Screening of Homologous Recombination Gene Polymorphisms in Lung Cancer Patients Reveals an Association of the *NBS1*-185Gln Variant and *p53* Gene Mutations[1]

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Abstract

Carcinogens present in tobacco smoke lead to several types of DNA damage in bronchial cells. In lung cancer, karyotype, allelotype, and fluorescence *in situ* hybridization analyses have demonstrated the common presence of aneuploidy, although its severity varies considerably among tumors. Deficiencies in the DNA-double strand break (DSB) repair system may be critical in the generation and persistence of chromosomal gains or losses during lung tumorigenesis. Therefore, we examined whether specific DSB repair gene polymorphisms were associated with an increase in tobacco-induced DNA damage, including gene mutations (*p53* and *KRAS*) and chromosomal alterations. Nonsynonymous polymorphisms with a frequency higher that 0.1 at the *XRCC3, NBS1,* and *BRCA2* genes were selected for the study. A PCR-RFLP analysis was performed to identify the Met241Thr, Glu185Gln, and Asn372His polymorphisms in the *XRCC3, NBS1,* and *BRCA2* genes, respectively, in 109 lung cancer patients. Interestingly, the prevalence of *p53* mutations was significantly greater among individual homozygous for the *NBS1-1*85Gln allele (8 of 8, 100%) than among individuals for the wild-type allele (24 of 52, 46%). This increase in *p53* mutation frequency was largely attributable to an increased prevalence of G→T or C→A transversions among these patients ($P < 0.001$). In addition, the association between this type of mutation and the *NBS1-1*85Gln allele remained statistically significant after adjusting for age, smoking, and histological cell-type (odds ratio = 3.42 for heterozygous and odds ratio = 38.3 for *NBS1-1*85Gln homozygous). Germ-line variants in the *NBS1* gene may play a role in the lung carcinogenesis in cigarette smokers.

Introduction

The contribution of DNA repair deficiencies to sporadic cancer is not completely elucidated. Interestingly, individuals with homozygous germ-line mutations in several genes implicated in the repair of DSBs such as the *Ataxia Telangiectasia*, *BRCA1*, *BRCA2*, and *NBS1* genes develop syndromes that share, among other characteristics, a predisposition to several types of cancer and high levels of aneuploidy (Ref. 1 for review). These genetic syndromes reveal a clear cause/effect association between defects in the HR repair system and the development of cancer.

In lung cancer, cigarette smoking has been identified as the main causative agent in smokers (2, 3). The activated form of the tobacco compound benzo(*a*)pyrene, BPDE, can irreversibly damage DNA (4, 5) and selective BPDE adduct formation occur at guanine positions of codons 157, 248, and 273 of the *p53* gene and at codons 12 and 13 of the *KRAS* gene (6, 7), strongly supporting an active role of this chemical agent in lung carcinogenesis. In addition, chromosomal aberrations have been induced in lymphoblastoid cell lines after BPDE treatment, highlighting the role of tobacco carcinogens as the main cause of the widespread chromosomal abnormalities present in lung tumors (5). Stable covalent DNA adducts can induce single-strand breaks and one-strand DNA damage can generate DSBs in cells that are replicating their DNA, thus, contributing to several forms of chromosomal damage (5). In agreement with this hypothesis, several molecular alterations such as chromosomal losses or gene promoter hypermethylation have been observed in apparent normal bronchial epithelium and in sputum from smokers or ex-smokers compared with never-smokers (8–11). Moreover, we previously observed that lung tumors from smokers have a significantly increased number of chromosomal alterations compared with lung tumors from nonsmokers (12), additionally supporting that tobacco carcinogens are among the causes for chromosomal abnormalities arising in lung tumors.

