of the subject population, with 14 studies in whites, 8 studies in Asians, and 5 studies in populations comprising a mix of other ethnic groups, including whites, African Americans, and Mexican Americans. Crude odds ratios and 95% confidence intervals were also estimated by source of controls (healthy or hospital) and then by both ethnicity and control source.

Summary odds ratios and 95% confidence intervals were calculated for all studies combined, as well as for each ethnic group (white, Asian, other), control source (healthy or hospital), and then by both ethnicity and control source. All analyses were performed by examining risk associated with carrying at least 1 valine allele compared with *Ile/Ile* genotypes at *GSTP1* exon 5 (Ile105Val, 313A → 313G). All meta-analyses were performed with the STATA software package (Stata Corporation, College Station, Texas).

To reduce the potential for confounding associated with ethnicity, the pooled analyses were performed separately for the 2 ethnic groups with the greatest number of studies and participants (whites and Asians). Chi-squared tests were conducted to test for Hardy-Weinberg equilibrium in the reported genotype frequencies among the controls in the pooled analysis, after stratification by ethnicity. Study-specific crude odds ratios and 95% confidence intervals for lung cancer and *GSTP1* genotype were estimated by using unconditional logistic regression models. As with the meta-analysis, odds ratios and 95% confidence intervals were calculated for individuals carrying 1 or 2 *105Val* alleles compared with individuals carrying 2 *Ile* alleles. Heterogeneity between studies was tested by using the Breslow-Day test for homogeneity. Crude and adjusted odds ratios were calculated for each ethnic group, as well as stratified by control source (healthy and hospital), smoking status (nonsmoker/ever smoker), and histologic type (adenocarcinoma, squamous cell carcinoma, and small cell carcinoma). Regarding studies that provided information on pack-years of smoking, this paper presents adjusted odds ratios and 95% confidence intervals for nonsmokers and by tertile of numbers of pack-years of smoking. Regression lines were fitted to test for linear trend between the odds ratios and amount smoked. To formally test for interactions between amount smoked and genotype, cross-product terms were created and tested in the logistic model. The cutpoints for the tertiles of amount smoked were calculated from the controls who smoked. All pooled analyses were performed by using SAS version 9.1 software (SAS Institute, Inc., Cary, North Carolina).

RESULTS

The genotype frequencies for the *GSTP1* exon 5 polymorphism varied according to ethnicity. When ethnicity-specific individual data from the controls were used in the pooled analysis, 46.9% of whites carried the *Ile/Ile* genotype, 41.8% carried the *Ile/Val* genotype, and 11.3% carried the *Val/Val* genotype (data not shown). In Asians, the respective percentages were 66.8%, 30.2%, and 3.0%. These frequencies are similar to those for the *GSTP1* exon 5 polymorphism found in other control populations for different cancer sites (70, 71). Among all controls in the pooled analysis, the genotype frequencies were in Hardy-Weinberg equilibrium for both Asians ($P = 0.38$) and whites ($P = 0.17$).

Meta-analysis

For all 27 studies combined, the meta–odds ratio was 1.04 (95% confidence interval (CI): 0.97, 1.10), with no apparent heterogeneity between the studies (P for Q test = 0.27) (data not shown). Thus, a fixed-effects model versus a random-effects model was used. Sensitivity analysis was conducted to examine the influence of each study. Exclusion of the study by Miller et al. (52) resulted in a summary odds ratio of 1.08 (95% CI: 1.00, 1.15) (data not shown).

Two tests were performed to detect publication bias. Publication bias was not identified when Begg's test was performed ($P = 0.10$), but the Egger et al. (69) regression asymmetry test, which tends to suggest the presence of publication bias more frequently than Begg's test, did suggest that publication bias was present ($P = 0.02$). To adjust for this bias, a trim and fill method developed by Duval and Tweedie (72) was implemented. Trimming was based on the fixed-effects model, and the adjusted estimate obtained by using a random-effects model was an odds ratio of 0.99 (95% CI: 0.91, 1.07). Thus, the overall conclusion that there is no association between lung cancer and carrying at least 1 valine allele remained unchanged.

Because of the ethnicity-specific differences in genotype frequency, study-specific crude odds ratios and 95% confidence intervals, stratified by ethnicity, are presented in Figure 1 for individuals carrying at least 1 *105Val* (*Ile/Val* and *Val/Val* vs. *Ile/Ile*) allele. Among studies of whites, only 1 study (64) reported a statistically significant association (odds ratio (OR) = 1.70, 95% CI: 1.13, 2.57). The remaining 13 studies were clustered around the null effect (6 below 1.0 and 7 above 1.0) and were not statistically significant. None reported a negative association. The overall odds ratio for studies in whites was 1.00 (95% CI: 0.93, 1.07). No heterogeneity was identified between the 14 studies included ($P = 0.23$, data not shown). Among the 8 studies in Asian populations, none were statistically significant, with 4 slightly below the null and 4 slightly above the null. The overall odds ratio in Asian studies was 1.09 (95% CI: 0.93, 1.28). No heterogeneity was identified between the 8 studies included ($P = 0.51$, data not shown). In the studies that reported other ethnicities, 4 of the 5 had odds ratio estimates above the null, and 1 was slightly below the null. The overall risk associated with lung cancer and carrying at least 1 valine allele was statistically significant in studies not limited to a single ethnicity, with an odds ratio of 1.19 (95% CI: 1.01, 1.40). No heterogeneity was identified between the 5 studies included ($P = 0.49$, data not shown).

No statistically significant differences were identified when studies were stratified by control source (healthy or hospital, data not shown). Stratifying by both ethnicity and control source resulted in sparse strata for "Asian and healthy" (2 studies) and "other and hospital" (1 study). For other strata, no statistically significant associations between genotype and lung cancer were identified, nor were

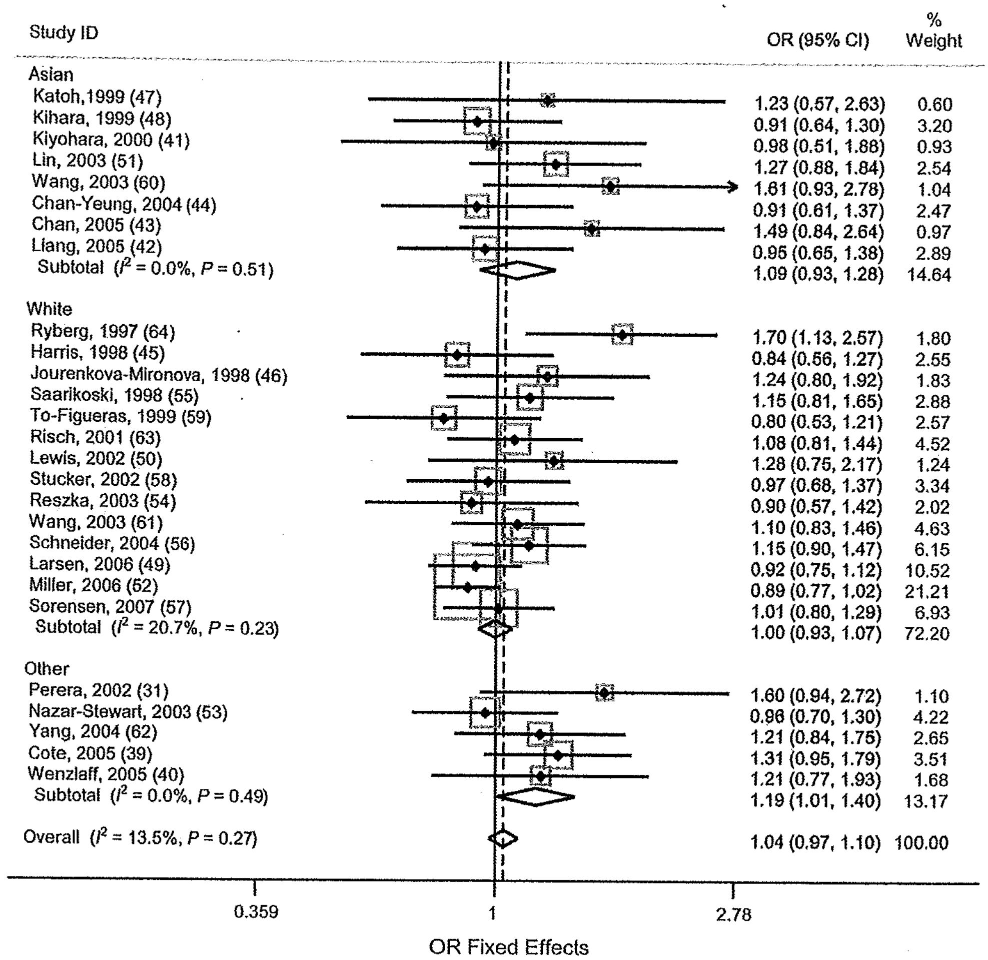

Figure 1. Study-specific (first author, year of publication (reference no.)) and meta-log odds ratios (ORs) with 95% confidence intervals (CIs) for a glutathione *S*-transferase pi gene (*GSTP1*) exon 5 (Ile105Val, 313A → 313G) polymorphism for individuals carrying at least 1 valine allele (*Ile/Val* and *Val/Val* vs. *Ile/Ile*), by ethnicity. The shading around the point estimate reflects the weight of the study in the meta-analysis. The dashed line indicates the overall OR. ID, an assigned study identifier for each study population; Ile, isoleucine.

there any significant differences between strata (data not shown).

