

Letter to the Editor

Alternate methods of nasal epithelial cell sampling for airway genomic studies

To the Editor:

Recent translational studies of airway inflammation have shown that nasal epithelial cells are a good surrogate for bronchial epithelial cells^{1,2} in asthmatic patients.^{3,4} However, the standard method of nasal sampling requires use of a nasal speculum and specialized training. In pediatric studies requiring longitudinal specimen collection, sampling by this method can be limited by subject refusal and technical challenges.

Alternate methods of nasal sampling have been proposed. Different instruments for collection have been used, ranging from polyester-tipped swabs to plastic curettes to cytology brushes. Different sampling locations have been proposed, such as beneath the inferior turbinate or the anterior nares, where respiratory epithelial cells are also located.⁵ Obtaining nasal epithelial cells beneath the inferior turbinate with a cytology brush has been the most commonly used method. These cells have been validated as a surrogate for bronchial epithelial cells² and have been shown to be clinically important in translational asthma studies.⁴ This method has also been shown in preliminary studies to be more difficult to tolerate.⁶ Whether a more comfortable method of sampling exists and whether this method can provide equivalent cytologic, gene expression, and epigenetic results is undetermined.

Here we compared nasal epithelial cells sampled from the anterior nares with either a polyester swab or a cytology brush with the standard collection method of cytology brush sampling from beneath the inferior turbinate. The benefit of the former method is that it does not require the use of a speculum to visualize nasal anatomy, is technically easy to perform, and, with the swab method, is already widely used in clinical practice for obtaining microbiologic samples.

Informed consent was obtained from 12 healthy adults. Four samples were collected from each subject; for each naris, paired inferior turbinate and anterior nares samples were collected. Inferior turbinate samples were collected with cytology brushes using nasal speculums for direct visualization. Anterior nares samples were obtained by inserting either a brush or swab inferior to the nasal bone and vigorously rubbing along the nares (see the [Methods](#) section in this article's Online Repository at www.jacionline.org). Each sample was immediately aliquoted for cytology and RNA and DNA extraction. Paired samples with sufficient nucleic acid yield for downstream microarray analysis were analyzed for whole-genome expression (Illumina HumanHT-12 v4 Expression BeadChip; Illumina, San Diego, Calif) and methylation (Illumina BeadChip Infinium HD array) levels. Subjects were asked to rate their discomfort level immediately after each collection using a numeric 0- to 10-point rating scale.

The largest observed difference between sample collection methods for measured parameters was between the location of sampling (inferior turbinate vs anterior nares) rather than the use of a cytology brush versus swab ([Table I](#)). Average discomfort levels were significantly higher for inferior turbinate compared with anterior nares sampling (median, 3.5 [interquartile range [IQR], 2.9-7.0] vs 1 [IQR, 0.4-2.0]; $P < .001$). Inferior turbinate

TABLE I. All measured parameters comparing inferior turbinate with anterior nares sampling locations

Parameter	Inferior turbinate	Anterior nares	<i>P</i> value*
Discomfort†	3.5 (2.9-7.0)	1.0 (0.4-2.0)	<.001
Proportion of respiratory epithelial cells (%)	99.2 (92.2-100.0)	65.4 (46.8-84.7)‡	<.001
RNA yield (ng)	4620 (2364-6993)	156 (66-606)§	<.001
RNA integrity number	8.9 (8.6-9.5)	2.2 (1.0-3.9)	<.001
DNA yield (ng)	4288 (2081-7600)	875 (181-2375)¶	<.001
Gene expression (correlation)#	0.94 (0.93-0.96)	0.91 (0.78-0.94)	—
Gene expression (average relative error [%])#	6.1 (3.0-6.0)	9.1 (3.7-15.3)	—
Methylation, all genes (correlation)#	0.97 (0.96-0.98)	0.93 (0.81-0.98)	—
Methylation, all genes (average relative error [%])#	6.1 (5.2-7.4)	7.0 (4.7-8.8)	—
Methylation, asthma genes (correlation)#	0.98 (0.97-0.99)	0.95 (0.88-0.93)	—
Methylation, asthma genes (average relative error [%])#	5.8 (5.0-6.1)	7.6 (3.1-10.4)	—

Parameters are expressed as medians (IQRs). Forty-eight samples were collected from 12 subjects. Twenty-four samples from 8 subjects underwent downstream whole-genome expression and methylation analysis.