To define whether some gene variants at the HR repair pathway contribute to lung carcinogenesis and genetic damage

Received 12/30/02; revised 3/21/03; accepted 5/8/03.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
[1] This research work was supported by an institutional star-up fund grant (to M. S-C.) and, in part, by Spanish Ministerio de Ciencia y Tecnología Grant SAF2002-01595. P. P. M. is recipient of a fellowship from the Comunidad Autonoma of Madrid, and M. S-C. is recipient of a Ramon y Cajal Contract from the Spanish Ministry of Science and Technology.
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[3] The abbreviations used are: DSB, double strand break; NBS, Nijmegen breakage syndrome; HR, homologous recombination; BPDE, benzo(*a*) pyrene diol epoxide; NER, nucleotide excision repair; NSCLC, non-small cell lung cancer; FAL, fractional allelic losses; SCC; squamous cell carcinoma; AC, adenocarcinoma; bAC, bronchoalveolar adenocarcinoma; HWE, Hardy-Weinberg Equilibrium.

in lung tumors, we studied the *XRCC3*-Thr241Met, *NBS1*-Glu185Gln and *BRCA2*Asn372His gene polymorphisms and their correlation with clinical, pathological, and genetic characteristics of NSCLC.

Patients and Methods

Patient Samples, DNA Extraction, and Genetic Characterization of NSCLC. Primary tumors and blood were obtained from 109 patients with NSCLC at The Johns Hopkins University School of Medicine. Informed consent was obtained from each patient. The characteristics of the patients included in the study for which we had data available were as follows: median age, 65 years (range, 31–84 years); gender, 38 males and 58 females; race, 29 blacks and 68 whites; smoking history, 95 smokers and 13 nonsmokers; tumor histology, 36 SCCs, 49 ACs, and 18 bACs; and stage, 39 stage I, 38 stage II, 18 stage III, and 5 stage IV. Smokers and nonsmokers were as defined previously (12, 13). DNA was extracted from tumor and normal lymphocytes as described previously (14, 15). *KRAS* and *p53* mutations and FALs for these tumors have been determined and reported previously (12–15). All *p53* mutations were of the missense, nonsense, or frameshift type, except for one nucleotide change in the intron/exon boundary that will presumably disrupt splicing (13–15).

Gene Polymorphism Analysis. We searched databases and previously published data (16–18) and selected gene polymorphisms at *XRCC3*, *NBS1*, and *BRCA2* genes (*XRCC3*-Thr241Met, *NBS1*-Glu185Gln and *BRCA2*-Asn372His).[4,5] These genes were chosen among those that participate in DSBs repair and all of the variants leaded to amino acid changes and had a high prevalence (allele frequency higher than 0.1). In addition, *NBS1* and *BRCA2* are implicated in genetic syndromes leading to high predisposition to cancer. These polymorphisms were identified in lymphocyte DNA using PCR/RFLP assays. PCR reactions were performed in a total reaction volume of 25 μl containing: 30 ng of DNA; 6 ng/μl of each primer; 200 μM of each deoxynucleotide triphosphate; 0.75 units of DNA polymerase (Biotools); and 2 mM $MgCl_2$. The *XRCC3*-Thr241Met gene polymorphism was determined using the following primers: forward, 5′-GTGTGTGAATAAGAAGGTCC-3′ and reverse, 5′-GTGTGTGAATAAGAAGGTCC-3′. The PCR cycling conditions consisted of initial denaturation at 94°C for 2 min followed by 35 cycles of denaturation (30s at 94°C), annealing (30s at 58°C), and elongation (30s at 72°C). After the last cycle, a final extension (5 min at 72°C) was added. The product of 253 bp was digested with *Hsp92* II (Promega, Madison, WI) and resolved on 2% agarose gels. The three possible genotypes are identified by distinct banding patterns: (*a*) Thr/Thr (210 and 43 bp); (*b*) Thr/Met (210, 105, 105, and 43 bp); and (*c*) Met/Met (105, 105, and 43 bp).