Pooled analysis

Table 2 describes the 15 studies included in the pooled analysis. Crude odds ratios and 95% confidence intervals for the association between carrying at least 1 *105Val* allele at *GSTP1* exon 5 are presented. The overall pooled odds ratio associated with carrying at least 1 valine allele is 1.11 (95% CI: 1.03, 1.21) (Table 2).

Table 3 presents ethnicity-specific crude and adjusted odds ratios and 95% confidence intervals for the association between carrying at least 1 *105Val* allele at *GSTP1* exon 5 and lung cancer. In the crude analysis or after adjusting for study ID (an assigned study identifier for each study population), age, sex, or smoking status (ever/never), no increased risk of lung cancer was seen for whites carrying at least 1 valine allele compared with those with the *Ile/Ile* genotype. We found no differences in estimates based on whether the control source was healthy or hospital based. There were also no statistically significant differences in risk when subjects were stratified by ever smoker or nonsmoker status; after adjusting for study ID, age, and sex among nonsmokers; and after adjusting for study ID, age, sex, and number of pack-years of smoking among participants who had ever smoked (Table 3).

Regarding Asian study subjects, those who carried at least 1 *GSTP1 105Val* allele compared with those with the *Ile/Ile* genotype were shown to be at increased risk of lung cancer in both crude analysis (OR = 1.34, 95% CI: 1.07, 1.67) and after adjustment for study ID, age, sex, and smoking status (OR = 1.35, 95% CI: 1.07, 1.70) (Table 3). With the exception of 4 cases and 10 controls, these findings were all based

Table 2. Description of Studies Included in the Pooled Analysis: Study-Specific and Overall Crude Odds Ratios and 95% Confidence Intervals for the Association Between *GSTP1* Exon 5 (Ile105Val, 313A → 313G) Polymorphisms and Lung Cancer, by Continent, Country, and Year of Publication

First Author, Year (Reference No.)	No. of Cases	No. of Controls	Country of Study Origin	Source of Controls	Val/Val and Ile/Val vs. Ile/Ile	
					Crude OR	95% CI
Asia						
Wang, 2003 (60)	112	119	China	Hospital	1.61	0.93, 2.78
Liang, 2005 (42)	227	227	China	Hospital	1.22	0.84, 1.78
Kiyohara, 2000 (41)	62	80	Japan	Hospital	1.18	0.58, 2.42
Lin, 2003 (51)	198	332	Taiwan	Hospital	1.27	0.88, 1.84
Australia						
Larsen, 2006 (49)	1,095	626	Australia	Hospital	0.92	0.75, 1.12
Europe						
Sorensen, 2007 (57)	429	765	Denmark	Healthy	1.01	0.80, 1.28
Saarikoski, 1998 (55)	199	293	Finland	Healthy	1.49	1.03, 2.16
Jourenkova-Mironova, 1998 (46)	150	172	France	Hospital	1.24	0.80, 1.92
Schneider, 2004 (56)	480	630	Germany	Hospital	1.09	0.86, 1.39
Butkiewicz, 1999 (32)	165	326	Poland	Healthy	0.94	0.65, 1.37
Reszka, 2003 (54)	217	251	Poland	Hospital	0.93	0.64, 1.33
To-Figueras, 1999 (59)	173	202	Spain	Healthy	1.01	0.67, 1.51
Lewis, 2002 (50)	93	151	United Kingdom	Hospital	1.28	0.75, 2.17
North America						
Yang, 2004 (62)	235	234	United States	Healthy	1.22	0.85, 1.76
Cote, 2005 (39)	447	624	United States	Healthy	1.31	1.02, 1.69
Total	4,282	5,032			1.11	1.03, 1.21

Abbreviations: CI, confidence interval; *GSTP1*, glutathione *S*-transferase pi gene; Ile, isoleucine; OR, odds ratio; Val, valine.

on studies with hospital-recruited controls; thus, in this paper, these data are not presented for healthy controls. After we stratified by ever/never smoking status, the association between genotype and lung cancer was significant among only Asian nonsmokers (OR = 1.52, 95% CI: 1.09, 2.13) after we adjusted for study ID, age, and sex (Table 3).

Risk of lung cancer and GSTP1 *exon 5 polymorphism by amount smoked.* The large sample of whites for whom we had individual pack-year information (92.3% of the cases and 79.7% of the controls) allowed us to perform an analysis stratified by number of pack-years of cigarette smoking. Table 4 shows the relation among amount smoked, genotype, and lung cancer risk. For nonsmokers, no statistically significant association was seen between carrying at least 1 *105Val* allele and lung cancer risk after we adjusted for study ID, sex, and age (OR = 1.17, 95% CI: 0.92, 1.50). For participants who smoked for 1–28 pack-years, the odds of having lung cancer were 1.23-fold higher among those carrying at least 1 valine allele compared with those with the *Ile/Ile* genotype (95% CI: 1.00, 1.52) after we adjusted for study ID, sex, and age. Among moderate smokers who smoked for 28.01–48 pack-years, risk was not increased (OR = 1.01, 95% CI: 0.82, 1.24), and, among those with the heaviest reported pack-years of smoking (≥48.01), carrying at least 1 *105Val* allele was associated with a decreased risk of lung cancer compared with that for those with *Ile/Ile* genotypes after adjustment for study ID, age, and sex (OR = 0.83, 95% CI: 0.67, 1.03). We found no statistically significant linear trend between risk of lung cancer due to genotype and amount smoked ($P = 0.16$) when nonsmokers were included. There was a statistically significant trend between amount smoked and risk of lung cancer due to genotype ($P = 0.05$) when only ever smokers were examined. The overall effect of interactions between amount of smoking and genotype on lung cancer risk was marginally significant ($P = 0.08$, data not shown). When we used nonsmoking and *Ile/Ile* as the reference group, the group of heavy smokers with *Ile/Val* or *Val/Val* had a significantly increased risk ($P = 0.03$, Table 4).

Risk of lung cancer and GSTP1 *exon 5 polymorphism by histologic type and ethnicity.* Crude and adjusted odds ratios and 95% confidence intervals for the association between carrying at least 1 valine allele and lung cancer risk are presented stratified by histologic type and ethnicity in Table 5. Among whites, there were no statistically

Table 3. Odds Ratios and 95% Confidence Intervals for the Association Between a *GSTP1* Exon 5 (Ile105Val, 313A → 313G) Polymorphism (Ile/Val and Val/Val vs. Ile/Ile) and Lung Cancer in the Pooled Analysis, Stratified by Smoking Status and Ethnicity

Ethnic Group	No. of Cases	No. of Controls	OR	95% CI
Asian				
All studies: unadjusted	603	768	1.34	1.07, 1.67
All studies: adjusted[a]	603	768	1.35	1.07, 1.70
Hospital controls: unadjusted	599	758	1.32	1.06, 1.65
Hospital controls: adjusted[a]	599	758	1.32	1.05, 1.68
Smoker: unadjusted	287	298	1.23	0.88, 1.72
Smoker: adjusted[b]	287	298	1.17	0.83, 1.66
Smoker: adjusted[c]	48	49	1.06	0.41, 2.75
Nonsmoker: unadjusted	254	390	1.49	1.08, 2.07
Nonsmoker: adjusted[b]	254	390	1.52	1.09, 2.13
White				
All studies: unadjusted	3,538	4,098	1.07	0.98, 1.17
All studies: adjusted[a]	3,490	4,077	1.05	0.95, 1.16
Healthy controls: unadjusted	1,503	2,268	1.12	0.98, 1.27
Healthy controls: adjusted[a]	1,497	2,254	1.15	0.99, 1.33
Hospital controls: unadjusted	2,035	1,830	1.02	0.90, 1.16
Hospital controls: adjusted[a]	1,993	1,823	1.00	0.87, 1.14
Smoker: unadjusted	3,044	2,737	1.02	0.93, 1.14
Smoker: adjusted[b]	3,035	2,729	1.05	0.95, 1.17
Smoker: adjusted[c]	2,809	2,181	1.00	0.89, 1.13
Nonsmoker: unadjusted	465	1,352	1.18	0.95, 1.45
Nonsmoker: adjusted[b]	455	1,348	1.17	0.92, 1.49

