Causes and Consequences of Vascular Pathologies in CKD

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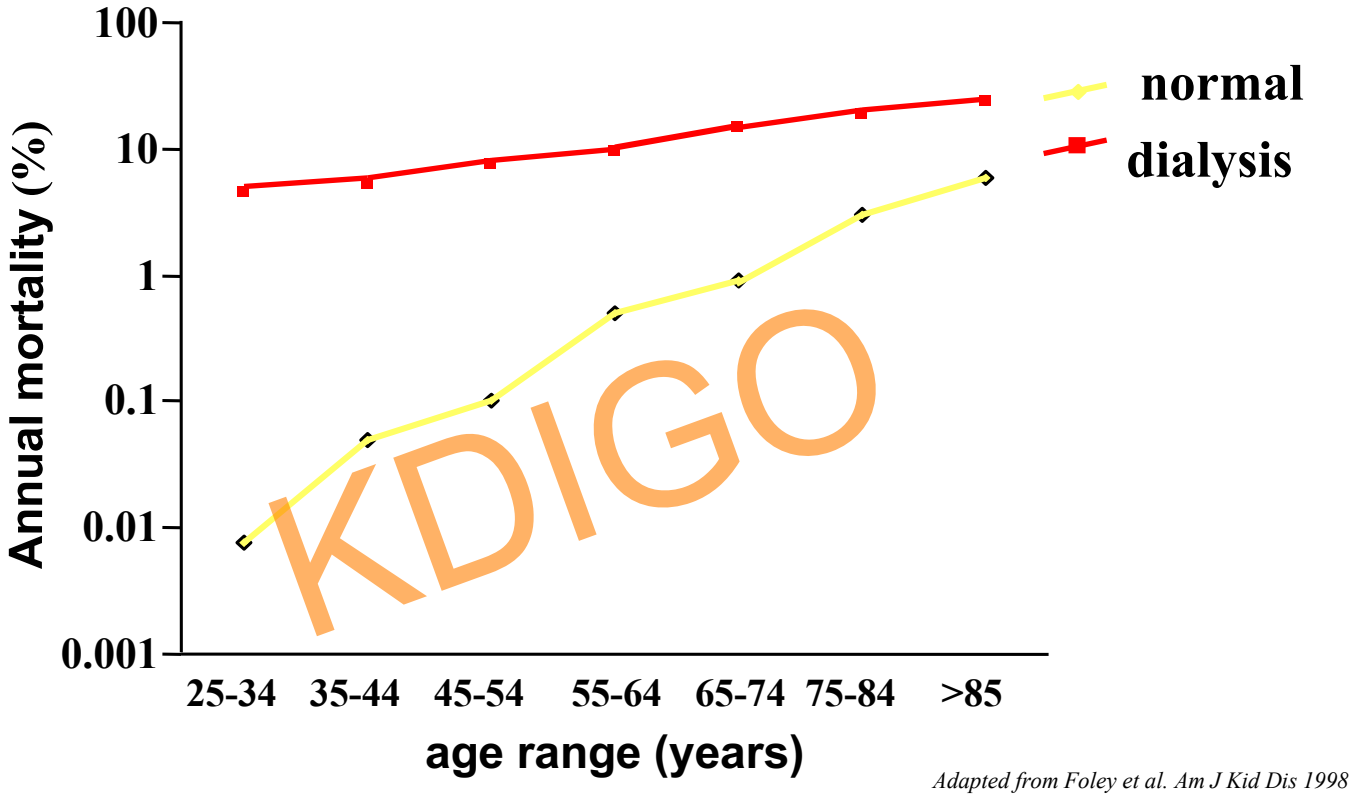
Cardiovascular mortality in CKD patients



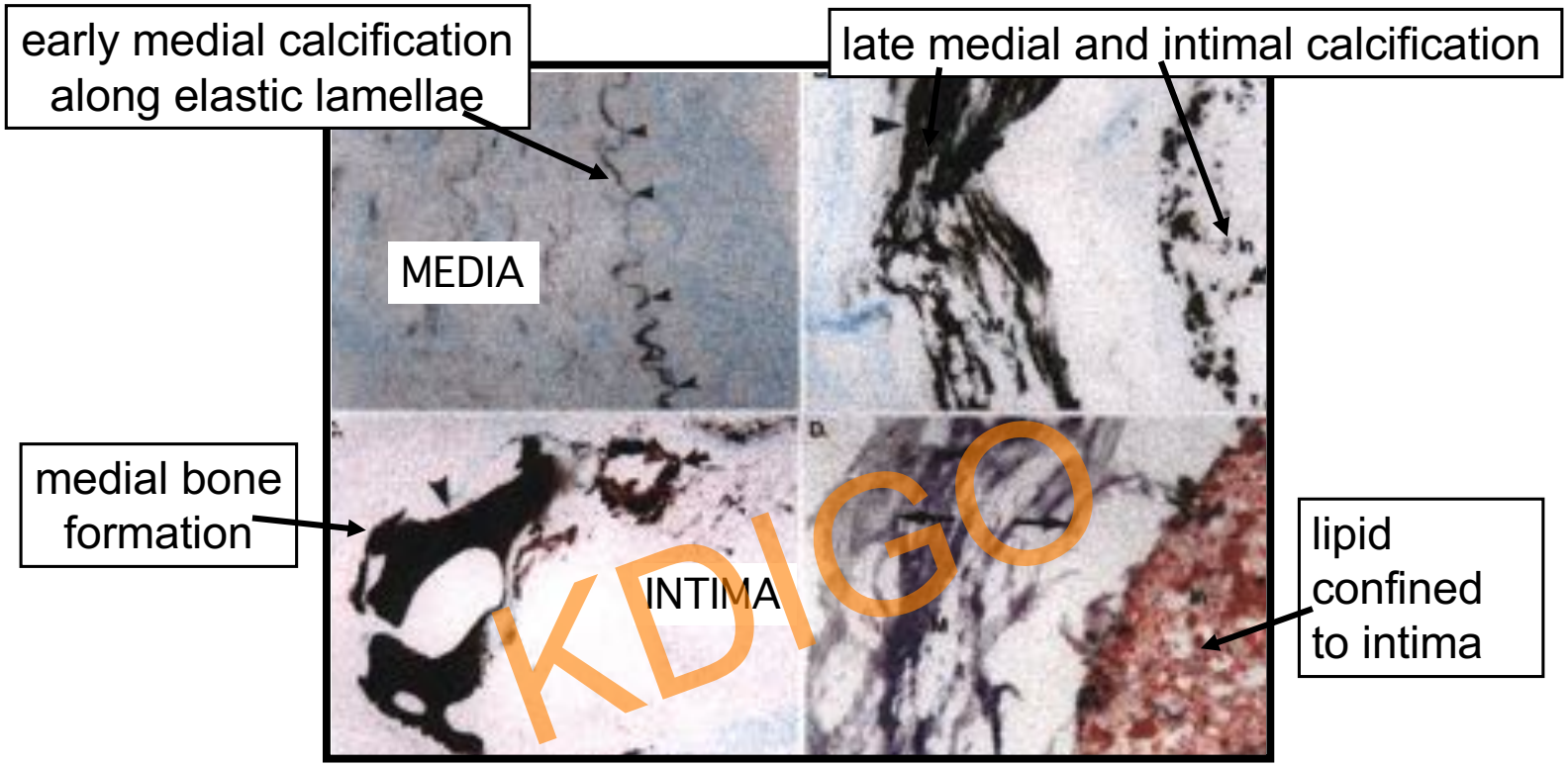
Adapted from Foley et al. Am J Kid Dis 1998

Adolescents and young adult with CKD:

- structural and functional abnormalities in the large vessels
- present even in the second decade of life
- linked to disorders in mineral metabolism

Goodman, NEJM, 2000; Litwin, JASN, 2005;
Mitsnefes, JASN 2005; Goldsmith, NDT, 2006

Is Vascular Calcification A Major Cause Of Cardiovascular Mortality in Renal Failure Patients?



MEDIAL CALCIFICATION

organised along elastic lamellae
bone formation common
VSMCs only
little lipid

→ Ageing
Uremia
Diabetes

INTIMAL CALCIFICATION

punctate, disorganised
bone formation uncommon
macrophages + VSMCs
lipid always present

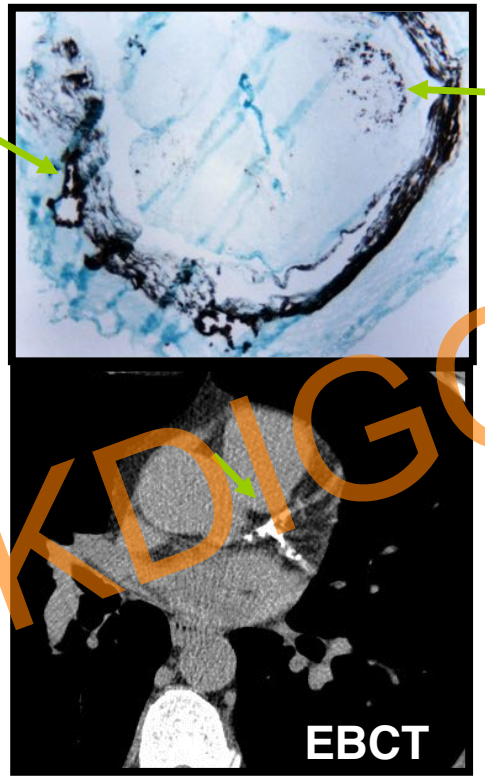
Atherosclerosis

Vascular calcification occurs at two sites

MEDIAL

Diabetes
Renal Disease
Ageing

Stiffness



INTIMAL

Atherosclerosis

Plaque Rupture

CAD?

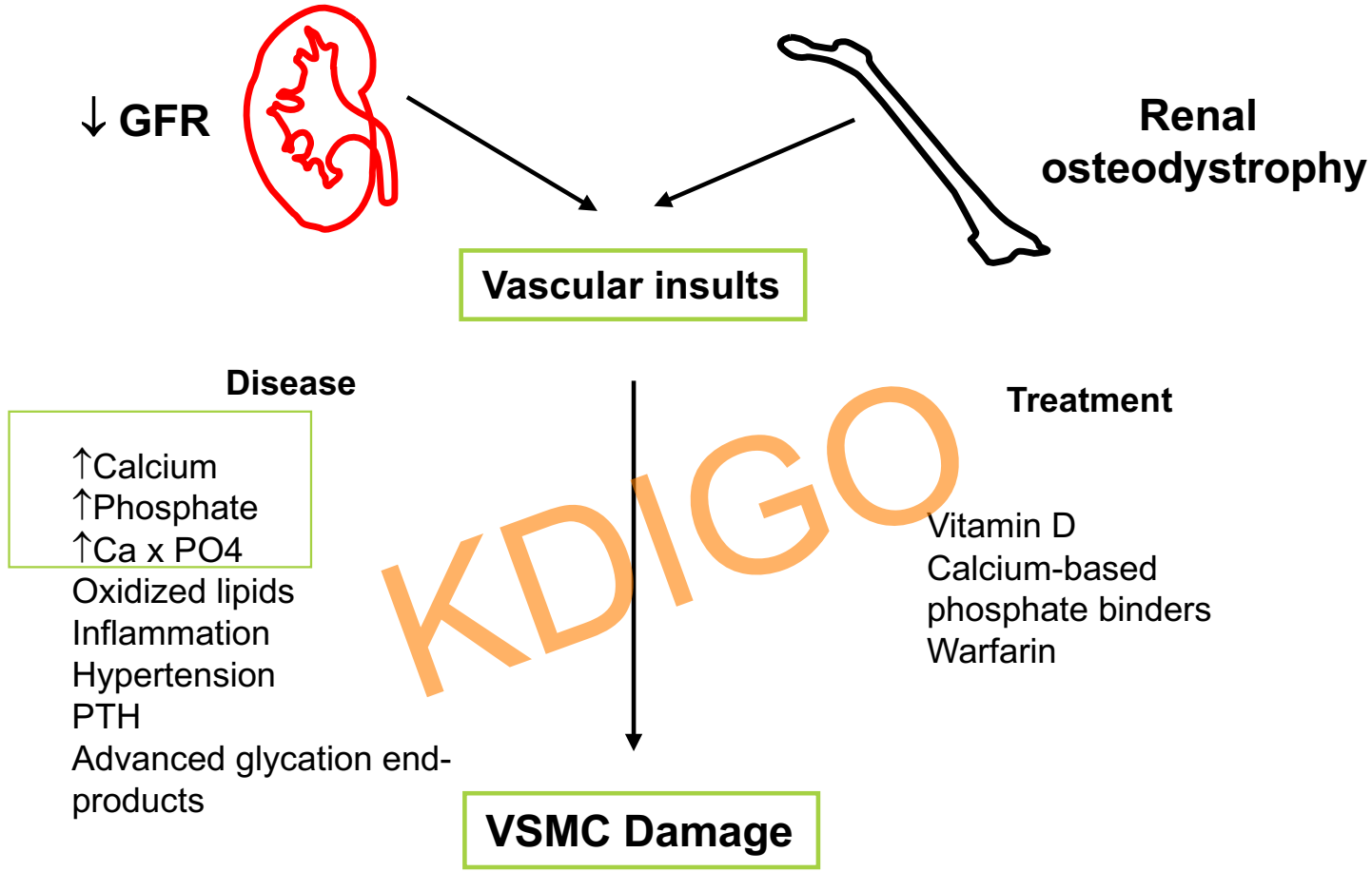
- **Major cause of cardiovascular mortality in CKD**
- **Increased risk of myocardial infarction and all cause mortality**
- **Surgical complications and amputations**
- **Valve calcification**

Vascular Calcification is a

Regulated Process similar to

bone calcification

So Reflects Disease Processes?