The *NBS1*-Glu185Gln gene polymorphism was PCR-amplified using the following primers: forward, 5′-GGATGTAAACAGCCTCTTTG-3′ and reverse, 5′-CACAGCAACTATTACATCCT-3′. The PCR cycling conditions were identical to the *XRCC3* gene. The product of 290-bp was digested with *Hin*fI restriction enzyme (Promega) and resolved on 2% agarose gels. The three possible genotypes are identified by distinct banding patterns: (*a*) Glu/Glu (170 and 120 bp); (*b*) Glu/Gln (290, 170, and 120 bp); and (*c*) Gln/Gln (290 bp). Finally, the *BRCA2*-Asn372His gene polymorphism was determined using the following primers: forward, 5′-CTACAAAAAGTAAGAACTAGCAAGACTAG-3′ and reverse, 5′-GTCACTTCCACTCTCAAAGGGCTTCTCAT-3′ (the underlined base introduces a restriction site for the *Hsp92* II enzyme). The PCR cycling conditions consisted of initial denaturation at 94°C for 2 min followed by 10 cycles of denaturation (30s at 94°C), annealing (30s at 50°C), and elongation (30s at 72°C) and 25 cycles of denaturation (30 s at 94°C), annealing (30 s at 45°C), and elongation (30 s at 72°C). After the last cycle, a final extension (5min at 72°C) was added. The product of 190 bp was digested with *Hsp92* II (Promega) and resolved on 3% agarose gels. The three possible genotypes are identified by distinct banding patterns: (*a*) Asn/Asn (147 and 43 bp); (*b*) Asn/His (147, 119, 43, and 28 bp); and (*c*) His/His (119, 43, and 28 bp).

As quality control, the three assays were repeated on 30% of the samples, and the replicates were 100% concordant. PCR products from the *BRCA2* and *XRCC3* genes have an additional restriction site that was used as internal control for complete restriction reaction. As control for the complete restriction digestion of the *NBS1* gene, we used a sample with a known homozygous 185Glu genotype.

Statistical Analysis. Hardy-Weinberg equilibrium in the prevalence of *XRCC3*, *NBS1*, and *BRCA2* gene polymorphisms among lung cancer patients was tested using the likelihood ratio test. Association between genotype frequencies of these polymorphisms with demographic and tumor characteristics was assessed by χ^2 test and Fisher's exact test. For quantitative variables, the nonparametric Wilcoxon test was used. The relationship between the *NBS1* genotype and G→T or C→A mutations at *p53* was additionally explored using logistic regression taking into account age, histological type, and smoking habit. Finally, differences in survival according the studied genotypes were analyzed using Kaplan-Meier and the log-rank test.

Results

Prevalence of *XRCC3*, *NBS1*, and *BRCA2* Gene Variants. One hundred nine NSCLC patients were genotyped for these variants in the *XRCC3*, *NBS1*, and *BRCA2* genes. Frequencies for each gene polymorphism and patient clinical characteristics are presented in Table 1. None of the genotype distributions differed significantly from the HWE, although *P*s for *XRCC3* and *BRCA2* were rather low because of the distinct frequencies among blacks and whites. No statistically significant differences in the distribution of the polymorphisms according to smoking history or lifetime cigarette consumption were observed (data not shown). Representative examples of PCR and restriction analysis are depicted in Fig. 1.

Gene Variants at the *XRCC3*, *NBS1*, and *BRCA2* Genes and Their Association with the Pathological and Genetic Characteristics of Lung Tumors. We also examined the distribution of gene polymorphims among different tumor characteristics such as tumor histology (SCC, AC, and bAC) and tumor genetic background (*KRAS* and *p53* gene mutations and FAL). Patients with the *NBS1*-185Gln gene variant had an increased frequency of lung tumors carrying *p53* gene mutations (Table 2). The prevalence of *p53* mutations was significantly higher among individuals homozygous for the *NBS1*-185Gln allele (8 of 8, 100%) than among individuals homozygous for the wild-type allele (24 of 52, 46%, $P = 0.018$). In addition, G→T or C→A transversions were clustered in individuals with the Gln/Gln genotype ($P < 0.001$), whereas the frequency of *p53* mutations other than G→T or C→A was not related with this

[4] Internet address: http://www.genome.utah.edu.
[5] Internet address: http://www.ncbi.nih.gov.

Table 1 Distribution of gene polymorphisms according to the different patient characteristics

