Joint Meeting of Coronary Revascularization (JCR2011), in PUASN, Korea

# Progress in Atherosclerosis Research-Impact on Treatments and Biomarkers

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Disclosures (None)

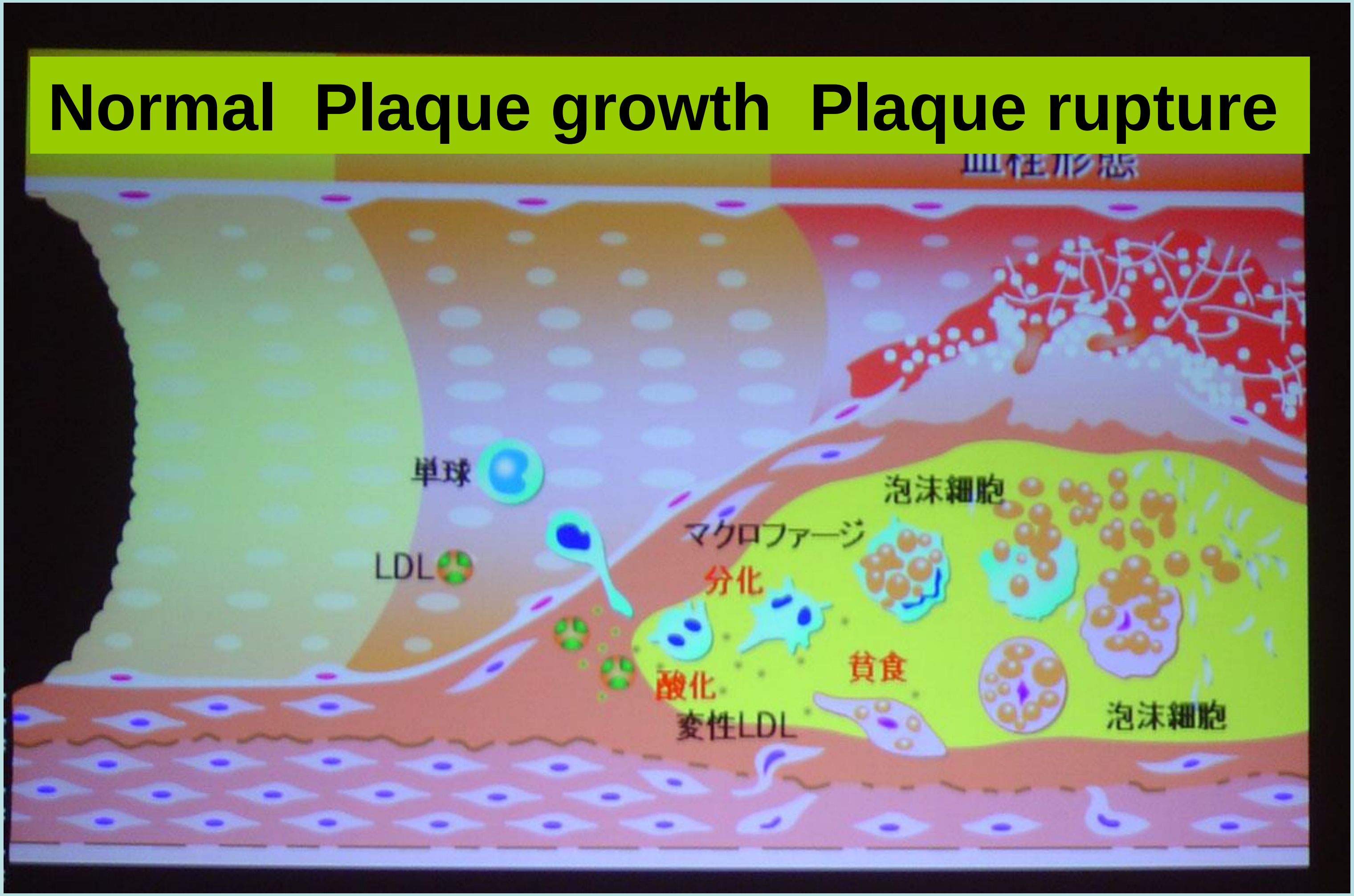
December 9, 2011

# ***Outline***

- Angiotensin type 1 receptor (AT1R) antagonism on atherosclerosis-associated vasa vasorum.

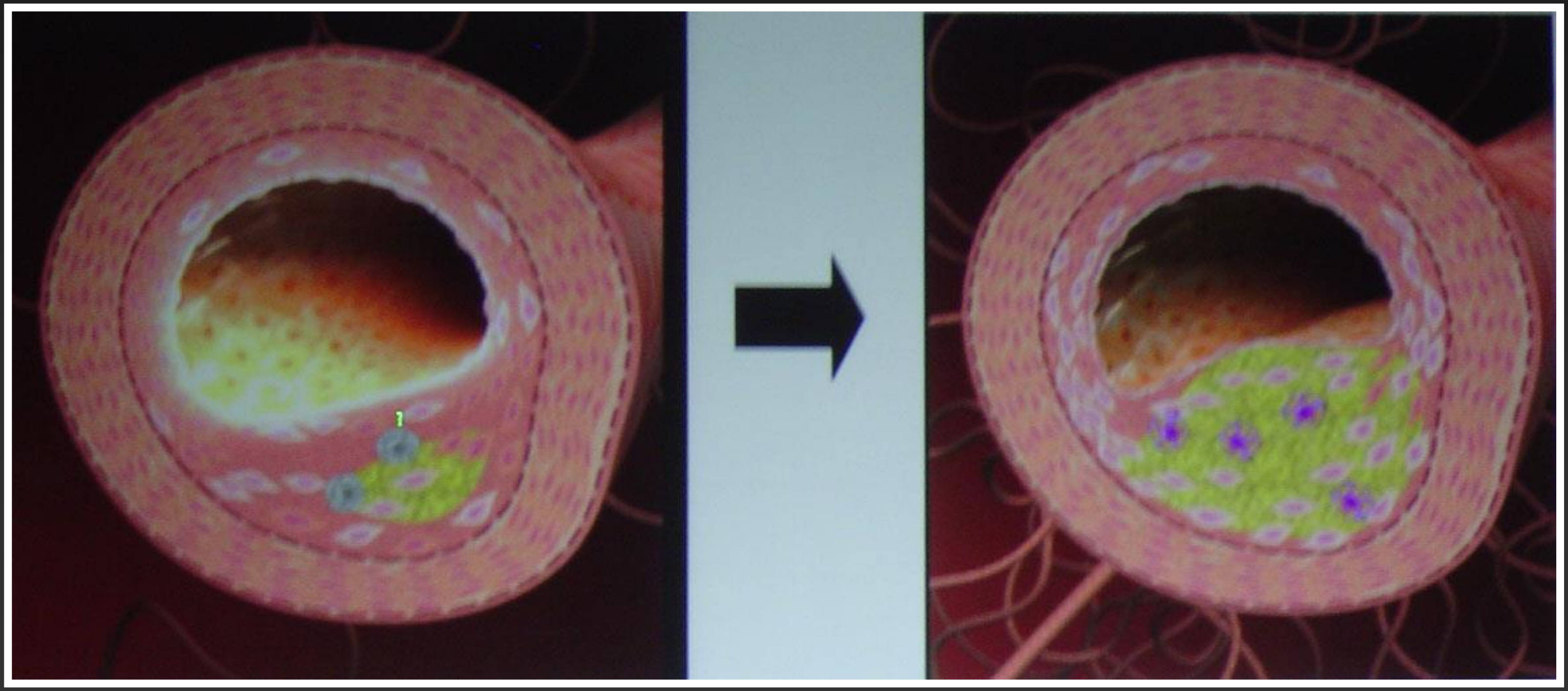
- Identification of new biomarker for atherosclerosis-based coronary artery disease (CAD).

# Atherosclerotic plaque growth and rupture

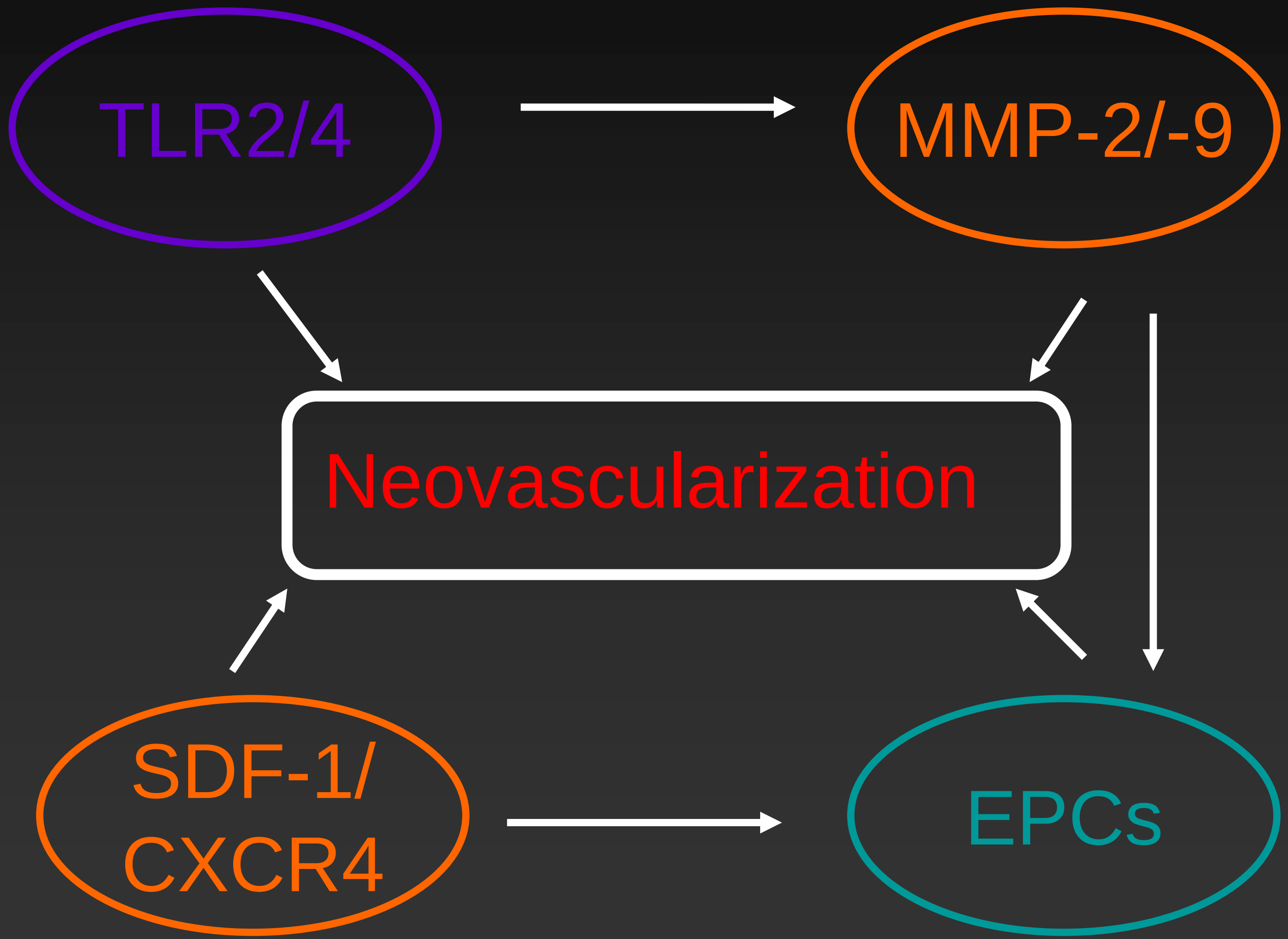


# Atherosclerotic plaque and vasa vasorum

Stable | Vulnerable

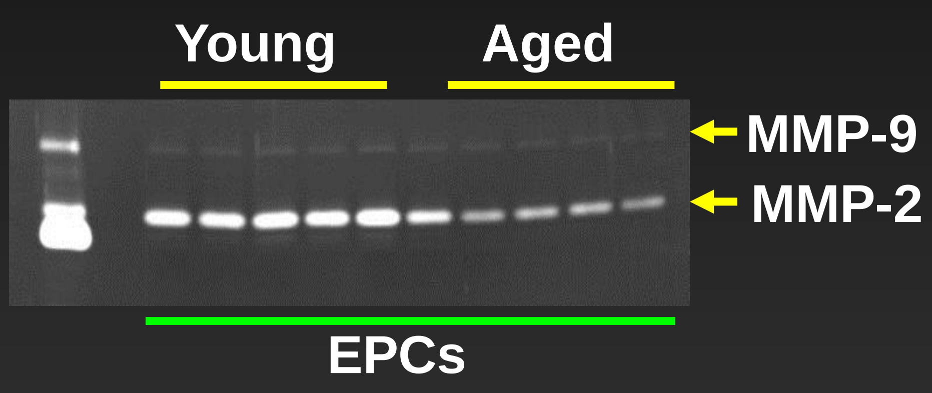


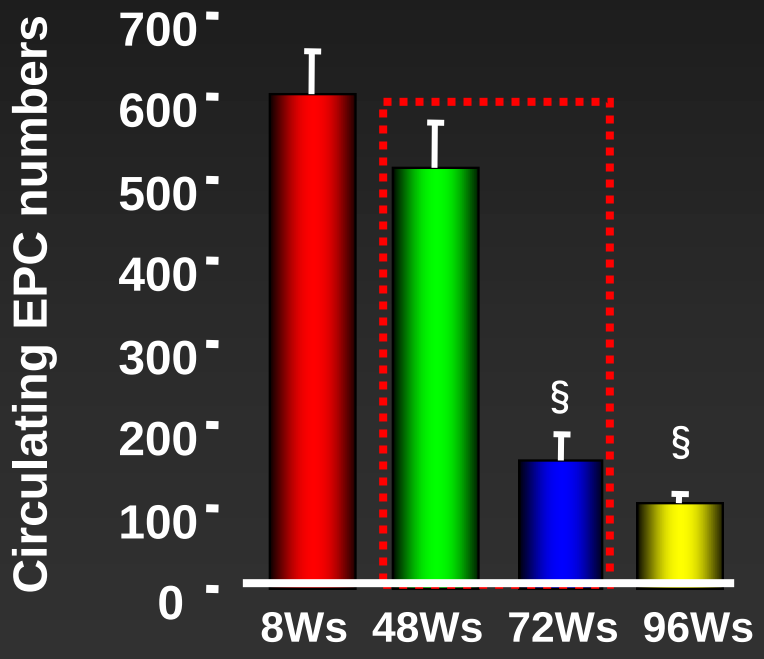
# Key players in neovascularization processes




