Association of Ile462Val (Exon 7) Polymorphism of Cytochrome P450 IA1 with Lung Cancer in the Asian Population: Further Evidence from a Case-Control Study in Okinawa[1]

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Abstract

Okinawa, a group of islands that lie between the East China Sea and the Pacific Ocean, 2000 km south of the Japanese main islands, has a different profile of diseases, ethnicities, and cultures than does the rest of Japan. We examined an Ile462Val polymorphism (*CYP1A1*2* allele) of cytochrome P450 IA1 in a hospital-based case-control study of lung cancer patients (247 cases and 185 controls) in Okinawa to ascertain the association of this variant with lung cancer. In addition, the distribution of this genotype was studied in populations from different areas of Japan, including Tokyo (n = 69) and Iwate (northern part of Japan; n = 81), as well as in a Chinese group from the Jiangsu province (n = 39) and in an Australian Caucasian group (n = 146). Genotype frequency in controls was not significantly different from area to area in Japan. In Okinawa, however, the genotype encoding Val/Val was associated with a significantly higher risk of lung cancer (odds ratio = 3.32, P = 0.013), especially of squamous cell carcinoma and small cell carcinoma (odds ratio = 4.85 and 9.35, respectively). The Val-encoding allele was less frequent in the Chinese population and was rare in Australian Caucasians. Thus, this study gives support to the value of the cytochrome P450 IA1 Ile462Val polymorphism as a practical high-risk marker of lung cancer in populations, especially those in southeast Asia, in which this variant is more common.

Introduction

The Ile462Val polymorphism of CypIA1[3] is one of the most extensively studied markers among the putative high-risk alleles for human lung cancer (1–13). The significance of its contribution appears to vary between populations and reports, indicating the need for a meta-analysis. The reports from Scandinavia (6) and an early report from North America (11) suffer from an extremely low prevalence of the mutant genotype in these populations, making it statistically difficult to obtain a significant difference upon comparison of lung cancer with control groups. Reports from Japan have suggested significantly increased odds ratios for lung cancer, especially in relation to population frequency of smoking and in tobacco-related histological categories (4, 5, 14). However, even in Japanese populations, the data are not completely consistent, and further expansion and evaluation are required (7). Genotype frequencies in healthy populations in southeast Asia have been reported recently (15, 16), but their association with lung cancer has not been studied. No studies have been performed on mainland China. Okinawa, the islands of which lie 2000 km southwest of Honsyu (the main island of Japan) and north of Taiwan, has a different profile of genetic markers, such as blood type and HLA (17–19). The culture and lifestyle of Okinawa's inhabitants, as well as their profile of cancer incidence, are different from those on the Japanese mainland (20). Although there have been many epidemiological studies in Okinawa on unique environmental factors, including food and nutrition (21–23), no work has thus far been performed on genetic markers related to cancer susceptibility. We, therefore, considered it important to determine the association of the CypIA1 polymorphism with lung cancer risk in this isolated population and to compare it with that on the Japanese mainland. Here, we report a large contribution of Ile462Val polymorphism of CypIA1 for lung cancer risk in a hospital-based case-control study in Okinawa. This was especially evident in relation to the Val/Val genotype and squamous and small cell carcinoma.

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[3] The abbreviations used are: CypIA1, cytochrome P450 IA1; BI, Brinkman index.

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Table 1 Age distribution of cases and controls

Age (yr)	Cases		Controls	
	No.	%	No.	%
30–39	1	0.4	6	3.2
40–49	9	3.6	17	9.2
50–59	32	13.0	54	29.2
60–69	90	36.4	60	32.5
70–79	94	38.1	38	20.5
≥80	21	8.5	10	5.4
Total	247	100	185	100

