

## Association between Cigarette Smoking and *FHIT* Gene Alterations in Lung Cancer<sup>1</sup>

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### Abstract

Epidemiologic data have strongly indicated that cigarette smoking is linked to the development of lung cancer. However, little is known of the molecular targets of carcinogens contained in tobacco smoke. To identify genetic lesions characteristic of tobacco damage, we undertook a molecular analysis of microsatellite alterations within the *FHIT* gene and *FRA3B*, as well as at an independent locus on chromosome 10, *D10S197*, in lung tumors from heavy smokers and in tumors from never smokers. Loss of heterozygosity affecting at least one locus of the *FHIT* gene was observed in 41 of 51 tumors in the smokers group (80%) but in only 9 of 40 tumors in nonsmokers (22%). The comparison between the frequency of losses in *FHIT* in smokers and nonsmokers was statistically significant ( $P = 0.0001$ ), whereas no difference in loss of heterozygosity rate was observed at *D10S197* locus. These findings suggest that *FHIT* is a candidate molecular target of carcinogens contained in tobacco smoke.

### Introduction

Smoking is recognized as a major cause of cancer-related death worldwide (1). Lung cancer, which represents the most common tumor type in men, is directly associated with tobacco smoking (2), and lung tumors in never smokers account for only 5–10% of all lung cancer. Carcinogens in cigarette smoke may leave “fingerprints” in the bronchial tissue in the form of specific mutations that initiate cancer development. The recently cloned *FHIT* gene at 3p14.2 contains the most common fragile site of the human genome, *FRA3B* (3). *FHIT*, a 5′,5′-P<sub>1</sub>P<sub>3</sub>-triphosphate hydrolase (4), is a putative tumor suppressor gene. Given the concordance between the occurrence of LOH<sup>3</sup> affecting microsatellite markers within the *FHIT* gene and abnormal *FHIT* transcripts in tobacco-related cancers such as lung (5) and head and neck tumors (6, 7), loss of one *FHIT* allele is likely to be a crucial step leading to loss of function of the gene. Abnormalities of the *FHIT* gene in carcinogen-related tumors provided the first molecular evidence linking the instability of fragile sites to cancer. Here, we undertook a molecular study of *FHIT* and *FRA3B* microsatellite alterations in lung tumors from heavy smokers and in tumors developed in never smokers to seek genetic damage attributable to tobacco smoking.

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<sup>3</sup> The abbreviations used are: LOH, loss of heterozygosity; B(a)P, benzo(a)pyrene.

### Patients and Methods

**Patient and Sample Collection.** Tumor specimens were obtained from surgically resected lung cancer patients at Istituto Nazionale Tumori (Milan, Italy) and Università di Pisa (Pisa, Italy). The tumors were classified according to the WHO Histological Typing of Lung Tumors (8) and staged according to the TNM classification of malignant tumors defined by the International Union against Cancer.

Among tumors in smokers, 31 were in stage I, 9 were in stage II, and 11 were in stage III, whereas in nonsmokers, 16 tumors were in stage I, 9 were in stage II, and 15 were in stage III. The mean ages of patients at presentation were 62 in smokers and 58 in nonsmokers.

Matched normal lung parenchyma tissue samples were taken at a most distant site of the tumor or in a different segment or lobe as a source for the normal DNA.

**LOH Analysis.** DNAs were extracted from frozen tumor and normal tissues using standard methods (9). Analysis of allelic losses was performed using a PCR-based approach (5). Primers that amplify polymorphic microsatellite markers were used for the following loci: *D3S4103*, *D3S1300*, and *D3S1234*, all internal to the *FHIT* gene, and *D10S197* on the short arm of chromosome 10. The sequences of all nucleotide primers are available through the Genome Data Base. We carried out 22 cycles of amplification at 57–60°C annealing temperature, as appropriate for each primer. Products were separated in 6% urea-polyacrylamide gels, and autoradiography was then performed. For informative cases, allelic loss was scored if the autoradiographic signal of one allele was approximately 50% reduced in the tumor DNA, compared with the corresponding normal allele. The loci displaying microsatellite instability were not scored for allelic loss.

### Results and Discussion

LOH at *D3S1300* and *D3S4103* microsatellite markers, located in the epicenter of the fragile region encompassing exon 5 and intron 5 of the *FHIT* gene (Fig. 1), and at *D3S1234*, in the more distal 3′ end of the gene, was analyzed in tumor tissues. To test the effect of smoking at a genomic region other than 3p14.2, we scored LOH at the *D10S197* locus on the short arm of chromosome 10.