**P* value comparing parameters from inferior turbinate sampling versus anterior nares sampling.

†Subjects were asked to rate discomfort level on a standardized 0- to 10-point numeric scale, where 0 indicates no discomfort and 10 indicates maximum discomfort. For the anterior nares location, there were no significant differences between the brush (1.0 [0.63-2.8]) and swab (1.3 [0.3-2.0]) methods ($P = .51$).

‡For the anterior nares, there were no statistically significant differences between the brush (82.4% [59.6% to 84.9%]) and swab (50.3% [42.3% to 71.5%]) methods ($P = .28$). For the anterior nares, the brush method (11.0% [5.8% to 39.3%]) had a lower proportion of squamous epithelial cells compared with the swab method (49.7% [24.4% to 50.9%]; $P < .001$).

§For the anterior nares location, there were no statistically significant differences between the brush (192 [81-615]) and swab (156 [60-588]) methods ($P = .58$).

||For the anterior nares location, the brush method (3.9 [2.7-5.3]) had a higher RNA integrity number compared with the swab method (1.1 [1-1.8]; $P = .047$).

¶For the anterior nares location, there were no statistically significant differences between the brush (850.0 [175.0-2343.8]) and swab (875.0 [212.5-2281.3]) methods ($P = .87$).

#Correlation and average relative error were calculated between the left and right inferior turbinate samples from the same subject and between inferior turbinate and anterior nares samples from the same subject.

samples had significantly more respiratory epithelial cells than anterior nares samples (median, 99.2% [IQR, 92.2% to 100.0%] vs 65.4% [IQR, 46.8% to 84.7%]; $P < .001$; see [Fig E1](#) in this article's Online Repository at www.jacionline.org); proportions for squamous epithelial cells were reversed. Regardless of the sampling method, there were very few inflammatory cells in all samples (median, 0%; IQR, 0% to 1.2%).

Inferior turbinate samples had higher RNA yields (4620 vs 156 ng, $P < .001$) and higher RNA integrity numbers (8.9 vs 2.2, $P < .001$) than anterior nares samples ([Table I](#)). The same was true for DNA yields (4288 vs 875 ng, $P < .001$). Importantly, 91.7% of inferior turbinate samples but only 33.3% of anterior nares samples yielded sufficient RNA, and

