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Roles of the Genetic Polymorphisms of Alcohol-Metabolizing Enzymes on the Immunology in High-Risk Drinkers

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Alcohol metabolism involves several enzymes and the individual genetic variations in the alcohol metabolism are related to the absorption, distribution, and elimination of alcohol and metabolites such as acetaldehyde. Therefore, the genetic variations of alcohol-metabolizing enzymes are responsible for the different toxicity of alcohol in several organs like liver and immunological systems. The purpose of this study was to evaluate if the life styles such as drinking and smoking and the genetic variations of alcohol-metabolizing enzymes (ADH2, ALDH2, CYP2E1, and CAT) were associated with the immunological biomarkers. In this study, 105 high-risk drinkers and 102 low-risk drinkers who were excluded from the immune-related diseases and other critical diseases were enrolled to evaluate the immunological functions. Counts of white blood cells, mononuclear cells, and lymphocyte subpopulations, and liver and immunological function tests were measured. Genotypes of alcohol-metabolizing enzymes were assayed by a real-time PCR and PCR-restriction fragment length polymorphism. Generally, the activity of aspartate aminotransferase (AST) was higher than that of alanine aminotransferase (ALT) in alcoholics; however, the activities of AST and ALT were simultaneously elevated in general hepatitis except for alcohol-induced hepatitis. Thus, the higher ratio of AST/ALT was used to be a marker for the alcohol-induced abnormal liver function. Glutamyltransferase (GGT) is produced by the liver cell microsomes and is a useful laboratory marker as an indicator of early liver cell damage. An increase in GGT concentration has been regarded as a marker of alcohol consumption or liver disease. In addition, the synergistic effects of smoking and drinking on the count of white blood cell (WBC) and mononuclear cells were found to be significant. Furthermore, there were higher OR to become high-risk drinkers in subjects with the combination of ALDH2 (*1/*1) genotype and either genotype of ADH2 or CYP2E1 than the others with other combinations of genotypes. Additionally, there were more abnormal immunological tests in the subjects with higher activity of ADH2 and lower activity of ALDH2. Our results suggested that the habits of drinking, smoking, and betel chewing, and genetic variations of alcohol metabolism were associated with the immunological biomarkers.

Key Words: **high-risk drinkers; alcohol-metabolizing enzymes; genetic variations; immunology.**

Excessive alcohol intake and smoking frequently co-occur and are associated with varieties of adverse health, social consequences and many disorders, such as liver cirrhosis and nervous system disorders (Lieber, 2000; McBride *et al*., 2002). Alcohol is also known to modulate the immune system in complicated ways and increases the risk for susceptibility of infectious diseases (Crews *et al*., 2006; Nicolaou *et al*., 2004; Schleifer *et al*., 2006; Szabo, 1997). Additionally, alcohol may also reduce the ability of lymphocytes to proliferate and differentiate adequately after being activated by a foreign antigen (Szabo, 1997) and cigarette smoking may alter bronchial mucosal immunity in asthma patient (Tsoumakidou *et al*., 2007). Alcohol metabolism involves several enzymes as shown in Figure 1. Alcohol dehydrogenase (ADH) metabolizes most alcohol in the cytoplasm within the liver cells in a major pathway; other enzymes in the minor pathways, such as cytochrome P4502E1 (CYP2E1) and catalase (CAT); also contribute to the production of acetaldehyde from alcohol oxidation. Acetaldehyde is more toxic for the liver, but also shows systemic toxicity and then is degraded to acetate by the aldehyde dehydrogenase (ALDH), which mainly located in hepatocyte mitochondria.

Genetic polymorphisms of alcohol-metabolizing enzymes have been reported in different ethnic groups (Goulas *et al*., 2002; Sun *et al*., 1999). As shown in Figure 1, individuals with the different alleles of alcohol-metabolizing enzymes oxidize alcohol and acetaldehyde variably. Among the alcohol-metabolizing pathways, individuals with higher activity of ALDH2 will be able to tolerate excessive alcohol intake because they can quickly metabolize acetaldehyde to acetic

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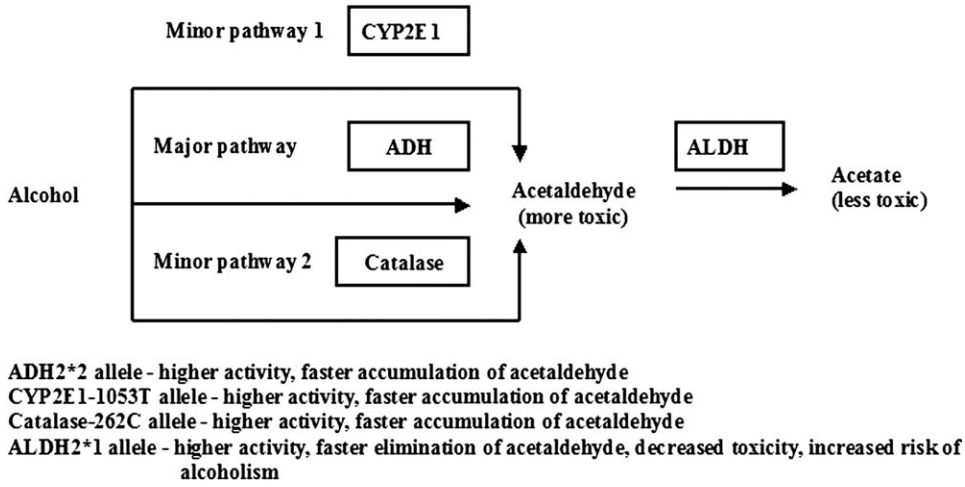

FIG. 1. Alcohol metabolism related enzymes in the major and minor pathways and the accumulative rates of acetaldehyde in different alleles of alcohol-metabolizing enzymes.

acid, leading to less acetaldehyde-induced toxicity; however, it may increase the risk of alcoholism because individuals with higher activity of ALDH2 are not discomfortable after drinking and will unconsciously over drink.

In our previous reports (Tseng *et al*., 2007, 2008b), we found that the genetic variations of alcohol-metabolizing enzymes may play an important role in trauma patients at the emergency department (ED); furthermore, in another study of ours (Tseng *et al*., 2008a) we found that a high dose of alcohol may affect the antioxidant status in human peripheral blood mononuclear cells and the imbalance of antioxidant statuses may be involved in immune-related diseases. The effects of alcohol on the immunity are diverse, including the personal body constitutions. The polymorphisms of alcohol-metabolizing enzymes may be associated with the susceptibility of alcoholism. In this study, therefore, our main purposes were to evaluate the associations between drinking habits and immunological biomarkers and the associations between immunological biomarkers and combined genotypes of alcohol-metabolizing enzymes.

MATERIALS AND METHODS

Study population and questionnaire. The participants, including hospital employers, general blue-collar laborers, and white-collar workers, who were excluded from the immunorelated diseases and other critical diseases, included 207 subjects in this study. With informed consent, each participant signed and completed questionnaires, including the data about his/her age, weight, height, education level, lifestyle, and self-reported alcohol intake status.

