at the time of emergent PCI

Serial blood samples were obtained from arrival to at discharge from the hospital and at the later outpatient visit (0h, 2h, 6h, 9h, 12h, 24h, 48h,72h after emergent PCI and later outpatient visit)

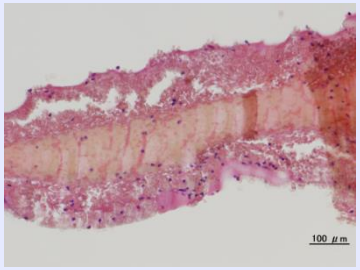
We also underwent immunostaining of aspirated samples

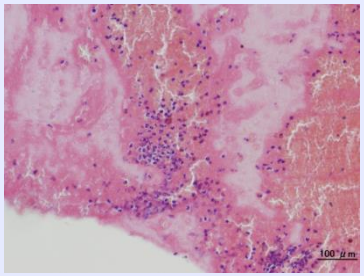
Osaka Univ. IRB and Saiseikai Senri Hospital IRB approved this study : UMIN000004241

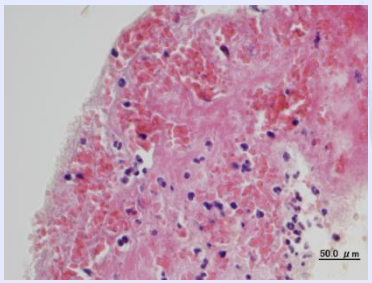
PGRN Was Mainly Expressed in Macrophages

