Carcinogenesis vol.27 no.12 pp.2448–2454, 2006
doi:10.1093/carcin/bgl095
Advance Access publication June 14, 2006

Association of polymorphisms in the *MTH1* gene with small cell lung carcinoma risk

Takashi Kohno[1,2,†], Tokuki Sakiyama[1,†], Hideo Kunitoh[3], Koichi Goto[4], Yutaka Nishiwaki[4], Daizo Saito[5], Hiroshi Hirose[6,7], Takashi Eguchi[7], Noriko Yanagitani[8], Ryusei Saito[8], Rumie Sasaki-Matsumura[2], Sachiyo Mimaki[1], Kaoru Toyama[2], Seiichiro Yamamoto[9], Aya Kuchiba[9,10], Tomotaka Sobue[9], Tsutomu Ohta[1], Misao Ohki[1] and Jun Yokota[1,2,*]

[1]Center for Medical Genomics and [2]Biology Division, National Cancer Center Research Institute, Tokyo, [3]Division of Thoracic Oncology and [5]Department of Endoscopy and Gastrointestinal Oncology, National Cancer Center Hospital, Tokyo, [4]Division of Thoracic Oncology, National Cancer Center Hospital East, Chiba, [6]Health Center and [7]Department of Internal Medicine, Keio University School of Medicine, Tokyo, [8]First Department of Internal Medicine, Gunma University School of Medicine, Gunma, [9]Statistics and Cancer Control Division, Research Center for Cancer Prevention and Screening, National Cancer Center, Tokyo and [10]Department of Biostatistics/Epidemiology and Preventive Health Sciences, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan

*To whom correspondence should be addressed. Tel: +81 3 3547 5272; Fax: +81 3 3542 0807;
Email: jyokota@gan2.ncc.go.jp

Fifty single-nucleotide polymorphisms (SNPs) associated with amino acid changes in 36 genes involved in diverse DNA repair pathways were assessed for associations with risk for small cell lung carcinoma (SCLC) by a case–control study consisting of 211 SCLC cases and 685 controls. An SNP, Val83Met, in the *MTH1* (mutT homolog 1) gene encoding a triphosphatase that hydrolyzes pro-mutagenic oxidized nucleoside triphosphates, such as 8-hydroxy-dGTP and 2-hydroxy-dATP, showed the strongest and a significant association with SCLC risk [odds ratio (OR) = 1.6, 95% confidence interval (CI): 1.2–2.2, $P = 0.004$], while three other SNPs in the *TP53, BLM* and *SNM1* genes, respectively, also showed marginal associations ($0.05 < P < 0.1$). Another SNP, which causes a nucleotide change in the 5′-UTR of *MTH1* transcripts leading to alternative translation initiation, was additionally examined and the SNP also showed a significant association (OR = 1.7, 95% CI: 1.2–2.3, $P = 0.002$). The two SNPs in the *MTH1* gene were in linkage disequilibrium, and the OR for carrying a copy of the haplotype consisting of both the risky SNP alleles was 2.0 (95% CI: 1.2–3.2, $P = 0.002$). The present results indicate that inter-individual differences in MTH1 activities due to SNPs are involved in susceptibility to SCLC.

Abbreviations: ADC, adenocarcinoma; CI, confidence interval; LCC, large cell carcinoma; *MTH1*, mutT homolog 1; NSCLC, non-small cell lung carcinoma; OR, odds ratio; SCLC, small cell lung carcinoma; SNPs, single-nucleotide polymorphisms; SQC, squamous cell carcinoma.

[†]These authors contributed equally to this work.

Introduction

Lung cancer is the leading cause of cancer-related deaths in the world, and is comprised of a group of four histologically distinct types: adenocarcinoma (ADC), squamous cell carcinoma (SQC), large cell carcinoma (LCC) and small cell lung carcinoma (SCLC) (1). SCLC accounts for ∼20% of all lung cancer cases and has clinical and biological characteristics distinct from non-small cell lung carcinoma (NSCLC). More than 90% of patients at the time of diagnosis are stage III or stage IV owing to its early and wide dissemination. Although, in most cases tumors initially respond to chemotherapy, >95% of patients eventually die from the cancer. Accordingly, the prognosis of patients with SCLC is poor, and 5-year survival of SCLC is <10% (1–3). Thus, SCLC is the most aggressive type of lung cancer. Genes responsible for the susceptibility to SCLC have been searched for to establish novel and efficient ways of preventing the disease. On the basis of the fact that smoking contributes to SCLC development, polymorphisms in metabolic genes encoding enzymes that activate or detoxify carcinogens in tobacco smoke are being studied for association with SCLC risk by case–control studies. Up to the present, a few metabolic genes, such as *CYP1A1, CYP2A6* and *GSTM1*, have been found to be associated with SCLC risk (4–7). Thus, it is possible that polymorphisms in several metabolic genes are involved in SCLC susceptibility.

