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Variants in *ALOX5*, *ALOX5AP* and *LTA4H* are not associated with atherosclerotic plaque phenotypes: The Athero-Express Genomics Study

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ABSTRACT

Background: The eicosanoid genes *ALOX5*, *ALOX5AP* and *LTA4H* have been implicated in atherosclerosis. We assessed the impact of common variants in these genes on gene expression, circulating protein levels, and atherosclerotic plaque phenotypes.

Methods: We included patients from the Stockholm Atherosclerosis Gene Expression study (STAGE, N = 109), and the Athero-Express Biobank Study (AE, N = 1443). We tested 1453 single-nucleotide variants (SNVs) in *ALOX5*, *ALOX5AP* and *LTA4H* for association with gene expression in STAGE. We also tested these SNVs for association with seven histologically defined plaque phenotypes in the AE (which included calcification, collagen, cellular content, atheroma size, and intraplaque vessel density and hemorrhage).

Results: We replicate a known *cis*-eQTL (rs6538697, $p = 1.96 \times 10^{-6}$) for *LTA4H* expression in whole blood of patients from STAGE. We found no significant association for any of the SNVs tested with serum levels of ALOX5 or ALOX5AP ($p > 5.79 \times 10^{-4}$). For atherosclerotic plaque phenotypes the strongest associations were found for intraplaque vessel density and smooth muscle cells in the *ALOX5AP* locus ($p > 1.67 \times 10^{-4}$).

Conclusions: We replicate a known eQTL for *LTA4H* expression in whole blood using STAGE data. We found no associations of variants in and around *ALOX5*, *ALOX5AP* and *LTA4H* with serum ALOX5 or ALOX5AP levels, or plaque phenotypes. On the supposition that these genes play a causal role in atherosclerosis, these results suggest that common variants in these loci play a limited role (if any) in influencing advanced atherosclerotic plaque morphology to the extent that it impacts atherosclerotic disease.

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1. Introduction

Three genes in the inflammation mediating eicosanoid pathway (Fig. 1) have been a focal point of atherosclerotic genomic research. Various studies have shown that the expressions of both arachidonic 5-lipoxygenase (*ALOX5*, OMIM: *152390) and its upstream regulator, arachidonic 5-lipoxygenase-activating protein (*ALOX5AP*, OMIM: *603700) are elevated in atherosclerotic plaques of murine models and human specimens [1–4]. Other studies have found a relation between a variable number of tandem repeats (VNTR) and *ALOX5* expression in circulating cells [5–8]. Different polymorphisms in the *ALOX5AP*, *ALOX5*, and leukotriene A4 hydrolase (*LTA4H*, OMIM: *151570) loci were also associated with carotid intima-media thickness (cIMT) [9], restenosis after percutaneous intervention (PCI) [10], myocardial infarction (MI), coronary artery disease (CAD) and ischemic stroke [11–14]. One of the end-products of the *ALOX5-ALOX5AP-LTA4H* axis in this pathway is leukotriene B4 (LTB4, Fig. 1). Through binding to the leukotriene B4 receptors 1 and 2 (BLT1 and BLT2) [15], it has been proposed that LTB4 regulates the production of interleukin 8 (IL8) [16], monocyte chemoattractant protein-1 (MCP1) [17], and interleukin 6 (IL6) [15]. IL6 in turn is thought to facilitate the recruitment of inflammatory cells [18]. The pro-inflammatory effects of MCP1 and IL6 are believed to modulate the inflammatory response in the vessel wall during atherogenesis [19].

These findings support the hypothesis that the eicosanoid pathway might be involved in atherogenesis and clinical outcome. However, recent genome-wide meta-analyses of CAD and ischemic stroke by the CARDIoGRAM [20] and METASTROKE [21] consortia have reported no genome-wide significant associations between the *ALOX5*, *ALOX5AP* or *LTA4H* loci and risk of disease. Moreover, a substantial number of studies report ambiguously on the role of these loci in cardiovascular disease, exemplified by a meta-analysis involving *ALOX5AP* variants that showed significant heterogeneity [22].

One possible explanation for the observed heterogeneity is an imprecise or noisy phenotype definition, such as MI versus CAD (which includes MI and coronary interventions). Unlike case-control studies, studies examining quantitative traits are more powered and are less affected by misclassification. Another possible explanation might be the incomplete coverage of genetic studies performed to date. Multiple causal variants could exist in these genes and may not be sufficiently tagged by previous studies, thus leading to ambiguous associations. Indeed, genome-wide studies (including CARDIoGRAM and METASTROKE) have almost exclusively focused on common variants in HapMap Phase 2, even though the catalog of known variants has dramatically expanded through the 1000 Genomes Project and similar efforts [23]. Moreover, as opposed to modulating risk, the effect of genetic variants in *ALOX5*, *ALOX5AP*, and *LTA4H* could be limited to the onset of disease or atherosclerotic plaque morphology.