Aspirated sample

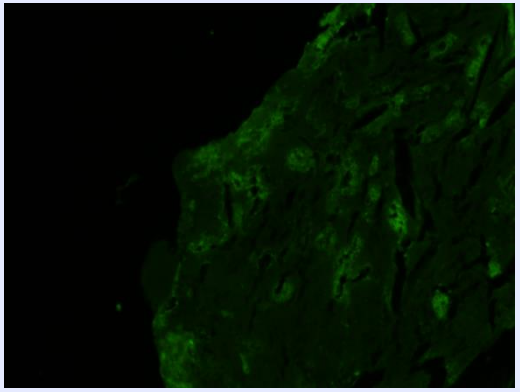
HE staining



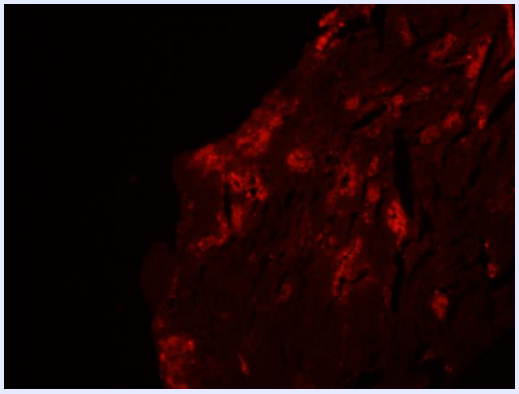




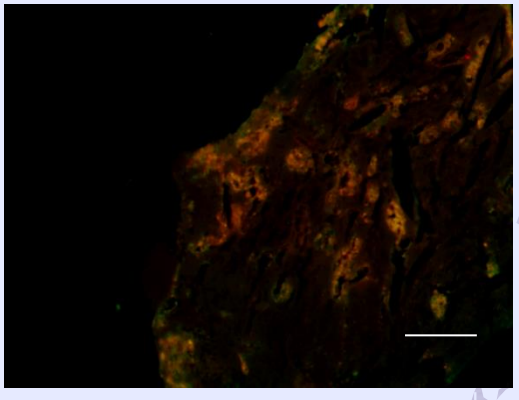
PGRN



CD68



Merge

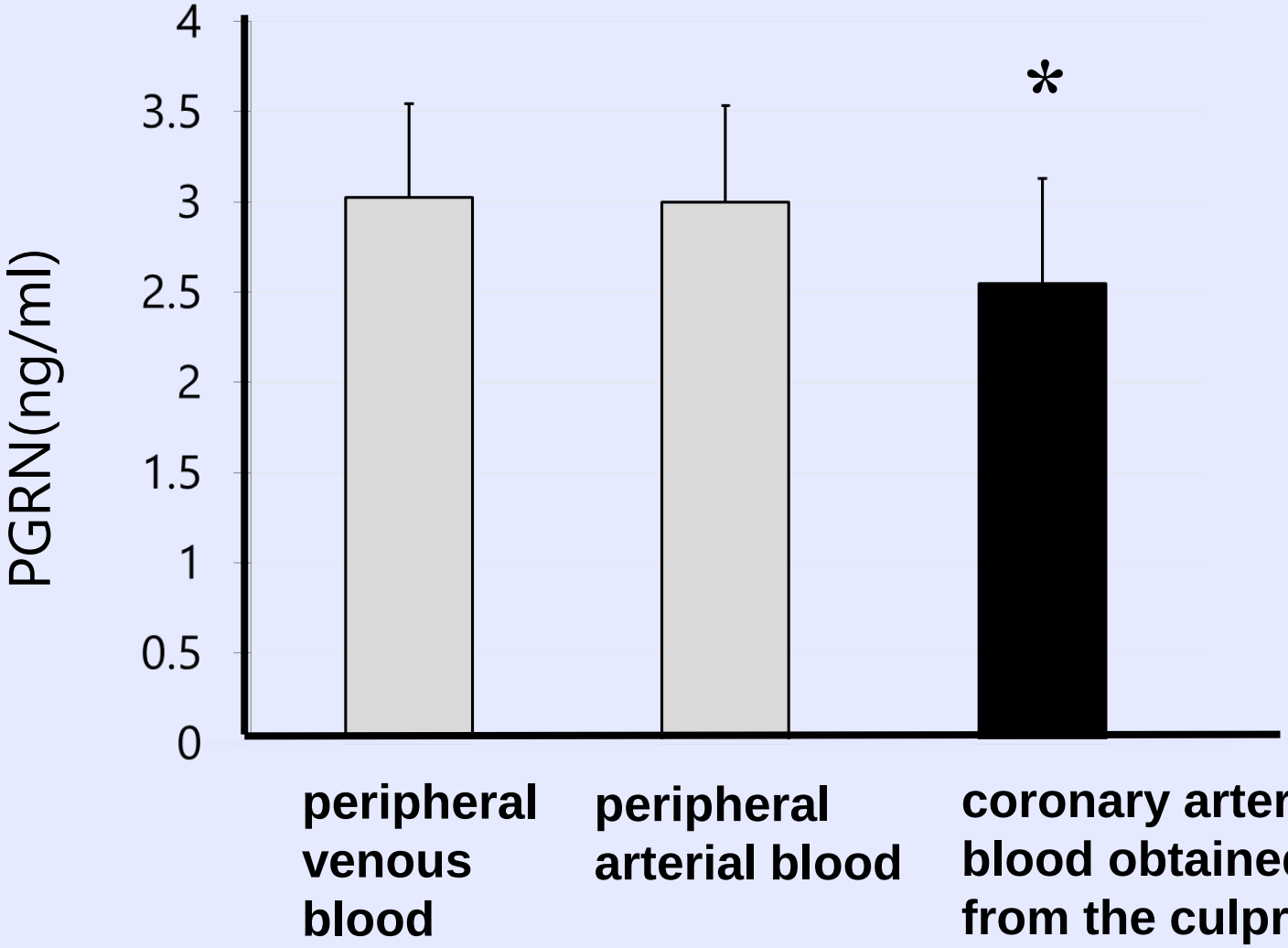


Bar:100μm

Immunostaining of aspirated samples at the culprit region showed that PGRN was mainly expressed in macrophages