Polymorphisms in DNA repair genes have been considered to be involved in susceptibility to cancers, since they are thought to cause inter-individual differences in the capacity for preventing mutagenesis (8–12). In fact, single-nucleotide polymorphisms (SNPs) in several DNA repair genes have been shown to be associated with the risk for several types of cancers (12,13). Carcinogens in cigarette smoke are thought to cause a variety of pro-mutagenic DNA adducts, including benzo[*a*]pyrene-diol-epoxide (BPDE) and 8-hydroxyguanine (8OHG), which are repaired by nucleotide excision repair (NER) and base excision repair (BER) (12). Lung cancer patients were indicated to have lower activities of NER and BER than healthy individuals (9,14). Mice deficient in BER were reported to predispose to lung cancer (15). These results support the fact that inter-individual variations of DNA repair activity are involved in lung cancer susceptibility. We recently identified 50 non-synonymous (associated with amino acid change) SNPs in 36 DNA repair genes involved in diverse intracellular processes that maintain genome

integrity (13) (see Table II). These 50 SNPs were examined for association with NSCLC risk in a case–control study consisting of 752 ADC cases, 250 SQC cases and 685 controls, and four of them, LIG4-Ile658Val, TP53-Arg72Pro, POLI-Thr706Ala and REV1-Phe257Ser, were found associated with NSCLC risk. The results suggested that polymorphisms in genes involved in a variety of DNA repair pathways contribute to NSCLC susceptibility. However, to our knowledge, association of SNPs in DNA repair genes with SCLC risk has not been extensively investigated; therefore, their involvements in SCLC susceptibility is unknown. Thus, in the present study, allele distributions for 50 SNPs in 36 DNA repair genes were examined in 211 SCLC cases to investigate association of the SNPs with SCLC risk. Furthermore, DNA repair genes commonly or specifically involved in susceptibility to SCLC and NSCLC were investigated by comparing the present results with our previous results on NSCLC.

Subjects and method

Case–control study

All cases and controls were Japanese. The cases consisted of 211 SCLC patients of four hospitals located in the Kanto area of Japan (i.e. Tokyo and surrounding prefectures) from 1999 to 2004. The hospitals were the National Cancer Center Hospital (NCCH) (113 patients), the National Cancer Center Hospital East (NCCHE) (81 patients), the National Nishigunma Hospital (NNGH) (16 patients) and the Gunma Prefecture Cancer Center Hospital (1 patient). All SCLC cases, from whom informed consent as well as blood samples were obtained, were consecutively included in this study without any particular exclusion criteria. All the cases were diagnosed by cytological and/or histological examinations according to WHO classification (16). From each individual, a 10 or 20 ml whole-blood sample was obtained. Genomic DNAs for all the cases and the controls were isolated from the samples, and 10 ng of genomic DNA was subjected to genotyping by pyrosequencing as described previously (13). Information on the primer sequences and conditions for PCR were described previously (13).

Genotypes for the 50 SNPs of 685 controls had been determined by the same method as used in the present study (13). The information on the controls was described previously (13). Briefly, the controls consisted of patients of two hospitals, NCCH and NNGH, in which SCLC cases were enrolled, and 302 healthy volunteers of Keio University, located in Tokyo. All of the control subjects were selected with a criterion of no history of any cancer.

Smoking history of cases and controls was obtained via interview using a questionnaire. Smoking habit was expressed by pack-years, which was defined as the number of cigarette packs smoked daily multiplied by years of smoking, both in current smokers and former smokers. Smokers were defined as those who had smoked regularly for 12 months or longer at any time in their life, while non-smokers were defined as those who had not. The study was approved by the institutional review boards of the National Cancer Center, the Nishigunma Hospital, the Gunma Prefecture Cancer Center and Keio University.

Statistical analysis

Differences in the allele distributions for the 50 SNPs between the cases and controls were tested by the χ^2-test. Hardy–Weinberg equilibrium (HWE) tests were performed using the TFPGA software (http://bioweb.usu.edu/mpmbio/). Calculation of the D' and r^2 values and haplotype estimation were undertaken using the EM algorithm. The strength of association of *MTH1* (mutT homolog 1) genotypes and haplotypes with SCLC risk was measured as crude odds ratios (ORs), and ORs were adjusted for gender, age (49, 50–59, 60–69, 70) and smoking dosage (pack-years: 0, 1–49, 50) with 95% confidence intervals (CIs) by unconditional logistic regression analysis (17). ORs for carrying a copy of a haplotype were also calculated by the bootstrap method with 5000 resampling. All the statistical analyses were performed using the SAS version 9 software (SAS Institute, NC, USA).

Results

We conducted a case–control study consisting of 211 SCLC cases and 685 controls (Table I). The SCLC cases consisted of patients enrolled in four hospitals in Tokyo and surrounding prefectures. The 685 controls consisted of patients/outpatients and healthy volunteers without a history of cancer enrolled in two hospitals and a university in the same area. Most of the SCLC cases were males and had a smoking habit, as has been reported (18,19). Therefore, the SCLC cases showed a higher fraction of males and smokers than the controls, and the mean smoking dosage of the SCLC cases was larger than that of the controls.

All the 685 controls were genotyped for the 50 SNPs with a success rate of 99.98% in our previous study (13). The 211 SCLC cases were genotyped for the same 50 SNPs in the present study, and the success rate was 99.94% (Table II). The allele distribution in the SCLC cases was compared with that in the 685 controls. None of the 50 SNPs deviated from HWE in cases and controls ($P \geq 0.05$). A significant difference in the allele distribution between the controls and cases was observed in one of the 50 SNPs, MTH1-Val83Met (OR for the MTH1-83Met allele = 1.6, 95% CI: 1.2–2.2, $P = 0.004$) (Table II). Marginally significant ($0.5 \leq P < 0.1$) allele differentiations were observed in three SNPs, SNM1-His317Asp, TP53-Arg72Pro and BLM-Thr298Met. Allele distributions for the other 46 SNPs were not significantly or were marginally significantly different between the controls and cases.

