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Alternate methods of nasal epithelial cell sampling for airway genomic studies

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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 asthma[3, 4]. The standard method of nasal sampling, however, requires use of a nasal speculum and specialized training. In pediatric studies requiring longitudinal specimen collection, sampling by this method may be limited by subject refusal and technical challenges.

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Alternate methods of nasal sampling have been proposed. Different instruments for collection have been used, ranging from polyester tipped swabs, 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]. Nasal epithelial cells obtained with use of a cytology brush beneath the inferior turbinate has been the most commonly used method; it has been validated as a surrogate for bronchial epithelial cells[2], and has 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 using either a polyester swab or a cytology brush with the standard collection method - 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 nare, paired inferior turbinate and anterior nare samples were collected. Inferior turbinate samples were collected with cytology brushes using nasal speculums for direct visualization. Anterior nare samples were obtained by inserting either a brush or swab inferior to the nasal bone, and vigorously rubbing along the nares (detailed methods in Online Repository). Each sample was immediately aliquoted for cytology, 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) and methylation (Illumina Beadchip Infinium HD array) levels. Subjects were asked to rate their discomfort level immediately after each collection using a numerical 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 vs. swab (Table 1). Average discomfort levels were significantly higher for inferior turbinate compared to anterior nares sampling (median 3.5 [IQR 2.9-7.0] vs. 1 [0.4-2.0], $p < 0.001$). Inferior turbinate samples had significantly more respiratory epithelial cells than anterior nares samples (median 99.2 [IQR 92.2 - 100.0] % vs 65.4 [46.8- 84.7] %, $p < 0.001$, Supplemental Figure E1); proportions for squamous epithelial cells were reversed. Regardless of sampling method, there were very few inflammatory cells in all samples (median 0, IQR 0–1.2%).

Inferior turbinate samples had higher RNA yields (4,620 vs. 156 ng, $p < 0.001$) and higher RNA integrity numbers (8.9 vs 2.2, $p < 0.001$) than anterior nares samples (Table 1). The same was true for DNA yields (4,288 vs. 875 ng, $p < 0.001$). Importantly, 91.7% of inferior turbinate samples but only 33.3% of anterior nares samples yielded sufficient RNA, and 100% of inferior turbinate samples but only 50% of anterior nares samples yielded sufficient DNA, for downstream microarray analysis.

24 samples were selected for paired whole genome expression and methylation analysis. 7 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 remaining anterior nares samples, average expression intensity was highly correlated in expressed genes when comparing inferior turbinate with anterior nares samples from the same individual (correlation 0.91 [0.78-0.94], Figure 1A). To determine the accuracy of the two sampling techniques, we calculated the relative error averaged over all genes. Average relative error comparing anterior nares to inferior turbinate samples was not significantly different from comparing left and right inferior turbinate samples from the same individual (9.1 [3.7-15.3] % vs. 6.1 [3.0-6.0] %, p = 0.19).

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 individual (correlation 0.93 [0.81-0.98], Figure 1B). Average relative error was also not significantly different (6.1 [5.2-7.4] % vs. 7.0 [4.7-8.8] %, p=0.67). When we looked only at genes previously reported to be associated with asthma (Supplemental Table E1), methylation was highly correlated between inferior turbinate and anterior nares samples (Supplemental Figure E2, correlation 0.95 [0.88-0.93]).

High dimensional genomics studies have recently focused on the use of nasal epithelial cells as a noninvasive surrogate for bronchial epithelial cells in asthma, with the hope that prognostic biomarkers discovered can ultimately be translated to the bedside. While 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. Nasal sampling using polyester swabs, however, 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 vs 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 way 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 limits the use of this method for expression studies. In methylation studies involving children, or in biomarker discovery studies targeted towards clinical practice, anterior nares sampling represents a promising alternative and should be explored in future asthma trials.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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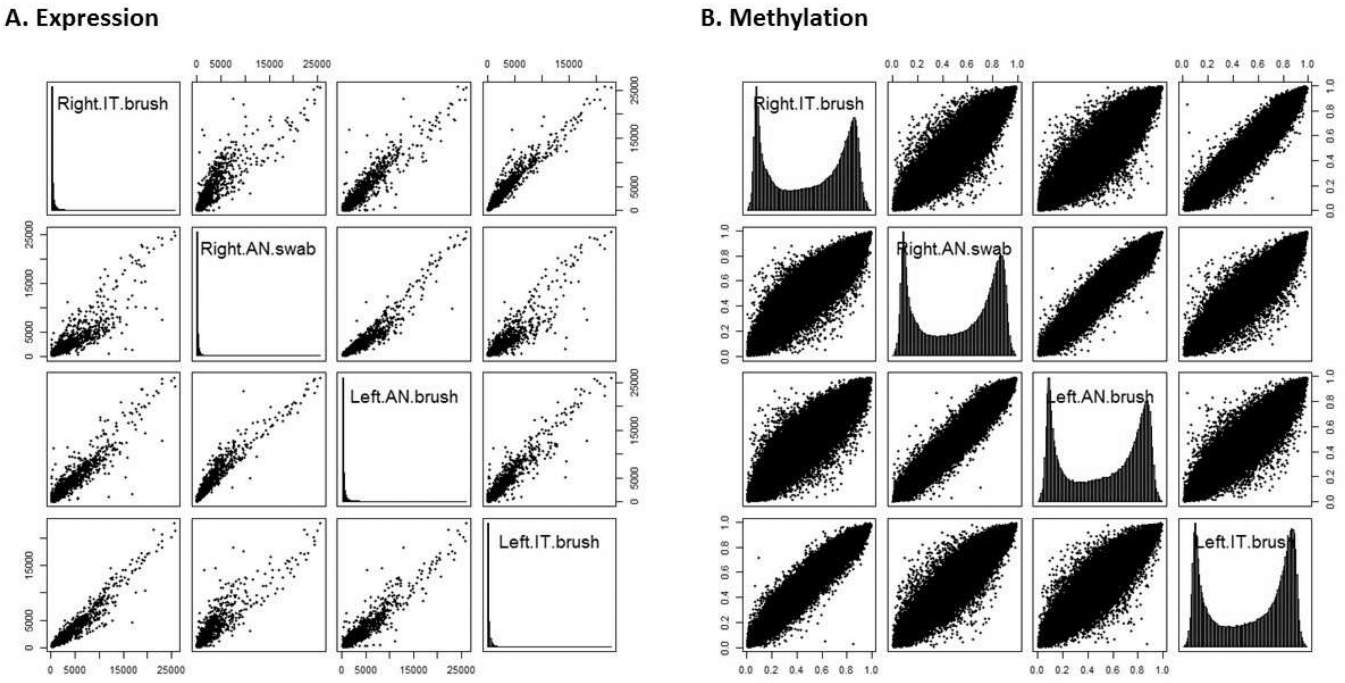

