Online Repository

Supplemental Methods:

Sample collection:

Informed consent was obtained from healthy adults, and the protocol was approved by the Institutional Review Board of Boston Children's Hospital. All samples were collected by the same trained physician (PL). Inferior turbinate samples were collected by rotating a cytology brush (Medical Packing Corporation, Camarillo, CA) beneath the inferior turbinate, with visualization aided by a nasal speculum and head lamp. Anterior nares samples were collected by inserting either a cytology brush or a sterile polyester tipped applicator (Puritan, Guildford, ME) in the anterior nares until the tip is just inferior to the nasal bone, and vigorously rotating the brush or swab along the nares, avoiding the nasal septum.

Four samples were collected from each subject by the same trained physician (PL); for each nare, paired inferior turbinate brush with anterior nare (either brush or swab) samples were collected. The order of collection of each sample differed by subject. For example, for subject 1, samples were collected in the following order: right anterior nares brush, right inferior turbinate brush, left inferior turbinate brush, and left anterior nares swab. For subject 2, samples were collected in the following order: right inferior turbinate brush, right anterior nares swab, left anterior nares brush, left inferior turbinate brush. Subjects were asked to rate their discomfort level immediately after each discrete sample was collected using a numerical 0 to 10 point rating scale.[1]

Sample processing:

The brush or swab was immediately immersed in 700 microliters of sterile saline; 100 microliters was aliquoted for cytology, and 3000 microliters of RNAprotect (Qiagen, Valencia, CA) added to the remainder. Cytology samples were prepared using a cytospin to deposit samples on a glass slide, then stained using a rapid chrome Papanicolaou staining kit (Thermo Scientific, Waltham, MA). Differential cell counts were obtained by taking the average of manual counts (1000x under oil immersion, 3 high power fields). RNA and DNA was simultaneously extracted from the remaining aliquot using the Qiagen All-prep micro kit. Nucleic acid yield was quantified using a Nanodrop spectrophotometer. Paired samples (inferior turbinate with anterior nares sampling from the same individual) with sufficient nucleic acid yield for downstream microarray analysis were sent for quality control and whole genome profiling with the Illumina Human HT-12 BeadChip for gene expression, and the Illumina Beadchip Infinium HD for methylation levels at the Center for Genetic Medicine at the Feinberg School of Medicine. Sufficient nucleic acid yield was defined for RNA as at least 150 nanograms, and for DNA as at least 750 nanograms.

Statistical approach:

Correlation and relative error was calculated comparing anterior nares and inferior turbinate samples from the same individual across all genes for gene expression, and across all methylation sites for methylation data. For methylation data, statistical analyses were performed only on variable methylation sites (defined as those that had greater than 10% or less than 90% methylation). Correlation was calculated using the cor() function in R. Average relative error was calculated using custom code as follows: for comparing anterior nares to inferior turbinate samples from the same individual, relative error for methylation was defined as: (|methylation beneath inferior turbinate - methylation in anterior nares|/methylation beneath inferior turbinate)*100. For comparing left and right inferior turbinate samples from the same individual, this was defined as: (|methylation beneath left inferior turbinate - methylation beneath right inferior

turbinate|/methylation beneath left inferior turbinate)*100. The mean was taken of these measures across genes/methylation sites to obtain the average relative error. A similar approach was taken to calculate average relative error for expression data. All statistical analyses were performed in R 3.1.0. The Bioconductor package *lumi* was used for expression and methylation analysis.

Supplemental Tables

Table E1. List of gene symbols for genes previously implicated in asthma.

