

# Lung Cancer FRONTIERS

The Forum for Early Diagnosis and Treatment of Lung Cancer



*Steinn Jonsson, MD, FCCP, is Associate Professor of Medicine, University of Iceland Hospitals, in Reykjavik, Iceland. He is an expert on lung cancer genetics and member of the Lung Cancer Frontiers Editorial Board.*

The purpose of **Lung Cancer Frontiers** is to acquire and disseminate new knowledge about lung cancer and how it can be most quickly and effectively diagnosed and treated.

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## Nicotine Receptors and the Risk of Lung Cancer

By Steinn Jonsson, MD, FCCP

It is estimated that 5 million premature deaths occur annually in the world as a result of tobacco smoking<sup>1</sup> and 1 million of those are due to lung cancer (LC). Smoking-related diseases are perhaps the largest public health issue in the western world. The study of diseases such as LC and COPD has been seriously under funded with respect to their importance.

Although the causal relationship between smoking and LC is no longer in doubt, the important contribution of genetic predisposition has been brought to the forefront in recent years<sup>2</sup>. The familial risk of LC in relatives of LC patients has now been established to be on the order of 2.0-3.5, depending on age of diagnosis<sup>3</sup>. The first report of a genetic locus linked to LC used linkage analysis and materials from 52 U.S. families with cancer of the lung or upper airways and found a locus of genome-wide significance on chromosome 6q23-25<sup>4</sup>. Further analysis of these findings, with mapping of the involved genes, is said to be under way.

As is true with most complex diseases, familial LC susceptibility is likely due to multiple genetic factors. The study of this complex relationship has now been greatly facilitated by the development of Genome Wide Association (GWA) analysis, which involves genotyping systems and specialized software that map hundreds of thousands of single nucleotide polymorphisms (SNPs) with a high throughput assay.

Recently, three research groups, working independently of each other, discovered sequence variants on chromosome 15 that are strongly linked to nicotine dependence and LC. This region contains a cluster of nicotinic acetylcholine receptor genes. The results of the three studies were published simultaneously in *Nature*<sup>5,6</sup> and *Nature Genetics*<sup>7</sup>.

Amos and co-workers<sup>7</sup> used material from 1,154 current or former smokers of European ancestry and 1,137 controls from Texas and analyzed 315,450 tagging SNPs with Illumina HumanHap 300 BeadChips. Using a substantial replication cohort from the U.K., they then evaluated the ten SNPs most significantly associated with LC and found that two SNPs, rs1051730 and rs8034191, mapped to a region of strong linkage disequilibrium on 15q25.1. This area contains *PSMA4*

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## Nicotine Receptors and the Risk of Lung Cancer (continued)

and the nicotinic acetylcholine subunit genes *CHRNA3* and *CHRNA5*. The combined analysis for both SNPs yielded an odds ratio of 1.32 ( $P < 1 \times 10^{-17}$ ). They concluded that variation in the region of 15q25.1 containing the nicotinic acetylcholine receptor genes contributes to LC risk.

Hung and co-workers<sup>6</sup>, representing the International Agency for Research on Cancer (IARC) in France and a large number of collaborating institutes, initially used materials from 1,989 LC cases and 2,625 controls from six central European countries. They found a locus on chromosome 15 that was strongly associated with LC ( $P = 9 \times 10^{-10}$ ). The two SNPs most strongly associated with LC were rs1051730 and rs8034191 on chromosome 15q25, the same SNPs reported by Amos and colleagues. These findings were then replicated in 2,513 additional LC cases and 4,752 controls from five other, separate LC studies. In the combined data set, the  $P$  value was  $5 \times 10^{-20}$ . The odds ratio for heterozygous carriers of rs8034191, the most significant marker, was 1.21 (95% CI: 1.11-1.31), and for homozygous carriers it was 1.77 (95% CI: 1.58-2.00). The population attributable risk was calculated to be 14% of all LC cases.

The risk did not appear to be dependent on smoking status or propensity to smoke cigarettes in this study. Furthermore, the study looked at the risk of head and neck cancer and cancer of the esophagus in the same data set and did not find added risk for those diseases. From these findings, the investigators concluded the variants conferred an increased risk of LC that was specific and independent of nicotine dependence. They pointed out that the chromosomal region involved is large and contains a number of potential disease genes, the most notable of which are genes that code for the nicotinic acetylcholine receptor subunits (*CHRNA5*, *CHRNA3* and *CHRNA4*). These genes encode proteins that form receptors for nicotine in neurons, but also other tissues, such as alveolar epithelial cells, pulmonary neuroendocrine cells and some LC cell lines. There is already considerable evidence that nicotine may be involved in neoplastic transformation and induction of angiogenesis through the binding of nitrosamines<sup>8</sup>. The inhibition of such binding would suggest a possible chemopreventive mechanism<sup>9</sup>.