Polymorphism	All subjects $n = 109$	HWE	P	Gender[b]		P	Ethnicity		P	Age[b]			P	Smoking[b]		P
				F[a]	M		B	W		<60	60–69	70		Sk	Nsk	
XRCC3-(Thr241Met)																
Genotype																
Thr/Thr	50 (46%)	43%		33 (57%)	12 (32%)		20 (69%)	25 (37%)		16 (55%)	16 (57%)	13 (32%)		41 (43%)	9 (69%)	
Thr/Met	43 (39%)	45%	0.19[c]	18 (31%)	19 (50%)	0.05	7 (24%)	30 (44%)	0.013	11 (38%)	8 (29%)	18 (45%)	0.15	40 (42%)	3 (23%)	0.2
Met/Met	16 (15%)	12%		7 (12%)	7 (18%)		2 (69%)	13 (19%)		2 (7%)	4 (14%)	9 (23%)		14 (15%)	1 (8%)	
Thr/Met and Met/Met	59 (54%)			25 (43%)	26 (68%)	0.021	9 (31%)	43 (63%)	0.004	13 (45%)	12 (43%)	27 (68%)	0.07	54 (57%)	4 (31%)	0.14
NBS1-(Glu185Gln)																
Genotype																
Glu/Glu	58 (53%)	52%		31 (53%)	21 (55%)		19 (69%)	34 (50%)		16 (55%)	15 (54%)	22 (55%)		49 (52%)	9 (69%)	
Glu/Gln	41 (38%)	40%	0.49[c]	23 (40%)	12 (32%)	0.5	9 (24%)	26 (38%)	0.26	11 (38%)	12 (43%)	12 (30%)	0.5	37 (39%)	3 (23%)	0.48
Gln/Gln	10 (9%)	8%		4 (7%)	5 (13%)		1 (69%)	8 (12%)		2 (7%)	1 (4%)	6 (15%)		.9 (9%)	1 (8%)	
Glu/Gln and Gln/Gln	51 (47%)			27 (47%)	17 (45%)	1.00	10 (31%)	34 (50%)	0.19	13 (45%)	13 (46%)	18 (45%)	0.99	46 (48%)	4 (31%)	0.25
BRCA2-(Asn372His)																
Genotype																
Asn/Asn	59 (54%)	51%		31 (53%)	21 (55%)		22 (76%)	20 (37%)		16 (55%)	15 (54%)	21 (52%)		53 (56%)	5 (38%)	
Asn/His	38 (35%)	40%	0.14[c]	21 (36%)	13 (34%)	0.98	7 (24%)	27 (45%)	0.007	10 (35%)	8 (29%)	16 (40%)	0.69	33 (35%)	5 (38%)	0.27
His/His	12 (11%)	8%		6 (10%)	4 (11%)		0 (0%)	11 (18%)		3 (10%)	5 (18%)	3 (8%)		.9 (9%)	3 (23%)	
Asn/His and His/His	50 (46%)			27 (47%)	17 (45%)	1.00	7 (24%)	38 (63%)	0.007	13 (45%)	13 (46%)	19 (48%)	0.98	42 (44%)	8 (62%)	0.37

[a] F, females; M, males; B, blacks; W, whites; Sk, smokers; Nsk, nonsmokers.
[b] Calculated for those patients with available information.
[c] HWE among ethnicity: *XRCC3* was $P = 0.28$ and $P = 0.46$ in B and W, respectively; *NBS1* was $P = 0.96$ and $P = 0.39$ in B and W, respectively; *BRCA2* was $P = 0.46$ and $P = 0.26$ in B and W, respectively.