- Time on dialysis
- Pre-existing vascular calcification (once present rapidly progresses)

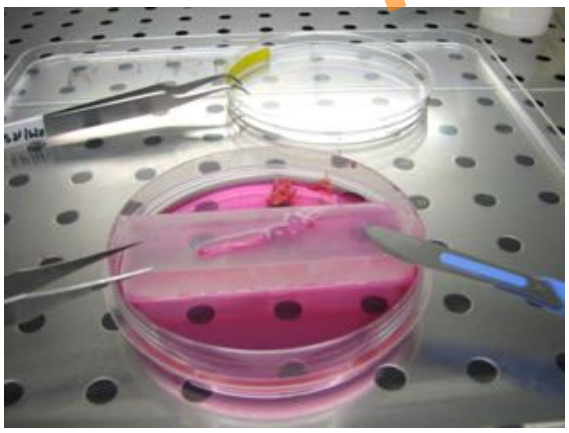
Vessel Rings from Children *in vivo* and *ex vivo*

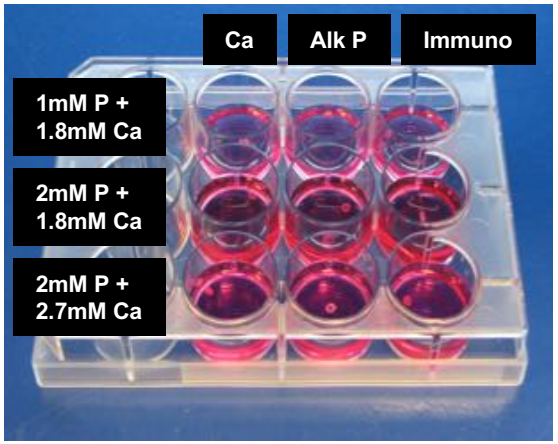
Studied vessels from children on dialysis who develop rapid medial vascular calcification

- pristine vessels -no atherosclerosis
- Intact - vascular matrix structure maintained

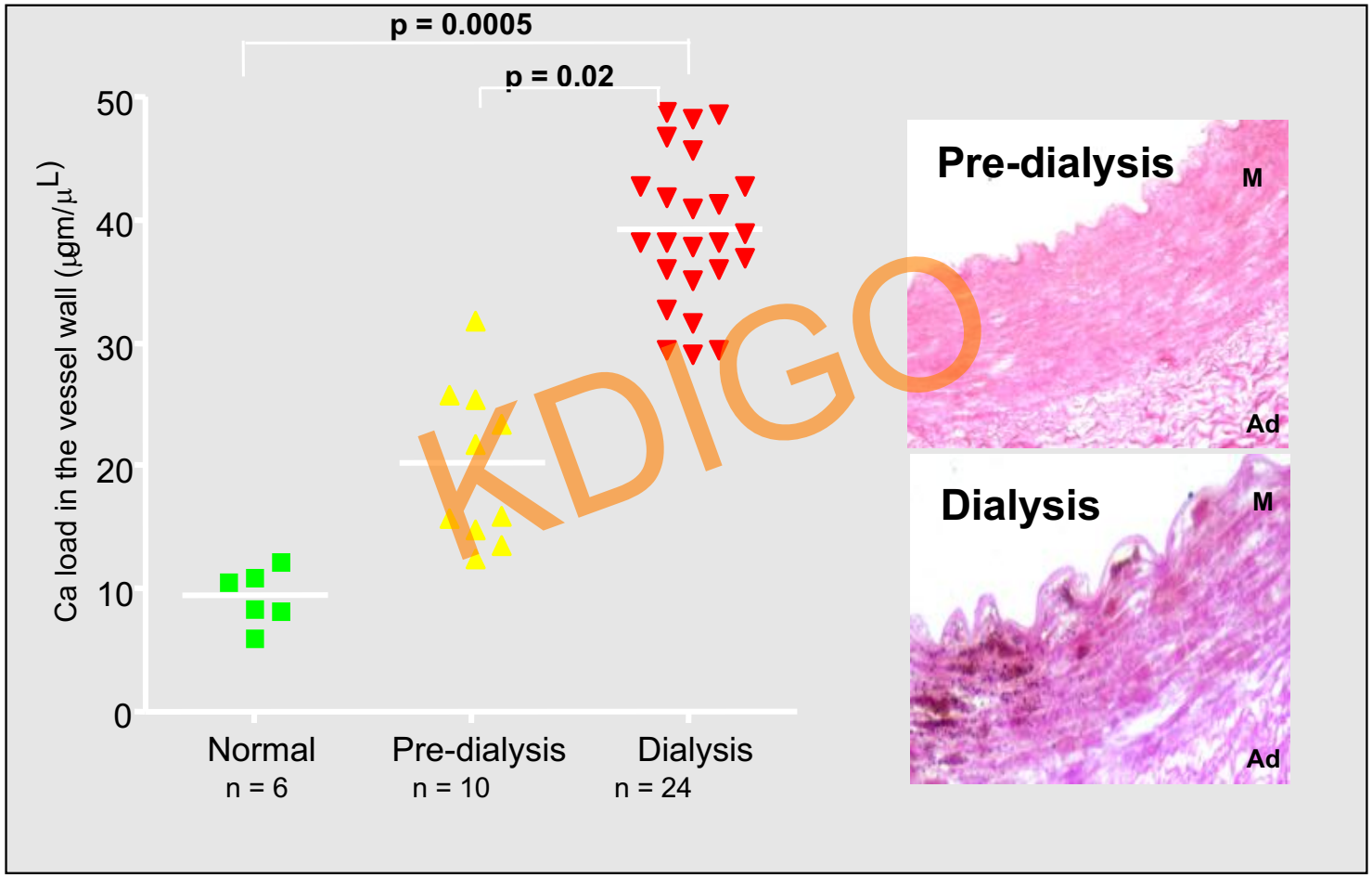
Measured: CALCIUM LOAD
VESSEL HISTOLOGY

Correlated with : VASCULAR MEASURES
BIOCHEMICAL DATA



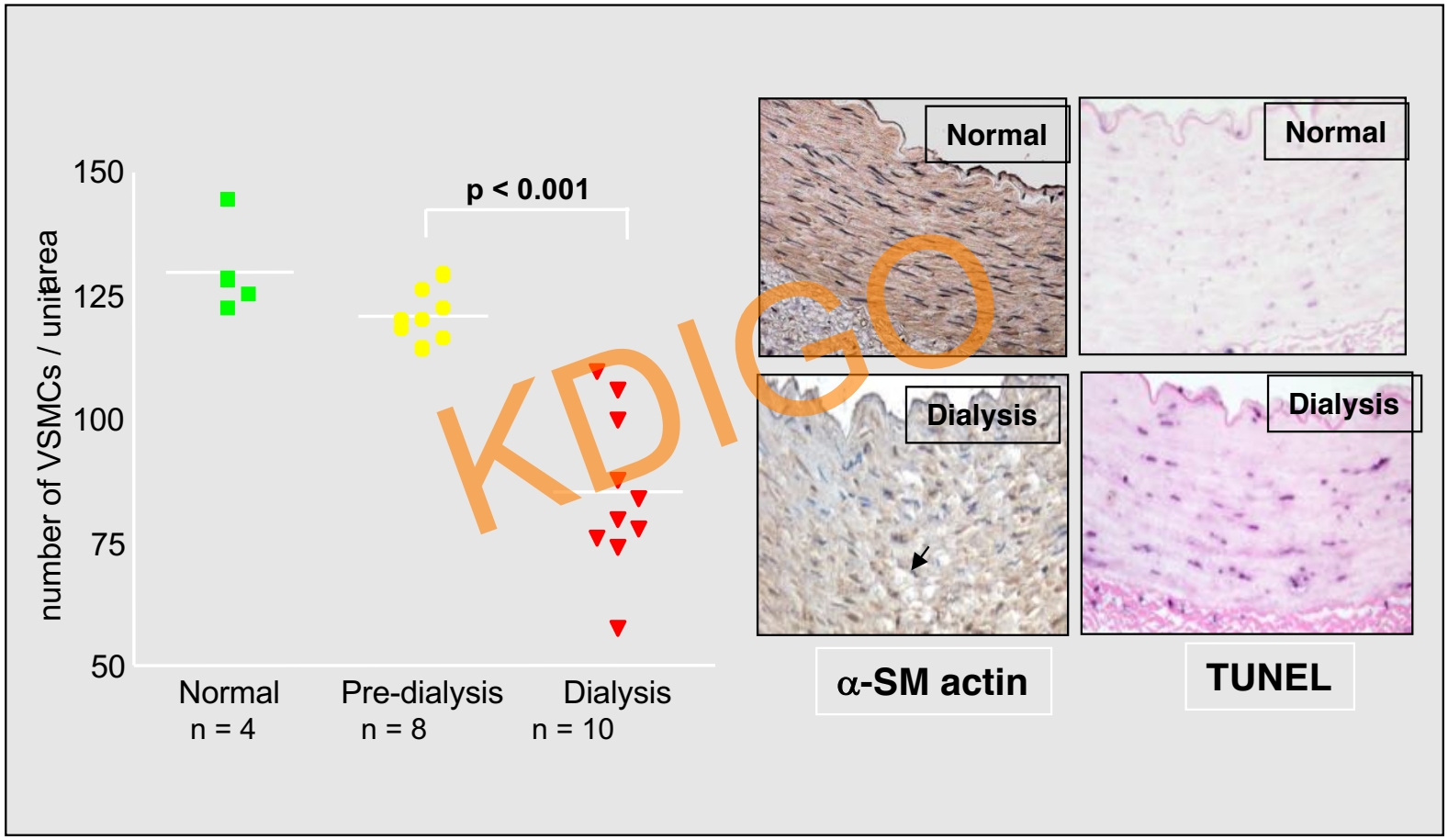


Children on Dialysis develop rapid medial calcification

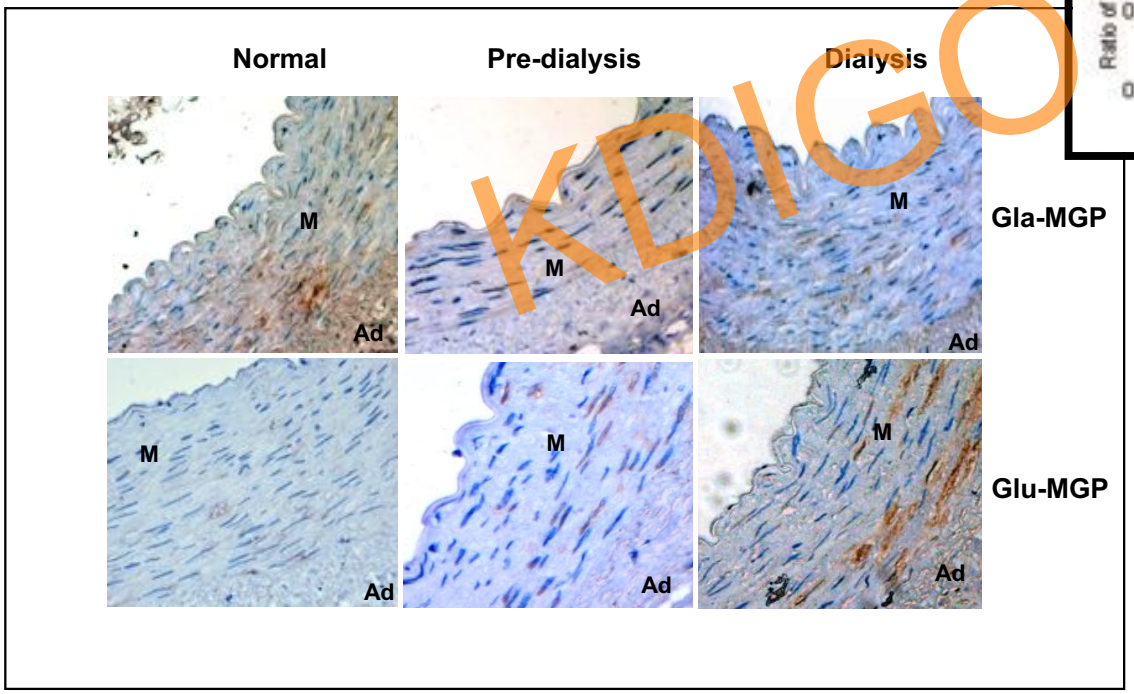


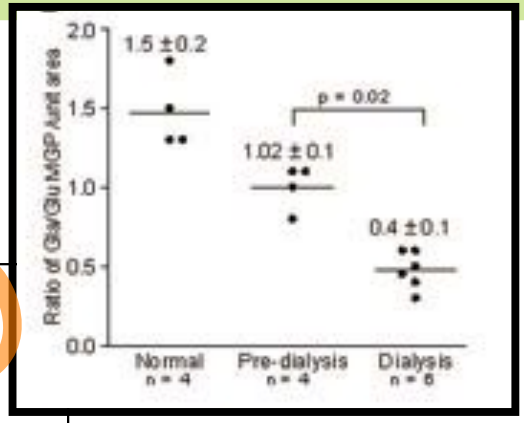
High Circulating Phosphate Levels, Transient Hypercalcemia?

Calcification correlates with VSMC loss via apoptosis

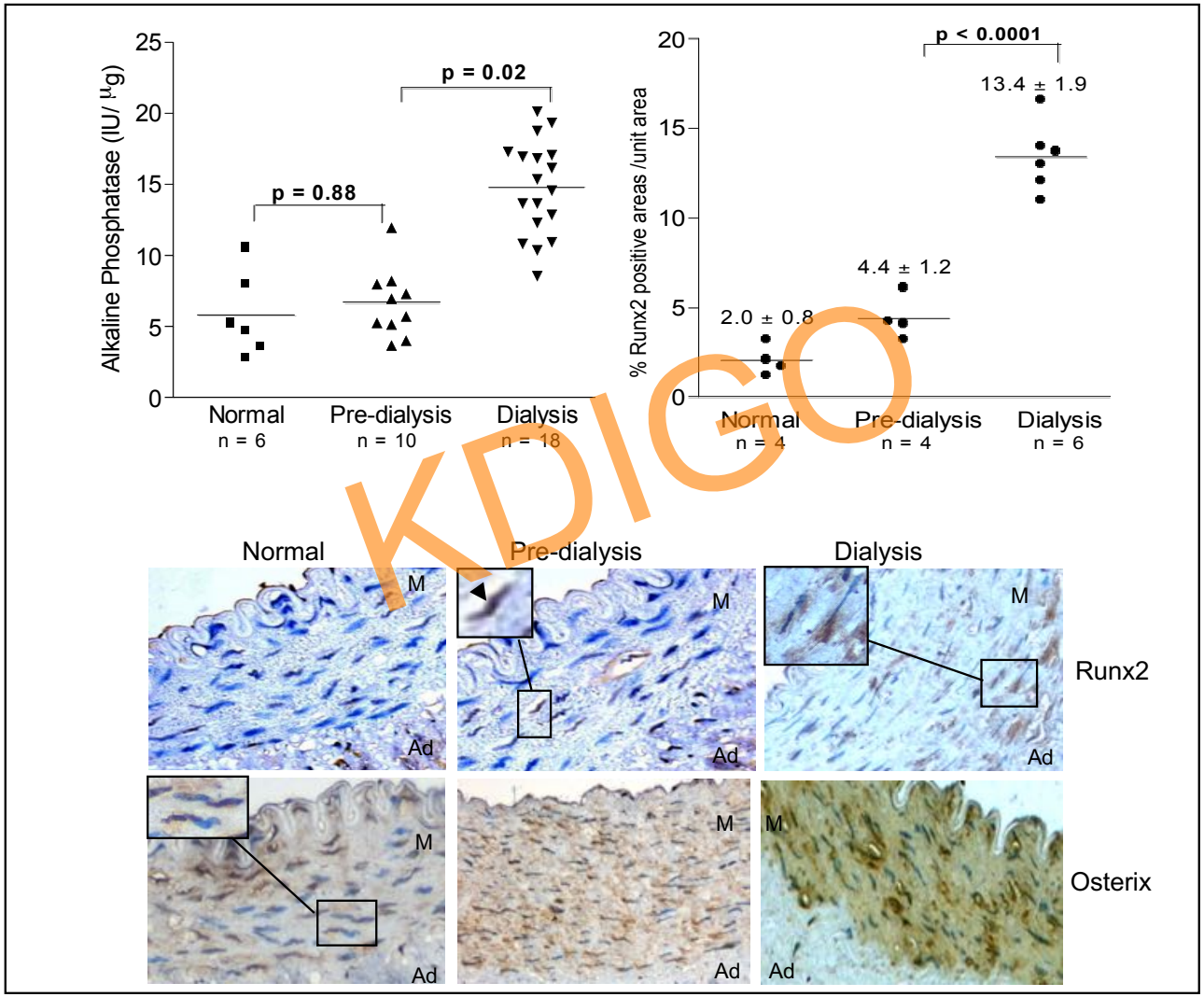


Loss of Calcification Inhibitors
Non-functional Glu-MGP predominates in Dialysis vessels