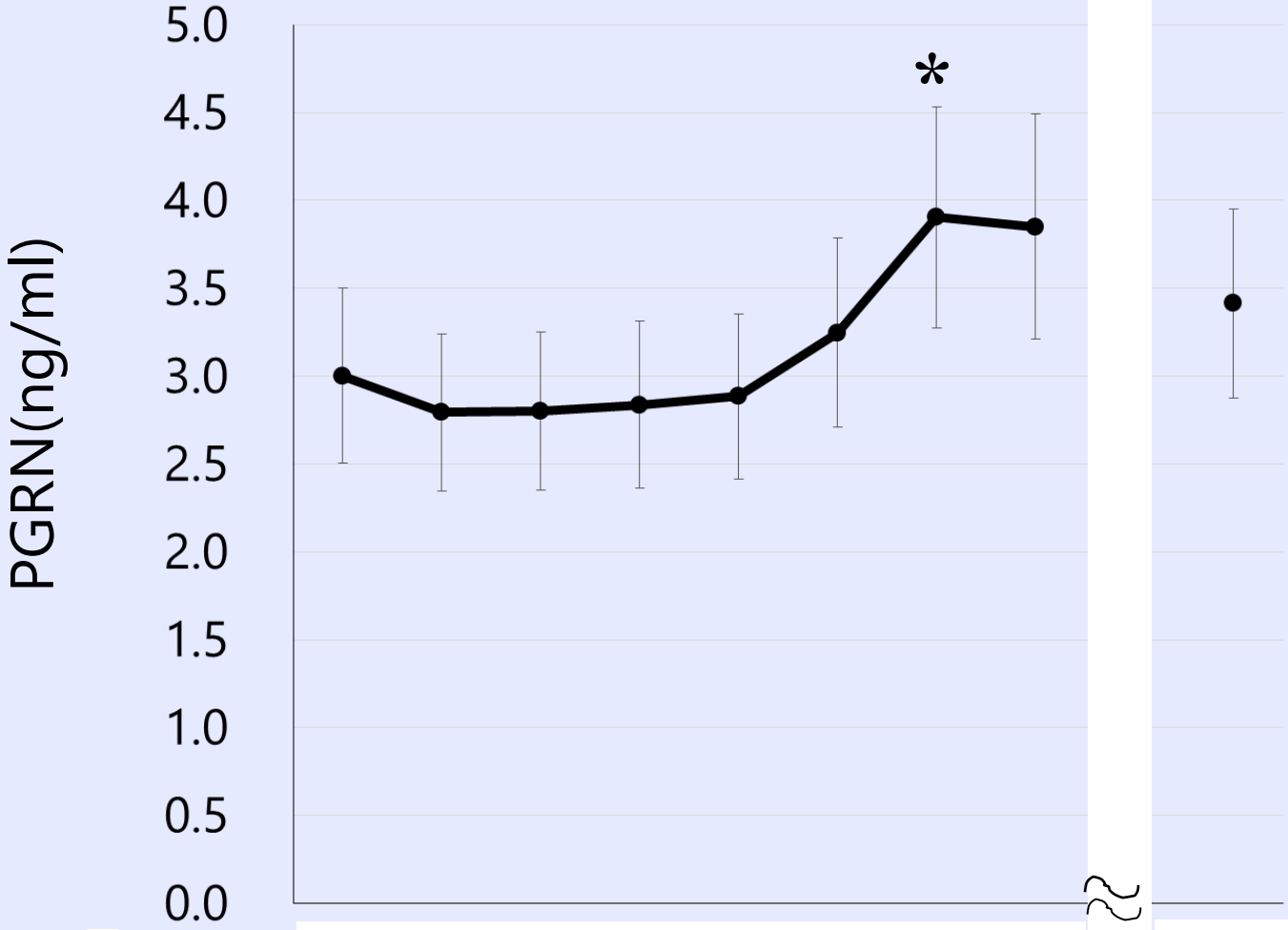
PGRN Concentration in Coronary Arterial Samples Was Significantly Lower