Subjects and Methods

Case-Control Study. This hospital-based case-control study was conducted in the National Okinawa Hospital, Ginowan City, Okinawa, Japan, from 1993 to 1996. All cases and controls were males. A total of 247 patients with lung cancer, the diagnosis of which was confirmed by cytological and histological examination, were recruited from the National Okinawa Hospital. In this hospital, patients come from all of the surrounding small islands as well as from all over the Okinawan main island, and it is the referral hospital for lung cancer (21). All male lung cancer patients referred during that period were included, and there were not any particular exclusion criteria. For hospital controls, 185 patients without lung cancer were randomly selected from in-patients at the same hospital (Table 1). The distribution of clinical diagnoses among the controls was as follows: bronchitis or pneumonia, 17 cases; tuberculosis, 11 cases; old tuberculosis, 7 cases; lung abscess, 8 cases; chronic obstructive lung disease, 6 cases; bronchogenic cyst, 5 cases; other respiratory disease, 17 cases; diabetes mellitus, 14 cases; other cancer, 7 cases (including 2 liver cancer cases and 1 case each of gastric, pancreatic, esophageal, thyroid, and renal cancer); benign tumor, 6 cases; neuromotor disease, 19 cases; benign liver disease, 11 cases; peptic ulcer, 5 cases; other gastrointestinal tract disease, 13 cases; bone fracture, 9 cases; other orthopedic disease, 14 cases; other miscellaneous condition, 14 cases; and no symptoms, 2 cases. The exclusion of a diagnosis of lung cancer in these controls was based on the absence of clinical signs and symptoms, in addition to negative X-rays and negative cytological examinations. Smoking history, including dosage (numbers of cigarettes smoked), years, and ages at beginning and cessation of smoking habits, were obtained from the participants at interview using a standardized questionnaire. Ages of the participants were computed from their date of birth. Informed consent was taken before blood sampling, and the study was approved by the ethical committee of the National Okinawa Hospital and Hamamatsu University School of Medicine.

Reference Groups. As reference groups, blood samples of randomly selected healthy people aged 40–49 years in Okinawa, Tokyo (center of Honsyu Island), and Iwate prefecture (700 km north of Tokyo) were also investigated (24). All these subjects were informative for smoking history and the other diseases and were male. In addition, a healthy Chinese population in Jiangsu province, China (Fig. 1), and randomly sampled DNAs from Australian Caucasians with hypertension (25) were also analyzed to compare the distributions of CypIA1 genotypes.

Histopathological Categorization. Pathological examination, including histopathology (biopsy and/or operation) and cytopathology, confirmed the diagnosis of lung cancer. Subcategories of lung cancer were standardized by two independent pathologists according to the WHO classification (26).

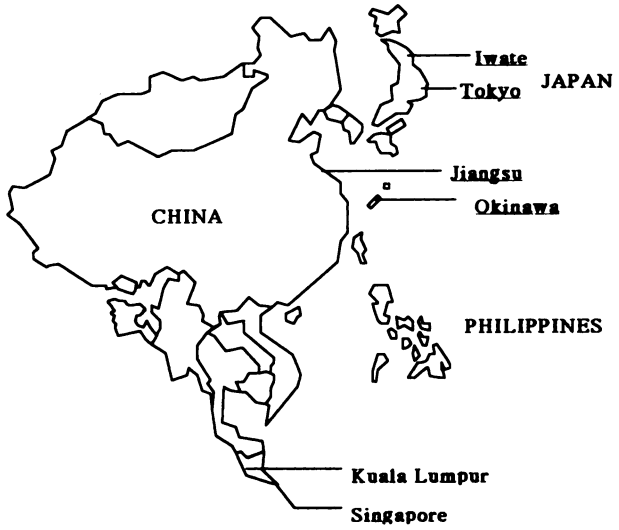

Fig. 1. Geographical map of the locations of populations referred to herein. *Underlined locations*, places where DNA was collected for genotype analysis.

Genotyping of CypIA1. Whole blood (5 ml) was collected into EDTA-coated tubes and centrifuged for 15 min, and the buffy coat layer was isolated. DNA was extracted as previously reported (27), and genotypes that specify the Ile462Val polymorphism of CypIA1 [*CYP1A1*2* according to Cascorbi *et al.* (10)] were determined as described previously (2). Silver-stained migration differences were recognized as Ile/Ile, Ile/Val, or Val/Val genotypes and were confirmed by sequencing of the PCR products in representative samples.

Statistical Analysis. The strength of the association between lung cancer and the CypIA1 polymorphism was measured as odds ratios. The odds ratios were obtained by using unconditional logistic regression analysis, adjusted for age and smoking history (28). Crude odds ratios, odds ratios adjusted for age, and those adjusted for age and smoking habits were calculated in comparison to the Ile/Ile genotype. Odds ratios adjusted for age and smoking habits were not computed when analysis of smoking dosage was performed. When the interaction between smoking dosage and the Cyp1A1 polymorphism was examined, interaction terms were included in the logistic models. χ^2 test was used to test for deviation of genotype distributions from Hardy-Weinberg equilibrium (29).