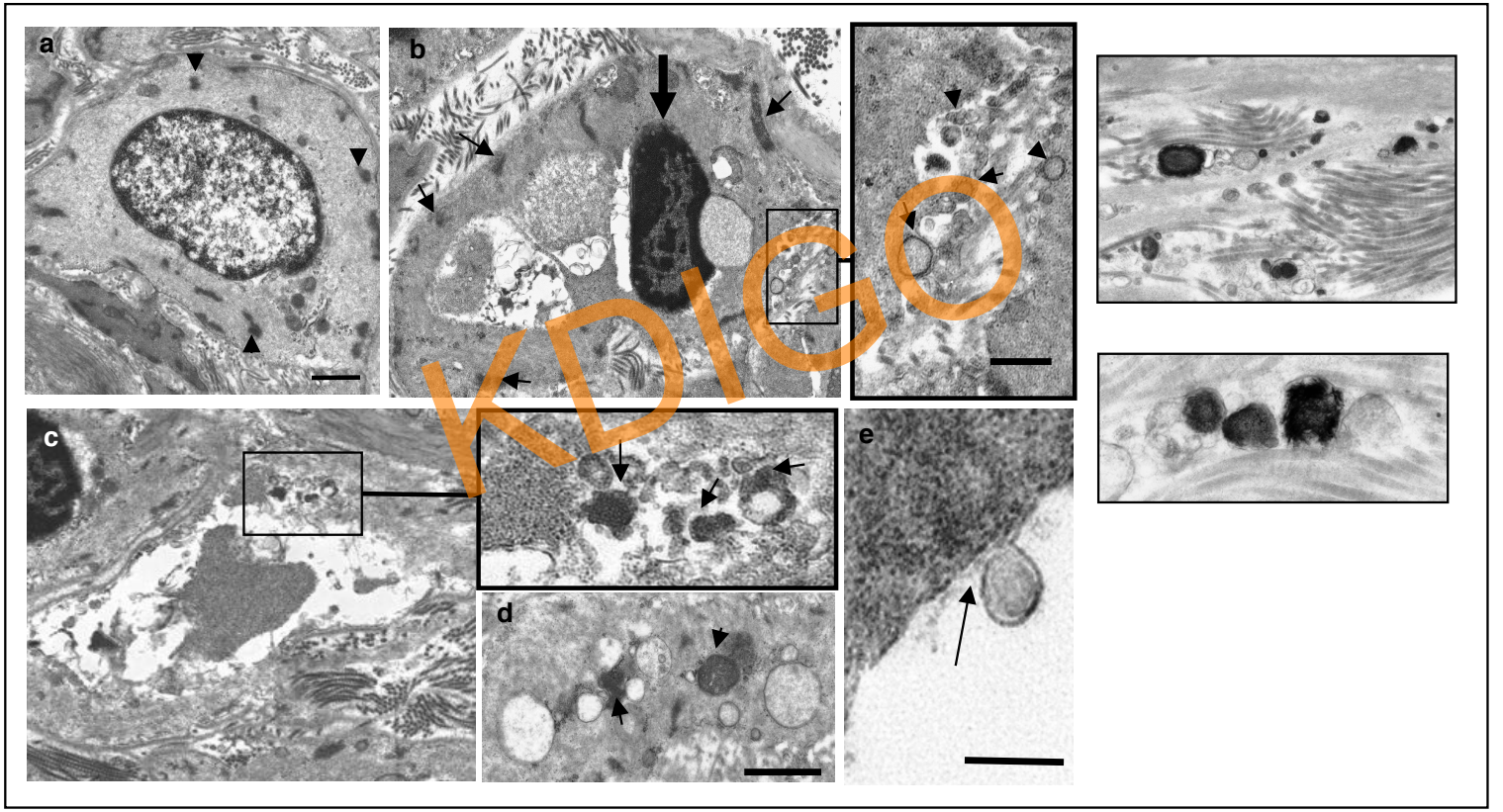


Dialysis vessels show increased osteogenic differentiation



(Shroff et al 2008,Circulation)

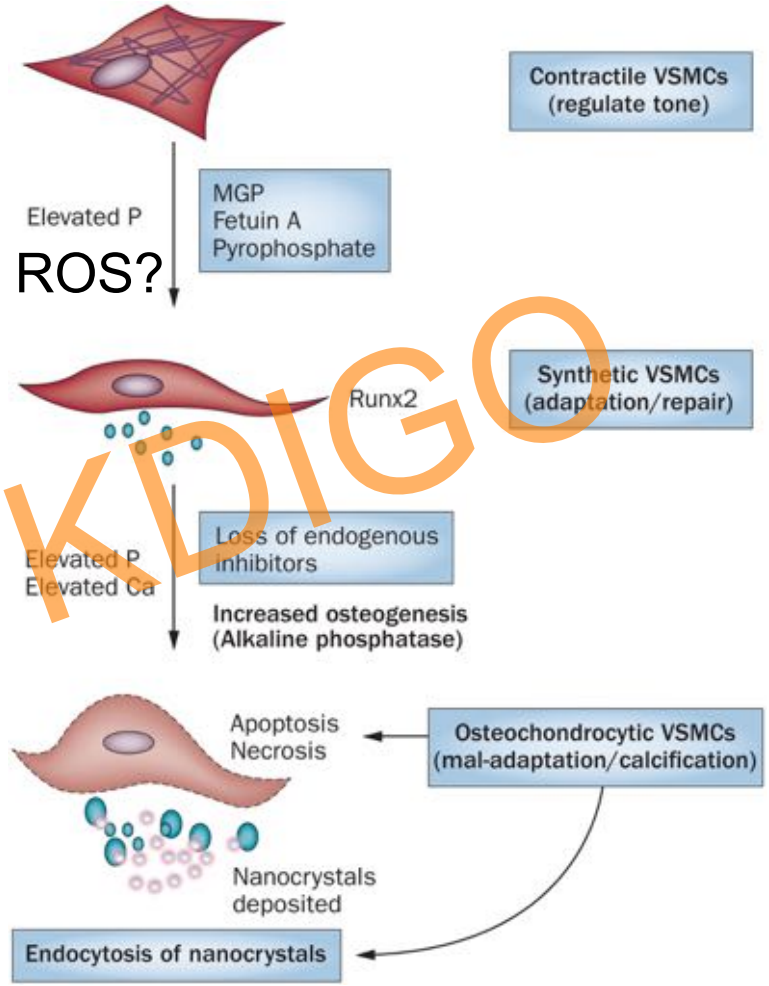
Ca load is associated with increased vesicle deposition by VSMCs



(Shroff et al 2008, *Circulation*)

Mechanisms of Vascular Smooth Muscle Cell Calcification

CKD
Diabetes
Ageing

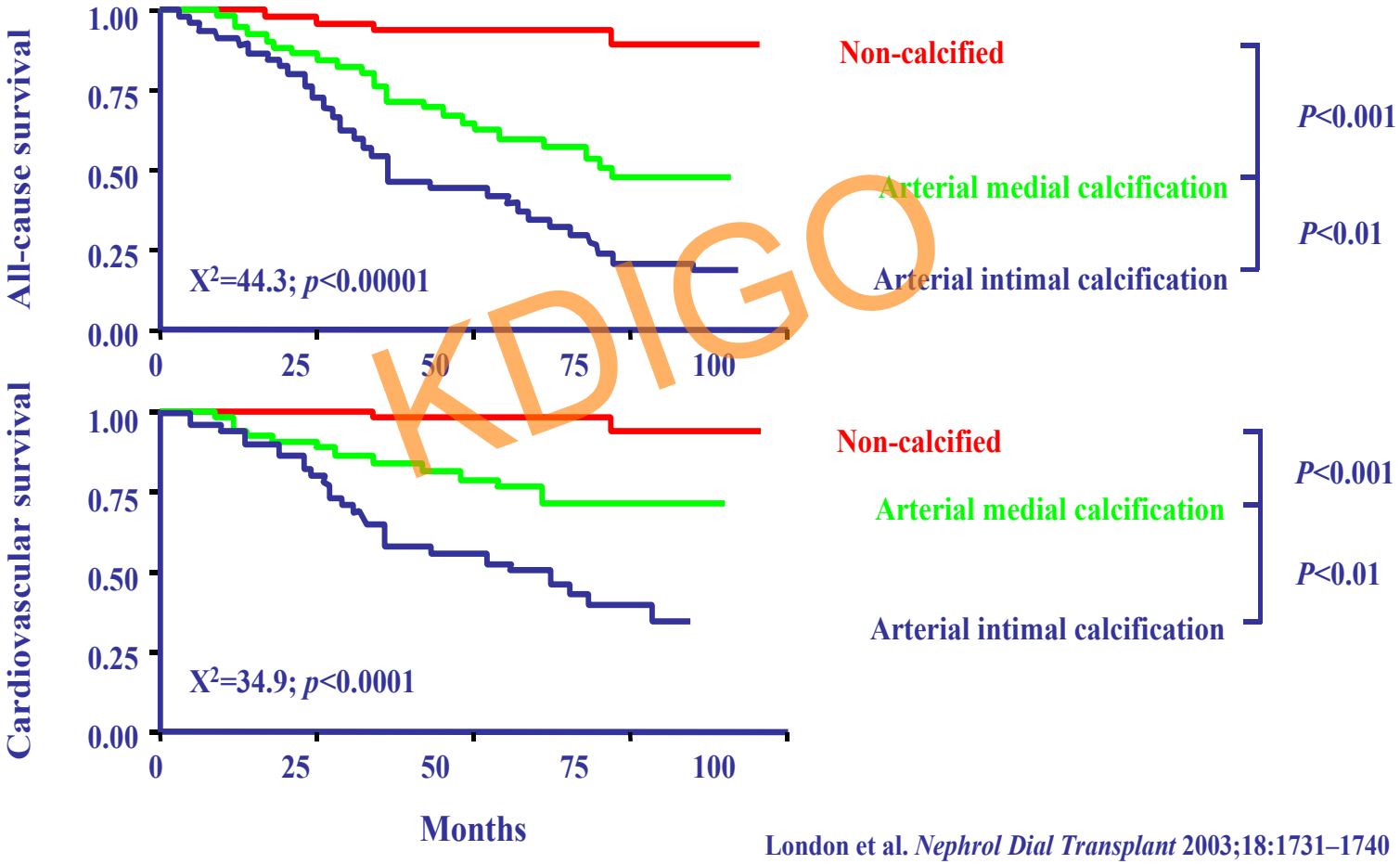


Shanahan, C. M. (2013)

Why is Calcification Important Clinically?

The Clinical Consequence Medial Calcification is Arterial Stiffening

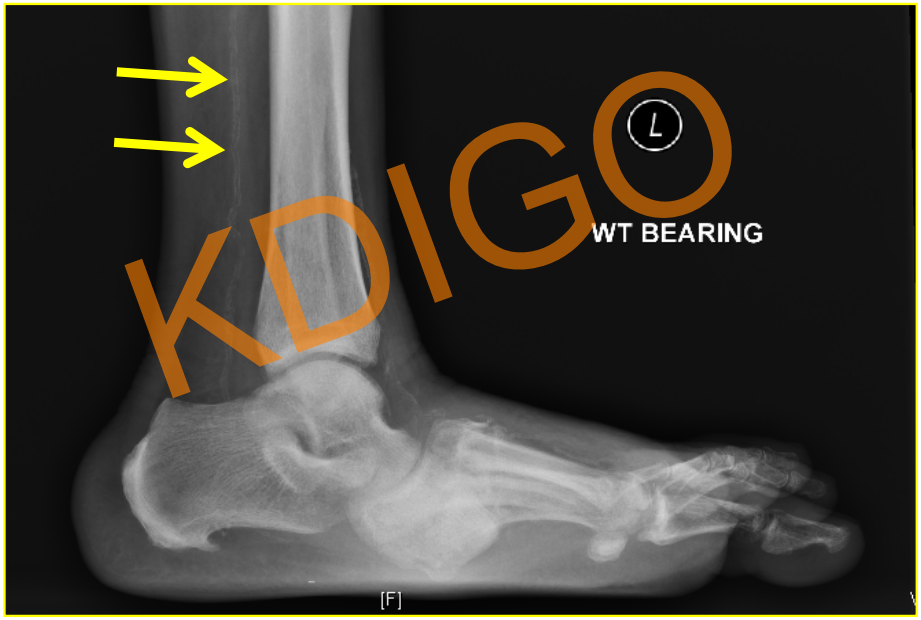
Impact on all-cause and CV mortality of arterial Calcification in CKD



London et al. *Nephrol Dial Transplant* 2003;18:1731–1740

Diabetes is Associated with a high Prevalence of Vascular Calcification in Peripheral Arteries

