Microsatellite DNA Instability in COPD*

Nikolaos M. Siafakas, MD, PhD, FCCP; Eleni G. Tzortzaki, MD; George Sourvinos, MSc; Demosthenes Bouros, MD, FCCP; Nikolaos Tzanakis, MD; Antony Kafatos, MD; and Dimitrios Spandidos, PhD

Study objectives: **Cigarette smoking is the prime cause of COPD; however, only a few smokers develop the disease. In a previous study, we demonstrated that microsatellite DNA instability (MSI) is a detectable phenomenon in sputum cells of COPD patients. Therefore, we hypothesize that this genetic alteration may indicate susceptibility to COPD.**
Design: **In order to investigate this hypothesis, we compared smokers who developed COPD with smokers who did not develop COPD (referred to as non-COPD smokers).**
Setting: **Seven highly polymorphic microsatellite markers were targeted on the DNA of sputum cells and of WBCs.**
Patients and participants: **We studied 60 non-COPD smokers and 59 severe COPD patients with a similar smoking history (mean ± SD) of 48 ± 25 and 54 ± 33 pack-years, respectively ($p = 0.77$). Non-COPD smokers were tested once; COPD smokers were tested twice, with an interval of 24 months between tests.**
Results: **MSI was detected in 14 COPD patients (24%) but in none of the non-COPD smokers. In 10 COPD patients, MSI was exhibited by one microsatellite marker; in the remaining 4 COPD patients, MSI was exhibited by two different alleles. The most commonly affected marker was *THRA1* on chromosome 17 (43%). No significant differences were found between MSI-positive and MSI-negative COPD patients for clinical or laboratory parameters, survival, and development of lung cancer. No change in the microsatellite alleles was found between the tests performed with a 24-month interval.**
Conclusions: **This study demonstrated that MSI was found exclusively in the sputum cells of smokers with COPD. The results support the hypothesis that MSI could be part of the complex genetic basis of COPD, and it could be a marker of the genetic alteration caused by smoking that allows COPD to develop.** ***(CHEST 1999; 116:47–51)***

Key words: chronic bronchitis; COPD; emphysema; genome; microsatellite markers; polymerase chain reaction; recombination; smoking; sputum; tobacco smoke

Abbreviation: MSI = microsatellite DNA instability

COPD is a leading cause of morbidity and mortality among the adult population.[1,2] The major risk factor of COPD is, undoubtedly, cigarette smoking.[3,4] However, < 20% of smokers develop clinically significant COPD. Thus, in addition to exposure to exogenous factors, host factors are important in determining whether smokers develop lung disease. It has not been established whether these "susceptible" smokers who develop COPD are genetically predetermined or whether environmental (type of tobacco), dietary, or other factors affect the development of the disease.[5] In addition, the genetic basis of COPD is poorly understood.[6]

Microsatellites of DNA are very short tandem nucleotide repeats, and they are found scattered throughout the human genome of eukaryotes.[7] The instability of tandem repeat DNA sequences (microsatellite DNA instability [MSI]) has been correlated with a high mutational rate and has been reported in various malignancies.[8–14]

A study from our department[15] has shown that a genetic defect characterized by MSI is a detectable phenomenon in the sputum cells of COPD patients; it was postulated that MSI is either a marker of potential malignancy or a genetic defect requirement

*From the Department of Thoracic Medicine (Drs. Siafakas, Tzortzaki, Bouros, and Tzanakis); the Laboratory of Virology (Mr. Sourvinos and Dr. Spandidos); and the Preventive Medicine and Nutrition Clinic (Dr. Kafatos), Medical School, University of Crete, Heraklion, Greece.
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Correspondence to: Nikolaos M. Siafakas MD, PhD, FCCP, Professor of Thoracic Medicine, Medical School, University of Crete, 711 10 Heraklion, Greece; e-mail: Siafak@cc.uch.gr

for the development of COPD. However, the design of that study did not allow us to come to conclusions, because we did not follow up the patients long enough and we did not compare them with non-COPD smokers.[15]

The present protocol was designed to study MSI in smokers who develop COPD and in non-COPD smokers. We hypothesized that if MSI is a potential marker of malignancy, this marker should be rather evenly distributed among non-COPD smokers and COPD patients, whereas if MSI is a genetic alteration leading to COPD, it would be more common in COPD patients. The results of this study indicate that MSI could be part of the complex genetic basis of COPD. To the best of our knowledge, this is the first report of such a genetic alteration in smokers with COPD.

