Universidad de Alcalá

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AGING-RELATED ILK LEVELS ARE ASSOCIATED TO CALCIFIED AORTIC VALVE

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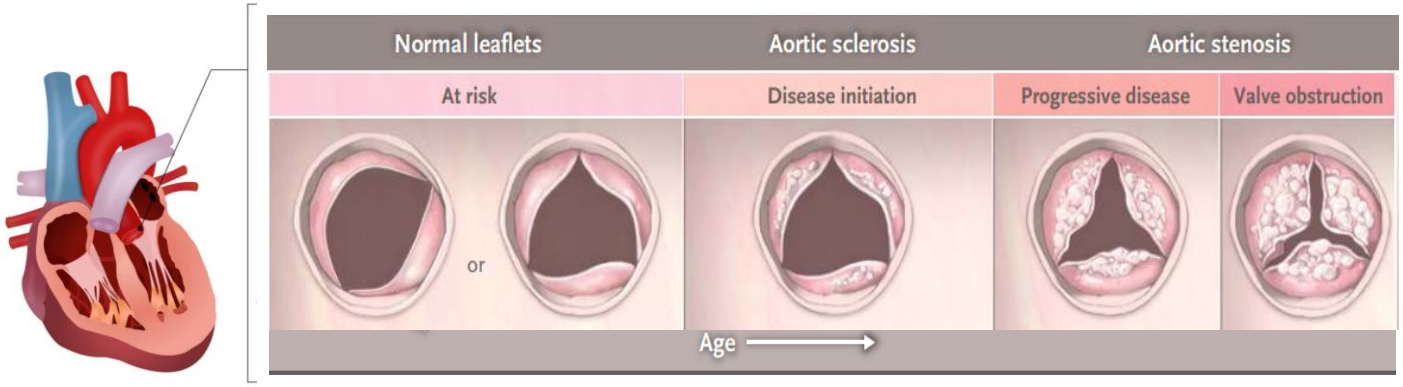
WHAT IS CALCIFIC AORTIC VALVE DISEASE OR CAVD?

CAVD is a slow, progressive and highly active ossification process, ranging from mild leaflet thickening (aortic sclerosis) to severe calcification (aortic stenosis).

Aging-related disease: affecting patients >65 years of age.

Treatment: **surgical replacement**

Increases the risk of myocardial infarction and heart failure.



Adaptado de Otto, C. M., & Prendergast, B. (2014).

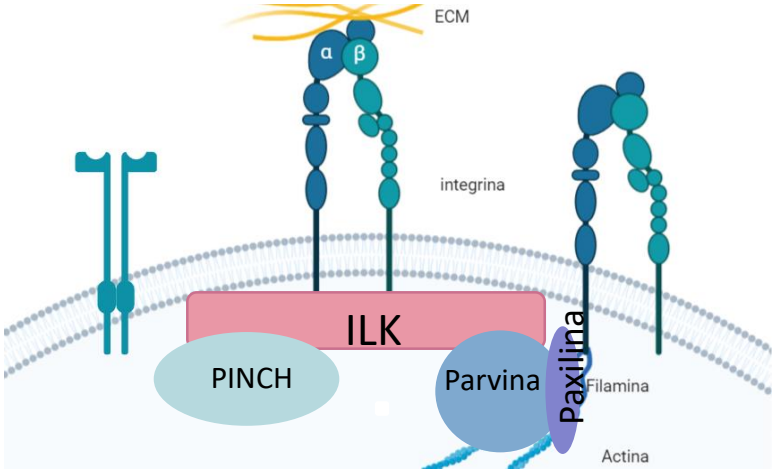
Recent studies suggest that the mechanisms of CAVD are initiated in the valvular endothelium → **Endothelial dysfunction**

Shares some characteristics with atherosclerotic disease.