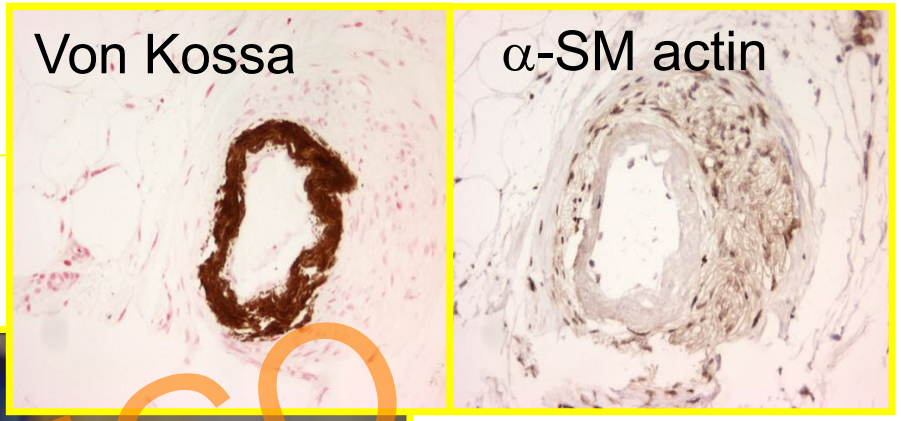
Peripheral Artery Calcification in Diabetes

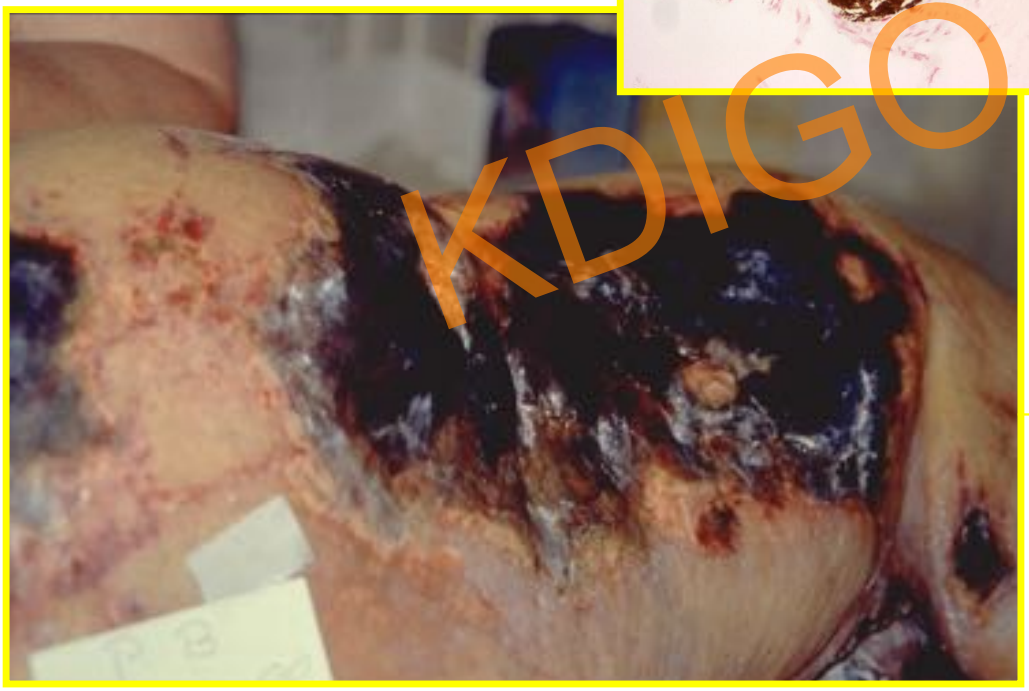


Associated with increased CV mortality, amputation and ulcers, surgical complications

Calciphylaxis in CKD

Medial calcification of small arterioles





Progressive Gangrene
High Mortality

Is there medial calcification in the coronary arteries of patients with ESRD?

Detailed analysis of calcified areas using the Kossa stain showed that calcification of the coronary lesions was predominantly located in the intima, i.e. the arterial plaque, whereas we could hardly find any media calcification in the coronary arteries (Fig. 1).

?

N=25 CKD patients

Watcher et al, Histol Histopathol, 2018

Vascular Calcification is Associated with an increased risk of Plaque Rupture.

Does calcification cause plaque rupture?

Is the type of calcification Important?

Small Calcium deposits are associated with plaque instability

Spotty Calcification Typifies the Culprit Plaque in Patients With Acute Myocardial Infarction

An Intravascular Ultrasound Study

Shoichi Ehara, MD; Yoshiki Kobayashi, MD; Minoru Yoshiyama, MD; Kenei Shimada, MD; Yoshihisa Shimada, MD; Daiju Fukuda, MD; Yasuhiro Nakamura, MD; Hajime Yamashita, MD; Hiroyuki Yamagishi, MD; Kazuhide Takeuchi, MD; Takahiko Naruko, MD; Kazuo Haze, MD; Anton E. Becker, MD; Junichi Yoshikawa, MD; Makiko Ueda, MD

Background—Calcification is a common finding in human coronary arteries; however, the relationship between calcification patterns, plaque morphology, and patterns of remodeling of culprit lesions in a comparison of patients with acute coronary syndromes (ACS) and those with stable conditions has not been documented.

Methods and Results—Preinterventional intravascular ultrasound (IVUS) images of 175 patients were studied, 61 with acute myocardial infarction (AMI), 70 with unstable angina pectoris (UAP), and 47 with stable angina pectoris (SAP). The frequency of calcium deposits within an arc of less than 90° for all calcium deposits was significantly different in culprit lesions of patients with AMI, UAP, and SAP ($P<0.0001$). Moreover, the average number of calcium deposits within an arc of <90° per patient was significantly higher in AMI than in SAP ($P<0.0005$; mean±SD, AMI 1.4±1.3, SAP 0.5±0.8). Conversely, calcium deposits were significantly longer in SAP patients ($P<0.0001$; mean±SD, AMI 2.2±1.6, UAP 1.9±1.8, and SAP 4.3±3.3 mm). In AMI patients, the typical pattern was spotty calcification, associated with a fibrofatty plaque and positive remodeling. In ACS patients showing negative remodeling, no calcification was the most frequent observation. Conversely, SAP patients had the highest frequency of extensive calcification.

Conclusions—Our observations show that IVUS allows the identification of vulnerable plaques in coronary arteries, not only by identifying a fibrofatty plaque and positive remodeling, but also by identifying a spotty pattern of calcification.

(*Circulation*. 2004;110:3424-3429.)

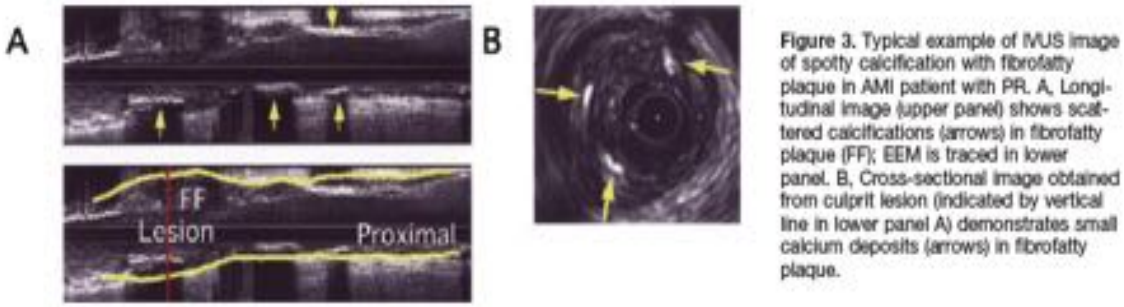
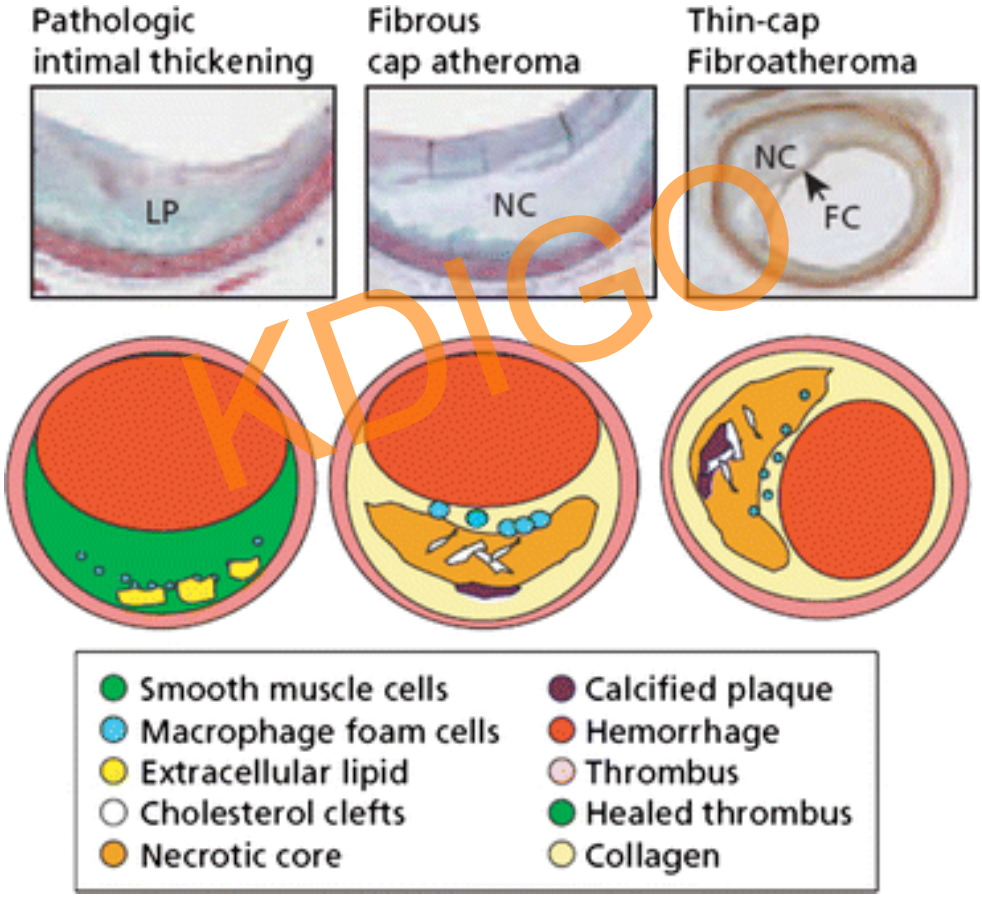


Figure 3. Typical example of IVUS image of spotty calcification with fibrofatty plaque in AMI patient with PR. A, Longitudinal image (upper panel) shows scattered calcifications (arrows) in fibrofatty plaque (FF); EEM is traced in lower panel. B, Cross-sectional image obtained from culprit lesion (indicated by vertical line in lower panel A) demonstrates small calcium deposits (arrows) in fibrofatty plaque.

Calcium Crystals Cause VSMC Death and Inflammation and Plaque Rupture?

- Nanocrystals induce VSMC death
  (Ewence et al Circ Res 2008)
- Nanocrystals cause macrophage Inflammation
  (Nadra et al Cir Res 2006)
- Changes in plaque response to mechanical forces
  (Richardson et al, Lancet 1989)
- Nanocrystals cause rupture of the fibrous cap
  (Kelly-Arnould, et al Weinbaum, PNAS 2013)

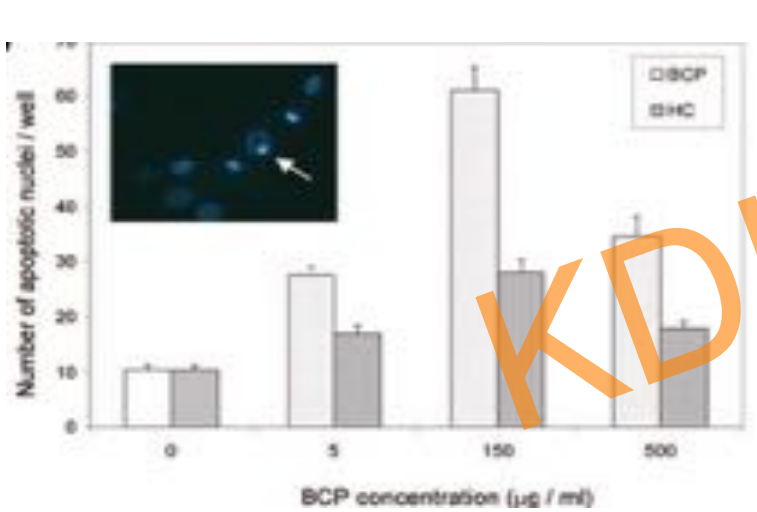
Plaque Rupture is Associated with Thinning of the FC but Plaque Erosion can also occur

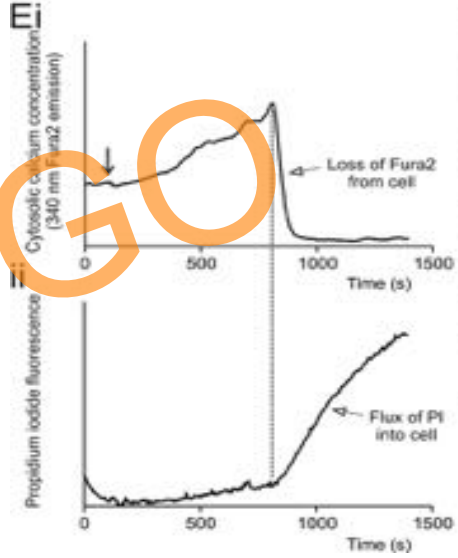


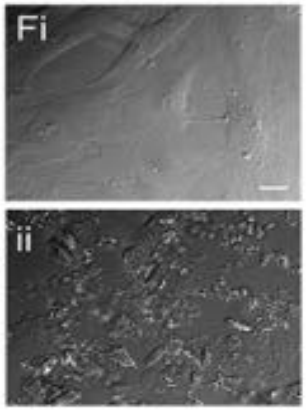
Calcium Phosphate Crystals Induce Cell Death in Human Vascular Smooth Muscle Cells

A Potential Mechanism in Atherosclerotic Plaque Destabilization

Alexandra E. Ewence, Martin Bootman, H. Llewelyn Roderick, Jeremy N. Skepper, Geraldine McCarthy, Matthias Epple, Markus Neumann, Catherine M. Shanahan, Diane Proudfoot







Nano-crystals from plaques induce VSMC death. Small crystals most potent.

Small crystals also activate the Infammasome and IL1a signalling.

Proudfoot et al 2018

VSMC death is induced by intracellular Ca overload due to phagocytosis and lysosomal degradation of nano-crystals.