《Samples at emergent PCI》



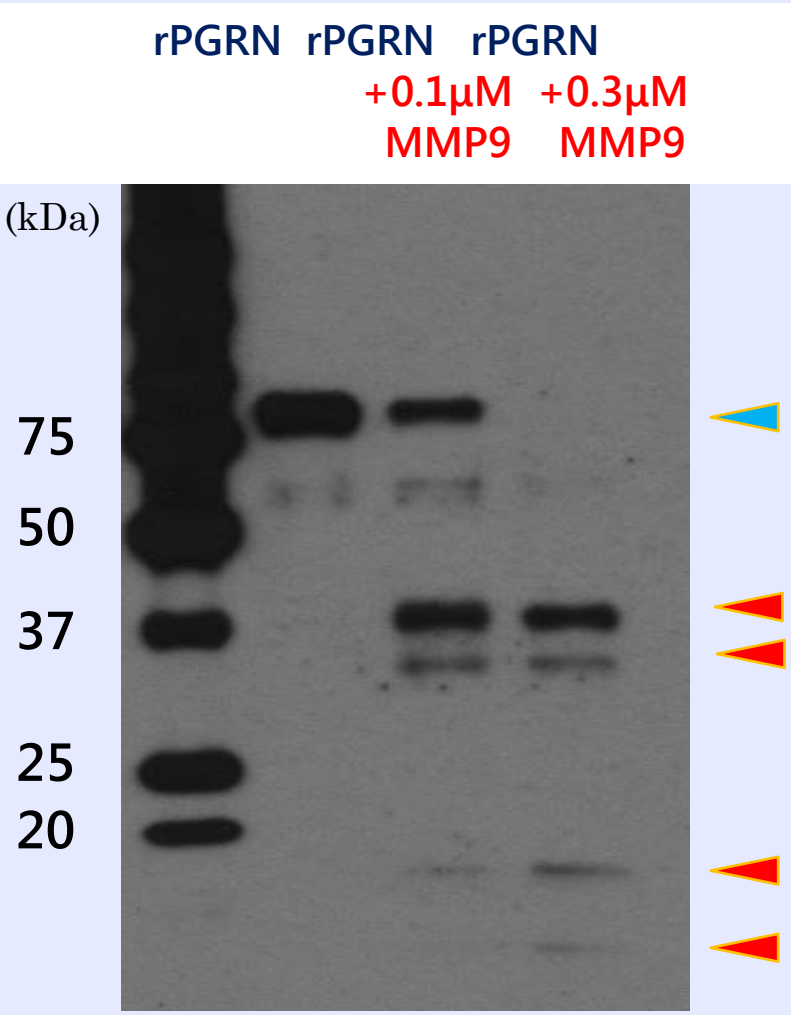
PGRN Concentration Gradually Increased After PCI