For the estimation of alcohol intake, alcohol conversion factors differ by country but generally are about 3–5% for beer, about 10–14% for wine, and about 30–40% for distilled spirits. Thus, the pure alcohol content for a bottle of beer might be calculated as (330 ml) × (0.04) = 13.2 ml of alcohol. 13.2 ml of alcohol was converted into about 10 g of pure alcohol. In this study, a bottle (330 ml) of beer, 100 ml of wine, or 40 ml of distilled spirits was estimated as about 10 g of pure alcohol. The participants were divided into two groups, the low-risk drinkers and the high-risk drinkers, according to the amounts of weekly alcohol intake through the questionnaires and documents from World Health Organization (WHO, 2000). Briefly, high-risk drinkers was defined as pure alcohol intake of either (I) exceeding 100 g for men and 50 g for women weekly, or (II) more than 40 g a time for men, and 20 g for women at least once a week. On the contrary, pure alcohol intake that did not exceed the above-mentioned amounts was considered to be low-risk drinkers. According to the questionnaires, a total of 105 high-risk drinkers and 102 low-risk drinkers were enrolled in this study.

The questionnaire of alcohol use disorders identification test (AUDIT) and cut down, annoyed, guilty, eye opener (CAGE) are used for assessment of alcohol abuse. CAGE is a brief and short evaluation, which includes four questions in order to differentiate individuals with alcohol use disorders (Gul *et al*., 2005). Point of intersection is recommended as two. The AUDIT is a self-rated 10-item questionnaire with each item scored 0–4, giving a total score of 40. The questions of AUDIT include frequency and amounts of alcohol intake, alcohol dependence, and the problems caused by alcohol. AUDIT has been developed as a screening test by WHO in order to determine harmful and risky alcohol intake in the first stage health facilities. Point of intersection is assumed as 8 or 9 (Gul *et al*., 2005). The Institutional Review Board of the Kaohsiung Veterans General Hospital approved the study protocol and informed consent was obtained from the participants after the aims and objectives of the study had been explained.

Measurements. Participants were asked to comply with a minimum fasting period of 12 h before blood collection (12 ml). We adopted an automated hematology analyzer (XE 2100, Sysmex Co., Japan) to analyze complete blood count. Liver function tests (AST, ALT, GGT, total protein, albumin, and globulin), lipids profiles (cholesterol [CHOL] and triglyceride [TG]), complement 3, complement 4, and immunoglobulins (IgG, IgM, and IgA) were performed on a chemistry analyzer (Vitros Fusion 5.1, Ortho Clinical Diagnostics, Johnson & Johnson Co., Rochester, NY). Analysis of lymphocyte subpopulations ($CD3^+$, $CD4^+$, $CD8^+$, and $CD19^+$) was performed on a flow cytometric analyzer (Coulter Epics XL, TM; Beckman Coulter, Fullerton, CA). The inflammatory cytokines (TNF-α, IL-2R, IL-6, and IL-8) and immunoregulatory cytokine (IL-10) were measured with a chemistry analyzer (Immulite, DPC/Siemens, Germany).

Genotyping of alcohol-metabolizing enzymes. Blood samples (2–4 ml) were obtained from the participants. DNA was extracted from buffy coat preparations by using a commercial kit (QIAmp DNA Blood Mini Kit; Qiagen, Germany). The polymorphisms of ADH2 Arg48His (rs1229984), ALDH2 Glu504Lys (rs671), and CYP2E1-1053C>T (rs2070674) were genotyped by

a Real-Time PCR thermocycler (MJ Chromo4, Bio-Rad, Hercules, CA). All of the assays were done in 96-well PCR plates.

For exon 3 of the ADH2 gene, reactions were performed in 25 μl of final volume containing: 2 μl of template DNA, a 10 μl of iQ Multiplex Power Mix buffer (Bio-Rad), 0.75 μl of each primer, and 0.4 μl of the each probe. Thermal cycling was initiated with a first denaturation step of 3 min at 95°C, and then by 40 cycles of 15 s at 95°C and 60 s at 55.2°C.

For exon 12 of the ALDH2 gene, reactions were performed in 25-μl final volume containing: 1 μl of template DNA, a 12.5 μl of iQ Multiplex Power Mix buffer, 0.75 μl of each primer, and 0.5 μl of the each probe. Thermal cycling was initiated with a first denaturation step of 3 min at 95°C, and then by 40 cycles of 15 s at 95°C and 60 s at 55.1°C.

For the 5′ *flanking* region of the CYP2E1 gene, reactions were performed in 25-μl final volume containing: 1 μl of template DNA, a 12 μl of iQ Multiplex Power Mix buffer, 1 μl of each primer, and 0.5 μl of the each probe. Thermal cycling was initiated with a first denaturation step of 3 min at 95°C, and then by 40 cycles of 15 s at 95°C and 60 s at 58.6°C.

Genotyping for the CAT −262C>T was analyzed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis (Forsberg *et al*., 2001). A total of 1 μl of genomic DNA was mixed with 1 μl of each primer, which targeted portions of the CAT gene promoter region in a total volume of 50 μl, which contained 10mM of deoxy-nucleotidyl triphosphate, a 10× buffer, 1 U *Taq* DNA polymerase. PCR amplification of the CAT gene promoter region was performed with a touchdown program designed as follows: 92°C for 30 s, 70°C for 40 s, −0.5°C per cycle (19 cycles), then 92°C for 30 s, 60°C for 40 s, 1 s/cycle (19 cycles). The final elongation step was 10 min at 72.8°C. Then, the 10 μl of PCR products was digested with 0.4 μl *Sam I* for 2 h at 25°C, electrophoresed on 4% agarose gel, and viewed with the aid of ethidium bromide staining. When a *Sam I* restriction site was presented, the 185-bp fragment was digested into two fragments, 155- and 30-bp lengths. The T (−262) variant yields an undigested product of 185 bp, relative to the digested C (−262) variant, which was visualized as a 155 and 30-bp fragment.

Statistical analysis. Data were analyzed using SPSS software for Windows (version 10.0, SPSS, Inc., Chicago, IL). Differences between the two groups were evaluated by using the Chi-Square test for discontinuous variables and Student's *t*-test for continuous variables and the univariate analyses were performed using the interaction test for categorical data and analysis of variance for continuous data. Fisher's exact test was used in the analysis of categorical data where sample sizes were small. Data were presented as mean ± SD. Probability differences of $p < 0.05$ were considered statistically significant.

RESULTS

The general characteristics of the study population are shown in Table 1. In this study, our purpose was to compare the immunological biomarkers and genetic variations of the major alcohol-metabolizing enzymes between the heavy drinkers and light drinkers. Thus, we adopted the documents of the WHO, which defined that a person who fit the descriptions of either criterion I or criterion II might be regarded as a high-risk drinker. According to the criteria, a total of 105 high-risk drinkers and 102 low-risk drinkers were enrolled in this study and they completed both the CAGE and AUDIT questionnaires. Among the 105 high-risk drinkers, 60 of them fit the descriptions of Criterion I, and 45 of them fit those of Criterion II, and there were not significant differences between the two subgroups. The body mass index (BMI), the proportion of smoking and betel chewing habits, and the scores of CAGE and AUDIT in the high-risk drinkers were significantly higher than those of the low-risk drinkers. On the contrary, there were significantly higher education levels in the low-risk drinkers than those in the high-risk drinkers. Additionally, for the liver function enzymes, lipid and protein levels, there were higher activities of AST and GGT, and the ratio of AT/ALT and higher levels of TG; however, there were lower levels of protein (total protein and globulin) and the ratio of A/G in the high-risk drinkers.

