

A Nose for Diagnosing Asthma

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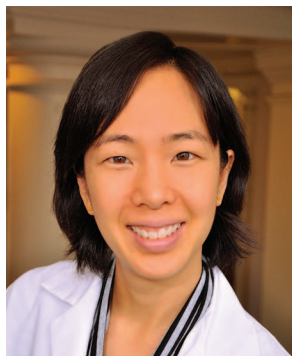
Asthma is a staple diagnosis for primary care providers across the world, with its workup being outside the laboratory and squarely in the clinic. Until now, perhaps: Investigators at the Icahn School of Medicine at Mount Sinai in New York, led by Dr. Supinda Bunyavanich, are exploring the use of an RNA test of nasal brushings to diagnose asthma.

Bunyavanich, Associate Director of the Jaffe Food Allergy Institute and Associate Professor at Mount Sinai, did not have to go far for inspiration. She runs a pediatric allergy and immunology clinic where every week she confronts the realities of treating asthma. “I see patients who struggle with our current tools. Trying to get objective data usually depends on spirometry, which can be difficult. In many primary care settings, you don’t have spirometry on the spot,” she explains.

And spirometry has its challenges. It requires trained staff and demands a precise performance by patients to arrive at adequate measurements. “Most patients just find spirometry difficult to do,” Bunyavanich concludes.

Dr. Raj Kapoor, a pulmonologist and specialist in critical care and sleep medicine, supports this view. “The old adage, ‘Not all that wheezes is asthma,’ attributed to physician Chevalier Jackson, has never been more relevant than today,” he says, citing reports that at least 30% of physicians’ asthma diagnoses are incorrect.

“The overdiagnosis of allergic or nonallergic asthma can lead to weeks, months, or even years of unnecessary albuterol and controller drug treatment,” says Kapoor.



Dr. Supinda Bunyavanich

What Is the Innovation?

Since her fellowship training, Bunyavanich has been thinking about the relationship between the biology of the upper and lower airways.

The field has struggled with a concrete definition of asthma, given the number of overlapping conditions and the reliance on clinical presenta-



Dr. Raj Kapoor

tion, which can be varied. Although conventional instruction has been that asthma is an obstructive disease of the lower airways, there is greater appreciation for the role of the upper airways—notably the nose. Inflammation and irritation of the nose or rhinitis presents in at least half of patients with asthma.

“Establishing a correct diagnosis of asthma cannot rely on the detection of symptoms alone because the symptoms of wheezing, cough, and dyspnea on exertion are all nonspecific,” says Kapoor. “Furthermore, pulmonary function test results are often normal in asthma and, thus, cannot distinguish between asthma, chronic obstructive pulmonary disease, and asthma–chronic obstructive pulmonary disease overlap syndrome when they are abnormal,” he explains.

Enter the nasal biomarker of asthma. A simple way to diagnose asthma via nasal brush would provide an alternative to some of the other innovations—exhaling nitric oxide or the cellular analysis of an induced sputum, for example—that have failed to gain traction. “We wanted something that can be easily accessible,” says Bunyavanich. “It takes a few seconds to do the nasal brush.”

How Does It Work?

A patient sits down and a provider introduces a cytology brush into the patient’s nares to collect material. The RNA is sequenced, and algorithms predict whether the patient has asthma.



Dr. Augusto Litonjua

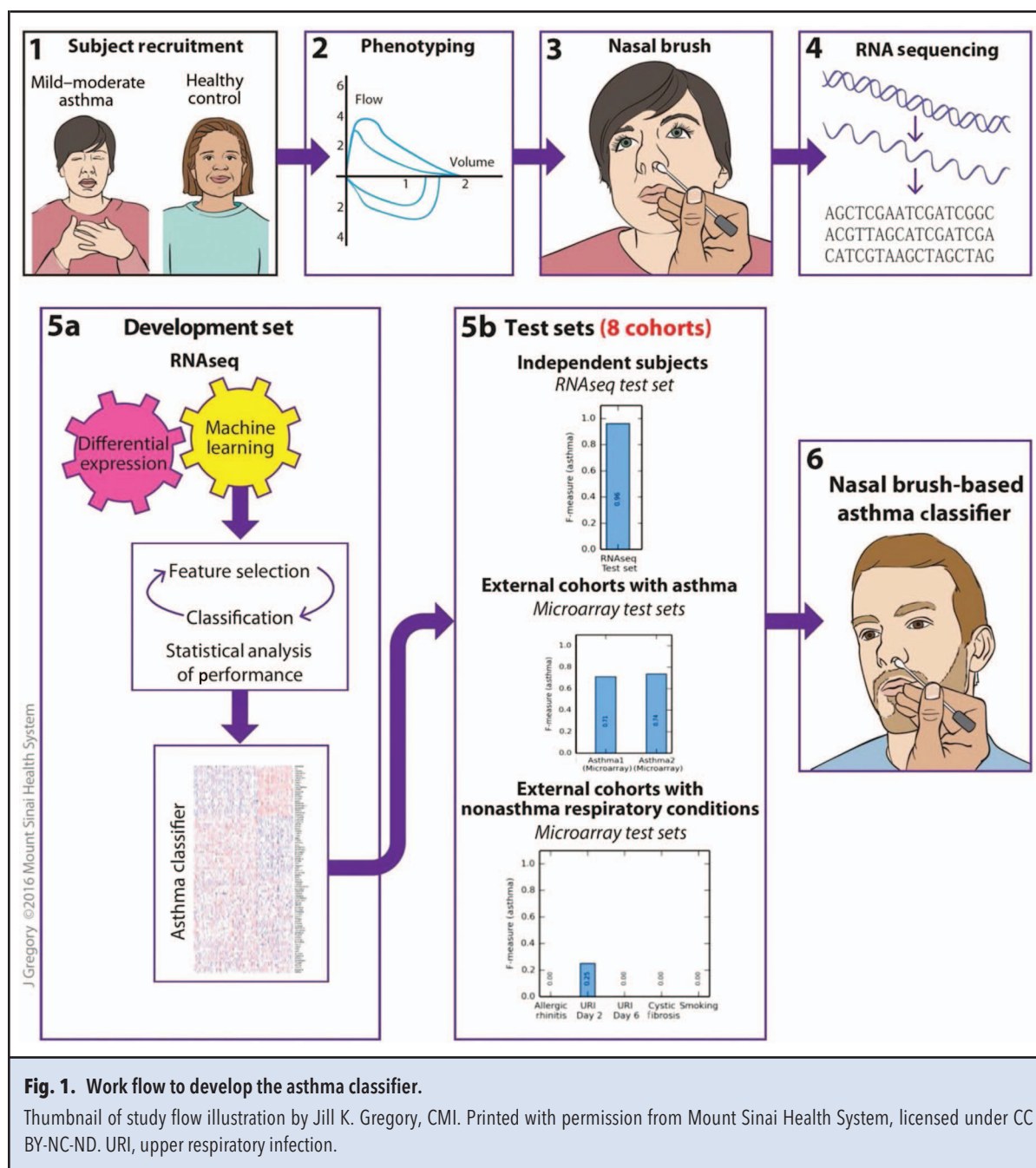
To develop their model, the investigators took samples from 66 individuals with mild to moderate asthma and 124 controls without asthma (Fig. 1). They focused on nonsevere asthma, as the diagnostic challenges lay