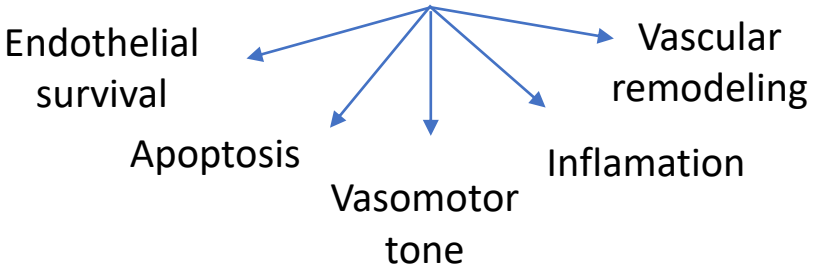
Pathological pathways involved in the onset, progression of CAVD remain unknown.

INTEGRIN-LINKED KINASES (ILK)

ILK: Serine/threonine kinase that interacts with Integrins β1. Highly expressed in the cardiovascular system.



It is a **mechanosensitive** protein that controls the production of nitric oxide (NO) in the endothelium by regulating



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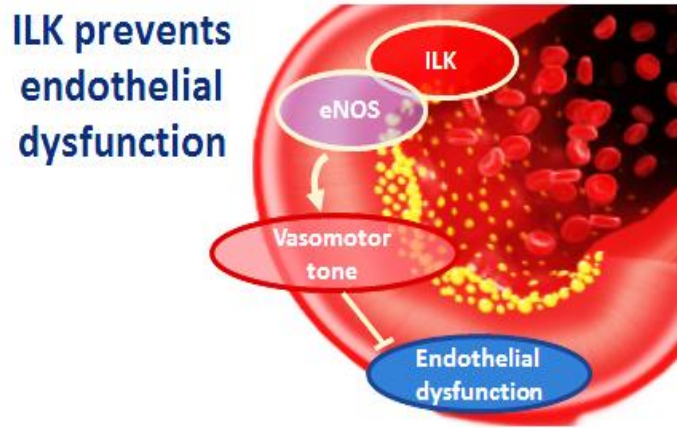
Integrin-Linked Kinase Regulates Vasomotor Function by Preventing Endothelial Nitric Oxide Synthase Uncoupling : Role in Atherosclerosis
Beatriz Herranz, Susana Marquez, Brenda Guijarro, Enrique Aracil, Clara Aicart-Ramos, Ignacio Rodriguez-Crespo, Manuel Rodríguez-Puyol, Carlos Zaragoza and Marta Saura



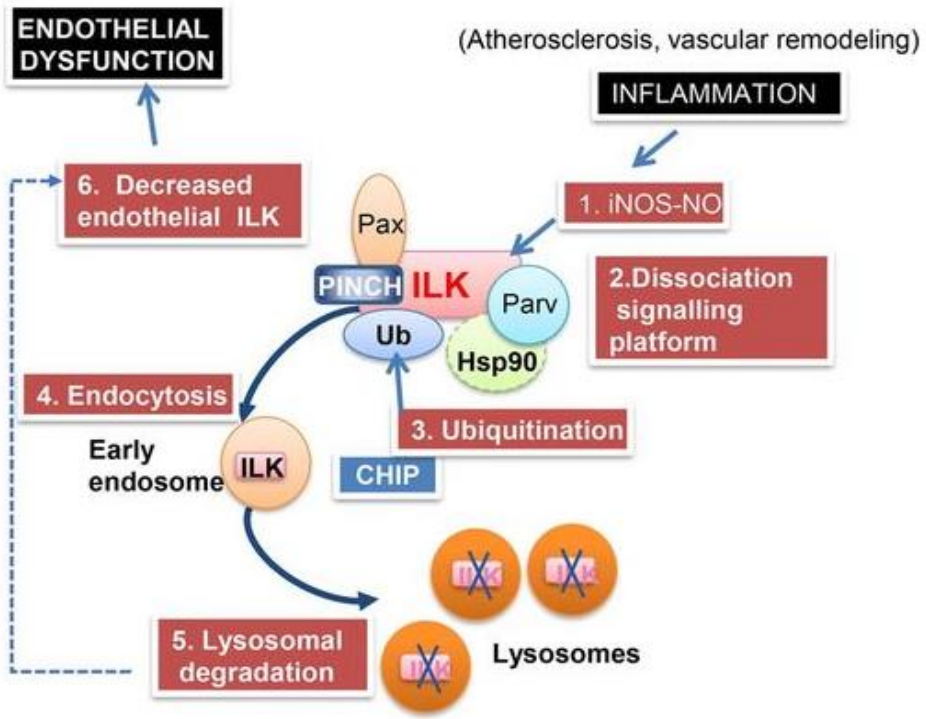
ILK in the endothelium preserves vasomotor tone by preventing eNOS uncoupling and **endothelial dysfunction.**