TLR: toll-like receptor; EPC: endothelial progenitor cell; CXCR4, CXC chemokine receptor
SDF-1: stromal cell-derived factor-1; MMP: matrix metalloproteinase

# Aging reduces MMP-2 expression and EPC recruitment in response to ischemia

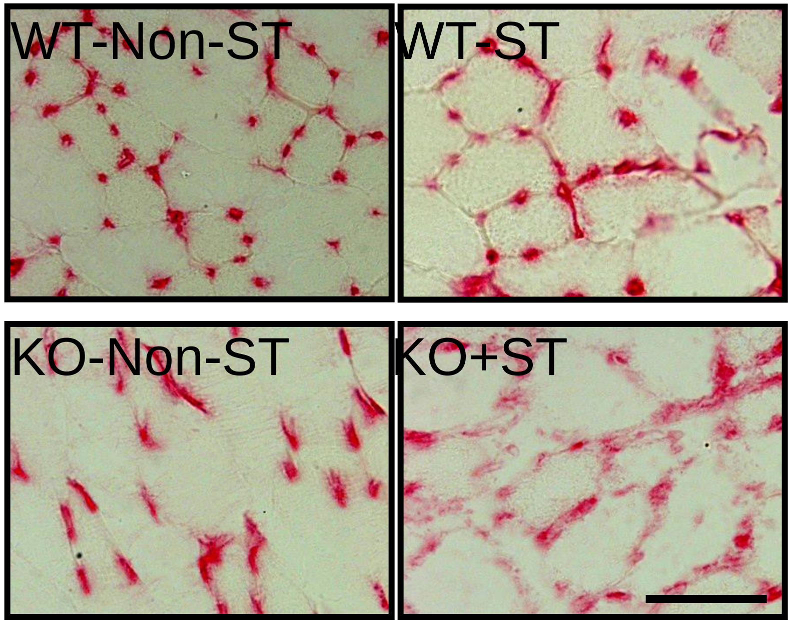


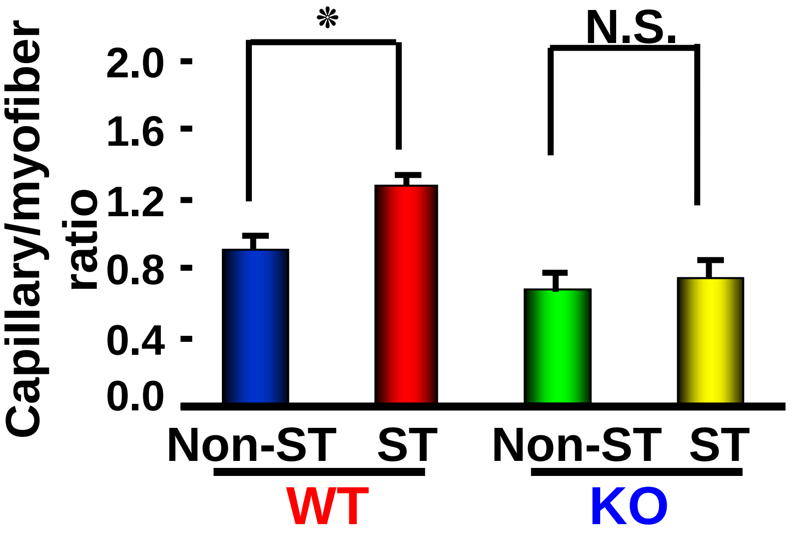




§ P < 0.01, † P < 0.01
vs. corresponding controls

*Cheng XW Murohara T et al*. Circ Res 2007; CEPP 2010

# MMP-2 deficiency impairs exercise-induced neovascularization in advanced age

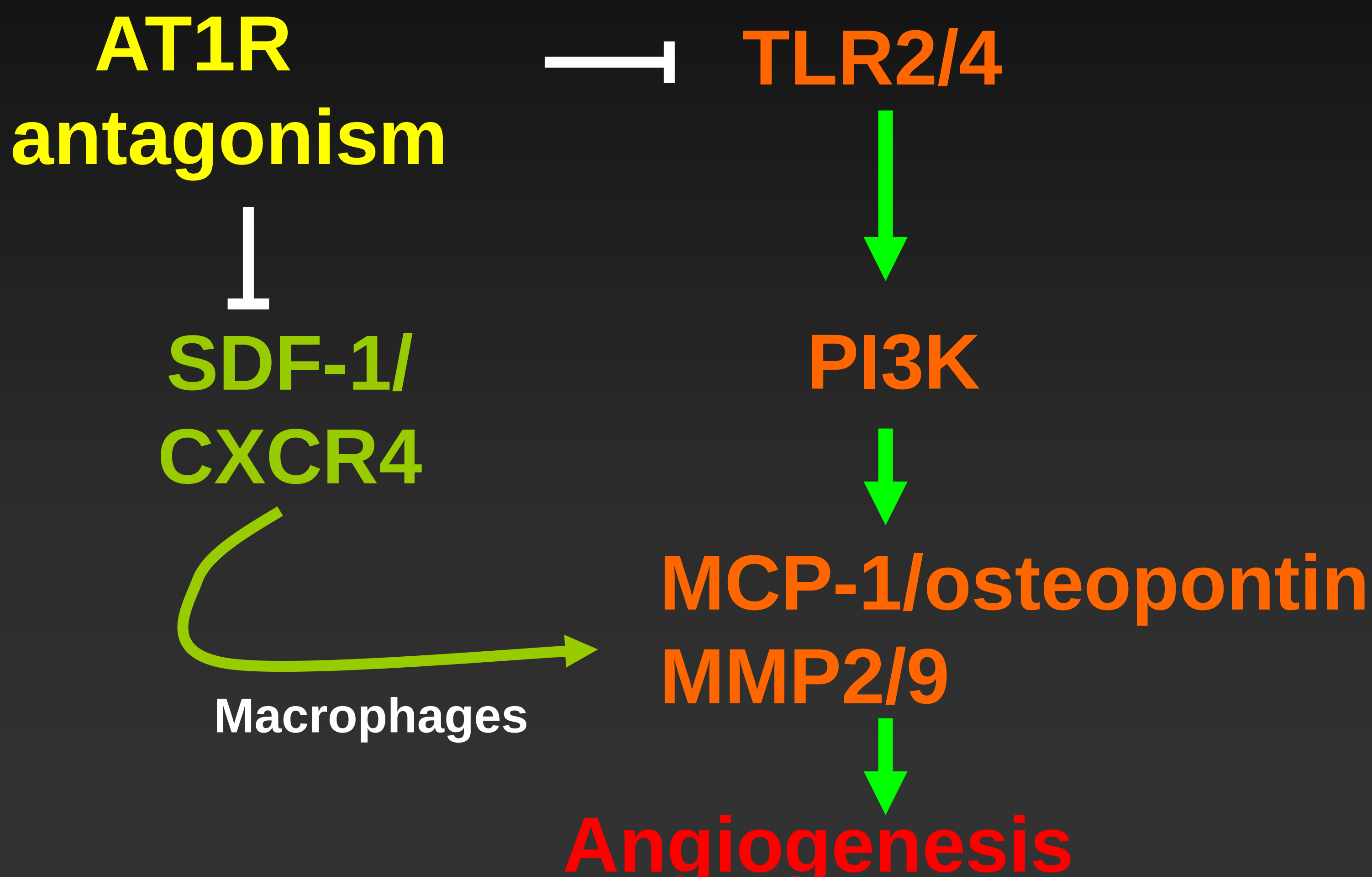



- **P < 0.05 vs. corresponding controls**
- **N.S.: no significant difference**

*Cheng XW Murohara T et al*. Circulation 2010

# ***Hypothesis***




AT1R: angiotensin II type 1 receptor;
MCP-1: macrophage chemoattractant protein-1; PI3K: phosphatidylinositol 3-

# Goal

- To investigate the protective effects and the mechanism of action of angiotensin II type 1 receptor antagonism therapy on atherosclerotic plaque growth and instability in apolipoprotein E -deficient ($ApoE^{-/-}$) mice with a special focus on plaque neovescularization.

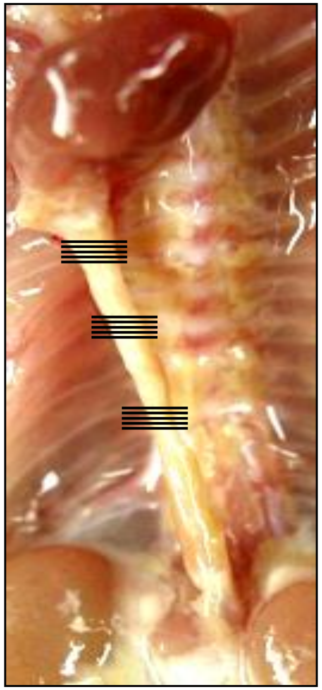
# Exp: Protocol (1):

## Early atherosclerotic lesion formation

**ApoE$^{-/-}$ mice (*n* = 26)**


① 0.5% Carboxymethyl cellulose (CMC, CONT)
② Olmesartan (1 mg/kg/d: OLM)

