

Online Supplementary Text

The H Antigen at Epithelial Surfaces is Associated with Susceptibility to Asthma Exacerbation.

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FUT2 genotyping. Peripheral blood DNA was collected from subjects to enable assay for the G428A mutation in *FUT2*. G428A is a nonsense mutation that results in an early stop codon, yielding a truncated α 1,2 FucT protein that is non-functional. The G428A mutation in *FUT2* was genotyped using iPLEX reagents and protocols from Sequenom (San Diego). Five multiplexed assays containing between 24 and 36 individual SNPs each were designed using Sequenom's Assay Designer software (version 3.0). Multiplexed PCR was performed in 5- μ l reactions on 384-well plates containing 5 ng of genomic DNA. Reactions contained 0.5 U HotStar Taq polymerase (QIAGEN), 100 nM primers, 1.25X HotStar Taq buffer, 1.625 mM MgCl₂, and 500 μ M dNTPs. Following enzyme activation at 94 °C for 15 min, DNA was amplified with 45 cycles of 94 °C x 20 sec, 56 °C x 30 sec, 72 °C x 1 min, followed by a 3-min extension at 72 °C. Unincorporated dNTPs were removed using shrimp alkaline phosphatase (0.3 U, Sequenom). Single-base extension primers were added (0.625 μ M [low MW primers] to 1.25 μ M [high MW primers]) using iPLEX enzyme and buffers in 9- μ l reactions. Reactions were desalted and SBE products measured using the MassARRAY Compact system, and mass spectra analyzed using TYPER software (Sequenom), to generate genotype calls and allele frequencies.

SECRETOR STATUS AND EXPRESSION OF BLOOD GROUP ANTIGENS IN THE AIRWAY EPITHELIUM

To confirm that histoblood group antigens are expressed by airway epithelial mucins in secretors but not in non-secretors, we examined the expression of H antigens using *Ulex europaeus* agglutinin Type 1 (UEA-1), a lectin specific for the H antigen, in sections of bronchial biopsies obtained from asthmatic subjects during research bronchoscopy

and available from the UCSF Airway Tissue Bank. These asthmatic subjects had mild-to-moderate asthma and had not been taking inhaled corticosteroids (ICS); their secretor status had been determined using Lewis antigen testing in peripheral blood.

After removal of paraffin and rehydration of the tissue sections, endogenous peroxidase was inhibited by 20 minute incubation in 3.0% H₂O₂ in methanol. Sections were rinsed twice for 5 minutes in PBS/0.3% Tween-20. Quenching of non-specific binding was achieved by addition of 200-400µl of 1% BSA/PBS to the sections for 30 minutes at room temperature. The 1% BSA/PBS was replaced by 200-400µl of peroxidase-labeled UEA-I (Sigma, catalog # L8146) (1:400 dilution of 2mg/mL). The sections were incubated for 1 hour, rinsed in PBS/Tween-20, 3x for 5 minutes, and AEC substrate (Vector Laboratories) added for 15 minutes before addition of hematoxylin counterstain (all steps at room temperature).