The relative risks of the genotypes for the four SNPs, which showed significant or marginally significant allele differentiations, were calculated as crude and adjusted ORs. Heterozygotes, homozygotes for the MTH1-83Met allele and carriers of the allele showed significantly increased

Table I. SCLC cases and controls for case–control study

Subject	Institution[a]	No.	Gender (%)		Age	Smoking habit (%)			Pack-years of smokers
			Male	Female	(Mean ± SD)	Non-smoker	Smoker	Unknown	(Mean ± SD)
Case	NCCH	113	88 (78)	25 (22)	61 ± 10	8 (7)	105 (93)	0 (0)	62 ± 31
	NCCHE	81	68 (84)	13 (16)	65 ± 8	0 (0)	77 (95)	4 (5)	57 ± 30
	NNGH[b]	17	16 (94)	1 (6)	68 ± 8	0 (0)	17 (100)	0 (0)	55 ± 25
	Total	211	172 (82)	39 (18)	63 ± 9	8 (4)	199 (94)	4 (2)	59 ± 30
Control	NCCH	242	129 (53)	113 (47)	60 ± 16	138 (57)	102 (42)	2 (1)	36 ± 32
	NNGH	141	100 (71)	41 (29)	65 ± 14	46 (33)	91 (65)	4 (3)	46 ± 35
	KEIO	302	254 (84)	48 (16)	48 ± 10	202 (67)	94 (31)	6 (2)	22 ± 20
	Total	685	483 (71)	202 (29)	55 ± 13	386 (56)	287 (42)	12 (2)	35 ± 31

[a]NCCH, National Cancer Center Hospital; NCCHE, National Cancer Center Hospital East; NNGH, National Nishigunma Hospital; KEIO, Keio university.
[b]Including a case of Gunma Prefecture Cancer Center Hospital.

Table II. Allele frequencies of 50 SNPs in 36 DNA repair genes in controls and cases

DNA repair	Gene	SNP	Amino acid change	dbSNP ID	Minor allele frequency[a]		
					Control[b]	Case	
BER	*PARP/ADPRT*	T2444C	Val762Ala	rs1805412	0.40	0.37	
		A2978G	Lys940Arg	rs1136471	0.05	0.04	
	APEX/APE1	A395G	Ile64Val	rs2307486	0.04	0.05	
		T649G	Asp148Glu	rs3136820	0.38	0.41	
	MBD4	G1212A	Glu346Lys	rs140693	0.35	0.36	
	MTH1/NUDT1	G273A	Val83Met	rs4866	0.09	0.15	($P = 0.004$)
	OGG1	C2243G	Ser326Cys	rs1052133	0.48	0.46	
	XRCC1	C685T	Arg194Trp	rs1799782	0.33	0.30	
		G944A	Arg280His	rs25489	0.09	0.08	
		G1301A	Arg399Gln	rs25487	0.25	0.24	
NER	*XPG/ERCC5*	C3507G	His1104Asp	rs17655	0.42	0.46	
	CSB/ERCC6	G1275A	Gly399Asp	rs2228528	0.45	0.43	
	XPC	A2655C	Lys939Gln	rs2228001	0.40	0.38	
	XPD/ERCC2	G1615A	Asp312Asn	rs1799793	0.04	0.04	
		A2932C	Lys751Gln	rs1052559	0.05	0.05	
Mismatch repair	*MLH1*	A676G	Ile219Val	rs1799977	0.05	0.03	
	MLH3	C2645T	Pro844Leu	rs175080	0.18	0.16	
		C2939T	Thr942Ile	rs17102999	0.05	0.06	
	MSH2	C91T	Thr8Met	rs17217716	0.02	0.02	
	MSH3	A3122G	Thr1036Ala	rs26279	0.24	0.27	
	MSH6	G203A	Gly39Glu	rs1042821	0.32	0.31	
DNA double-strand break repair	*BRCA2*	A1342C	Asn372His	rs144848	0.22	0.21	
	SNM1/KIAA0086	C1867G	His317Asp	rs3750898	0.26	0.30	($P = 0.08$)
	LIG4	A2245G[c]	Ile658Val	rs2232641	0.04	0.06	
	NBS1	C605G	Gln185Glu	rs1805794	0.50	0.46	
	RAD51L3/RAD51D	G501A	Arg165Gln	rs4796033	0.04	0.03	
	RAD54L	A551G	Lys151Glu	rs2295466	0.02	0.01	
	RINT-1	G33C	Glu4Gln	rs818620	0.07	0.09	
	XRCC3	C1075T	Thr241Met	rs861539	0.09	0.09	
DNA damage response	*TP53*	G466C[c]	Arg72Pro	rs1042522	0.33	0.38	($P = 0.097$)
DNA polymerase	*POLD1*	G409A	Arg119His	rs1726801	0.20	0.22	
	POLH/XPV/RAD30	A1840G	Lys535Glu	-	0.03	0.04	
	POLI/RAD30B	A2180G[c]	Thr706Ala	rs8305	0.25	0.24	
	POLL	C1683T	Arg438Trp	rs3730477	0.01	0.012	
	REV1	T982C[c]	Phe257Ser	rs3087386	0.33	0.32	
	A1330G	Asn373Ser	rs3087399	0.04	0.04		
	POLZ/REV3	C4259T	Thr1146Ile	rs462779	0.35	0.37	
Other pathways	*BLM*	C967T	Thr298Met	rs28384991	0.09	0.12	($P = 0.09$)
		G4035A	Val1321Ile	rs7167216	0.04	0.04	
	FANCA	G827A	Ala266Thr	rs17232400	0.03	0.03	
		G1080A	Arg350Gln	rs17233497	0.01	0.01	
		A1532G	Ser501Gly	rs2239359	0.17	0.16	
		A2457G	Asp809Gly	rs7195066	0.03	0.03	
		C3294T	Ser1088Phe	rs7190823	0.02	0.02	
	FANCE	G451T	Arg89Leu	-	0.01	0.00	
		G1213A	Arg343Gln	-	0.04	0.04	
	FANCF	A983G	Lys324Glu	-	0.003	0.002	
	FANCG/XRCC9	C1382T	Thr297Ile	rs2237857	0.12	0.13	
	WRN	C2573T	Thr781Ile	rs17847568	0.03	0.03	
		T4330C	Cys1367Arg	rs1346044	0.09	0.07	

