ANTICANCER RESEARCH 27: 3497-3500 (2007)

Effectiveness of Concurrent Radiation Therapy with UFT or TS-1 for T2N0 Glottic Cancer in Japan

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Abstract. *The aim of this retrospective study was to compare concurrent radiation therapy (RT) combined with peroral chemotherapy (UFT or TS-1) with conventional RT for T2N0 glottic cancer. Between 1974 and 2005, 153 patients with T2N0 glottic cancer were treated with radiation alone or radiation combined with peroral (UFT or TS-1) or intravenous chemotherapy. All except one patient were treated with 2 Gy per fraction, 5 fractions per week, totaling 60 Gy; and the remaining patient was treated with 1.8 Gy per fraction, 5 fractions per week, totaling 61.2 Gy. Eighty-three patients were concurrently given UFT, 24 were given TS-1, 23 intravenous chemotherapy (mainly cisplatin; the Pt Group), and 23 had no chemotherapy. The 5-year local control rate was 83.4%. Stratified by RT alone (the RT group) and concurrent chemoradiation therapy (the CCRT group), 5-year local control rates of the RT and CCRT groups were 82.7% and 83.4%, respectively (p=NS). Stratified by chemotherapy regimens of the CCRT group, 3-year local control rates of the UFT, TS-1 and Pt groups were 90.1%, 100.0% and 73.4%, respectively. Concurrent chemoradiation therapy using UFT or TS-1 for T2N0 glottic cancer is one of the standard treatments in Japan. Concurrent use of TS-1 could be a breakthrough treatment for T2N0 glottic cancer.*

At diagnosis, about 60% of head and neck cancer is found to be stage III-IV disease, because effective screening examinations to detect early-stage head and neck cancer have not been established yet (1). On the other hand, laryngeal cancer, especially glottic cancer, is mostly detected in the early stage owing to the concomitant symptom of hoarseness. Thus, the strategy for glottic cancer differs from other head and neck carcinomas.

Early-stage glottic cancer is usually treated with radiation therapy (RT) alone, voice preservation partial-pharyngectomy or altered fractionated RT (1-5). RT alone is effective for T1N0 squamous cell carcinoma of the glottic larynx, is not burdensome for the patients and enables patients to maintain the same voice quality as before treatment. In Japan, RT alone is often used to treat T1N0 glottic cancer. The 5-year overall survival rate and 5-year local control rate of T1N0 glottic cancer has been reported to be 79%-82% and 80%-93% (6-10), respectively (1). However, the treatment outcome of RT alone for T2N0 glottic cancer is unsatisfactory. The 5-year overall survival rate and local control rate of T2N0 glottic cancer has been reported to be 77% and 67%-88% (6-10), respectively (1). Surgery is effective, however, the quality of voice preservation is much worse than radiation therapy (11). Altered fractionated RT may have the possibility to be superior to conventional RT (12-15), although relatively few conclusive reports have been published and acute morbidity might be exacerbated.

Recently, Akimoto *et al.* have demonstrated that concurrent chemoradiotherapy (CCRT) of T2N0 laryngeal cancer had better local control rate than conventional RT alone (16). The 5-year local control rates of CCRT and of RT alone were 89% and 61%, respectively. Nonetheless, there are a few remaining obstacles to overcome in order to apply this strategy to medical practice. The CCRT method of Akimoto *et al.* is a combination of RT and intravenous chemotherapy, which means that patients are treated with CCRT in hospital, increasing the patients' physical, psychological and economical burdens. Most patients prefer peroral rather than intravenous chemotherapy when the treatment outcomes are the same (17). Peroral chemotherapy enables patients to be treated in outpatient clinics.

This study was presented at the 43rd Annual Meeting of the American Society of Clinical Oncology (ASCO), June 1-5, 2007, Chicago, Illinois, U.S.A.

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Key Words: Chemoradiotherapy, T2N0 glottic cancer, peroral chemotherapy, UFT, TS-1.

0250-7005/2007 $2.00+.40

The current study were retrospectively reviewed 153 T2N0 glottic cancer patients treated at the Kitasato University Hospital to compare concurrent RT combined with chemotherapy (mostly, peroral anticancer drugs: UFT or TS-1 Taiho Pharmaceutical, Tokyo, Japan, approved by the Japanese government) with conventional RT.

Patients and Methods

From 1974 to December, a total of 153 patients, 151 males and 2 females (median: age 64 years; range: 45-86 years) with T2N0 (according to the 1994 UICC TNM classification) glottic cancer had been treated with RT alone or RT combined with peroral (UFT or TS-1) or intravenous chemotherapy at the Kitasato University Hospital, Kanagawa, Japan. The local control and overall survival rates were retrospectively analyzed and comparisons were made between cases receiving peroral chemotherapy combined with RT and those receiving RT alone.

Regarding the RT protocol, all except one had been treated with 2 Gy per fraction, 5 fractions per week and a total of 60 Gy using 4MV X-ray and lateral paralleled opposed fields, the remaining one patient had been treated with 1.8 Gy per fraction, 5 fractions per week and a total of 61.2 Gy. The reference point for irradiation selected for glottic cancer at the Kitasato University Hospital is the half point of the isocenter and the posterior irradiation field line (the Kitasato Method). However, the usual reference point for glottic cancer is the isocenter. Dose-distribution is superior in the former protocol to the latter one. Paired wedge filters had been used when radiation oncologists considered it to be required for compensation of dose inhomogeneity.

For the chemotherapy protocol 83 patients had concurrently taken UFT, 600-800 mg per day totaling 9,000-30,000 mg (median: 24,000 mg), during RT (the UFT group). Twenty-four patients had taken TS-1, 50-100 mg per day (median: 80 mg per day), totaling between 1,250-2,500 mg (median: 2,000 mg) with a 1- or 2-week break during the RT (the TS-1 group). Twenty-three patients had ungergone intravenous chemotherapy using mainly cisplatin (the Pt group). The remaining 23 patients had undergone no chemotherapy.

The analysis was performed in January 2006. Survival and local control rates were calculated from the first day of RT using the Kaplan-Meier method, and the log-rank sum test was used for comparing variables.

Results

The median follow-up period was 61 months (range: 3-372 months). The 3- and 5-year local control rates of all patients were 86.8% and 83.4%, respectively (Figure 1). The 3- and 5-year local control rates for RT along (the RT group) and concurrent chemoradiotherapy (the CCRT group) were 82.7% and 82.7%, and 87.7%, and 83.4%, respectively (p=NS) (Figure 2). Stratified by chemotherapy regimens within the CCRT group, the 3-year local control rates of the UFT, TS-1 and Pt groups were 90.1%, 100.0% and 73.4%, respectively (Figure 3).

The 3- and 5-year overall survival rate of all patients was 94.5% and 87.9%, respectively (Figure 4). The 3- and 5-year overall survival rates of the RT and CCRT groups were 82.9% and 78.3%, and 96.1% and 90.1%, respectively (Figure 5). These differences were statistically significant (p=0.04). Furthermore, stratified by chemotherapy regimens within the CCRT group, the 3-year overall survival rates of the UFT, TS-1 and Pt groups were 96.1%, 100.0% and 94.7%, respectively (Figure 6). These differences were not statistically significant.

As for morbidity, 2 patients in the UFT group experienced severe enteritis, so-called "UFT enteritis." However, no patients in the TS-1, Pt or RT groups experienced severe enteritis. No other severe morbidities were recognized in the present study.

Discussion

UFT and TS-1 are 5-fluorouracil (5-FU) prodrugs approved by the Japanese government for head and neck carcinomas and are administered perorally not intravenously. The effectiveness of UFT and TS-1 are reported to be almost the same or somewhat superior to 5-FU in gastric, colon, and lung carcinomas (18-20). The standard treatment of unresectable advanced head and neck carcinoma is concurrent chemoradiation therapy (1). The chemotherapeutic regimens contain platinum drugs, such as cisplatin (21) and nedaplatin (22), and a combination of platinum drugs with 5-FU (21, 22) or docetaxel (23). T2N0 glottic cancer is and early stage cancer usually treated with RT alone or the combination of radiation therapy with platinum drugs, plus 5-FU and docetaxel is regarded as an over-treatment. Therefore, the combination of radiation therapy with UFT or TS-1 for T2N0 glottic cancer was administered in the current study.

Recently, Akimoto *et al.* have reported that the 5-year local control rate of T2N0 laryngeal cancer treated with CCRT was 89% (16). This was the first clinical demonstration of CCRT for T2N0 laryngeal cancer that mostly consisted of glottic cancer. However, their method adopted the chemotherapeutic regimens of cisplatin and/or docetaxel administered in an intravenous infusion. Liu *et al.* reported that patients preferred peroral rather than intravenous chemotherapy when the treatments outcomes were the same (17).

In the current study, the 5-year local control rates were 82.7% and 83.4% for the RT and CCRT groups, respectively. These are nearly the same. However, stratified by the UFT, TS-1 and Pt groups, the 3-year local control rates were 90.1%, 100.0% and 73.4%, respectively, which suggested that peroral chemotherapy was not inferior to intravenous chemotherapy but even somewhat superior. In particular, the 3-year local control rate and overall survival rate of the TS-1 group were both 100%, even though the follow-up period was 4-41 months (median: 19 months).

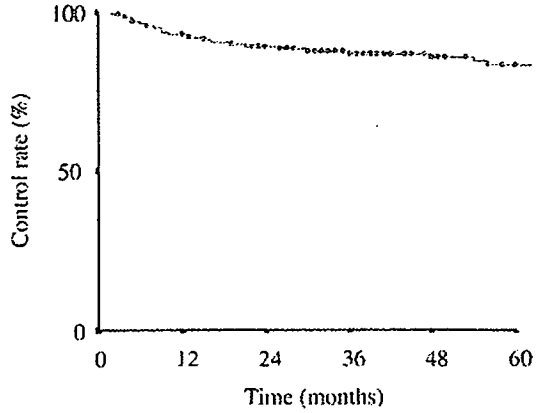

Figure 1. *Local control curve of all patients.*

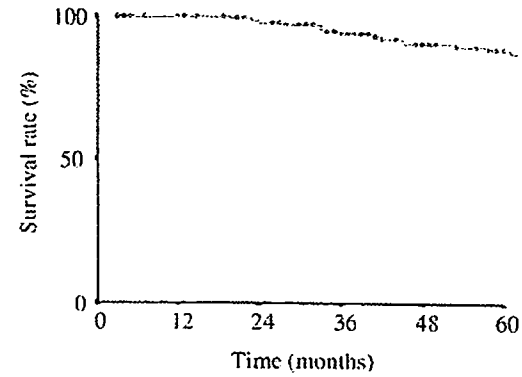

Figure 4. *Overall survival curve of all patients.*

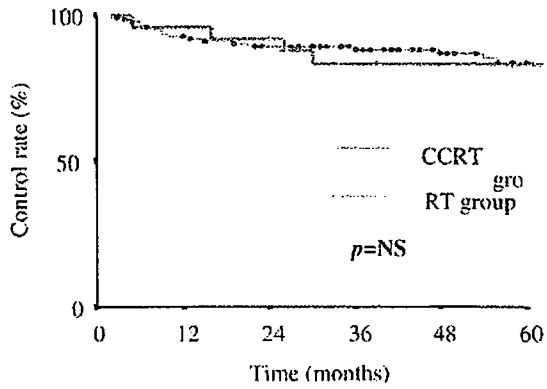

Figure 2. *Local control curves stratified by therapy. RT: radiation therapy alone, CCRT: concurrent chemoradiotherapy.*

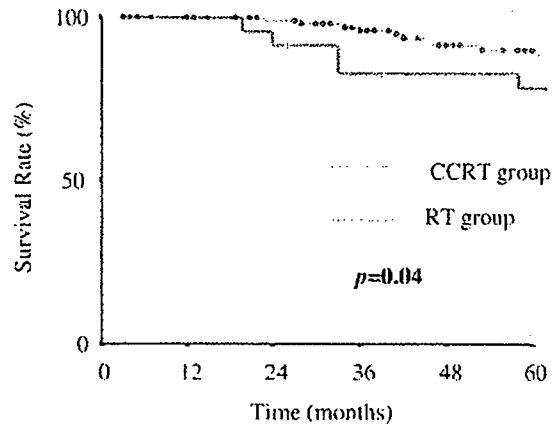

Figure 5. *Overall survival curves stratified by therapy. RT: radiation therapy alone, CCRT: concurrent chemoradiotherapy.*