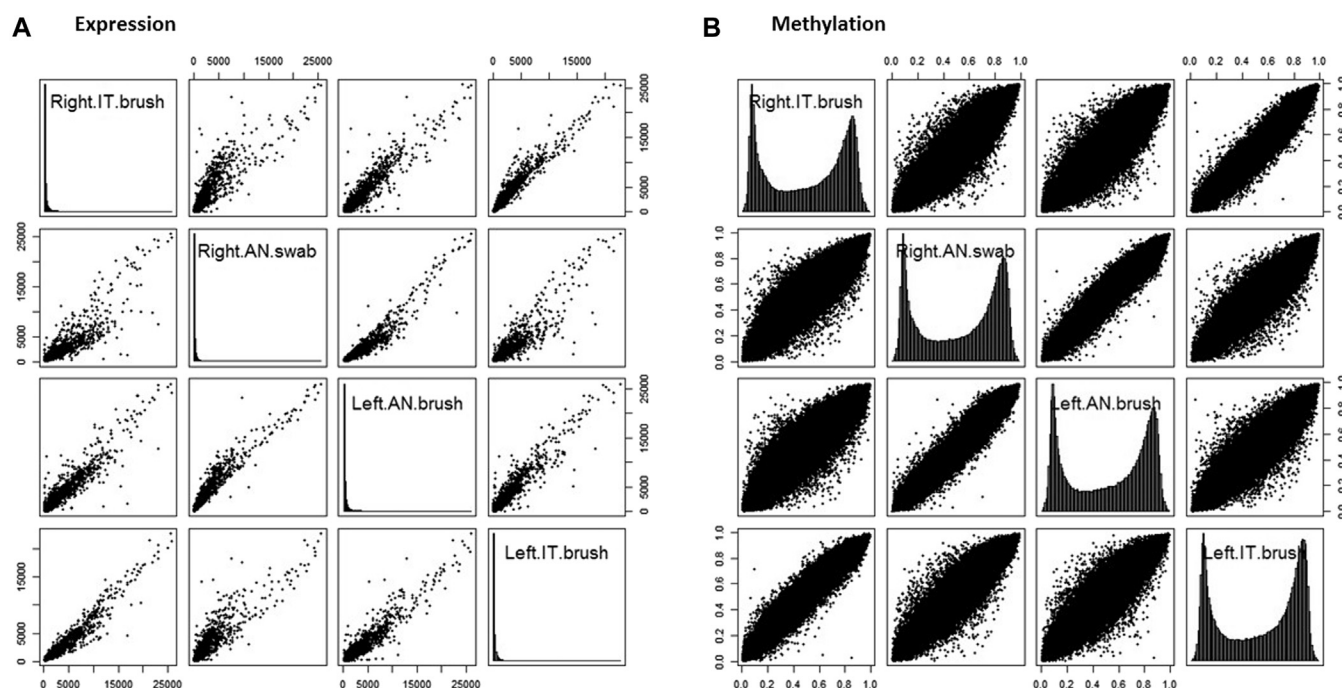


FIG 1. Correlation of expression and methylation patterns from different sampling locations. The figure depicts samples obtained from subject 10 as a representative figure. **A**, Scatterplot of gene-level expression intensities from the Illumina HumanHT-12 v4 Expression BeadChip array. Nasal samples from inferior turbinate (IT) and anterior nares (AN) locations collected with cytology brushes or polyester swabs are shown. Expression intensities for each sample are plotted in histograms on the diagonal. **B**, Scatterplot of all varying methylation sites from Illumina BeadChip Infinium HD array. Note that anterior nares and inferior turbinate samples from the same subject are highly correlated for both gene expression and methylation.

100% of inferior turbinate samples but only 50% of anterior nares samples yielded sufficient DNA for downstream microarray analysis.

Twenty-four samples were selected for paired whole-genome expression and methylation analysis. Seven failed to hybridize to the expression microarray; of these, all were anterior nares samples with low RNA yields. Despite the low RNA integrity number in the remaining anterior nares samples, average expression intensity was highly correlated in expressed genes when comparing inferior turbinate with anterior nares samples from the same subject (correlation, 0.91 [IQR, 0.78-0.94]; Fig 1, A). To determine the accuracy of the 2 sampling techniques, we calculated the relative error averaged over all genes. Average relative errors comparing anterior nares with inferior turbinate samples were not significantly different from those comparing left and right inferior turbinate samples from the same subject (9.1% [IQR, 3.7% to 15.3%] vs 6.1% [IQR, 3.0% to 6.0%]; $P = .19$).

All 24 DNA samples were successfully hybridized to the methylation array. Among all variable methylation sites, methylation was again highly correlated between inferior turbinate and anterior nares samples from the same subject (correlation, 0.93 [IQR, 0.81-0.98]; Fig 1, B), although the average relative error was also not significantly different (6.1% [IQR, 5.2% to 7.4%] vs 7.0% [IQR, 4.7% to 8.8%], $P = .67$). When we looked only at genes previously reported to be associated with asthma (see Table E1 in this article's Online Repository at www.jacionline.org), methylation was highly correlated between inferior turbinate and anterior nares samples

(correlation, 0.95 [IQR, 0.88-0.93]; see Fig E2 in this article's Online Repository at www.jacionline.org).