The third group was led by Thorgeirsson and colleagues<sup>5</sup> at deCODE Genetics and collaborating institutes in Reykjavik, Iceland. The Icelandic genetics project has involved the study of genetic susceptibility to a wide variety of complex diseases in

recent years, including smoking-related diseases such as LC, COPD and vascular diseases. In the course of these studies, thousands of smokers have been studied within these various disease categories. It was, however, within the nicotine addiction project that the discoveries of interest for LC were made<sup>5</sup>.

The initial dataset involved material from 10,995 Icelandic smokers from whom a detailed smoking history and DNA samples were available. A GWA scan was performed using Infinium HumanHap300 SNP chips from Illumina and the findings were correlated with smoking quantity (SQ) and other indices of smoking behavior and nicotine dependence. Seven SNPs in the nicotinic acetylcholine receptor cluster on chromosome 15q25 reached genome-wide significance in association with SQ ( $P < 2 \times 10^{-7}$ ), but allele T of the SNP rs1051730 was most strongly associated with SQ ( $P = 5 \times 10^{-16}$ ). The variant affected the amount smoked but did not influence smoking initiation when compared to controls. It was concluded that the variant is strongly associated with nicotine dependence.

Because of the high risk of LC and vascular disease among smokers, the variant was also assessed as a risk factor for LC and peripheral vascular disease. The LC study utilized 1,024 LC cases and 32,244 controls from Iceland, Spain and the Netherlands. Among the Icelandic LC cases, the variant was associated with increased risk for LC with an odds ratio of 1.27 ( $P = 4.1 \times 10^{-5}$ ) when compared to controls. When combined with the cases from Spain and the Netherlands, the odds ratio for LC was 1.31 (95% CI: 1.19-1.44) where, based on the effect of smoking alone, the expected odds ratio for the variant with respect to LC was 1.05. Thus, a substantially increased risk for LC was associated with the variant, amounting to a population attributable risk of 18%. The variant also conferred added risk for peripheral vascular disease with an odds ratio of 1.19.

It is likely that some of the added risk for LC attributable to the variant may be due to more tobacco consumption, since nicotine dependence leads to heavier and more prolonged smoking. But the results of these studies also raise the possibility that the increased risk for LC associated with the variant may be due, in part, to increased susceptibility to the carcinogenic effect of tobacco smoke through mechanisms that involve the nicotinic acetylcholine receptor. In order to reach definitive conclusions on this question, more research is required with extensive information on the intensity and duration of tobacco smoke exposure.

## Nicotine Receptors and the Risk of Lung Cancer (continued)

The concordant findings described in these studies have now established a highly significant association between genetic variants on chromosome 15, nicotine dependence, and LC that accounts for up to 18% of the population attributable risk. This information could be put to use directly in risk assessment and counselling. In conjunction with smoking history, pulmonary function testing and family history, this genetic information may also be used for patient selection into screening trials and smoking cessation intervention. There may also be exciting possibilities in chemoprevention through nicotinic acetylcholine receptor blockade. Much further work remains to investigate the biological consequences of these genetic variants on smoking behavior and carcinogenesis.

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## Interventional Bronchoscopy in Lung Cancer Diagnosis and Staging

By Ali I. Musani, MD, FCCP

### Part 1: Ultrasound-guided transbronchial needle aspiration

Transbronchial needle aspiration (TBNA) is a time-tested and effective technique for sampling lymph nodes in the diagnosis and staging of lung cancer. In experienced hands, TBNA, with or without ultrasound guidance, facilitates the diagnosis and staging of lung cancer in a single outpatient procedure.

Prior to TBNA, a chest CT scan with contrast is reviewed to visualize the relationship between lymph nodes, airways, and vascular structures. In the case of known or potential malignancy, a strategic plan is made to sample the lymph node that would stage the disease at its highest level. For example, in a patient with a 1 cm left upper lobe nodule (T1) and adenopathy at the left hilar, subcarinal, and right paratracheal node stations, the first target would be the right paratracheal lymph node (N3) which, if positive, would make the disease stage IIIB. Sampling only the subcarinal (N2) or left hilar (N1) nodes would result in a lower stage (IIIA or IIA, respectively).