ROLE OF INTEGRIN-LINKED KINASES (ILK) IN AGED-RELATED DISEASES: **ATHEROSCLEROSIS**

iNOS-Derived Nitric Oxide Induces Integrin-Linked Kinase Endocytic Lysosome-Mediated Degradation in the Vascular Endothelium

Paula Reventun, Matilde Alique, Irene Cuadrado, Susana Marquez, Rocio Toro, Carlos Zaragoza, Marta Saura

Arterioscler Thromb Vasc Biol is available at http://atvb.ahajournals.org DOI: 10.1161/ATVBAHA.117.309560

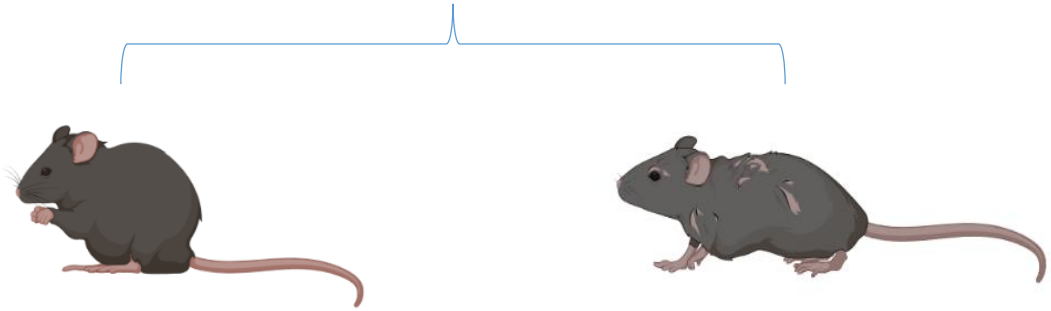


As part of the inflammatory response during **atherosclerosis**, cytotoxic NO levels from iNOS produced by immune foam cells and smooth muscle cells, contribute to oxidative and nitrosative stress by promoting **eNOS uncoupling through lysosomal degradation of ILK in the vascular endothelium**

HIPOTHESIS AND METHODS

Endothelial dysfunction and arteriosclerosis are two aging complications in which ILK may play a significant role. However, the contribution of ILK in the pathogenesis of CAVD, still remain to be elucidated.

We hypothesize that ILK expression in the endothelium is decreased with age and this loss is crucial for the onset of aging-associated pathologies where the endothelium plays a key role such as **Aortic Valve Calcification.**

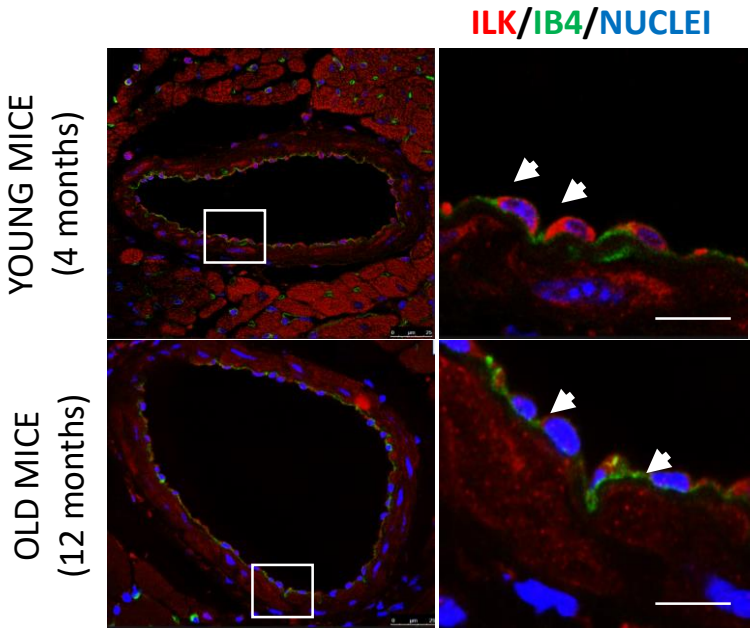


Young mice
(4 months old)