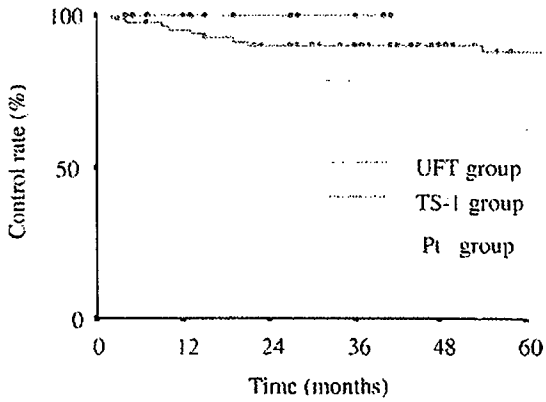

Figure 3. *Local control curves stratified by chemotherapy regime. UFT: peroral UFT, TS-1: peroral TS-1, Pt: intravenous chemotherapy.*

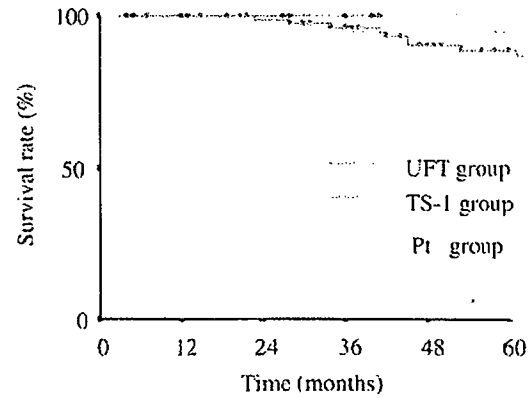

Figure 6. *Overall survival curves stratified by chemotherapy regimen. UFT: peroral UFT, TS-1: peroral TS-1, Pt: intravenous chemotherapy.*

Conclusion

Concurrent radiation therapy combined with chemotherapy could be one of the standard treatment options for T2N0 glottic cancer. Moreover, concurrent radiation therapy combined with TS-1 may prove to be a breakthrough treatment for T2N0 glottic cancer in terms of improving local control rate, overall survival and patients' burdens of treatment.

Acknowledgements

The authors thank Uemae M RT, Egawa T RT, Shudo N RT for their maintenance of the quality of the daily radiation therapy.

Conflict of interest: The authors declare there is no conflict of interest in this study.

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Received April 4, 2007
Revised June 20, 2007
Accepted June 27, 2007

Carcinogenesis vol.28 no.8 pp.1718–1725, 2007
doi:10.1093/carcin/bgm104
Advance Access publication April 29, 2007

Impact of one-carbon metabolism-related gene polymorphisms on risk of lung cancer in Japan: a case–control study

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There is substantial evidence that the decreased risk of lung cancer with high intake of vegetables and fruits is linked to folate as a specific nutrient. Functional polymorphisms in genes encoding one-carbon metabolism enzymes, methylenetetrahydrofolate reductase (*MTHFR* C677T and A1298C), methionine synthase (*MTR* A2756G), methionine synthase reductase (*MTRR* A66G) and thymidylate synthase, influence folate metabolism and thus might be suspected of impacting on lung cancer risk. We therefore conducted a case–control study with 515 lung cancer cases newly and histologically diagnosed and 1030 age- and sex-matched non-cancer controls to clarify associations with these five polymorphisms according to lung cancer subtype. Gene–environment interactions with smoking and drinking habit and folate consumption were also evaluated by logistic regression analysis. None of the polymorphisms showed any significant impact on lung cancer overall risk by genotype alone, but on histology-based analysis increase in *MTHFR* 677T and 1298C alleles was associated with reduced risk of squamous/small cell carcinoma ($P = 0.029$), especially among heavy smokers ($P = 0.035$), whereas the *MTHFR* 677TT genotype was linked to decreased risk for these subtypes among heavy drinkers (odds ratio = 0.17, 95% confidence interval: 0.03–0.98). In addition, we found interactions between the *MTRR* A66G polymorphism and smoking ($P = 0.015$) and the *MTHFR* A1298C polymorphism and alcohol consumption ($P = 0.025$) for risk of lung cancer overall. In conclusion, the results suggest that *MTHFR* polymorphisms contribute to risk of squamous/small cell carcinomas of the lung, along with possible interactions among folate metabolism-related polymorphisms and smoking/drinking habits. Further evaluation is warranted.

Introduction

Lung cancer, with its four major histological types (adenocarcinoma, squamous cell carcinoma, large cell carcinoma and small cell carcinoma), currently claims >55 000 lives annually in Japan and has become the leading cause of cancer death (1). Despite rapid advances in treatment over recent decades, the prognosis has not greatly improved. Therefore, efforts toward primary prevention in addition to early detection have come under the spotlight.

Abbreviations: CI, confidence interval; FFQ, food frequency questionnaire; 5,10-methylene THF, 5,10-methylenetetrahydrofolate; MTHFR, methylenetetrahydrofolate reductase; MTR, methionine synthase; MTRR, methionine synthase reductase; OR, odds ratio; PCR, polymerase chain reaction; 2R, two repeat; TS, thymidylate synthase; VNTR, variable number of tandem repeat.

Many epidemiological studies have provided evidence that high consumption of vegetables and fruits is associated with a reduced risk of lung cancer (2–4). Folate is one of the constituents found in vegetables and fruits, and dietary folate may be one of the micronutrients that provide protection against lung carcinogenesis (5–7).

Biological functions of folate within so-called 'one-carbon metabolism' are to facilitate *de novo* deoxynucleoside triphosphate synthesis and to provide methyl groups required for intracellular methylation reactions. Folate deficiency is thought to increase the risk of cancer through impaired DNA repair synthesis and disruption of DNA methylation that may lead to proto-oncogene activation (8–10).

Methylenetetrahydrofolate reductase (MTHFR), methionine synthase (MTR), methionine synthase reductase (MTRR) and thymidylate synthase (TS) play important and interrelated roles in folate metabolism (Figure 1). The MTHFR reduces 5,10-methylenetetrahydrofolate (5,10-methylene THF) to 5-methyl THF, the primary circulating form of folate (11). The TS catalyzes the conversion of deoxyuridine monophosphate to deoxythymidine monophosphate using 5,10-methylene THF (12). The MTHFR product, 5-methyl THF, is the methyl group donor for the remethylation of homocysteine to methionine catalyzed by MTR (13). MTR activity is maintained by MTRR (14). Polymorphisms in the genes for *MTHFR* C677T and A1298C, *MTR* A2756G, *MTRR* A66G and *TS* 28 bp variable number of tandem repeat (VNTR) in the promoter region are known to have functional relevance (15). Thus, they might play roles in the etiology of lung cancer in combination with environmental factors such as folate consumption. Since information for this area of lung cancer is limited (16–22), we conducted the present case–control study, taking tobacco smoking, alcohol drinking and intake of folate into consideration.

Materials and methods

Subjects

The cases were 515 patients who were newly and histologically diagnosed as having lung cancer and not having any earlier history of cancer. Controls ($n = 1030$) were randomly selected and matched by age (±3 years) and sex to cases with a 1:2 case–control ratio from among the 2395 cancer-free individuals. All the subjects were recruited in the framework of the Hospital-based Epidemiologic Research Program at Aichi Cancer Center, as described elsewhere (23,24). In brief, information on lifestyle factors was collected using a self-administered questionnaire, checked by a trained interviewer, from all first-visit out-patients at Aichi Cancer Center Hospital aged 18–79 who were enrolled in Hospital-based Epidemiologic Research Program at Aichi Cancer Center between January 2001 and November 2005. Out-patients were also asked to provide blood samples. Each patient was asked about his or her lifestyle when healthy or before the current symptoms developed. Approximately 95% of eligible subjects complete the questionnaire and 60% provide blood samples. The data were loaded into a Hospital-based Epidemiologic Research Program at Aichi Cancer Center database and routinely linked with the hospital-based cancer registry system to update the data on cancer incidence. All participants gave written informed consent and the study was approved by Institutional Ethical Committee of Aichi Cancer Center.

Genotyping of MTHFR, MTR, MTRR and TS

DNA from each subject was extracted from the buffy coat fraction using BioRobot EZ1 and an EZ1 DNA Blood 350 ml Kit (Qiagen, Tokyo, Japan). The genotyping method was described in our previous reports with the polymerase chain reaction (PCR) TaqMan method using the GeneAmp PCR System 9700 or the 7500 Fast Real-Time PCR system (Applied Biosystems, Foster City, CA). Briefly, for the *MTHFR* C677T (dbSNP ID: rs677) and A1298C (rs1801131), as well as *MTR* A2756G (rs1805087) and *MTRR* A66G (rs1801394) polymorphisms, extracted DNA was amplified with validated probes (assay IDs: C__11975651_10, C__850486_20, C__12005959_10 and C__3068176_10, respectively; Applied Biosystems). The *TS* VNTR polymorphism was defined by PCR using 5′-CGTGGCTCCTGCGTTTCC-3′ and 5′-GAGCCGGCCACAGGCAT-3′ primers. In our laboratory, quality of genotyping is routinely assessed statistically using the Hardy–Weinberg test.

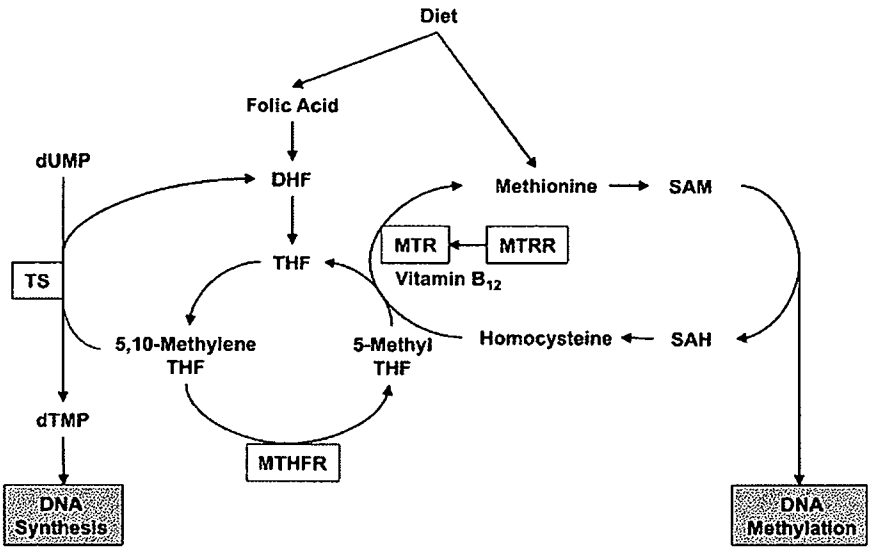

Fig. 1. Overview of folate metabolism. Enzymes with polymorphisms investigated in this study are boxed. THF, tetrahydrofolate; DHF, dihydrofolate; dUMP, deoxyuridine monophosphate; dTMP, deoxythymidine monophosphate; SAM, *S*-adenosylmethionine and SAH, *S*-adenosylhomocysteine.

When allelic distributions for controls depart from the Hardy–Weinberg frequency, genotyping is assessed using another method.

Intake assessment for folate and other nutrients

The consumption of folate and other nutrients was determined using a food frequency questionnaire (FFQ), described in detail elsewhere (25,26). Briefly, the FFQ consisted of 47 single food items with frequencies in the eight categories. We estimated the average daily intake of nutrients by multiplying the food intake (in grams) or serving size by the nutrient content per 100 g of food as listed in standard tables of food composition. Consumption of folate and other vitamins from supplements was not considered in total consumption because the questionnaire for multivitamins was not quantitative. Energy-adjusted intake of nutrients was calculated by the residual method (27). The FFQ was validated by referring to a 3-day weighed dietary record as a standard, which showed reproducibility and validity to be acceptable (28). The deattenuated correlation coefficients for energy-adjusted intakes of folate were 0.36 in men and 0.38 in women.

Consumption of tobacco and alcohol

Cumulative smoking dose was evaluated as pack-years, the product of the number of packs consumed per day and years of smoking. Smoking habit was entered for four categories of never, former and current smokers of <40 and ≥40 pack-years. Former smokers were defined as those who quit smoking at least 1 year before the survey. Consumption of each type of beverage (Japanese sake, beer, shochu, whiskey and wine) was determined by the average number of drinks per day, which was then converted into a Japanese sake (rice wine) equivalent. One drink equates to one 'go' (180 ml) of Japanese sake, which contains 23 g of ethanol, equivalent to one large bottle (633 ml) of beer, two shots (60 ml) of whiskey and two and a half glasses of wine (200 ml). One drink of 'shochu' (distilled spirit), which contains 25% ethanol, was rated as 108 ml. Total amount of alcohol consumption was estimated as the summarized amount of pure alcohol consumption (gram per drink) of Japanese sake, beer, shochu, whiskey and wine among current regular drinkers. Drinking habit was entered for four categories of never, former, current moderate and heavy drinkers. Heavy drinkers were defined as those currently drinking alcoholic beverages 5 days or more per week in a daily amount of 46 g (two Japanese drinks) or more, whereas moderate drinkers were defined as those currently consuming less frequently than 5 days/week, in lower amounts, or both. Former drinkers were defined as those who quit drinking at least 1 year before the survey. Former or current smokers and drinkers were categorized as 'smokers' and 'drinkers', respectively.

