Abstract form

Title: GWAS identifies a KLKB1 promoter SNP that regulates serum PLAUR levels in asthma and COPD

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Abstract

Rationale: The urokinase plasminogen activator receptor (PLAUR, also known as uPAR) has been identified as an asthma susceptibility gene with single nucleotide polymorphisms (SNPs) associated with BHR, lung function decline and serum PLAUR in asthma patients. An elevated level of serum PLAUR has been detected in asthma and chronic obstructive pulmonary disease (COPD) and is thought to modulate the activity of the membrane bound receptor. In this study, we aimed to identify novel genetic determinants of serum PLAUR levels using a genome wide association (GWA) and determine the mechanism underlying this association.

Methods: 480 asthmatic subjects along with 104 non-asthmatic controls were recruited from the Netherlands. Baseline characteristics of the populations identify a mean age of 40 years for asthmatics and 50 years for controls with a percentage predicted pre-bronchodilator FEV1 of 81% and 100% and an FEV1/VC of 0.70 and 0.80 respectively. A GWAS on log10-transformed serum PLAUR levels was carried out in the asthma cases and controls separately, using linear regression in PLINK (additive model) with population substructure in asthmatic population. The top eight hits ($P<1 \times 10^{-4}$) were then investigated in a COPD cohort (n=381) (Mean Age: 67 years; percentage predicted FEV1 78%; FEV1/VC 0.45). The relationship between human Plasma Kallikrein (KLKB1) in serum from the Asthmatic, Control and COPD subjects (n=20 each), stratified according to the KLKB1 promoter SNP genotype was detected using a fluorescence based activity assay. Proteolytic activity of KLKB1 on recombinant PLAUR was identified by Western Blotting using a PLAUR Domain 1 specific monoclonal and a polyclonal PLAUR antibody. The effect of KLKB1 driven disruption of the receptor structure on the functional effects of sPLAUR, was investigated using a scratch wound model and MTT assay in a cultured human bronchial epithelial cell population.

Results: A SNP within the promoter of the KLKB1 gene was significantly associated with serum PLAUR levels; Genome wide significant after a combined analysis of asthma cases and unaffected controls (Mean serum PLAUR levels: C:C=6920pg/ml, T:C=5898pg/ml, T:T=4481pg/ml; fixed effect combined $P=1.13 \times 10^{-7}$, heterogeneity $I^2$ index=0). Independent evidence for a role for this SNP was obtained in the COPD cohort (Mean Age: 67 years; percentage predicted FEV1 pre-bronchodilator 78%; FEV1/VC 0.45). The relationship between human Plasma Kallikrein (KLKB1) in serum from the Asthmatic, Control and COPD subjects (n=20 each), stratified according to the KLKB1 promoter SNP genotype was detected using a fluorescence based activity assay. Proteolytic activity of KLKB1 on recombinant PLAUR was identified by Western Blotting using a PLAUR Domain 1 specific monoclonal and a polyclonal PLAUR antibody. The effect of KLKB1 driven disruption of the receptor structure on the functional effects of sPLAUR, was investigated using a scratch wound model and MTT assay in a cultured human bronchial epithelial cell population.
Conclusions: Serum *PLAUR* levels are modulated by a *KLKB1* promoter SNP in multiple control and disease populations, with the *KLKB1* genotype associated with decreased *KLKB1* activity and subsequently elevated serum *PLAUR*. The mechanism underlying this association involves proteolytic cleavage of *PLAUR* by *KLKB1* which is enhanced in carriers of the *KLKB1* T:T genotype. Proteolytic cleavage of scPLAUR effects cellular functions important to airway remodelling. Therefore a novel regulatory mechanism for sc PLAUR has been detected, which may be of importance in the modulation and progression of a number of disease including, but not limited to, asthma and COPD.