Nano-crystals activate inflammatory NfκB signalling in macrophages

ELSEVIER

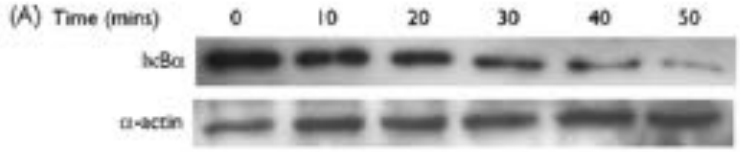
ATHEROSCLEROSIS

Atherosclerosis 196 (2008) 98–105

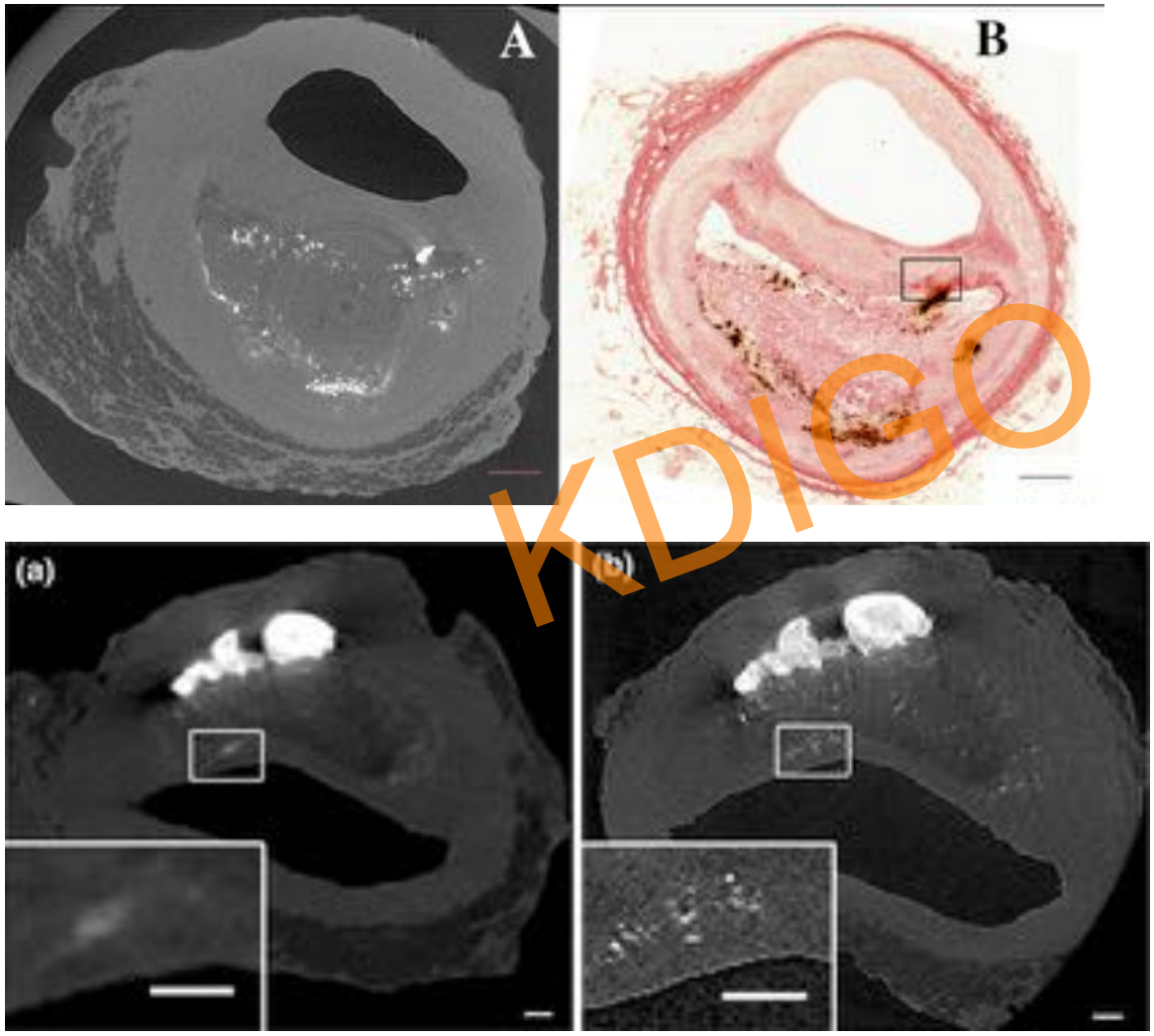
www.elsevier.com/locate/atherosclerosis

Effect of particle size on hydroxyapatite crystal-induced tumor necrosis factor alpha secretion by macrophages

Imad Nadra[a], Aldo R. Boccaccini[b], Pandelis Philippidis[a], Linda C. Whelan[c], Geraldine M. McCarthy[c], Dorian O. Haskard[a], R. Clive Landis[a,*]



Micro CT shows microcalcifications in the Fibrous Cap

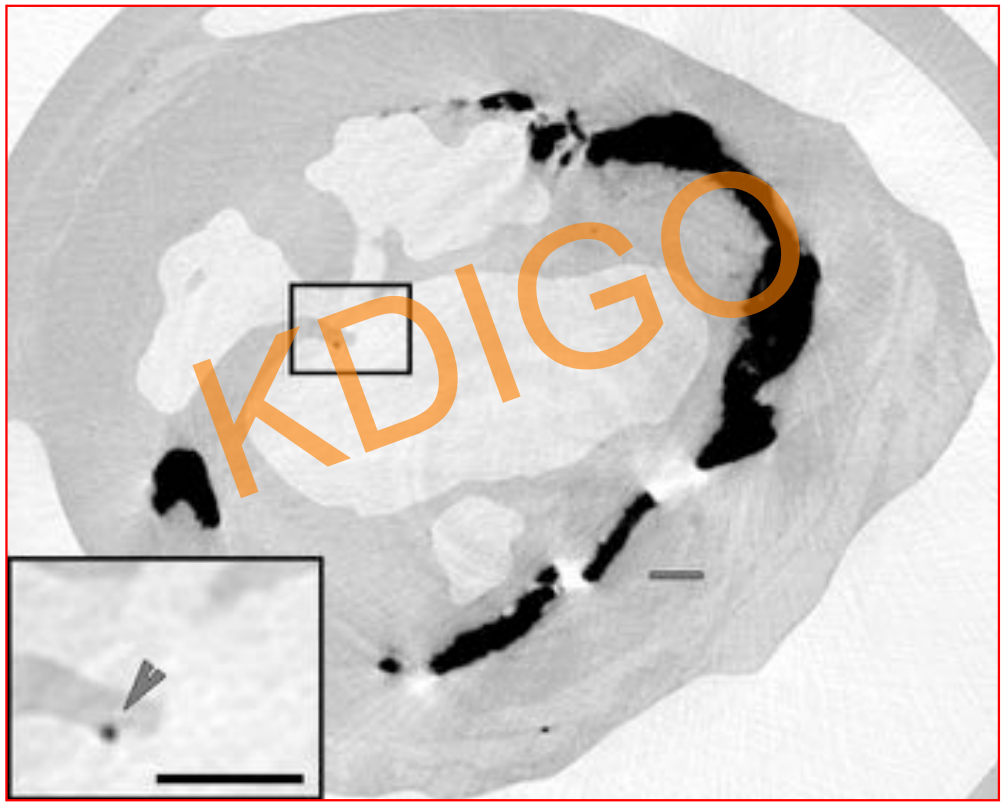


Plaque Erosion?

Vermani

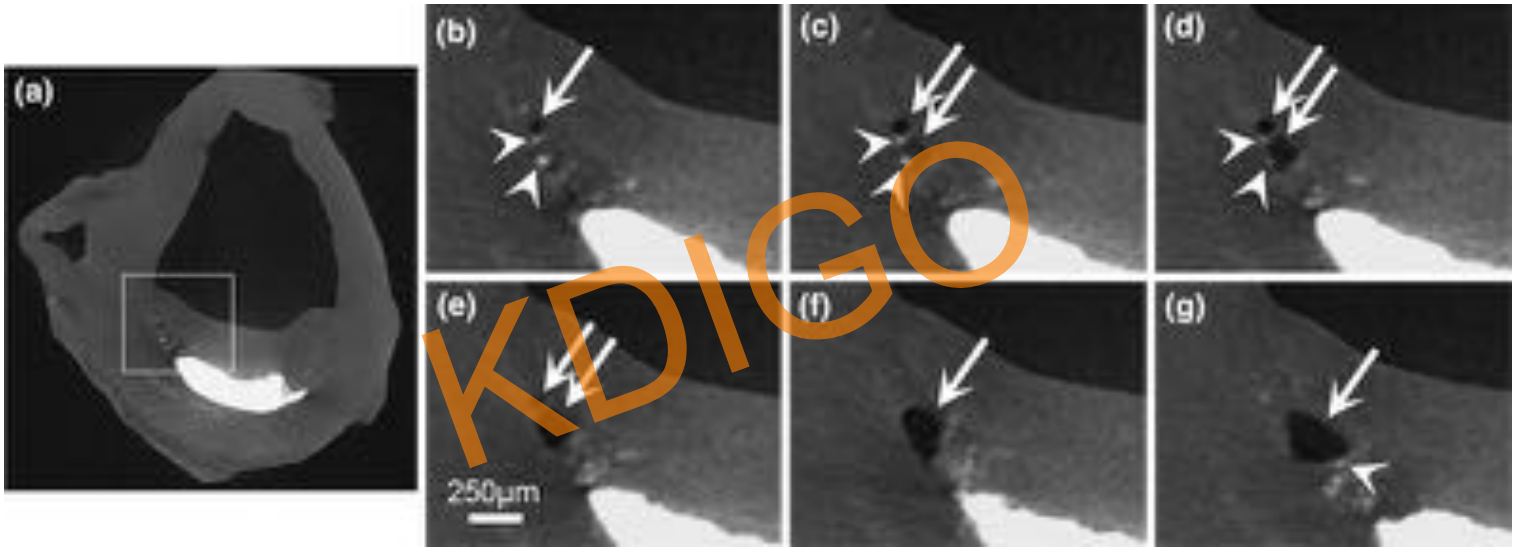
Maldonado et al 2012

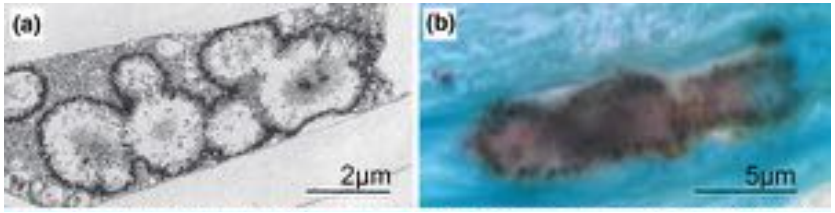
Plaques Rupture at Sites of Micro-calcifications



Why?

Microcalcifications are associated with Voids in the Extracellular Matrix

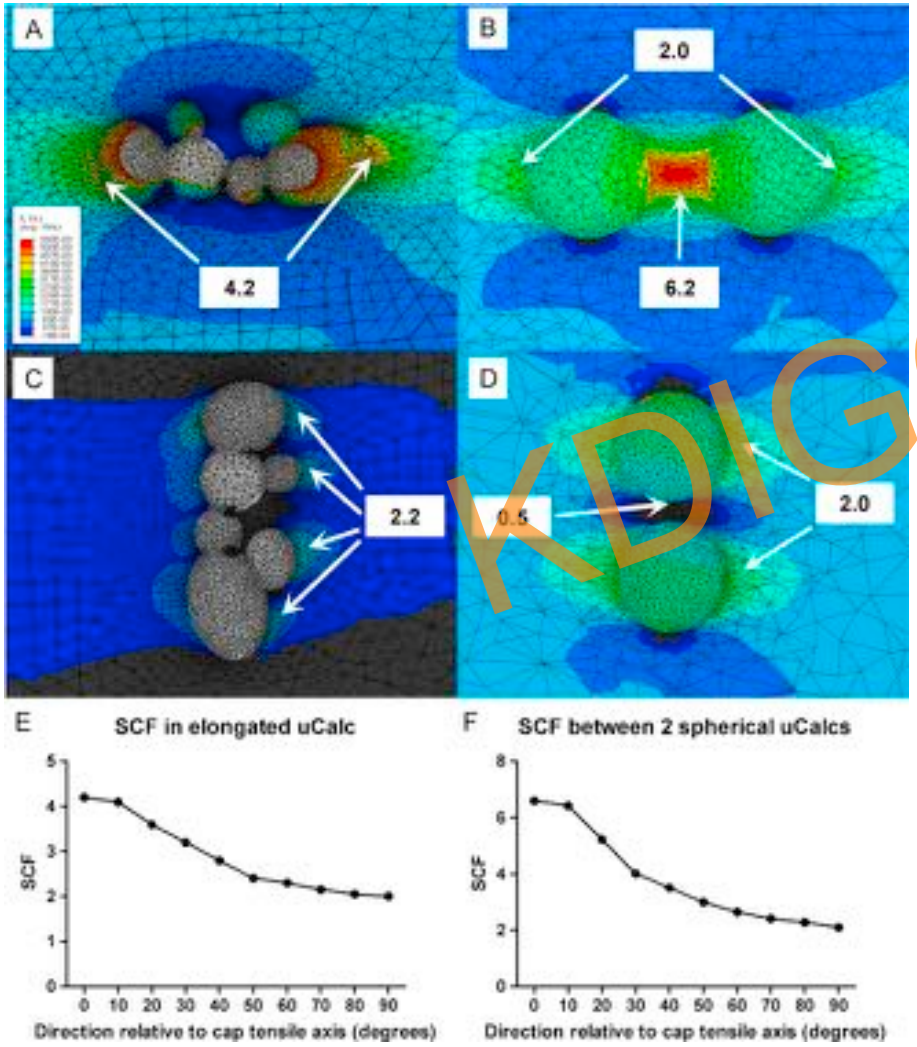




Exosomes (matrix vesicles) contain Matrix metalloproteinases that can degrade collagen – create a void.

Aikawa Lab

Modelling of Material Properties of Mineral/Matrix Interface



Predicts Material Stress at these Sites.

Sheldon Weinbaum

Maldonado et al 2012

The Holy Grail of Atherosclerosis Research!

How can Unstable Atherosclerotic Plaque Be Detected?

