Wnt5a contributes to human atherosclerosis via novel USP17 redox signalling

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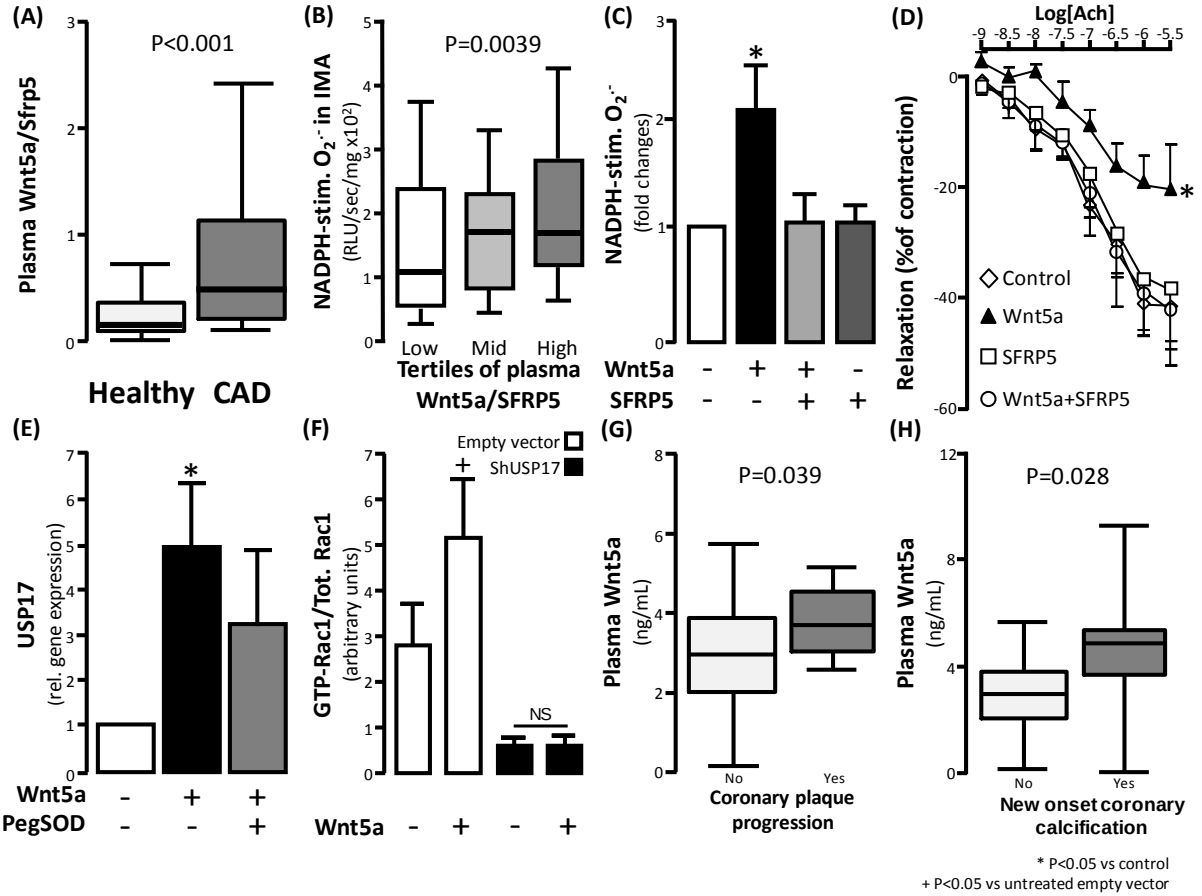
Background: Wnt5a is a non-canonical Wnt ligand with potential vascular effects, but its mechanistic role in atherosclerosis progression and the underlying downstream mechanisms are poorly explored.

Purpose: To address the hypothesis that Wnt5a induces vascular NADPH-oxidases activity, endothelial dysfunction and detrimental downstream redox signalling which could propagate atherosclerosis in humans.

Methods: Study 1 included 70 patients with coronary artery disease (CAD) versus age- and sex-matched non-CAD controls. Study 2 included 1,003 CAD patients undergoing cardiac surgery; internal mammary artery (IMA) and saphenous vein (SV) segments were harvested and used for *ex vivo* experiments. Study 3 included 68 individuals undergoing two cardiac computed tomography scans 3-5 years apart; calcified plaque burden was assessed by coronary calcium score (CCS). Superoxide ($O_2^{\cdot-}$) generation was measured by lucigenin chemiluminescence with NADPH 100μM stimulation as indicator of NADPH-oxidases activity. Activation of Rac1, a key NADPH-oxidases subunit, was evaluated by a commercially available kit. Primary vascular smooth muscle cells (VSMCs) and HeLa cells were used for *in vitro* experiments. Circulating Wnt5a and Sfrp5 (a Wnt5a antagonist) were measured by ELISA in fasting plasma samples.

Results: In Study 1, the presence of CAD was independently linked with increased circulating Wnt5a bioavailability (A), which was, in turn, associated with increased IMA NADPH-oxidases activity in Study 2 (B). Recombinant Wnt5a directly stimulated NADPH-oxidases activity (C) via Rac1 activation (not shown) in human IMA, while inducing endothelial dysfunction evidenced by impaired SV endothelium-dependent acetylcholine (Ach) vasorelaxations (D). Transcriptomic analysis in Wnt5a-treated primary VSMCs versus controls identified USP17, a deubiquitinating enzyme implicated in Rac1 activation, as the top differentially regulated hit (not shown). Indeed, Wnt5a stimulated USP17 upregulation in VSMCs which was reversed by PEGylated superoxide dismutase (peg-SOD) 300U/mL (E), suggesting a redox sensitive effect. USP17 knockdown abolished the ability of Wnt5a to induce Rac1 activation in HeLa cells (F). At a clinical level, plasma Wnt5a was a predictor of plaque progression (defined as ΔCCS≥1, G) and new onset calcification (H) in Study 3.

Conclusions: We demonstrate for the first time that Wnt5a is elevated in CAD and causally associated with increased vascular oxidative stress and endothelial dysfunction in humans. We further reveal USP17 to be a novel, previously undescribed, link between Wnt5a, Rac1 activation and NADPH-oxidase activity induction in humans. We finally propose that circulating Wnt5a may have a clinically relevant role in predicting atherosclerosis progression. Our findings identify Wnt5a as rational therapeutic target in vascular disease.



* P<0.05 vs control
\+ P<0.05 vs untreated empty vector