Additional candidates to conventional genes susceptible for lung cancer and changing trend in Japan (Review)

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Abstract. The polymorphism of *CYP1A1*2A* or *CYP1A1*2B*, and the linkage of *CYP1A1*2A*, *CYP1A1*2B*, *GSTM1* and *GSTT1* polymorphisms have been established as susceptible genes or gene-gene interactions of tobacco-related lung cancer. New candidate genes susceptible for lung cancer such as *NQO1* (NAD(P)H:quinine oxidoreductase), *NAT2* (N-acetyltransferase 2), and several others have been reported. In the present review we focus on new candidate genes susceptible for lung cancer, then examine all Japanese references by meta-analysis on susceptible genes over the past 20 years, and discuss whether new candidates and changing trend in Japan could be caused by environmental change.

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Abbreviations: CYP, cytochrome P-450; ETS, environmental tobacco smoke; GST, glutathione S-transferase; MPO, myeloperoxidase; NAT2, N-acetyltransferase 2; NQO1, NAD(P)H: quinine oxidoreductase; OR, odds ratio; SULT, sulfotransferase

Key words: lung cancer, susceptible genes, gene polymorphism, smoking, meta-analysis

1. Introduction

Cytochrome P-450 (CYP) enzymes have been reported to participate in chemical carcinogenesis and to form reactive intermediates which can then covalently bind to DNA. Formed DNA adducts show strong carcinogenic properties (1-3). This process is called phase I chemical reaction for carcinogenesis, whereas in phase II reaction DNA adducts are detoxified by glutathione S-transferase (GST) or other enzymes (1,3-5). Polymorphism of these CYPs shows different activities of aryl hydrocarbon hydroxylase to form DNA adducts (DNA-binding diolepoxide) (1-5). Benzo(a) pyrene, one of the carcinogenic agents in cigarettes, as well as other carcinogens are metabolized by CYP1A1, CYP1A2, CYP2A6, CYP2E1 and CYP2D6 to form benzo(a)pyrene DNA adducts (epoxide), resulting in very active carcinogens (1-5). The polymorphism of CYP1A1 have been shown at three sites, the Msp1 recognition site in intron (*CYP1A1*2A: MspI)*, the isoleucine-to-valine substitution site in the heme-binding region of the enzyme (*CYP1A1*2B: Ile/Val*) and the threonine-to-asparagine substitution site in exon 7 of the enzyme (*CYP1A1*4*) (4-7). The distribution of gene polymorphism in both *CYP1A1*2A* and *CYP1A1*2B* was not the same between Japanese and Caucasian populations (6-9). Since the study of Kawajiri *et al* (10), the Japanese population showed a type of *CYP1A1* gene polymorphism significantly susceptible to tobacco-related lung cancer (11-16). On the other hand, Caucasians did not show significant distribution of either type of gene polymorphism of *CYP1A1* for non-small cell lung cancer (8,9,17). This ethnic difference may be considered due to the fact that the allelic frequency of *CYP1A1*2A(m2/m2)* and *CYP1A1*2B (Val/Val)* among Caucasians was about 10-fold less than among Japanese (7,8). Recent reports, however, based on pooled analysis of individual data of lung cancer cases and controls from the International Collaborative Study on Genetic Susceptibility to Environmental Carcinogenesis revealed significantly higher cancer patients with *CYP1A1*2A* variant gene than controls (6,7,18,19). Next, the associated variant genes of *CYP1A1*2A*, *CYP1A1*2B*, *GSTM1* and *GSTT1* showed the high risk (13,19,20), and the gene-gene interactions of

*CYP1A1*2A*, *CYP1A1*2B*, *GSTM1* and *GSTT1* were clearly demonstrated to pose significantly high risk in the non-smoking persons of a large pooled studies by the detailed polymorphism analysis of their genes (7,21). Moreover *NQO1*, *NAT2* and several other genes have been reported as new susceptible genes of lung cancer (22-36). The present review focuses on new candidates, then examines all Japanese references by meta-analysis on susceptible genes over the past 20 years, and finally discusses whether new candidates and changing trend in Japan could be caused by environmental change.