Abbreviations: CI, confidence interval; *GSTP1*, glutathione *S*-transferase pi gene; Ile, isoleucine; OR, odds ratio; Val, valine.
[a] Adjusted for study ID (an assigned study identifier for each study population), age, sex, and smoking status (ever/never).
[b] Adjusted for study ID, age, and sex.
[c] Adjusted for study ID, age, sex, and pack-years of smoking.

significant differences in risk associated with the *GSTP1* gene polymorphism and adenocarcinoma, squamous cell carcinoma, or small cell carcinoma. In Asian populations, individuals carrying at least 1 valine allele were at increased risk of adenocarcinoma (OR = 1.33, 95% CI: 1.02, 1.74) after adjustment for study ID, age, sex, and smoking status. Risk of squamous cell lung cancer was increased for Asians carrying at least 1 valine allele (OR = 1.44, 95% CI: 1.05, 1.98), but the odds ratio was no longer statistically significant after adjusting for study ID, age, sex, and smoking status (OR = 1.36, 95% CI: 0.95, 1.94).

DISCUSSION

The meta-analysis found no association (OR = 1.04, 95% CI: 0.97, 1.10) between lung cancer risk and carrying 1 or more *GSTP1 105Val* alleles. This finding is similar to that of another meta-analysis of 25 studies with a combined 6,221 cases and 7,602 controls, which reported an unadjusted odds ratio of 1.04 (95% CI: 0.99, 1.09) for the association between lung cancer and carrying the *GSTP1* exon 5 Ile105Val variant (15). After stratifying by the ethnicity of study subjects, studies that included subjects of various ethnic backgrounds (i.e., the study had both African-American and white participants) reported an increase in risk associated with carrying at least 1 *105Val* allele compared with those with *Ile/Ile* genotypes.

The pooled analysis did identify an overall statistically significant increase in lung cancer risk associated with carrying at least 1 valine allele, with an odds ratio of 1.11 (95% CI: 1.03, 1.21). When the studies were stratified by subject ethnicity, this association was seen among Asian subjects but not among white subjects. A pooled analysis of whites diagnosed with early-onset lung cancer (under age 60 years) also reported no association between lung cancer risk and the *GSTP1* exon 5 genotype (30). Unlike the white control populations, who were recruited through either population-based methods or hospitals, almost all Asian controls were recruited by using hospital-based methods. Among Asians, this association between the *Ile/Val* and *Val/Val* genotypes and lung cancer risk was strongest among nonsmokers and those with adenocarcinoma. The higher prevalence of adenocarcinoma of the lung in Asians, particularly among women nonsmokers, was identified decades ago (73) and has received more attention recently with the success of using epidermal growth factor receptor tyrosine kinase inhibitors in these populations (74).

The results from the meta-analyses suggest no association between lung cancer risk and the *GSTP1* exon 5 polymorphism, either overall or stratified by race/ethnicity, whereas the results from the pooled analysis suggest risk of carrying at least 1 *105Val* allele is associated with increased risk of lung cancer overall and also in Asians. Examination of the 95% confidence intervals associated with the risk estimates suggests that these apparently discrepant results are not statistically significant. In addition, the pooled analysis did not contain subjects from all studies included in the meta-analysis, and vice versa, and the pooled analysis allowed for individual adjustment by age, sex, and smoking status. It has been suggested that results from individual subject data that allow for adjustment of confounders, such as the pooled analysis presented here, may best summarize results of multiple studies (75).

We found an interaction between *GSTP1* exon 5 genotype and personal smoking history. Among whites, those classified as "light" smokers (1–28.00 pack-years) were at increased risk of lung cancer if they carried the *Ile/Val* or *Val/Val* genotype compared with those with the *Ile/Ile* genotype. Conversely, heavy-smoking (≥48.01 pack-years) whites carrying the *Ile/Val* or *Val/Val* genotypes were at decreased risk compared with those with the *Ile/Ile* genotype. This interaction may explain some of the variability seen between populations with different recruitment criteria (i.e. early-onset cases who likely do not have as extensive smoking histories) and highlights the need to investigate the gene-environment interactions between genotype and amount smoked (i.e., pack-years).

Table 4. Odds Ratios and 95% Confidence Intervals for the Association Between a *GSTP1* Exon 5 (Ile105Val, 313A → 313G) Polymorphism (Ile/Val and Val/Val vs. Ile/Ile) and Lung Cancer in Whites in the Pooled Analysis, Stratified by Pack-years of Smoking

Smoking Status	No. of Cases		No. of Controls		OR[a]	95% CI	*P* Trend	*P* Value[b]
	Ile/Ile	Ile/Val or Val/Val	Ile/Ile	Ile/Val or Val/Val				
Nonsmoker	204	251	656	692	1.17	0.92, 1.50	0.16[c]	Reference
1–28 pack-years	279	386	459	539	1.23	1.00, 1.52		0.09
28.01–48 pack-years	490	557	292	334	1.01	0.82, 1.24		0.57
≥48.01 pack-years	505	592	228	329	0.83	0.67, 1.03	0.05[d]	0.03

Abbreviations: CI, confidence interval; *GSTP1*, glutathione *S*-transferase pi gene; Ile, isoleucine; OR, odds ratio; Val, valine.
[a] Adjusted for study ID (an assigned study identifier for each study population), age, and sex.
[b] *P* value for nonsmokers and with Ile/Ile as the reference group, adjusted for study ID, age, and sex.
[c] *P*-value test for trend among all 4 categories.
[d] *P*-value test for trend among smokers only.

Laboratory evidence suggests that carrying a *105Val* allele results in reduced GSTP1 enzymatic activity in the cell (11–13). These characterizations, while important for developing a hypothesis about the biologic mechanisms through which carcinogenesis evolves, do not necessarily represent what is occurring in the environment of the human lung. The seemingly protective effect of the *Val/Val* or *Ile/Val* genotype in heavy smokers identified in this pooled analysis does not directly support reduced activity associated with carrying the *Val* allele because it would be expected that those with reduced GSTP1 activity would be at increased risk of malignant transformation after exposure to carcinogens. The continuous assault from heavy smoking may change cellular activity in ways we are currently unable to assess in the human lung. A recent murine model, using GSTP-null mice, found a significantly higher number of adenomas in null mice compared with wild-type mice after exposure to 3 PAHs (76). When adducts in these mice were examined, there were significant differences in the number of adducts formed depending on the PAH they were exposed to, with 1 PAH resulting in no increase in adducts, suggesting that an alternative protective pathway in response to this specific exposure exists. While this study in mice is the first known in vivo model showing the importance of GSTP1 in

Table 5. Odds Ratios and 95% Confidence Intervals for the Association Between a *GSTP1* Exon 5 (Ile105Val, 313A → 313G) Polymorphism (Ile/Val and Val/Val vs. Ile/Ile) and Lung Cancer in the Pooled Analysis, Stratified by Histologic Type and Ethnicity

Ethnic Group	Unadjusted				Adjusted[a]			
	No. of Cases	No. of Controls	OR	95% CI	No. of Cases	No. of Controls	OR	95% CI
White								
Adenocarcinoma	1,048	4,098	1.08	0.95, 1.24	1,043	4,077	1.041	0.90, 1.21
Squamous cell carcinoma	1,394	4,098	1.00	0.86, 1.09	1,377	4,077	0.994	0.87, 1.14
Small cell carcinoma	399	4,098	1.11	0.90, 1.36	393	4,077	1.103	0.91, 1.41
Asian								
Adenocarcinoma	384	768	1.32	1.02, 1.70	384	768	1.33	1.02, 1.74
Squamous cell carcinoma	206	768	1.44	1.05, 1.98	206	768	1.36	0.95, 1.94
Small cell carcinoma	5	768	0.50	0.06, 4.52	5	768	0.44	0.04, 4.56
Total								
Adenocarcinoma	1,496	5,032	1.05	0.94, 1.18	1,491	5,009	1.11	0.98, 1.26
Squamous cell carcinoma	1,617	5,032	1.02	0.91, 1.14	1,600	5,009	1.03	0.91, 1.16
Small cell carcinoma	412	5,032	1.22	1.00, 1.49	406	5,009	1.12	0.90, 1.39

Abbreviations: CI, confidence interval; *GSTP1*, glutathione *S*-transferase pi gene; Ile, isoleucine; OR, odds ratio; Val, valine.
[a] Adjusted for study ID (an assigned study identifier for each study population), sex, smoking (ever/never), and age.

lung carcinogenesis, it is also apparent that the role of glutathione *S*-transferase has yet to be fully elucidated. In this study, we were not able to explore the other major roles that GSTP1 is thought to have in the cell, including resistance to chemotherapy (77), because we did not have information on chemotherapy or other exposures that might help clarify this gene-environment interaction.