High-dimensional genomics studies have recently focused on the use of nasal epithelial cells as a noninvasive surrogate for bronchial epithelial cells in asthmatic patients, with the hope that prognostic biomarkers discovered can ultimately be translated to the bedside. Although nasal epithelial cell collection from beneath the inferior turbinate has been the standard collection method, it requires specialized training and equipment, can be uncomfortable, and is unlikely to be broadly implemented given these challenges. However, nasal sampling with polyester swabs is already widely used by clinicians for microbiologic testing. This is the first study to comprehensively evaluate cytologic, expression, and methylation patterns in epithelial cells obtained from inferior turbinate versus anterior nares sampling.

Although one limitation of our study is that it was performed in healthy subjects, we identified anterior nares sampling as a promising alternative method to obtain nasal epithelial cells. Both expression and methylation markers are highly correlated between anterior nares and inferior turbinate samples, with comparable relative error. RNA quantity and degradation in anterior nares samples likely limit the use of this method for expression studies. In methylation studies involving children or in biomarker discovery studies targeted to clinical practice, anterior nares sampling represents a promising alternative and should be explored in future asthma trials.

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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 (P.S.L.). Inferior turbinate samples were collected by rotating a cytology brush (Medical Packing, Camarillo, Calif) 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, Guilford, Me) in the anterior nares until the tip was 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 (P.S.L.); for each naris, paired inferior turbinate brush with anterior naris (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, and left inferior turbinate brush. Subjects were asked to rate their discomfort level immediately after each discrete sample was collected using a numeric 0- to 10-point rating scale.^{E1}

Sample processing

The brush or swab was immediately immersed in 700 μ L of sterile saline; 100 μ L was aliquoted for cytology, and 3000 μ L of RNeasy Protect (Qiagen, Valencia, Calif) was added to the remainder. Cytology samples were prepared with a cytospin to deposit samples on a glass slide and then stained with a rapid chrome Papanicolaou staining kit (Thermo Scientific, Waltham, Mass). Differential cell counts were obtained by taking the average of manual counts ($\times 1000$ magnification under oil immersion, 3 high-power fields). RNA and DNA were simultaneously extracted from the remaining aliquot by using the Qiagen AllPrep Micro Kit (Qiagen, Hilden, Germany). Nucleic acid yield was

quantified with a NanoDrop spectrophotometer (Thermo Scientific). Paired samples (inferior turbinate with anterior nares sampling from the same subject) 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 ng and for DNA as at least 750 ng.

Statistical approach

Correlation and relative error were calculated comparing anterior nares and inferior turbinate samples from the same subject 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 with $>10\%$ or $<90\%$ methylation). Correlation was calculated by using the `cor()` function in R. Average relative error was calculated by using custom code. For comparing anterior nares with inferior turbinate samples from the same subject, relative error for methylation was defined as follows:

$$\left(\frac{\text{Methylation beneath inferior turbinate} - \text{Methylation in anterior nares}}{\text{Methylation beneath inferior turbinate}} \right) * 100.$$

For comparing left and right inferior turbinate samples from the same subject, relative error for methylation was defined as follows:

$$\left(\frac{\text{Methylation beneath left inferior turbinate} - \text{Methylation beneath right inferior turbinate}}{\text{Methylation beneath left inferior turbinate}} \right) * 100.$$

The mean was taken of these measures across genes/methylation sites to obtain the average relative error. A similar approach was taken to calculate the 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.