2. Conventional susceptible genes of tobacco-related lung cancer

Kawajiri *et al* (10) were the first to demonstrate a strong association of the *CYP1A1*2A* genotype with the risk of lung cancer. In particular, in patients with squamous cell carcinoma the frequency of *CYP1A1*2A (m2/m2)* was 30.4%, while in healthy controls it was 10.6%. Subsequent studies (#2,4-7 in Table I) confirmed their study (10). Hayashi *et al* (#3 in Table I) also demonstrated an association of the *CYP1A1*2B* (*Val/Val)* with the risk of lung cancer, especially strong association in squamous cell carcinoma. This association was confirmed in three subsequent studies (#4,6,7 in Table I).

The significant association of these genes had not been reported in Caucasians people before the recent pooled analysis reports (7,8,18,19). There was an ethnic difference in the frequency of *CYP1A1* gene polymorphism in lung cancer, and the reason was considered due to the fact that the homozygous rare allele (C*YP1A1*2A* or *CYP1A1*2B*) in Caucasians occurs approximately ten times less frequently than in Japanese (6-9). However, recent studies based on pooled analysis of individual data of lung cancer cases and controls from the International Collaborative Study on Genetic Susceptibility to Environmental Carcinogenesis revealed significant data of the higher frequency of C*YP1A1*2A* rare genotype in lung cancer compared with that in controls (7,8,18,19). The recent studies on lung cancer in Asian peoples naturally supported the significant data of the Kawajiri *et al* originals (37-39). The polymorphism of *CYP1A1* has been considered to involve in the phase I chemical reaction for carcinogenesis related with tobacco-related lung cancer.

The next conventional susceptible gene group is glutathione S-transferase that can detoxify formed DNA adducts in phase II reaction as mentioned above (1,3-5). Five classes of soluble glutathione-S-transferase (GSTs), alpha (A), mu (M), pi (P), theta (T) and zeta (Z), have been reported (5,40). GSTM1 is involved in degradation of active metabolites of polycyclic aromatic hydrocarbons (41), and GSTT1 in detoxification of small hydrocarbons such as monohalometanes and ethylene oxide in tobacco smoke (42). Null-type polymorphisms of *GSTM1* and *GSTT1* have been shown to cause deletion of glutathione S-transferase activity in the phase II chemical reaction which involves in tobacco-related lung cancer. It was reported not only in Caucasians (24,43,44) but also in Asian people (13,14,45,46), although *GSTT1* is still inconsistent (46,47).

Nakachi *et al* (13) found that the linkage of *CYP1A1* variants (*m2/m2* or *Val/Val*) and *GST M1 null* genotype showed a high susceptibility to lung cancer (nos. 3-5 in Table I). Since then positive reports were published (17,19-21,39). In a community-based study in Greece (47) the subjects carrying simultaneously three specific genotype combination of *CYP1A1*, *GSTM1* and *GSTT1* deviated from the common genotype in more than one gene, were over-represented in lung cancer patients. Hung *et al* (19) demonstrated the odds ratio (OR) of the combination of *CYP1A1*2B* variant gene and *GSTM1 null* genotype was 4.67 (95% CI 2.00-10.9) compared with the concurrent presence of the CYP1A1 wild-type and *GSTM1 non-null* genotype in a pooled analysis of 14 case-control studies on lung cancer in Caucasian non-smokers. Recently Vineis *et al* (7) showed definite evidence of the gene-gene interactions of the polymorphisms of *CYP1A1*, *GSTM1* and *GSTT1* in a large pooled analysis of reported studies. Their significant data were observed in the non-smokers with OR of 3.71 (1.70-8.07), 3.99 (0.49-32.20), 4.51 (0.72-28.35) and 16.19 (1.90-137.65) for the combination of *GSTM1-GSTT1* double deletions with *CYP1A1*1*, *CYP1A1*2A*, *CYP1A1*2B* and *CYP1A1*4*, respectively (P=0.13) (reference *CYP1A1 *1* and no deletion) (7). Non-smokers represent a population at the low level of exposure to carcino-gens such as environmental tobacco smoke (ETS) and they were often overlooked because of the small number of cases (19). The association with smoking was notably higher for squamous cell carcinomas than for adenocarcinoma (7,18). Aforementioned Greek study showed strong positive association in heavy smokers (47). Thus, the gene-environment interactions in cases with these specific genotype combination have been reported.