[a]*P*-values by χ^2-test against the control population are shown, when they are <0.1.
[b]Determined in our previous study (12).
[c]Significantly associated with SQC and/or ADC risks in our previous study (12).

ORs, when homozygotes for the 83Val allele were used as a reference, respectively (Table III). On the other hand, ORs of genotypes for the remaining three SNPs, SNM1-His317Asp, TP53-Arg72Pro and BLM-Thr298Met, did not show significant increases or decreases in SCLC cases (data not shown); therefore, these SNPs were not further investigated in the present study.

The *MTH1* gene, whose SNP, Val83Met, showed a significant association as described above, encodes a triphosphatase that hydrolyzes oxidized purine nucleoside triphosphates, such as 8-hydroxy-dGTP and 2-hydroxy-dATP (20). The activity of the MTH1-83Met protein was reported to be more thermolabile than that of the MTH1-83Val protein (20–22). The mitochondrial translocation of the MTH1-83Met protein was reported to be less efficient than that of the MTH1-83Val protein (23). Thus, it was suggested that the MTH1-83Met protein is less active than the MTH1-83Val protein. Previously, another SNP was found in a non-coding exon of *MTH1* (i.e. the T/C SNP in exon 2) 7.0 kb distal to the MTH1-Val83Met SNP, and the C allele in exon 2 leads to the production of an additional translation start site, resulting in the production of a longer MTH polypeptide in addition to commonly produced MTH polypeptides (21). This T/C SNP of the *MTH1* gene was

Table III. MTH1 genotypes and SCLC risk

SNP	Genotype	No. of controls (%)	No. of cases (%)	Crude OR (95% CI, P)	Adjusted OR[a](95% CI, P)
Val83Met	Val/Val	558 (82)	154 (73)	Reference	Reference
	Val/Met	117 (17)	53 (25)	1.6 (1.1–2.4, 0.009)	1.7 (1.1–2.7, 0.03)
	Met/Met	6 (1)	4 (2)	2.4 (0.7–8.7, 0.2)	6.5 (1.3–32.1, 0.02)
	Val/Met + Met/Met	123 (18)	57 (27)	1.7 (1.2–2.4, 0.005)	1.8 (1.2–2.9, 0.01)
T/C in exon 2	T/T	560 (82)	154 (73)	Reference	Reference
	T/C	118 (17)	53 (25)	1.6 (1.1–2.4, 0.009)	1.7 (1.1–2.7, 0.03)
	C/C	3 (0)	4 (2)	4.8 (1.1–21.9, 0.04)	15.7 (2.5–100.6, 0.004)
	T/C + C/C	121 (17)	57 (27)	1.7 (1.2–2.5, 0.004)	1.9 (1.2–3.0, 0.008)

[a]Adjusted for gender, age and smoking dosage.

not included in our 50 SNP set, because it was located in a non-coding exon. However, the above result prompted us to genotype this SNP in the same SCLC and control subjects. Since genotype data for the MTH1-Val83Met SNP were obtained from 681 of the 685 controls and all 211 cases, the genotypes for the T/C SNP were also determined for the same 681 controls and the 211 SCLC cases. Significant allele differentiations were also observed in the T/C SNP (OR for the C allele = 1.7, 95% CI: 1.2–2.3, $P = 0.002$). ORs of the heterozygotes, homozygotes for the exon 2-C allele and carriers of the allele were also significantly increased, when homozygotes for the exon 2-T allele were used as a reference, respectively (Table III).

Since both the case and control subjects in the present case–control study were enrolled in several institutions, it was possible that differences in the institutions lead to the observed allele differentiations due to population stratification. Therefore, we compared allele frequencies for the MTH1-Val83Met and exon 2-T/C SNPs among SCLC cases and controls of each institution (Figure 1). Allele frequencies for the MTH1-Val83Met SNP had been also reported in two other populations consisting of healthy Japanese volunteers (21,24); therefore, the frequencies in those studies were also compared. Frequencies of the 83Met and exon 2-C alleles in any SCLC populations were higher than those in any of the control populations. Allele frequencies for these SNPs were not significantly different among control populations and among case populations ($P > 0.05$ by χ^2-test). We also compared the frequencies of genotypes for the two SNPs, and they were not significantly different among control populations and among case populations, either ($P > 0.05$ by χ^2-test). Thus, it was indicated that the 83Met and exon 2-C alleles were associated with the SCLC risk beyond institutional differences.