Results

Age distributions for cases and controls are shown in Table 1 and indicate a slightly lower profile for the control group. The average ages (± SD) of the cases and the controls were 67.8 ± 9.1 and 62.1 ± 11.2 years, respectively. The histological subtypes of cases were: squamous cell carcinoma, 49.4% of cases (n = 122); adenocarcinoma, 31.6% of cases (n = 78); small cell carcinoma, 11.3% of cases (n = 28); adenosquamous cell carcinoma, 4.5% of cases (n = 11); others, 1.2% of cases (n = 3); and unclassified, 2.0% of cases (n = 5). Squamous cell carcinoma, especially well-differentiated squamous cell carcinoma of particular histology, as reported elsewhere (30), was more prevalent in Okinawa than it was on the Japanese main-

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Table 2 Odds ratios of the CypIA1 genotype in the Okinawa case-control study

Genotype	No. of cases/ no. of controls	Crude			Adjusted for age			Adjusted for age and smoking habits		
		OR[a]	95% CI	*P*	OR	95% CI	*P*	OR	95% CI	*P*
Total										
Ile/Ile	125/94	1.00			1.00			1.00		
Ile/Val	94/84	0.84	0.57–1.25	0.395	0.87	0.57–1.31	0.493	0.89	0.58–1.38	0.611
Val/Val	28/7	3.01	1.26–7.18	0.013	2.98	1.22–7.24	0.016	3.32	1.29–8.56	0.013
Squamous cell carcinoma										
Ile/Ile	61/94	1.00			1.00			1.00		
Ile/Val	46/84	0.84	0.52–1.37	0.491	0.92	0.55–1.54	0.762	0.88	0.51–1.53	0.645
Val/Val	15/7	3.30	1.27–8.57	0.014	3.37	1.24–9.19	0.018	4.85	1.44–16.3	0.011
Small cell carcinoma										
Ile/Ile	10/94	1.00			1.00			1.00		
Ile/Val	13/84	1.46	0.61–3.49	0.401	1.49	0.62–3.59	0.377	1.42	0.57–3.53	0.446
Val/Val	5/7	6.71	1.79–25.1	0.005	6.38	1.69–24.1	0.006	9.35	2.08–42.0	0.004
Adenocarcinoma										
Ile/Ile	43/94	1.00			1.00			1.00		
Ile/Val	27/84	0.70	0.40–1.24	0.220	0.72	0.41–1.28	0.267	0.72	0.40–1.28	0.261
Val/Val	8/7	2.50	0.85–7.33	0.096	2.44	0.82–7.28	0.110	2.37	0.77–7.32	0.133
Nonadenocarcinoma										
Ile/Ile	80/94	1.00			1.00			1.00		
Ile/Val	64/84	0.90	0.58–1.39	0.623	0.95	0.60–1.52	0.839	0.92	0.56–1.53	0.756
Val/Val	20/7	3.36	1.35–8.35	0.009	3.36	1.31–8.62	0.012	4.70	1.58–14.0	0.005

[a] OR, odds ratio; CI, confidence interval.

Table 3 Odds ratio of CypIA1 Ile-Val polymorphism in relation to degree of smoking

Genotype	No. of cases/ no. of controls	Crude			Adjusted for age		
		OR[a]	95% CI	*P*	OR	95% CI	*P*
Total							
Cigarette-years <800, excluding never-smokers							
Ile/Ile	39/33	1.00			1.00		
Ile/Val	20/28	0.60	0.29–1.26	0.181	0.62	0.29–1.33	0.220
Val/Val	7/1	5.92	0.69–50.6	0.104	5.02	0.57–44.1	0.145
Cigarette-years ≥800							
Ile/Ile	82/23	1.00			1.00		
Ile/Val	65/25	0.73	0.38–1.40	0.344	0.76	0.39–1.47	0.409
Val/Val	20/3	1.87	0.51–6.85	0.345	2.00	0.54–7.42	0.299
Nonadenocarcinoma							
Cigarette-years <800, excluding never-smokers							
Ile/Ile	21/33	1.00			1.00		
Ile/Val	15/28	0.84	0.37–1.94	0.685	0.85	0.39–1.47	0.727
Val/Val	4/1	6.29	0.66–60.2	0.111	4.56	0.45–46.3	0.200
Cigarette-years ≥800							
Ile/Ile	58/23	1.00			1.00		
Ile/Val	46/25	0.73	0.37–1.45	0.368	0.76	0.38–1.53	0.446
Val/Val	15/3	1.98	0.52–7.50	0.313	2.11	0.55–8.09	0.277

[a] OR, odds ratio; CI, confidence interval.

land. However, further classification of squamous cell carcinoma was not performed.