# Sampling procedure



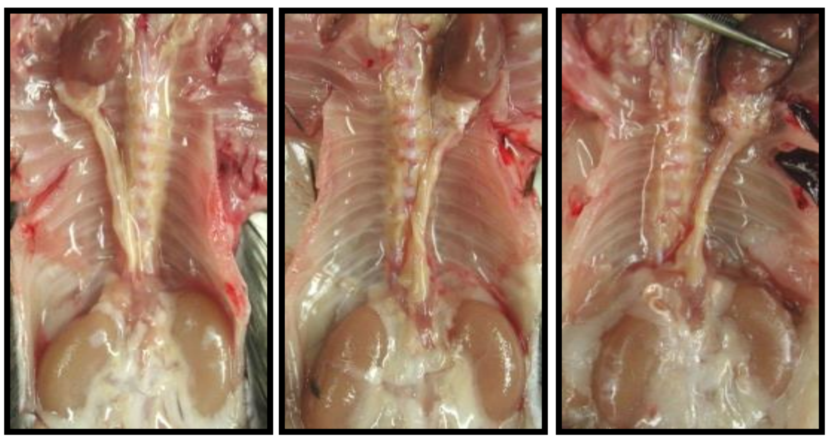
**Aortic sinus**

**Thoracic**

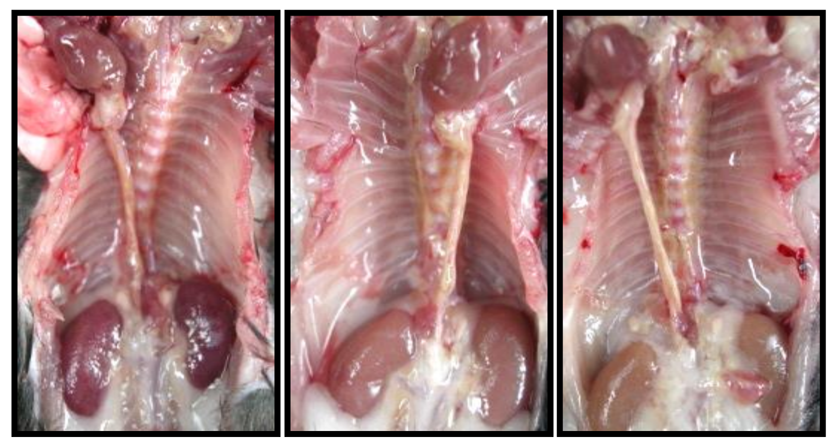
**Abdominal**

# Microscopy images show whole aortas of $ApoE^{-/-}$ mice treated with or without olmesartan

CONTROL

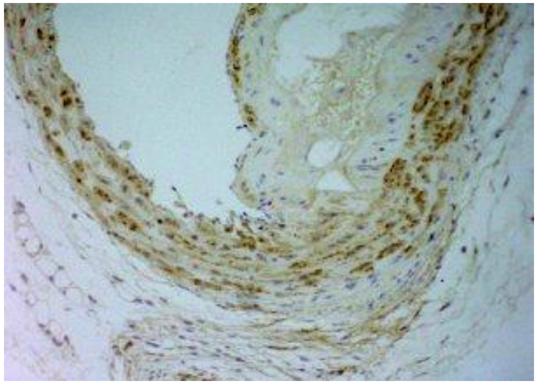


OLMESARTAN

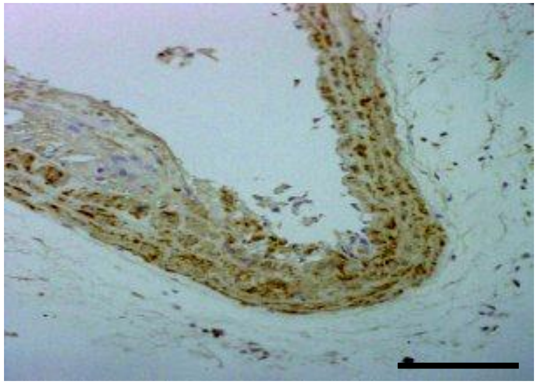


# Olmesartan inhibits atherosclerotic lesion formation

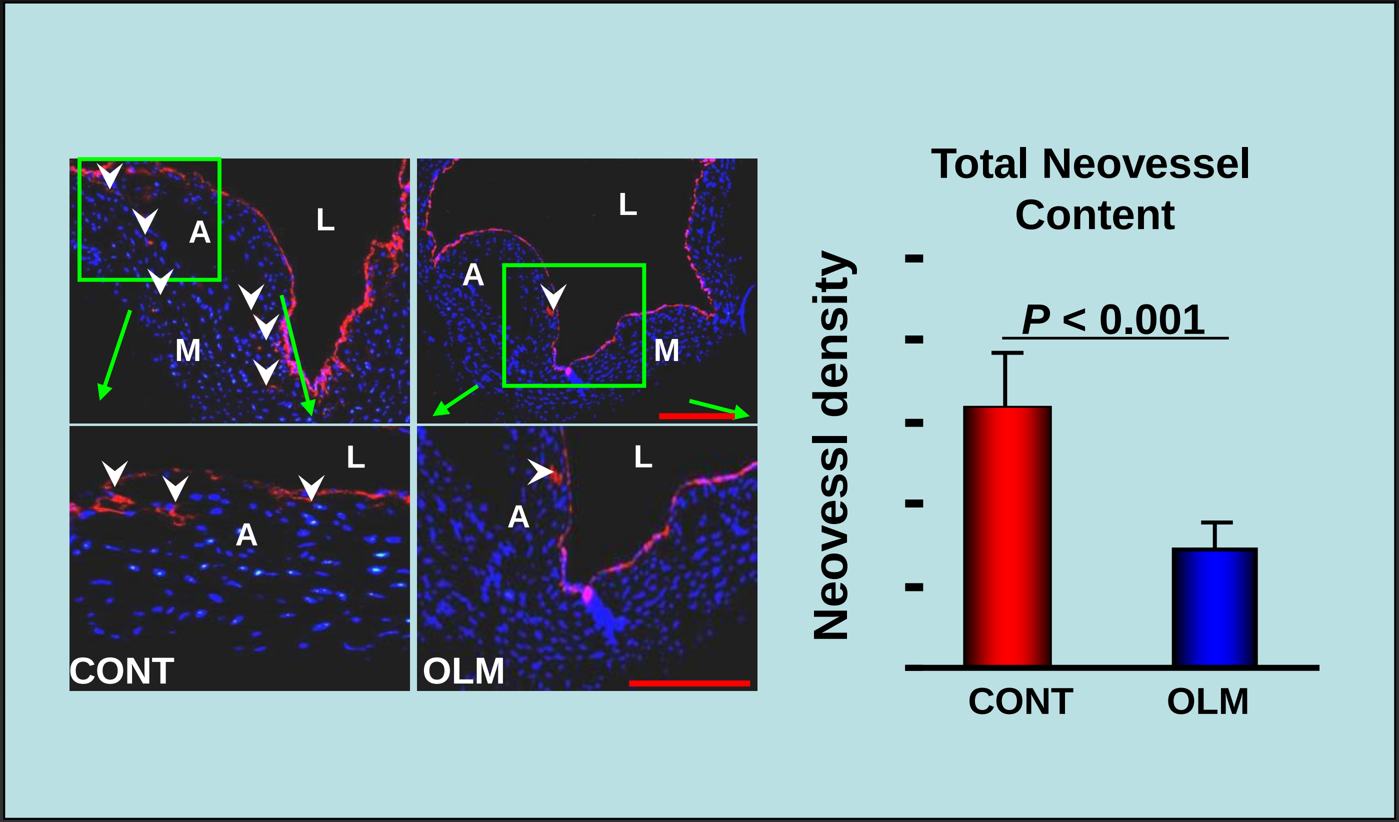
Control



Olmesartan

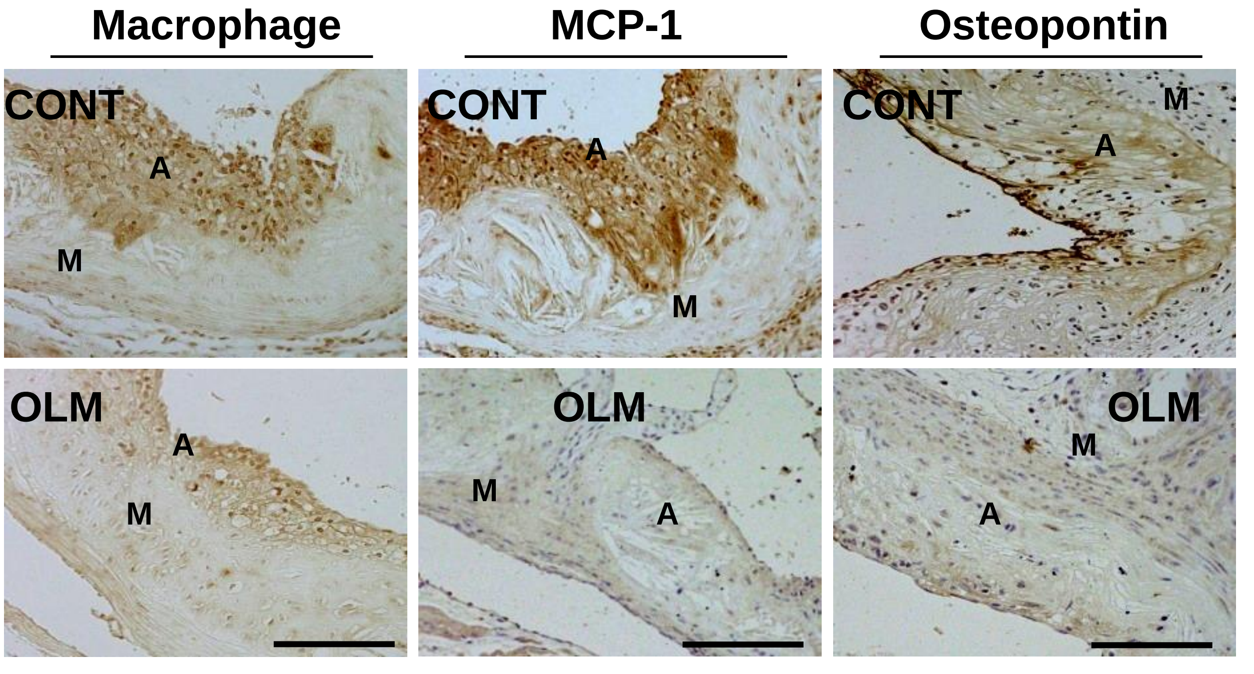


# OLM treatment reduces neovessel in the atherosclerotic plaques of ApoE$^{-/-}$ mice

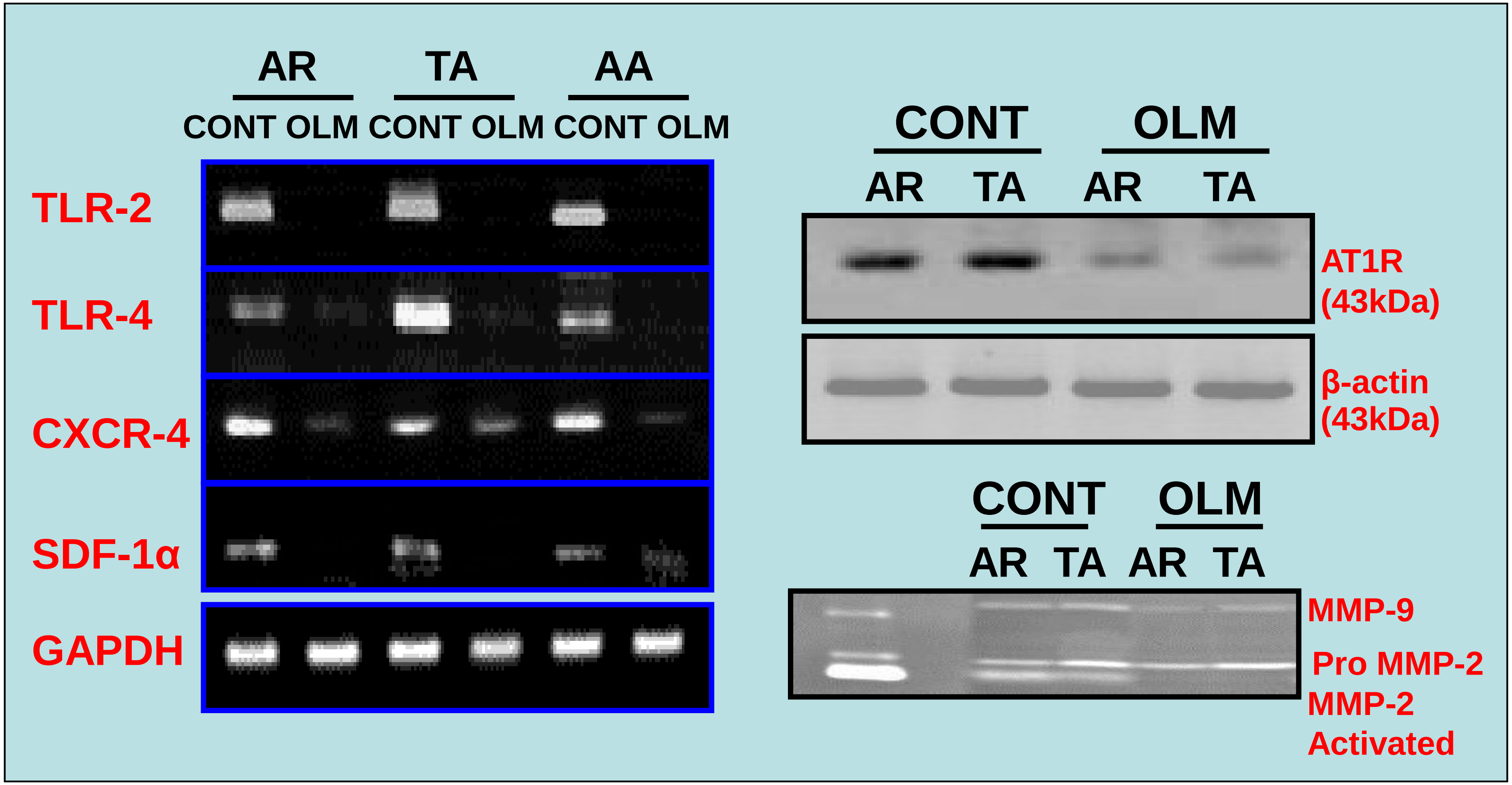