The variation in risk associated with lung cancer and *GSTP1* exon 5 genotypes between Asians and whites is likely due to a number of factors, including different exposures in the populations. For example, studies in Asian women nonsmokers suggest that exposure to the carcinogens found in cooking oils increases risk of lung cancer (78, 79). Further studies of gene-environment interactions in lung cancer should also include occupational risk factors such as ionizing radiation (through radon exposure), asbestos, chromium, and arsenic (25, 80). The ability to examine only a small number of potential confounding variables is a limitation to both pooled and meta-analysis studies. It is also possible that publication bias accounts for some of the difference in risk seen between the Asian and white populations. It has been shown that a large proportion of Chinese literature does not reach PubMed and that studies that do are more likely than non-Chinese studies to be statistically significant and report larger measures of effect (i.e., odds ratios) (81). Thus, our findings may be a result of this publication bias, and the inability to include the entire collection of literature is a limitation of this analysis.

Other potential limitations include the presence of heterogeneity between studies. We tested for heterogeneity and performed a sensitivity analysis to determine whether a particular study or studies were a source of heterogeneity. Various amounts of data regarding smoking behaviors were collected, so we were unable to examine number of years of smoking or number of cigarettes smoked per day; therefore, our analyses were restricted to use of the 2 most commonly collected variables: pack-years of use or dichotomous classification as never or ever smokers. Additionally, pack-years of smoking was missing for approximately 20% of individuals identified as ever smokers. There were also differences in how the subjects were identified (hospital based or population), the histologic types of lung cancers included in the studies, the types of tissues used to extract DNA, and genotyping methods. When possible, we stratified by source of controls and histologic type of cancer. It was not feasible to control for the variation in pathologic reports of histology type that may occur by region or country.

LABORATORY TESTS

The methods used for determining *GSTP1* exon 5 genotypes are described in each article. The majority of the studies included in the analyses used genomic DNA extracted from blood, although 3 studies included DNA extracted from blood or lung tissue (32, 43, 49); 2 studies included DNA extracted from blood, lung tissue, or buccal samples (39, 40); and 1 study used blood and bronchial lavage (50). Polymerase chain reaction–based restriction fragment length polymorphism methods were the most frequently cited technique to determine *GSTP1* exon 5 genotypes.

POPULATION TESTING

The evidence to date regarding the polymorphism in *GSTP1* exon 5 and lung cancer risk is insufficient to suggest testing at the population level.

CONCLUSIONS AND RECOMMENDATIONS FOR RESEARCH

Overall, the meta-analysis found no significant association between lung cancer and the *GSTP1* exon 5 (Ile105Val) polymorphism for individuals carrying at least 1 *105Val* allele. No association was seen when we stratified by ethnicity in white or Asian populations. In the 5 studies that included more than 1 ethnic group, the meta-analysis suggested that an association between lung cancer and carrying at least 1 valine allele (*Ile/Val* and *Val/Val* vs. *Ile/Ile*) was statistically significant, with an odds ratio of 1.19 (95% CI: 1.01, 1.40), although this finding may be the result of population stratification.

In the pooled analysis, there was a statistically significant, mild overall association (OR = 1.11, 95% CI: 1.03, 1.21) between lung cancer and the *GSTP1* exon 5 (Ile105Val) polymorphism for individuals carrying a *Val/Val* or *Ile/Val* genotype compared with those carrying the *Ile/Ile* genotype. After stratification by ethnicity and adjustment for study ID, age, sex, and smoking status, increased risk associated with the *Val/Val* or *Ile/Val* genotypes and lung cancer was seen in Asian populations only. Among Asians, this risk was highest for nonsmokers and those with adenocarcinoma of the lung.

There is evidence for interaction among amount of smoking (i.e., pack-years), the *GSTP1* exon 5 (Ile105Val) polymorphism, and risk of lung cancer in whites. The odds of lung cancer associated with carrying at least 1 valine allele appear to decrease as the amount of pack-years of smoking increases, with heavy smokers who carry a *Ile/Val* or *Val/Val* genotype at decreased risk of lung cancer compared with their heavy-smoking counterparts with the *Ile/Ile* genotype. This finding highlights the importance of context when studying gene-environment interactions and clearly shows the need to collect detailed exposure information on all study participants, because gene expression, and risk of lung cancer, may differ by the environmental exposure.

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ORIGINAL ARTICLE

Alcohol Drinking May Not Be a Major Risk Factor for Fatty Liver in Japanese Undergoing a Health Checkup

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Abstract The question of whether alcohol drinking is a risk factor for fatty liver as shown by ultrasonography was investigated by both cross-sectional and longitudinal approaches in Japanese undergoing a health checkup. In this cross-sectional study, 32,438 males (49.0 ± 11.9 years old) and 31,009 females (48.2 ± 11.6 years old) receiving a health checkup from 2000 to 2005 were included. Longitudinally, 5,444 males (49.8 ± 10.7 years old) and 4,980 females (50.4 ± 9.3 years old) participating in both 2000 and 2005 were included. Multiple logistic regression analyses were performed for both sexes, adjusted for age, BMI, and smoking. The prevalence of fatty liver in non-, occasional, daily moderate, and daily heavy drinkers was 28.5, 27.5, 18.7, and 19.1% in men and 12.4, 7.7, 5.4, and 6.7% in women, respectively (inverse association, $P \leq 0.05$ for both). Occasional, daily moderate, and daily heavy drinking in men and occasional and daily moderate drinking in women were inversely associated with fatty liver in the cross-sectional study. Daily moderate and heavy drinking appeared protective in men in the longitudinal study. Alcohol drinking may not be a major risk for fatty liver in Japanese undergoing a health checkup.

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Abbreviations

BMI Body mass index
OR Odds ratio
FBG Fasting blood glucose

Introduction

Fatty liver due to intrahepatic accumulation of lipids is a widely recognized disease, thought to be linked to obesity and alcohol consumption [1–3]. Non-alcoholic fatty liver is recognized as the hepatic consequence of the metabolic syndrome, characterized by abdominal obesity, hypertriglycemia, hyperglycemia, and hypertension [4–6].

It has been controversial whether alcohol drinking causes obesity, although consumption was associated with a greater waist-to-hip ratio, overweight, and fatty liver [7–12]. Alcohol abuse and obesity were found to be equally strong risk factors for fatty liver in the Guangzhou area of China [13]. On the other hand, alcohol drinking may not increase the risk of obesity among US adults, drinking frequency further being inversely associated with the increase in waist circumference and obesity [9–11].

Low to moderate alcohol drinking may lower the risk of type 2 diabetes as well as the metabolic syndrome and cardiovascular mortality [14–19]. Protective effects of low to moderate alcohol drinking on type 2 diabetes may be related to improved insulin sensitivity [20–23]. It is possible that low to moderate alcohol drinking may therefore reduce the fatty liver, which is closely related to insulin resistance [5, 24]. Moderate alcohol drinking may also be a weaker risk factor for fatty liver than obesity from results for the general population of Northern Italy [25]. Low alcohol drinking, less than 20 g alcohol/day, did not increase the risk for fatty liver in Japanese at a health checkup [26]. Low to moderate alcohol drinking attenuated liver steatosis and non-alcoholic steatohepatitis in severely obese individuals in the USA, possibly by reducing insulin resistance [27]. Moreover, modest wine drinking decreased the prevalence of non-alcoholic fatty liver disease in the Third National Health and Nutrition Survey [28].

Most earlier studies excluded subjects with regular alcohol consumption of more than 20 g/day. Some 54–70% of men and 13% of women in Japan consume more than 23 g alcohol/day [29, 30], drinking behavior being to some extent determined by genetic polymorphisms of alcohol metabolism genes and alcohol-induced liver damage being influenced by the genetic variation of cytochrome P4502EI and alcohol dehydrogenase [31–33]. Therefore, exclusion and selection of categories of drinkers may give rise to misleading results.

In the present cross-sectional and longitudinal investigation, we therefore included all alcohol drinkers in an assessment of risk factors including alcohol drinking for fatty liver assessed by ultrasonography. Adjustment was made for age, body mass index (BMI), and smoking in Japanese undergoing a health checkup.