TABLE 1
General Characteristics of the Study Population

	High-risk drinkers ($n = 105$)	Low-risk drinkers ($n = 102$)
Age ranges (years)	22-77	22–61
Gender (males/females)	82/23	49/53
Average alcohol consumption (g/week)	174 ± 139	7 ± 8
BMI	25.1 ± 3.6**	23.2 ± 3.0
Education levels@		
Primary school or below	6 (5.7%)	1 (1.0%)
High school	58 (55.2%)	30 (29.4%)
College or above	41 (39.0%)	71 (69.6%)
Smoking@		
Yes	57 (54.3%)	13 (12.7%)
No	48 (45.7%)	89 (87.3)
Betel Chewing@		
Yes	21 (20.0%)	2 (2.09%)
No	84 (80.0%)	100 (98.0%)
Screens for alcoholism		
CAGE score	1.9 ± 1.1**	0.4 ± 0.8
AUDIT score	13.2 ± 6.7**	1.6 ± 1.8
Liver functions and protein levels		
AST (U/l)	28 ± 17**	23 ± 8
ALT (U/l)	33 ± 19	30 ± 18
AST/ALT	0.9 ± 0.3*	0.8 ± 0.2
GGT (U/l)	68 ± 113**	26 ± 12
Total protein (g/dl)	7.2 ± 0.6##	7.5 ± 0.6
Albumin (g/dl)	4.5 ± 0.3	4.5 ± 0.4
Globulin (g/dl)	2.8 ± 0.5##	3.0 ± 0.5
Ratios of A/G	1.7 ± 0.3	1.6 ± 0.3
Lipids		
CHOL (mg/dl)	212 ± 38	209 ± 39
TG (mg/dl)	155 ± 110**	109 ± 55

Note. *$p < 0.05$, **$p < 0.01$ higher than those of the low-risk drinkers; #$p < 0.05$, ##$p < 0.01$ lower than those of the low-risk drinkers; @$p < 0.01$ Comparisons between the high-risk drinkers and low-risk drinkers by the Chi-square test. p value adjusted for gender.

Table 2 shows the comparisons of parameters involved in the immunological biomarkers between the high-risk drinkers and low-risk drinkers. For the lymphocyte subpopulation, there were significantly higher percentages of $CD4^+$ T lymphocytes and ratios of $CD4^+/CD8^+$, but lower percentages of $CD8^+$ T lymphocytes in the high-risk drinkers than those in the low-risk drinkers. However, there was no significant difference in the percentage of $CD19^+$ B lymphocyte between the two groups. Additionally, there were lower IgG and IgM levels, but

TABLE 2
Comparisons of Immunological Biomarkers

Parameters	High-risk drinkers ($n = 105$)	Low-risk drinkers ($n = 102$)
WBC counts ($\times 10^3/\mu l$)	6.3 ± 1.4	6.0 ± 1.3
Mononuclear cells (%)	42.1 ± 7.2	41.4 ± 7.4
Mononuclear cells ($\times 10^3/\mu l$)	2.6 ± 0.7	2.5 ± 0.6
Lymphocyte subpopulations		
$CD3^+$ T lymphocytes (%)	66.0 ± 9.4	64.0 ± 8.2
$CD4^+$ T helper cells (%)	36.0 ± 9.1**	32.1 ± 8.0
$CD8^+$ T cytotoxicity cells (%)	22.5 ± 6.7#	24.7 ± 6.3
Ratios of $CD4^+/CD8^+$	1.8 ± 0.8**	1.4 ± 0.5
$CD19^+$ B lymphocytes (%)	11.2 ± 4.3	11.9 ± 4.5
Levels of immunoglobulin		
IgG (mg/dl)	1123 ± 237#	1208 ± 252
IgM (mg/dl)	89 ± 43##	110 ± 55
Cytokine		
IL-2R (U/l)	372 ± 137	361 ± 144
IL-6 (ng/ml)	4.0 ± 2.0*	3.3 ± 1.9
IL-8 (ng/ml)	10.3 ± 5.0	9.1 ± 5.8
IL-10 (ng/ml)	1.9 ± 1.0	1.8 ± 1.2
TNF-α (pg/ml)	9.7 ± 7.8	9.9 ± 8.0

Note. *$p < 0.05$, **$p < 0.01$ higher than those of the low-risk drinkers; #$p < 0.05$, ##$p < 0.01$ lower than those of the low-risk drinker.

higher level of IL-6 in the high-risk drinkers. In this study, we also evaluated the levels of IgA, complement 3, and complement 4 between two groups, but there were not significant differences (data not shown).

To investigate the effects of the interaction of smoking and drinking on the immunological biomarkers, we compared the parameters as shown in Table 3. The synergistic effects of smoking and drinking on the count of WBC, especially mononuclear cells, have been found statistically significant ($p = 0.01$ and $p = 0.009$, respectively). In the high-risk drinkers, there was significantly higher WBC count, especially mononuclear cell count in subjects with smoking. Furthermore, there were lower levels of IgG and IgM in the low-risk drinkers with smoking habits. On the contrary, there was a higher IL-10 level in the low-risk drinkers with smoking habits. Furthermore, Table 4 showed that there was no interaction between the betel chewing and drinking on the immunological biomarkers.

In this study, the frequencies of the ADH2*1/*1, ADH2*1/*2, and ADH2*2/*2 genotypes in the study subjects were 23 (11%), 76 (37%), and 108 (52%), respectively. Gene frequencies of ADH2*1 and ADH2*2 inferred from the Hardy-Weinberg's equilibrium were 0.29 and 0.71, respectively. The deviation from the Hardy-Weinberg's expectation was not statistically significant ($\chi^2 = 2.82$, $p > 0.05$). Frequencies of the ALDH2*1/*1, ALDH2*1/*2, and ALDH2*2/*2 genotypes were 138 (67%), 60 (29%), and 9 (4%), respectively. Gene frequencies of ALDH2*1 and ALDH2*2 inferred from the Hardy-Weinberg's equilibrium were 0.81 and 0.19, respectively. The deviation from the Hardy-Weinberg's expectation was statistically significant ($\chi^2 = 0.564$, $p > 0.05$). Frequencies of the CYP2E1C/C, CYP2E1C/T, and CYP2E1T/T genotypes in the study subjects were 128 (61%), 65 (31%), and 16 (8%), respectively. Gene

TABLE 3
Effects of Interaction of Smoking and Drinking on the Immunological Biomarkers