*Cheng XW Murohara T, et al*. Hypertension 2011

# OIM inhibits macrophage infiltration and inflammatory chemokine expressions

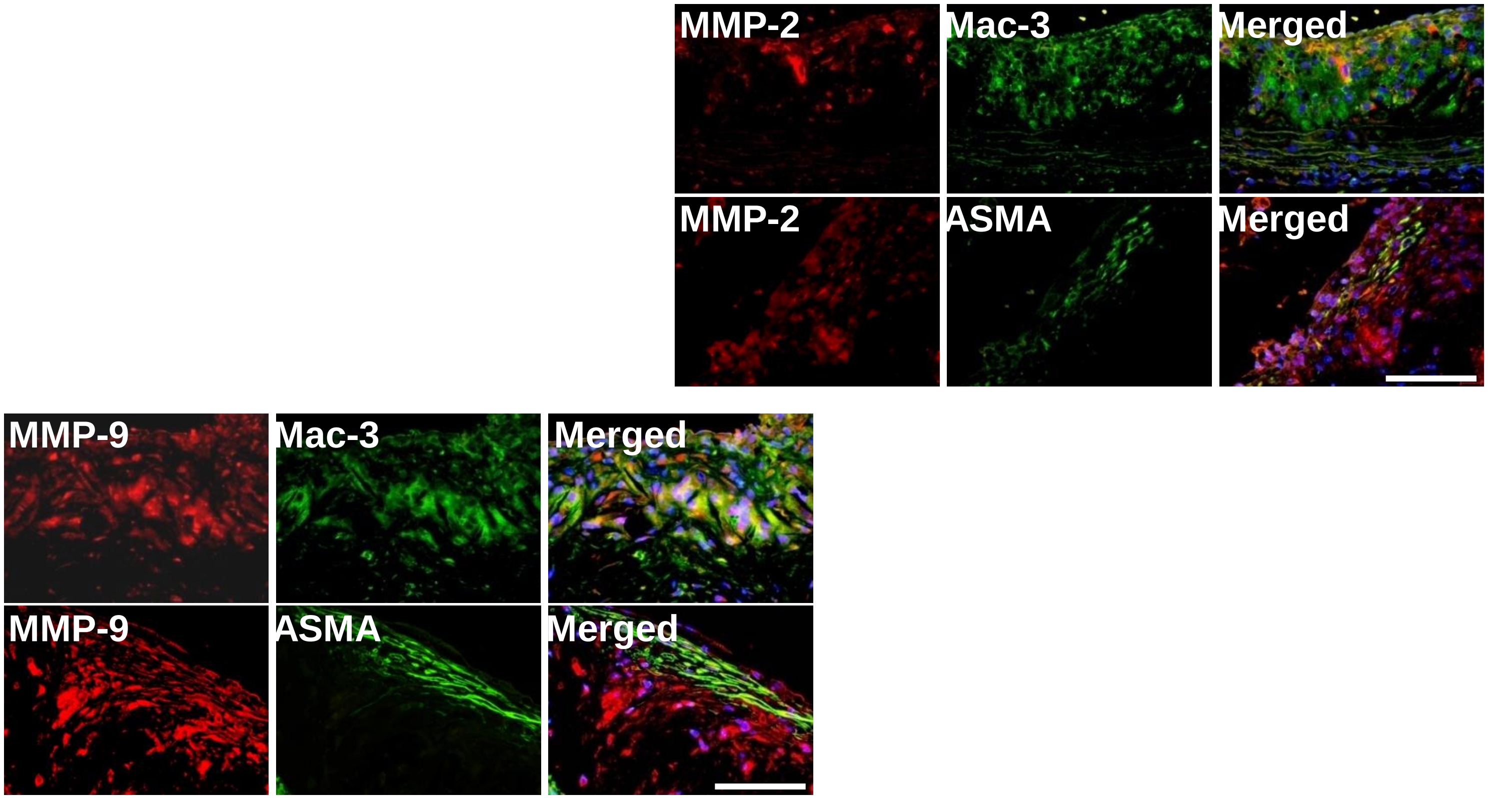


# OLM inhibits targeted gene expressions in atherosclerotic plaques

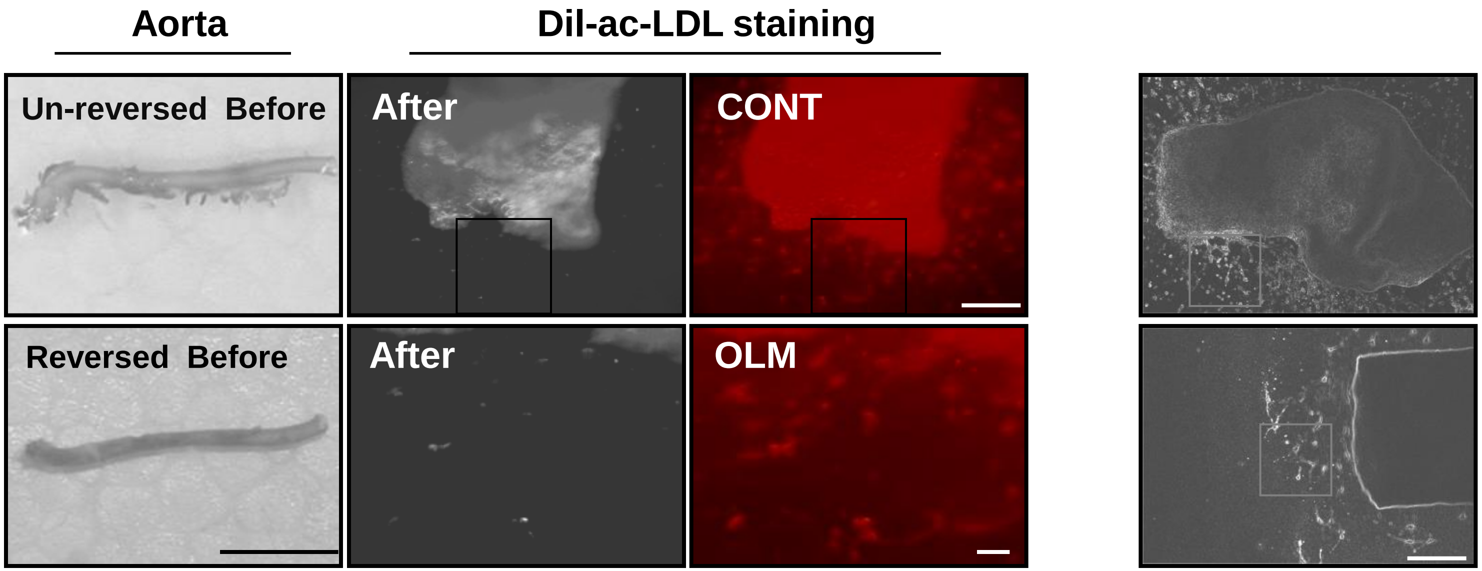


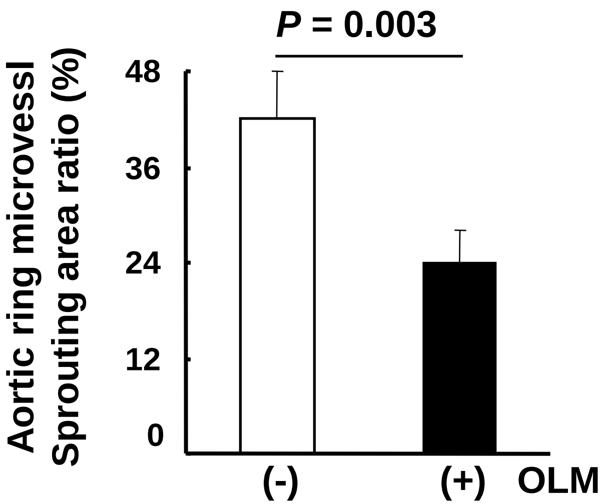

AR, aortic root; TA, thoracic aorta; AA, abdominal aorta; TLR, toll-like receptor
SDF-1, stromal-derived factor-1; CXCR4, CXC chemokine receptor

# Localization of MMP-2/-9 in macrophages and SMCs of atherogenic plaques

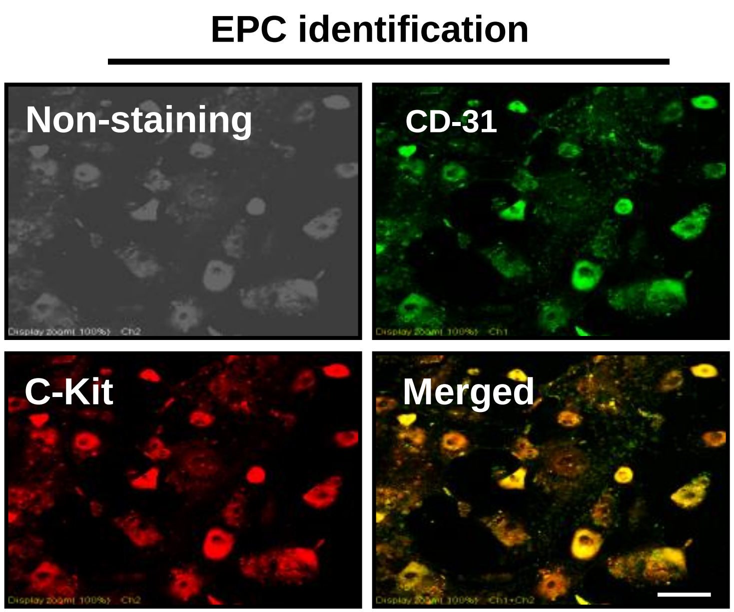


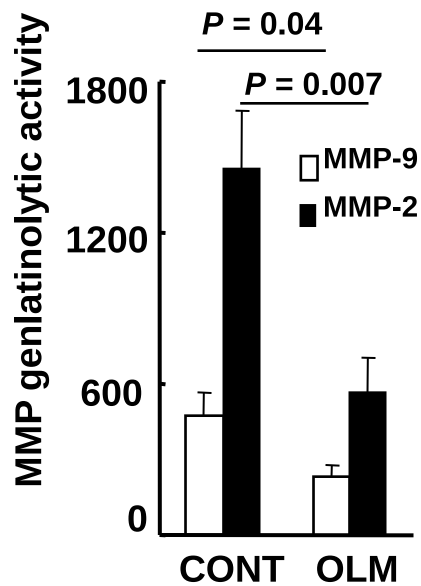
# OLM inhibits aorta-ring angiogenic action