CLEC16A	CLEC4G	TLR11	NLRP7
CLEC4GP1	CLEC4F	TLR12	NLRP8
CLEC4E	CLEC2B	TLR13	NLRP9
CLEC4M	CLEC17A	CIITA	NLRP10
CLEC18C	CLEC5A	NAIP	NLRP11
CLEC3A	CLEC7A	NOD1	NLRP12
CLEC18A	CLEC2D	NOD2	NLRP13
CLEC12B	CLECL1	NLRC3	NLRP14
CLEC4A	CLEC2A	NLRC4	F2R
CLEC18B	CLEC1B	NLRC5	F2RL1
CLEC4C	TLR1	PAR1	F2RL3
CLEC11A	TLR2	PAR2	GPR11
CLEC4D	TLR3	PAR3	TSLP
CLEC12A	TLR4	PAR4	CSF2
CLEC1A	TLR5	NLRP1	IL25
CLEC10A	TLR6	NLRP2	IL33
CLEC3B	TLR7	NLRP3	IL13
CLEC9A	TLR8	NLRP4	
CLEC2L	TLR9	NLRP5	
CLEC14A	TLR10	NLRP6	

Supplemental Figure Legends

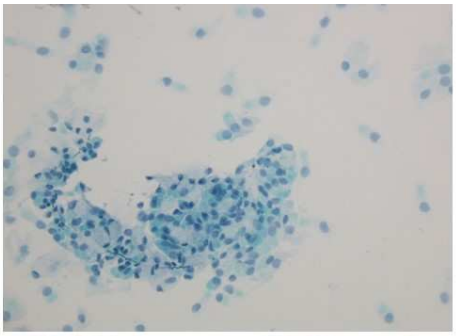
Figure E1. Cytology from different nasal sampling locations. All nasal samples obtained from subject 10 and stained using a rapid Papanicolaou staining kit. Keratin is stained pink. Images at 10*20x. Note almost uniform population of respiratory epithelial cells from inferior turbinate (IT) sampling locations, vs. a more heterogeneous population of squamous and respiratory epithelial cells from anterior nares (AN) sampling locations.

Figure E2. Correlation of methylation in asthma genes from different sampling locations. Scatterplot of all varying asthma-related methylation sites from Illumina Beadchip Infinium HD array of nasal samples from different sampling locations obtained from subject 10.

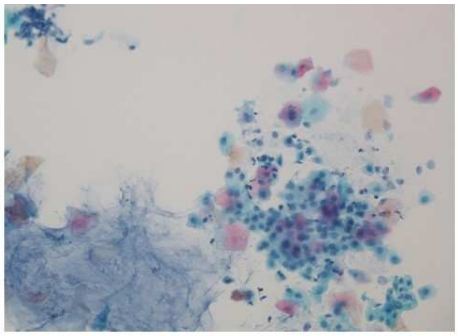
References cited:

1. Ferreira-Valente MA, Pais-Ribeiro JL, Jensen MP. Validity of four pain intensity rating scales. Pain 2011; 152:2399-404.