To perform TBNA, the bronchoscopist inserts a fine-gauge needle via the working channel of the bronchoscope, through the airway

Illustration by Boyd Jacobson

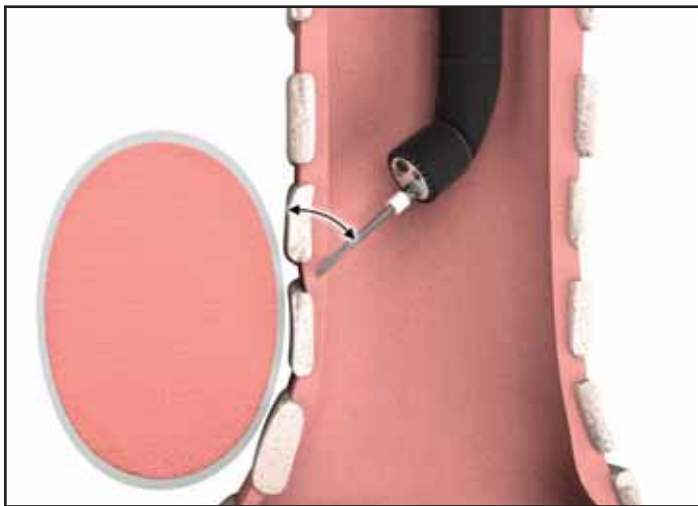


Figure 1: Transbronchial needle aspiration, without ultrasound guidance. The lymph node lying outside of the airway wall is sampled blindly.



Ali I. Musani, MD, FCCP

wall, into a mediastinal or hilar lymph node (*Fig. 1*). Significant bleeding is rare. Several passes are made with the needle to obtain cells and tissue. At National Jewish Health, a pathologist examines the aspirate immediately, in the bronchoscopy suite. If a diagnosis is confirmed, then additional aspirates are not needed, thus reducing the risk of complications and the duration of the procedure.



Figure 2: The EBUS radial probe (UM-BS20-26R) extends from the bronchoscope's working channel. The balloon is filled with saline. Photo courtesy of Olympus Corporation.

## Interventional Bronchoscopy in Lung Cancer Diagnosis and Staging (continued)

### Endobronchial ultrasound-guided TBNA

In the early 1990s, the miniaturization of the ultrasound probe made its use possible in bronchoscopy. Initially, the endobronchial ultrasound (EBUS) probe was a 10 MHz probe that passed through the working channel of a bronchoscope (*Fig. 2*). A balloon sheath over the probe was inflated with saline to allow sonic coupling between the probe and the airway wall. Its 360-degree rotation allowed a circumferential view of vessels and lymph nodes adjacent to the airway. Once the image was recorded, the EBUS probe was pulled out of the bronchoscope to allow for the needle to pass through the working channel. As a result, the bronchoscopist had to develop a mental image of the needle insertion site in reference to surrounding structures, a “handicap” which limited the method’s utility and popularity.

In 2000, the EBUS probe was modified and integrated into the bronchoscope itself (XBF-UC260F-OL8, Olympus Corporation, Tokyo, Japan). The EBUS scope (*Fig. 3*) provides real-time ultrasound images (*Fig. 4A, 4B*) of the structures around the airways while performing routine video bronchoscopy. The convex surface of the transducer is placed in direct contact with the airway wall, or the small balloon covering the transducer is filled with saline. The needle exits the working channel of the EBUS scope at a 45-degree angle, which allows the needle to clear the balloon and penetrate the airway wall at

close to a 90-degree angle (*Fig. 3*). Because EBUS scope set-up and operation are so different from conventional bronchoscopy, dedicated training and practice are required in order to maximize the yield of EBUS-TBNA.

The lymph nodes easily sampled by EBUS-TBNA include the highest mediastinal station (station 1), upper paratracheal (stations 2R and 2L), lower paratracheal (stations 4R and 4L), subcarinal (station 7), hilar (station 10), interlobar (station 11), and lobar (station 12). If station 8 must be sampled, it is possible to approach these lymph nodes using an endoscopic ultrasound probe in the esophagus and transesophageal needle aspiration. Similarly, for stations 5 and 6, anterior mediastinoscopy (Chamberlain procedure) or extended mediastinoscopy could be used.