	High-risk drinkers		Low-risk drinkers		
Parameters	Smoking ($n = 57$)	Nonsmoking ($n = 48$)	Smoking ($n = 13$)	Nonsmoking ($n = 89$)	p Value for interaction
WBC ($\times 10^3/\mu l$)	6.94 ± 1.41**	5.50 ± 1.04	6.26 ± 0.85	6.00 ± 1.36	0.010
Mononuclear cells (%)	41.8 ± 7.3	42.5 ± 7.2	39.2 ± 5.4	41.7 ± 7.7	N.S.
Mononuclear cells ($\times 10^3/\mu l$)	2.9 ± 0.6**	2.3 ± 0.6	2.4 ± 0.4	2.5 ± 0.6	0.009
Lymphocyte subpopulations					
$CD3^+$ T lymphocytes (%)	66.4 ± 9.9	65.6 ± 9.1	62.8 ± 4.5	64.2 ± 8.6	N.S.
$CD4^+$ T helper cells (%)	35.8 ± 8.8	36.3 ± 9.6	32.6 ± 6.7	32.1 ± 8.2	N.S.
$CD8^+$ T cytotoxicity cells (%)	22.8 ± 6.3	22.1 ± 7.1	24.6 ± 7.0	24.7 ± 6.2	N.S.
Ratios of $CD4^+/CD8^+$	1.7 ± 0.7	1.8 ± 0.8	1.4 ± 0.6	1.4 ± 0.5	N.S.
$CD19^+$ B lymphocytes (%)	11.1 ± 4.5	11.2 ± 4.0	9.8 ± 3.1	12.2 ± 4.6	N.S.
Levels of immunoglobulin					
IgG (mg/dl)	1086 ± 240	1168 ± 226	1106 ± 109##	1223 ± 264	N.S.
IgM (mg/dl)	84 ± 43	95 ± 43	83 ± 30##	114 ± 57	N.S.
Cytokine					
IL-2R (U/l)	395 ± 141	346 ± 128	397 ± 109	356 ± 149	N.S.
IL-6 (ng/ml)	4.1 ± 2.1	3.9 ± 2.0	3.3 ± 2.3	3.3 ± 1.9	N.S.
IL-8 (ng/ml)	10.2 ± 5.2	10.6 ± 4.6	8.9 ± 5.6	10.9 ± 7.3	N.S.
IL-10 (ng/ml)	2.0 ± 1.0	1.9 ± 1.0	2.5 ± 1.3*	1.7 ± 1.2	N.S.
TNF-α (pg/ml)	10.2 ± 8.7	9.0 ± 6.1	12.3 ± 13.1	10.1 ± 8.2	N.S.

Note. *$p < 0.05$, **$p < 0.01$ higher than those of the nonsmoking group; #$p < 0.05$, ##$p < 0.01$ lower than those of the nonsmoking group. N.S.: Not Significant

TABLE 4
Effects of Interaction of Betel Chewing and Drinking on the Immunological Biomarkers

	High-risk drinkers		Low-risk drinkers		
	Betel chewing ($n = 21$)	Nonbetel chewing ($n = 84$)	Betel chewing ($n = 2$)	Nonbetel chewing ($n = 100$)	p Value for interaction
WBC ($\times 10^3/\mu l$)	6.62 ± 1.52	6.20 ± 1.42	6.34 ± 0.13	6.03 ± 1.32	N.S.
Mononuclear cells (%)	42.0 ± 6.6	42.1 ± 7.4	39.6 ± 9.8	41.4 ± 7.4	N.S.
Mononuclear cells ($\times 10^3/\mu l$)	2.8 ± 0.8	2.6 ± 0.6	2.5 ± 0.7	2.5 ± 0.6	N.S.
Lymphocyte subpopulation					
T helpers (CD4%)	38.2 ± 6.1	35.4 ± 9.7	36.1 ± 14.4	32.1 ± 7.9	N.S.
T cytotoxicity (CD8%)	21.9 ± 6.4	22.7 ± 6.7	34.3 ± 22.6	24.5 ± 5.8	N.S.
Ratios of CD4/CD8	1.9 ± 0.6	1.7 ± 0.8	1.5 ± 1.4	1.4 ± 0.5	N.S.
Total protein (g/dl)	7.1 ± 0.6	7.2 ± 0.6	7.2 ± 0.8	7.5 ± 0.6	N.S.
Albumin (g/dl)	4.5 ± 0.4	4.4 ± 0.3	4.5 ± 0.6	4.5 ± 0.4	N.S.
Globulin (g/dl)	2.6 ± 0.3#	2.8 ± 0.5	2.7 ± 0.2	3.0 ± 0.5	N.S.
Levels of immunoglobulin					
IgG (mg/dl)	1051 ± 256	1141 ± 229	1021 ± 174	1212 ± 253	N.S.
IgM (mg/dl)	78 ± 30	92 ± 46	75 ± 31	111 ± 55	N.S.
Cytokine					
IL-6 (ng/ml)	4.1 ± 1.9	3.9 ± 2.1	4.7 ± 3.7	3.2 ± 1.9	N.S.
IL-10 (ng/ml)	2.1 ± 1.1	1.9 ± 1.0	2.0 ± 2.2	1.8 ± 1.2	N.S.
TNF-α (pg/ml)	15.4 ± 13.7*	8.3 ± 4.4	16.8 ± 8.4	9.7 ± 8.0	N.S.
Complement C3 (mg/dl)	132 ± 21*	119 ± 22	164 ± 17**	121 ± 22	N.S.

Note. * $P < 0.05$, ** $P < 0.01$ higher than those of the nonbetel chewing group; # $P < 0.05$, ## $P < 0.01$ lower than those of the nonbetel chewing group. N.S.: Not Significant

frequencies of CYP2E1C and CYP2E1T inferred from the Hardy-Weinberg's equilibrium were 0.77 and 0.23, respectively. The deviation from the Hardy-Weinberg's expectation was not statistically significant ($\chi^2 = 3.39$, $p > 0.05$).

Table 5 showed that the frequencies of alleles, genotypes, and phenotypes of alcohol-metabolizing enzymes between the high-risk and low-risk drinkers. There were significant differences in alleles and genotypes of ALDH2 between the two drinker groups. On the contrary, there were no significant differences in alleles and genotypes of ADH2, CYP2E1, and CAT between the two drinker groups.

Because the drinking habit behavior can be affected by genetic variation of alcohol-metabolizing enzymes, we assessed the estimated odds ratios of the three alcohol-metabolizing pathways in the high-risk drinkers and low-risk drinkers. As shown in Table 6, in the major pathway and minor pathway 1, there were significantly higher odds ratios (ORs) in the subjects with ALDH2 (*1/*1) than in the reference group no matter which genotypes of ADH2 and CYP2E1 the subjects combined with. In the minor pathway 2, there were marked increased OR values in the subjects with ALDH2 (*1/*1) combined with either genotypes of CAT, although there was no statistical significance.

Because alcohol was mainly metabolized in the major pathway (Fig. 1), we compared the immunological biomarkers among subjects with different combined genotypes of ADH2 and ALDH2 in alcohol-metabolizing major pathway (Table 7). Among subjects with combined ADH2 (*1/*1+*1/*2) + ALDH2 (*1/*2 + *2/*2) genotypes (Group I), there were significantly lower levels of IgG in the high-risk drinkers. Among subjects with combined ADH2 (*1/*1 + *1/*2) + ALDH2 (*1/*1) genotypes (Group II), there were lower level of IgM in the high-risk drinkers. Furthermore, subjects with combined ADH2 (*2/*2) + ALDH2 (*1/*2 + *2/*2) genotypes (Group III), there were higher percentages of mononuclear cells, $CD3^+$ and $CD4^+$ T lymphocytes, but lower levels of IgM in the high-risk drinkers. Finally, subjects with combined ADH2 (*2/*2) + ALDH2 (*1/*1) genotypes (Group IV) had higher ratios of $CD4^+/CD8^+$, but lower levels of IgG in the high-risk drinkers.