The distribution of the Ile462Val polymorphism and the odds ratios of each genotype for lung cancer are summarized in Table 2. Ile462Val genotype frequency was similar to that in Honsyu (5) and was consistent with Hardy-Weinberg equilibrium.

The relative risk, obtained as an odds ratio for lung cancer of each genotype, was calculated by logistic regression analysis, adjusted for age and smoking history, and is shown in Table 2. For all lung cancers combined, the odds ratio after adjustment of age and smoking dosage was 3.3 (significantly increased) for the Val/Val genotype and 0.89 (not significant) for the Ile/Val genotype.

The histological breakdown of the cases and contribution of the Ile462Val genotype in each subcategory is also shown in Table 2. When all lung cancers were combined, there was a statistically significant association between Ile462Val genotype

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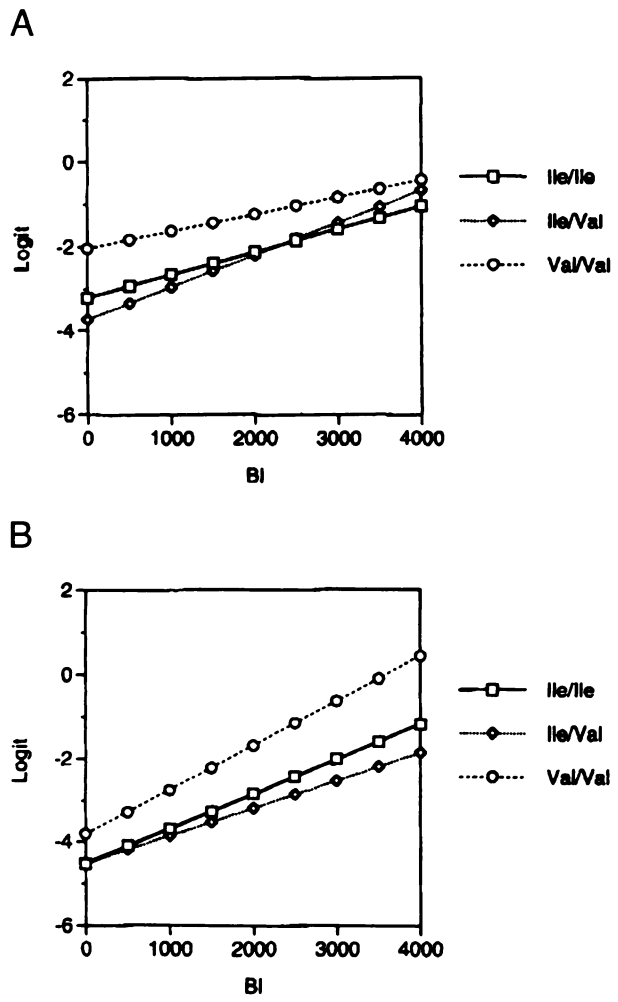

Fig. 2. Odds ratios according to smoking dosage are shown for each genotype (logistic model). Never-smokers were excluded from the analysis. *Horizontal axis*, numbers of cigarettes smoked × years of smoking (BI). *A*, total lung cancers; *B*, total lung cancers, excluding adenocarcinoma. A tendency for a lower odds ratio for the Val/Val genotype in heavier smokers was not detected.

and lung cancer, especially squamous cell carcinoma and small cell carcinoma, in relation to Val/Val. In Ile/Val heterozygotes, odds ratios were not significantly altered. There was no statistically significant association between Val/Val and adenocarcinoma of the lung.

BIs (numbers of cigarettes smoked × years of smoking ± SD) of the cases and the controls were 1057 ± 679 and 582 ± 612, respectively. When the never-smokers were excluded, BIs were 1121 ± 646 and 845 ± 566, respectively. We divided the cases into subgroups according to number of cigarettes smoked (Table 3), excluding never-smokers. In the group that smokes least (cigarette-years < 800), apparent odds ratios were greater than those among heavier smokers (cigarette-years ≥ 800). However, this difference was not actually significant when the interaction between genotype and odds ratio was investigated; the trends in odds ratios in the three genotypes according to smoking dosage, both in total lung cancers and lung cancers and excluding adenocarcinomas, were not largely different, as shown in Fig. 2.