In this study, we set out to test the role of common variants at the *ALOX5*, *ALOX5AP* and *LTA4H* candidate loci on advanced plaque morphology. We used serum samples and atherosclerotic plaques from patients undergoing carotid endarterectomy (CEA) who are enrolled in the Athero-Express Biobank Study (AE) in order to

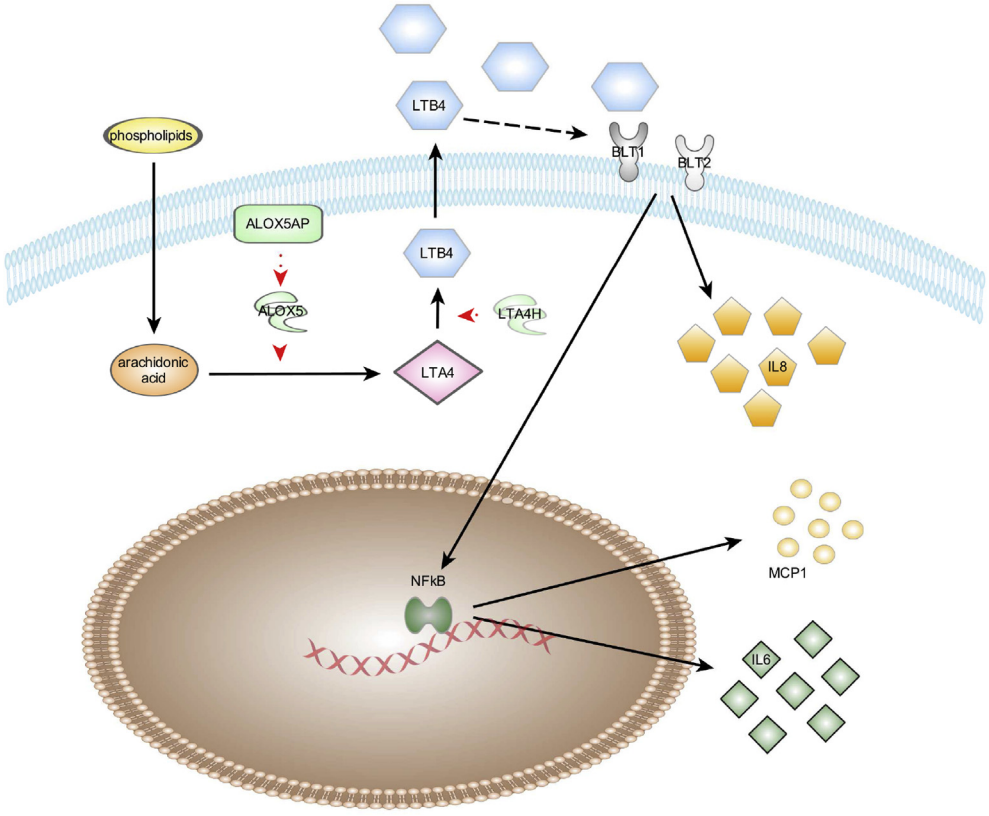

Fig. 1. The eicosanoid pathway. Phospholipids are taken up by the cell and metabolized into arachidonic acid (AA) which is the basis for many derivatives, one of which is leukotriene A4 (LTA4) that is metabolized by leukotriene A4 hydrolase (LTA4H) into leukotriene B4 (LTB4). The membrane-bound arachidonic 5-lipoxygenase-activating protein (ALOX5AP) activates arachidonic 5-lipoxygenase (ALOX5), which transforms AA into LTA4. Leukotriene B4 receptor 1 and 2 (BLT1 and BLT2 respectively) are transmembrane receptors of LTB4. Upon exocytosis and through binding of its receptors (BLT1 and BLT2), it is thought that LTB4 activates the NFκB pathway leading to the expression of interleukin 6 (IL6) and monocyte chemoattractant protein-1 (MCP1). The receptor binding also correlates with increased interleukin 8 (IL8) production by an unknown mechanism in neutrophils. Thus LTB4 is thought to facilitate chemotaxis of inflammatory cells, angiogenesis, and cell proliferation. Made and adapted using Ingenuity® Systems [43] and literature.