DISCUSSION

To our knowledge, our study was the first report which described the effects of alcohol-metabolizing enzyme polymorphisms on the immunological biomarkers. It is well known that alcohol can interfere with both cell-mediated and humoral immunities in human (Szabo, 1997).

In the studies of Bataille *et al*. (2003), they indicated that the CAGE and AUDIT questionnaires can be better devices for the detection of heavy drinkers than any of the biochemical markers and can be effectively used to detect people with formal alcohol disorders and those having hazardous alcohol intake. In this study, there were higher scores of CAGE or AUDIT in the high-risk drinkers, which was consistent with the

TABLE 5
Frequencies of Allele and Genotype Polymorphisms of Alcohol-Metabolizing Enzymes in the Study Population

Genetic polymorphisms		High-risk drinkers (n = 105)	Low-risk drinkers (n = 102)	Odds ratio (95% CI)	p Value
ADH2 (Ex3 Arg47His)					
Alleles	*1	67/210	55/204	1	N.S.
	*2	143/210	149/204	0.79 (0.50–1.23)	
Genotypes					
Weak activity	(*1/*1)	16	7	1	N.S.
Intermediate activity	(*1/*2)	35	41		
Normal activity	(*2/*2)	54	54	0.88 (0.49–1.59)	
Phenotypes	*1	51	48	1	N.S.
	*2	89	95	0.88 (0.53–1.48)	
ALDH2 (Ex12 Lys504Glu)					
Alleles	*1	191/210	145/204	1	< 0.001
	*2	19/210	59/204	0.24 (0.13–0.44)	
Genotypes					
Normal activity	(*1/*1)	87	51	1	< 0.001
Intermediate activity	(*1/*2)	17	43	0.21 (0.10–0.40)	
Weak activity	(*2/*2)	1	8		
Phenotypes	*1	104	94	1	< 0.001
	*2	18	51	0.32 (0.17–0.61)	
CYP2E1 (−1053C>T)					
Alleles	C	161/210	156/204	1	N.S.
	T	49/210	52/204	0.91 (0.57–1.46)	
Genotypes					
Weak activity	(C/C)	62	64	1	N.S.
Intermediate activity	(C/T)	37	28	1.17 (0.64–2.12)	
Normal activity	(T/T)	6	10		
Phenotypes	C	99	92	1	N.S.
	T	43	38	1.05 (0.60–1.83)	
CAT (−262C>T)					
Alleles	C	203/210	198/204	1	N.S.
	T	7/210	6/204	1.14 (0.34–3.89)	
Genotypes					
Weak activity	(C/C)	98	96	1	N.S.
Intermediate activity	(C/T)	7	6	1.14 (0.33–4.01)	
Normal activity	(T/T)	0	0		
Phenotypes	C	105	102	1	N.S.
	T	7	6	1.13 (0.33–3.96)	

Note. N.S.: not significant.

study of Dawson *et al*. (2005). In addition, we found that there were approximate 79% of the participants' scores of AUDIT in the high-risk drinkers were over intersection score while only 2% of those were so in the low-risk drinkers. Meanwhile, the average score of AUDIT was greater than the point of intersection and the average score of CAGE was closely to the point of intersection in the high-risk drinkers. Furthermore, there was higher activity of GGT in the high-risk drinkers; thus, this result showed that the classification of drinking from the modified questionnaires is adequate in this study.

In this study, the number of females was smaller than that of males in the high-risk drinkers. In Taiwan, there have always been more male drinkers than female ones; therefore, there were bound to be more high-risk male drinkers than female ones in our samples, which we also made gender adjustment statistically. In addition, we found that there were higher levels of BMI in the high-risk drinkers with the combination of smoking and betel chewing habits. In southern Taiwan, the betel chewing is a special living habit, which promotes spirits during work among laborers, and their education levels were usually lower than those of the white-collar groups. Generally speaking, alcohol intake usually accompanies high-fat food intake; moreover, the synthesis of fatty acid is increased after the excessive alcohol intake and then causes a higher level of TG in blood. In our study, there were higher levels of TG in the high-risk drinkers and this result is consistent with the previous studies by Pennington (Pennington *et al*., 2002; Pownall *et al*., 1999), which indicated that concurrent alcohol intake and especially alcohol plus high-fat intake markedly increased serum TG levels in humans. Therefore, it may be a reason for the high BMI in the high-risk drinkers.

In general, the effects of alcohol on the immunological functions include the inflammatory reaction and the development of immunity to the pathogens, thus, we have investigated the biomarkers involved in both cell-mediated and humoral immunity. In the study of Cook (1998), they indicated that abnormalities of immune functions could be accompanied by alterations in the percentages of lymphocyte subsets, and they also demonstrated that the ratio of $CD4^+/CD8^+$ was normal or elevated in chronic alcoholics. Our results are consistent with the study of Cook (1998). In our study, the mean ratio of $CD4^+/CD8^+$ T lymphocytes in the high-risk drinkers was higher than that of the low-risk drinkers. In addition, there were significantly higher in the percentages of $CD4^+$ and $CD8^+$ T lymphocytes in the high-risk drinkers than those of the low-risk drinkers, but there was no significant difference of the percentages of B lymphocyte between the high- and low-risk drinkers. However, in another study by Arosa *et al*. (2000), there was no significant difference of the percentages of $CD4^+$ or $CD8^+$ T lymphocytes between the controls and the heavy alcohol drinkers. Therefore, other factors are apparently involved in the abnormalities of lymphocyte subsets in alcoholics or heavy alcohol drinkers. Furthermore, levels of the protein and immunoglobulins related to liver functions were reduced in the high-risk drinkers, meanwhile, we found that the levels of total protein in the betel chewers were lightly lower than those of the nonbetel chewers. These results may reflect the poorer nutrition status in those who were both

TABLE 6
Estimated Odds Ratios of the Three Alcohol-metabolizing Pathways

Combined genotypes			High-risk drinkers (*n*)	Low-risk drinkers (*n*)	Odds ratio (95% CI)	*p* Value
Major pathway						
Groups	ADH2	ALDH2				
I	*1/*1 + *1/*2	*1/*2 + *2/*2	10	26	Reference	
II	*1/*1 + *1/*2	*1/*1	41	22	4.85 (1.82–13.15)	< 0.001
III	*2/*2	*1/*2 + *2/*2	8	25	0.83 (0.25–2.78)	N.S.
IV	*2/*2	*1/*1	46	29	4.12 (1.61–10.78)	< 0.001
Minor pathway 1						
Groups	CYP2E1	ALDH2				
A	C/T + T/T	*1/*2 + *2/*2	6	20	Reference	
B	C/T + T/T	*1/*1	37	18	6.85 (2.11–23.27)	< 0.001
C	C/C	*1/*2 + *2/*2	12	31	1.29 (0.37–4.64)	N.S.
D	C/C	*1/*1	50	33	5.05 (1.68–15.86)	< 0.001
Minor pathway 2						
Groups	CAT	ALDH2				
1	C/T + T/T	(*1/*2 + *2/*2) + (*1/*1)	7	6	Reference	
2	C/C	*1/*2 + *2/*2	16	47	0.29[a] (0.07–1.16)	N.S.
3	C/C	*1/*1	82	49	1.43[a] (0.40–5.11)	N.S.