Table 1—*Clinical and Laboratory Data Taken From Non-COPD and COPD Smokers

Patient Data	Smokers		p Value
	Non-COPD	COPD	
No. of patients	60	59	
Male/female	51/9	54/5	
Age, yr	58.6 ± 15.6	63.1 ± 15.7	NS
Smoking, pack-yr	48 ± 25	54 ± 33	NS
Duration of illness, yr	–	7.5 ± 4.2	
FEV_1, % predicted	84.8 ± 25	33.9 ± 10.4	< 0.001
FVC, % predicted	88.2 ± 9.5	48.5 ± 10.7	< 0.001
FEV_1/FVC, %	82.3 ± 7.5	54.6 ± 10.3	< 0.001
Pa_{O_2}, mm Hg	–	60.5 ± 7.9	
Pa_{CO_2}, mm Hg	–	49.4 ± 10.4	
pH	–	7.37 ± 0.05	
HCO_3, mEq/L	–	28.4 ± 3.4	

*Values are mean ± SD. NS = not significant.

Materials and Methods

Subjects

Two groups of smokers were studied. The first group consisted of 59 stable but severe COPD patients with a mean (± SD) smoking history of 54 ± 33 pack-years. The inclusion criteria for COPD and assessment of severity were in accordance with a recent consensus statement.[1] Briefly stated, in addition to a history of smoking, the patients had a clinical history of cough and sputum, breathlessness on exertion, nonreversible obstruction on spirometry after β_2-agonist administration, and no history of atopy or asthma. The patients had to be stable for at least 4 weeks prior to this investigation. The demographic parameters, smoking history, mean spirometric values, and arterial blood gas measurements are shown in Table 1.

The COPD cohort of patients was matched for gender, age, and smoking intensity to a second group consisting of 60 smokers without clinical, physical, or laboratory evidence of COPD (referred to as non-COPD smokers). The non-COPD smokers had normal physical examinations and chest radiographs, and their spirometric values were within normal limits (Table 1). A detailed investigation of the non-COPD smokers revealed a mean (± SD) smoking history of 48 ± 25 pack-years. The control subjects were derived from a large group of bank employees participating in an annual check-up.

All subjects were followed up for at least 24 months after the initial investigation for survival and for the development of clinically detectable lung cancer. The latter was investigated with annual physical examinations and chest radiographs.

Methods

We investigated the presence of MSI in sputum cells in comparison to that of peripheral WBCs in the same individual[14,15] using an extraction kit (IsoQuick; ORCA Research Incorporated; Bothell, WA) for DNA isolation. Seven microsatellite markers were used to assess MSI, namely *THRA1*, *ANK1*, *HRM*, *D6S344*, *D3S1210*, *D17S250*, and *D13S71*.[16] These microsatellite markers were located on several chromosomal arms (Table 2). We chose to investigate the above MSI markers because they were the MSI-positive markers in our previous study,[15] and they are the markers commonly investigated in malignancies.[8–14,16]

The polymerase chain reaction technique was used to amplify DNA sequences. The MSI was scored by comparing the electrophoretic pattern of the microsatellite markers amplified from the paired DNA preparations (sputum/WBCs), demonstrating a shift of one or both of the alleles in the pathologic DNA specimen or the generation of novel alleles. The shift was indicated by the addition or the deletion of one or more repetitive units. Photographic films of the electrophoretic gels were scanned for further (computer-aided) analysis if needed. Two independent readings were performed by scientists who were unaware of the clinical characteristics of the subjects. The MSI-positive samples were tested twice using fresh DNA, showing a 100% reproducibility. The microsatellite analysis was performed once in the non-COPD smokers; in the COPD patients, the analysis was performed twice, with an interval of 24 months between the tests. The study was approved by the Medical Research Ethics Committee of our hospital.

Statistical Analysis

The differences in the mean values of the quantitative measurements were tested using the Student's *t* test or the Mann-Whitney test, and the χ^2 test was used to compare percentages.

Table 2—*Number of Non-COPD and COPD Smokers With Microsatellite Instability According to Markers and the Corresponding Chromosome

Chromosome	Microsatellite Marker	Microsatellite Instability	
		Non-COPD	COPD
8	*ANK1*	0	1 (7%)
3	*D3S1210*	0	2† (14%)
6	*D6S344*	0	1 (7%)
13	*D13S71*	0	2† (14%)
17	*D17S250*	0	3† (21.4%)
11	*HRM*	0	3 (21.4%)
17	*THRA1*	0	6† (42.8%)

*Values in parenthesis show the number of cases of MSI expressed as the percentage of the total number of MSI cases.

†This marker was found in combination with others in the same case. See text for further details.

Survival was studied using the Kaplan-Meier method and the log-rank test. A p value < 0.05 was considered statistically significant.