Statistical analysis

To assess the strength of the associations between polymorphic genes involved in folate metabolism and risk of lung cancer, odds ratio (ORs) with 95% confidence intervals (CIs) were estimated using age- and sex-matched conditional logistic models adjusted for potential confounders. For stratified and interaction analysis by smoking and drinking habit and folate intake, an unconditional logistic regression model was used because the matching was not retained after stratification by smoking and drinking habit and folate intake. Folate and other nutrient intakes were categorized into three groups as: first, second and third tertiles of dietary intake among controls. Potential confounders considered in the multivariate analyses were age, sex, smoking habit (never smokers, former smokers, current smokers of <40 or ≥40 pack-years), drinking habit (never drinkers, former drinkers, moderate drinkers or heavy drinkers), body mass index (<18.5, 18.5–24.9 or ≥25.0), total energy intake (as a continuous variable), dietary carotene intake (μg/day, tertiles), dietary vitamin C intake (mg/day, tertiles), dietary vitamin E intake (mg/day, tertiles), dietary folate intake (μg/day, tertiles), multivitamin use (at least once per week for 1 year or longer: yes or no) and referral pattern (patient's discretion, family recommendation, referral from other clinics, secondary screening after primary screening or others). Missing values for each covariate were treated as an additional category in the variable and were included in the logistic model.

For the histology-based analysis, we combined squamous cell carcinoma and small cell carcinoma, because tumors of these subtypes were small in number and both are consistently more related with smoking as compared with adenocarcinomas. Considering potential effects of two polymorphisms (*MTHFR* C677T and *MTHFR* A1298C) on lung risk, we evaluated associations with their combined genotypes. Trend of genotype was assessed by score test applying score for each genotype (0, homozygous for reference allele or combined reference genotypes; 1, heterozygote or one reference genotype and 2, homozygous non-reference allele or non-reference genotype).

Gene–environment interactions between smoking and drinking habit and folate intake and genotypes in each polymorphism were evaluated under the multiplicative assumption. Products of scores for genotype (0, homozygous; 1, heterozygote and 2, homozygous or 0, referent alleles and 1, non-referent alleles) and smoking habit (0, non-smoker and 1, smoker), drinking habit (0, non-drinker and 1, drinker), folate intake (0, tertile 1 and 1, tertile 2 + 3) or combined smoking–drinking habit (0, non-smoker and non-drinker; 1, smoker and non-drinker or drinker and non-smoker and 2, smoker/drinker) were included as interaction terms. Differences in categorized demographic variables between the cases and controls were tested by the Chi-squared test. Mean values for age and total energy intake were compared for cases and controls by the Student's *t*-test. Accordance with the Hardy–Weinberg equilibrium was checked for controls using the Chi-squared test and the exact *P*-value was used to assess any discrepancies between genotypes and allele frequencies. A *P*-value <0.05 was considered statistically significant. All analyses were performed using STATA version 9 (Stata Corp., College Station, TX).

Results

Data from 515 lung cancer cases, comprising 316 (61.4%) adenocarcinomas, 91 (17.7%) squamous cell carcinomas, 55 (10.7%) small

cell carcinomas, 40 (7.8%) large cell carcinomas and 13 (2.5%) others, and 1030 controls were available for analysis. Table I shows the distribution of cases and controls by background characteristics. Age and sex were appropriately matched. Smoking habits differed to a large extent between cases and controls. The proportion of 40 pack-years or more current smokers in cases was significantly higher than controls. Heavy drinkers in the cases were significantly higher than for the controls. Among cases, the proportion of lower body mass index was higher, consistent with previous study (29). Total energy intake did not differ between cases and controls. Significant lower intake of dietary carotene was found among the cases. For other nutrients lower proportions of the highest intake group among the cases also were found, including for folate, but these were not statistically significant. With regard to referral pattern, referral from other clinics was frequent, whereas patient discretion and secondary screening after primary screening were less common among the case group than the control group.

Table II shows genotype distributions for *MTHFR*, *MTR*, *MTRR* and *TS* and their ORs and 95% CIs for lung cancer risk according to histological subtypes. The genotype frequencies for all the polymorphisms were in accordance with the Hardy–Weinberg law in controls: *MTHFR* C677T (P = 0.17), *MTHFR* A1298C (P = 0.51), *MTR* A2756G (P = 0.17), *MTRR* A66G (P = 0.85) and *TS* VNTR (P = 0.51). On analysis of lung cancer overall, a slightly reduced risk was observed with the *MTHFR* 677TT genotype, but without statistical significance. The genotype frequencies for *TS* VNTR were quite varied; however, two repeat (2R) and three repeat alleles were dominant. The 2R/2R genotype showed decreased risk of lung cancer as compared with the non-2R homozygous, although again this was not significant. On subanalysis according to histological subtypes, the combination of *MTHFR* C677T and A1298C polymorphisms showed a significant decreased risk of squamous/small cell carcinoma among individuals with two or more *MTHFR* 677T and/or 1298C alleles (OR = 0.34, 95% CI: 0.13–0.92, trend P = 0.029), compared with those with *MTHFR* 677CC and 1298AA genotypes. In contrast, none of the polymorphisms showed any significant impact on adenocarcinoma risk.

To further evaluate the impact of *MTHFR* polymorphisms with regard to squamous/small cell carcinoma, we conducted stratified analysis by smoking and drinking habit (Table III). Among heavy drinkers, the *MTHFR* 677TT genotype conferred a significant decreased risk (OR = 0.17, 95% CI: 0.03–0.98, trend P = 0.041). A significant decreased risk among 40 pack-years or more current smokers was observed as number of *MTHFR* 677T or 1298C alleles increased (trend P = 0.035). No clear association was found for lung cancers overall or for adenocarcinomas in the stratified analysis (data not shown).

Table IV shows data for the combinations of gene and environmental factors with reference to lung cancer overall risk. The interaction with smoking was significant for the *MTRR* A66G genotype (P = 0.015). Among non-smokers, risk was reduced with increase in the number of *MTRR* G alleles, whereas a trend for increased risk was observed among smokers. A significant interaction between drinking habits and the *MTHFR* A1298C genotype was found (P = 0.025). These two interactions were especially noteworthy for adenocarcinomas when histology-based analyses were conducted (data not shown). We were not able to analyze the smoking interaction for squamous/small cell due to insufficient number of non-smokers in this category. No obvious interaction was found between folate intake and the polymorphisms.

Considering the possible effects of both tobacco smoking and alcohol drinking on folate, we further examined the impact of four-way combinations of these two factors, folate intake and the polymorphisms on lung cancer risk (Table V). The *MTRR* A66G genotype showed a significant interaction among the subjects with tertiles 2 or 3 of folate intake (P = 0.023). The risk with the *MTRR* 66GG was consistently decreased among non-smoker/non-drinker subjects with adequate folate intake (OR = 0.20, 95% CI: 0.04–0.91).

Table I. Characteristics of cases and controls

	Cases (n = 515), n(%)	Controls (n = 1030), n(%)	P-value
Age			
<50	53 (10.3)	108 (10.5)	
50–59	142 (27.6)	283 (27.5)	
60–69	193 (37.5)	389 (37.8)	
70–79	127 (24.7)	250 (24.3)	1.00
Mean age ± SD	61.9 ± 9.9	61.8 ± 9.8	0.87
Sex			
Male	381 (74.0)	762 (74.0)	
Female	134 (26.0)	268 (26.0)	1.00
Smoking status			
Never	129 (25.0)	401 (38.9)	
Former[a]	111 (21.6)	310 (30.1)	
Current (pack-years)			
0–39	71 (13.8)	149 (14.5)	
≥40	197 (38.3)	161 (15.6)	<0.01
Unknown	7 (1.4)	9 (0.9)	
Drinking status			
Never	196 (38.1)	378 (36.7)	
Former[a]	15 (2.9)	56 (5.4)	
Current			
Moderate[b]	192 (37.3)	454 (44.1)	
Heavy[c]	98 (19.0)	119 (11.6)	<0.01
Unknown	14 (2.7)	23 (2.2)	
BMI			
<18.5	38 (7.4)	55 (5.3)	
18.5–24.9	381 (74.0)	720 (69.9)	
≥25.0	94 (18.3)	249 (24.2)	0.03
Unknown	2 (0.4)	6 (0.6)	
Mean total energy ± SD, kcal/day	1670 ± 372	1677 ± 352	0.73
Carotene (μg/day)			
Tertile 1 (1331.2–2305.9)	200 (38.8)	341 (33.1)	
Tertile 2 (2306.0–3312.6)	149 (28.9)	341 (33.1)	
Tertile 3 (3312.7–12801.4)	158 (30.7)	341 (33.1)	0.04
Unknown	8 (1.6)	7 (0.7)	
Vitamin C (mg/day)			
Tertile 1 (26.8–74.5)	188 (36.5)	342 (33.2)	
Tertile 2 (74.6–102.0)	161 (31.3)	342 (33.2)	
Tertile 3 (102.1–364.5)	159 (30.7)	341 (33.1)	0.15
Unknown	7 (1.4)	5 (0.5)	
Vitamin E (total α-mg/day)			
Tertile 1 (1.5–4.8)	193 (37.5)	342 (33.2)	
Tertile 2 (4.9–6.3)	168 (32.6)	342 (33.2)	
Tertile 3 (6.4–17.1)	151 (29.3)	342 (33.2)	0.29
Unknown	3 (0.6)	4 (0.4)	
Folate intake (μg/day)			
Tertile 1 (139.5–274.5)	191 (37.1)	342 (33.2)	
Tertile 2 (274.6–354.9)	156 (30.3)	342 (33.2)	
Tertile 3 (355.0–1481.0)	162 (31.5)	341 (33.1)	0.18
Unknown	6 (1.2)	5 (0.5)	
Multivitamin use (at least once per week for 1 year or longer)			
Yes	111 (21.6)	253 (24.6)	
No	380 (73.8)	721 (70.0)	0.30
Unknown	24 (4.7)	56 (5.4)	
Referral pattern to our hospital			
Patient's discretion	52 (10.1)	306 (29.7)	
Family recommendation	86 (16.7)	195 (18.9)	
Referral from other clinics	287 (55.7)	300 (29.1)	
Secondary screening after primary screening	83 (16.1)	214 (20.8)	
Others	2 (0.4)	10 (1.0)	<0.01
Unknown	5 (1.0)	5 (0.5)	

SD: standard deviation, BMI: body mass index.
[a]Former smokers and drinkers were defined as subjects who had quit smoking and drinking at least 1 year previously.
[b]Moderate drinker means <46 g ethanol/drink and/or <5 days/week.
[c]Heavy drinker means ≥46 g ethanol/drink and ≥5 days/week.