Note. CI: confidence interval. N.S.: not significant.
[a]Fisher exact.

high-risk drinkers and betel chewers; therefore, they had insufficient production of effective antibodies to fight against infectious diseases. It is well known that B and T lymphocytes communicate with each other and with other immune cells by secreting numerous cytokines that can influence various components of both nonspecific and specific immune responses. In this study, levels of IL-6 in the high-risk drinkers were higher than those in the low-risk drinkers, which meant that excessive cytokines levels might cause tissue damages. This result was consistent with the study of Brown *et al*.

TABLE 7
Comparisons of Immunological Biomarkers in Different Combined Genotypes of ADH2 and ALDH2

	I		II		III		IV	
Parameters	High-risk drinkers ($n = 10$)	Low-risk drinkers ($n = 26$)	High-risk drinkers ($n = 41$)	Low-risk drinkers ($n = 22$)	High-risk drinkers ($n = 8$)	Low-risk drinkers ($n = 25$)	High-risk drinkers ($n = 46$)	Low-risk drinkers ($n = 29$)
WBC ($\times 10^3/\mu l$)	6.8 ± 1.2	6.1 ± 1.2	6.3 ± 1.5	5.7 ± 1.4	6.5 ± 1.5	6.3 ± 1.1	6.1 ± 1.5	6.0 ± 1.5
Mononuclear cells (%)	37.8 ± 4.7	40.5 ± 7.2	42.4 ± 7.7	42.9 ± 9.0	45.1 ± 4.3*	40.7 ± 4.5	42.2 ± 7.4	41.5 ± 8.5
Mononuclear cells ($\times 10^3/\mu l$)	2.6 ± 0.5	2.4 ± 0.5	2.7 ± 0.8	2.4 ± 0.8	2.9 ± 0.6	2.5 ± 0.5	2.5 ± 0.6	2.4 ± 0.7
$CD3^+$ T lymphocytes (%)	64.8 ± 8.7	64.2 ± 8.3	66.1 ± 7.1	62.5 ± 10.2	72.3 ± 7.3*	65.5 ± 6.5	65.0 ± 11.3	63.8 ± 8.0
$CD4^+$ T helper cells (%)	32.8 ± 8.7	32.4 ± 8.7	35.3 ± 10.0	31.8 ± 8.5	39.0 ± 4.9*	32.2 ± 7.0	36.2 ± 9.2	32.2 ± 8.1
$CD8^+$ T cytotoxicity cells (%)	22.4 ± 6.4	24.1 ± 5.6	22.1 ± 6.1	25.7 ± 8.3	24.5 ± 6.2	26.1 ± 6.4	22.5 ± 7.4	23.1 ± 4.8
Ratios of $CD4^+/CD8^+$	1.7 ± 0.6	1.4 ± 0.5	1.8 ± 0.9	1.4 ± 0.6	1.8 ± 0.9	1.3 ± 0.5	1.8 ± 0.8*	1.5 ± 0.5
$CD19^+$ B lymphocytes (%)	11.2 ± 4.8	12.5 ± 4.7	11.9 ± 3.	11.9 ± 4.6	11.8 ± 5.4	12.9 ± 4.5	10.4 ± 4.4	10.6 ± 4.3
IgG (mg/dl)	1099 ± 118#	1295 ± 258	1155 ± 249	1155 ± 252	986 ± 217	1120 ± 192	1124 ± 245#	1247 ± 271
IgM (mg/dl)	112 ± 66	113 ± 58	81 ± 34#	113 ± 43	70 ± 20#	100 ± 38	95 ± 45	113 ± 71
Cytokine								
IL-2R (U/l)	331 ± 66	398 ± 169	391 ± 173	362 ± 160	356 ± 50	344 ± 123	367 ± 120	342 ± 125
IL-6 (ng/ml)	3.6 ± 2.4	2.7 ± 1.0	4.0 ± 2.2	3.1 ± 1.9	3.9 ± 2.1	3.3 ± 2.1	4.1 ± 1.9	3.8 ± 2.4
IL-8 (ng/ml)	9.6 ± 3.7	8.8 ± 3.9	11.0 ± 6.0	9.6 ± 6.2	9.1 ± 2.2	9.2 ± 6.8	10.2 ± 4.6	9.1 ± 6.1
IL-10 (ng/ml)	1.6 ± 0.6	1.7 ± 1.0	2.1 ± 1.2	1.6 ± 1.2	1.5 ± 0.6	2.1 ± 1.8	1.9 ± 1.0	1.7 ± 0.7
TNF-α (pg/ml)	7.6 ± 2.8	9.5 ± 7.2	9.3 ± 8.3	11.7 ± 10.4	8.6 ± 3.2	8.4 ± 6.0	10.8 ± 8.5	10.0 ± 8.2

Note. Group I: ADH2 (*1/*1 + *1/*2) + ALDH2 (*1/*2 + *2/*2), Group II: ADH2 (*1/*1 + *1/*2) + ALDH2 (*1/*1). Group III: ADH2 (*2/*2) + ALDH2 (*1/*2 + *2/*2), Group IV: ADH2 (*2/*2) + ALDH2 (*1/*1). *$p < 0.05$, **$p < 0.01$ higher than those of the low-risk drinkers; #$p < 0.05$, ##$p < 0.01$ lower than those of the low-risk drinkers.

(2006), which indicated that in chronic alcohol abusers, particularly those with alcoholic liver diseases, the levels of IL-6 were significantly elevated. In addition, the study of Crews *et al.* (2006) indicated that chronic alcohol use in humans is associated with increased proinflammatory cytokines; and there were massive increases in proinflammatory cytokines including tumor necrosis factor alpha (TNF-α), IL-1, and IL-6, and the chemokine IL-8 by the activation of monocytes and macrophages in alcoholic hepatitis (McClain and Cohen, 1989; McClain *et al.*, 1999). However, there were no significant differences of cytokines, except IL-6 in our study, which may be due to the fact that the participants were just high-risk drinking without alcoholic hepatitis. Furthermore, we analyzed the statistics of the relationship between IL-6 levels and the activities of either GGT or AST in the high-risk drinkers with/without the habit of betel chewing and we found that there were no significant differences. However, there was a significant positive relationship between the GGT activity and IL-6 levels between the high- and low-risk drinkers (Pearson correlation = 0.279, p value = 0.005).