Results

Figure 1 shows representative specimens exhibiting differences in mobility of the microsatellite alleles (MSI-positive) in various chromosomes. Fourteen COPD patients (24%) exhibited MSI in their sputum samples. In 10 patients, one microsatellite DNA marker was found to be unstable; in the remaining 4 patients, instability was detected in two different microsatellite markers. The results of MSI in COPD patients are summarized in Table 2. The most commonly affected microsatellite marker was *THRA1* on chromosome 17 (43%; Table 2). In specimens that exhibited MSI in two alleles, the *D17S250* marker was found in three of the four cases.

The microsatellite DNA analysis of sputum specimens from the non-COPD smokers showed that none exhibited MSI in any of the seven microsatellite alleles tested (Table 2). Two subgroups of COPD patients (MSI-positive and MSI-negative) were compared; no significant differences were found in age, duration of illness, smoking habits, modes of treatment, and spirometric and arterial blood gas measurements (Table 3).

The Kaplan-Meier analysis shows no significant difference in 2-year survival between MSI-positive and MSI-negative COPD patients ($p = 0.95$). During the 24-month follow-up period, two patients from the COPD group developed squamous cell lung cancer. One of them had initially exhibited MSI in the *D13S17* and *D17S250* markers, and the other was MSI negative. No cancer was found in the non-COPD smoker group.

In the COPD group, no change in the microsat-

Table 3—*Comparison of Clinical and Laboratory Data Taken From COPD Patients With and Without MSI

Patient Data	COPD		p Value†
	MSI-Positive	MSI-Negative	
No. of patients	14	45	–
Age, yr	65 ± 25	62 ± 9	0.21
Duration of illness, yr	7.7 ± 5.1	7.4 ± 4.0	0.92
Smoking, pack	60 ± 25	54 ± 34	0.14
Inhaled treatment, % of cases			
β_2-agonist	100	95.5	0.42
Anticholinergics	86	88.9	0.75
Corticosteroids	93.3	93.3	0.95
Oral treatment, % of cases			
Corticosteroids	14.3	15.5	0.91
Lung cancer, case/24 mo	1	1	
FEV_1, % predicted	35.4 ± 7.8	33.4 ± 11	0.53
FVC, % predicted	52.8 ± 5.7	47.2 ± 11.6	0.09
FEV_1/FVC, %	52 ± 8.8	55.4 ± 10.6	0.23
Pao_2, mm Hg	60.3 ± 6.9	60.6 ± 8.2	0.89
$Paco_2$, mm Hg	48 ± 12.1	49.8 ± 9.9	0.46
pH	7.38 ± 0.06	7.37 ± 0.04	0.35
HCO_3^-, mEq/L	28.7 ± 3.4	28.6 ± 3.7	0.95

*Values are mean ± SD unless indicated otherwise.
†Values are not significant.

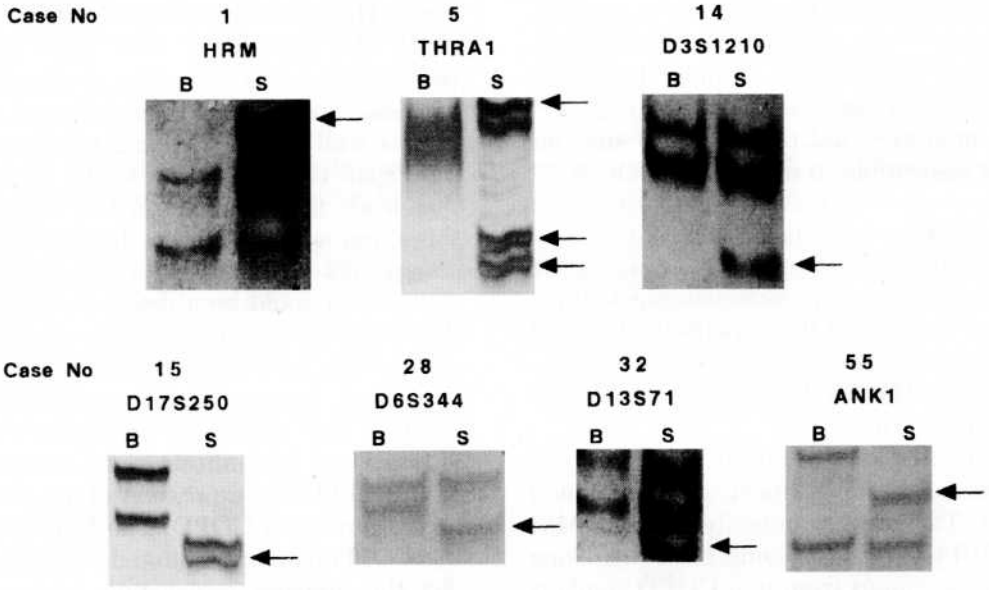

Figure 1. Representative electrophoretic patterns of MSI (arrows) of the seven markers tested. B = blood cells; S = sputum cells.

ellite alleles of DNA was observed in sputum cells during the repeated examination made after 24 months. The patients who exhibited MSI during the initial test were also positive to the same marker during the second study, and the patients who were negative initially remained negative. This was also true for the two patients who developed lung cancer.

