Urine proteome analysis reflects atherosclerotic disease in an ApoE-/- mouse model and allows the discovery of new biomarkers in mouse and human atherosclerosis

Constantin Von Zur Muhlen¹, YC Chen², Eric Schiffer³, Christine Sackmann¹, Petra Zarbig⁴, Harald Mischak³, Christoph Bode¹, Karlheinz Peter²
¹ University Hospital Freiburg
² Baker IDI Heart & Diabetes Institute, Melbourne, Australia
³ Mosaiques Diagnostics GmbH, Hannover, Germany

Introduction: Non-invasive diagnosis of atherosclerosis via single biomarkers has been attempted but remains elusive. The aim of the current study is to use urine proteomics in ApoE-/- mice to discover proteins with pathophysiological roles in atherogenesis and to identify urinary polypeptide patterns reflecting early stages of atherosclerosis.

Methods and Results: Urine of ApoE-/- mice either on high fat diet (HFD) or chow diet (CD) was collected over 15 weeks. Capillary electrophoresis coupled to mass spectrometry (CE-MS) of samples identified 16 polypeptides specific for ApoE-/- mice on HFD. In a blinded test set, these polypeptides allowed identification of atherosclerosis at a sensitivity of 95% and specificity of 90%. Sequencing of the discovered polypeptides identified fragments of α₁-antitrypsin, EGF, kidney androgen regulated protein (KAP) and collagen. Using immunohistochemistry, α₁-antitrypsin, EGF and collagen type I was shown to be highly expressed in atherosclerotic plaques of ApoE-/- mice on HFD. Urinary excretion levels of collagen and α₁-antitrypsin fragments also significantly correlated with intraplaque collagen and α₁-antitrypsin content, mirroring plaque protein expression in the urine proteome. To provide further confirmation that the newly identified proteins are relevant in humans, presence of collagen type I, α₁-antitrypsin, and EGF was also confirmed in human atherosclerotic disease.

Conclusions: Urine proteome analysis in mice exemplifies the potential of a novel multimarker approach for non-invasive detection of atherosclerosis and monitoring of disease progression. Furthermore, this approach represents a novel discovery tool for the identification of proteins relevant in murine and human atherosclerosis and thus also defines potential novel therapeutic targets.