The study of Wu *et al.* (2001) reported that habits of consuming cigarettes, alcohol and areca were the major risk factors for developing esophageal squamous cell carcinoma in Taiwan. In this study, we found that synergistic effects of smoking and drinking on the immunological biomarkers were the counts of WBC and mononuclear cells (Table 3). It meant that the immune system might be impaired in the subjects with habits of both the drinking and smoking. In addition, smoking habit-induced effects on immunity may be attributed to the alteration of humoral immunity. In this study, we found that the elevated anti-inflammatory cytokine IL-10 might play an important role on the immune system in the subject with combination of low-risk drinking and smoking. IL-10 has been identified to diminish the extent or persistence of some acquired or innate immune responses and inhibit the production of proinflammatory cytokines (Grimbaldeston *et al.*, 2007), but the exact mechanism still need to be further studied.

Chang *et al.* (2005) has indicated that the component of areca nut (AN) could result in the alterations of some cytokine secretion and the cell-mediated immunity. Previous studies have indicated that the habit of betel chewing is associated with increased risks of cirrhosis as well as hepatocellular cancer (Tsai *et al.*, 2001; Wu *et al.*, 2009). In this study, there was no interaction between betel chewing and drinking on the immunological biomarkers (data shown as Table 4); however, we found that the levels of TNF-α and C3 in the groups with betel chewing were higher than those of the nonbetel chewing groups regardless of whether they were high-risk or low-risk drinkers. Therefore, whether the higher levels of TNF-α and C3 were the factors involved in the alterations of liver functions and immunological functions and whether the impact on the immunity by smoking or betel chewing is greater than those of alcohol or not required further studies for confirmation.

In our previous studies (Tseng *et al.*, 2007, 2008b), we have found that there was association between the genetic variations of alcohol-metabolizing enzymes and increased risk of traumatic occasions at ED. Among the alcohol-metabolizing enzymes, the polymorphisms of major enzymes such as ADH2 and ALDH2 were involved in the rate of alcohol oxidation and acetaldehyde elimination, and minor enzymes such as catalase was also involved in the elimination of reactive oxygen species, which can result in damages of cells. Additionally, chronic alcohol intake can induce the production of CYP2E1, leading to the generation of ROS and enhancement of procarcinogen activation (Zhang *et al.*, 2007). In this study, we found that there was significantly higher OR in subjects with the combination of ALDH2 (*1/*1) genotype and either genotype of ADH2 and CYP2E1. In other words, the participants with the ALDH2 (*1/*1) genotype were predisposed to becoming high-risk drinkers. However, there were no such findings in the groups with the combination of ALDH2 (*1/*1) and CAT; although there were higher ORs in the Groups 1 and 4, there were no significant differences. It may be due to the small samples in the participants with CAT T carrier.

The faster elimination of acetaldehyde by the normal activity of ALDH2 (*1/*1) may cause the over-drinking behavior and more adverse effects, including impairment of immunity. Therefore, we further examined whether different combinations of ADH2 and ALDH2 genotypes in major alcohol-metabolizing pathway affected immunological biomarkers. Previous studies showed that the roles of the ADH2 and ALDH2 genotypes on immunity were controversial (Kaliappan *et al.*, 2008; Matsuo *et al.*, 2006; Sakamoto *et al.*, 2006). As shown in Table 5, owing to higher activity of ADH2, synthesis of acetaldehyde in Groups III and IV was more rapid than that in the Groups I and II, which would result in more abnormal immunological functions. Furthermore, we found that there were lower levels of immunoglobulins and higher percentages of mononuclear cells and $CD3^+$ and $CD4^+$ T lymphocytes in Group III than those in the other groups. The quicker synthesis and slower degradation of acetaldehyde in Group III may result in more toxic effects on immunological functions.

In this study, we think that the genomic combination based on alcohol-metabolizing pathways is logical; however, this causes significant reduction in the statistical power because there is only a small number of an individual under each group. Therefore, statistical power appears to be the major limitation of the research. Besides, there are other limitations, which include (1) the number of females was smaller than that of males in the high-risk drinkers due to the social fact about the gender of drinkers in Taiwan, and (2) the different genomic combinations of ADH2 and ALDH2 in the major alcohol-metabolizing pathway result in significant reduction in the statistical power because there is only a small number of an individual under each group. Furthermore, our previous study (Tseng *et al.*, 2007) showed that the frequency of ADH2 was different from the results of the present study, but the frequencies of ALDH2 and CYP2E1

were similar to the results of the present study. In that study (Tseng *et al*., 2007), the major study population was trauma patients with excessive alcohol intake in an ED; however, the major study population in this study was general workers who were hospital employers, general blue-collar laborers, and white-collar workers. Therefore, we thought that the major cause was due to the difference of the study populations.

In conclusion, our results suggested that the habits such as drinking, smoking, betel chewing, and genetic variations of alcohol metabolism were associated with immunological biomarkers. However, the mechanisms of alcohol-associated alterations and the genetic variations on immunity are still incompletely understood; therefore, further studies will be needed.

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REFERENCES

Arosa, F. A., Porto, G., Cabeda, J. M., Lacerda, R., Resende, D., Cruz, E., Cardoso, C., Fonseca, M., Simoes, C., Rodrigues, P., *et al*. (2000). Expansions of CD8+CD28- and CD8+TcRVbeta5.2+ T cells in peripheral blood of heavy alcohol drinkers. *Alcohol Clin. Exp. Res.* **24,** 519–527.

Bataille, V., Ruidavets, J. B., Arveiler, D., Amouyel, P., Ducimetiere, P., Perret, B., and Ferrieres, J. (2003). Joint use of clinical parameters, biological markers and CAGE questionnaire for the identification of heavy drinkers in a large population-based sample. *Alcohol Alcohol* **38,** 121–127.

Brown, L. A., Cook, R. T., Jerrells, T. R., Kolls, J. K., Nagy, L. E., Szabo, G., Wands, J. R., and Kovacs, E. J. (2006). Acute and chronic alcohol abuse modulate immunity. *Alcohol Clin. Exp. Res.* **30,** 1624–1631.

Chang, M. C., Chiang, C. P., Lin, C. L., Lee, J. J., Hahn, L. J., and Jeng, J. H. (2005). Cell-mediated immunity and head and neck cancer: with special emphasis on betel quid chewing habit. *Oral Oncol.* **41,** 757–775.

Cook, R. T. (1998). Alcohol abuse, alcoholism, and damage to the immune system—A review. *Alcohol Clin. Exp. Res.* **22,** 1927–1942.

Crews, F. T., Bechara, R., Brown, L. A., Guidot, D. M., Mandrekar, P., Oak, S., Qin, L., Szabo, G., Wheeler, M., and Zou, J. (2006). Cytokines and alcohol. *Alcohol Clin. Exp. Res.* **30,** 720–730.

Dawson, D. A., Grant, B. F., Stinson, F. S., and Zhou, Y. (2005). Effectiveness of the derived Alcohol Use Disorders Identification Test (AUDIT-C) in screening for alcohol use disorders and risk drinking in the US general population. *Alcohol Clin. Exp. Res.* **29,** 844–854.

Forsberg, L., Lyrenas, L., de Faire, U., and Morgenstern, R. (2001). A common functional C-T substitution polymorphism in the promoter region of the human catalase gene influences transcription factor binding, reporter gene transcription and is correlated to blood catalase levels. *Free Radic. Biol. Med.* **30,** 500–505.