We found LOH affecting at least one locus of the *FHIT* gene in 41 of 51 (80%) tumors in the smokers group (80%), whereas only 9 of 40 nonsmokers (22%) showed *FHIT* allelic losses in tumor DNA (Fig. 2). The comparison between the frequency of losses in smokers and nonsmokers was statistically significant (80 versus 22%;  $P = 0.0001$ ).

The results did not change following adjustment for histological type (73 versus 22% of losses in adenocarcinoma from smokers and nonsmokers, respectively;  $P = 0.0001$ ). All the tumors with loss of one *FHIT* marker had lost all the informative (heterozygous) markers (Fig. 2), suggesting that the tumor cells had lost an entire *FHIT* allele.

No difference in the LOH rate was found at locus *D10S197* between smokers (7 of 33 informative cases; 21%) and nonsmokers (5 of 27 informative cases, 19%). These observations indicate that the

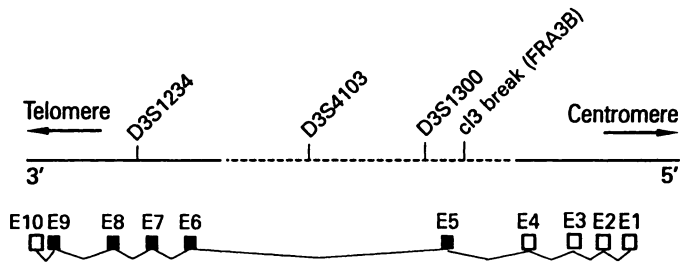


Fig. 1. *FHIT* gene organization showing the position of the internal microsatellite markers and a FRA3B site represented by the hybrid clone 3 (*c13*) break. ■, *FHIT* protein-coding exons; □, untranslated exons.

preferential involvement of the *FHIT* gene in smokers is a specific event and not a result of a more general genotoxic effect of tobacco smoke.

These data indicate that FRA3B is a preferential target of tobacco smoke damage at a molecular level, although we can not exclude involvement of other 3p loci because we have not delineated the extent of the deletion on the short arm of chromosome 3. It is well

known that LOH on 3p in lung cancer generally involves most of 3p, complicating identification of specific target regions. The level of LOH at the *FHIT* gene in lung cancer from heavy smokers, among the highest observed for other markers on 3p and for other tumor suppressor genes, and the extent of allelic losses, involving not only markers in the epicenter of the fragile region (10), but also the more distal *D3S1234* marker (Fig. 1), strongly implicate *FHIT* as a target of carcinogens contained in tobacco smoke.

Notably, an accurate history of smoking exposure in six of eight nonsmoker patients with *FHIT* abnormalities revealed a significant exposure to passive smoke, either at home or at work.

The lower incidence of *FHIT* genetic alterations in lung tumors from never smokers indicates that different somatic or inherited genetic mechanisms could underlie cancer development in these patients. On the other hand, the frequency of mutations in the *p53* gene among lung tumors in smokers was similar to that reported among nonsmokers (11–13). However, a significant relationship between *p53* mutation and cigarette smoke is indicated by the type of mutations detected in smokers' tumors, G:C to T:A transversions, whereas C:T to A:T transitions are more frequent in tumors from nonsmokers (14),