3. New candidates of susceptible genes

The phase I chemical metabolites by CYP1A2, CYP2E1, and other CYPs may be involved in the increase in adenocarcinoma with the decrease in squamous cell carcinoma in lung cancer (23). CYP1A2 can catalyze the N-oxidation of several amines including heterocyclic amines in tobacco smoke or consuming well cooked meat or fish (5,23), and A/A genotype of *CYP1A2*1F* and *G/G* genotype of *CYP1A2*1C* increase the CYP1A2 activity with the association of lung adenocarcinoma (23).

CYP2E1 Rsa1/Pst1 polymorphism was shown to be a decreased risk factor for the developing lung cancer among Asians (48). On the other hand, *CYP2E1 c1/c1* genotype was associated with a significant increased risk for lung cancer in smokers with alcohol drinking (49). The induction of CYP2E1 is influenced by alcohol metabolism and this effect participates in the metabolic activation of various carcinogens such as N-nitrosodimethylamine (5,50). High consumption of alcohol beverage was known to be associated with increased lung cancer risk, whereas modest consumption was inversely associated with risk (51-53). *CYP2E1*1D* might involve in the development of alcohol and nicotine dependence (54).

N-acetyltransferases 2 (*NAT2*) and Sulfotransferase (*SULT*) *1A1* are included in phase II chemical reaction. *NAT* 2 participates in the detoxification of aromatic amines and is

Table I. Reports on gene polymorphism of susceptible genes for lung cancer in Japan.

No.	Author(s) (year)	Subjects	Summary	Refs.
1	Kawajiri *et al,* (1990)	68 lung cancer/104 healthy controls 23 squamous cell carcinoma/104 healthy controls	*CYP1A1*2A* (OR 3.09) (23.5%/10.6% *m2/m2*) *CYP1A1*2A* (OR 4.64) (30.4%/10.6% *m2/m2*)	10
2	Nakachi *et al,* (1991)	91 lung cancer/375 healthy controls (matched[a] 45/135) 60 adenocarcinoma/375 healthy controls	*CYP1A1*2A* (26.4%/10.6% *m2/m2*, p<0.001) (OR 7.31 lower-dose smokers, 1.13 *higher-dose smokers) *CYP1A1*2A* (13.3%/10.6% *m2/m2*, not significant)	11
3	Hayashi *et al,* (1992)	212 lung cancer/358 healthy controls 67 squamous cell carcinoma/ 358 healthy controls 96 adenocarcinoma/358 healthy controls	*CYP1A1*2B* and *GSTM1 null* (OR 5.83; 12.3%/4.7% *Val/Val)* *CYP1A1*2B* and *GSTM1 null* (OR 9.07; 14.9%/4.7% *Val/Val)*	12
4	Nakachi *et al,* (1993)	85 squamous cell carcinoma/matched[a] 170 healthy controls	*CYP1A1*2A* and *GSTM1 null* (OR 16.0 lower-dose smokers; 20.0 higher-dose smokers) *CYP1A1*2B* and *GSTM1 null* lower-dose smokers; 27.3 heavy)	13
5	Kihara *et al,* (1995)	97 male lung cancer (61 squamous and 36 small cell)/185 male smoker controls	*CYP1A1*2A* and *GSTM1 null* (OR 8.3 at 0<SI<800; OR 21.9 at SI>800)	14
6	Nakachi *et al,* (1995)	125 adenocarcinoma (71 males, 54 females; 55 current, 25 ex-, 45 never smoked)/matched[a] 160 healthy controls	*CYP1A1*2A* (current or ex-; OR 3.25) *CYP1A1*2B* (poorly differentiated; OR 4.09) *CYP1A1*2A* (current or ex-; OR 3.22) *CYP1A1*2B* (poorly differentiated; OR 3.22)	15
7	Kiyohara *et al,* (1998)	108 lung cancer (56 adenocarcinoma, 30 squamous, 6 large cell, 16 small cell)/ 95 healthy male controls	*CYP1A1*2A* (OR 2.93) *CYP1A1*2B* (OR 3.45) AHH inducibility 7.0<(OR 12.4)	16
8	Sunaga *et al,* (2002)	198 lung adenocarcinoma (124 males 74 females)/152 hospital controls (108 males, 44 females) Non-smokers 75 (37.9%)/49 (32.2%) Smokers 65 (32.8%)/47 (30.9%)	*NQO1* (OR 2.15), *GSTT1 null* (OR 1.61), linkage of both genes (OR 4.61) and more evident in smokers than non-smokers *CYP1A1*2B* (not significant), *GSTM1* (not significant), *OGG1* (not significant)	22
9	Kiyohara *et al,* (2003)	158 lung cancer female patients (140 adenocarcinoma, 10 squamous)/ matched 259 hospital non-smoking women	*CYP1A1*2A* (not significant) *GSTM1 null* (OR 1.37), *GSTM1 null* and high-dose ETS* (OR2.27)	57
10	Osawa *et al,* (2007)	113 lung cancer (68 adenocarcinoma 35 squamous; 74 males, 37 females)/ matched 121 healthy controls (73 males, 48 females), never smoked 32/55; light 21/18; heavy 58/43; unknown 2/5	*NAT2* (light smokers; OR 10.9) *NAT2* and *CYP1A2*1F A/A* (never smoked; OR 4.95) *CYP1A1*2A/*2B* (not significant), *CYP1A1* and *GSTM1* (not significant)	23