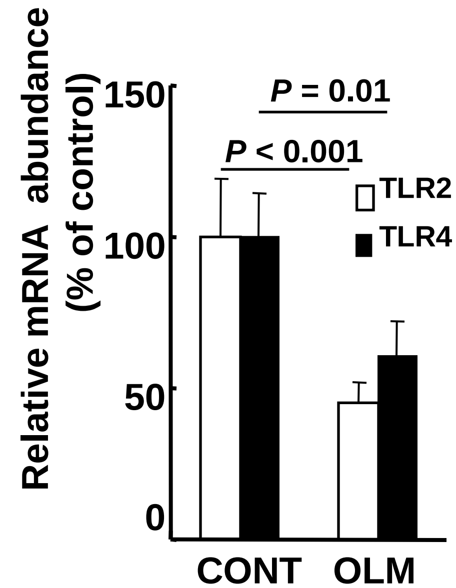


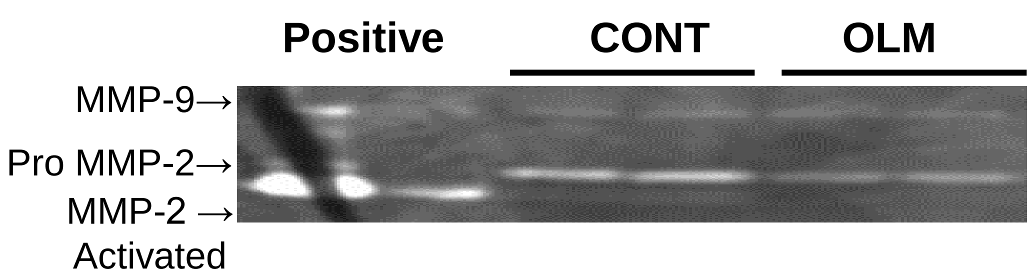



# OLM inhibits the levels of MMP-2/-9 mRNAs in bone marrow-derived EPCs



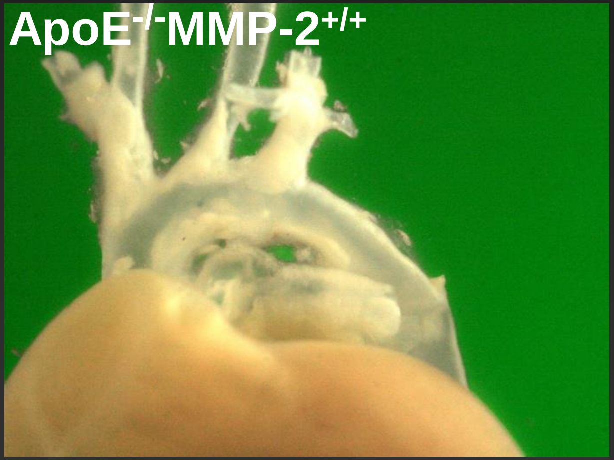



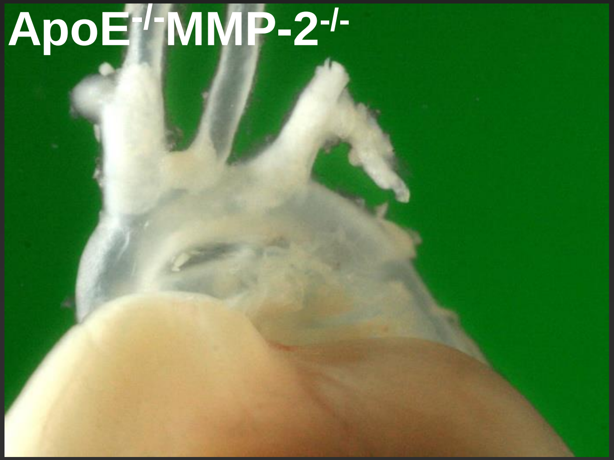



# Exp: Protocol (2)

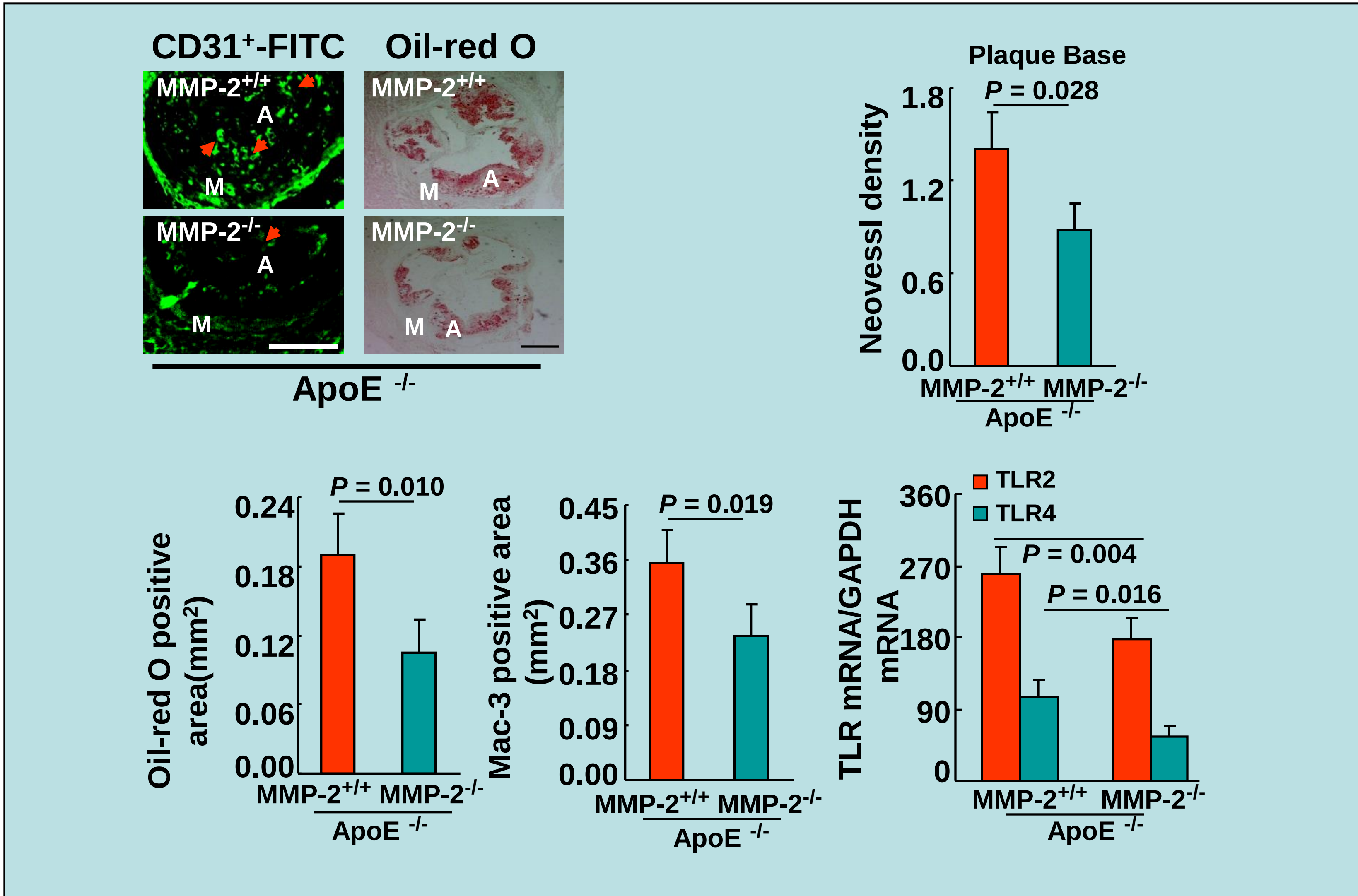

① ApoE$^{-/-}$/MMP-2$^{+/+}$ ($n$ = 13)
② ApoE$^{-/-}$/MMP-2$^{-/-}$ ($n$ = 12)

# MMP-2 deficiency reduced fat accumulation around aortas in ApoE$^{-/-}$ mice

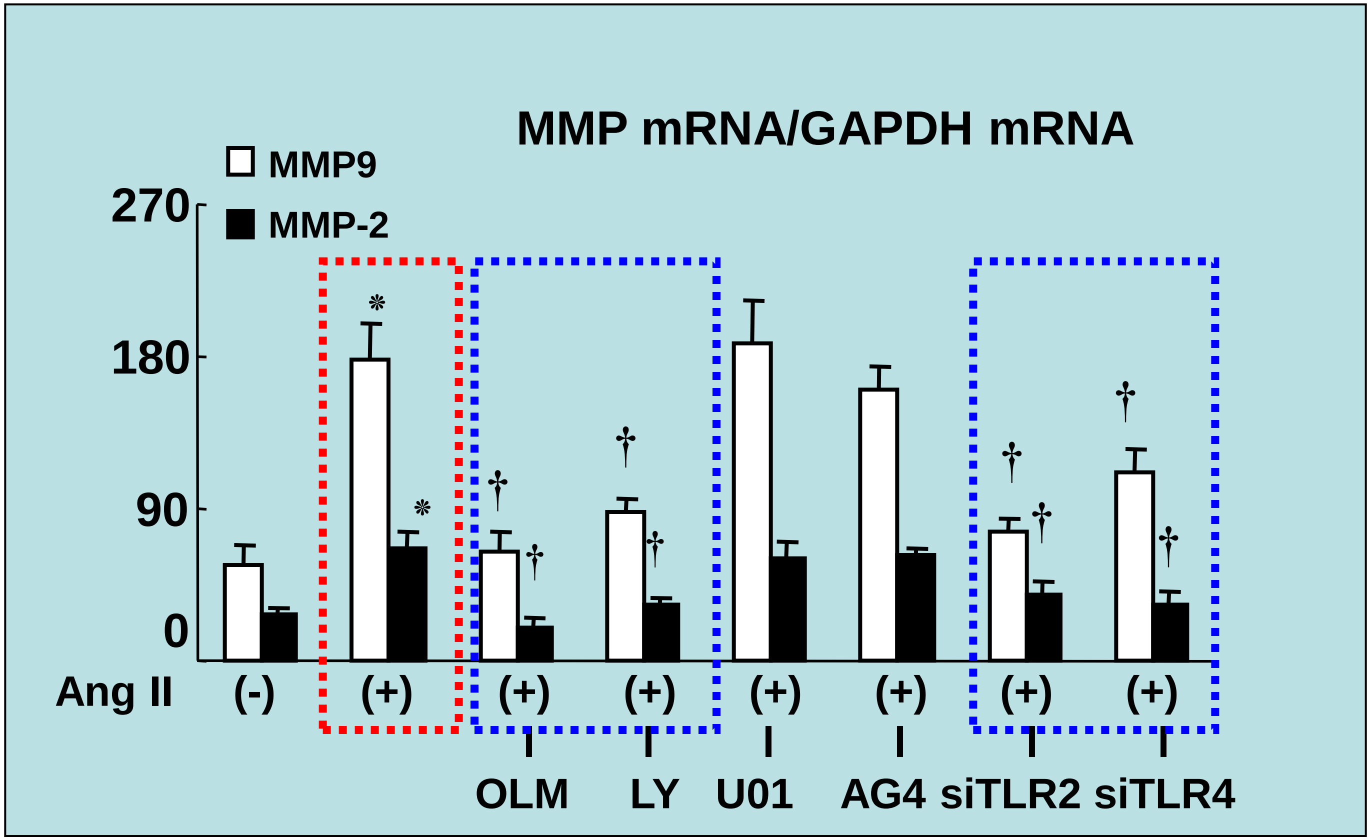


# MMP-2 deficiency reduces atherosclerotic plaques and neovessel formation



# OLM inhibits Ang II-induced MMP expression via TRL signaling pathway in HUVECs



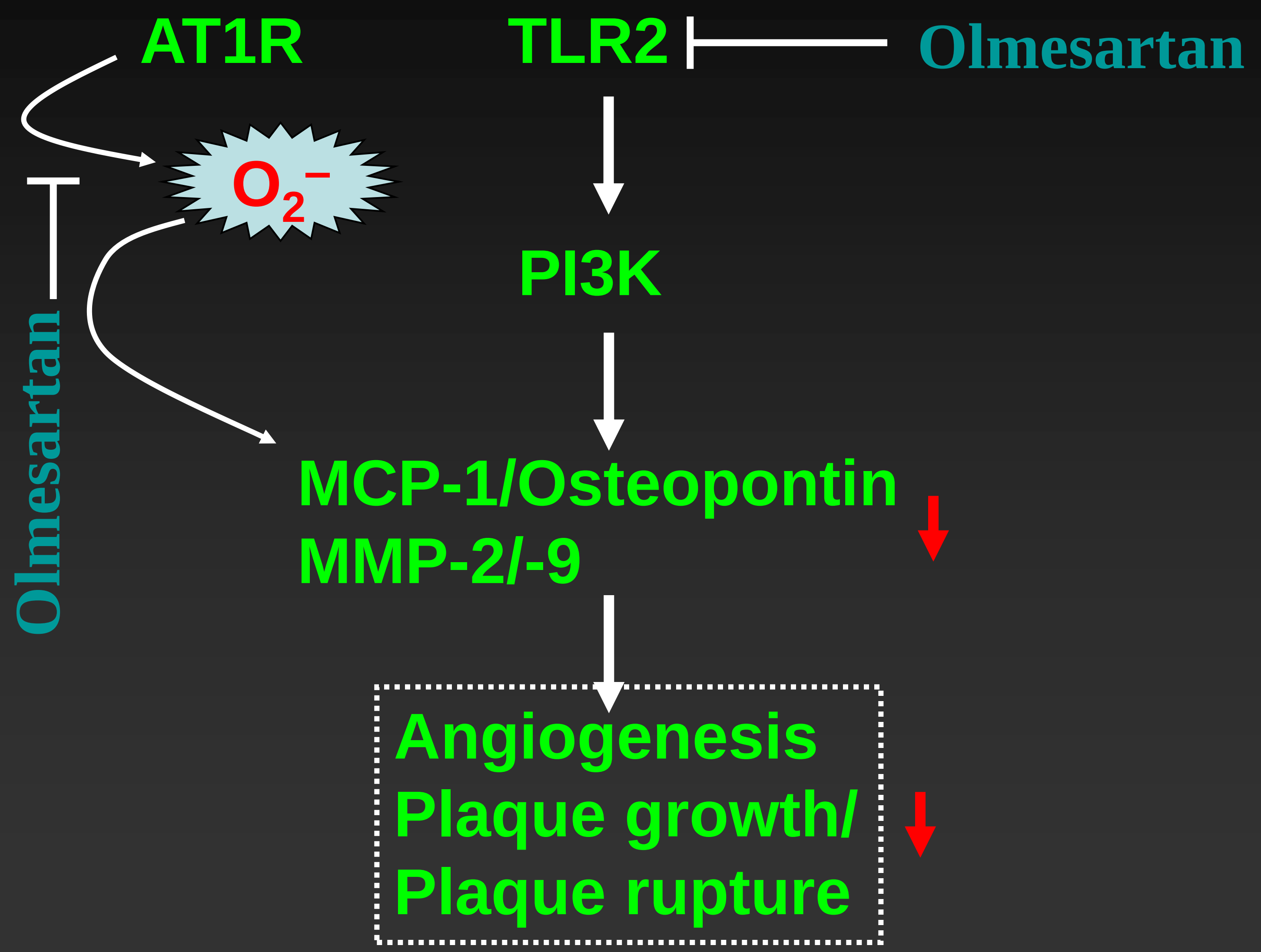

**HUVEC**, human umbilical vein endothelial cell; **Ang II**, angiotensin II; **LY** LY294002, PI3K inhibitor; **U1024**, extracellular signal-regulated kinase inhibitor **AG490**, janus kinase/sinal transducer and activator of transcription 3 inhibitor.

# Observations

- Olmesartan lessened the levels of TLR2/4 and SDF-1/CXCR4 genes and MMP-2/-9 protein and activity in the atherosclerotic plaques.

- Olmesartan reduced diet-induced atherosclerotic plaque neovessel density and plaque instability in *Apo E*$^{-/-}$ mice.

# Proposed mechanisms




*Cheng XW, Murohara T*. Hypertension 2011

# Conclusion

Our findings suggest that olmesartan exerts inhibitory effect on TLR2-mediated MMP-2/-9 expression and activity and angiogenic action, leading to the enhancement of atherogenic plaque stability and protection of its disruption in *ApoE*$^{-/-}$ mouse model without lipid lowing effect.

# ***Outline***

- Exercise rescues vascular action in response to hypoxia in aged animals and humans.

- Identification of new biomarker for atherosclerosis-based coronary artery disease (CAD).