Table II. *MTHFR*, *MTR*, *MTRR* and *TS* genotype distributions, and ORs for lung cancer according to histology

	All			Adenocarcinoma			Squamous + small cell carcinoma		
	Cases (n = 515), n (%)	Controls (n = 1030), n (%)	ORs[a] (95% CIs)	Cases (n = 316), n (%)	Controls (n = 632), n (%)	ORs[a] (95% CIs)	Cases (n = 146), n (%)	Controls (n = 292), n (%)	ORs[a] (95% CIs)
MTHFR (C677T)									
CC	182 (35.3)	379 (36.8)	1.00 (ref.)	109 (34.5)	237 (37.5)	1.00 (ref.)	54 (37.0)	103 (35.3)	1.00 (ref.)
CT	256 (49.7)	474 (46.0)	1.05 (0.81–1.37)	158 (50.0)	288 (45.6)	1.01 (0.72–1.41)	72 (49.3)	134 (45.9)	0.83 (0.43–1.58)
TT	77 (15.0)	177 (17.2)	0.75 (0.52–1.09)	49 (15.5)	107 (16.9)	0.85 (0.53–1.34)	20 (13.7)	55 (18.8)	0.44 (0.16–1.18)
P_{trend}[b]			0.260			0.567			0.129
MTHFR (A1298C)									
AA	341 (66.2)	652 (63.3)	1.00 (ref.)	210 (66.5)	416 (65.8)	1.00 (ref.)	94 (64.4)	175 (59.9)	1.00 (ref.)
AC	149 (28.9)	322 (31.3)	0.85 (0.65–1.13)	90 (28.5)	189 (29.9)	0.94 (0.67–1.33)	46 (31.5)	99 (33.9)	0.84 (0.42–1.68)
CC	22 (4.3)	45 (4.4)	1.01 (0.56–1.83)	14 (4.4)	22 (3.5)	1.46 (0.68–3.16)	5 (3.4)	14 (4.8)	0.40 (0.07–2.28)
UK[c]	3 (0.6)	11 (1.1)		2 (0.6)	5 (0.8)		1 (0.7)	4 (1.4)	
P_{trend}[b]			0.428			0.822			0.348
MTHFR C677T and A1298C combined Number of Variants									
0	76 (14.8)	174 (16.9)	1.00 (ref.)	43 (13.6)	118 (18.7)	1.00 (ref.)	23 (15.8)	41 (14.0)	1.00 (ref.)
1	273 (53.0)	471 (45.7)	1.19 (0.83–1.71)	171 (54.1)	293 (46.4)	1.46 (0.93–2.27)	76 (52.1)	130 (44.5)	0.52 (0.19–1.40)
≦2	163 (31.7)	374 (36.3)	0.84 (0.58–1.24)	100 (31.6)	216 (34.2)	1.10 (0.68–1.77)	46 (31.5)	117 (40.1)	0.34 (0.13–0.92)
UK[c]	3 (0.6)	11 (1.1)		2 (0.6)	5 (0.8)		1 (0.7)	4 (1.4)	
P_{trend}[b]			0.110			0.819			0.029
MTR (A2756G)									
AA	319 (61.9)	698 (67.8)	1.00 (ref.)	192 (60.8)	423 (66.9)	1.00 (ref.)	100 (68.5)	195 (66.8)	1.00 (ref.)
AG	175 (34.0)	291 (28.3)	1.23 (0.94–1.60)	109 (34.5)	184 (29.1)	1.26 (0.91–1.75)	42 (28.8)	84 (28.8)	0.80 (0.42–1.52)
GG	21 (4.1)	40 (3.9)	1.04 (0.55–2.00)	15 (4.7)	25 (4.0)	1.35 (0.62–2.91)	4 (2.7)	13 (4.5)	0.49 (0.07–3.38)
UK[c]	0 (0)	1 (0.1)							
P_{trend}[b]			0.227			0.146			0.364
MTRR (A66G)									
AA	235 (45.6)	484 (47.0)	1.00 (ref.)	148 (46.8)	294 (46.5)	1.00 (ref.)	63 (43.2)	136 (46.6)	1.00 (ref.)
AG	226 (43.9)	446 (43.3)	1.02 (0.79–1.31)	139 (44.0)	275 (43.5)	0.93 (068–1.28)	64 (43.8)	131 (44.9)	1.18 (0.60–2.31)
GG	54 (10.5)	100 (9.7)	0.96 (0.62–1.47)	29 (9.2)	63 (10.0)	0.91 (0.52–1.58)	19 (13.0)	25 (8.6)	1.11 (0.38–3.21)
P_{trend}[b]			0.939			0.638			0.718
TS VNTR									
Non-2R/non-2R	372 (72.2)	721 (70.0)	1.00 (ref.)	236 (74.7)	434 (68.7)	1.00 (ref.)	101 (69.2)	212 (72.6)	1.00 (ref.)
2R/non-2R	132 (25.6)	278 (27.0)	0.96 (0.73–1.27)	73 (23.1)	181 (28.6)	0.81 (0.57–1.13)	43 (29.5)	69 (23.6)	1.26 (0.61–2.59)
2R/2R	10 (1.9)	31 (3.0)	0.63 (0.29–1.39)	6 (1.9)	17 (2.7)	0.62 (0.22–1.73)	2 (1.4)	11 (3.8)	0.23 (0.03–1.62)
UK[c]	1 (0.2)	0 (0)		1 (0.3)	0 (0)				
P_{trend}[b]			0.394			0.137			0.653

[a]ORs were matched for age and sex and adjusted for smoking habit, drinking habit, body mass index, total energy intake, carotene intake, vitamin C intake, vitamin E intake, multivitamin use and referral pattern to our hospital.

[b]Trend of genotype was assessed by score test applying score for each genotypes (0, homozygous for reference allele or combined reference genotypes; 1, heterozygote or one reference genotype and 2, homozygous non-reference allele or non-reference genotype).

[c]UK denotes genotype unknown.

Table III. Stratification analysis by smoking and drinking habit for the *MTHFR* polymorphisms in squamous/small cell carcinoma

	Smoking status						Drinking status							
	Smokers		0–39 pack-years		40 ≥ pack-years		Non-drinkers		Drinkers		Moderate drinkers		Heavy drinkers	
	Cases/ controls	ORs[a] (95% CIs)	Cases/ controls	ORs[a] (95% CIs)	Cases/ controls	ORs[a] (95% CIs)	Cases/ controls	ORs[a] (95% CIs)	Cases/ controls	ORs[a] (95% CIs)	Cases/ controls	ORs[a] (95% CIs)	Cases/ controls	ORs[a] (95% CIs)
MTHFR (C677T)														
CC	53/229	1.00 (ref.)	7/65	1.00 (ref.)	35/58	1.00 (ref.)	15/143	1.00 (ref.)	39/236	1.00 (ref.)	22/168	1.00 (ref.)	14/38	1.00 (ref.)
CT	72/293	0.94 (0.61–1.45)	10/65	1.83 (0.40–8.47)	41/73	0.85 (0.43–1.67)	23/176	0.89 (0.36–2.21)	49/298	1.08 (0.63–1.84)	26/206	1.02 (0.48–2.16)	14/61	0.37 (0.09–1.63)
TT	20/107	0.81 (0.44–1.51)	4/19	5.63 (0.60–53.25)	11/30	0.50 (0.20–1.29)	3/59	0.52 (0.09–3.00)	17/118	0.90 (0.44–1.84)	8/80	0.85 (0.30–2.43)	5/20	0.17 (0.03–0.98)
P_{trend}[b]		0.528		0.151		0.179		0.519		0.871		0.817		0.041
MTHFR (A1298C)														
AA	93/406	1.00 (ref.)	15/106	1.00 (ref.)	57/97	1.00 (ref.)	29/232	1.00 (ref.)	65/420	1.00 (ref.)	36/293	1.00 (ref.)	18/74	1.00 (ref.)
AC	46/186	1.04 (0.68–1.61)	5/33	0.84 (0.15–4.63)	26/55	0.68 (0.35–1.33)	11/123	0.67 (0.26–1.77)	35/199	1.17 (0.69–2.01)	18/139	0.99 (0.46–2.11)	13/40	3.61 (0.87–14.96)
CC	5/30	0.80 (0.28–2.26)	0/8	NA[d]	4/9	0.84 (0.21–3.31)	1/20	0.30 (0.02–3.82)	4/25	1.05 (0.31–3.51)	2/17	1.03 (0.19–5.74)	2/5	1.05 (0.08–13.61)
UK[c]	1/7		1/2				0/3		1/8		0/5			
P_{trend}[b]		0.916		0.381		0.362		0.263		0.649		0.998		0.300
MTHFR C677T and A1298C combined Number of Variants														
0	22/105	1.00 (ref.)	3/37	1.00 (ref.)	15/22	1.00 (ref.)	6/64	1.00 (ref.)	17/110	1.00 (ref.)	13/81	1.00 (ref.)	4/15	1.00 (ref.)
1	76/295	1.08 (0.61–1.92)	11/72	0.63 (0.10–3.98)	47/73	0.97 (0.39–2.43)	28/169	1.02 (0.30–3.50)	48/302	1.13 (0.57–2.27)	22/211	0.57 (0.23–1.44)	17/58	0.93 (0.12–6.96)
≦2	46/222	0.88 (0.48–1.63)	6/38	2.40 (0.30–18.97)	25/66	0.44 (0.17–1.17)	7/142	036 (0.08–1.56)	39/232	1.15 (0.56–2.36)	21/157	0.82 (0.32–2.13)	12/46	0.30 (0.04–2.55)
UK[c]	1/7		1/2				0/3		1/8		0/5			
P_{trend}[b]		0.557		0.397		0.035		0.109		0.751		0.860		0.148

Data were not available in non-smokers because of absence of subjects in this category.

[a]ORs were adjusted for age, sex, smoking habit, drinking habit, body mass index, total energy intake, carotene intake, vitamin C intake, vitamin E intake, folate intake, multivitamin use and referral pattern to our hospital.

[b]Trend of genotype was assessed by score test applying score for each genotypes (0, homozygous for reference allele; 1, heterozygote and 2, homozygous non-reference allele).

[c]UK denotes genotype unknown.

[d]NA indicates not available because of absence of subjects in this category.

Table IV. Interaction between *MTHFR, MTR, MTRR* and *TS* polymorphisms and smoking and drinking habit and folate intake for lung cancer risk

	Smoking habit			Drinking habit			Folate intake		
	Non-smoker	Smoker	*P* interaction[b]	Non-drinker	Drinker	*P* interaction[b]	Tertile 1 (139.5–274.5 μg/day)	Tertile 2 + 3 (274.6–1481.0 μg/day)	*P* interaction[b]
	ORs[a] (95% CIs)	ORs[a] (95% CIs)		ORs[a] (95% CIs)	ORs[a] (95% CIs)		ORs[a] (95% CIs)	ORs[a] (95% CIs)	
MTHFR (C677T)									
CC	1.00 (ref.)	2.59 (1.61–4.17)		1.00 (ref.)	1.02 (0.67–1.55)		1.00 (ref.)	0.82 (1.34–0.82)	
CT	1.09 (0.69–1.73)	2.84 (1.79–4.52)		1.17 (0.77–1.78)	1.07 (0.72–1.60)		1.03 (1.58–1.03)	0.93 (1.50–0.93)	
TT	0.68 (0.36–1.29)	2.55 (1.48–4.40)	0.430	1.21 (0.68–2.14)	0.74 (0.45–1.23)	0.207	0.98 (1.82–0.98)	0.69 (1.22–0.69)	0.851
MTHFR (A1298C)									
AA	1.00 (ref.)	2.80 (1.88–4.18)		1.00 (ref.)	0.76 (0.56–1.04)		1.00 (ref.)	0.81 (1.24–0.81)	
AC	0.88 (0.55–1.39)	2.61 (1.67–4.07)		0.73 (0.48–1.12)	0.77 (0.53–1.11)		0.73 (1.14–0.73)	0.80 (1.27–0.80)	
CC	1.60 (0.61–4.19)	1.84 (0.85–3.96)	0.464	0.36 (0.12–1.04)	1.20 (0.58–2.47)	0.025	1.72 (4.44–1.72)	0.52 (1.18–0.52)	0.824
MTR (A2756G)									
AA	1.00 (ref.)	3.08 (2.07–4.57)		1.00 (ref.)	0.93 (0.68–1.28)		1.00 (ref.)	0.93 (1.40–0.93)	
AG	1.65 (1.05–2.59)	3.49 (2.26–5.40)		1.55 (1.03–2.33)	1.12 (0.77–1.61)		1.56 (2.40–1.56)	1.08 (1.70–1.08)	
GG	0.90 (0.27–2.94)	3.08 (1.45–6.53)	0.348	0.76 (0.27–2.14)	1.18 (0.54–2.56)	0.798	1.24 (3.45–1.24)	0.85 (1.92–0.85)	0.285
MTRR (A66G)									
AA	1.00 (ref.)	2.04 (1.31–3.16)		1.00 (ref.)	1.21 (0.83–1.76)		1.00 (ref.)	0.73 (1.15–0.73)	
AG	0.70 (0.45–1.09)	2.31 (1.49–3.58)		1.53 (1.02–2.27)	0.90 (0.61–1.32)		0.75 (1.14–0.75)	0.83 (1.31–0.83)	
GG	0.46 (0.20–1.09)	2.60 (1.49–4.56)	0.015	0.88 (0.45–1.73)	1.18 (0.67–2.06)	0.212	1.41 (2.82–1.41)	0.58 (1.09–0.58)	0.907
TS VNTR									
Non-2R/non-2R	1.00 (ref.)	2.62 (1.78–3.85)		1.00 (ref.)	0.93 (0.69–1.26)		1.00 (ref.)	0.88 (1.32–0.88)	
2R/non-2R	0.84 (0.51–1.37)	2.52 (1.64–3.88)		1.01 (0.65–1.55)	0.85 (0.58–1.24)		1.02 (1.60–1.02)	0.80 (1.28–0.80)	
2R/2R	0.60 (0.15–2.36)	1.70 (0.64–4.53)	0.668	0.82 (0.19–3.53)	0.44 (0.17–1.12)	0.550	0.66 (2.62–0.66)	0.48 (1.29–0.48)	0.673

[a]ORs were adjusted for age, sex, smoking habit, drinking habit, body mass index, total energy intake, carotene intake, vitamin C intake, vitamin E intake, folate intake, multivitamin use and referral pattern to our hospital.
[b]Interaction was modeled as a product of smoking habit (0, non-smoker and 1, smoker), drinking habit (0, non-drinker and 1, drinker), folate intake in score (0, tertile 1 and 1, textile 2 + 3) and genotype in score.