Table 4 CypIA1 Ile-Val genotype distribution of reference populations

Genotype	Population				
	Okinawa	Tokyo	Iwate	Jiangsu	Australian Caucasian
Ile/Ile	56 (60%)	41 (59%)	53 (65%)	28 (72%)	127 (87%)
Ile/Val	31 (33%)	23 (33%)	25 (31%)	11 (28%)	18 (12%)
Val/Val	7 (7%)	5 (7%)	3 (4%)	0	1 (<1%)

The distributions of the Ile462Val genotypes in five reference populations, including Chinese Asians and Australian Caucasians, are listed in Table 4. The Val-containing genotype is lower in Chinese (Jiangsu) and very rare in Australian Caucasian populations. The distributions of genotype frequencies in these groups were also in accordance with Hardy-Weinberg equilibrium.

Discussion

The association of CypIA1 polymorphism and lung cancer susceptibility is currently one of the most important issues in molecular epidemiology (1–7, 9–11, 13, 31–33). There are conflicting data even within nations, such as Japan [Nakachi *et al.* (5) and Kihara *et al.* (4) *versus* Ikawa *et al.* (7)] and North America [Shields *et al.* (11) *versus* Xu *et al.* (13)]. A pooled analysis is in progress.[4] Here, we provide the first CypIA1 genotype and lung cancer data for an Asian population outside the main islands of Japan. This was of interest in view of the known genetic differences between Okinawan and mainland Japanese populations. A particularly interesting finding was the high odds ratio of the Val/Val genotype in small cell carcinoma patients. Nakachi *et al.* (5) and Kihara *et al.* (4) repeatedly reported the contribution of genotypes of a *Msp*I polymorphism and the Ile462Val polymorphism, which are in linkage disequilibrium with each other, to tobacco-related lung cancer, after adjusting for cigarette dosage. However, data from elsewhere in Japan, as well as Scandianvian countries and North America, have not revealed a statistically significant association of this genotype with lung cancer susceptibility. Recently, Hamada *et al.* (2) showed an association of the Ile462Val polymorphism with lung cancer in Brazil, which was the first study to implicate this CypIA1 genotype in lung cancer outside of Japan. An association of heterozygosity for the *Msp*I polymorphism with lung cancer has also been noted recently in North America (13), where, previously, only negative findings had been reported (11). Even at a mechanistic level, recently, the investigators demonstrated recombinant proteins with Ile and Val catalyzed ethoxyresoruffin (Val > Ile) and benzo(*a*)pyrene (Ile > Val) differently (34). Thus, the working hypothesis itself may be challenged. These observations prompted us to expand the evaluation of this genotype to other populations. Our study disclosed a high contribution of Val/Val genotype to the risk of squamous and small cell lung carcinoma. Val/Val genotype is assumed to be linked to *Msp*I site present homozygous type as reported, and high odds ratios of *Msp*I site present homozygote were reported for squamous and small cell carcinoma in Japanese lung cancer (4, 5). Our data further support that CypIA1 polymorphism is associated with tobacco-related carcinogenesis. As for the relation to smoking dosage (Fig. 2 and Table 3),

[4] E. Taioli and the Collaborative Group on Genetic Susceptibility to Environmental Carcinogenesis, personal communication.

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we may be able to interpret a weak tendency of increased risk for lighter smokers, which is also consistent with some of the previous reports (4, 5). Because the simple analysis of the relationship of Val/Val to lung cancer risk have not been described explicitly, that is, many investigators found a significant increase in the odds ratio after combining the glutathione *S*-transferase null genotype and *Msp*I site polymorphism, we cannot directly assess our data in comparison with theirs (4, 5). The contribution of *Msp*I polymorphism and glutathione *S*-transferase genotype would be interesting to study in our subjects as well. Data on the Ile462Val polymorphism are available for Asian populations in Kuala Lumpur, with Malays showing frequencies of 54, 30, and 16% for the Ile/Ile, Ile/Val, and Val/Val genotypes, respectively, whereas in Indians, the Val allele was less frequent (16). In Chinese populations in Singapore, the respective frequencies were 53, 37, and 10% (15). The Chinese population is heterogeneous, and our study is the first to report CypIA1 Ile462Val polymorphism frequency in mainland China, and a difference between Singapore and mainland China was indicated, with a lower Val allele frequency in the latter. Although CypIA1 genotype frequency is still largely unknown in southeast Asia, the limited data that are available suggest a higher Val allele prevalence. Such regions are likely to be an increasing focus for cancer mortality, and it will be interesting to see whether such statistics follow the higher Val allele prevalence.

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