Goulas, A., Fidani, L., Kotsis, A., Mirtsou, V., Petersen, R. C., Tangalos, E., and Hardy, J. (2002). An association study of a functional catalase gene polymorphism, -262C–>T, and patients with Alzheimer's disease. *Neurosci. Lett.* **330,** 210–213.

Grimbaldeston, M. A., Nakae, S., Kalesnikoff, J., Tsai, M., and Galli, S. J. (2007). Mast cell-derived interleukin 10 limits skin pathology in contact dermatitis and chronic irradiation with ultraviolet B. *Nat. Immunol.* **8,** 1095–1104.

Gul, S., Akvardar, Y., Tas, G., and Tuncel, P. (2005). [The diagnostic validity of screening tests and laboratory markers in alcohol use disorders]. *Turk Psikiyatri Derg.* **16,** 3–12.

Kaliappan, S., Jha, P., Lyzogubov, V. V., Tytarenko, R. G., Bora, N. S., and Bora, P. S. (2008). Alcohol and nicotine consumption exacerbates choroidal neovascularization by modulating the regulation of complement system. *FEBS Lett.* **582,** 3451–3458.

Lieber, C. S. (2000). Alcoholic liver disease: new insights in pathogenesis lead to new treatments. *J. Hepatol.* **32,** 113–128.

Matsuo, K., Wakai, K., Hirose, K., Ito, H., Saito, T., Suzuki, T., Kato, T., Hirai, T., Kanemitsu, Y., Hamajima, H., and Tajima, K. (2006). A gene-gene interaction between ALDH2 Glu487Lys and ADH2 His47Arg polymorphisms regarding the risk of colorectal cancer in Japan. *Carcinogenesis* **27,** 1018–1023.

McBride, W. J., Le, A. D., and Noronha, A. (2002). Central nervous system mechanisms in alcohol relapse. *Alcohol Clin. Exp. Res.* **26,** 280–286.

McClain, C. J., Barve, S., Deaciuc, I., Kugelmas, M., and Hill, D. (1999). Cytokines in alcoholic liver disease. *Semin. Liver Dis.* **19,** 205–219.

McClain, C. J., and Cohen, D. A. (1989). Increased tumor necrosis factor production by monocytes in alcoholic hepatitis. *Hepatology* **9,** 349–351.

Nicolaou, C., Chatzipanagiotou, S., Tzivos, D., Tzavellas, E. O., Boufidou, F., and Liappas, I. A. (2004). Serum cytokine concentrations in alcohol-dependent individuals without liver disease. *Alcohol* **32,** 243–247.

Pennington, J. S., Shuvaeva, T. I., and Pennington, S. N. (2002). Maternal dietary ethanol consumption is associated with hypertriglyceridemia in adult rat offspring. *Alcohol Clin. Exp. Res.* **26,** 848–855.

Pownall, H. J., Ballantyne, C. M., Kimball, K. T., Simpson, S. L., Yeshurun, D., and Gotto, A. M., Jr. (1999). Effect of moderate alcohol consumption on hypertriglyceridemia: A study in the fasting state. *Arch Intern. Med.* **159,** 981–987.

Sakamoto, T., Hara, M., Higaki, Y., Ichiba, M., Horita, M., Mizuta, T., Eguchi, Y., Yasutake, T., Ozaki, I., Yamamoto, K., *et al*. (2006). Influence of alcohol consumption and gene polymorphisms of ADH2 and ALDH2 on hepatocellular carcinoma in a Japanese population. *Int. J. Cancer* **118,** 1501–1507.

Schleifer, S. J., Keller, S. E., and Czaja, S. (2006). Major depression and immunity in alcohol-dependent persons. *Brain Behav. Immun.* **20,** 80–91.

Sun, F., Tsuritani, I., Honda, R., Ma, Z. Y., and Yamada, Y. (1999). Association of genetic polymorphisms of alcohol-metabolizing enzymes with excessive alcohol consumption in Japanese men. *Hum. Genet.* **105,** 295–300.

Szabo, G. (1997). Alcohol's contribution to compromised immunity. *Alcohol Health Res. World* **21,** 30–41.

Tsai, J. F., Chuang, L. Y., Jeng, J. E., Ho, M. S., Hsieh, M. Y., Lin, Z. Y., and Wang, L. Y. (2001). Betel quid chewing as a risk factor for hepatocellular carcinoma: A case-control study. *Br. J. Cancer* **84,** 709–713.

Tseng, Y. M., Chen, S. Y., Chen, C. H., Jin, Y. R., Tsai, S. M., Chen, I. J., Lee, J. H., Chiu, C. C., and Tsai, L. Y. (2008a). Effects of alcohol-induced human peripheral blood mononuclear cell (PBMC) pretreated whey protein concentrate (WPC) on oxidative damage. *J. Agric. Food Chem.* **56,** 8141–8147.

Tseng, Y. M., Hu, B. W., Tsai, S. M., Chen, I. J., Jin, Y. R., Lee, J. H., Huang, F. D., Wu, S. H., Yeh, F. L., and Tsai, L. Y. (2007). Distribution of alcohol-metabolizing enzyme genotypes in trauma patients with excessive alcohol consumption in the emergency department. *Clin. Biochem.* **40,** 370–376.

Tseng, Y. M., Jin, Y. R., Chen, I. J., Huang, F. D., Wu, S. H., Ma, H., Chen, S. Y., Tsai, L. Y., Tsai, S. M., and Lee, J. H. (2008b). Roles of the genetic variation of alcohol-metabolizing enzymes on biomarkers in trauma patients with excessive alcohol intake at emergency department. *Clin. Chim. Acta* **389,** 14–18.

Tsoumakidou, M., Elston, W., Zhu, J., Wang, Z., Gamble, E., Siafakas, N. M., Barnes, N. C., and Jeffery, P. K. (2007). Cigarette smoking alters bronchial mucosal immunity in asthma. *Am. J. Respir. Crit. Care Med.* **175,** 919–925.

World Health Organization (WHO). (2000). *International Guide for Monitoring Alcohol Consumption and Related Harm*. World Health Organization, Switzerland.

Wu, G. H., Boucher, B. J., Chiu, Y. H., Liao, C. S., and Chen, T. H. (2009). Impact of chewing betel-nut (Areca catechu) on liver cirrhosis and hepatocellular carcinoma: A population-based study from an area with a high prevalence of hepatitis B and C infections. *Public Health Nutr.* **12,** 129–135.

Wu, M. T., Lee, Y. C., Chen, C. J., Yang, P. W., Lee, C. J., Wu, D. C., Hsu, H. K., Ho, C. K., Kao, E. L., and Lee, J. M. (2001). Risk of betel chewing for oesophageal cancer in Taiwan. *Br. J. Cancer* **85,** 658–660.

Zhang, F. F., Hou, L., Terry, M. B., Lissowska, J., Morabia, A., Chen, J., Yeager, M., Zatonski, W., Chanock, S., and Chow, W. H. (2007). Genetic polymorphisms in alcohol metabolism, alcohol intake and the risk of stomach cancer in Warsaw, Poland. *Int. J. Cancer* **121,** 2060–2064.