These results suggest that MSI is exclusively found in smokers with COPD, at least for the markers tested. In addition, our findings indicate that there is no association between the severity of COPD, survival, and the occurrence of MSI.

Discussion

Despite the significant association of smoking and airway obstruction in COPD, there is a marked individual variation in the response to tobacco smoke, with < 20% of smokers developing symptomatic COPD.[3,4,17] This suggests that additional genetic or environmental factors contribute to the development of COPD.[5,6] Epidemiologic and clinical studies[18–22] demonstrated a hereditary susceptibility to COPD. The finding of Laurell and Eriksson[23] that α_1-antitrypsin was very low in patients with emphysema was the first indication of a genetic risk factor for COPD. Although there is a clear association of homozygosity for the gene controlling α_1-antitrypsin and the development of emphysema, its prevalence in the homozygous state is very low in the general population, and it explains only a very small fraction (< 1%) of the genetic susceptibility to emphysema among cigarette smokers.[5,6] Even less convincing evidence was found in the case of other genetic alterations, such as those on the α_1-antichymotrypsin gene,[24,25] the cystic fibrosis transmembrane regulatory gene,[26] the vitamin D-binding protein,[27] the α_2-macroglobulin regulatory gene,[28] blood group antigens,[29] and the effects of smoking on individuals susceptible to developing COPD.

In a previous study,[15] we have shown that a genetic alteration affecting the sequencing of MSI is detectable in the sputum cells of COPD patients (23%). The aim of the present study was to investigate these findings further and to test the hypothesis that MSI is a marker of susceptibility to COPD or of carcinogenesis. Until now, MSI has been implicated only in the development of cancer.[8–14]

The results of this study indicate that MSI was found in sputum cells only in smokers who exhibited severe COPD. The most commonly affected MSI marker was *THRA1* of chromosome 17 (43%). None of the sputum specimens from non-COPD smokers exhibited MSI. During the 24-month follow-up period, none of the non-COPD smokers had evidence of lung carcinoma. In contrast, two of the COPD patients developed squamous cell lung carcinoma within a period of 24 months. One patient was MSI positive, and the other was MSI negative.

In order to assess whether MSI is an index of the severity of COPD, the two COPD subgroups (MSI-positive and MSI-negative) were compared. The results suggested that MSI is not related to the severity, survival, or treatment of the disease (Table 3).

Although great care was taken to match the smoking intensity of the two groups of subjects (Table 1), the two groups may not have matched in terms of other behavioral (diet), environmental (air pollution), and genetic (blood type) variables. This possible mismatch, however, is unlikely, because all of our subjects lived in Crete, and there was no specific selection procedure. A second criticism could be that the 24-month follow-up period was too short to find significant differences in the occurrence of lung cancer. However, during this period, two patients with COPD developed lung cancer, whereas this was not the case in any of the non-COPD smokers. The results of this study do not exclude the possibility that MSI could be involved in the pathogenesis of both COPD and lung cancer, especially since both diseases were detected in a relatively small sample of the patients tested.

Sputum specimens were retested for MSI in all COPD patients who were alive 24 months after the initial investigation, and no changes were found. The same was true in the two patients who developed squamous carcinoma of the lung. This suggests that this genetic alteration occurs early in the development of the disease, and that it is not an epiphenomenon. However, the present results could not exclude the possibility that MSI could be the consequence of COPD. Thus, further studies including tissue specimens are needed to clarify this issue.

It is well known that cigarette smoke causes a significant oxidative stress to the airway epithelium that leads to inflammation and injury.[30] Although this stress is equivalent to the intensity of smoking, almost 20% of smokers develop COPD. A possible explanation could be a defect in the repair process that leads to an inappropriate remodeling of the airways (the American hypothesis).[31] It is known that the repair process is extremely complex, and it includes cell migration, premitosis differentiation, mitosis, and postmitosis redifferentiation.[32] Thus, MSI could be an expression of an abnormality of a repair gene(s) in COPD. The fact that only 24% of the COPD patients exhibited MSI does not contradict the previous explanation, because only seven microsatellite markers were investigated. An examination of sputum specimens with additional markers

might increase the percentage of COPD patients who exhibit MSI. Further longitudinal studies are needed to support the hypothesis of a link between MSI and airway remodeling (the American hypothesis),[31] because the results could be affected by confounding or population substructure factors.

In conclusion, the results of this study showed that MSI of sputum cells is strongly associated with smoking that causes COPD. Therefore, MSI may be a useful marker of the genetic alteration leading to COPD.

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