# *The properties of cysteine protease: cathepsins (Cats)*

- Cats generally known as functioned in lysosomes, were discovered in the half of the 20th century. There are 11 human Cats (B,C,F,H,K,L,O,S,V,W,and X) that belong to papain subfamily of cysteinyl proteases. Cystatin C (CystC) is one of the major endogenous inhibitor o cathepsins.

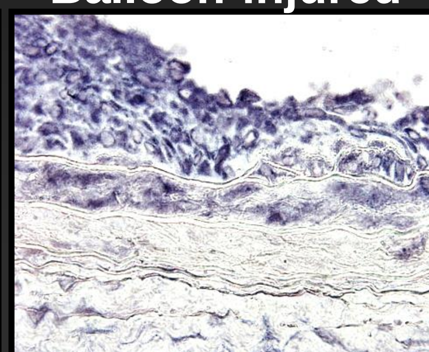
- Previously, we have reported that CatK, which is one of the most potent mammalian collagenase, was overexpressed in the failing myocardium of humans and rats with hypertension.

# *CatK expression in the balloon-injured carotid artery and failing myocardium of rat*

**In situ hybridization**

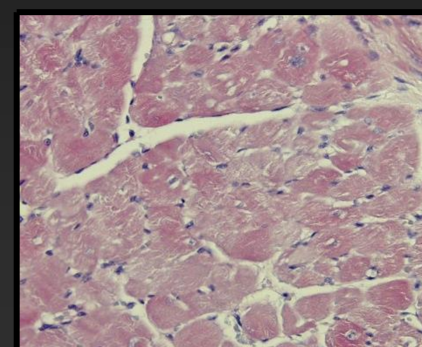
**Control**

**Balloon-injured**

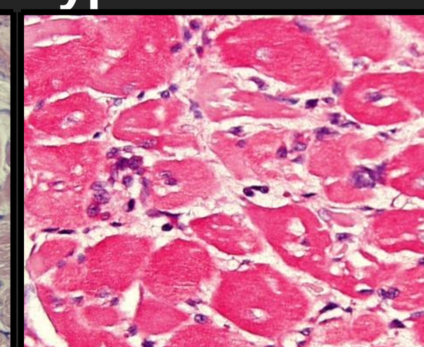


**Immunostaining**

**Control**



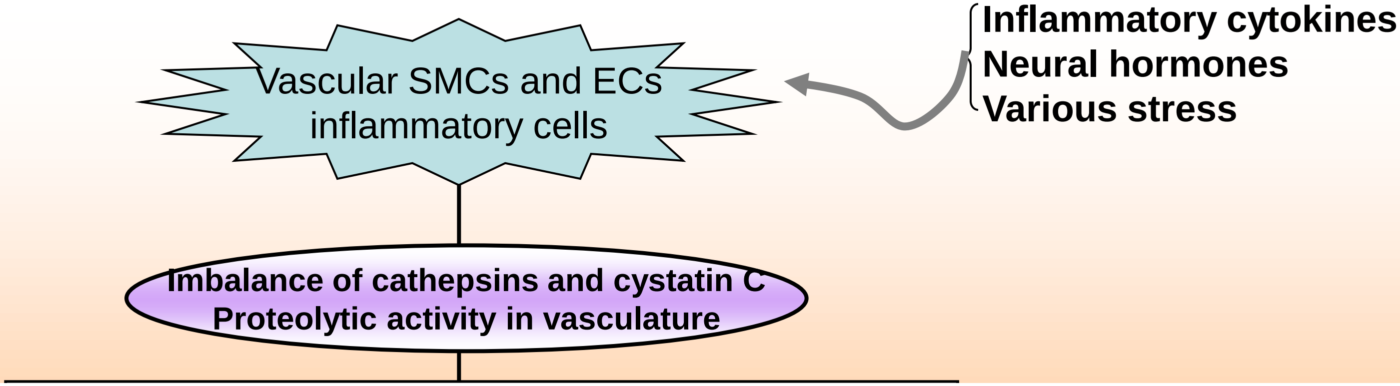
**Hypertensive-HF**



***Rat***

**Cheng XW, et al. *Am J Path 2004, 2006, 2008; Hypertension 2006.***

# *Illustration of cathepsin function in pathogenesis of atherosclerosis-based vascular disease and its implications*





**December 2011**
(next issue online December 14)

**Editor's Picks** FREE

Cysteine Protease Cathepsins in Atherosclerosis-Based Vascular Disease and Its Complications.

Full Text | PDF

**Cheng XW and Murohara T *Hypertension 2011.***

# *Hypothesis*

- Circulating CatK levels might represent a novel marker of patients with CAD predict potential atherosclerotic plaque.

# Study Protocol: 3

## Subjects:

***257 CAD*** ***vs*** ***100 controls***

(admitted for scheduled RFCA between Mar. 2009 - Dec. 2010)

### Definition

- Coronary angiography (at least one major artery 50% > stenosis)

### Exclusion criteria

- Dilated or hypertrophic cardiomyopathy
- Congenital heart disease
- Congestive heart failure
- Valvular heart diseases
- Renal failure on hemodialysis

# Methods

## *Laboratory measurements*

- CatK, CystC
- Intact procollagen type I N-terminal propeptide (I-PINP), Carboxy-terminal telopeptide of collagen type I (ICTP, either as an index of collagen synthesis or degradation, respectively)
- High-sensitivity C-reactive protein (hs-CRP)
- Interleukin (IL)-1β level

## *Echocardiography*

- Left atrial (LA) dimension
- Left ventricular ejection fraction (LVEF)

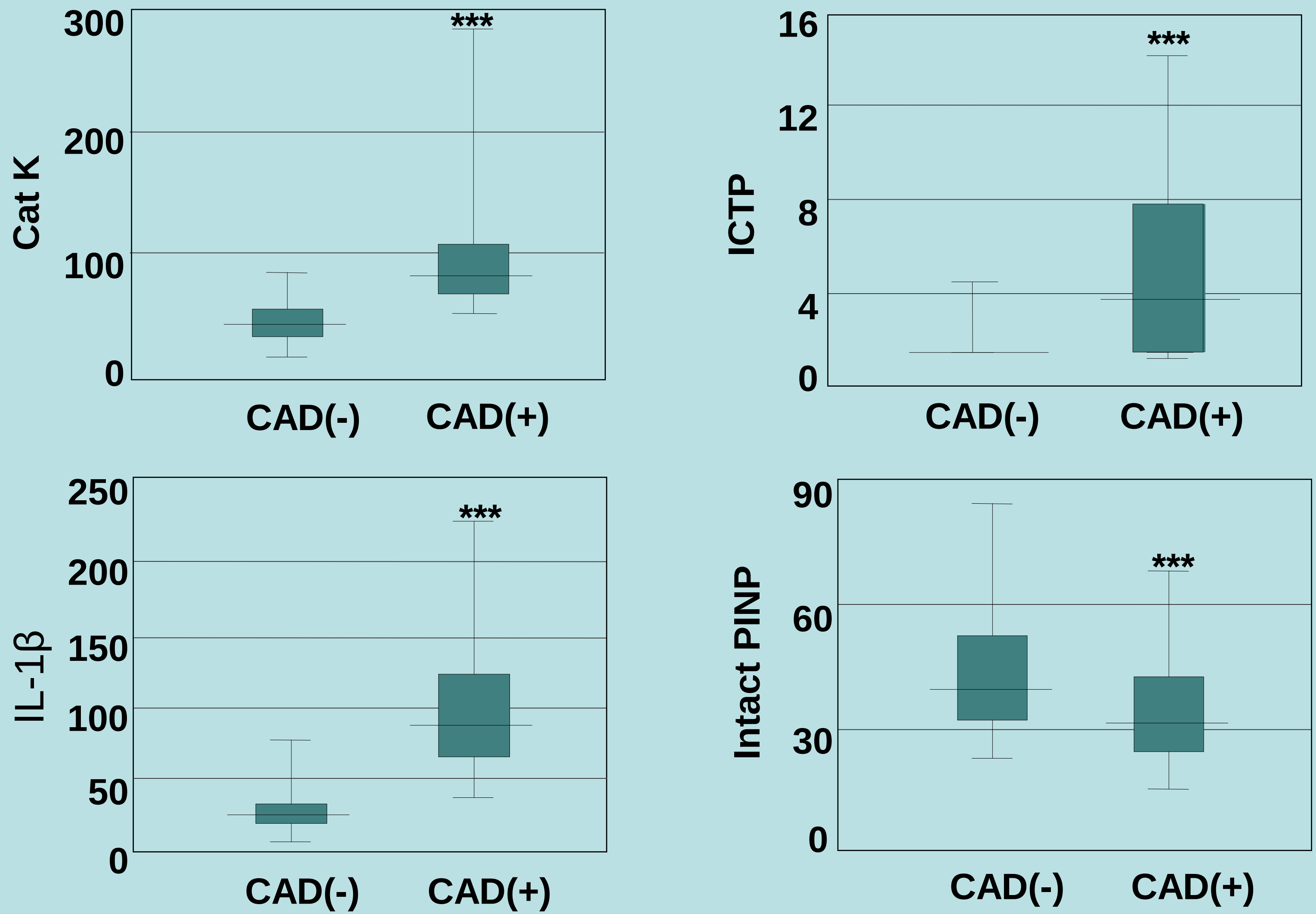
# CAD study

## Patients Characteristics

| | CAD (-) (n=100) | CAD (+) (n=257) | *p* |
|---|---|---|---|
| Age (yrs) | 60.1±5.08 | 62.8±6.96 | N.S. |
| Male (%) | 61 | 67.5 | N.S. |
| BMI | 23.5±2.42 | 24.3±5.21 | N.S. |
| HT (%) | 16.0 | 30.2 | *** |
| DM (%) | 13.0 | 57.5 | *** |
| Smoker (%) | 29.0% | 28.3 | N.S. |
| LDL (mg/dl) | 101.2±25.6 | 123.0±27.6 | *** |
| Statins (%) | 8.0 | 35.2 | *** |
| ARBs (%) | 5.0 | 24.5 | *** |
| CCB (%) | 7.0 | 29.1 | *** |
| ACEI (%) | 3.0 | 7.5 | *** |
| Insulin (%) | 0.0 | 25.1% | *** |

BMI, body mass index; HT, Hypertension; DM, diabetes mellitus; LDL, low-density lipoproteins; ARB, angiotensin II receptor blocker; CCB, calcium channel blocker; ACEI, angiotensin-converting enzyme inhibitor.

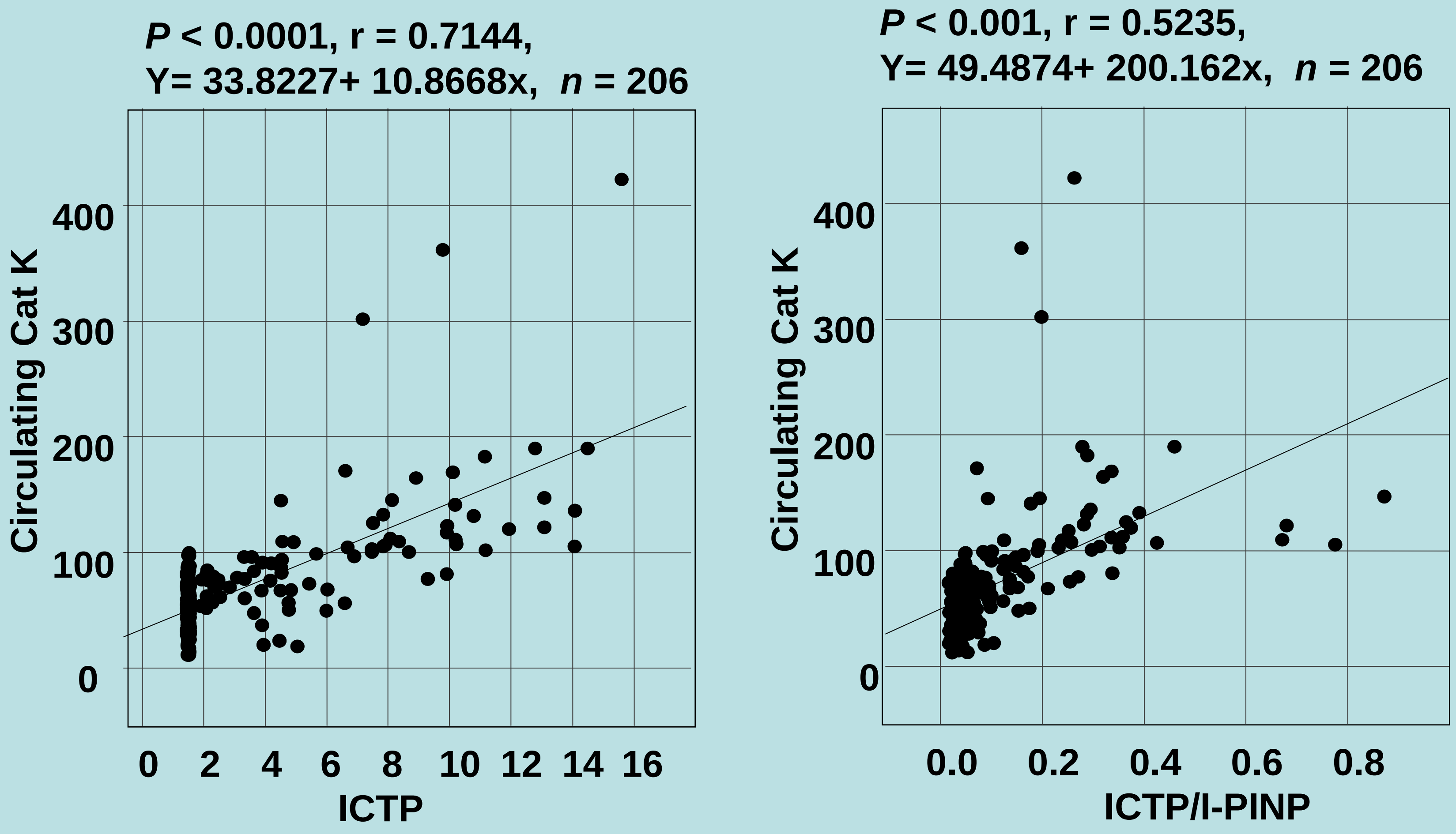
# The levels of serum Cat K, ICTP, I-PINP, and IL-1β in CAD and non-CAD patients