OR, odds ratio; SI, smoking index; ETS, environmental tobacco smoke; NAT2, N-acetyltransferase 2; NQO1, NAD(P)H:quinone oxidoreductase; OGG1, a DNA glycosylase for 8-hydroxyguanine. [a]sex and age-matched case control study.

involved in N-acetylation (deactivation) and O-acetylation (activation) of a variety of polycyclic aromatic hydrocarbons (23). The slow genotype (23) and the fast genotype (25) among the polymorphisms of *NAT2* were shown to be related with risk of lung cancer. Vineis *et al* (24) reported that *NAT2* slow genotype showed a higher odds ratio, though not significant, in subjects exposed to ETS in European Prospective Investigation into Cancer and Nutrition. *SULT 1A1* has been

known to participate in the detoxification of hydroxylated metabolites of polycyclic aromatic hydrocarbons and aromatic amines, and the variant allele of *SULT 1A1* was reported to be a risk factor for lung cancer in smokers (26).

Besides the phase I and phase II genes mentioned above, new candidate genes involved in oxidative stress have been investigated in the carcinogenesis of lung cancer (24). *NQO1* is a flavoenzyme in xenobiotic metabolism and protects cells from oxidative damage (24). *NQO1 -Pro/Pro* and *Pro/Ser* genotypes have been reported as a significant susceptible gene for lung cancer in Mexican-Americans and African-Americans (27) and Taiwanese (28), although the specific histologic subtypes of lung cancer were not assessed. Subsequently the *NQO1-Pro/Pro* genotype has a higher enzymatic activity and was associated with the risk of lung adenocarcinoma (22,29). The 690C>TSNPs of *NQO1* has been associated with lower enzyme activity against protection and was significantly associated with lung cancer among never smokers (55) and in pooled analysis study of European Prospective Investigation into Cancer and Nutrition (24).

Myeloperoxidase (MPO) is a lysosomal enzyme in neutrophils and activates procarcinogens in tobacco smoke. The variant A allele is related with low metabolic activation and subsequently with low risk of lung cancer (30). The combination of C*YP1A1*2B* rare genotype and *MPO G/G* genotype showed significantly increased risk of lung adenocarcinoma (31), although Vineis *et al* did not show *MPO* genotypes as the susceptible gene for lung cancer in a nested case-control study for non-smokers (24). At present time this candidate is still controversial.