Both the SNPs of *MTH1* were found to be in linkage disequilibrium with each other ($D' = 0.97$, $r^2 = 0.91$). Thus, we further evaluated the haplotype differentiation between the SCLC cases and the controls (Table IV). The haplotype consisting of the two risky alleles (i.e., haplotype #2 consisting of the 83Met and C alleles in Table IV) was significantly over-represented in the SCLC population (OR = 1.7, 95% CI: 1.2–2.4, $P = 0.001$), and the OR for haplotype #2 was similar to those for individual 83Met and C allele, respectively. In addition, by taking into account the estimation error of haplotype frequency, crude and adjusted ORs for carrying one copy of haplotype #2 were calculated on the basis of the estimated number of haplotypes for each subject by the bootstrap method, and they were 1.8 (95% CI: 1.2–2.5, $P = 0.0004$) and 2.0 (95% CI: 1.2–3.2, $P = 0.002$), respectively.

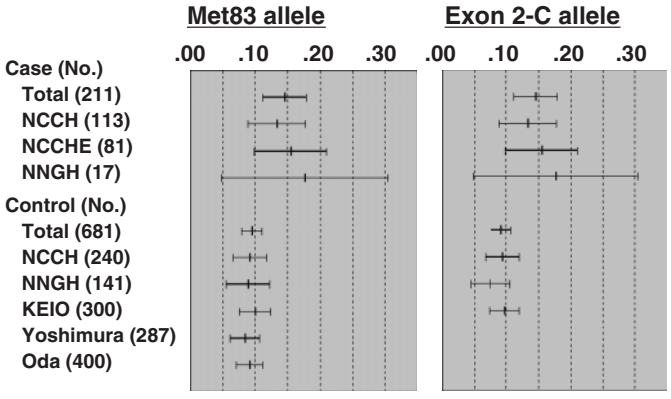

Fig. 1. Frequencies of the MTH-83Met and exon 2-C alleles in cases and controls. Allele frequency is shown with its sampling variations estimated by 95% CI. Frequencies of the MTH-83Met allele in two control populations reported by Yoshimura *et al*. (24) and Oda *et al*. (21) are also shown.

We next assessed the effect of smoking on the contribution of the MTH1-Val83Met and exon 2-T/C SNPs to the SCLC risk. ORs in light (PY < 50) smokers and heavy (PY $\geq$ 50) smokers were compared (Table V). The number of non-smokers in the case subjects was small (i.e. $N < 10$); therefore, they were excluded from the analysis. Increases of ORs for the 83Met and exon 2-C alleles were more evident in light smokers than in heavy smokers, and the ORs were statistically significant in light smokers but not in heavy smokers. *P*-values for interaction of the Val83Met and exon 2-T/C genotypes on the SCLC risk with smoking were 0.15 and 0.11, respectively. *P*-value for interaction of haplotype #2 on the SCLC risk by smoking was calculated as being 0.095.

Discussion

The *MTH1* gene was cloned as a human homolog for the *Escherichia coli mutT* gene, encoding an enzyme hydrolyzing 8-hydroxy-dGTP, an oxidized dNTP causing A:T to C:G transversion (20). It has been shown that MTH1 protein hydrolyzes not only 8-hydroxy-dGTP but also several other oxidatively damaged dNTPs, such as 2-hydroxy-dATP, thereby preventing multiple mutations including A:T to C:G, G:C to T:A and G:C to A:T mutations (20). *Mth1* nullizygous mice are susceptible to tumor development in lung and other tissues (25). Thus, it has been assumed that inter-individual differences in *MTH1* activity are associated with risks for cancers by causing inter-individual differences

Table IV. Association of MTH1 haplotypes and SCLC risk

Haplotype	SNP		Haplotype frequency		OR (95% CI)	*P*
	Val83Met	T/C in exon 2	Control (95% CI)	Case (95% CI)		
1	Val	T	0.90 (0.89–0.92)	0.85 (0.82–0.89)	Reference	
2	Met	C	0.089 (0.073–0.10)	0.14 (0.11–0.18)	1.7 (1.2-2.4)	0.001
3	Met	T	0.0067 (0.0023–0.011)	0.0024 (0–0.0070)	0.4 (0.05–3.0)	0.3
4	Val	C	0.0030 (0–0.0059)	0.0024 (0–0.0070)	0.9 (0.1–7.7)	0.9

Table V. OR for MTH1 genotypes by smoking dosage and age

SNP	Stratification	No of controls (%)		No of cases (%)		Crude OR	Adjusted OR[a]	*P* for interaction[a]
		Major homozygote	Minor allele carrier	Major homozygote	Minor allele carrier	(95% CI, *P*)	(95% CI, *P*)	
Val83Met	py = 0	319 (83)	67 (17)	5 (63)	3 (38)	2.8 (0.7–12.2, 0.16)	2.9 (0.7–12.7, 0.16)	
	0 < py < 50	178 (82)	38 (18)	59 (69)	26 (31)	2.1 (1.2–3.7, 0.014)	2.3 (1.2–4.4, 0.011)	0.15
	py ≥ 50	54 (78)	15 (22)	88 (77)	26 (23)	1.1 (0.5–2.2, 0.87)	1.1 (0.5–2.3, 0.85)	
T/C in exon 2	py = 0	316 (82)	68 (18)	5 (63)	3 (38)	2.8 (0.7–12.0, 0.16)	2.8 (0.6–12.3, 0.17)	0.11
	0 < py < 50	181 (84)	35 (16)	59 (69)	26 (31)	2.3 (1.3–4.1, 0.006)	2.6 (1.3–4.9, 0.005)	
	py ≥ 50	54 (78)	15 (22)	88 (77)	26 (23)	1.1 (0.5–2.2, 0.87)	1.1 (0.5–2.3, 0.85)	