^{18}F-fluoride positron emission tomography for identification of ruptured and high-risk coronary atherosclerotic plaques: a prospective clinical trial

Nikhil V Joshi, Alex T Vesey, Michelle C Williams, Anoop S V Shah, Patrick A Calvert, Felicity H M Craighead, Su Ern Yeoh, William Wallace, Donald Salter, Alison M Fletcher, Edwin J R van Beek, Andrew D Flapan, Neal G Uren, Miles W H Behan, Nicholas L M Cruden, Nicholas L Mills, Keith A A Fox, James H F Rudd, Marc R Dweck, David E Newby**

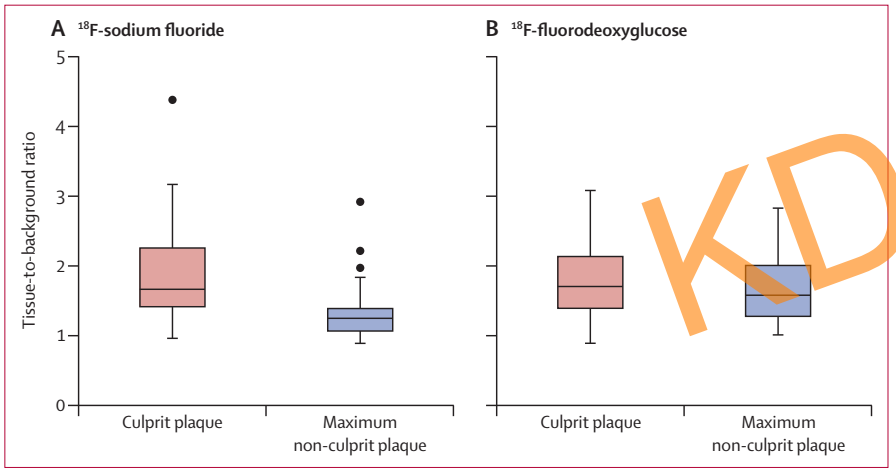


Figure 2: **^{18}F-fluoride and ^{18}F-fluorodeoxyglucose uptake in patients with myocardial infarction**
^{18}F-fluoride activity (maximum tissue-to-background ratio) was increased in the culprit plaque (red) compared with the maximum uptake in any of the non-culprit plaques (blue). By contrast, there was no difference in the activity of ^{18}F-fluorodeoxyglucose between these regions.

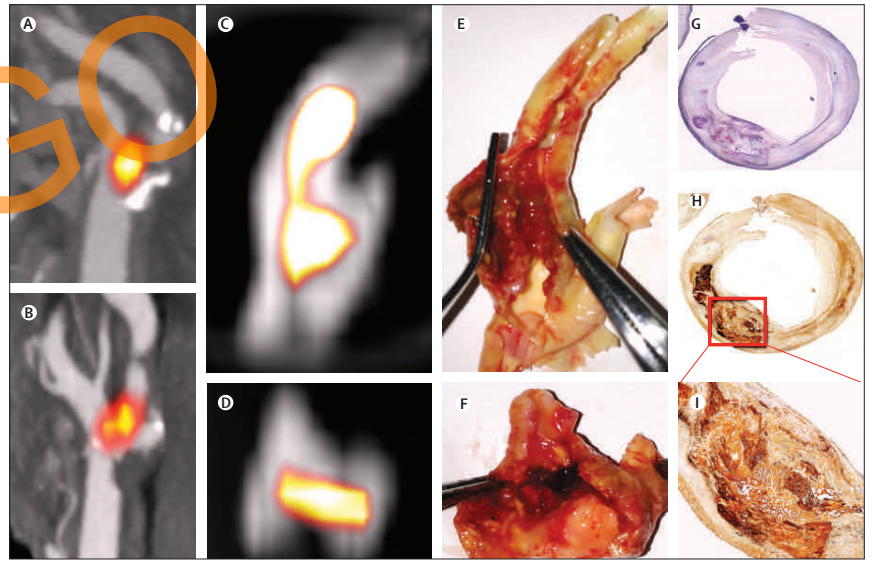
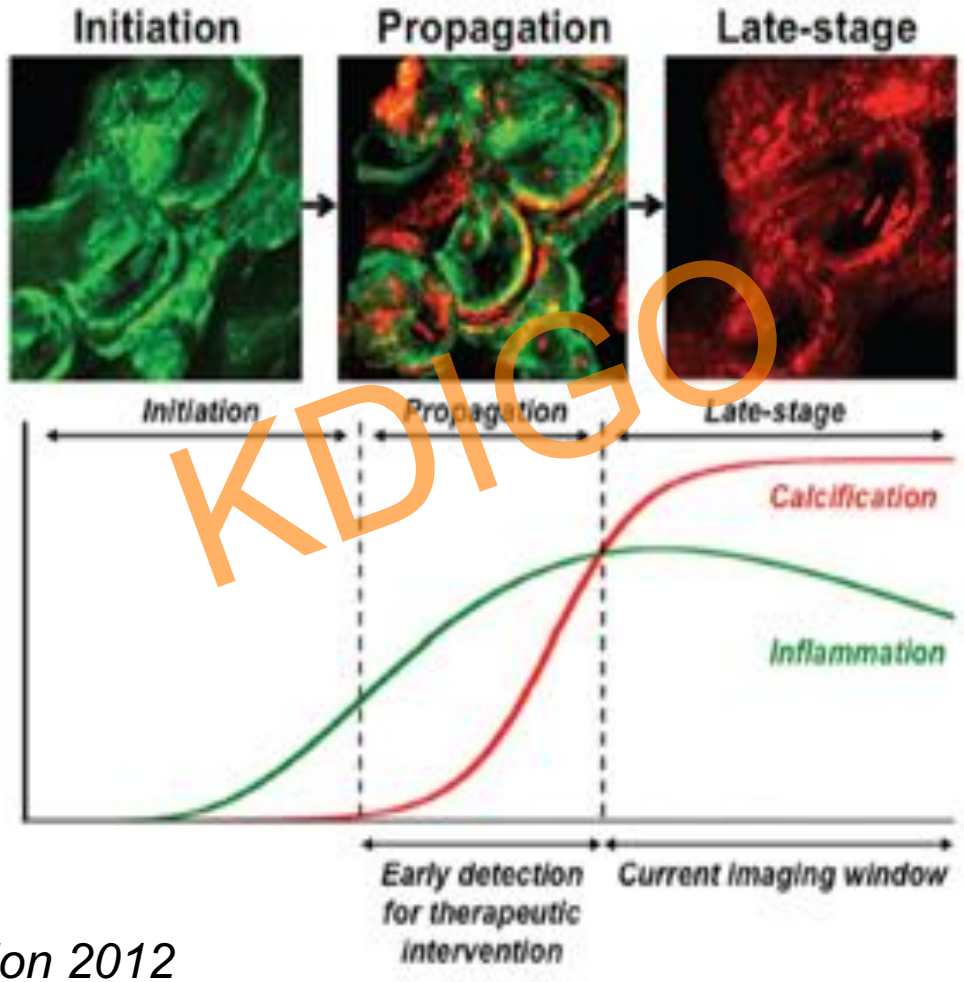


Figure 3: **Carotid ^{18}F-fluoride uptake and carotid plaque rupture**
In-vivo (A and B) and ex-vivo (C and D) positron emission and computed tomograms showing colocalisation of ^{18}F-fluoride (^{18}F-NaF) uptake (yellow-orange) to the site of plaque rupture with adherent thrombus on excised carotid endarterectomy tissue (E and F). Histology of the ^{18}F-NaF-positive region shows a large necrotic core (Movat's pentachrome, magnification 4×, G), within which increased staining for tissue non-specific alkaline phosphatase can be seen as a marker of calcification activity on immunohistochemistry (magnification 4×, H; magnification 10×, I).

Can and Should Calcification be Treated?

What is the Treatment Window

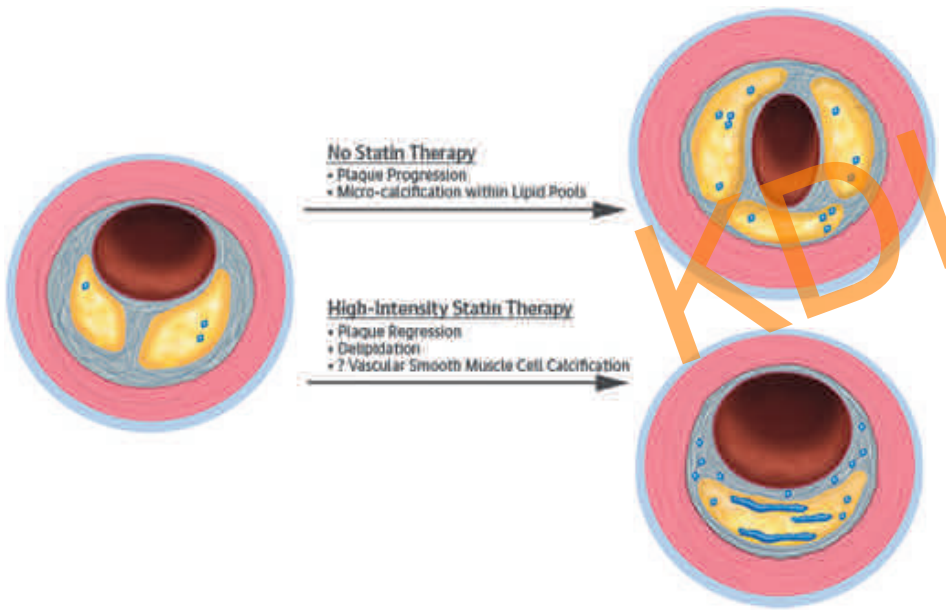


Aikawa and Otto, Circulation 2012

Impact of Statins on Serial Coronary Calcification During Atheroma Progression and Regression

Rishi Puri, MBBS, PhD,*† Stephen J. Nicholls, MBBS, PhD,‡ Mingyuan Shao, MS,* Yu Kataoka, MD,‡ Kiyoko Uno, MD, PhD,* Samir R. Kapadia, MD,† E. Murat Tuzcu, MD,† Steven E. Nissen, MD*†

CENTRAL ILLUSTRATION Plaque Calcification in the Setting of No-Statin Therapy or High-Intensity Statin Therapy



Puri, R. et al. J Am Coll Cardiol. 2015;65(13):1273-83.

Natural plaque progression likely involves lipid-pool expansion coupled with microcalcifications within lipid pools. Following long-term high-intensity statin therapy, plaque regression manifests as delipidation and probable vascular smooth muscle cell calcification, promoting plaque stability.

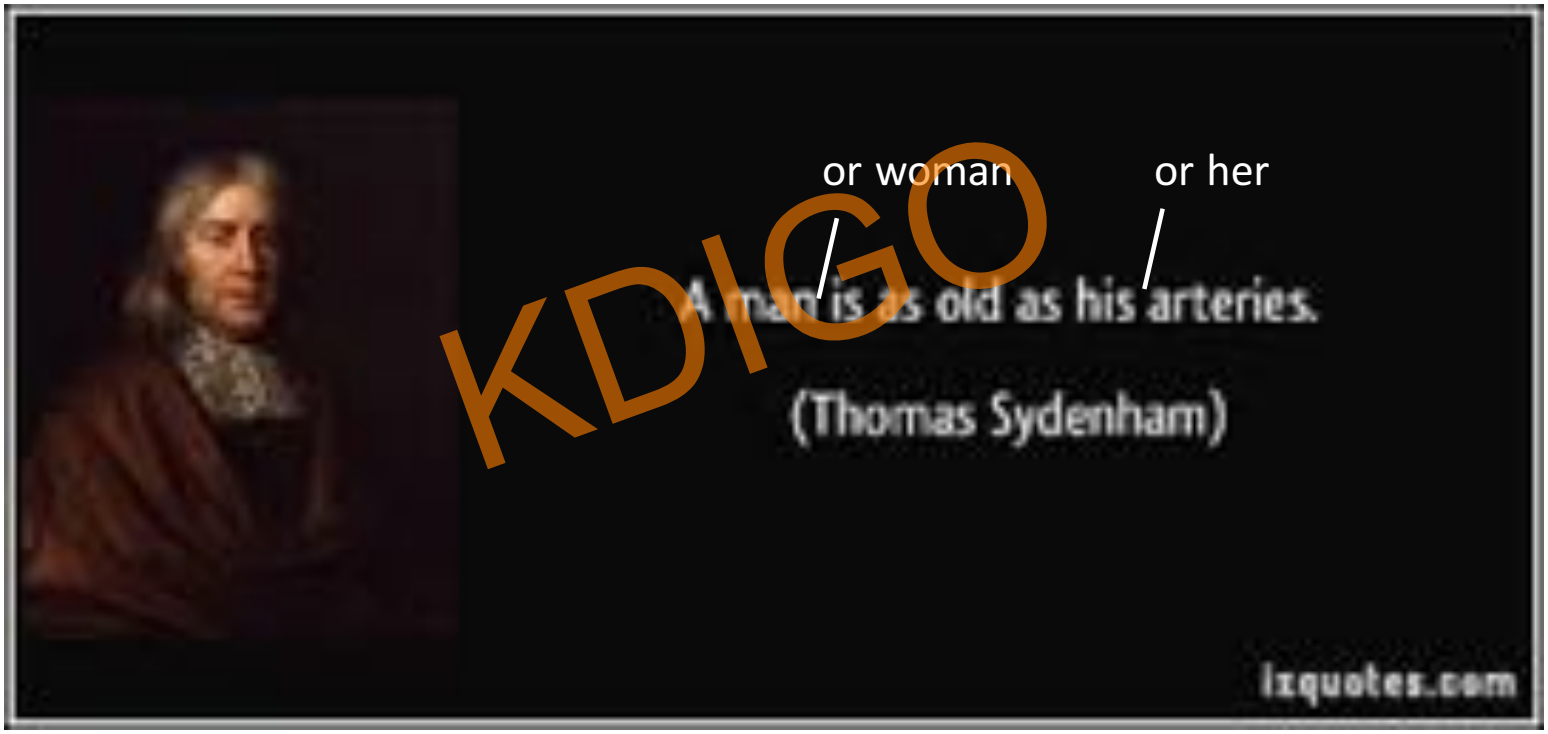
STATINS -The Gold Standard for Treating CAD
INCREASE calcification

Are statins effective in calcified renal patients?
Wanner et al NEJM 2005

What is the Nature of CAD in Renal Patients?