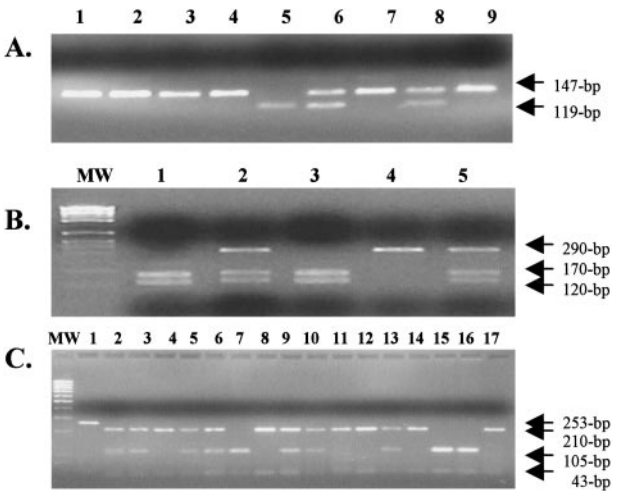

Fig. 1. PCR-RFLP of the different gene polymorphisms. *A*, *BRCA2*-Asn372His. *Lanes 1–4*, *7*, and *9* represent Asn/Asn, *Lane 5* represents His/His, and *Lanes 6* and *8* represent the heterozygous Asn/His; *B*, *NBS1*-Glu185Gln. *Lanes 1* and *3* represent the homozygous Glu/Glu, *Lane 4* represents homozygous Gln/Gln, and *Lanes 2* and *5* the heterozygous Glu/Gln and (*C*) *XRCC3*-Thr241Met. *Lane 1* is an uncut control. *Lanes 4*, *8*, *11*, *12*, *14*, and *17* represent homozygous Thr/Thr, *Lanes 2*, *3*, *5*, *6*, *9*, *10*, and *13* are heterozygous Thr/Met and *Lanes 7*, *15*, and *16* are homozygous Met/Met. MW, molecular weight marker.

polymorphism ($P = 0.2$; Table 2). The association between the Gln variant at *NBS1* with G→T or C→A transversions at *p53* gene was additionally examined using logistic regression (Table 3). The risk of G→T or C→A changes at *p53* was significantly higher among heterozygous and homozygous carriers of the 185Gln allele, after adjusting for age, smoking history, and histological cell type. These differences were not attributable to the unequal distribution of the *NBS1* genotypes among tumors with and without *p53* data available ($P = 0.40$; Fisher's exact test). No correlation was found between *KRAS* mutations considering only lung AC and bAC and the *XRCC3*, *NBS1*, and *BRCA2* gene polymorphisms.

The presence of DSB repair gene variants was also compared with the degree of chromosomal abnormalities in the tumors. The percentage of chromosomal alterations was calculated as the frequency of chromosomal gains/losses per chromosomal arms (FALs/gains, FAL; Ref. 12). Data on the FAL status was available in only 26 tumors from smokers. Tumors arising in individuals with the Gln/Glu and Gln/Gln genotypes for the *NBS1* gene had higher levels of FAL (mean, 34.9) compared with tumors from Glu/Glu individuals (mean, 24.3), although the differences were not statistically significant ($P = 0.182$). Similarly, tumors from individuals with Thr/Met and Met/Met genotypes for the *XRCC3* gene had higher levels of FAL (mean, 34.8) than those with the Thr/Thr genotypes (mean, 21.2), but differences did not reach statistical significance ($P = 0.119$). This preliminary observation was not because of differences in the distribution of the polymorphisms because it was comparable among patients with and without FAL data available: (*XRCC3*, $P = 0.60$; *NBS1*, $P = 1.00$, and *BRCA2*, $P = 0.84$; Fisher's exact test).

Discussion

Although the implication of DNA repair genes in cancer predisposition syndromes has been well established, their relevance in sporadic tumors is still under intense investigation.

The XRCC3 protein participates in the repair of DSBs throughout the HR system, contributing to the maintenance of genomic stability. However, the role of *XRCC3* gene in cancer development is still uncertain, and no cancer predisposition syndrome has been linked to germ-line mutations in the *XRCC3* gene. The *XRCC3*-241Met gene variant has been associated with predisposition to melanoma, bladder cancer, and in head

Table 2 Distribution of gene polymorphisms according to the histology and the genetic background of the lung tumors