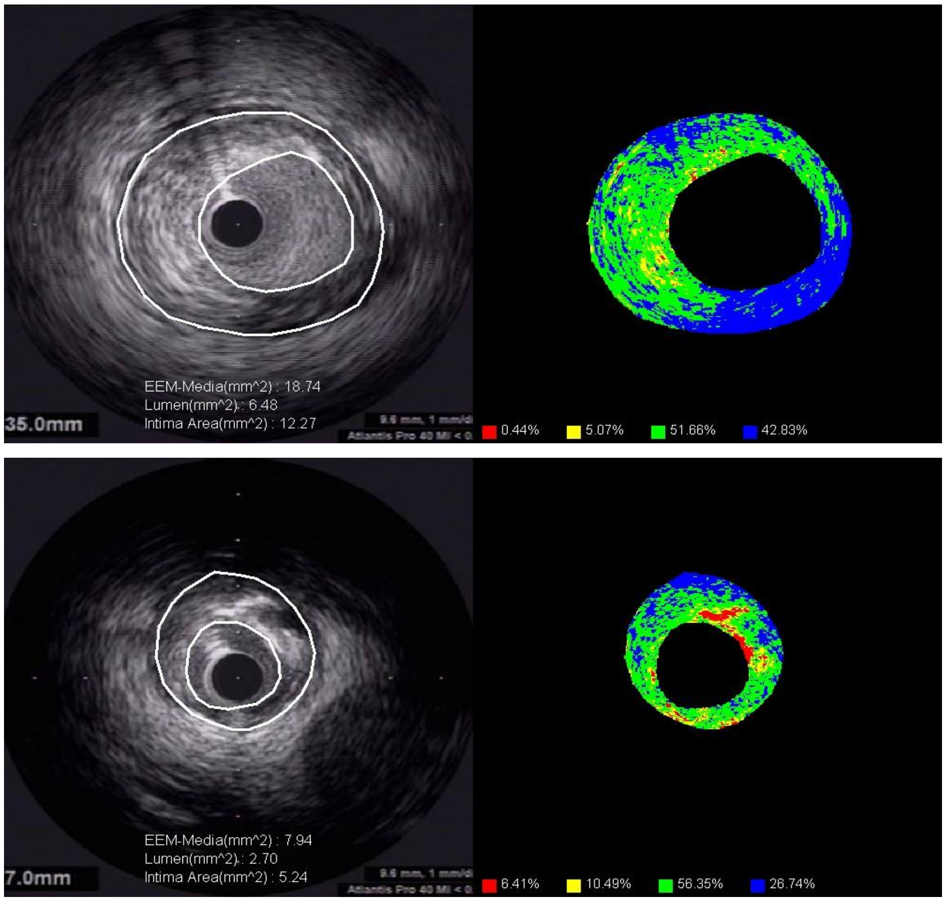

ICTP: linked carboxy-terminal telopeptide of collagen type I
I-PINP: intact procollagen type I N-terminal propeptide

***$P$ < 0.001, vs CAD(-)

# The correlations of circulating Cat K and ICTP and ICTP/I-PINP



# Representative images of serial conventional and integrated backscatter (IB) intravascular images

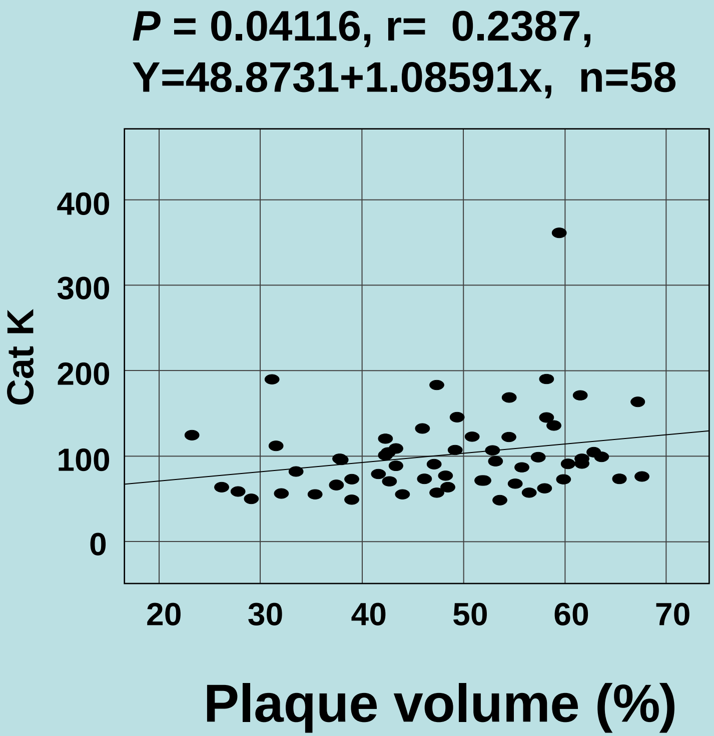


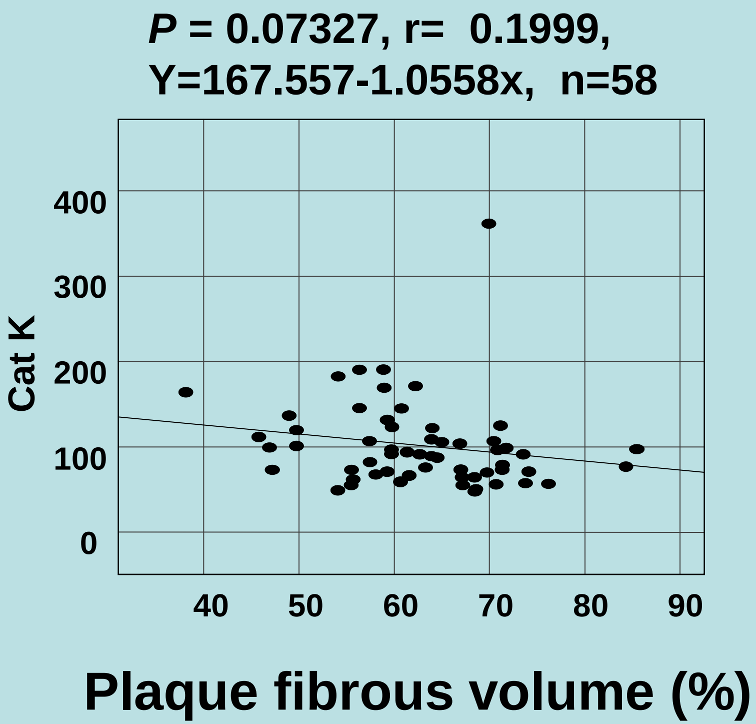

**Serum Cat K: 156 pg/ml)**

**Serum Cat K: 72 pg/ml)**

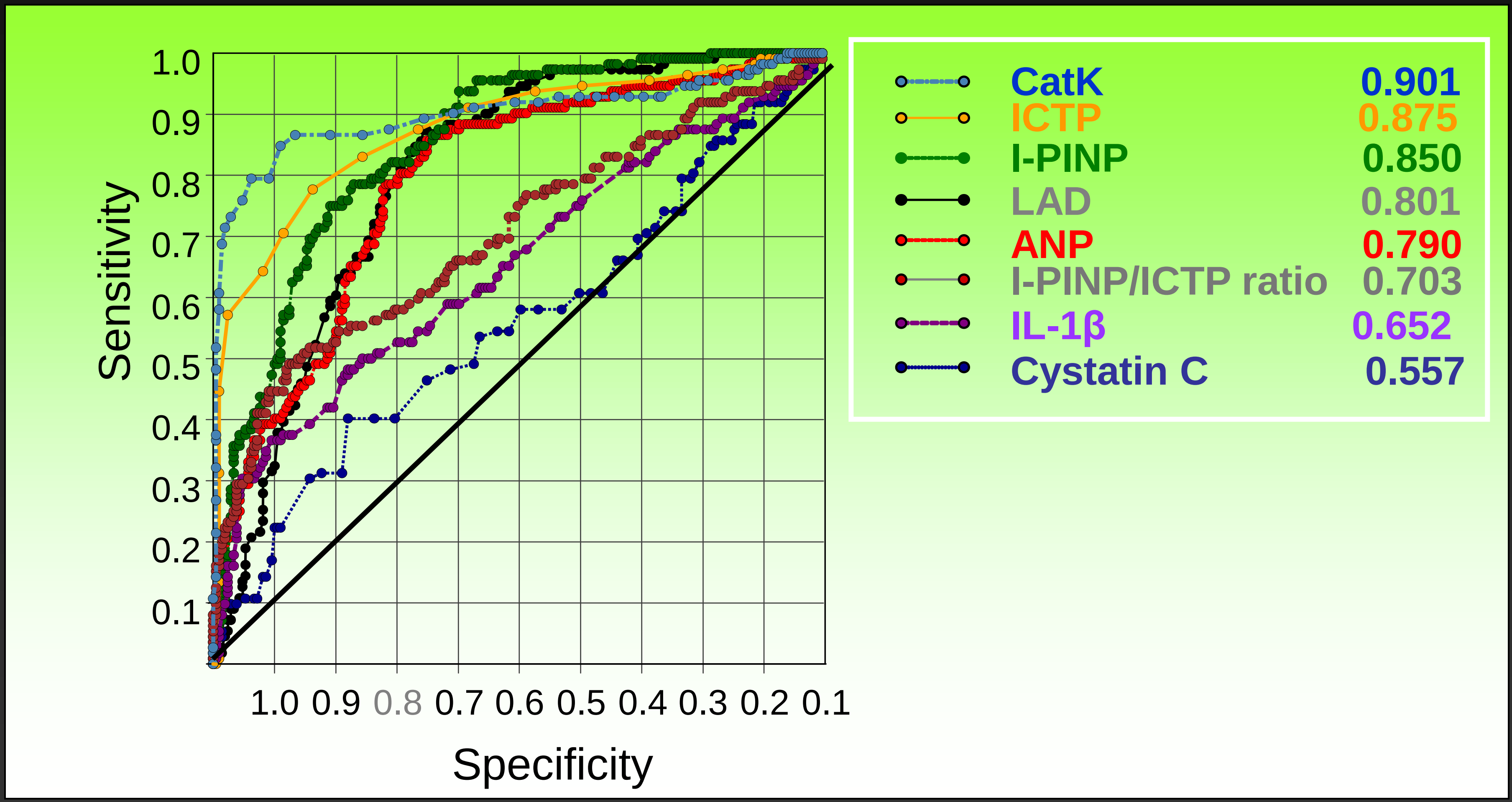
**Green: fibrous volume**
**Blue : lipid volume**
**Red : Calcification: red**