[a]Adjusted for gender and age.

in the capacity to prevent mutations of the cancer-related genes caused by incorporation of oxidatively damaged dNTPs during DNA replication (20). In the present study, SNPs in the *MTH1* gene were found to be associated with SCLC risk. To the best of our knowledge, SNPs in the *MTH1* gene were found for the first time as being associated with risks for human cancers by a case–control study. However, the possibility of false positives (type I statistical errors) must be considered. We performed 50 separate tests of significance in the analysis. A consecutive Bonferroni adjustment to yield an experiment-wide type I error rate of 0.05 would demand a test-wise *P*-value of 0.001. Therefore, the association of the MTH1-Val83Met SNP would not be considered significant on an experiment-wide level after Bonferroni adjustment. Thus, the association requires confirmation in other population samples, although the present study proposed *MTH1* as a candidate gene responsible for SCLC susceptibility.

The two *MTH1* SNPs, Val83Met and exon 2-T/C, examined in the present study were suggested to cause functional differences, although their effects on mutation suppression efficiency against oxidative DNA damages are unknown (20–22). These two SNPs were in linkage disequilibrium, and the risky allele of each SNP (i.e. the 83Met and exon 2-C alleles) was on the same haplotype (haplotype #2) in most of the Japanese population. Thus, at present, it is unclear whether both or one of the two SNPs are responsible for the SCLC susceptibility. It is also possible that other SNPs consisting of the haplotype are responsible. Further biological and genetic analyses of the *MTH1* SNPs will elucidate the issue.

Interestingly, ORs for carriers of the 83Met and C alleles were more evidently increased in light smokers than in heavy smokers. Tobacco smoke is known to cause oxidative damages on genomic DNA and nucleoside triphosphates (26). Therefore, individuals carrying the 83Met and C alleles might be more prone to acquiring gene mutations even by a low-dose exposure of carcinogens, and therefore, the effects of *MTH* SNPs might have more prominently appeared under the condition of a low-dose exposure of tobacco smoke. On the other hand, the effects of the SNPs might be masked under the condition of a high-dose exposure of tobacco smoke, since, under such a condition, environmental factors (i.e. carcinogens in tobacco smoke) rather than genetic factors predominantly determine the risk for SCLC. However, the interaction of *MTH1* SNPs with smoking on SCLC risk in the present study was not statistically significant; therefore, further case–control studies are necessary to elucidate how *MTH1* SNPs contribute to SCLC risk of smokers.

We previously examined the same 50 SNP set for associations with lung SQC and ADC risk using the same controls (13). In the study, frequencies of the MTH1-83Met allele in SQC and ADC cases, respectively, were slightly higher than that in controls. However, ORs of the carriers of the allele was not significantly increased (Figure 2). Thus, the MTH1-Val83Met SNP was thought to be associated with SCLC risk but not with NSCLC risk. In the previous study, an SNP, TP53-Arg72Pro, in the *p53* gene was associated with SQC risk, and the association remained significant after Bonferroni adjustment. Association of the SNP with NSCLC and overall lung cancer risks have been observed in several other case–control studies (28–31). The association was also supported by a report that TP53-72Pro protein has a weaker activity than TP53-72Arg protein in inducing apoptosis of human cells suffering from DNA damages (32). Interestingly, the TP53-72Pro allele was marginally significantly over-represented in SCLC cases in the present study. ORs of the homozygotes for the carriers of the TP53-72Pro allele were increased in SCLC cases, although the increase was not statistically significant (Figure 2). Thus, it is possible that the TP53-72Pro allele confers increased susceptibility both to SCLC and NSCLC. In the present study, marginally significant associations with SCLC risk were observed for two other SNPs, BLM-Thr298Met and SNM1-His317Asp. However, such associations were not detected in ADC and

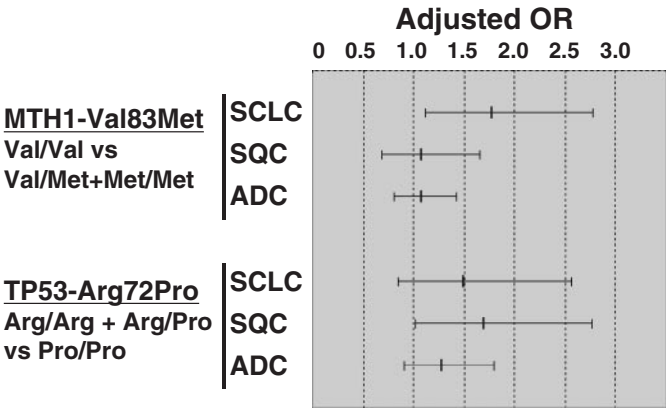

Fig. 2. ORs of the MTH-83Met allele carriers against homozygotes for the MTH-83Val allele and those of homozygotes for the TP53-72Pro allele against others. ORs adjusted for gender, age and smoking dosage are shown. ORs in SQC and ADC cases are from our previous report (13).

SQC (Table II). SNPs that showed association with SQC or ADC risk, such as LIG4-Ile658Val, POLI-Thr706Ala and REV1-Phe257Ser, were not associated with SCLC risk in this study. Thus, genes involved in the susceptibility might be overlapped but different between SCLC and NSCLC.