Polymorphism	Tumor histology[a]			*P*	*KRAS*[b]		*P*	*p53*[c]		*P*	*p53*		*P*
	AC	bAC	SCC		Mutant	WT		Mutant	WT		G→T/C→A	non-G→T/C→A	
Genotype													
XRCC3-(Thr241Met)													
Thr/Thr	24 (49%)	8 (44%)	15 (42%)		13 (59%)	18 (41%)		23 (44%)	23 (49%)		10 (50%)	13 (41%)	
Thr/Met	18 (37%)	8 (44%)	14 (39%)	0.90	6 (27%)	20 (45%)	0.32	23 (44%)	14 (30%)	0.24	7 (35%)	16 (50%)	0.54
Met/Met	7 (14%)	2 (11%)	7 (19%)		3 (14%)	6 (14%)		6 (12%)	10 (21%)		3 (15%)	3 (9%)	
Thr/Met and Met/Met	25 (51%)	10 (56%)	21 (58%)	0.79	9 (41%)	26 (59%)	0.20	29 (56%)	24 (51%)	0.84	10 (50%)	19 (59%)	0.57
Genotype													
NBS1-(Glu185Gln)													
Glu/Glu	24 (49%)	13 (72%)	19 (53%)		13 (59%)	23 (51%)		24 (46%)	28 (61%)		5 (24%)	19 (61%)	
Glu/Gln	20 (41%)	5 (28%)	13 (36%)	0.43	8 (36%)	17 (38%)	0.76	20 (38%)	18 (39%)	0.018	10 (48%)	10 (32%)	0.014 (<0.001[d] and 0.20[e])
Gln/Gln	5 (10%)	0 (0%)	4 (11%)		1 (5%)	5 (11%)		8 (15%)	0 (0%)		6 (29%)	2 (6%)	
Glu/Gln and Gln/Gln	25 (51%)	5 (28%)	17 (47%)	0.23	9 (41%)	22 (49%)	0.79	28 (54%)	18 (39%)	0.16	16 (76%)	12 (39%)	0.011 (0.008[d] and 1.00[e])
Genotype													
BRCA2-(Asn372His)													
Asn/Asn	26 (53%)	10 (56%)	21 (58%)		10 (45%)	25 (57%)		28 (54%)	25 (54%)		12 (60%)	16 (47%)	
Asn/His	19 (39%)	5 (28%)	11 (31%)	0.80	10 (45%)	14 (32%)	0.55	20 (8%)	14 (30%)	0.43	8 (40%)	12 (40%)	0.25
His/His	4 (8%)	3 (17%)	4 (11%)		2 (9%)	5 (11%)		4 (46%)	7 (15%)		0 (0%)	4 (13%)	
Asn/His and His/His	23 (47%)	8 (44%)	15 (42%)	0.89	12 (55%)	19 (43%)	0.44	24 (46%)	21 (46%)	1.00	8 (40%)	16 (53%)	0.57

[a] Calculated for those patients with available information.
[b] Calculated only in the ACs and bACs histological types for which we had *KRAS* data available.
[c] Calculated for individuals with *p53* mutational status available.
[d] *P* corresponding to the comparisons between *p53*-wild type tumors *versus* tumors with mutations G→T/C→A.
[e] *P* corresponding to the comparisons between *p53*-wild type tumors *versus* non-G→T/C→A mutations.

Table 3 Distribution of *NBS1* gene polymorphisms according to the type of *p53* mutations

Variable	*p53* status			Univariate analysis G→T/C→A *versus* non			Multivariate Analysis[b,c] G→T/C→A *versus* non		
	No.	G→T/C→A	Non[a]	OR	95% CI	*P*	OR	95% CI	*P*
NBS1-(Glu185Gln)									
Glu/Glu	52	5 (10%)	47 (90%)	1.00			1.00		
Glu/Gln	38	10 (26%)	28 (74%)	3.36	1.04–10.8	0.043	3.42	0.95–12.3	0.060
Gln/Gln	8	6 (75%)	2 (25%)	28.2	4.45–178.8	<0.001	38.3	4.76–307.6	0.001

[a] Comparing G→T/C→A mutations *versus* the rest of tumors (with or without *p53* mutations).
[b] OR adjusted by age, smoking, and histological type.
[c] When the same analysis was done, excluding the 13 nonsmokers, the results did not vary significantly (data not shown).

and neck SCCs from previous smokers but not to lung cancer (19–22). The *XRCC3*-241Met gene polymorphism has been associated with higher DNA adduct levels in WBCs from peripheral blood of healthy individuals (23), suggesting that the XRCC3 protein may participate in the repair of bulky DNA adducts and that the *XRCC3*-241Met gene variant could generate a protein with less efficient DNA repair ability. However, recent *in vitro* studies have demonstrated no difference in the homology-directed repair of DSBs between the variant XRCC3-241Met protein and the wild-type 241Thr protein (24). In our study, the *XRCC3* gene polymorphism did not cluster with any of the tumor parameters studied, suggesting that such gene variant does not significantly contribute to the genetic or pathological characteristics of NSCLC.