Methods

Design of Study

This study included both cross-sectional and retrospective longitudinal analyses to investigate whether alcohol consumption, determined by questionnaire, is associated with fatty liver, assessed by ultrasonography, in apparently healthy Japanese undergoing a health checkup. Informed consent was obtained from all participants.

Subjects of the Cross-Sectional Study

A total of 179,646 participants (men: 95,977, 51.7 ± 11.6 years old; women: 83,669, 51.4 ± 11.1 years old) underwent medical examinations including ultrasonography at Okazaki City Medical Association, Public Health Center, between April 2000 and March 2006. Since more than half of the participants repeatedly underwent medical checkups, the participants undergoing a checkup for the first time during this period were included. These comprised 34,593 men and 32,743 women. After exclusion of participants who had past or present histories of hepatic diseases induced by drugs, autoimmune conditions, or unknown etiology based on questionnaire and positive results for hepatitis virus, a total of 63,447 participants (men: 32,438, 49.0 ± 11.9 years old; women: 31,009, 48.2 ± 11.6 years old) were included.

Subjects of the Longitudinal Study

The numbers of participants undergoing medical checkups including ultrasonograpy in 2000 and 2005 were 26,247 (men: 14,627; women: 11,620) and 32,548 (men: 17,207; women: 15,341), respectively. After exclusion of participants who had past or present histories of hepatic diseases induced by drugs, autoimmune conditions, or unknown etiology based on questionnaire and positive results of hepatitis virus, a total of 12,453 participants in both 2000 and 2005 (men: 6,924, 49.5 ± 10.5 years old; women: 5,529, 50.7 ± 9.3 years old) were included. Since 2,029 cases (men: 1,480, 21.4%; women: 549, 9.9%) were assessed as having fatty liver in 2000 on ultrasonography, a total of 10,424 participants (men: 5,444, 49.8 ± 10.7 years old; women: 4,980, 50.4 ± 9.3 years old) without fatty liver in 2000 were longitudinally analyzed to determine risk factors for newly developed fatty liver on ultrasonography in 2005.

Questionnaire

Subjects provided data for alcohol consumption and smoking status in a self-administered questionnaire that was then checked during individual interview by expert nurses in the center. Alcohol consumption was recorded using questions on both frequency and quantity. Frequency of drinking was classified into occasional (1–6 days/week) and daily (7 days/week). One drink was defined as one bottle (500 ml) of beer containing 4–5% alcohol or 1 gou (180 ml) of Japanese sake containing 14% alcohol, which is equivalent to 23 g alcohol [29, 30]. Quantities of drinks were recorded as one, two, or three and more than three drinks per day. Amounts of alcohol consumed per week were estimated by assessing both frequency and numbers of drinks only in the daily drinkers since it was difficult to accurately determine amounts of alcohol in the occasional drinkers. The amounts of alcohol in the participants having daily one, two, and three or more than three drinks were estimated to be 161 g/week, 322 g/week, and 483 g or more than 483 g/week, respectively.

The drinkers were divided into three categories: occasional drinkers, daily moderate drinkers who have one drink (23 g alcohol) per day, and daily heavy drinkers who have two and three or more than three drinks (46 g and 69 g or more than 69 g alcohol, respectively) per day. These categories were determined according to the previous reports demonstrating that less than 30 g alcohol/day prevented cardiovascular diseases and the risk threshold for alcohol-induced liver disease was more than 30 g alcohol/day [34, 35].

Measurements

Body weight was measured to the nearest 0.1 kg and height to the nearest 0.1 cm. Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared. BMI was categorized into three categories: <25, 25–29.9, and ≤30 according to the criteria determined by the Japan Society for the Study of Obesity. Age was categorized into four categories: <40, 40–49, 50–59, and <60.

Blood samples were taken from each participant after overnight fasting. Fasting blood glucose (FBG) was measured by Hitachi autoanalyzer models 7600 and 7700 (Hitachi Medical, Co., Tokyo, Japan).

Fasting hyperglycemia was defined if serum FBG was ≤110 mg/dl. Elevated blood pressure or hypertension was diagnosed if resting blood pressures was ≤130/85 mmHg or if the participants had either a history of hypertension or antihypertensive medication, respectively.

Abdominal ultrasonographic examination was performed using convex-type real-time electronic scanners (SSA 250 and 300, Toshiba Medical, Co., Tokyo, Japan) by ten technicians lacking any information about the subjects, including alcohol history. All images were printed on sonograph paper and reviewed by other technicians and physicians. Fatty liver was assessed according to the modified criteria reported previously [36, 37]. These include a comparative assessment of liver brightness (diagnosed by a difference of more than 10 in the average liver and renal cortical echo amplitudes), attenuation of echo penetration, and decreased visualization of veins.

Statistical Analyses

Multiple logistic regression analyses were performed to determine the influence of drinking as a risk factor for fatty liver in both men and women, both adjusted for age and for age, BMI, and smoking in the cross-sectional and longitudinal studies. Adjustment was also made for age, BMI, smoking, and either FBG or elevated blood pressure and hypertension. The analyses were further performed after excluding daily heavy drinkers.

Statistical differences among groups were identified using one-way analysis of variance, followed by multiple comparisons using Bonferroni method. The $m \times n$ chi-square test and Fisher's test were used for comparison of prevalence of fatty liver. Logistic regression analyses were performed using computer software (SPSS version 13.0 for Windows). P values less than 0.05 were considered significant.

Results

Cross-Sectional Study

The percentages of occasional, daily moderate, and daily heavy drinkers were 32.9, 17.7, and 9.3% overall, 33.8, 27.6, and 16.5% for men, and 32.1, 7.4 and 1.8% for women, respectively. Age was significantly lower in occasional and daily drinkers than in non-drinkers in both sexes (Table 1). BMI was significantly higher in occasional drinkers and lower in daily drinkers than in non-drinkers in men and was significantly lower in occasional and daily drinkers than in non-drinkers in women. In addition, the overall prevalence of fatty liver was 23.9% in men and 10.3% in women, and the prevalence of fatty liver in daily

Table 1 Age, BMI, prevalence of fatty liver, and ever smoking rates due to drinking habits in the cross-sectional study

	Non-drinkers	Occasional drinkers	Daily moderate drinkers	Daily heavy drinkers
Men				
%	21.7	33.8	27.6	16.5
Age	50.9 ± 12.6	46.4 ± 12.1*	50.7 ± 11.2	49.1 ± 10.7*
BMI	23.1 ± 3.2	23.4 ± 3.1*	22.9 ± 2.8	23.0 ± 2.8
Fatty liver (%)	28.5	27.5	18.7	19.1
Ever smoking rates (%)	41.1	41.3	44.4	59.6
Women				
Number (%)	58.5	32.1	7.4	1.8
Age	50.6 ± 11.4	44.3 ± 11.2*	47.5 ± 10.0*	42.7 ± 10.1*
BMI	22.2 ± 3.3	21.7 ± 3.1*	21.4 ± 2.8*	21.2 ± 3.0*
Fatty liver (%)	12.4	7.7	5.4	6.7
Ever smoking rates (%)	5.9	11.6	17.3	52.4

* $P < 0.05$ compared with non-drinkers

drinkers was significantly lower than in non-drinkers in both sexes.

Multiple logistic regression analysis revealed that occasional and daily moderate drinking both adjusted for age and for age, BMI, and smoking was inversely associated with fatty liver in both sexes (Table 2). Daily heavy drinking fully adjusted for other factors was inversely associated with fatty liver in men, while this relation did not reach statistical significance in women.

Adding FBG or elevated blood pressure and hypertension, the ORs were not changed in both sexes. After removing the daily heavy drinkers (5,370 men and 563 women), the results were not essentially changed (data not shown).

Longitudinal Study

The percentages of occasional, daily moderate, and daily heavy drinkers were 30.6, 20.3, and 9.5% overall, 31.3, 32.3, and 17.0% for men, and 29.9, 7.0, and 1.2% for women, respectively. Age was significantly lower in occasional and daily heavy drinkers in men and in three groups of drinkers in women than in non-drinkers (Table 3). Fatty liver newly developed in 10.2, 12.1, 11.7, and 12.0% of non-, occasional, daily moderate, and daily heavy drinkers, respectively, overall within the 5-year period. Fatty liver was found in 16.4, 16.7, 12.9, and 12.4% of non-, occasional, daily moderate, and daily heavy drinkers in men, respectively, and in 8.2, 6.8, 5.7, and 6.7% of the women, respectively. The risk of newly developed fatty liver was significantly lower in daily moderate and heavy drinkers than non-drinkers in men.