Aged mice
(12 months old)

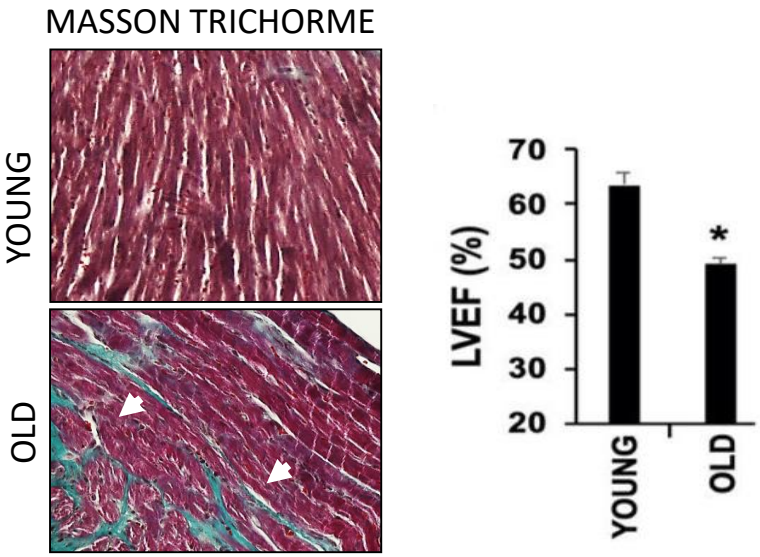
- **Cardiac function**: Ecocardiography
- **Cardiac endothelium study**: WB, IHC, IF, DHE stain
- **Cardiac valves study**: IF, Von Kossa stain, IHC

ENDOTHELIAL ILK EXPRESSION IS DOWNREGULATED IN OLD MICE COMPARE TO YOUNG MICE



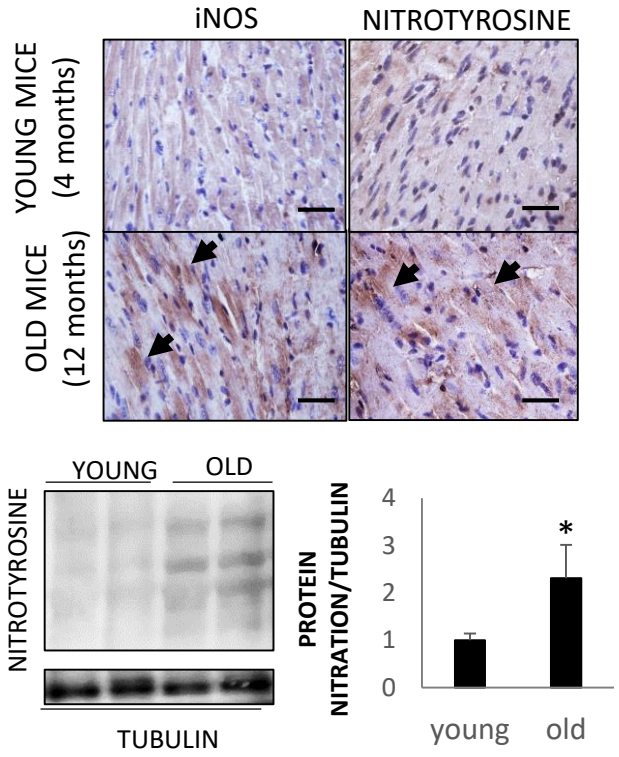
Immunofluorescence of ILK in vessels endothelium. ILK (red) colocalizes with endothelial marker IB4 (green) in young mice (4 month old) and is strongly express, whereas ILK staining in old endothelium (12 months old) is down-regulated.