Base excision repair (BER) genes, such as *OGG1 Ser326Cys*, *XRCC1 Arg194Try*, *XRcc1 Arg280His* and *XRCC1 Arg399Gln*, are considered to modulate DNA repair capacity and to be associated with the decrease or increase in risk of lung cancer (32,33). Among them the association between the O*GG1 Cys/Cys* genotype and adenocarcinoma risk and between *XRCC1 Arg194Trp* polymorphism and lung cancer risk among heavy smokers were observed (33).

Other recent studies (34-36) have revealed a strong and reliable association between genetic variation on chromosome 15 and risk of lung cancer. The association region contains several genes which encode nicotinic acetylcholine receptor subunits (*CHRNA5*, C*HRNA3*, and *CHRNB4*) although the presence of nicotine dependence was not consistent in three studies (34-36,56). That is, Thorgeirsson *et al* (35) pointed out that the variant has an effect on the number of cigarettes smoked per day in smokers and was significantly associated with nicotine dependence.

4. Changing trend of susceptible genes in Japan

Recent Japanese reports by Sunaga *et al* (#8 in Table I) and Osawa *et al* (23) neither showed any significant rare allele distribution of *CYP1A1* nor any significant linkage between *CYP1A1* and *GSTM1* for both smokers and those who had never smoked, but they did demonstrate novel types of gene polymorphism such as *NQO1* (8), *NAT2* for light smokers and linkage between *NAT2* and *CYP1A2*1F A/A* for those who had never smoked (10). The majority of cases in the three recent studies surveyed in Japan (8-10) were adenocarcinoma. The incidence of adenocarcinoma increased in the 1990s in Japan similarly to other developed countries (58-61). Four studies (nos. 2, 3, 6 and 7) published before 1998 had examined the frequency of *CYP1A1*2A* and *CYP1A1*2B* rare variants in cases with adenocarcinoma. Although one of four studies (no. 2) showed almost the same distribution of the *CYP1A1*2A* rare allele in healthy controls, the three other studies (nos. 3, 6 and 7) demonstrated a significant difference in the frequency of *CYP1A1*2A* and/or *CYP1A1*2B* rare variants of cases with lung adenocarcinoma compared with the controls. Among the references published after 2002, Kiyohara *et al* (57) investigated the association of ETS in non-smoking women with the occurrence of lung cancer and revealed a weak odds ratio for the *CYP1A1*2A* and *GSTM1* variants. Therefore, a different frequency of *CYP1A1*2A* and *CYP1A1*2B* variants in patients with adenocarcinoma was noted in the studies published before 1998, but not noted in the reports published after 2002.

Then we examined whether the polymorphisms of genes had changed among the ten studies listed in Table I, that is, whether gene(s) susceptible to lung cancer had changed over the past 20 years. Seven studies published before 2000 (#1-7 in Table I) were compared with the three recent references published after 2001 (#8-10). The odds ratios of the rare alleles of *CYP1A1*2A*, *CYP1A1*2B*, and *GSTM1* null type susceptible genes reported in the references (Table I) were investigated by the meta-analysis method described previously (62).

Summarized odds ratios and their 95% confidence intervals (CI) of *CYP1A1*2A (m2/m2)* vs. *CYP1A1*2A (m1/m1)* susceptible to all lung cancer among the studies are shown in the upper half of Table II. Summarized odds ratios 'before 2000' were 2.493, i.e., greater than 1. This means that the incidence of lung cancer for the population of *CYP1A1*2A (m2/m2)* was much greater than that of *CYP1A1*2A (m1/m1)*. The odds ratios (with 95% CI) of all references (eight studies) were 1.945 (1.258-3.006), and their p-value of homogeneity test was 0.009, in other words the odds ratios in the eight references were not homogeneous, but distributed diffusely (62,63). A comparison of summarized odds ratios between 'before 2000' and 'after 2001' groups showed large differences with significant p-value as well as homogeneity (62-64).

The lower half of Table II shows different results for *CYP1A1*2B (Val/Val)* vs. *CYP1A1*2B (Ile/Ile)*. Summarized odds ratios 'before 2000' were larger than those 'after 2001' with respective homogeneity p-values, but there were no significant differences in the two groups.