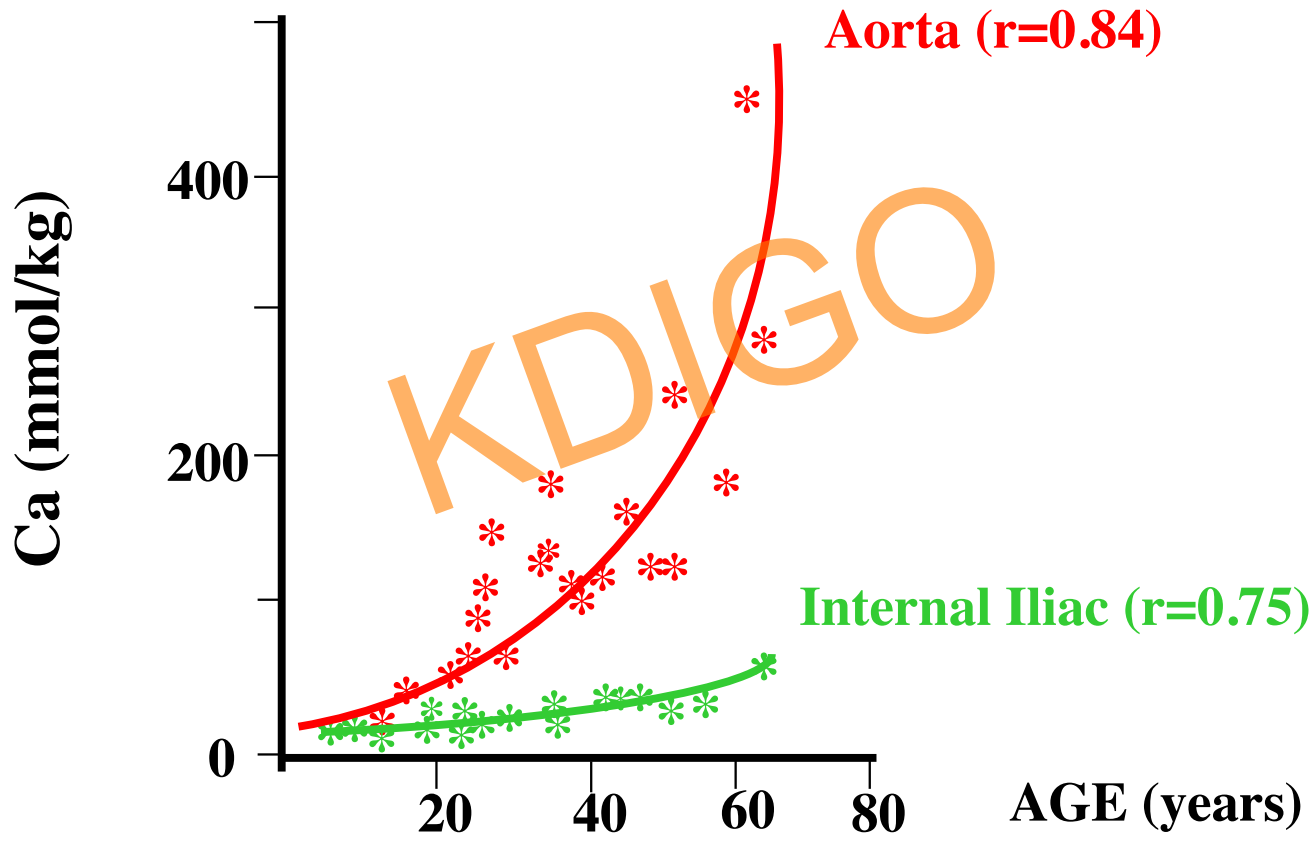
- Is the calcification medial or intimal.
- Is the calcification micro or macro?
- Are the lesions different from those seen in the 'general' population?
- Lipid, Inflammation?

Vascular Calcification – A Degenerative Unmodifiable Risk Factor that Predicts Disease and Death?



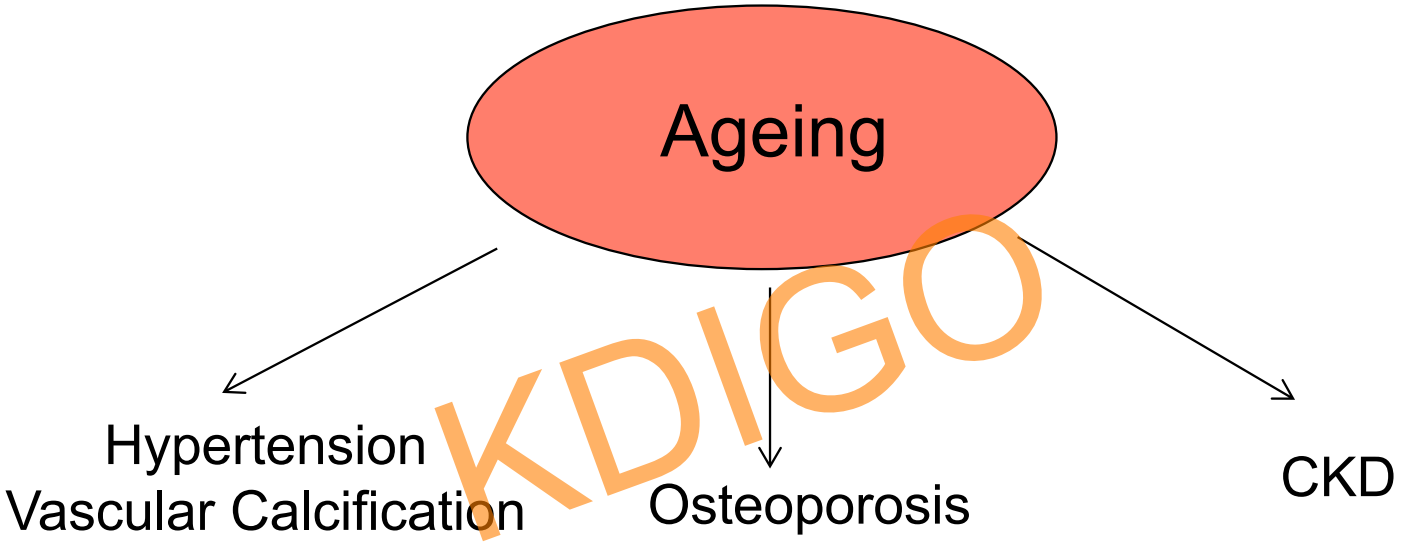
British Physician 1624-1689

Incidence of Vascular Calcification with Age



Ibels et al. Am J Med 1979

Ageing is the Strongest Risk Factor for Defects in Kidney-Bone-Vascular Axis Tissues

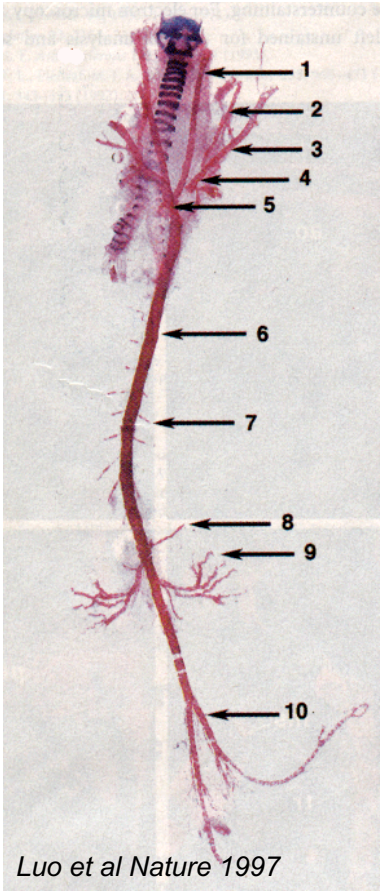


- Elevated Phosphate/FGF23/Klotho
- Low Vitamin D
- DNA damage
- Oxidative Stress
- Systemic Inflammation

Multiple Pathways Regulate Vascular Calcification

Mouse gene knockouts develop vascular calcification and bone defects (eg. osteoporosis).

- MGP (matrix Gla protein)
- Fetuin**
- Osteoprotegerin
- Klotho/ FGF23** - Phosphate and Vit D metabolism
- Pyrophosphate metabolism (ENPP1)
- carbonic anhydrase
- Smad 6



Luo et al Nature 1997

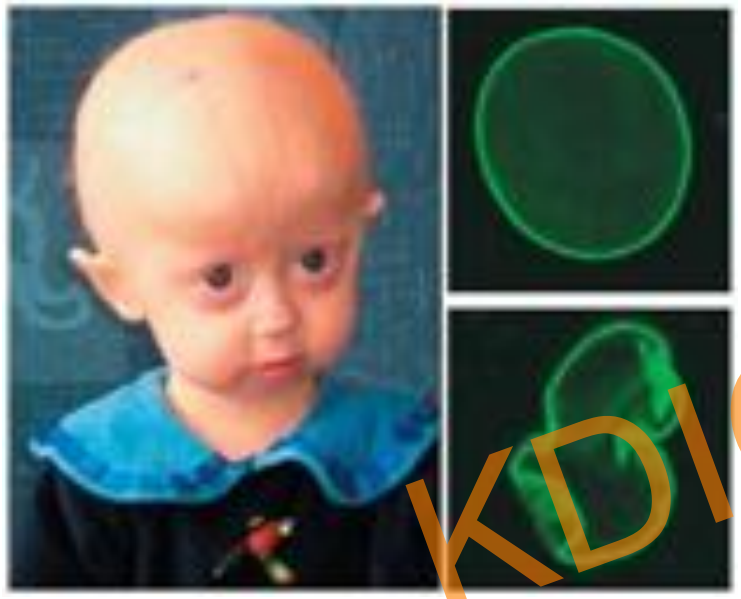
Human single gene defects

- Keutel Syndrome (MGP null)
- Idiopathic calcification of newborn (ENPP1)

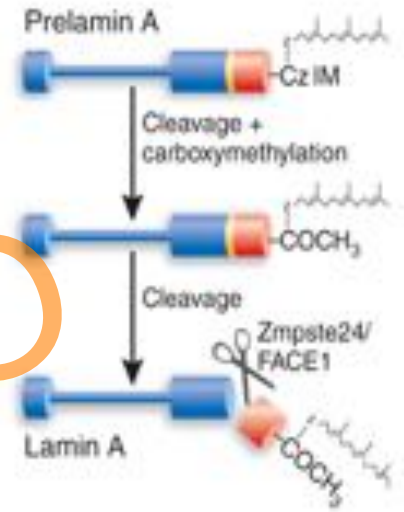
Develop vascular calcification

→ Genetic Component

Hutchinson-Gilford Progeria Syndrome (HGPS)



- VSMC loss
- Premature Atherosclerosis
- Calcification
- Vascular Stiffening
- Osteoporosis
- Death before age 16 of MI or stroke

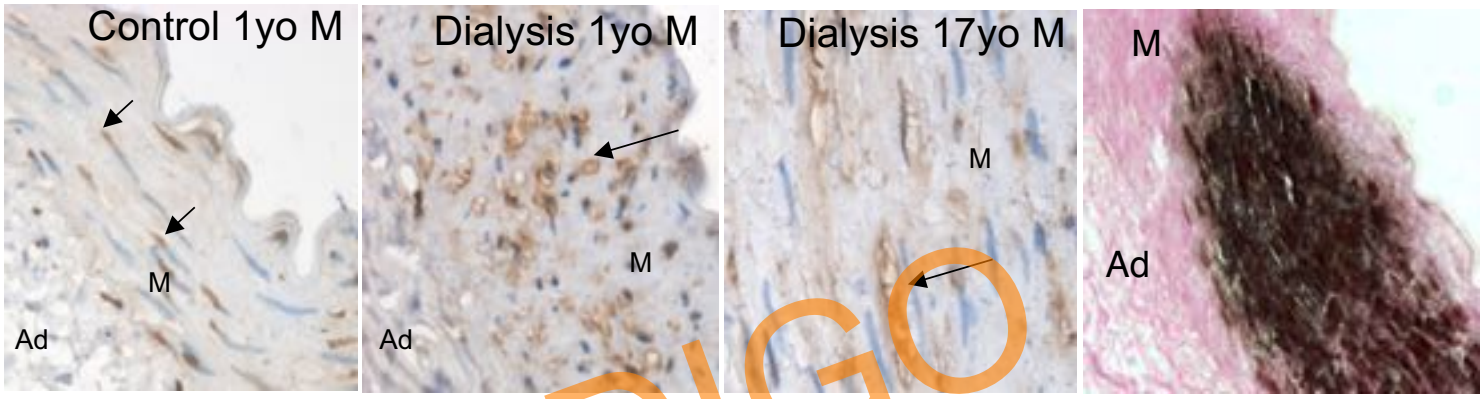


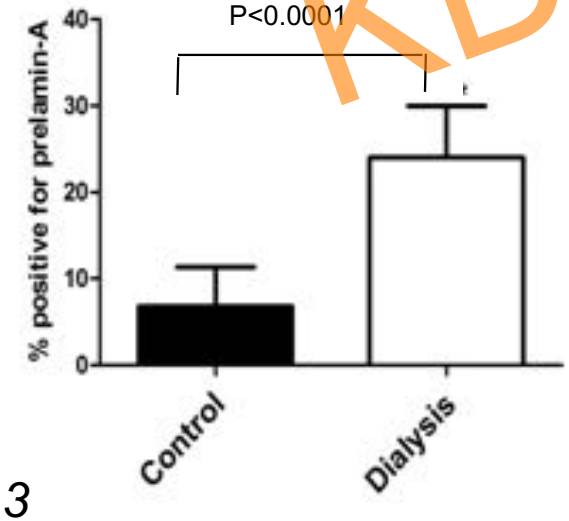
Due to accumulation of mutant or unprocessed Prelamin A

LMNA or *FACE1* mutations

Protein selectively accumulates in MSC populations

Is there evidence for this pathway in dialysis patients?