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greatest therein. Of these individuals, 150 were randomly assigned to the set used for development of the asthma classifier, and the remaining 40 individuals were assigned to the RNAseq test set. The investigators performed differential gene expression analysis to arrive at 2872 genes that were overexpressed or underexpressed in asthma compared with controls.

A limitation of differential expression analysis is that you are looking at 1 gene at a time. "That doesn't reflect

what happens. Genes interact. If you think about biology, you should think about the collective expression of genes," says Bunyavanich. To see whether a smaller set of genes, through their combined interaction, could better classify asthma, she ran a machine learning pipeline with her team. They first did feature selection to enhance the signal-to-noise ratio. To find these features, they iteratively picked features, in different numbers, to come up with a specific number of genes that most accurately pre-

dicted the asthma status in their training set. They next ran classification algorithms including logistic regression to build classification models with established thresholds and used statistics to pick the best performing model with the fewest number of features. The ultimate classifier was composed of 90 predictive genes and used a probability output >0.76 as its threshold to label an individual as having asthma.

The parsimonious model of 90 genes performed better than using all 2872 differentially expressed genes. “Genes could be contributing in ways not detectable. Traditional methods including differential expression are limited in that they are less able to integrate multiple layers of information,” says Bunyavanich.

To validate their classifier, they ran it across 8 test sets and showed impressive results. In a look at the 40 RNAseq test individuals, the classifier had a positive predictive value of 1.0 and a negative predictive value of 0.96 with an area under the curve of 0.994. It had 0 false-positive results when run on 5 cohorts of allergic rhinitis, upper respiratory infection, cystic fibrosis, and smokers. It did not perform as well on 2 sets of asthma-control microarray samples, with a positive predictive value of 0.93 or 0.61 and negative predictive value of 0.31 or 0.67. The investigators attribute this to “differences in study design and technological factors between RNAseq and microarray profiling” (1).

Where Can It Fit in the Clinic?

Kapoor, who is also Medical Director of Restful Night Sleep Center in Pennsylvania, is enthusiastic. “In a clinic, nasal brushing can be easily performed and does not require machinery for collection. Because it takes seconds for nasal brushing and the interpretation is automated, an instant accurate diagnosis of asthma will prove to be extremely helpful for the primary care providers,” he says.

Asthma expert Dr. Augusto A. Litonjua, Professor of Pediatrics and Medicine at the University of Rochester Medical Center, thinks the use of systems biology to develop an asthma classifier as Bunyavanich has done is “promising.”

“This would be solving a problem not currently addressed in the clinic. Asthma researchers have known for a long time that there are different forms of asthma, that

asthma is not 1 disease but likely a conglomeration of several conditions that have 1 final common phenotype. We have been attempting to classify asthma into different subphenotypes using phenotypic markers or blood biomarkers. This may be an easier way to obtain samples to classify different types of asthma,” he shares. “If validated, this could have wide application in the clinical setting.”

What Additional Validation Needs to Happen?

“Validation in different populations, using different forms of asthma (i.e., allergic vs nonallergic), and in different age-groups (i.e., childhood asthma vs adult asthma). In addition to classification, studies will need to be done using this method to see whether there is a signature that can accurately predict response to treatment,” says Litonjua.

Bunyavanich agrees that they need to do validation in larger cohorts and has been working with her institution to see how to move this technology forward.

“I was in the clinic yesterday,” she says. “Someone describes symptoms, we already have brushes, and could take a brush and swab the nose. It would take a few seconds. The algorithms can be automated to get an answer the same or next day for management and medication.”

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