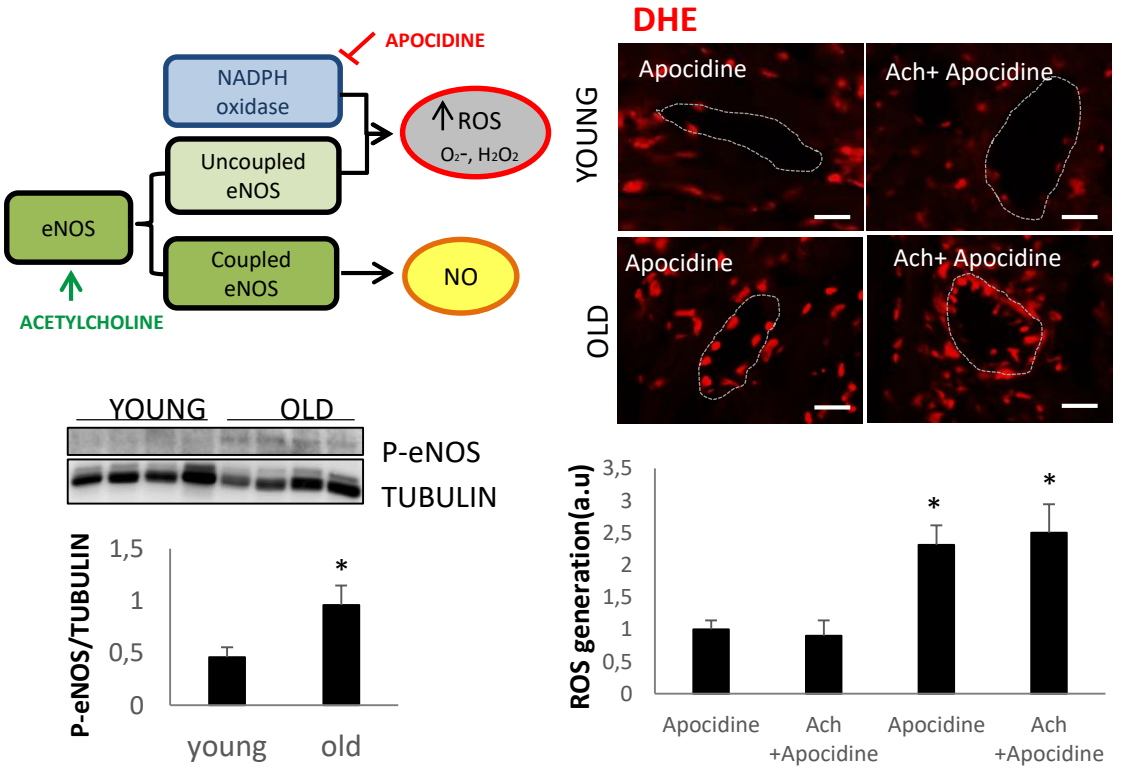
OLD MICE HEARTS SHOWS A SIGNIFICANT IMPAIRMENT OF CARDIAC FUNCTION ACCOMPANIED BY INCREASED INTERSTITIAL FIBROSIS



Cardiac function is impaired in aged mice. Masson's trichrome staining of heart sections shows interstitial fibrosis (blue). In addition, echocardiographic analysis shows a significant decrease in left ventricular ejection fraction (LVEF %) in old mice (12 months) (n=12 ; *p<0.05). Both intertitial fibrosis and decreased LVEF are signals of heart failure.

HEARTS OF OLD MICE SHOWED INFLAMMATORY INFILTRATES WITH INCREASED EXPRESSION OF INDUCIBLE NITRIC OXIDE SYNTHASE (iNOS) AND PERSISTENT ENOS PHOSPHORYLATION LEADING TO NISTROSATIVE AND OXIDATIVE STRESS