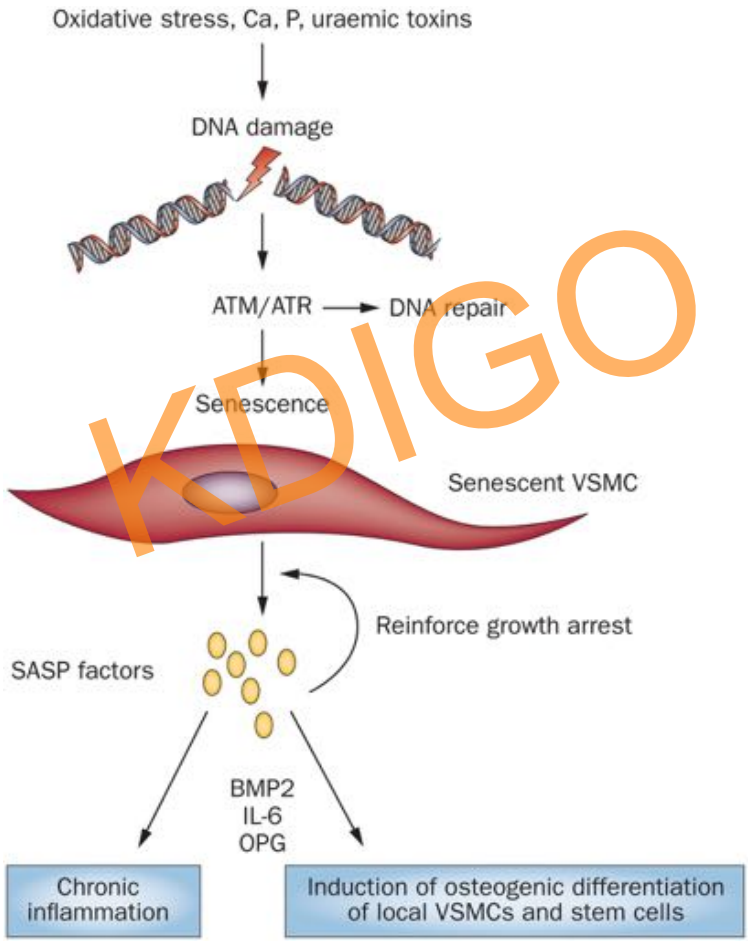




Children on dialysis show prelamin A accumulation and increased levels of p16 positive cells.

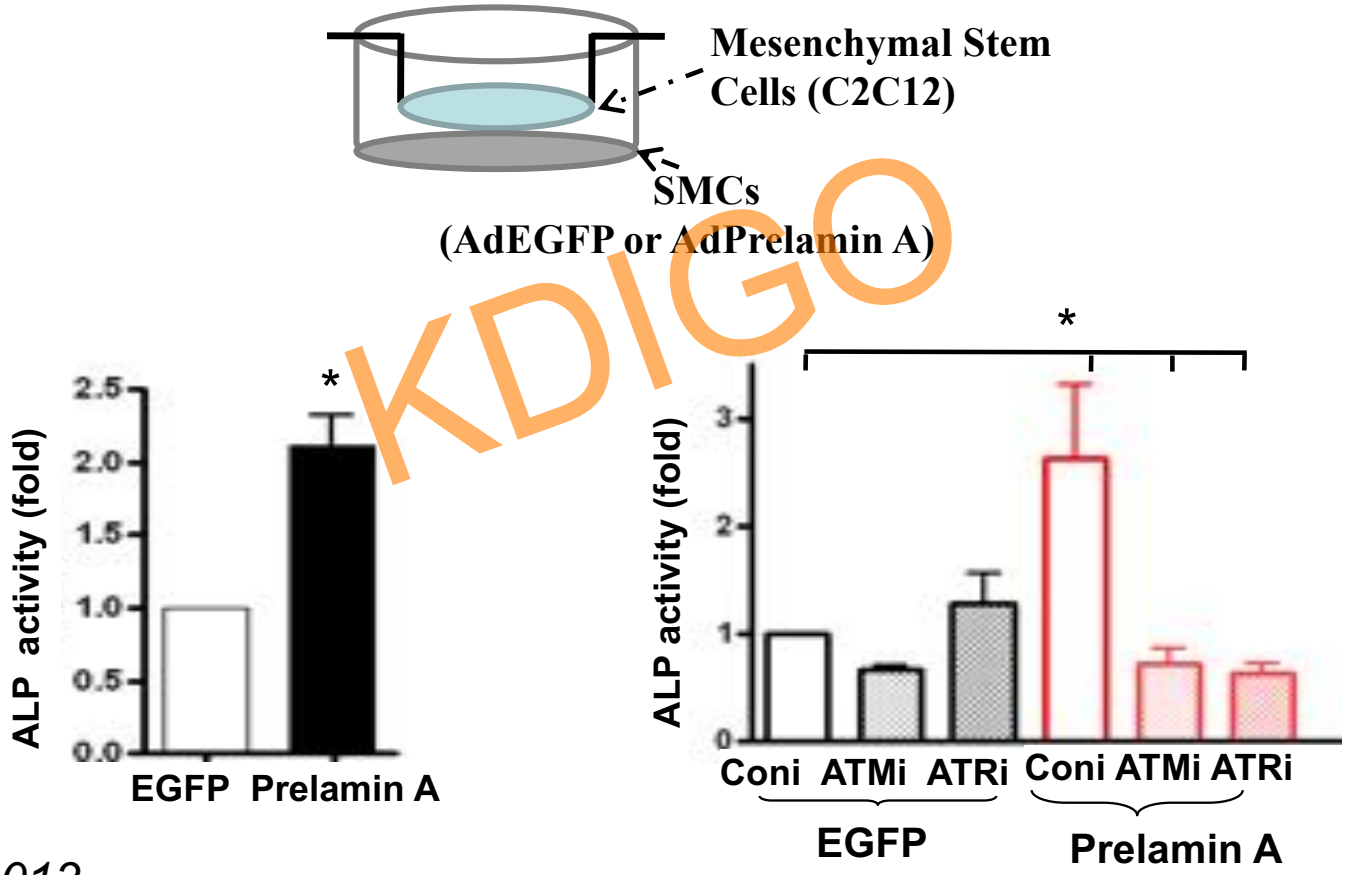
Liu et al Circ Res 2013

Ageing is Associated with Increased Inflammation Senescence Associated Secretory Phenotype (SASP)



Shanahan, C. M. (2013)

SMCs overexpressing prelamin A show osteogenic paracrine effects on surrounding cells *in vitro*



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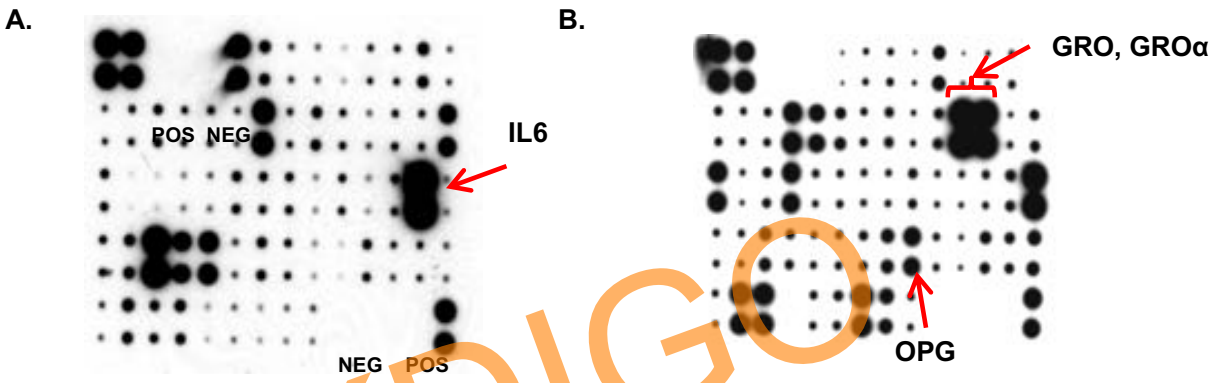
Array analysis shows VSMCs secrete pro-osteogenic cytokines in response to prelamin A accumulation



	SASP factors
Interleukins and chemokines	IL6, IL8, GRO, GROα, MCP-1, -2, -3, ENA78, GCP-2
Growth factors and regulators	Angiogenin, IGFBP-4, -6, VEGF, BMP2
Proteases	TIMP-1, -2
Soluble or shed receptors or ligands	OPG, Fas, uPAR, ICAM-1

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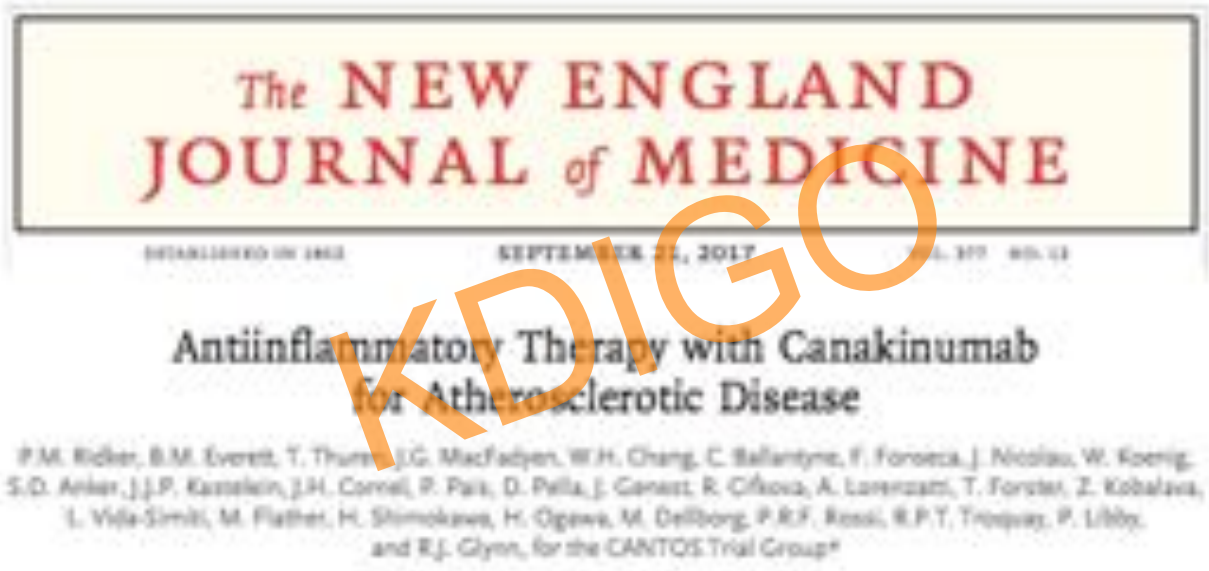
Same Inflammatory Profile seen in VSMCs from Children on Dialysis

Is Inflammation the Key?



KDIGO

Monoclonal antibody to Interleukin-1β
CANTOS trial

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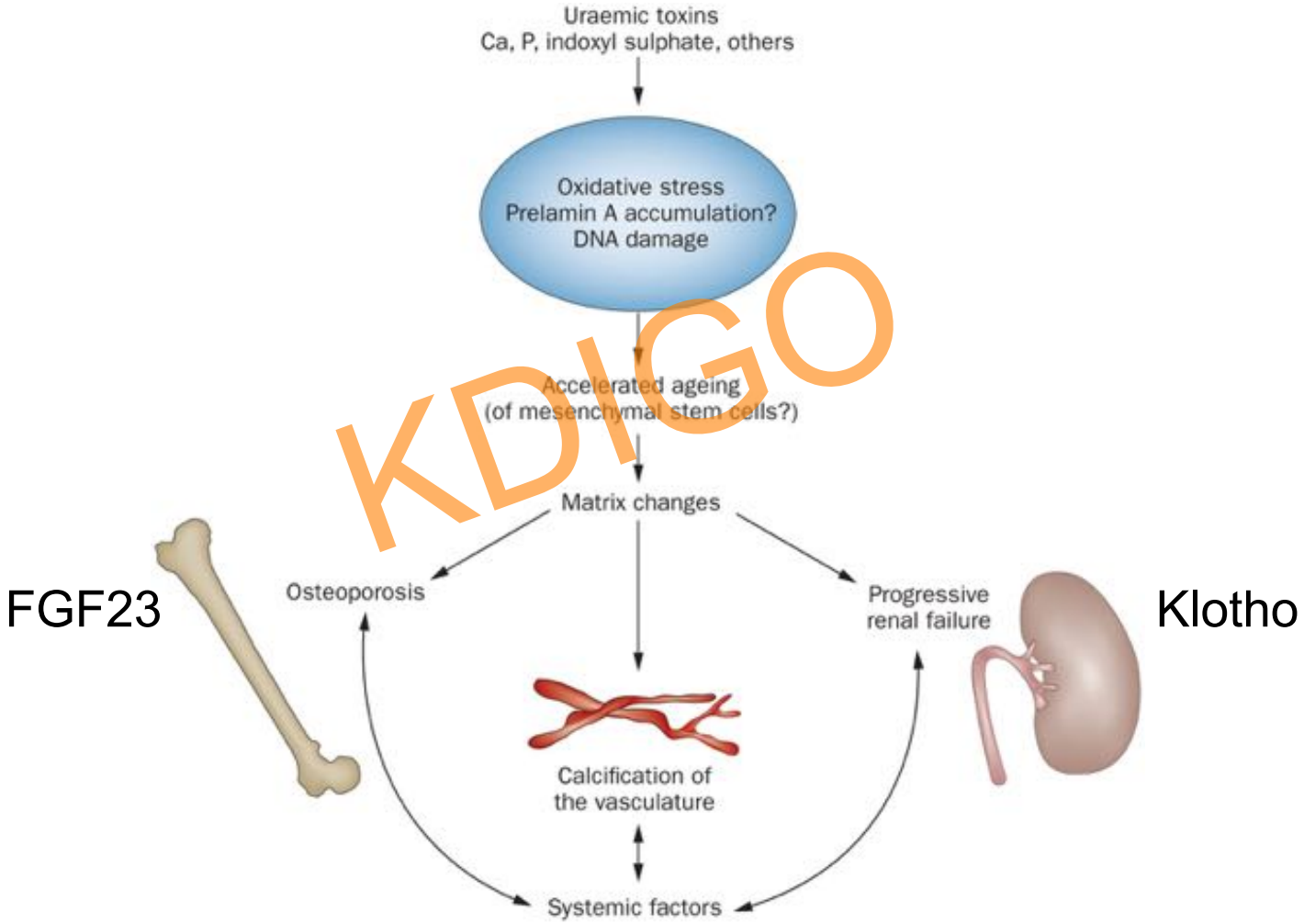
Calcium phosphate particles stimulate interleukin-1β release from human vascular smooth muscle cells: A role for spleen tyrosine kinase and exosome release

Yana Dautova[a], Alexander N. Kapustin[b], Kevin Pappert[c], Matthias Epple[c], Hanneke Okkenhaug[a], Simon J. Cook[a], Catherine M. Shanahan[b], Martin D. Bootman[d], Diane Proudfoot[a,*]

Tissue ageing is driven by DNA damage and inflammatory mediators released from senescent tissues



Summary

1. Calcification is a cell mediated process that reflects a disease process.
2. Calcification occurs at two sites with different clinical outcomes.
3. Calcification can be used to predict clinical events.
4. There are no treatments for vascular calcification.
5. The status of calcification in plaque stability remains controversial.
6. Inflammation may be a key process in CAD in renal failure
7. THE NATURE OF CAD IN RENAL PATIENTS REQUIRES FURTHER BASIC KNOWLEDGE