《Serial blood samples》



*:p<0.05

Progranulin Can Be Cleaved by MMP-9



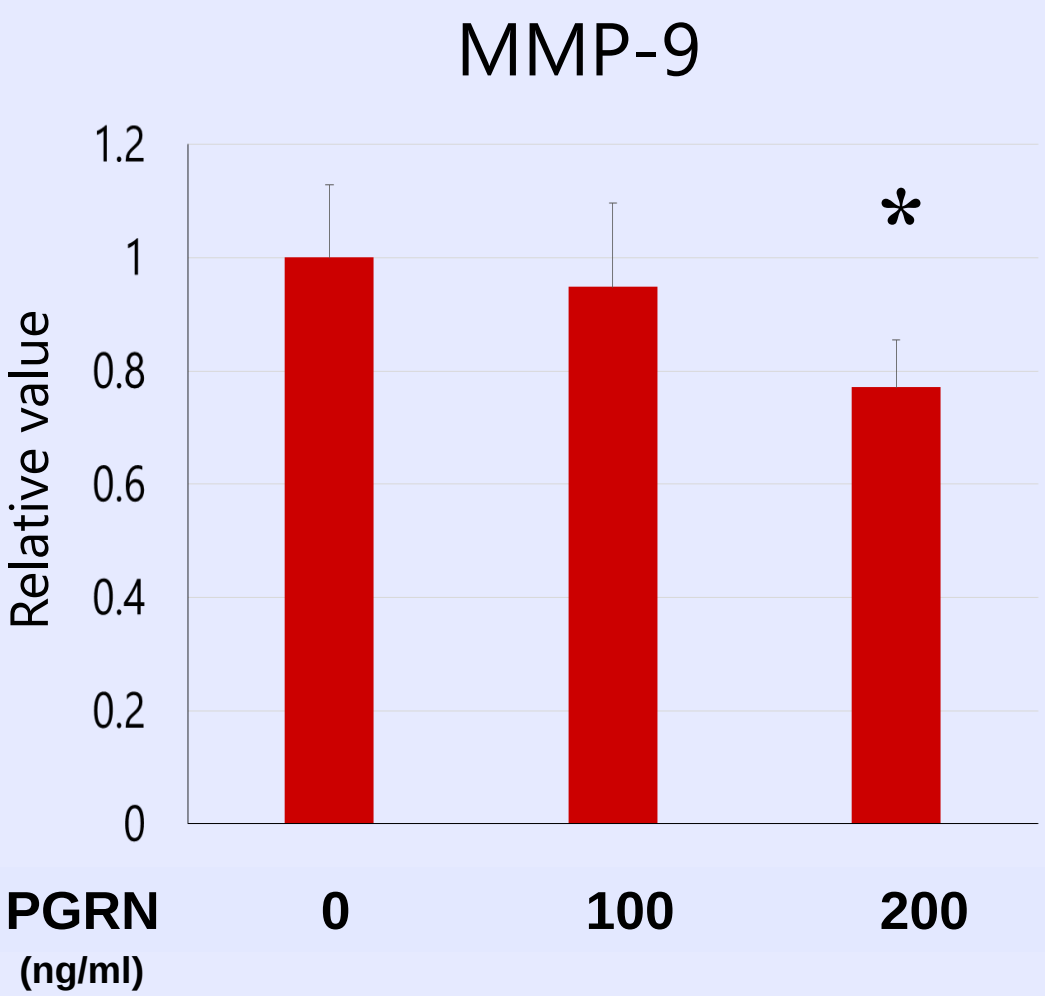
1st antibody: c-PGRN (Invitrogen)

progranulin (PGRN)