# IVUS analysis shows that the correlations of serum Cat K and plaque and fibrous volumes in CAD patients

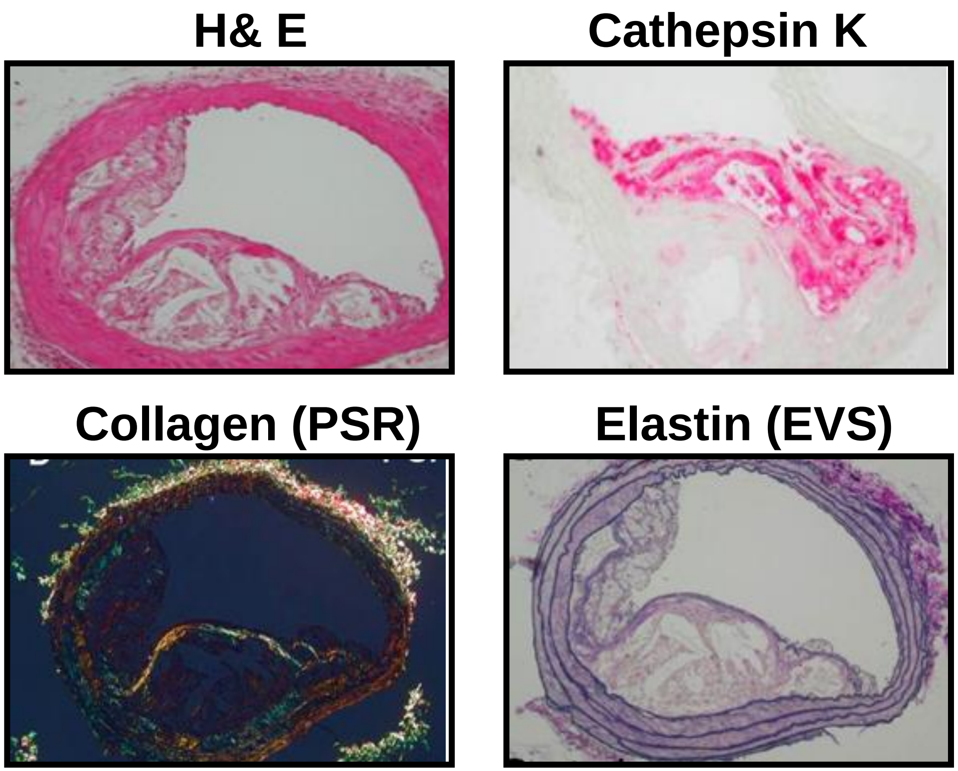


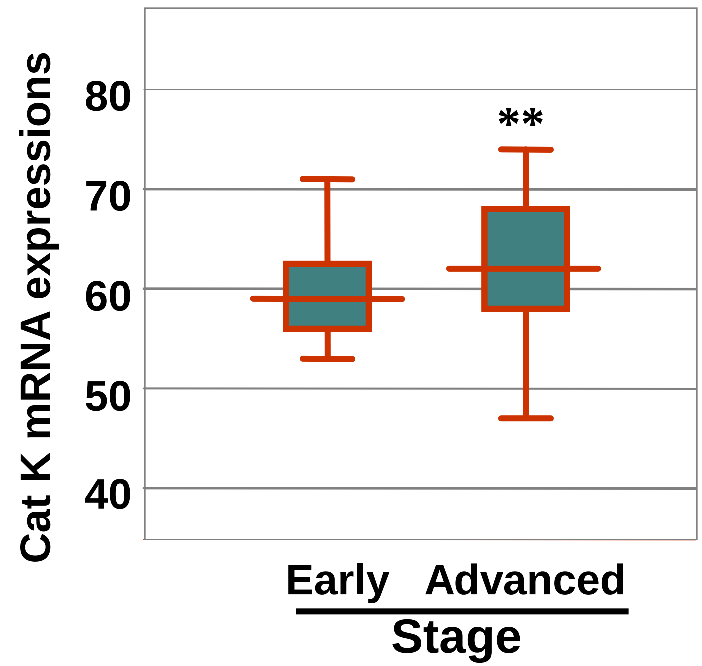



# Receiver Operator Characteristic (ROC) curve for logistic regression models



# The levels of Cat K, collagen, and elastin in the aortic plaques of mice



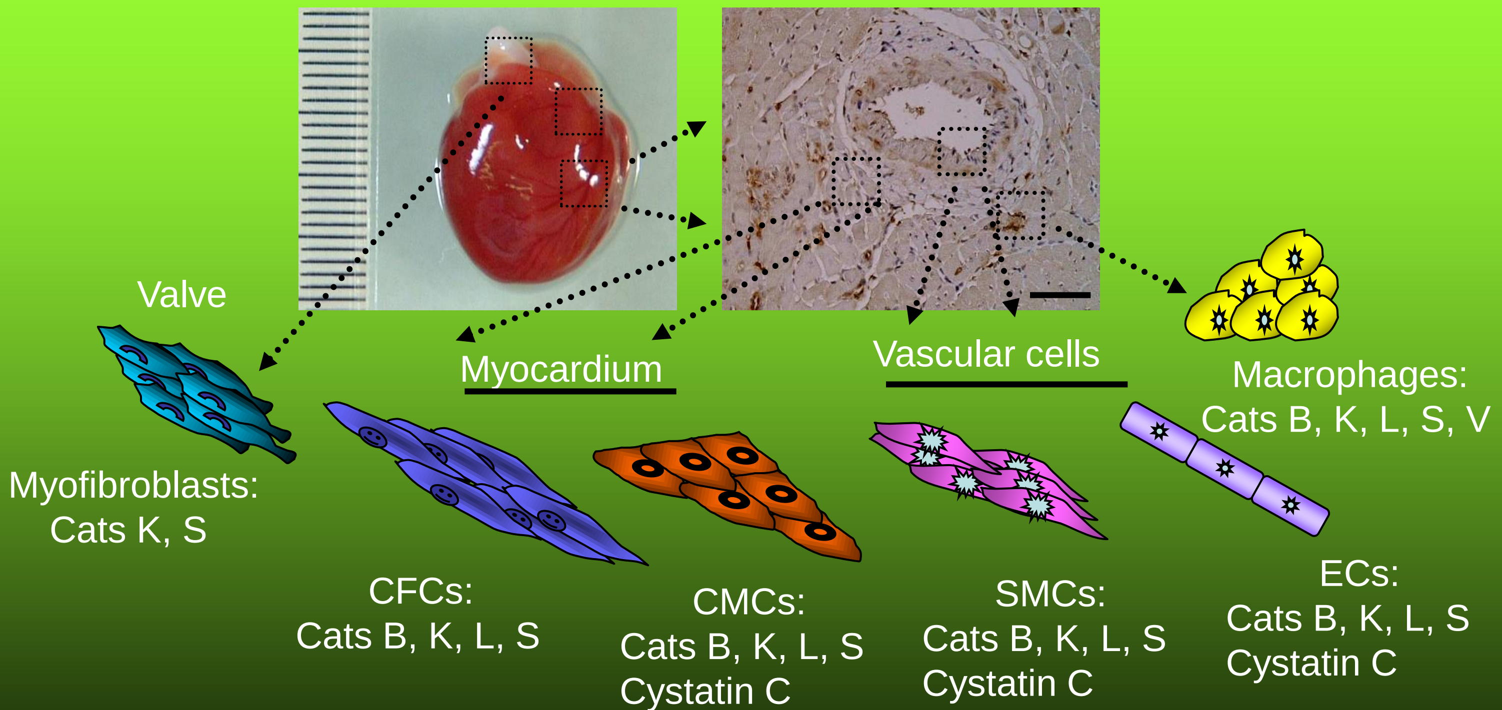

PSR: picrosirous red staining for collagen
EVG: elastica van Gieson staining for elastin

*Cheng XW et al*. (2011 AHA in Orlando: unpublished data)

# *Summary*

- Patients with CAD had significantly higher plasma CatK levels as well as IL-1β and ICTP levels than control subjects.
- Plasma CatK levels were correlated positively with ICTP, and IL-1β.
- Stepwise Logistic regression analysis revealed that, among age, gender, CatK, and collagen markers, CatK, and I-PINP/ICTP ratio were independently associated with CAD.

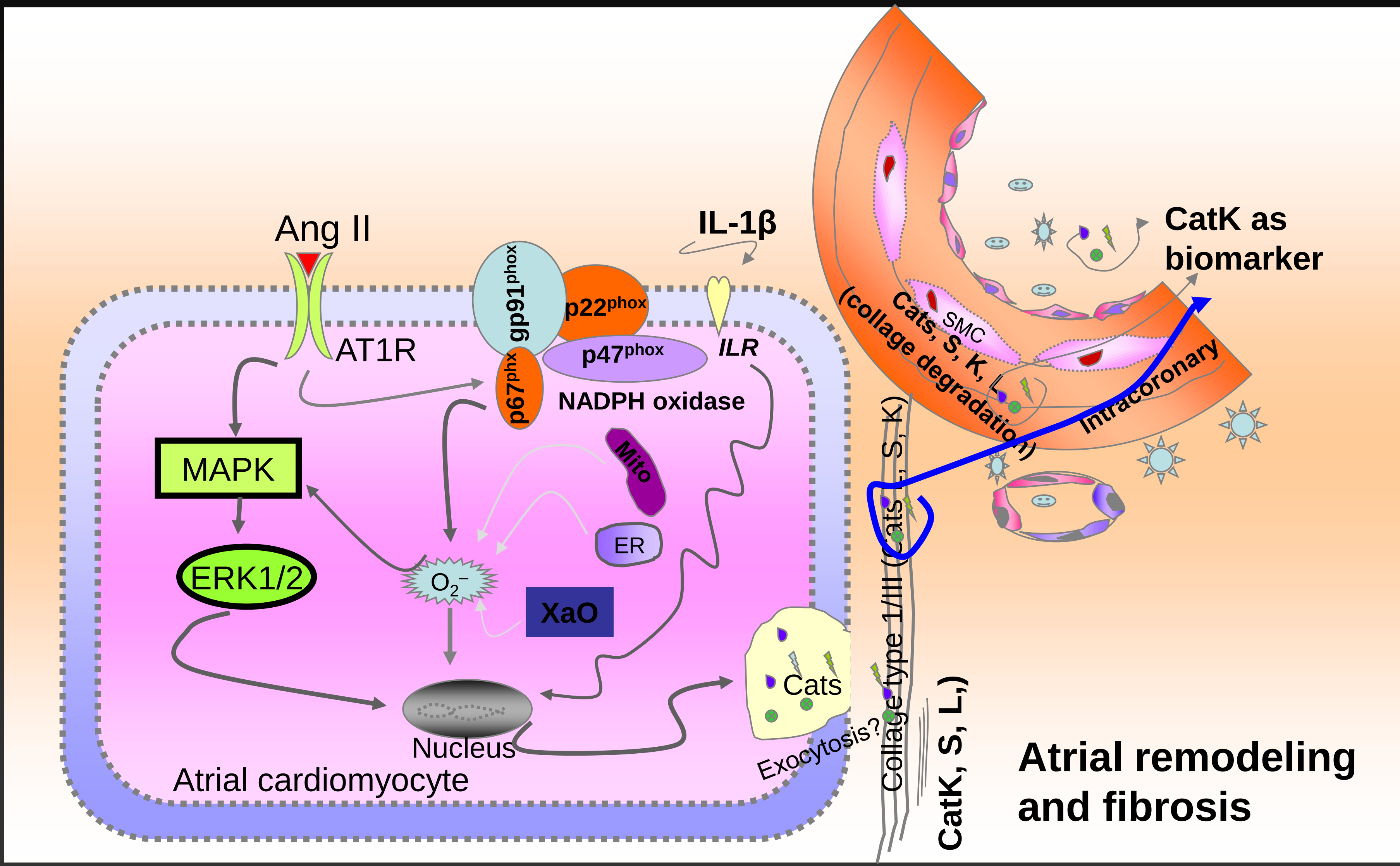
# Cat expressions in cardiovascular and valve cells




CFC, cardiac fibroblast; CMC, cardiomyocyte
SMC, smooth muscle cell; ECs, endothelial cell.

**Cheng XW, Murohara T (Review) Circulation 2011**

# Proposed mechanisms underlying the regulation of CatK expression and releasing in atrium with AF




Mito = mitochondria; ER = endoplasmic reticulum
Xao = xanthine oxidase

Cheng XW, Murohara T (Review) Circulation 2011 (accept)

# ***Conclusions***

- These finding suggest that serum CatK levels represent a novel marker of patients with CAD and predict potential atherosclerotic plaque.

# Collaborative Researchers

## Harvard University

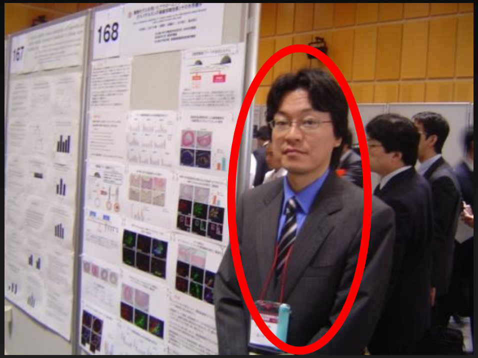
Peter Libby

## Nagoya University

Guo-Ping Shi

Masafumi Kuzuya

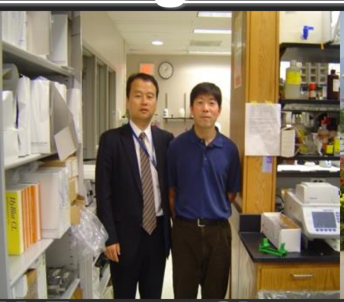
## Hamamatsu University



Takeshi Sasaki

Circ Res 2003, 2007; AJP 2004, 2006, 2008; Hypertension 2006, 2011; JCVP 2009; J Hyperten 2010 (2); Circulation 2010.

## Michigan University



Y. Eugene Chen

PPAR-γ and mesenteric artery aneurysm (on going)

Weonsam Kim

Zhao X et al. YMJ 2011

## Yanbian University

Like Guan

# Acknowledgments

## Nagoya University

**Cardiology**

Michitaka Tsuzuki
Toyoaki Murohara

**Cardiovascular Research Medicine**

Haizhen Song
Kenji Okumura

**Geriatrics**

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Lina Hu
Kae Nakamura
Wazuko Adachi
Masafumi Kuzuya

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