The meta-analysis on genotype of GSTM1 *null* to positive for all lung cancer showed that summarized odds ratios were 1.415 (1.104-1.813) 'before 2000' (three studies) and 1.287 (0.989-1.675) 'after 2001' (three studies) with no statistical differences, with respective homogeneity in p-values.

Next, meta-analysis was conducted focusing on squamous cell carcinoma and adenocarcinoma separately. However, sufficient data were not available to compare the summarized odds ratios before 2000 and after 2001. Linkage of genotypes of *CYP1A1*2A*, *CYP1A1*2B* and *GSTM1 null* type could not be obtained. AS *NQO1* and *NAT 2* appeared as candidate genes after 2001, we could not examine new candidate.

Table II. Summarized odds ratios and their 95% confidence intervals of genotypes of *CYP1A1*2A*, and *CYP1A1*2B* susceptible to all lung cancer among studies listed in Table I, and p-value of statistical test between two groups, one before 2000 and one after 2001.

	Before 2000	After 2001	All refs.
*CYP1A1*2A (m2/m2)* vs. *CYP1A1*2A (m1/m1)*			
Summarized odds ratios	2.493 (1.677-3.705)	0.899 (0.537-1.506)	1.945 (1.258-3.006)
No. of studies	6	2	8
No. of references in Table I	(1,2,4-7)	(9,10)	(1,2,4-7,9,10)
P-value of homogeneity test	0.151	0.807	0.009
P-value of test for the two groups	0.00259		
*CYP1A1*2B (Val/Val)* vs. *CYP1A1*2B (Ile/Ile)*			
Summarized odds ratios	2.199 (1.074-4.502)	1.278 (0.551-2.964)	1.887 (1.105-3.221)
No. of studies	4	2	6
No. of references in Table I	(3,5-7)	(8,10)	(3,5-8,10)
P-value of homogeneity test	0.176	0.521	0.220
P-value of test for the two groups	0.391		

Methods of meta-analysis: when the odds ratio and its 95% CI were given in an article, they were employed for the present meta-analysis. If the odds ratio and its 95% CI are not reported in an article but could be calculated from the contents of the article by the method described previously (62), the values calculated were used for the present meta-analysis. If the odds ratio and its 95% CI were not given in an article and the values could not be calculated from the contents of the article by any means, the article was removed from the present meta-analysis study. Summarized odds ratios and their 95% CIs were calculated by the method of Fleiss and Gross (63) as described previously (62). When odds ratios for meta-analysis were homogeneous, a fixed-effect model was used (62,63), and in other cases the random-effects model by DerSimonian and Laird (64) was used as described previously (62). The original method for *CYP1A1*2A* polymorphism is based on RFLPs of the CYP1A1 gene digested with MspI (10,65), but most of the cases in the first study of Kawajiri *et al* (10) were examined by the PCR-RFLP method developed by Kawajiri *et al* (66). Since then the PCR-RFLP method by Kawajiri *et al* (10) has been used in all references. *CYP1A1*2B* polymorphism analysis based on PCR by Hayashi *et al* (12) was used in all references. GSTM1 positive or null genotype based on the PCR method by Comstock *et al* (67) and Groppi *et al* (68) was used by Hayashi *et al* (12) and Nakachi *et al* (13). Other references (14,23,57) used the same PCR method, although two references cited other studiess (69,70). Therefore, these genotypes were in principal examined by the same PCR so the references could be compared for the meta-analysis.

The results of our meta-analysis study mentioned above suggest that *CYP1A1*2A (m2/m2)* is not a susceptible gene for lung cancer at present time, and susceptible genes for lung cancer might have changed during 2 decades. Our meta-analysis study, however, has not sufficient evidence because the numbers of studies after 2000 were few, the study sizes in Table I were not large, and the study designs were different. The controls used in two studies (nos. 1 and 3) were 104 and 358 healthy individuals over 40 years old with no information regarding sex or smoking. Controls matched for sex and age were reported in five studies (#2,4,6,8 and 10, Table I). Although cigarette doses were not matched, analysis was performed on cigarette doses in these five studies. Recent report of Asian pooled analysis (48) was not investigated chronologically and included all cases and controls in 11 Asian countries, different life styles and different environment. We should investigate the environment-modified polymorphism expression of susceptible genes for lung cancer in a large pooled analysis.