On the other hand, germ-line mutations at *BRCA2* and *NBS1* genes clearly predispose to cancer (1). Most publications on the *BRCA2*-Asn372His and the *NBS1*-Glu185Gln gene variants have focused on breast cancer risk assessment and not lung cancer (17, 25). Homozygous germ-line mutations at the *NBS1* gene lead to the NBS, a rare disease characterized by microcephaly, growth and mental retardation, radiosensitivity, immunodeficiency, high incidence of malignancies at an early age, and elevated rates of chromosomal abnormalities (26, 27).

Interestingly, our observations show that virtually all individuals homozygous for the Gln variant have tumors with *p53* mutations in contrast with only 46% of *p53* mutations in tumors from individuals homozygous for the Glu variant. Even more intriguing was the strong association between the *NBS1*-185Gln gene polymorphism and G→T or C→A transversions at *p53* but not with other types of *p53* mutations. However, these observations are still preliminary and additional studies, including larger number of patients are needed to confirm our findings. Compared with other tumor types, point mutations at *KRAS* and *p53* genes in lung tumors have an increased rate of G→T or C→A transversions. In tumors others than lung, the total frequency of G→T or C→A mutations at the *p53* gene is 15% compared with 33% in lung tumors (7, 28). This specific mutational spectra of *p53* mutations in lung cancer is likely to occur as a consequence of bulky adducts originated by the exposure to tobacco carcinogens such as BDPE and others being repaired through the NER pathway (1). Although most of the lung tumors included in our study were from smokers, it is unlikely that our observations apply also to tumors from nonsmokers because they harbor a significant lower proportion of *p53* mutations and G→T or C→A transversions (28).

The NBS1 protein is a member of the hMre11/hRad50 complex that actively participates in DSB repair but not in the NER pathway (27). However, it has been shown that the slow growth phenotype of *rad50* mutants can be suppressed by mutations in *RAD1*, which encodes a nuclease that mediates in the recognition and incision of DNA damage during NER (30). Such results may link the NER system with the Mre11/Rad50 complex and could help explain our observations. Moreover, recent studies have uncovered that the hMre11/hRad50 complex is also implicated in the checkpoint signaling and in DNA replication, additionally suggesting that the hMre11/hRad50 complex has a broader biological function outside the DSBs repair system (29, 30). To our knowledge, there is no information regarding changes in the activity of the NBS1-185Gln isoform. The region between amino acid 108–196 of the NBS1 protein constitutes a BRCA1 COOH-terminus domain that is presumably involved in cell-cycle checkpoints or in DNA repair. Most of the NBS patients (80%) harbor a 5-bp deletion, a mutation of Slavic origin, at nucleotide 657 (31). Other six types of mutations have been found in these families, restricted to the central region of the protein (between codons 233 and 385). All mutations lead to protein truncations. Thus, the mutational pattern of *NBS1* gene in NBS patients does not gives us clues about the functional relevance of the *NBS1*-185Gln gene polymorphism.

Previous studies have reported that *p53* mutations accumulate in lung tumors with high levels of chromosomal abnormalities (12, 32), suggesting that *p53* mutations may prevent the efficient repair of the chromosomal alterations caused by tobacco carcinogens. We failed to detect a significant correlation between the presence of the gene polymorphisms analyzed and higher levels of FAL, although there was a trend toward a high levels of FAL in tumors from individuals carrying either the *NBS1*-185Gln or *XRCC3*-241Met variant alleles. However, the number of samples for which we had FAL status available was too low to reach definitive conclusions.

Overall, our observations suggest that the NBS1-185Gln protein variant could affect repair of DNA adducts and would, thereby, allow the accumulation of G→T or C→A transversions in *p53*. Examples of the association between deficiencies in DNA repair genes and specific mutational profiles in important cancer genes have already been described. In colorectal and lung tumors, gene promoter hypermethylation at the DNA repair protein O^6-methylguanine-DNA methyltransferase, in charge of removing mutagenic adducts from the O6 position of guanine, correlates with G→A transitions at *KRAS* and *p53*, demonstrating how DNA repair deficiencies affect the status of important cancer genes (33, 34).

In conclusion, our results show that *NBS1*-185Gln gene polymorphism associates with *p53* mutations in NSCLC, suggesting that it may contribute to human lung carcinogenesis in smokers.

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Cancer Epidemiol Biomarkers Prev 2003;12:699-704.