In the multiple logistic regression analysis, daily moderate and heavy drinking was inversely associated with fatty liver adjusted for age, BMI, and smoking in men. Although similar inverse association was observed in women, this did not reach statistical significance (Table 4). Adding FBG or elevated blood pressure and hypertension did not alter the ORs (data not shown). After removing the daily heavy drinkers (928 men and 60 women), daily moderate drinking was the inverse risk factor for fatty liver (ORs 0.72, 95% CI 0.58–0.89) in men, while the results were not changed in women.

Table 2 Multiple logistic regression analysis for fatty liver in the cross-sectional study

	Age-adjusted OR	95% CI	Multivariate OR*	95% CI
Men				
Non-drinkers	1.00	References	1.00	References
Occasional drinkers	0.93	0.87–0.99	0.89	0.83–0.96
Daily moderate drinkers	0.56	0.52–0.60	0.58	0.53–0.63
Daily heavy drinkers	0.56	0.51–0.61	0.57	0.52–0.63
Women				
Non-drinkers	1.00	References	1.00	References
Occasional drinkers	0.74	0.68–0.81	0.77	0.70–0.85
Daily moderate drinkers	0.44	0.37–0.53	0.53	0.43–0.64
Daily heavy drinkers	0.70	0.50–0.98	0.85	0.60–1.23

* Adjusted by age, BMI, and smoking status

Table 3 Age, BMI, and ever smoking rates due to drinking habits in the longitudinal study

	Non-drinkers	Occasional drinkers	Daily moderate drinkers	Daily heavy drinkers
Men				
Number (%)	19.1	31.3	32.3	17.0
Age	51.4 ± 11.2	48.7 ± 11.1*	50.3 ± 10.5	49.0 ± 9.5*
BMI	22.2 ± 2.6	22.5 ± 2.5*	22.4 ± 2.4	22.4 ± 2.4
Ever smoking rates (%)	39.0	41.8	44.6	63.9
Women				
Number (%)	61.5	29.9	7.0	1.2
Age	51.8 ± 9.2	47.9 ± 9.2*	49.6 ± 8.6*	46.8 ± 9.0*
BMI	21.8 ± 2.6	21.8 ± 2.6	21.5 ± 2.5	21.5 ± 2.7
Ever smoking rates (%)	4.3	9.2	17.7	53.5

* $P < 0.05$ compared with non-drinkers

Table 4 Multiple logistic regression analysis for fatty liver in the longitudinal study

	Age-adjusted OR	95% CI	Multivariate OR*	95% CI
Men				
Non-drinkers	1.00	References	1.00	References
Occasional drinkers	0.97	0.78–1.19	0.95	0.77–1.17
Daily moderate drinkers	0.73	0.59–0.90	0.72	0.58–0.89
Daily heavy drinkers	0.67	0.52–0.87	0.65	0.50–0.85
Women				
Non-drinkers	1.00	References	1.00	References
Occasional drinkers	0.83	0.65–1.05	0.81	0.63–1.04
Daily moderate drinkers	0.67	0.42–1.07	0.71	0.44–1.16
Daily heavy drinkers	0.08	0.29–2.26	0.74	0.25–2.17

* Adjusted by age, BMI, and smoking status

Discussion

The present study demonstrated that alcohol drinking may not be a major risk factor for fatty liver as assessed by ultrasonography in Japanese undergoing a health checkup. Thus, the prevalence of fatty liver in both sexes was significantly lower in daily drinkers than in non-drinkers. Occasional, daily moderate, and daily heavy drinking in men and occasional and daily moderate drinking in women fully adjusted for other factors were inversely associated with fatty liver in the cross-sectional study. Daily moderate and heavy drinking exerted protective effects against the development of fatty liver in men in the longitudinal study.

The low to moderate amounts of alcohol found to reduce type 2 diabetes, metabolic syndrome, and cardiovascular diseases have ranged widely [14–23]. However, low to moderate amounts of alcohol were usually defined as less than 30 g alcohol/day [34, 35, 38]. Further, the risk for cardiovascular diseases is lower when alcohol consumption is low to moderate, and the risk is higher when alcohol consumption is high, resulting in a dose-response curve that is J- or U-shaped [38]. It was also demonstrated that the threshold for non-cirrhotic and cirrhotic liver damage was reported to be less than 30 g alcohol/day, and risk increased with increasing daily intake [35, 39]. We estimated that alcohol consumption of daily heavy drinkers ranged from 46 g alcohol/day to 69 g or more than 69 g alcohol/day in the present study. We also demonstrated that even daily heavy drinking was inversely associated with fatty liver and that exclusion of daily heavy drinkers did not essentially alter the trend in both cross-sectional and longitudinal studies. However, we do not encourage heavy alcohol drinking since we focused the effect on fatty liver, but not on liver injury, and more than 30 g alcohol/day has been reported to be injurious to the liver [35, 39].

Ethanol is known to impair fat oxidation and stimulate lipogenesis in the liver [2, 3]. Although there is conflicting evidence, alcohol intake is reported to be associated with fatty liver in apparently healthy adult men in Spain, with alcohol abuse and obesity being equally strong risk factors for fatty liver in the Guangzhou area of China [12, 13]. Alcohol drinking was found to be a weaker risk factor for fatty liver than obesity in another study [25].

Although our results appear paradoxical on the surface, we speculate that the discrepancy may be related to the different proportion of heavy alcohol drinkers. Our results are in line with other reports that low alcohol drinking did not increase the risk for fatty liver in health checkup participants in Japan and that low to moderate alcohol drinking reduced liver steatosis and non-alcoholic steatohepatitis found in the severely obese in the USA [26, 27]. Further, it was recently demonstrated that modest wine consumption was associated with a reduced prevalence of non-alcoholic fatty liver disease [28].

Adding FBG or elevated blood pressure and hypertension did not alter the ORs in both cross-sectional and longitudinal studies, suggesting that the relationship between alcohol drinking and fatty liver was not confounded by these factors and the effect of alcohol drinking on fatty liver may be independent of improved glucose metabolism and endothelial function. The mechanism by which low to moderate alcohol drinking reduces type 2 diabetes, cardiac ischemic diseases, and the metabolic syndrome may be, in part, related to increased insulin sensitivity [20–23]. Insulin resistance causes accumulation of fat in the hepatocytes through lipolysis and hyperinsulinemia [4, 40]. Although we did not measure insulin sensitivity in the present study, we speculate that this may be increased in our population by alcohol drinking, thereby attenuating fatty liver.

A major limitation of the present study was the cross-sectional and retrospective longitudinal design. The subjects were limited to the Japanese participants undergoing a health checkup. Although it would have been preferable to follow up all participants in 2000 to investigate the risk factor for fatty liver in 2005 in a cohort manner, only 42.5% of the participants in 2000 received the medical checkup in 2005. In addition, alcohol consumption was self-reported, and the drinkers were roughly divided into four groups according to the frequency of drinking for logistic regression analyses, which may result in inaccuracies. Finally, although histological diagnosis is more accurate, we had to rely on ultrasonography for the purposes of the present study. Ultrasonography cannot distinguish steatosis and steatohepatitis, with the result that it may be unclear if the participants drinking alcohol have liver damage. However, it has been widely used to assess fatty liver since it is a non-invasive procedure with relatively high sensitivity and specificity for screening purposes [1, 12, 13, 25, 26, 36, 37]. The prevalence of fatty liver, 23.9% in men and 10.3% in women in the present study, is consistent with values in a previous Japanese report [41].

In conclusion, alcohol drinking may not be a major risk factor for fatty liver on ultrasonography in Japanese undergoing a health checkup. However, we should be prudent, and the available data do not yet provide a rationale for encouragement of alcohol consumption. Future cohort studies assessing the influence of differing amounts of alcohol are necessary to confirm whether alcohol drinking may indeed not be a risk for fatty liver.