PGRN cleavage products

TNF-α-induced Expression of MMP-9 Is Supressed by PGRN

《in THP-1 cells》

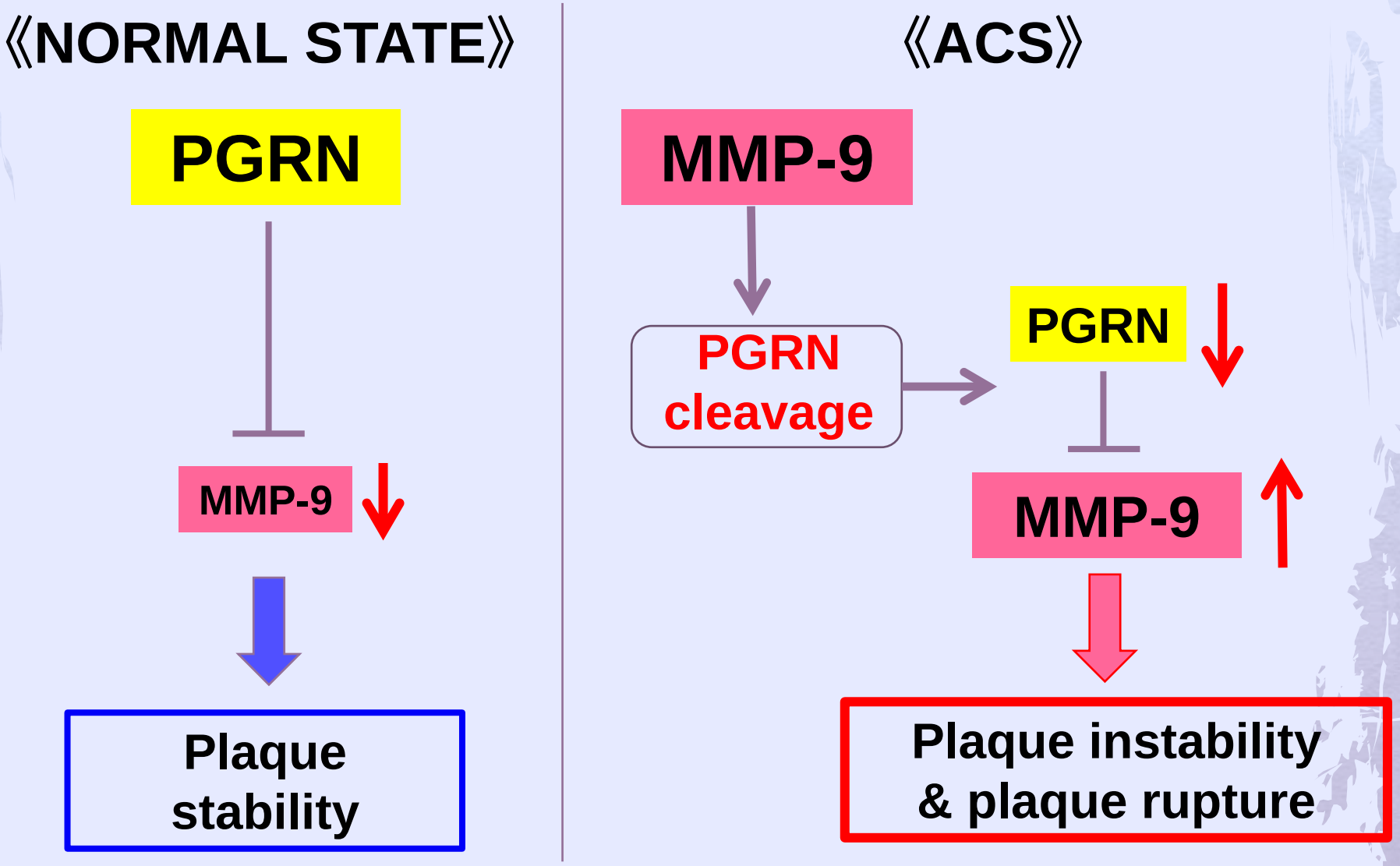


*:p<0.05

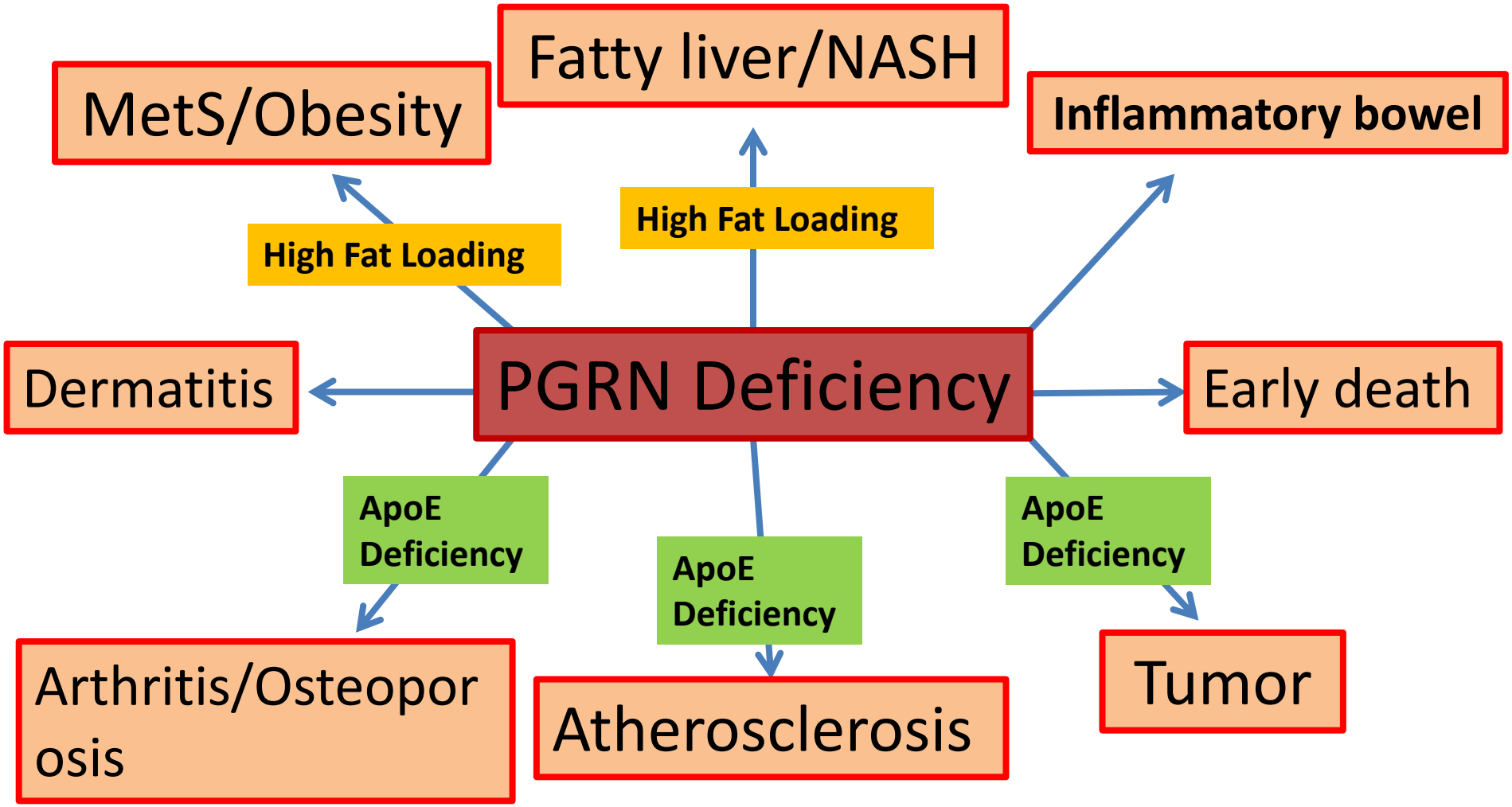
Summary (2)