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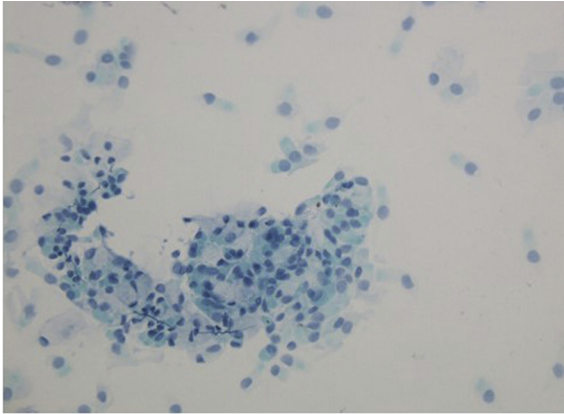
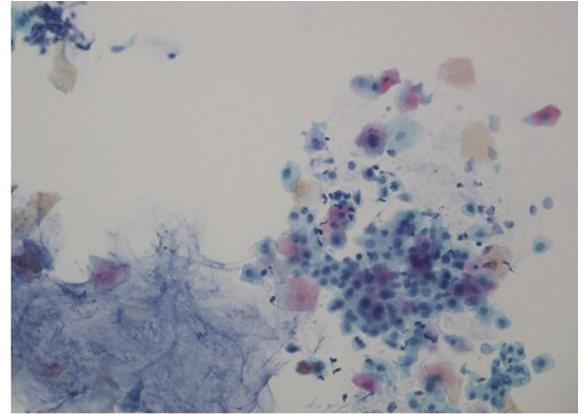
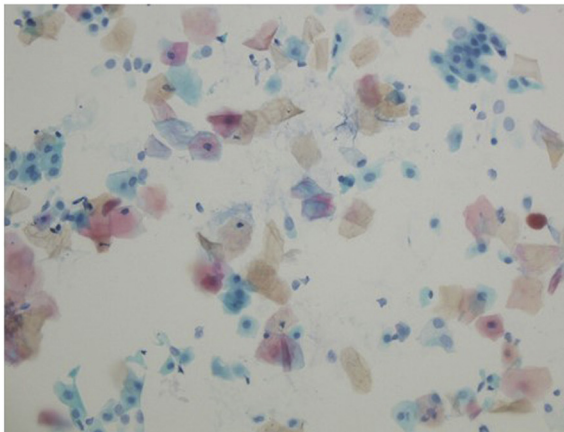
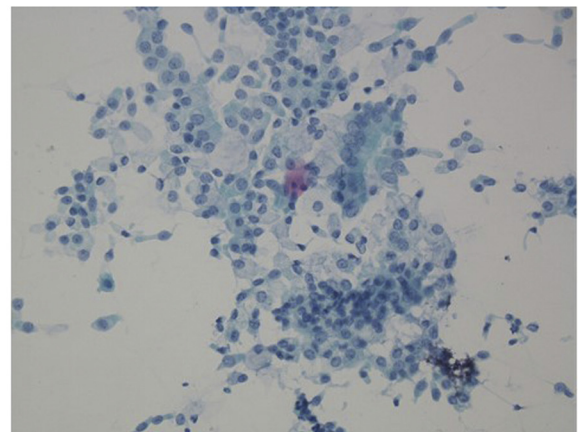
Right
IT
BrushLeft
AN
BrushRight
AN
SwabLeft
IT
Brush

FIG E1. Cytology from different nasal sampling locations. All nasal samples were obtained from subject 10 and stained with a rapid Papanicolaou staining kit. Keratin is stained pink. Images are at $\times 200$ magnification. Note the almost uniform population of respiratory epithelial cells from inferior turbinate (*IT*) sampling locations versus a more heterogeneous population of squamous and respiratory epithelial cells from anterior nares (*AN*) sampling locations.