Discussion

The present study showed a significant impact of *MTHFR* C677T and *MTHFR* A1298C in combination for risk of the most smoking related subtypes of lung cancer, squamous and small cell carcinomas. Moreover, this effect was prominent among heavy smokers. The *MTHFR* 677TT genotype was inversely associated with squamous/small cell carcinoma risk among heavy drinkers. In combination analysis of smoking, drinking and folate consumption, several potential gene–environment interactions were suggested, between (i) the *MTRR* A66G polymorphism and smoking and (ii) the *MTHFR* A1298C polymorphism and alcohol consumption.

High dietary intake of folate has been found to decrease the risk of lung cancer in several epidemiological studies (5–7). Although our result for folate did not reach statistically significance, the observed trend was accordant with other studies. Two small-sized clinical trials found folate and vitamin B_{12} supplementation to reverse atypia among patients with bronchial squamous metaplasia, a precursor of squamous cell carcinoma of the lung (30,31). One might therefore hypothesize a protective effect of folate on lung cancer, but there are also epidemiological studies providing no support for this concept (32–35). Considering the fact that functional polymorphisms in folate-related genes may contribute to alteration of folate metabolism (15), it is biologically plausible to hypothesize that the polymorphisms or the gene–environment interactions rather than the folate intake alone have the impact on lung cancer risk.

Hitherto, only a few studies have investigated associations between one-carbon metabolism-related gene polymorphisms and lung cancer risk. The *MTHFR* 677TT genotype has been reported to decrease risk of lung cancer in female Caucasians (20), but the results were inconsistent in other case–control studies (17,19). The *MTHFR* 1298CC and *MTRR* 66AG or GG genotypes were associated with significantly increased risk (20,21), whereas *MTR* and *TS* enhancer region polymorphisms in the Caucasians studies demonstrated no link (21,22). Our results of overall analysis added evidence for a null association in this controversial issue. However, of note in this study was the fact that *MTHFR* 677T and/or *MTHFR* 1298C alleles were associated with reduced risk of squamous/small cell carcinomas, especially among heavy smokers and drinkers. It has been shown that subjects with the *MTHFR* 677TT and *MTHFR* 1298CC genotypes have a reduction in enzyme activity compared with the wild-type homozygous, 677CC and 1298AA genotypes (36–38). This would lead to high 5,10-methylene THF concentrations, which may provide more one-carbon groups for thymidylate synthesis, thereby enhancing DNA synthesis and repair ability. Thus, it is biologically reasonable that individuals harboring the *MTHFR* 677T and *MTHFR* 1298C alleles among heavy smokers and drinkers have lower risk of squamous/small cell carcinoma development, given that carcinogenesis is strongly related with the accumulation of DNA damage. To our knowledge, this is the first indication of protective effects of combinations of *MTHFR* polymorphisms for this histologic subtype. These data provide support for the hypothesis of links between one-carbon metabolism and tobacco and alcohol influence on squamous/small cell carcinoma carcinogenesis. Regarding other body sites, our previous study on esophagus cancers, which are almost all squamous cell carcinomas in Japan, demonstrated that the *MTHFR* 677TT had the protective effects among heavy drinkers, consistent with the present study (39).

One difficulty exists in distinguishing effects of smoking and drinking on lung cancer risk. In the present study, of 33 heavy drinkers in squamous/small cell carcinoma cases, 24 (72.7%) cases were heavy smokers, so we may not conclude an independent protective effect of *MTHFR* 677TT genotypes among heavy drinkers, although adjustment for smoking habits was performed. On the other hand, all cases with squamous/small cell carcinomas were smokers except one and 60% (85/142) in this subtype were heavy smokers (40 pack-years or more). Alcohol drinking as well as tobacco smoking is considered to induce DNA damage and resultant modification of nucleotides (40,41). In addition, high intake of alcohol can lead to folate depletion

Table V. Impact of combination of smoking and drinking habit by folate intake and the polymorphisms on lung cancer risk

	Folate intake							
	Tertile 1 (139.5–274.5 μg/day)				Tertile 2 + 3 (274.6–1481.0 μg/day)			
	Non-smoker/non-drinker	Smoker/non-drinker or non-smoker/drinker	Smoker/drinker[a]	*P* interaction[c]	Non-smoker/non-drinker	Smoker/non-drinker or non-smoker/drinker	Smoker/drinke[a]	*P* interaction[c]
	ORs[b] (95% CIs)	ORs[b] (95% CIs)	ORs[b] (95% CIs)		ORs[b] (95% CIs)	ORs[b] (95% CIs)	ORs[b] (95% CIs)	
MTHFR (C677T)								
CC	1.00 (ref.)	1.33 (0.44–3.99)	1.60 (0.58–4.41)		1.00 (ref.)	1.82 (0.90–3.67)	2.89 (1.43–5.84)	
CT + TT	0.61 (0.19–1.98)	1.66 (0.62–4.48)	1.55 (0.58–4.18)	0.763	1.54 (0.83–2.89)	1.48 (0.79–2.80)	2.93 (1.50–5.73)	0.389
MTHFR (A1298C)								
AA	1.00 (ref.)	3.18 (1.20–8.42)	2.78 (1.06–7.27)		1.00 (ref.)	0.97 (0.57–1.65)	1.70 (0.97–3.00)	
AC + CC	1.68 (0.52–5.48)	1.63 (0.55–4.81)	2.33 (0.84–6.47)	0.701	0.56 (0.29–1.08)	1.04 (0.56–1.91)	2.04 (1.10–3.76)	0.078
MTR (A2756G)								
AA	1.00 (ref.)	2.13 (0.90–5.04)	1.95 (0.83–4.62)		1.00 (ref.)	1.11 (0.64–1.92)	2.74 (1.53–4.89)	
AG + GG	1.63 (0.46–5.72)	3.00 (1.12–8.00)	3.28 (1.34–8.02)	0.826	1.38 (0.74–2.55)	2.03 (1.09–3.77)	2.16 (1.15–4.05)	0.069
MTRR (A66G)								
AA + AG	1.00 (ref.)	2.17 (1.00–4.73)	2.27 (1.05–4.91)		1.00 (ref.)	1.14 (0.71–1.82)	1.98 (1.18–3.32)	
GG	3.96 (0.49–32.23)	3.19 (1.02–10.03)	2.77 (0.88–8.78)	0.384	0.20 (0.04–0.91)	0.67 (0.24–1.88)	2.34 (1.12–4.90)	0.023
TS VNTR								
Non-2R/non-2R	1.00 (ref.)	1.75 (0.74–4.16)	2.14 (0.92–4.96)		1.00 (ref.)	0.95 (0.56–1.60)	2.04 (1.17–3.55)	
2R/non-2R + 2R/2R	0.87 (0.24–3.07)	2.38 (0.92–6.18)	1.48 (0.57–3.82)	0.367	0.58 (0.28–1.20)	1.30 (0.71–2.39)	1.65(0.90–3.05)	0.769

[a]Subjects who are both smoker and drinker.
[b]ORs were adjusted for age, sex, body mass index, total energy intake, carotene intake, vitamin C intake, vitamin E intake, multivitamin use and referral pattern to our hospital.
[c]Interaction was modeled as a product of smoking/drinking habit (0, non-smoker/non-drinker; 1, smoker/non-drinker or drinker/non-smoker and 2, smoker/drinker) and genotype in score.

(42). Therefore, it is within expectation that the *MTHFR* 677T allele, associated with high 5,10-methylene THF concentrations, may have the potential to protect against squamous/small cell carcinomas in tobacco consumers drinking large amounts of alcohol.

It was previously reported that lung cancer risk is higher with the *MTRR* 66AG/GG genotypes than the *MTRR* 66AA genotype among former smokers, but this did not extend to never and current smokers (21). Here, interaction between this gene and smoking habit was observed. Furthermore, the *MTRR* GG genotype exhibited a protective effect in low-risk subjects (non-smokers/non-drinkers with adequate folate intake). Several cytogenetic biomarker studies suggested that some polymorphisms involved in metabolic activation/deactivation or in DNA repair have been expected to be of special importance in modulating tobacco and alcohol carcinogen effects (43). A recent study reported a positive association with the modulating effect of the *MTRR* polymorphism on micronucleus frequency in peripheral blood lymphocytes, one of the cytogenetic markers (44), which is probably to increase by smoking (45) and drinking (46). The higher micronucleus frequency recorded in *MTRR* 66GG genotype with respect to AG or AA genotype is suggestive of a role of this polymorphism in modulation of chromosome stability, so that the findings may be consistent with our results. Further studies on the underlying mechanisms of the *MTRR* polymorphism thus appear warranted.

We found an interaction between the drinking habit and *MTHFR* A1298C polymorphisms for lung cancer risk, with decreased risk among non-drinkers. A Caucasian study showed that the *MTHFR* 1298CC genotype elevated risk among both drinkers and non-drinkers but only in women (20). The *MTHFR* A1298C is associated with decreased enzymatic activity (37,38) and would be expected to exert a similar effect to *MTHFR* C677T, with mutant alleles more protective among drinkers (27,39). There is no clear biological explanation for our results, and we cannot rule out the possibility that our observations for *MTHFR* A1298C were due to chance. Replication in a future study is needed.

Several potential limitations of the present study warrant consideration. First, internal validity of this hospital-based study is a potential threat to causal inference. We used non-cancer patients at our hospital as controls, given the likelihood that our cases arose within this population base, but individuals selected randomly from our control population were earlier shown to be similar to the general population in terms of the exposure of interest (47). Equivalence in the genotype distribution for the *MTHFR* C677T polymorphism between our controls and the general population has also been reported (48). To account for variation between cases and controls, we adjusted for referral pattern to our hospital. Second, as with other case–control studies, this study may suffer from recall bias. Although the questionnaires were completed before the diagnosis in our hospital, in some cases, patients referred from other institutions might have known the diagnosis. Third, we used a self-administered questionnaire to evaluate the nutrients intake, including folate. Data obtained from FFQ may not reflect intake as accurately as those from other methods, such as biological markers. We could not find any association with intake of vitamin C and E or folate for lung cancer risk, contrasting with our previous demonstration using the same population of protective effects of vegetables and fruits (4). The estimation of consumption by FFQ may be one possible explanation for this apparent anomaly. However, the reproducibility and validity of the FFQ were acceptable (28). We could not consider consumption of folate from supplements in total consumption, but the proportion of user with folate supplement is very low in Japan (0.1%) (49). Lastly, the limited number of cases, especially in subanalysis, is another factor and replication of our findings in a larger study is warranted.

In conclusion, we observed significant associations between *MTHFR* C677T and combined *MTHFR* C677T/A1298C polymorphisms and squamous/small cell carcinoma risk among heavy smokers and drinkers. Moreover, interactions between *MTRR* polymorphisms and smoking as well as the *MTHFR* A1298C polymorphism and alcohol consumption were also suggested. Our results thus support the hypothesis that folate metabolism-related gene polymorphisms may play a role in the genesis of lung cancer in combination with environmental factors. Replication in large epidemiological studies as well as studies of the mechanisms of the metabolisms is to be recommended.

Acknowledgements

Authors are grateful to the staff of the Division of Epidemiology and Prevention at Aichi Cancer Center Research Institute for their assistance. The study was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, Culture and Technology of Japan and by a Grant-in-Aid for the Third Term Comprehensive 10-Year Strategy for Cancer Control from the Ministry of Health, Labour and Welfare of Japan.

Conflict of Interest statement: None declared.

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Received February 19, 2007; revised April 4, 2007; accepted April 21, 2007

and second-line and subsequent chemotherapy regimens.[5] The tumor response was evaluated according to Response Evaluation Criteria in Solid Tumors (RECIST) using existing images and graded as complete response, partial response, stable disease, progressive disease or not evaluable.

