Karolinska Institutet

**Department of Medicine**

# Identification of Inflammatory Genes Involved in the Pathogenesis of Human and Experimental Atherosclerosis

AKADEMISK AVHANDLING

som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligen försvaras i CMM L8:00, Centrum för Molekylär Medicin, Karolinska Universitetessjukhuset, Solna.

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av

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# ABSTRACT

Atherosclerosis is a chronic systemic inflammatory disease of large and medium sized arteries, developing slowly and silently over decades. The disease is usually not apparent until occurrence of a sudden clinical symptom, such as myocardial infarction (MI) or stroke. Several classical risk factors have been established to play a role in the progression of disease over a long period of time. However, markers recognizing vulnerable patients being at risk of having an event in the near future are lacking. Thus, more knowledge about the ongoing complex pathogenesis is needed for identification of potential biomarkers and therapeutic targets of atherosclerosis. Patients with carotid atherosclerosis experiencing cerebral symptoms within one month before undergoing carotid endarterectomy (CEA) are classified having vulnerable plaques.

Based on the classification above, I show in this thesis that mRNA levels of enzymes in the leukotriene 5-lipoxygenase pathway, 5-lipoxygenase (5-LO) and leukotriene A4 hydrolase (LTA4H), are associated with plaque vulnerability.

Gene expression can be investigated on a single target level using real-time PCR or by analyzing thousands of genes simultaneously, using global transcription microarrays. Based on correlations to microarrays we argue for using total RNA mass in normalization of real-time PCR data, when analyzing heterogeneous human specimen.

To identify new candidates of plaque vulnerability an unbiased approach was used - transcript profiles of symptomatic plaques were compared to asymptomatic plaques, demonstrating an increase of fatty acid binding protein 4 (FABP4), which was associated with vulnerability, independent of age or gender. FABP4 localize mainly to the numerous macrophages present in the atherosclerotic plaque. This study suggests FABP4 to play a role in plaque vulnerability and to be a potential valuable biomarker within the carotid atherosclerotic plaque.

To determine if any atherosclerosis-related changes can be detected in circulating cells the transcriptome of leukocytes in the circulation from an experimental atherosclerotic model *Apoe*$^{-/-}$was analyzed. Surprisingly, we also here identify FABP4 as a marker in neutrophils and monocytes reflecting atherosclerotic lesion progression. Moreover, I observe human monocytes and neutrophils from the circulation to be positive for FABP4. Our findings make FABP4 in circulating cells interesting for functional investigations, and an appealing and easy accessible biomarker target for potential future translation into clinical purposes.

In conclusion, I have studied inflammatory genes being involved in the pathogenic process during atherosclerosis using human and experimental models. In brief, we demonstrate that human vulnerable plaques display increased mRNA levels of 5-LO and LTA4H, and FABP4. In addition, the latter is shown in an experimental model, to be a potential valuable biomarker in circulating leukocytes reflecting the extent of atherosclerotic lesion. Our discoveries in the human plaque may be of future clinical relevance to identify vulnerable plaques, whereas FABP4 in leukocytes potentially could be useful for recognizing asymptomatic patients before onset of symptoms.

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