In expert hands, the sensitivity of EBUS-TBNA has been reported to be > 95% with a specificity of 100% and an accuracy > 90%<sup>1</sup>. Another study<sup>2</sup> compared the accuracy of CT, PET, and EBUS-TBNA for staging lung cancer. EBUS-TBNA showed a very high sensitivity of 92.3%, specificity of 100%, and accuracy of 98%. This was in contrast to the sensitivity, specificity and accuracy shown by CT (76.9%, 55.3%, and 60.8%, respectively) and PET scans (80%, 70.1%, and 72.5%, respectively). Thus, real-time EBUS-TBNA can provide a very high yield with minimal complications in staging and diagnosing lung cancer.

Photo by Barry Silverstein



Figure 3: The EBUS bronchoscope transducer tip, with saline-filled balloon, and needle extending from the working channel.

## Interventional Bronchoscopy in Lung Cancer Diagnosis and Staging (continued)

### Endobronchial ultrasound for peripheral lung lesions

This technique was pioneered by Dr. Noriaki Kurimoto of Tokyo, Japan in the late 1990's. A 10 MHz ultrasound probe is passed through the working channel of a bronchoscope into a peripheral lung lesion to study its structure and composition<sup>3</sup>. Fluoroscopy is often used to localize the lesion. A guide sheath can be placed through the working channel, over the ultrasound probe, which is left in place after the probe is removed. The guide sheath functions as an extended working channel for insertion of biopsy needles and forceps.

Endobronchial ultrasound analysis has been used with high accuracy to distinguish benign from malignant peripheral lung lesions<sup>4</sup>. However, success with this technique is very user-dependent, and these findings have not been replicated by other researchers.

In the next issue of *Lung Cancer Frontiers*, Part 2 of this series on interventional bronchoscopy will explore other advanced imaging techniques for the diagnosis and staging of lung cancer, including electromagnetic navigation bronchoscopy, narrow band imaging, optical tomography, and confocal bronchoscopy.

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*Ali I. Musani, MD, FCCP, is the Director of the Interventional Pulmonology program at National Jewish Health. Prior to National Jewish Health, he was the Associate Director of the Interventional Pulmonology Program at the University of Pennsylvania and Director of the Interventional Pulmonology Program at Presbyterian Medical Center of the University of Pennsylvania Hospital, two of the most active and respected programs in the nation. He is an international expert in the latest technologies for early lung cancer diagnosis and management of malignant endobronchial and pleural disease. His research interests include novel bronchoscopic techniques for airway imaging and for treatment of asthma and COPD. He is a member of the Editorial Boards of the **Journal of Bronchology** and **Lung Cancer Frontiers**.*

Photos by Ali I. Musani, MD



Figure 4A: A small mediastinal lymph node is seen on the EBUS monitor screen.



Figure 4B: The needle penetrates diagonally across the lymph node. The green dot in the upper right corner marks the entry point of the needle.

## Lung Cancer Meetings and Symposia

International Thoracic Oncology Congress  
 International Association for the Study of Lung Cancer  
 October 1-5, 2008      Dresden, Germany  
 Contact: prof.manegold@t-online.de

2008 Chicago Multidisciplinary Symposium in  
 Thoracic Oncology  
 Chicago/IASLC/ASCO/ASTRO  
 November 13-15, 2008      Chicago, IL  
 Contact: evokes@medicine.bsd.uchicago.edu

### Upcoming Continuing Medical Education Events at National Jewish Health

Rheumatologic Lung Disease  
 Symposium: Focus on ILD and  
 Pulmonary Hypertension  
 Featuring: Aryeh Fischer, MD and  
 Kevin Brown, MD  
**September 8, 2008**

The Denver TB Course  
 Featuring: Michael Iseman, MD  
**October 22-25, 2008**

The 31st Annual  
 National Jewish Health  
 Respiratory and Allergy Update  
 Keystone Resort  
 Keystone, CO  
**February 4-7, 2009**

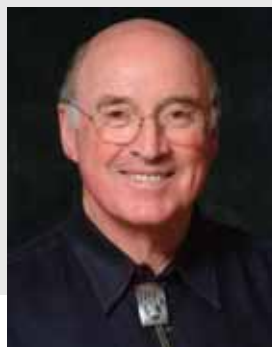
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