In the present and previous studies (13), we examined the associations of 50 SNPs in 36 DNA repair genes with SCLC and NSCLC risks. The studies led us to identify several DNA repair genes commonly or specifically involved in the susceptibility to SCLC and NSCLC. The results supported the idea that inherited variations in DNA repair genes are involved in susceptibility to lung cancer of each individual. Further examination of SNPs in DNA repair genes in the present and also in other sets of subjects will help us understand genetic factors responsible for the susceptibility to lung cancer. In addition, studies up to the present suggested that polymorphisms of genes involved in metabolism of carcinogens in cigarette smoke, such as *CYP1A1, CYP2A6* and *GSTM1*, are also responsible for the susceptibility to lung cancer (4–7). It is possible that such polymorphisms modify the effect of SNPs in DNA repair genes on risk for lung cancer. Therefore, combined effects of polymorphisms in DNA repair genes and metabolic genes on risks for SCLC and NSCLC should be also further investigated.

Acknowledgements

This work was supported by Grants-in-Aid from the Ministry of Health, Labour and Welfare for Research on Human Genome and Tissue Engineering and for Cancer Research (16S-1), and a Grant-in-Aid from the Program for Promotion of Fundamental Studies in Health Sciences of the Organization for Pharmaceutical Safety. We thank Dr Ikuo Saito, Dr Matsuhiko Hayashi and Dr Keiichi Hirao of the Keio University School of Medicine; Dr Teruhiko Yoshida, Dr Hiromi Sakamoto, Dr Kimio Yoshimura and Dr Shunpei Ohnami of the National Cancer Center Research Institute; and Ms Toyoko Matsumoto and Ms Fumiko Koh of the National Cancer Center Hospital East for their help in collecting blood samples in Keio University. We also thank Dr Kouichi Minato and Dr Shinichi Ishihara of Gunma Prefectural Cancer Center for their collection of blood samples from lung cancer patients. N.Y. was an awardee of a Research Resident Fellowship from the Foundation for Promotion of Cancer Research in Japan during the study. Funding to pay the Open Access publication charges for this article was provided by xxxxx.

Conflict of Interest Statement: None declared.

References

1. Jemal,A., Tiwari,R.C., Murray,T., Ghafoor,A., Samuels,A., Ward,E., Feuer,E.J. and Thun,M.J. (2004) Cancer statistics, 2004. *CA Cancer J. Clin.*, **54**, 8–29.
2. Travis,W., Nicholson,S., Hirsch,F.R. *et al.* (2004) Small Cell Carcinoma. In Travis,W.D., Brambilla,E., Muller-Hermelink,H.K. and Harris,C.C., (eds) *World Health Organization Classification of Tumors: Pathology and Genetics, Tumours of Lung, Pleura, Thymus and Heart*.pp. 31–34.
3. Jackman,D.M. and Johnson,B.E. (2005) Small-cell lung cancer. *Lancet*, **366**, 1385–1396.
4. Sugimura,H., Wakai,K., Genka,K. *et al.* (1998) Association of Ile462Val (exon 7) polymorphism of cytochrome P450 IA1 with lung cancer in the Asian population: further evidence from a case–control study in Okinawa. *Cancer Epidemiol. Biomarkers Prev.*, **7**, 413–417.
5. Bartsch,H., Nair,U., Risch,A., Rojas,M., Wikman,H. and Alexandrov,K. (2000) Genetic polymorphism of CYP genes, alone or in combination, as a risk modifier of tobacco-related cancers. *Cancer Epidemiol. Biomarkers Prev.*, **9**, 3–28.
6. Fujieda,M., Yamazaki,H., Saito,T. *et al.* (2004) Evaluation of CYP2A6 genetic polymorphisms as determinants of smoking behavior and tobacco-related lung cancer risk in male Japanese smokers. *Carcinogenesis*, **25**, 2451–2458.
7. Stucker,I., Hirvonen,A., de Waziers,I., Cabelguenne,A., Mitrunen,K., Cenee,S., Koum-Besson,E., Hemon,D., Beaune,P. and Loriot,M.A. (2002) Genetic polymorphisms of glutathione *S*-transferases as modulators of lung cancer susceptibility. *Carcinogenesis*, **23**, 1475–1481.
8. Shields,P.G. and Harris,C.C. (2000) Cancer risk and low-penetrance susceptibility genes in gene–environment interactions. *J. Clin. Oncol.*, **18**, 2309–2315.
9. Spitz,M.R., Wei,Q., Dong,Q., Amos,C.I. and Wu,X. (2003) Genetic susceptibility to lung cancer: the role of DNA damage and repair. *Cancer Epidemiol. Biomarkers Prev.*, **12**, 689–698.
10. Amos,C.I., Xu,W. and Spitz,M.R. (1999) Is there a genetic basis for lung cancer susceptibility? *Recent Results Cancer Res.*, **151**, 3–12.
11. Mohrenweiser,H.W., Wilson,D.M. III and Jones,I.M. (2003) Challenges and complexities in estimating both the functional impact and the disease risk associated with the extensive genetic variation in human DNA repair genes. *Mutat. Res.*, **526**, 93–125.
12. Goode,E.L., Ulrich,C.M. and Potter,J.D. (2002) Polymorphisms in DNA repair genes and associations with cancer risk. *Cancer Epidemiol. Biomarkers Prev.*, **11**, 1513–1530.
13. Sakiyama,T., Kohno,T., Mimaki,S. *et al.* (2005) Association of amino acid substitution polymorphisms in DNA repair genes TP53, POLI, REV1 and LIG4 with lung cancer risk. *Int. J. Cancer*, **114**, 730–737.
14. Paz-Elizur,T., Krupsky,M., Blumenstein,S., Elinger,D., Schechtman,E. and Livneh,Z. (2003) DNA repair activity for oxidative damage and risk of lung cancer. *J. Natl Cancer Inst.*, **95**, 1312–1319.
15. Sakumi,K., Tominaga,Y., Furuichi,M., Xu,P., Tsuzuki,T., Sekiguchi,M. and Nakabeppu,Y. (2003) Ogg1 knockout-associated lung tumorigenesis and its suppression by *Mth1* gene disruption. *Cancer Res.*, **63**, 902–905.
16. Brambilla,E., Travis,W.D., Colby,T.V., Corrin,B. and Shimosato,Y. (2001) The new World Health Organization classification of lung tumours. *Eur. Respir. J.*, **18**, 1059–1068.
17. Breslow,N.E. and Day,N.E. (1980) Statistical methods in cancer research. Volume I—The analysis of case–control studies. *IARC Sci. Publ.*, 5–338.
18. Yoshimi,I., Ohshima,A., Ajiki,W., Tsukuma,H. and Sobue,T. (2003) A comparison of trends in the incidence rate of lung cancer by histological type in the Osaka Cancer Registry, Japan and in the Surveillance, Epidemiology and End Results Program, USA. *Jpn. J. Clin. Oncol.*, **33**, 98–104.
19. Wynder,E.L. and Hoffmann,D. (1994) Smoking and lung cancer: scientific challenges and opportunities. *Cancer Res.*, **54**, 5284–5295.
20. Nakabeppu,Y. (2001) Molecular genetics and structural biology of human MutT homolog, MTH1. *Mutat. Res.*, **477**, 59–70.
21. Oda,H., Taketomi,A., Maruyama,R., Itoh,R., Nishioka,K., Yakushiji,H., Suzuki,T., Sekiguchi,M. and Nakabeppu,Y. (1999) Multi-forms of human MTH1 polypeptides produced by alternative translation initiation and single nucleotide polymorphism. *Nucleic Acids Res.*, **27**, 4335–4343.
22. Yakushiji,H., Maraboeuf,F., Takahashi,M., Deng,Z.S., Kawabata,S., Nakabeppu,Y. and Sekiguchi,M. (1997) Biochemical and physicochemical characterization of normal and variant forms of human MTH1 protein with antimutagenic activity. *Mutat. Res.*, **384**, 181–194.