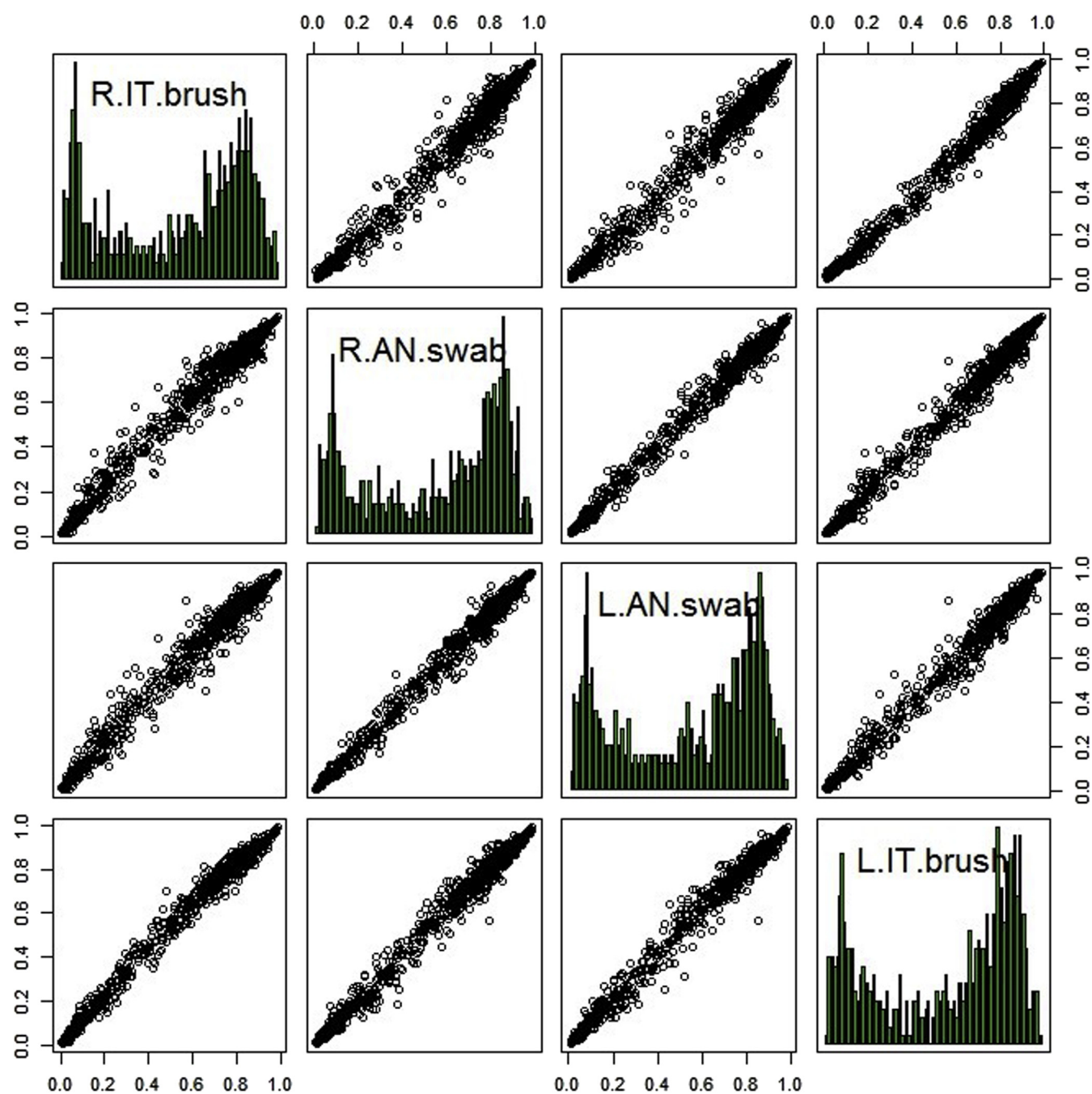


FIG E2. Correlation of methylation in asthma genes from different sampling locations. Scatterplots of all varying asthma-related methylation sites from the Illumina BeadChip Infinium HD array of nasal samples from different sampling locations obtained from subject 10 are shown.

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

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