Treatment. Patients received carboplatin and paclitaxel as first-line chemotherapy. Patients received paclitaxel 200 mg/m^2 as a 3-h intravenous infusion, followed by carboplatin AUC 6 (Calvert's setting) as a 1-h infusion on Day 1. Courses of treatment were repeated every 3 or 4 weeks for 4–6 cycles, until disease progression or severe toxicity. When a patient developed National Cancer Institute Common Toxicity Criteria (NCI-CTC) grade 3 non-hematological toxicity (except nausea and anorexia) after the start of treatment, the dose was reduced to carboplatin AUC 5 + paclitaxel 150 mg/m^2.

Statistical analysis. Kaplan-Meier plots were prepared for OS and PFS and median values were calculated. OS was measured from the first day of first-line treatment to the day of death or the day last seen alive (cut-off). PFS was measured from the first day of first-line treatment to the earliest observation of documented progressive disease, or the day of death if the patient died before observation of progressive disease. Univariate and multivariate analyses were performed for OS and PFS stratified by baseline factors. To identify factors influencing PFS and OS, multivariate analysis was performed with covariates including disease stage (IIIB versus IV), histology (adenocarcinoma versus other), smoking history (non-smoker versus smoker), gender (female versus male) and PS (0 versus 1). Multivariate analysis was performed by the stepwise regression method using a Cox proportional hazards model. To evaluate potential interaction between clinical variables such as smoking history or histology and EGFR-TKI treatment, patients who received second-line therapy were included in subsequent exploratory Cox analysis in which non-smokers and adnocarcinoma patients were divided by EGFR-TKI treatment, with smokers and nonadnocarcinoma patients set as references, respectively. Statistical analyses for this study were conducted using the Stat View software statistical tool.

Results

Patient characteristics. In total, 98 patients met the eligibility criteria and their demographic data are presented in Table 1. The majority of patients were male (64%), had a smoking history (69%), adenocarcinoma histology (76%), stage IV disease (70%) and PS 0 (57%). The median duration of first-line carboplatin/paclitaxel therapy was 3 cycles (range, 1–6 cycles). The median follow-up time was 24.8 months (range: 4.2–43.9). 57 patients died. 41 patients were still alive.

Table 1. Patient demographics ($n = 98$)

Gender *n* (%)	
Male	63 (64)
Female	35 (36)
Median (range) age, years	61 (34–78)
ECOG PS, *n* (%)	
0	56 (57)
1	42 (43)
Smoking history, *n* (%)	
Smoker	68 (69)
Non-smoker	30 (31)
Histology, *n* (%)	
Adenocarcinoma	74 (76)
Other	24 (24)
Stage, *n* (%)	
IIIB	29 (30)
IV	69 (70)

ECOG, European Cooperative Oncology Group; PS, performance status.

Table 2. Best overall objective response, *n* (%)

	Total population ($n = 98$)	By histology	
		Adenocarcinoma ($n = 74$)	Other ($n = 24$)
Partial response	20 (20)	15 (20)	5 (21)
Stable disease	53 (54)	42 (57)	11 (46)
Progressive disease	25 (26)	17 (23)	8 (33)

Efficacy. The overall response rate to first-line carboplatin/paclitaxel therapy was 20% (20/98), with outcomes similar in patients with adenocarcinoma and other histological subtypes (20% versus 21%, respectively) (Table 2). In the overall population, median PFS was 4.8 months and median OS 16.5 months, with a 1-year survival rate of 64%.

For PFS, only disease stage was a significant prognostic factor (Table 3). For OS, histology, smoking history and PS were significant prognostic factors (Table 3).

Multivariate analyses assessing the effects of histology and smoking history on PFS and OS were performed. No significant difference was observed for PFS between adenocarcinoma versus other histology ($P = 0.40$; Fig. 1) or non-smokers versus smokers ($P = 0.22$; Fig. 2). In contrast, OS differed significantly between adenocarcinoma versus other histology ($P = 0.0017$)

Table 3. Efficacy among patient subgroups: Cox regression analysis

Factor	Variable	PFS *P*-value HR (95% CI)	OS *P*-value HR (95% CI)
Histology	Adenocarcinoma versus other	0.2045 –	0.0020 0.410 (0.233–0.723)
Smoking	Non-smoker versus smoker	0.1351 –	<0.0001 0.222 (0.109–0.450)
Gender	Female versus male	0.2206 –	0.2691 –
PS	0 versus 1	0.9575 –	0.0109 0.499 (0.292–0.852)
Stage	IIIB versus IV	0.0074 0.536 (0.339–0.847)	0.2024 –

PFS, progression-free survival; OS, overall survival; HR, hazard ratio; CI, confidence interval; NS, not significant; PS, performance status.

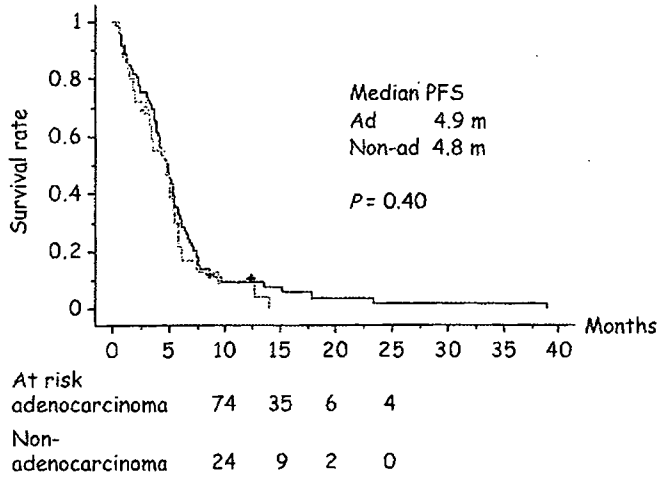

Fig. 1. Kaplan-Meier plot of progression-free survival (adenocarcinoma versus nonadenocarcinoma histology).

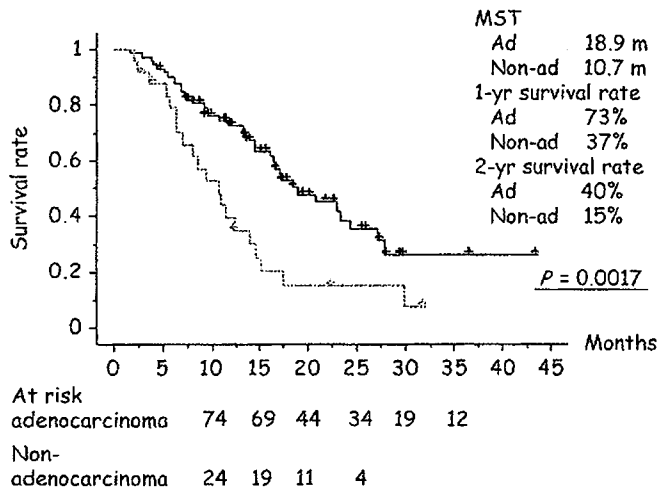

Fig. 2. Kaplan-Meier plot of progression-free survival (smoker versus non-smoker).

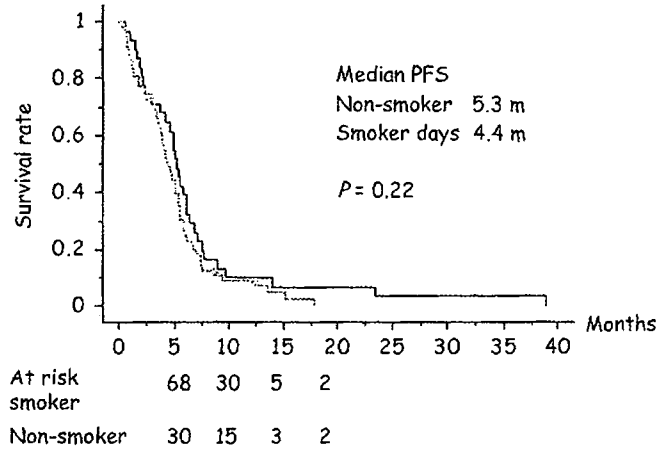

Fig. 3. Kaplan-Meier plot of overall survival (adenocarcinoma versus nonadenocarcinoma histology).

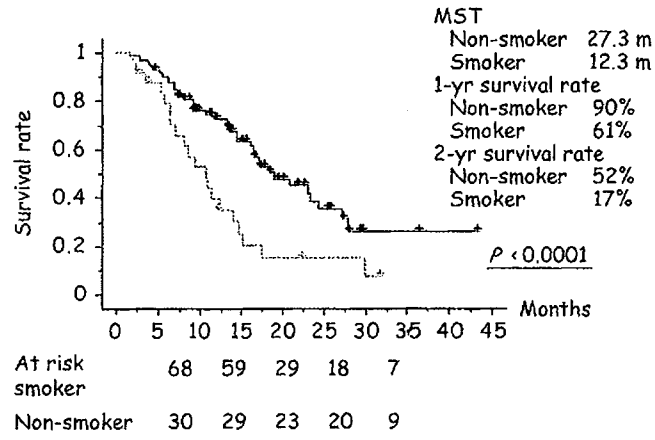

Fig. 4. Kaplan-Meier plot of overall survival (smoker versus non-smoker).

and between non-smokers versus smokers ($P < 0.0001$). Of particular note, median OS of smokers was 12.3 months, and non-smokers was 27.3 months. Two-year survival rates were 17% and 52% in smokers and non-smokers, respectively (Figs 3,4).

To identify factors influencing OS in patients who received second-line therapy ($n = 76$), multivariate analysis was performed with covariates including histology (adenocarcinoma versus other), smoking history (non-smoker versus smoker), PS (0 versus 1), docetaxel (use versus non-use) and EGFR-TKI (use versus non-use). The use of EGFR-TKI was identified as a significant prognostic factor associated with longer OS, together with non-smoking history and PS 0. The use of docetaxel was not associated with an increase in OS in this study (Table 4). When interaction terms between clinical variables and EGFR-TKI treatment were included in the model, no significant interaction was detected ($P = 0.354$ and 0.515 for smoking history × EGFR-TKI and histology × EGFR-TKI, respectively). In the exploratory Cox analysis, prognostic advantage for non-smoking history and adnocarcinoma histology was more prominent in patients who received EGFR-TKI treatment after adjustment for PS, suggesting a potential interaction between these favorable clinical variables and EGFR-TKI treatment. Compared with smokers, hazard ratio of non-smokers with or without EGFR-TKI was 0.961 (95% CI, 0.209–4.420) and 0.193 (0.083–0.449), respectively. Likewise, the hazard ratio of adenocarcinoma patients with or without EGFR-TKI was 0.429 (0.138–1.334) and 0.387 (0.187–0.800), respectively, compared with patients with other histologies.

Table 4. Cox regression analysis of prognostic factors for overall survival after second-line treatment: a stepwise forward procedure

Factor	Variable	*P*-value HR (95% CI)
Histology	Adenocarcinoma versus other	0.0639 –
Smoking	Non-smoker versus smoker	0.0052 0.325 (0.148–0.715)
PS	0 versus 1	0.0258 (0.258–0.917)
Docetaxel	– versus +	0.6720 –
EGFR-TKI	– versus +	0.0084 2.844 (1.306–1.823)

PS, performance status; EGFR-TKI, epidermal growth factor receptor-tyrosine kinase inhibitor; HR, hazard ratio; CI, confidence interval.

doi: 10.1111/j.1349-7006.2006.00379.x

Table 5. Historical comparison of outcomes in our study and the FACS study[2]

	FACS[1] (n = 145)	This study (n = 98)
Response rate (%)	32	20
Median PFS (months)	4.5	4.8
Median OS (months)	12.3	16.5
1-year survival rate (%)	51	64
Second-line therapy, n (%)	87 (60)	76 (78)
Docetaxel	25 (17)	42 (43)
EGFR-TKI	9 (6)	29† (30)
Other	58 (40)	5 (5)

†25 patients were treated with Gefitinib. FACS, Four-Arm Cooperative Study; PFS, progression-free survival; EGFR-TKI, epidermal growth factor receptor-tyrosine kinase inhibitor.