23. Sakai,Y., Oda,H., Yoshimura,D., Furuichi,M., Kang,D., Iwai,S., Hara,T. and Nakabeppu,Y. (2006) The GT to GC single nucleotide polymorphism at the beginning of an alternative exon 2C of human MTH1 gene confers an amino terminal extension that functions as a mitochondrial targeting signal. *J. Mol. Med.*, in press.
24. Yoshimura,K., Hanaoka,T., Ohnami,S., Kohno,T., Liu,Y., Yoshida,T., Sakamoto,H. and Tsugane,S. (2003) Allele frequencies of single nucleotide polymorphisms (SNPs) in 40 candidate genes for gene–environment studies on cancer: data from population-based Japanese random samples. *J. Hum. Genet.*, **48**, 654–658.
25. Tsuzuki,T., Egashira,A., Igarashi,H. *et al.* (2001) Spontaneous tumorigenesis in mice defective in the MTH1 gene encoding 8-oxo-dGTPase. *Proc. Natl Acad. Sci. USA*, **98**, 11456–11461.
26. Loft,S. and Poulsen,H.E. (1996) Cancer risk and oxidative DNA damage in man. *J. Mol. Med.*, **74**, 297–312.
27. Hou,S.M., Falt,S., Yang,K., Nyberg,F., Pershagen,G., Hemminki,K. and Lambert,B. (2001) Differential interactions between GSTM1 and NAT2 genotypes on aromatic DNA adduct level and HPRT mutant frequency in lung cancer patients and population controls. *Cancer Epidemiol. Biomarkers Prev.*, **10**, 133–140.
28. Kiyohara,C., Yoshimasu,K., Shirakawa,T. and Hopkin,J.M. (2004) Genetic polymorphisms and environmental risk of lung cancer: a review. *Rev. Environ. Health*, **19**, 15–38.
29. Wu,X., Zhao,H., Amos,C.I., Shete,S., Makan,N., Hong,W.K., Kadlubar,F.F. and Spitz,M.R. (2002) p53 genotypes and haplotypes associated with lung cancer susceptibility and ethnicity. *J. Natl Cancer Inst.*, **94**, 681–690.
30. Mechanic,L.E., Marrogi,A.J., Welsh,J.A., Bowman,E.D., Khan,M.A., Enewold,L., Zheng,Y.L., Chanock,S., Shields,P.G. and Harris,C.C. (2005) Polymorphisms in XPD and TP53 and mutation in human lung cancer. *Carcinogenesis*, **26**, 597–604.
31. Fan,R., Wu,M.T., Miller,D., Wain,J.C., Kelsey,K.T., Wiencke,J.K. and Christiani,D.C. (2000) The p53 codon 72 polymorphism and lung cancer risk. *Cancer Epidemiol. Biomarkers Prev.*, **9**, 1037–1042.
32. Dumont,P., Leu,J.I., Della Pietra,A.C. III, George,D.L. and Murphy,M. (2003) The codon 72 polymorphic variants of p53 have markedly different apoptotic potential. *Nat. Genet.*, **33**, 357–365.

Received March 2, 2006; revised May 14, 2006; accepted May 19, 2006