- PGRN is mainly expressed in macrophages and monocytes in aspiration samples from the culprit lesion
- Compared to peripheral venous and arterial samples, PGRN concentration in the coronary arterial samples was significantly lower in patients with ACS
- PGRN concentration gradually increased after PCI and became highest after 48 hours
- PGRN can be cleaved by MMP-9
- PGRN suppresses expression of MMP-9 in macrophages

Possible Roles of Progranulin in Acute Coronary Syndrome



Various Phenotypes of PGRN Knockout Mice

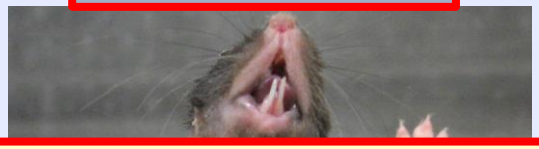


Various Phenotypes of Apo E/Progranulin Double Knockut Mice

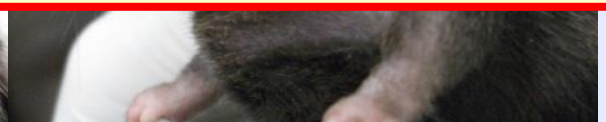
Dermatitis

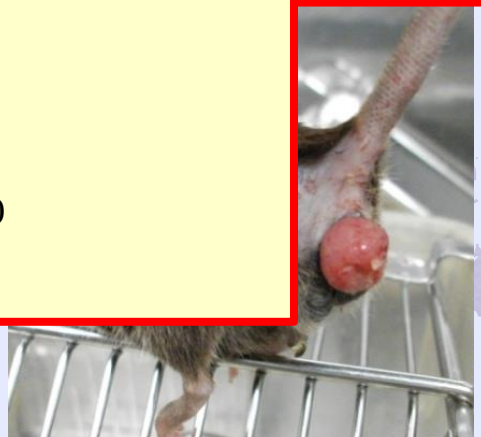
Arthritis

Tumor (Cancer)



Prolapse





Conclusion

Anti-inflammatory protein, progranulin, may be involved in the pathogenesis of atherosclerosis and stabilization of vulnerable coronary arterial plaques possibly through inhibition of MMP-9

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