Discussion

Since the results of a large meta-analysis revealed that platinum-based chemotherapy prolonged OS of patients with advanced NSCLC compared with best supportive care (BSC)[8] this therapy has been considered standard first-line treatment for advanced NSCLC worldwide. Median OS with carboplatin/paclitaxel – the most commonly used standard therapy outside Japan – has been reported to be 8–14 months[9–13] similar to the median OS (12.3 months) observed in the FACS trial conducted in Japan.[2,3]

Comparison of outcomes following carboplatin/paclitaxel treatment in our study with the results obtained with the same regimen in the FACS study, showed there was little difference in median PFS (4.8 versus 4.5 months), but median OS was approximately 4 months longer at our hospital (16.5 versus 12.3 months). With the recent approval of EGFR-TKIs, the use of these agents as second-line chemotherapy has increased since the FACS study was performed (30% of patients in this study versus 6% of patients in the FACS study) (Table 5). This observation suggests that the better treatment outcomes obtained in our study compared with those of FACS may be attributable to the effect of anticancer agents used in second-line and subsequent treatment, especially EFGR-TKI (gefitinib was used in most cases). In fact, the result of subgroup analysis by patient demographics in our study demonstrated a marked prolongation of OS for non-smokers and patients with adenocarcinoma, both of which are known to be factors associated with high responsiveness to EGFR-TKI. Furthermore, the multivariate analysis in patients receiving second-line treatment, revealed that EGFR-TKI use was an independent prognostic factor.

Generally, the prolongation of OS is the ultimate goal of anticancer therapy and an important clinical outcome in the evaluation of the effect of first-line treatment for NSCLC. With the emergence of potent anticancer agents in the second-line setting, therapy administered after the occurrence of progressive disease becomes a confounding factor in the interpretation of OS. To overcome this issue of confounding, there may be value in using prolongation of PFS as the primary outcome of first-line trials. Currently, the Food and Drug Administration (FDA) requires an applicant to demonstrate prolonged survival as an approval condition for new anticancer agents.[14] However, the European Agency for Evaluation of Medical Products (EMEA) has accepted PFS as the primary endpoint in some instances, and our present study result supports this view.[15]

The results of the BR21 trial showed that erlotinib significantly prolonged OS compared with placebo (6.7 versus 4.7 months, hazard ratio [HR] = 0.70). In the multivariate analysis, Asian origin (P = 0.01), adenocarcinoma histology (P = 0.004) and non-smoking status (P = 0.048) correlated with prolonged OS.[6] In the preplanned subgroup analysis in the ISEL trial, significantly longer survival was seen with gefitinib compared with placebo in patients of Asian origin (9.5 versus 5.5 months, HR = 0.66) and never-smokers (8.9 versus 6.1 months, HR = 0.67).[7] Although these two studies did not include Japanese patients, the findings might be extrapolated into Japanese populations. Since the reports of Paez *et al.*[16] and Lynch *et al.*[17] in April and May 2004, respectively, numerous studies of EGFR mutations have been conducted in a short period and studies conducted in Japan have reported a good correlation between OS and EGFR mutations in patients treated with gefitinib.[18–20] Moreover, the incidence of EGFR mutations is more frequent in women, patients with adenocarcinoma, never-smokers and Japanese patients[16,17] suggesting that there is a correlation between clinical and molecular factors and clinical benefit from EGFR-TKIs.

Although EGFR mutations are of interest as a biomarker that can be predictive of the effect of gefitinib, especially in patients of Asian or Japanese origin, their immediate clinical application for patient selection is not always possible, due to issues including method determination, cost and convenience. Correlation between response to gefitinib and EGFR copy number determined by fluorescence *in situ* hybridization (FISH) has attracted attention in the West as an alternative potential biomarker[21,22] and this needs to be further investigated in Japan. Acknowledging the need to pay close attention to future research trends, we believe further discussion into how to select those patient populations most likely to benefit from gefitinib in routine clinical practice is required. It is important to establish whether patients could be selected on the basis of biomarker data such as EGFR mutations, or EGFR over-expression, or clinical characteristics such as histological subtype and smoking history. Nevertheless, selection of appropriate patients for EGFR-TKI therapy is undoubtedly necessary, and we hope that future research will be able to identify possible methods as soon as possible. Once identified these will require validation in large-scale prospective clinical studies.

In conclusion, this retrospective study demonstrated a marked prolongation of overall survival in patients with adenocarcinoma and non-smoking history who received carboplatin/paclitaxel as first-line treatment. Our study results suggest that the use of EGFR-TKI (especially gefitinib) after first-line treatment may be associated with an improvement in overall survival.

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doi: 10.1111/j.1349-7006.2006.00379.x

Irinotecan pharmacokinetics/pharmacodynamics and *UGT1A* genetic polymorphisms in Japanese: roles of *UGT1A1*6* and **28*

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Objectives **SN-38, an active metabolite of irinotecan, is detoxified by glucuronidation with UGT1A isoforms, 1A1, 1A7, 1A9, and 1A10. The pharmacogenetic information on *UGT1A* haplotypes covering all these isoforms is important for the individualized therapy of irinotecan. Associations between *UGT1A* haplotypes and pharmacokinetics/pharmacodynamics of irinotecan were investigated to identify pharmacogenetic markers.**

Methods **Associations between *UGT1A* haplotypes and the area under concentration curve ratio (SN-38 glucuronide/SN-38) or toxicities were analyzed in 177 Japanese cancer patients treated with irinotecan as a single agent or in combination chemotherapy. For association analysis, diplotypes of *UGT1A* gene segments [(*1A1*, *1A7*, *1A9*, *1A10*), and Block C (common exons 2–5)] and combinatorial haplotypes (*1A9-1A7-1A1*) were used. The relationship between diplotypes and toxicities was investigated in 55 patients treated with irinotecan as a single agent.**

Results **Among diplotypes of *UGT1A* genes, patients with the haplotypes harboring *UGT1A1*6* or **28* had significantly reduced area under concentration curve ratios, with the effects of *UGT1A1*6* or **28* being of a similar scale. A gene dose effect on the area under concentration curve ratio was observed for the number of haplotypes containing **28* or **6* (5.55, 3.62, and 2.07 for 0, 1, and 2 haplotypes, respectively, $P<0.0001$). In multivariate analysis, the homozygotes and double heterozygotes of **6* and **28* (**6/*6*, **28/*28* and **6/*28*) were significantly associated with severe neutropenia in 53 patients who received irinotecan monotherapy.**

Conclusions **The haplotypes significantly associated with reduced area under concentration curve ratios and neutropenia contained *UGT1A1*6* or **28*, and both of them should be genotyped before irinotecan is given to Japanese and probably other Asian patients. *Pharmacogenetics and Genomics* 17:497–504 © 2007 Lippincott Williams & Wilkins.**

Pharmacogenetics and Genomics 2007, 17:497–504

Keywords: diplotypes, genetic polymorphism, haplotype, irinotecan, SN-38, UGT1A1

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Received 15 August 2006 Accepted 15 November 2006

Introduction

Irinotecan, an anticancer prodrug, is widely applied for colorectal, lung, stomach, ovarian, and other various cancers. It is activated by carboxylesterases to SN-38 (7-ethyl-10-hydroxycamptothecin), which shows antitumor activity by inhibiting topoisomerase I [1,2]. SN-38 is subsequently glucuronidated by uridine diphosphate glucuronosyltransferases (*UGTs*) to form an inactive metabolite, SN-38 glucuronide (SN-38G) [3]. Dose-limiting toxicities of irinotecan are diarrhea and leukopenia [4], and reduced activity for SN-38G formation is closely related to severe toxicities [5]. Among UGT isoforms, UGT1A1 is abundant in both the liver and intestine and is thought to be mainly responsible for inactivation of SN-38 [3,6]. Genetic polymorphisms of *UGT1A1* result in reduced enzyme activity and increased toxicity by irinotecan. A significant association of *UGT 1A1*28*, a repeat polymorphism of the TATA box (-40_-39insTA) [3,7], with severe irinotecan-induced diarrhea/leukopenia was first reported in a retrospective study of Japanese cancer patients [8]. Subsequent pharmacogenetic studies in Caucasians have shown close associations of **28* with reduced glucuronidation of SN-38 and/or severe neutropenia/diarrhea [9–12]. These

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studies have clearly indicated that **28* is a good genetic marker for individualized irinotecan therapy. On the basis of these observations, the Food and Drug Administration of the United States has approved an amendment of the label for Camptosar (irinotecan HCl) and added a warning to consider a reduction in the starting dose of irinotecan for **28* homozygous patients (NDA 20-571/S-024/S-027/S-028).

There is significant racial difference in *UGT1A1* polymorphisms among Asians, Caucasians, and Africans [13]. Although the association of *UGT1A1*28* with toxicities by irinotecan was first described in Japanese patients, its frequency in Japanese is one-third of that in Caucasians. Another low-activity allele **6* [211G > A(G71R)], which is not detected in Caucasians or Africans, is as frequent as the **28* allele in Japanese. Moreover, the area under concentration curve (AUC) ratio of SN-38G to SN-38 was decreased in patients having **6* haplotypes [14].

In addition to UGT1A1, recent studies have suggested possible contributions to SN-38G formation by UGT1A7, 1A9, and 1A10 [15–17], which are expressed in the gastrointestinal tract, the liver and intestine, and extrahepatic tissues, respectively [18]. Altered activity resulted from genetic polymorphisms of these isoforms, including *1A7*3* [387T > G(N129K), 391C > A(R131K), 622T > C(W208R)], *1A9*22* (-126_-118T_9 > T_{10}), *1A9*5* [766G > A(D256N)], and *UGT1A10*3* [605C > T(T202I)], but clinical relevance of these polymorphisms is yet to be elucidated [16,19–24]. Moreover, close linkages among *1A9*, *1A7*, and *1A1* polymorphisms were found in Caucasians and Asians in an ethnic-specific manner [20,25–27]. Therefore, comprehensive investigation that covers these genes, along with linkages among the polymorphisms, is needed, in each ethnic population, to evaluate associations between the genetic polymorphisms and pharmacokinetics, as well as clinical outcomes of irinotecan therapy.

Recently, we have analyzed the segmental and block haplotypes of *1A8*, *1A10*, *1A9*, *1A7*, *1A6*, *1A4*, *1A3* and *1A1*, and the common exons 2–5 (Block C) in a Japanese population, including the 177 cancer patients treated with irinotecan, and showed close linkages between the haplotypes, that is, *1A9*22* and *1A7*1*, *1A7*3* and *1A1*6*, and *1A7*3* and *1A1*28* [28]. Preliminary results of *UGT1A1* pharmacogenetics on 85 of these cancer patients were reported previously [14]. In the current study, we investigated the pharmacogenetics of irinotecan, focusing on diplotypes of the *UGT1A* complex covering *1A1*, *1A7*, *1A9*, *1A10*, and Block C (exons 2–5) of 177 patients, so as to elucidate haplotypes or genetic markers associated with altered glucuronidation of SN-38 and toxicities.

Methods

Patients and treatment schedule

Patients with cancers who started chemotherapy with irinotecan at two National Cancer Center Hospitals (Tokyo and Kashiwa, Japan) were eligible if they had not received irinotecan previously. Other eligibility criteria included bilirubin ≤ 2 mg/dl, aspartate aminotransferase (GOT) ≤ 105 IU/l, alanine aminotransferase (GPT) ≤ 120 IU/l, creatinine ≤ 1.5 mg/dl, white blood cell count ≥ 3000/μl, performance status of 0–2, and at least 4 weeks after the last chemotherapy (2 weeks for radiotherapy). Exclusion criteria were diarrhea, active infection, intestinal paralysis or obstruction, and interstitial pneumonitis. The ethics committees of the National Cancer Center and the National Institute of Health Sciences approved this study, and written informed consent was obtained from all participants.

Irinotecan was administered as a single agent or in combination chemotherapy at the discretion of attending physicians. Doses and schedules were according to approved usage in Japan; intravenous 90-min infusion at a dose of 100 mg/m^2 weekly or 150 mg/m^2 biweekly. In terms of combination chemotherapy, the dose of irinotecan was reduced according to clinical protocols.