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REGULAR ARTICLE

Abdominal circumference should not be a required criterion for the diagnosis of metabolic syndrome

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Abstract
Background Metabolic syndrome (MetS) is an established concept. However, it is characterized by a number of different definitions as well as different cut-off points (COPs) for waist circumference (WC) and different modes for incorporating WC into the diagnostic criteria.
Methods Abdominal ultrasonography was performed in 2,333 subjects who also underwent comprehensive medical examinations between April and July 2006. The odds ratios for the number of MetS components were calculated by taking central obesity status into account and considering concurrent fatty liver as an independent variable. We compared the areas under the receiver operating characteristic (ROC) curves for fatty liver and MetS using several MetS criteria.
Results Regardless of the WC criterion selected, we observed a strong linear trend for an association (trend $P < 0.0001$) between MetS and the number of components. The odds ratio (OR) of subjects without central obesity but with all three MetS components was 9.69 (95% confidence interval 3.11–30.2) in men and 55.3 (6.34–483) in women. The COP for the largest area under the curve in men and women was ≥82 cm (OR 0.701) and ≥77 cm (OR 0.699), respectively, when WC was considered as a component. When WC distribution is taken into consideration, practical and appropriate COPs should be ≥85 cm for men and ≥80 cm for women.
Conclusion We suggest that a WC of ≥85 cm for men and ≥80 cm for women would be optimal COPs for the central obesity criteria in the Japanese population. In addition, central obesity should be incorporated as a component of MetS rather than an essential requirement for the diagnosis of MetS.

Keywords Central obesity · Diagnostic criteria · Metabolic syndrome · ROC curve

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Introduction

The prevention of metabolic syndrome (MetS), for which visceral fat accumulation and insulin resistance are considered upstream factors, has recently attracted the attention of the medical world as a useful approach to protect against lifestyle-related diseases typified by arteriosclerotic diseases [1–8]. Visceral fat accumulates for many reasons, including hyperalimentation and inadequate exercise, among others, and causes the abnormal functioning of fat cells and excessive secretion of hormones that are involved in various pathological conditions [9, 10]. Excessive

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secretion of these hormones is thought to act in combination with other factors to cause arteriosclerotic and other serious diseases, such as renal failure, blindness, lower limb amputation, cerebral apoplexy, cardiac arrest, and cerebrovascular diseases. The progression of conditions, from obesity into serious diseases, is sometimes referred as the metabolic domino effect [11, 12], and includes fatty liver disease.

Diagnostic criteria for MetS have been published by the World Health Organization [13], American National Cholesterol Education Programs, Adult Treatment Panel III (NCEP–ATP III) [14], and International Diabetes Federation (IDF) [15] for Asian countries, including Japan [16]. In Japan, the Examination Committee for Criteria of MetS introduced diagnostic criteria for Japanese metabolic syndrome (JMetS) [16], which are similar to the ones defined by IDF. The criteria essentially include central obesity and several other components, such as hypertension, hyperglycemia, and abnormal lipid metabolism. In Japan, the most prominent difference between the IDF and Examination Committee criteria for evaluating central obesity is in the cut-off point (COP) for waist circumference (WC), especially that for women: in all countries of the world, with the exception of Japan, the COP for WC is larger for men than that for women.

The relative newness of the MetS concept necessitates that the diagnostic criteria be updated as and when needed. The association between the diagnosis of MetS and downstream diseases in the metabolic domino needs to be addressed in prospective studies. In the study reported here, we applied several criteria to examine the association between metabolic status and concurrent fatty liver, which we used as a specific example of a disease in the metabolic domino. Our aim was to identify preliminary criteria and COPs for WC that can be used in diagnosing MetS.

Subjects and methods

Height, weight, and WC were measured, and abdominal ultrasonography was performed in 2,333 subjects (1,195 men and 1,138 women) of 2,428 subjects aged 40–79 years. These subjects underwent comprehensive medical examinations at the Kasugai City Medical Center during a 3-month period between April and July 2006. Patients receiving drug treatment(s) for liver diseases, hypertension, diabetes mellitus, or hyperlipidemia were excluded from the study. Height and weight were measured using an automatic scale (Tanita BF-220). The WC was measured in standing subjects with a tape measure placed horizontally at the level of the navel while the subject was gently exhaling. If the abdomen was protuberant and the navel was deviated downwards, the tape measure was placed at the midpoint level between the lower intercostal border and the anterior superior iliac spine.

Fatty liver was diagnosed after discussion with medical technologists (including ultrasound technicians), radiology technologists, and physicians and by taking fatty liver scores (as shown in Table 1) obtained at Kasugai City Medical Center into consideration. These scores were based on previous studies [17–20].

Blood pressure was measured on the right arm using a mercury sphygmomanometer; the subject was in a lying position and had rested for at least 5 min prior to the measurement. Venous blood samples were collected in the morning from subjects after a fasting period of 12 h. Triglyceride (TG) and serum high-density lipoprotein cholesterol (HDL-C) were measured by the direct enzymatic method, and fasting plasma glucose (FPG) was measured by the glucose oxidase method. Their concentrations were measured using an automated analyzer (model 7170S; Hitachi, Japan).

Current JMetS criteria require a central obesity (visceral adipose tissue area $\geq$100 cm^2 or WC $\geq$85 cm for men and $\geq$90 cm for women) and two or more of the following three components: (1) high blood pressure, based on a systolic blood pressure $\geq$130 mmHg and/or diastolic blood pressure $\geq$85 mmHg; (2) hyperglycemia, based on FPG $\geq$110 mg/dl; (3) abnormal lipid metabolism, based on TG $\geq$150 mg/dl and/or HDL-C $<$40 mg/dl [16]. The Examination Committee for Criteria of MetS in Japan also defined a "risk group for MetS" (yobi-gun) consisting of people who have central obesity and one of the three components listed above (high blood pressure, hyperglycemia, or abnormal lipid metabolism). In our study, as in most epidemiological studies, only WC was considered in our evaluation of central obesity; the visceral adipose tissue area was not assessed.

Our primary aim was to identify and propose new MetS criteria based on our results. Our suggested criteria (our criterion 1) considers central obesity not to be an essential requirement for MetS but as only one of the components of MetS. Accordingly, we defined our patients as having MetS when they demonstrated three or more components of

Table 1 Fatty liver score

Condition	Points
Bright echo pattern	0 or 1
Hepatorenal or hepatosplenic contrast	0 or 1 or 2
Unclear vessels	0 or 1
Deep attenuation	0 or 1 or 2
Fatty bandless sign	0 or 1
Liver swelling	0 or 1

A total score of $\geq$3 points is considered to indicate fatty liver

MetS, regardless of their central obesity status. Similarly, the risk group for MetS consisted of those individuals who demonstrated two components.

Taking the number of MetS components listed above in consideration, we first calculated the odds ratios of fatty liver according to central obesity status in men and women by logistic regression. We then constructed receiver operating characteristic (ROC) curves to assess the detecting power of MetS criteria for concurrent fatty liver and calculated the areas under the curve (AUC) for diagnostic criteria. These procedures were repeated using the IDF COP for WC in the Japanese population, i.e., ≥90 cm for men and ≥80 cm for women (our criterion 2). We also calculated the COP for the largest AUC and suggested an optimal COP for men and women based on the study results. Statistical analyses were performed using the SAS system for Windows (release 9.1.3; SAS Institute, Cary, NC), and the AUC value was obtained to refer to the c statistic in PROC LOGISTIC output. All statistical tests were two-sided, and a P value <0.05 was considered to be significant. The study was approved by the ethics committee of Nagoya City University.

Results

Table 2 shows the number of subjects diagnosed with MetS according to the JMetS criteria and our newly proposed criteria, respectively. This diagnosis was based on the number of MetS components, other than central obesity, calculated by WC status in both men and women. Only 8.4% of the women satisfied the central obesity criterion of JMetS, whereas 26.7% men satisfied the criterion. When the COP for central obesity was changed to ≥80 cm, 36.6% of women satisfied the criterion. Among the 13 men and six women who were newly diagnosed with MetS based on our criteria using the same WC COP, seven men (53.8%) and five women (83.3%) had fatty liver. The

Table 2 Criteria of metabolic syndrome and number of subjects

Number of components[a]	Criteria of JMetS	Our criteria	Number of patients diagnosed with MetS	Criteria of JMetS	Our criteria	Number of patients diagnosed with MetS
Men						
	Waist circumference <85 cm			*Waist circumference ≥85 cm*		
0	Normal	Normal	391 (32.7%)	Normal	Normal	93 (7.8%)
1	Normal	Normal	357 (29.9%)	Risk MetS	Risk MetS	152 (12.7%)
2	Normal	Risk MetS	115 (9.6%)	MetS	MetS	61 (5.1%)
3	Normal	MetS	13 (1.1%)	MetS	MetS	13 (1.1%)
Total			876 (73.3%)			319 (26.7%)
	Waist circumference <90 cm			*Waist circumference ≥90 cm*		
0	–	Normal	453 (37.9%)	–	Normal	31 (2.6%)
1	–	Normal	457 (38.2%)	–	Risk MetS	52 (4.4%)
2	–	Risk MetS	151 (12.6%)	–	MetS	25 (2.1%)
3	–	MetS	20 (1.7%)	–	MetS	6 (0.5%)
Total			1,081 (90.5%)			114 (9.5%)
Women						
	Waist circumference <90 cm			*Waist circumference ≥90 cm*		
0	Normal	Normal	603 (53.0%)	Normal	Normal	28 (2.5%)
1	Normal	Normal	357 (31.4%)	Risk MetS	Risk MetS	45 (4.0%)
2	Normal	Risk MetS	76 (6.7%)	MetS	MetS	18 (1.6%)
3	Normal	MetS	6 (0.5%)	MetS	MetS	5 (0.4%)
Total			1,042 (91.6%)			96 (8.4%)
	Waist circumference <80 cm			*Waist circumference ≥80 cm*		
0	–	Normal	458 (40.2%)	–	Normal	173 (15.2%)
1	–	Normal	211 (18.5%)	–	Risk MetS	191 (16.8%)
2	–	Risk MetS	49 (4.3%)	–	MetS	45 (4.0%)
3	–	MetS	4 (0.4%)	–	MetS	7 (0.6%)
Total			722 (63.4%)			416 (36.6%)