5. The possibility of genotype expression modified by environmental change

Environmental change such as air pollution, indoor pollution, the chemical contents of cigarettes, food and other substances might affect the transcription of *CYP1A1*2A* followed by increased protein levels of CYP1A1 related with AHH activity, more than that of *CYP1A1*2B*, over the past few decades in Japan if the conventional susceptible genes have changed in patients with lung cancer. The reports of NQO1 and NAT2 may suggest the involvement of oxidative stress *in vivo* in the occurrence of lung cancer. The metabolites of CYP1A2, CYP2E1, and other CYPs may be involved in the increase in adenocarcinoma with the decrease in squamous cell carcinoma in lung cancer (5,23). CYP1A2 involves the metabolism of N-oxidation of several amines such as the heterocyclic amines formed when meat and fish are cooked well. The amines are also formed in tobacco smoke (1-5). The effect of heavy or moderate alcohol drinking with/without smoking may cause a complicated condition on CYP2E1 activity induced by alcohol drinking, and CYP2E1 is also involved in the metabolic activation of various N-nitrosamines including the potent tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (71).

In Japan habitual male smokers decreased from 53.1% in 1990 to 39.4% in 2007, while females increased from 9.7 to 11.0% (72). Environmental changes also took place over the past two decades in Japan. As for lifestyle, housing, food, clothes, cosmetics, perfumes, and drugs have not changed much except for increased consumption of meat and instant or conserved food. Increased meat consumption may cause induction of NAT2 (22), and may also be consistent

with the remarkable recent increase in the incidence of breast cancer as well as colon cancer in Japan (72). Another noticeable change is less pollution of air, water, sand and roads. Strict monitoring for dioxin is also conducted for industrial air and water. Recent trends in Japan including less pollution and increased meat consumption may have changed the CYP1A1 gene polymorphism susceptible to tobacco-related lung cancer.

The increase in incidence of adenocarcinoma has been considered to be related to the increased consumption of filtered cigarettes, which contain low tar and nicotine but increased nitrate (0.5-1.3%) (30,73). In Japan low-tar under 15 mg and low-nicotine under 1 mg cigarettes accounted for over a 50% share of consumption in 1983, while low-tar under 11 mg and low-nicotine under 1 mg cigarettes accounted for over 50% after 1991 (74).

The occurrence of adenocarcinoma in the lung may involve the oxidative stress *in vivo* with injury to the alveolar cells, which result in the uncontrolled proliferation of lung alveolar cancer stem cells (75,76). In this process many chemical reactants including tobacco-smoke, other chemical particles stimulate macrophages and neutrophiles to produce proteinases such as matrix metalloproteinases, elastase and others as well as cytokines which destroy alveolar cells. If the inflammation or damage to alveolar cells continues, bronchoalveolar stem cells may transform to cancer cells (75,76). Tobacco smoke is one of the most important carcinogens, but we do not think tobacco-smoke is alone responsible for lung cancer. We should follow new susceptible candidate genes in patients with lung cancer in order to prevent the most prevailed lung cancer.

6. Future problems from the aspect of preventive medicine

Excellent studies based on pooled analysis of individual data of lung cancer cases and controls from the International Collaborative Study on Genetic Susceptibility to Environmental Carcinogenesis (6,7,19,21,24) did not show the chronological change of susceptible genes. We should clarify the possibility of new candidate susceptible genes from the aspects of preventive medicine. For this reason we propose the need for a prospective study in the selected cities in the world for the genes susceptible to lung cancer related with environmental change from the chronological aspect.

The change of our life-styles may add new susceptible gene candidates to lung cancer beyond the conventional susceptible genes. We should investigate whether the possibility of environment-modified genotype expression occurs in future.

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