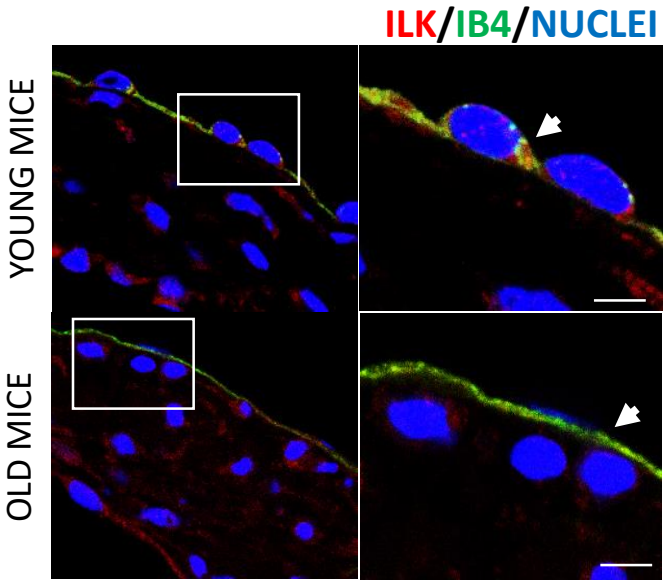




Activation of inducible Nitric Oxide Synthase (iNOS) leads to nitrosative stress. Upper panel. Immunostaining of iNOS and Nitrotyrosine in heart sections of young (4months) and aged mice (12 months). Lower panel. Western blot of cardicac lysates (n=6 ; *p<0.05).

Persistent phosphorylation of endotelial Nitric Oxie Syntase (eNOS) leads to ROS generation and oxidative stress. Upper left panel. Schematic image of eNOS function. Lower left panel. Western blot of eNOS phosphorilation in cardiac lysates (n=10 ; *p<0.05). Right panel. ROS staining with DHE of cardiac sections treated with the NADPH oxidase inhibitor apocidine or with apocidine + achetylcholine (eNOS activator). Representation of DHE fluorescence intensity (n=4; *p<0.05).

DOWN-REGULATION OF ENDOTHELIAL ILK IS ALSO FOUND IN THE ENDOTHELIUM OF OLD VALVES AND CORRELATES WITH VALVE CALCIFICATION



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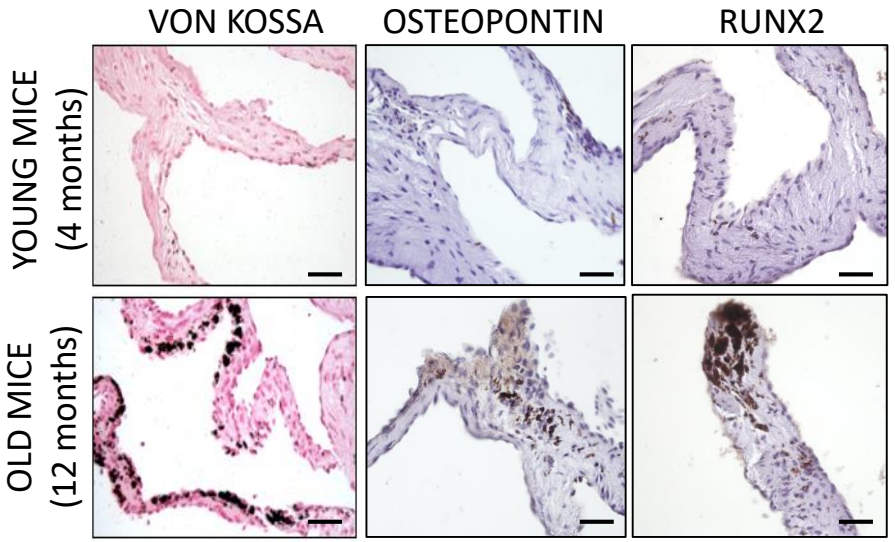

Aged mouse valves shows calcification. Left panel. Von Kossa staining shows calcium deposits (black) in aged valves. Middle and right panel. Immunostaining of two different calcification markers, Osteopontin and Runx2 respectively, in valves of aged mice (12 months old).

CONCLUTIONS

Endothelial ILK is significantly decrease in aged mice heart, including valve endothelium, leading to impaired cardiac functions.

The hearts of aged animals show signs of inflammation such as the presence of infiltrates, iNOS activation and persistent nitrosative stress.

In addition, the vascular endothelium shows signs of endothelial dysfunction such as persistent eNOS phosphorylation and oxidative stress.

Down-regulation of valve endothelial ILK correlates with calcification markers

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