JMetS Japanese metabolic syndrome, *Risk MetS* individuals with central obesity and one of three components (high blood pressure, hyperglycemia, or abnormal lipid metabolism), as defined by the Examination Committee for Criteria of MetS in Japan, *MetS* individuals with MetS

[a] Number of the components of MetS other than abdominal obesity

prevalence of fatty liver was much higher than the total prevalence of fatty liver in men and women, i.e., 27.1 and 16.5%, respectively.

Table 3 shows the characteristics of the subjects diagnosed with MetS based on the application of several criteria. The prevalence of MetS using the JMetS criteria was 6.2% in men and 2.0% in women; based on our criteria using the JMetS COP for central obesity, MetS prevalence was 7.3 and 2.5%, respectively. When we applied the criterion for ≥80 cm COP for central obesity in women using our criteria, the prevalence of fatty liver increased to 4.9%. Similarly, the application of the COP increased the prevalence among the MetS risk group to 21.1%, which was close to that observed in men according to our criteria which include the ≥85 cm COP for central obesity. Since central obesity is an essential criterion for determining JMetS or the JMetS risk group, the subjects in these categories are much more obese than those falling in the normal category. The difference in WC and BMI between subjects in the MetS group and the normal group was 12.1 cm and 3.5 kg/m^2, respectively, in men and 17.6 cm and 5.7 kg/m^2 in women. When our criteria were used, these differences decreased to 10.4 cm and 3.0 kg/m^2, respectively, in men and 14.5 cm and 5.0 kg/m^2 in women.

Table 3 Characteristics of the subjects by MetS status

Characteristics	Men			Women		
	Normal	Risk MetS	MetS	Normal	Risk MetS	MetS
Criteria of JMetS (cut-off of WC)	**(85 cm)**			**(90 cm)**		
Number (row%)	969 (81.1%)	152 (12.7%)	74 (6.2%)	1,070 (94.0%)	45 (4.0%)	23 (2.0%)
Fatty liver prevalence (%)	20.6%	46.1%	73.0%	14.5%	40.0%	65.2%
Age (years)	63.0 ± 8.8	63.3 ± 8.4	63.4 ± 7.9	61.6 ± 8.0	65.8 ± 8.1	64.4 ± 6.7
BMI (kg/m^2)	22.3 ± 2.4	25.8 ± 2.4	25.8 ± 2.5	21.7 ± 2.6	27.2 ± 3.4	27.4 ± 3.1
WC (cm)	77.8 ± 6.4	89.6 ± 5.3	89.9 ± 4.9	76.5 ± 7.5	95.0 ± 5.1	94.1 ± 3.7
Systolic blood pressure (mmHg)	122.6 ± 15.1	126.5 ± 16.0	136.2 ± 12.4	122.2 ± 17.0	132.0 ± 13.8	142.3 ± 14.7
Diastolic blood pressure (mmHg)	71.9 ± 8.6	75.0 ± 8.9	79.9 ± 8.2	70.5 ± 9.3	74.6 ± 8.3	79.3 ± 7.0
Triglycerides (mg/dl)	114.3 ± 70.8	142.7 ± 71.6	196.2 ± 150.0	97.5 ± 49.8	120.7 ± 52.7	204.5 ± 101.1
HDL-cholesterol (mg/dl)	62.1 ± 16.4	53.3 ± 12.6	51.9 ± 14.8	72.2 ± 17.1	64.7 ± 14.3	54.1 ± 13.6
Fasting glucose (mg/dl)	96.0 ± 17.2	100.0 ± 18.1	122.9 ± 48.1	92.4 ± 15.8	95.1 ± 13.5	117.3 ± 30.9
Our criteria 1 (cut-off of WC)	**(85 cm)**			**(90 cm)**		
Number (row%)	841 (70.4%)	267 (22.3%)	87 (7.3%)	988 (86.8%)	121 (10.6%)	29 (2.5%)
Fatty liver prevalence (%)	17.6%	43.1%	70.1%	12.7%	35.5%	69.0%
Age (years)	62.7 ± 8.9	63.9 ± 8.1	64.2 ± 8.0	61.3 ± 8.0	64.7 ± 7.9	64.8 ± 6.8
BMI (kg/m^2)	22.2 ± 2.5	24.5 ± 2.7	25.2 ± 2.6	21.7 ± 2.6	24.0 ± 3.7	26.7 ± 3.2
WC (cm)	77.6 ± 6.6	84.9 ± 7.2	88.0 ± 6.7	76.4 ± 7.6	84.3 ± 10.1	90.9 ± 7.6
Systolic blood pressure (mmHg)	120.5 ± 14.1	130.8 ± 15.9	137.1 ± 11.9	120.7 ± 16.3	137.2 ± 14.0	143.9 ± 14.4
Diastolic blood pressure (mmHg)	71.0 ± 8.3	76.1 ± 9.0	79.4 ± 8.0	69.9 ± 9.1	76.8 ± 8.7	79.9 ± 7.5
Triglycerides (mg/dl)	103.8 ± 50.2	159.2 ± 103.1	197.4 ± 140.9	91.2 ± 40.2	152.1 ± 76.4	207.7 ± 92.4
HDL-cholesterol (mg/dl)	63.2 ± 16.0	54.4 ± 15.0	51.3 ± 14.8	73.0 ± 16.8	63.3 ± 16.7	55.2 ± 14.0
Fasting glucose (mg/dl)	93.2 ± 12.2	105.3 ± 24.5	124.5 ± 45.0	91.2 ± 13.9	100.3 ± 21.6	121.9 ± 33.2
Our criteria 2 (cut-off of WC)	**(90 cm)**			**(80 cm)**		
Number (row%)	941 (78.7%)	203 (17.0%)	51 (4.3%)	842 (74.0%)	240 (21.1%)	56 (4.9%)
Fatty liver prevalence (%)	19.6%	50.2%	74.5%	10.3%	27.9%	60.7%
Age (years)	62.8 ± 8.8	64.0 ± 8.2	63.9 ± 7.9	60.6 ± 8.0	64.9 ± 7.2	64.6 ± 7.7
BMI (kg/m^2)	22.5 ± 2.5	24.3 ± 3.0	25.6 ± 3.3	21.3 ± 2.5	23.9 ± 3.1	25.4 ± 2.9
WC (cm)	78.6 ± 6.9	84.3 ± 8.2	88.8 ± 8.6	75.0 ± 7.4	84.3 ± 7.4	87.8 ± 6.6
Systolic blood pressure (mmHg)	121.2 ± 14.4	133.4 ± 15.5	138.2 ± 11.5	118.1 ± 14.9	135.5 ± 15.2	143.1 ± 14.8
Diastolic blood pressure (mmHg)	71.5 ± 8.4	76.9 ± 9.1	80.2 ± 7.9	68.8 ± 8.6	76.1 ± 8.8	79.8 ± 8.0
Triglycerides (mg/dl)	107.6 ± 53.0	172.0 ± 112.7	211.2 ± 172.3	87.0 ± 36.5	126.3 ± 56.4	195.5 ± 102.1
HDL-cholesterol (mg/dl)	62.2 ± 15.9	54.7 ± 16.1	50.1 ± 14.9	74.6 ± 16.6	64.2 ± 15.7	56.4 ± 13.3
Fasting glucose (mg/dl)	93.9 ± 13.4	109.7 ± 28.2	131.1 ± 49.9	90.8 ± 14.4	95.4 ± 14.7	115.5 ± 30.7

Data are given as the mean ± standard deviation (SD)

WC Waist circumference, *BMI* body mass index, *HDL* high-density lipoprotein