Genetic polymorphisms of UGT1As and pharmacokinetics

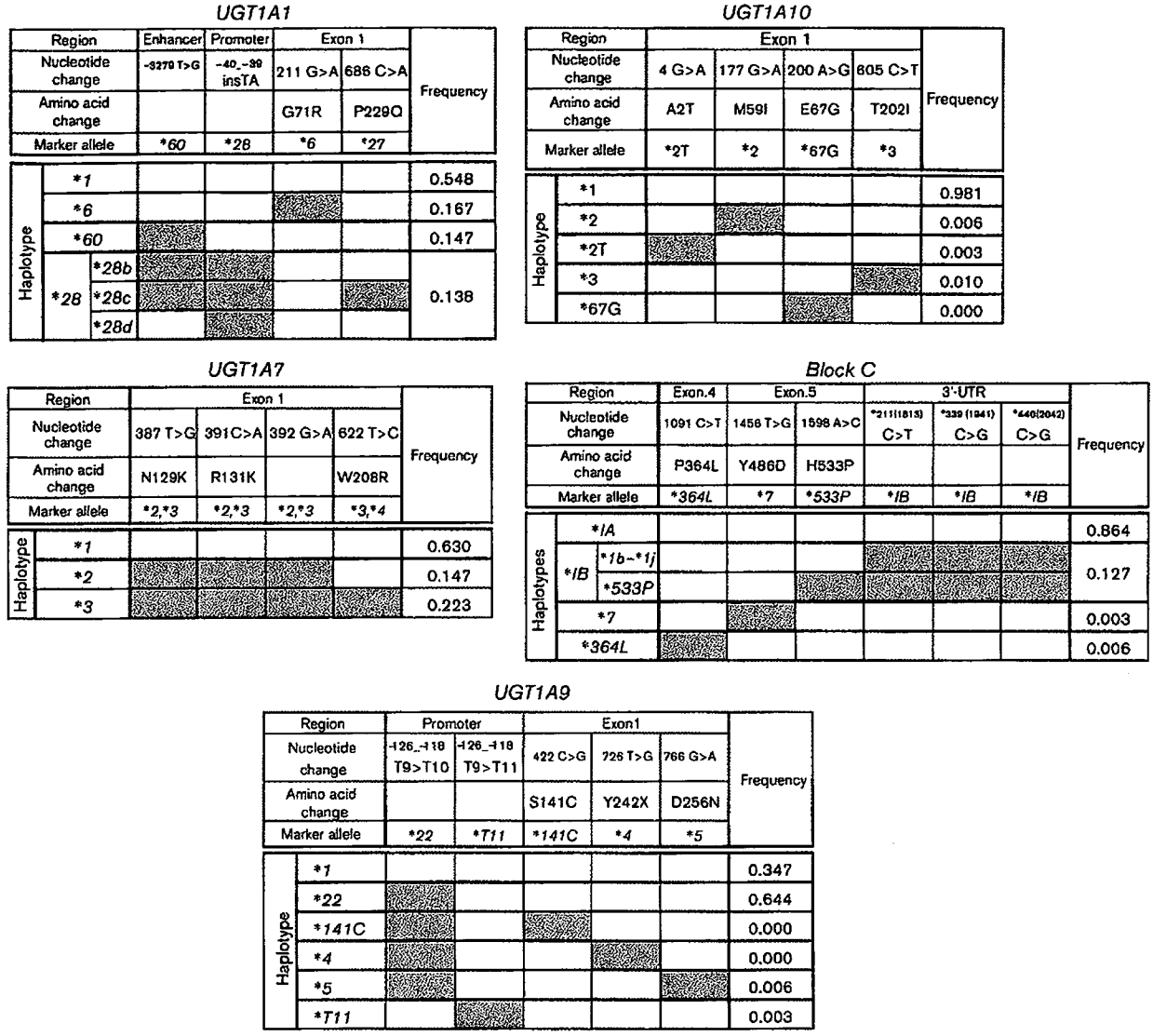
Detailed assay methods for genotypes of the *UGT1A* gene complex were reported previously [14,28]. In this study, we focused on the genetic variations in *UGT1A1*, *1A7*, *1A9*, and *1A10* and common exons 2–5, as they have been reported to contribute to the SN-38 glucuronidation. Haplotype analysis covering these regions was performed in our previous study [28], and haplotypes of each *UGT1A* segment [exon 1 for *1A1*, *1A7*, *1A9*, or *1A10*; and Block C (common exons 2–5)] are summarized in Fig. 1.

Pharmacokinetic analysis for irinotecan was performed as described previously [14]. Briefly, heparinized blood was collected before administration of irinotecan, as well as 0 and 20 min, and 1, 2, 4, 8, and 24 h after termination of the first infusion of irinotecan. Plasma concentrations of irinotecan, SN-38 and SN-38G were determined by the high-performance liquid chromatography [29], and AUC was calculated by the trapezoidal method using WinNonlin version 4.01 (Pharsight Corporation, Mountain View, California, USA). Associations between genotypes and the AUC ratio (AUC of SN-38G/AUC of SN-38) were evaluated in 176 patients.

Monitoring and toxicities

A complete medical history and data on physical examinations were recorded before the irinotecan therapy. Complete blood cell counts with differentials and platelet counts, as well as blood chemistry, were measured once a week during the first 2 months of irinotecan treatment. Toxicities were graded according to the Common Toxicity Criteria of National Cancer Institute version 2. Association of genetic factors with irinotecan toxicities was analyzed primarily in patients who received irinotecan as a single agent.

Fig. 1



Haplotypes of *UGT1A* gene segments (*UGT1A1*, *1A7*, *1A9*, *1A10*, and Block C) in 177 Japanese cancer patients. The tagging variations and haplotypes are shown. Variant alleles are indicated in grey. Definition of Block C haplotypes in our previous paper ([14]) (corresponding to Block 2) were slightly modified.

Statistical analysis

Statistical analysis on the differences in the AUC ratios (SN-38G/SN-38) among *UGT1A* genotypes was performed using the Kruskal–Wallis test, followed by nonparametric Dunnett's multiple comparison test, or with Wilcoxon test. Analysis of a gene–dose effect of each haplotype was performed using the Jonckheere–Terpestra test in the SAS system, version 5.0 (SAS Institute, Cary, North Carolina, USA). Relationship of *UGT1A* genetic polymorphisms to the toxicities of irinotecan was assessed by the χ^2 test via the use of using Prism version 4.0 (GraphPad Prism Software, San Diego, California, USA). The *P*-value of 0.05 (two-tailed) was set as a significant level, and the multiplicity adjustment was conducted for pharmacokinetics data with the false discovery rate [30].

To identify factors associated with the log-transformed AUC ratio of SN-38G/SN-38, multiple regression analysis was performed using age, sex, body surface area, dosage of irinotecan, history of smoking or drinking, performance status, coadministered drugs, serum biochemistry parameters at baseline, and *1A9-1A7-1A1* and Block C haplotypes (five or more chromosome numbers) or '*1A1*6* or **28*'. For multiple regression analysis of neutropenia, variables included the absolute neutrophil count at baseline and the dosing interval, in addition to

the other patient background factors described above. The multivariate analyses were performed by using JMP version 6.0.0 software (SAS Institute). The variables in the final models for both AUC ratio and neutropenia were chosen by forward and backward stepwise procedures at significance levels of 0.25 and 0.05, respectively.

Results

Patients and UGT1A haplotypes

Patient demographics and information on the treatment are summarized in Table 1. In addition to UGT1A1, UGT1A7, 1A9, and 1A10 were also reported to glucuronidate SN-38 [15–17]. In our previous study, haplotype analysis covering the *1A9* to *1A1* (5′–3′) gene segments was conducted, and the combinatorial diplotypes (*1A9-1A7-1A1*) of the patients were determined. It must be noted that close linkages between *1A9*22* and *1A7*1*, between *1A7*2* and *1A1*60*, and between *1A7*3* and *1A1*6* or *1A1*28* were observed as described previously [28]. To clarify the linkages between these segmental haplotypes (*1A9*, *1A7*, and *1A1*), we grouped the combinatorial (*1A9-1A7-1A1*) haplotypes into four categories (A–D) based on the *1A1* haplotypes (**1*, **6*, **60*, and **28*). Each group was further divided into the subgroups based on the previously defined Block 9/6 (including *1A9*, *1A7*, and *1A6*) haplotypes (Table 2). The frequency of Group B haplotypes (*B1-B4*) harboring *1A1*6* was 0.167 and higher than that of Group D haplotypes (*D1-D6*) with **28* (0.138) in this population.

Association of 1A9-1A7-1A1 diplotypes to SN-38G formation

When relationship between the *UGT1A* diplotypes (*1A9-1A7-1A1*) and the SN-38G/SN-38 AUC ratio was analyzed in the 176 cancer patients the AUC ratio for the diplotypes of *B2/B2*, *D2/A1*, and *D1/B2* was statistically significantly lower than the *A1/A1* diplotype (Fig. 2). These diplotypes harbored *1A1*6*, **28* or both. Significant gene–dose effects of *B2* (among *A1/A1*, *B2/A1*, and *B2/B2*) and *C3* (among *A1/A1*, *C3/A1*, and *C3/C3*) were also observed (Fig. 2). As no significant differences in AUC ratios were observed between *D1/A1* and *D2/A1*, *D1/C3* and *D2/C3*, and *D1/B2* and *D2/B2*, the haplotype combination *1A9*1-1A7*3* or *1A9*22-1A7*1* was not influential on the AUC ratio.

As the effect of diplotypes harboring *UGT1A1* polymorphism was prominent, we grouped the whole gene (*1A9-1A7-1A1*) diplotypes according to the *1A1* diplotypes (the upper part of Fig. 2). Patients with **6* or **28* (except for **28/*28*) haplotypes had significantly lower AUC ratios than the wild-type (**1/*1*), and significant gene–dose effects were observed for **28* (among **1/*1*, **28/*1*, and **28/*28*) and **6* (among **1/*1*, **6/*1* and **6/*6*). A significant additive effect of **6* and **28* on the decreased AUC ratio was also observed when the values for **28/*1* were compared with those for **28/*6* (Fig. 2 and Table 3).

Regarding other polymorphisms, a statistically nonsignificant tendency to decrease the AUC ratio was observed for **60*

Table 1 Characteristics of Japanese cancer patients in this study

		No. of participants	
Age			
Mean/range	60.5/26–78	177	
Sex			
Male/female		135/42	
Performance status	0/1/2	84/89/4	
Combination therapy and tumor type (initial dose of irinotecan; mg/m^2)			
Irinotecan monotherapy	Lung (100)	21	
	Colon (150)	28	
	Others (100)	7	
With platinum-containing drug[a]	Lung (60)	58[b]	48 [60][c]
	Stomach (70)	9	9 [80][c]
	Others (60)	5	5 [80][c]
With 5-fluorouracil (including tegafur)	Colon (100 or 150)	34	
	Others (90 or 100)	2	
With mitomycin-C	Stomach (150)	10	
	Colon (150)	1	
With amrubicin	Lung (60)	2	
Previous treatment			
Surgery	Yes/no	85/92	
Chemotherapy	Yes/no	97/80	
Radiotherapy	Yes/no	26/151	
Smoking history	Yes/no	29/148	

[a]Cisplatin, cisplatin plus etoposide or carboplatta.
[b]Two and eight patients received cisplatin and etoposide and carboplatin, respectively.
[c]Number of cisplatin-administered patients [initial dose of cinlatin (mg/m^2) is shown in brackets].

Table 2 Combinatorial haplotypes covering *UGT1A9*, *UGT1A7*, and *UGT1A1*

	Block haplotype[a]			Combination of segmental haplotypes	Cancer patients	
Haplotype	Block 9/6	Block 4	Block 3/1	1A9-1A7-1A1	*N*[b]	Frequency
A1[c]	*I	*1	*I	*22-*1-*1	189	0.534
	*I	*3	*I			
A3	*III	*1	*I	*1-*2-*1	2	0.006
A2	*II	*1	*I	*1-*3-*1	1	0.003
A4	*IV	*1	*I	*22-*3-*1	1	0.003
A5				*T11-*1-*1	1	0.003
B2[c]	*II	*1	*III			
	*II	*1	*VI	*1-*3-*6	47	0.133
	*II	*4	*VI			
B4	*IV	*1	*III	*22-*3-*6	6	0.017
B1	*I	*1	*III	*22-*1-*6	5	0.014
	*I	*1	*VI			
B3	*III	*1	*III	*1-*2- *6	1	0.003
C3[c]	*III	*3	*IV			
	*III	*1	*IV			
	*III	*3	*V	*1-*2-*60	44	0.124
	*III	*1	*V			
C1	*I	*3	*IV	*22-*1-*60	5	0.014
	*I	*1	*IV			
C2	*II	*3	*IV	*1-*3-*60	2	0.006
C7	*VII	*3	*V	*22-*2-*60	1	0.003
D1	*I	*1	*IIa	*22-*1-*28	23	0.065
	*I	*1	*IIc			
D2	*II	*1	*IIa			
	*II	*3	*IIa	*1-*3-*28	22	0.062
	*II	*1	*IIc			
D6	*VI	*1	*IIb	*1-*2-*28	4	0.011
				Total	354	1.000

[a]Block haplotypes described in Ref. [28] are shown for reference. *1A9* and *1A7* are included in block 9/6 and *1A1* is included in block 3/1.
[b]Number of chromosomes.
[c]Major combinatrial haplotypes.