Figure 1. Correlation of expression and methylation patterns from different sampling locations

Figure depicts samples obtained from subject 10 as a representative figure. **2A**. Scatterplot of gene-level expression intensities from Illumina HumanHT-12 v4 Expression BeadChip array. Nasal samples from inferior turbinate (IT) and anterior nares (AN) locations collected using cytology brushes or polyester swabs. Histogram of expression intensities for each sample plotted on the diagonal. **2B**. Scatterplot of all varying methylation sites from Illumina Beadchip Infinium HD array. Note that anterior nares and inferior turbinate samples from the same individual are highly correlated for both gene expression and methylation.

Table 1

All measured parameters comparing inferior turbinate to anterior nares sampling locations. Parameters expressed as median (interquartile range). 48 samples were collected from 12 subjects. 24 samples from 8 subjects underwent downstream whole genome expression and methylation analysis.

Parameter	Inferior turbinate	Anterior nares	p-value[a]
Discomfort [b]	3.5 [2.9 – 7.0]	1.0 [0.4 – 2.0]	<0.001
Proportion respiratory epithelial cells (%)	99.2 [92.2 – 100.0] %	65.4 [46.8 – 84.7] % [c]	<0.001
RNA yield (ng)	4620 [2364 – 6,993] ng	156 [66 – 606] ng [d]	<0.001
RNA integrity number (RIN)	8.9 [8.6 – 9.5]	2.2 [1.0 – 3.9] [e]	<0.001
DNA yield (ng)	4,288 [2,081 – 7,600] ng	875 [181 – 2375] ng [f]	<0.001
Gene expression (correlation) [g]	0.94 [0.93 – 0.96]	0.91 [0.78 – 0.94]	-
Gene expression (average relative error) [g]	6.1 [3.0 – 6.0] %	9.1 [3.7 – 15.3] %	-
Methylation, all genes (correlation) [g]	0.97 [0.96 – 0.98]	0.93 [0.81 – 0.98]	-
Methylation, all genes (average relative error) [g]	6.1 [5.2 – 7.4] %	7.0 [4.7 – 8.8] %	-
Methylation, asthma genes (correlation) [g]	0.98 [0.97 – 0.99]	0.95 [0.88 – 0.93]	-
Methylation, asthma genes (average relative error) [g]	5.8 [5.0 – 6.1] %	7.6 [3.1 – 10.4] %	-

[a] p-value comparing parameters from inferior turbinate sampling vs. anterior nares sampling

[b] Subjects asked to rate discomfort level on a standardized 0–10 numerical rating scale, where 0 indicates no discomfort and 10 indicates maximum discomfort. For anterior nares location, no significant differences between brush (1.0 [0.63 – 2.8]) and swab (1.3 [0.3 – 2.0]) method, p=0.51.

[c] For anterior nares location, no statistically significant differences between brush (82.4 [59.6 – 84.9]) and swab (50.3 [42.3 – 71.5]) method, p=0.28. For anterior nares location, brush (11.0 [5.8 – 39.3]) had lower proportion of squamous epithelial cells compared to swab (49.7 [24.4 – 50.9]) method ($p < 0.001$).

[d] For anterior nares location, no statistically significant differences between brush (192 [81 – 615]) and swab (156 [60 – 588]) method, p=0.58.

[e] For anterior nares location, brush (3.9 [2.7 – 5.3]) with higher RIN compared to swab (1.1 [1 – 1.8]) method, p=0.047.

[f] For anterior nares location, no statistically significant differences between brush (850.0 [175.0 – 2343.8]) and swab (875.0 [212.5 – 2281.3]) method, p=0.87.

[g] Correlation and average relative error calculated between left and right inferior turbinate samples from same subject, and between inferior turbinate and anterior nares samples from same subject.