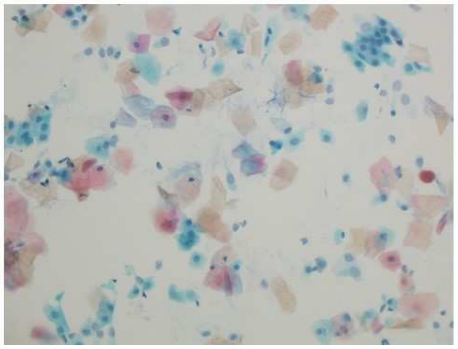
Right
IT
Brush



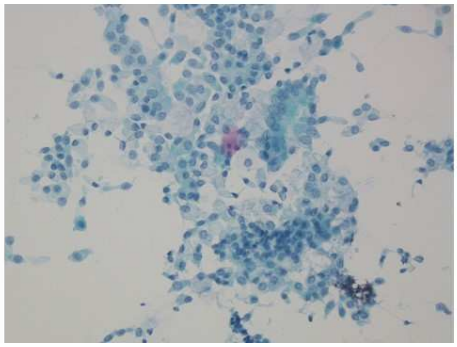
Left
AN
Brush

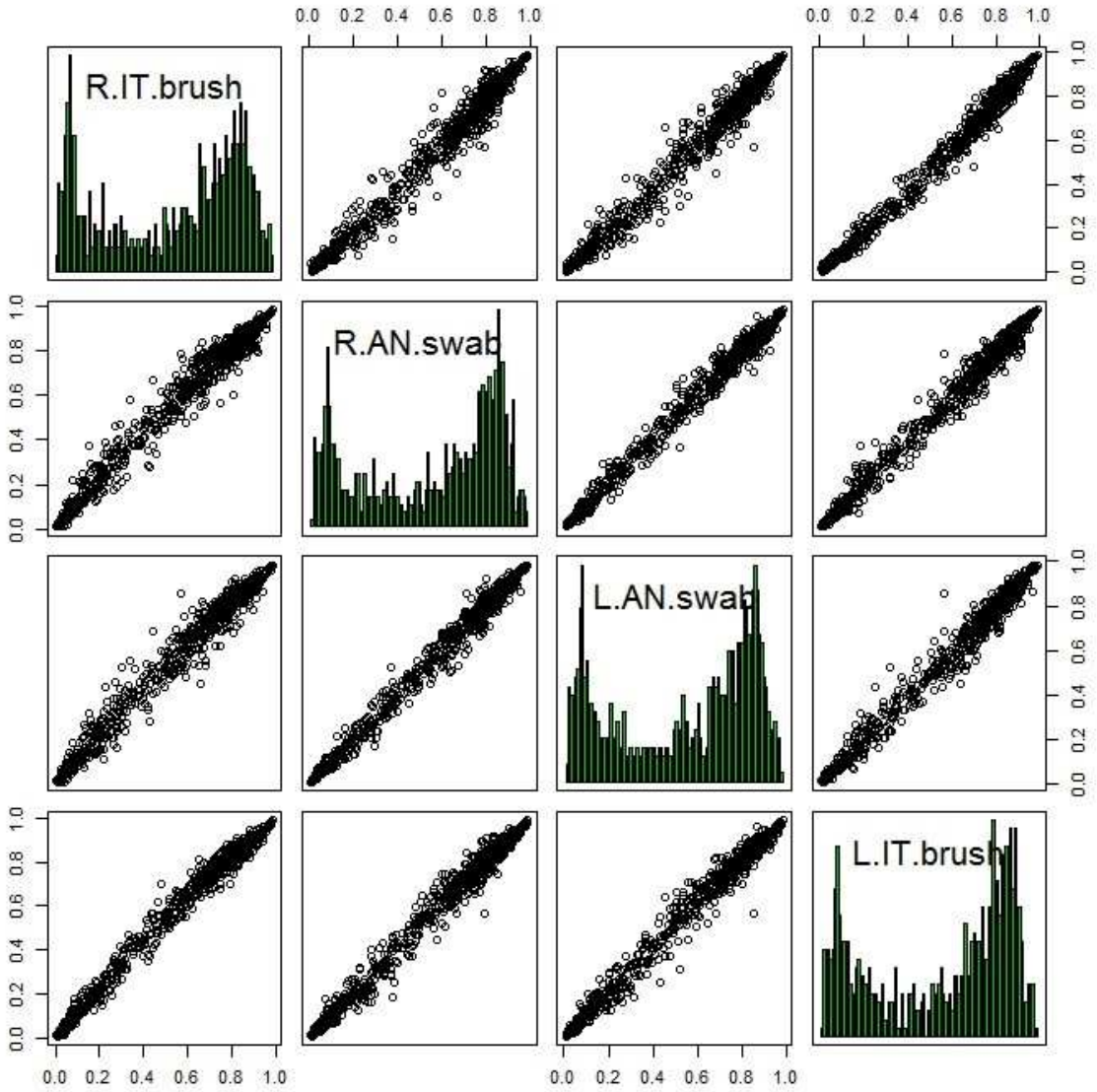


Right
AN
Swab



Left
IT
Brush




R.IT.brush
R.AN.swab
L.AN.swab
L.IT.brush
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