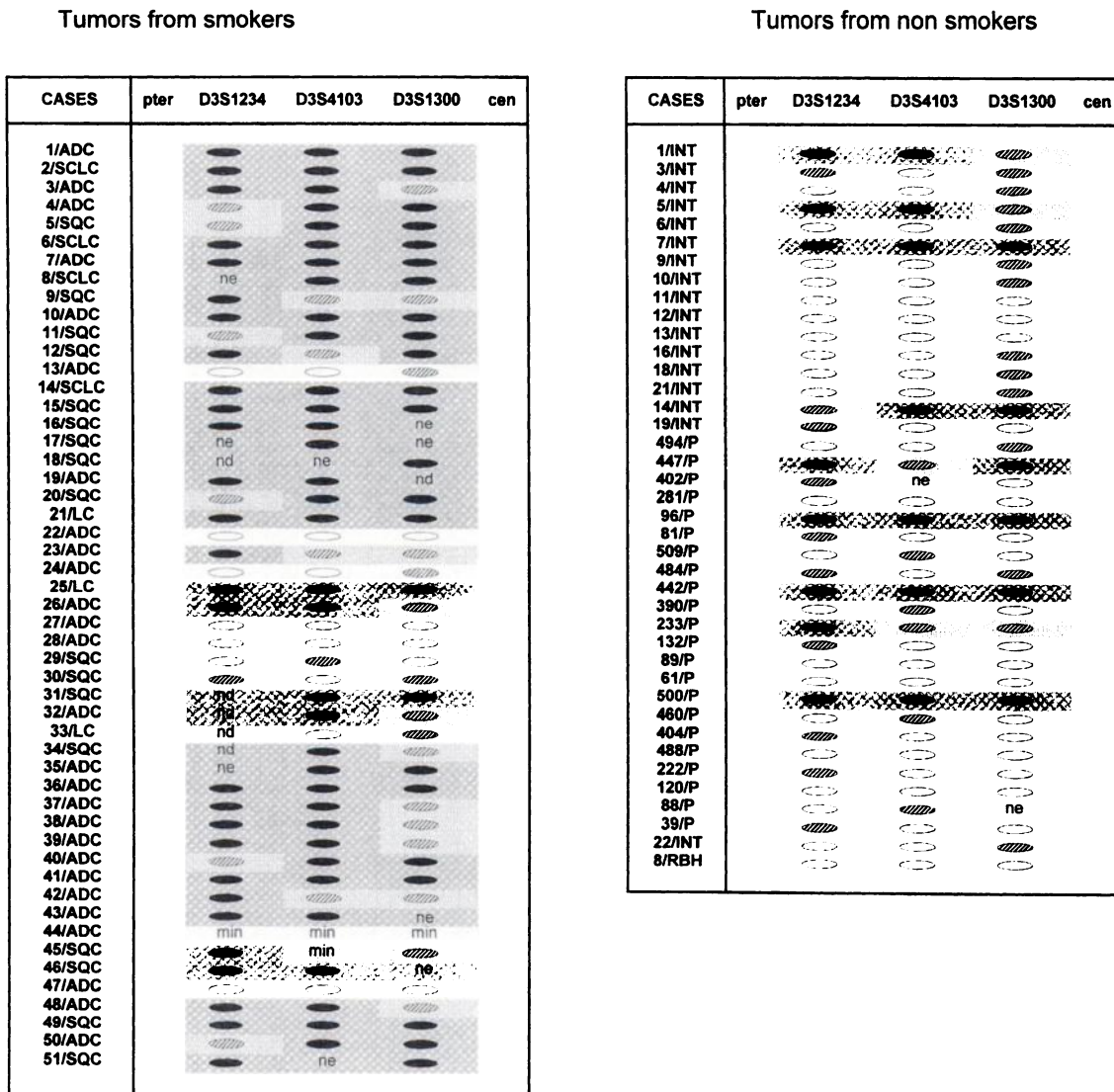


Fig. 2. Results of microsatellite analysis of the 51 lung tumors in smokers and of the 40 lung tumors in nonsmokers with three polymorphic markers internal to the *FHIT* gene. *Min*, microsatellite instability, *ne*, not evaluable, *nd*, not done; ○, heterozygous; ●, LOH; ⊗, not informative.

suggesting that mutational mechanisms involving DNA polymerase infidelity, deamination of 5-methylcytosine, and spontaneous depurination could play a role in lung tumor development in nonsmokers.

Tobacco smoke contains a mixture of highly mutagenic compounds such as polycyclic aromatic hydrocarbons; in particular, B(a)P, a major constituent of tobacco smoke, has been reported to be one of the most potent carcinogenic compounds *in vivo* and *in vitro* (15). Benzo(a)pyrene diol-epoxide, the ultimate carcinogen of B(a)P and the most reactive with DNA, specifically binds guanine-rich sequences of active genes and induces fragile sites (16). *In vitro* evidence for B(a)P-induced formation of DNA adducts at the major mutational hot spots of the *p53* gene in human lung cancer has recently been provided (17). Taken together, these results provide a direct link between specific genetic alterations and exposure to tobacco carcinogens.

In lung cancer, aberrant *FHIT* transcripts, lacking key coding exons, and LOH, affecting microsatellite markers within the *FHIT* gene, have been detected in >70% of all types of lung cancer (5). Moreover, the occurrence of LOH at 3p14.2 (18, 19) and *FHIT* gene alterations in precancerous lesions and nonneoplastic bronchial mucosa<sup>4</sup> argue in favor of *FHIT* deletion as an early molecular event in lung carcinogenesis. The ability to perform routine microsatellite analyses of cytological specimens will allow use of these genetic changes as early molecular indicators of damage related to tobacco smoke in screening high-risk individuals, such as those belonging to the heavy smokers category.

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